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PHASE II STUDY OF COMBINED TEMOZOLOMIDE AND TARGETED P53 GENE THERAPY (SGT-53) FOR TREATMENT OF PATIENTS WITH RECURRENT GLIOBLASTOMA

PROTOCOL NUMBER: SGT53-02-2

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This study will be performed in compliance with Good Clinical Practices (GCP) and applicable regulatory requirements, including the archiving of essential documents. Information contained in this protocol is

confidential in nature, and must not be used, divulged, published or otherwise disclosed to others except to the extent necessary to obtain approval of the Institutional Review Board or Independent Ethics Committee, or as required by law. Persons to whom this information is disclosed must be informed that this information is confidential and may not be further disclosed without the express permission of SynerGene.

SIGNATURES OF AGREEMENT FOR PROTOCOL

I, the undersigned, have read this protocol and confirm that to the best of my knowledge it accurately describes the planned conduct of the study.

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1. INVESTIGATOR APPROVAL STATEMENT

I have read this protocol and agree to conduct this clinical trial as outlined herein. I will ensure that all subinvestigators and other study staff members have read and understand all aspects of this protocol. I agree to cooperate fully with SynerGene and any appointed CRO during the study. I will adhere to the Declaration of Helsinki and its amendments, the International Conference on Harmonisation (ICH) principles of Good Clinical Practice (GCP; including archiving of essential study documents), and all US FDA regulations (for US sites) or European Directives (for European sites) and other applicable regulations and guidelines of the country in which the study is conducted.

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3. PROTOCOL SYNOPSIS

Title	PHASE II STUDY OF COMBINED TEMOZOLOMIDE AND TARGETED P53 GENE THERAPY (SGT-53) FOR TREATMENT OF PATIENTS WITH RECURRENT GLIOBLASTOMA
Study Number	SGT53-02-2
Clinical Study Phase	Phase II
Clinical Sites	MD Anderson Cancer Center, Texas, USA China Medical University Hospital, Taichung, Taiwan
Study Objectives	Primary: • To evaluate the 6 month PFS in patients treated with SGT-53 in combination with Temozolomide. Secondary: • To assess safety of the combination of SGT-53 and Temozolomide. • To evaluate progression free survival (PFS), and overall survival (OS) in patients in patients with recurrent or progressive GBM tumors treated with SGT-53 in combination with Temozolomide. • To determine the anti-tumor activity of the combination of SGT-53 and Temozolomide based upon the RANO criteria in patients with recurrent or progressive GBM tumors. • To assess nanoparticle delivery to tumor site by analysis of the presence of exogenous wt p53 in the tumor (optional procedure). • To assess induction of apoptosis by SGT-53 in the tumor by flow cytometry or histology (optional procedure).
Study Design	This is a single arm study wherein SGT-53, at a dose of 3.6 mg DNA per infusion, will be administered. SGT-53 will be administered twice weekly [every 3rd or 4th day] in a 28 day cycle (total of 6 infusions)] starting on Day 1 (cycle 1), Day 29 (cycle2) and Day 57 (cycle 3). In those patients who undergo the optional surgical resection, the 1st cycle of SGT-53 and TMZ will start 14-21 days post operatively after having recovered from the effects of

surgery. Temozolomide will be administered by mouth daily on days 9-13 of each cycle at 150mg/m2 (cycle 1), and 200mg/m2 as tolerated for cycle 2 and beyond provided the patient meets the criteria for non-hematological toxicity, ANC and platelet counts.. Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycles of SGT-53/Temozolomide therapy at investigator's discretion. Alternatively, they may continue on Temozolomide alone at investigator's discretion.

Optional Surgical Resection for Tumor Analysis:

Surgical resection of recurrent or progressive tumor for tumor analysis is an optional procedure. In these individuals SGT-53, at a dose of 3.6 mg DNA per infusion, will be administered twice (on days -1 and -3) in the week prior to surgery. Surgical resection is Day 0. 14-21 days post operatively and having recovered from the effects of surgery, the patients will then start cyclical TMZ with

Study Subject Population

The study population is subjects with confirmed glioblastoma or gliosarcoma who have proven tumor recurrence or progression. Study participants must have measurable disease on imaging studies and Eastern Oncology Cooperative Group (ECOG) performance status 0-2 (Karnofsky performance status [KPS] $\geq$ 60). Tumors amenable to surgical resection is not a requirement.

Study Procedures

After screening, SGT-53 study drug will be administered by iv infusion twice weekly , beginning on Day 1 of a 28 day cycle as described above with Temozolomide administered orally daily on days 9-13 of each cycle at 150mg/m2 (cycle 1), and 200mg/m2 as tolerated from cycle 2 onwards provided the patient meets the criteria for non-hematological toxicity, ANC and platelet counts. . In those patients who have undergone the optional surgical resection, the 1st cycle of SGT-53 and TMZ will commence 14-21 days post operatively and after having recovered from the effects of surgery. Following first infusion of study agent on Day1, participants will be evaluated weekly for a total of at least 12 weeks. At these weekly study visits, participants will have a physical examination, routine chemistry, and complete blood count.

- After screening, SGT-53 will be administered by iv infusion twice during the week prior to surgical resection of the tumor. 14-21 days post operatively and having recovered from the effects of surgery, SGT-53 study drug will be administered by iv infusion twice weekly beginning on Day 1 of a 28 day cycle as described above.
- A sample of the tumor obtained at resection will be employed to assess levels of exogenous wild type (wt) p53 cDNA to validate targeting.

Primary Study Outcome Measures

- To evaluate the 6 month PFS in patients treated with SGT-53 in combination with Temozolomide.

Secondary Study Outcomes

- To assess safety of the combination of SGT-53 and Temozolomide by analysis of adverse experiences, clinical laboratory tests and physical examinations.
- To evaluate PFS and OS in patients in patients with recurrent or progressive GBM tumors treated with SGT-53 in combination with Temozolomide.
- To determine the anti-tumor activity of the combination of SGT-53 and Temozolomide based upon the RANO criteria in patients with recurrent or progressive GBM tumors.
- To assess induction of apoptosis by SGT-53 in the tumor by flow cytometry or histology (optional procedure).
- To assess nanoparticle tumor delivery by analysis of the presence of exogenous wt p53 in the tumor (optional procedure).

Sample Size

- Approximately 26 subjects will be enrolled.

Study Duration

- Approximately 12-24 months.

Statistical Analysis

- Safety data will be analyzed using summary descriptive statistics for the total study population. The primary outcome of this phase II study is clinical response using PFS6 as the indicator.

4. INTRODUCTION

4.1 Background

Primary brain tumors, and particularly gliomas, are one of the most difficult cancers to treat. It is expected that in the U.S. more than 22,000 will be diagnosed in 2012 (1), about 50% of which will be glioblastoma multiforme (GBM). GBM is one of the most lethal types of brain cancer, with a median survival of about 12 months (2). In addition to primary tumors, metastatic brain cancer from a variety of primary sources [predominately lung (60%), breast (20%) and melanoma (10%)], is diagnosed in over 150,000 patients a year (3). Thus, there is a critical need for improved therapies for brain cancers, which is confirmed by the fact that the NCI has made brain cancers one of its top 5 funding priorities. Such improved therapies would have significant commercial potential.

The current standard of therapy for GBM is surgical resection, followed by radiotherapy and chemotherapy with Temozolomide (TMZ). TMZ, a second-generation alkylating (methylating) agent causing cytotoxic DNA lesions, is also approved for treatment of anaplastic astrocytoma (AA) and is in clinical trials for treatment of brain metastases from other non-CNS solid tumors. The mechanism of action and pharmacological properties have been recently reviewed (2, 4). TMZ is relatively well tolerated (5), however myelosuppression, neutropenia and thrombocytopenia are among its side effects and therapeutic dosages are limited by these. Extended TMZ dosing regimens were also found to provoke lymphocytopenia and opportunistic infections (4). The extensive tissue distribution that results from the non-tumor specific uptake of the orally administered TMZ is a major causality for these side effects. Thus, tumor-targeting delivery of TMZ could help reduce these adverse events.

Temozolomide

4-Methyl-5-oxo-2,3,4,6,8-pentazabicyclo[4.3.0]nona-2,7,9-triene-9-carboxamide, (Temozolomide) a prodrug, is a monofunctional alkylating agent that readily crosses the blood-brain barrier (6). It is chemically related to dacarbazine and is the 3-methyl derivative of the experimental anticancer drug, mitozolomide. Unlike dacarbazine, temozolomide does not require hepatic metabolism to the intermediate species methyltriazen-1-yl imidazole-4-carboxamide (MTIC) but spontaneously hydrolyzes to MTIC above pH7. MTIC degrades to a highly reactive cation that methylates guanines in DNA at the O^6 position, causing base pair mismatch. Unsuccessful cycles of mismatch repair eventually lead to breaks and permanent nicks in the daughter strand preventing mitotic division and the cell undergoes apoptosis.

TMZ has shown survival benefit in a subset of GBM patients, however this median increase is only 2.5 months compared to radiation alone (7). Recent studies have also indicated that 60-75% of GBM patients and 50% of AA patients do not benefit from TMZ (7). The failure of chemotherapy can be attributed to a number of factors including, short half-life in circulation, efflux of drugs from the tumor by p-glycoprotein, resistance of the tumors to the drug and failure to cross the blood-brain barrier. The primary mechanism of resistance to TMZ is overexpression of O^6-methylguanine-DNA-methyl transferase (MGMT), which repairs the TMZ-induced DNA lesion by removing the O^6-guanine adducts (2). Thus, a means to down modulate MGMT activity would enhance the therapeutic effect of TMZ. A number of reports have indicated that

increasing wtp53 expression could down-regulate expression of DNA repair genes such as MGMT and increases the sensitivity of tumor cells to alkylating agents (8-10).

SGT-53

SynerGene Therapeutics, Inc. has developed a unique investigational product, SGT-53, that is a complex of cationic liposome encapsulating a normal human wtp53 DNA sequence in a plasmid backbone. The liposome is decorated on its surface with an anti-transferrin receptor (TfR) single chain antibody fragment (scFv) that is designed to target cancer cells through binding of the TfRscFv to the transferrin receptor. This complex has been shown to efficiently and specifically deliver the p53 cDNA to the tumor cells via receptor-mediated endocytosis of the cationic liposomal complex (11, 12). Introduction of the p53 cDNA sequence is expected to restore wtp53 function in the apoptotic pathway. P53 restoration has been shown most effective in enhancing cytotoxicity in combination with an agent which results in DNA damage or initiates apoptosis (13, 14).

Use of Wild-type (wt) p53 as an Anti-cancer Therapeutic Agent

The therapeutic agent in SGT-53 is a DNA sequence encoding normal human p53. The p53 gene is a vital tumor suppressor gene in humans. Abnormalities in the p53 tumor suppressor gene have been reported in over 60% of human cancer, including brain cancer (15, 16). The p53 protein has a diverse range of functions including regulation of cell cycle checkpoints, cell death (apoptosis), senescence, DNA repair, maintenance of genomic integrity, and control of angiogenesis. Abnormalities of the p53 gene may impact the efficacy of standard anticancer treatments such as radiation and chemotherapy. The development of somatic gene therapy has created the potential to restore wild type function of p53.

Currently, approximately 37 gene therapy clinical trials employing p53 have been initiated or conducted in the USA (>75 world wide) and this number is likely to increase over the coming years. Introduction of wtp53 by various viral gene therapy delivery systems, in particular retroviral and adenoviral vectors, has been reported to suppress the growth of various types of malignancies in both *in vitro* and in xenografts in mouse models. The types of malignancies studied include leukemia, prostate, head and neck, colon, cervical, glioblastomas, breast, liver, ovarian, kidney and lung tumor (17-19).

Gene Therapy Delivery

One of the major obstacles to gene therapy is the development of an effective gene delivery system specific to cancer cells. Drawbacks of viral delivery systems include immunogenicity, integration problems, the potential for self-replication and/or creation of new classes of infectious pathogens as well as lack of specificity for cancer cells. Gene therapy systems using cationic liposomes share the lack of specificity of viral vectors and have relatively low transfection efficiency. However, SynerGene Therapeutics, Inc. has devised a strategy to use cationic liposomes that target tumor cells with a high transfection efficiency.

Most tumor cells, including pancreatic, prostate, ovarian, oral, colon, and breast cancer, express both increased folate and transferrin receptors and have increased receptor recycling (11, 12). SynerGene Therapeutics, Inc. has demonstrated that addition of either the folate or transferrin ligand to the cationic liposome facilitates the entry of the liposome-DNA complex into the tumor cells via receptor-mediated endocytosis. Preferential delivery of genes into tumors using either the folate or transferrin ligands has been shown in both *in vitro* and *in vivo* mouse models (20-22).

In addition to the use of ligands that bind to receptors on tumor cells, specific monoclonal antibodies (mAb) also can be attached to the liposome surface enabling them to target specific tumor surface antigens (including but not limited to receptors). These "immunoliposomes" can also deliver therapeutic drugs or genes to a specific target cell population (23). These immunoliposomes were found to be internalized efficiently by receptor-mediated endocytosis via the coated pit pathway and also possibly by membrane fusion. Immunoliposomes are being developed for a variety of therapeutic uses. Currently, they are primarily being used in drug therapy, delivering drugs, pro-drugs and enzymes capable of activating pro-drugs.

There are drawbacks and limitations to the use of the mAb-based agents. Full-sized mAbs exhibit a prolonged circulation time that can produce unacceptable myelotoxicities following bone marrow exposure, particularly when the mAb is coupled with radioisotopes. Adverse toxic reactions also can occur due to interactions between Fc receptors on normal tissues and the mAb carrying radioisotopes or toxins. Moreover, the large size of the intact mAb (approximately 155kDa) can limit their ability to diffuse from the capillaries into the solid tumor and thus their potential therapeutic effect in treating cancer.

SGT-53

The therapeutic component of SGT-53 is the DNA sequence for the normal human p53, situated within a plasmid backbone. When taken up by tumor cells, this DNA sequence is expressed as the wild-type p53 protein, and is functional as a tumor suppressor regulating tumor cell growth, cell death (apoptosis) and regulation of angiogenesis.

The delivery component of SGT-53 is a cationic liposome that encapsulates the plasmid-wtp53 DNA. Liposomes can be made from a wide variety of lipids, which have very different properties and characteristics. Many liposomes, including the commercially available liposome: chemotherapeutic agents Doxil, DaunoXome and AmBisome, contain cholesterol, which has been shown to be a stimulator of complement. Administration of these lipid-drug complexes can induce a "pseudoallergic" infusion reaction in some patients, presumably due to the presence of cholesterol in the liposome. The liposome component of SGT-53 is comprised of two different types of lipids (DOTAP and DOPE), and does not contain cholesterol.

The component of SGT-53 that allows targeting to tumor tissues after systemic administration is a single chain antibody fragment to the human transferrin receptor (TfRscFv) which decorates the surface of the liposome. Progress in biotechnology has allowed the derivation of specific recognition domains from monoclonal antibodies (mAb). The recombination of the variable regions of heavy and light chains and their integration into a single polypeptide provides the

possibility of employing single-chain antibody derivatives (designated scFv) for targeting purposes. A scFv based on an anti-TfR mAb, contains the complete antibody binding site for the epitope of the transferrin receptor (TfR) recognized by this mAb as a single polypeptide chain of approximate molecular weight 28,000. The TfRscFv is formed by connecting the component VH and VL variable domains from the respective heavy and light chains with an appropriately designed linker peptide. The binding site of an scFv can replicate both the affinity and specificity of its parent antibody combining site. For a number of reasons TfRscFv has advantages in human use over the Tf molecule itself or an entire mAb in targeting liposomes to cancer cells with elevated TfR levels :

1. The size of the scFv (~28kDa) is much smaller than that of the Tf molecule (~80kDa) or the parental mAb (~155kDa), thus the scFv-liposome-DNA complex may exhibit better penetration into small capillaries characteristic of solid tumors.
2. The smaller scFv also has practical advantages related to its production as a recombinant protein (large scale production of the TfRscFv will be required for clinical use).
3. The scFv is a recombinant molecule (not a blood product like Tf) and therefore presents no issues related to potential contamination by blood borne pathogens.
4. Without the Fc region of the mAb, the problem of non-antigen-specific binding through Fc receptors is eliminated.

We have already shown that such an anti-TfR single chain antibody molecule can target an intravenously administered cationic liposome-DNA (wtp53) complex preferentially to tumors (24, 25), and, in combination with chemotherapeutic agent docetaxel, results in increased survival in a mouse model of human metastatic breast cancer (26). In preclinical studies, the TfRscFv targeting moiety has shown significant enhancement of selective delivery to tumor cells (both primary and metastatic). In addition, the makeup of the complex with respect to choice and ratios of cationic and neutral lipids, ratio of ligand to liposome and liposome to DNA also enhances the uptake of the SGT-53 by the target tumor tissues.

4.2 Preclinical Data with SGT-53

SGT-53 is a novel liposome encapsulating the wtp53 gene and complexed with an anti-transferrin receptor single chain antibody fragment (TfRscFv) as the targeting entity. Pre-clinical studies with systemic (intravenous) delivery of the SGT-53 complex have demonstrated significant activity, resulting in tumor growth inhibition and even long-term regression, in a variety of solid tumor preclinical models, both as a single agent and in combination with chemotherapy and radiation [21, 22]. Further, these studies have demonstrated preferential tumor targeting capabilities of the TfRscFv liposome containing the wtp53 gene for both primary tumors and metastases. Tumor specific targeting has been observed in a variety of solid tumors including head and neck, prostate, breast, melanoma, pancreatic and brain tumor cells. For this phase II clinical study, the study population will be subjects with confirmed glioblastoma or gliosarcoma who have radiographically proven tumor recurrence or progression. Patients must also have evidence of tumor recurrence or progression but do not need to be surgical candidates. For those patients who have measureable disease on imaging studies amenable to surgical resection, they may also consider to participate in the optional surgical resection for tumor analysis.

Preclinical and clinical investigations of the safety of liposome-DNA complexes have reported that cationic lipid-DNA complexes are potent activators of the innate immune system, resulting in an inflammatory response in animals and in humans likely due to the DNA sequences. Investigations in mice have reported mononuclear cell infiltrates in the lung and activation of T cells and NK (natural killer) cells (27, 28). In at least 2 phase 1 clinical studies, subjects reported fever and/or muscle/joint pain. In the one phase 1 study that looked for them, no antibodies to the liposome or the DNA were detected. All of the responses were transient and resolved within a few days.

There are at least two reports of clinical trials where participants receiving IV liposome-DNA complexes experienced acute hypersensitivity reactions, including low-grade fever, chills, and nausea/vomiting, where premedication successfully alleviated these symptoms (29-31).

In addition, Protiva Biotherapeutics reported at the 2005 American Society of Gene Therapy Annual Meeting that in a Phase I Clinical Trial of an IV administered PEG-liposome-DNA complex, premedication with antiemetics, antipyrogenics and a steroid successfully prevented the onset of severe infusion reactions. Thus, the growing body of evidence suggests that for patient safety, premedication should be administered to offset the inflammatory response triggered by IV administration of liposome-DNA complexes.

The NCI contractor, Batelle Memorial Institute, Columbus, Ohio, performed the initial toxicity studies in rats of the liposome to be used in this clinical study. No toxicity (on clinical observation, body weight, clinical and gross pathology) was evident even after multiple high dose injections. The animals received a maximum dose of 27 uM three times per day on days 1, 4, and 7 and once a day on days 10, 13, 16, and 19. In addition, in six separate *in vivo* preclinical studies evaluating the efficacy of SGT-53, a total of 55 female athymic nude mice received 9 - 16 i.v. injections of SGT-53. In one study, the animal received 10 ug DNA while in the remainder of the studies, animals received 10 – 20 ug DNA. During infusions, no deaths or sustained weight loss due to toxicity were observed.

A formal GLP preclinical study evaluating the toxicity of SGT-53 in male and female Balb/c mice (total of 152 mice) was performed by the Department of Veterinary Sciences at the University of Texas, M.D. Anderson Cancer Center. This study evaluated 12 μg SGT-53 dose in mice equivalent to approximately 3.0 mg per injection in humans (60 kg) administered for five or ten injections. None of the animals receiving SGT-53 died or exhibited observable side effects even after ten injections. In addition, two follow-up studies were conducted (total of 140 mice). The first study evaluated 12 μg and 30 μg SGT-53 per injection; the latter dose is equivalent to a dose of 7.5 mg per infusion in a 60 kg human. The maximum dose to be evaluated in the proposed phase 1 study is 7.2 mg SGT-53 per injection. The second study compared the safety of 30 μg lyophilized /reconstituted and liquid (freshly prepared) SGT-53 administered twice weekly over five weeks. Both studies also included a control group of mice that were injected with 10% sucrose. No animals died of demonstrable test article related causes in these studies. Five mice died during the two follow-up studies, however none of the deaths could be attributed to effects of the study agent.

In the preclinical toxicity studies, marked elevation of the liver transaminases, AST and ALT, as well as marked elevation in glucose levels were noted in both the SGT-53 treated and control (10% sucrose) mice. Some effect was observed in all groups (control and SGT-53 treated) on levels of creatinine, phosphorus, urea nitrogen, white blood cells, lymphocytes, monocytes, red blood cells, platelets, segmented neutrophils, hemoglobin, hematocrit, total bilirubin and total protein. These effects were variable and not consistent between studies.

Increases in spleen size and follicular hyperplasia and expansion of hematopoietic cell populations in the spleen and lymph nodes were noted in mice receiving SGT-53. These changes were diminished or resolved in animals who had not received SGT-53 for two to six weeks.

In the preclinical studies, accumulation of mononuclear cells was noted on histopathological examination of the liver and lung with both 12 μg and 30 μg SGT-53 and with both formulations (freshly prepared and lyophilized/reconstituted). In the pulmonary tissue, scattered, mild multifocal infiltrates of mononuclear cells were noted in the perivascular, interstitial and subpleural pulmonary tissue. At six weeks following discontinuation of SGT-53, fewer changes on histopathology examination of both the liver and pulmonary tissue were noted indicating ongoing resolution.

In general, these histopathological findings are consistent with the literature that describes transient accumulation of liposomes in the lung and liver and to a lesser extent in the spleen in animal biodistribution studies. Overall, results from the pre-clinical studies to date indicate that the incidence of potential toxic side effects with administration of SGT-53 at doses comparable to the maximal dose proposed to be evaluated in humans is very low.

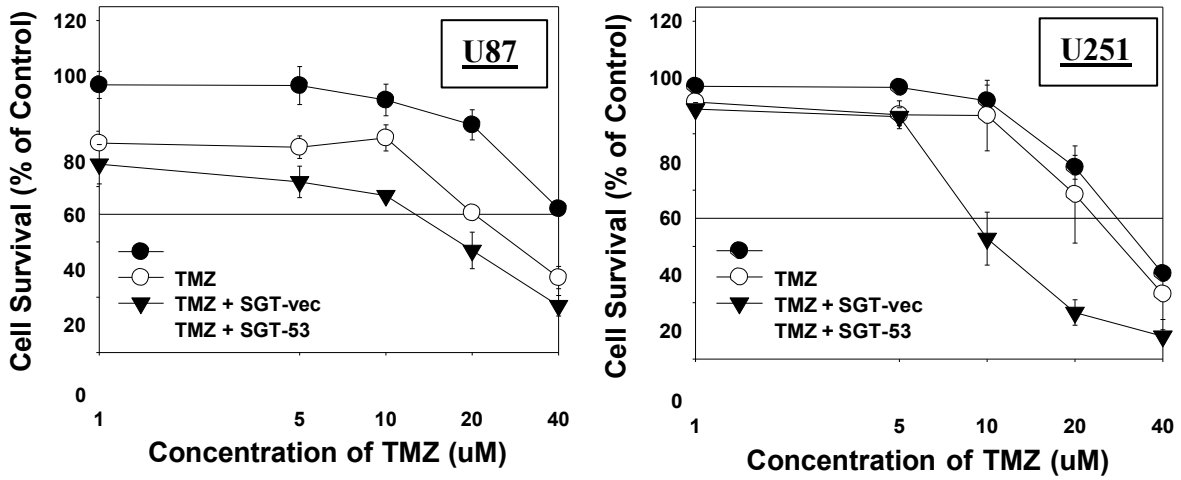
4.3 Pre-clinical data of SGT-53 with Temozolomide (TMZ) in Brain Tumors

The below *in vitro* and *in vivo* data has been published (see Reference 33)

4.3.1 *In vitro* studies

To assess the ability of scL delivered wtp53 (SGT-53) to sensitize brain tumor cells to first-line chemotherapeutic agent TMZ, human brain tumor derived U87 and U251 cells were

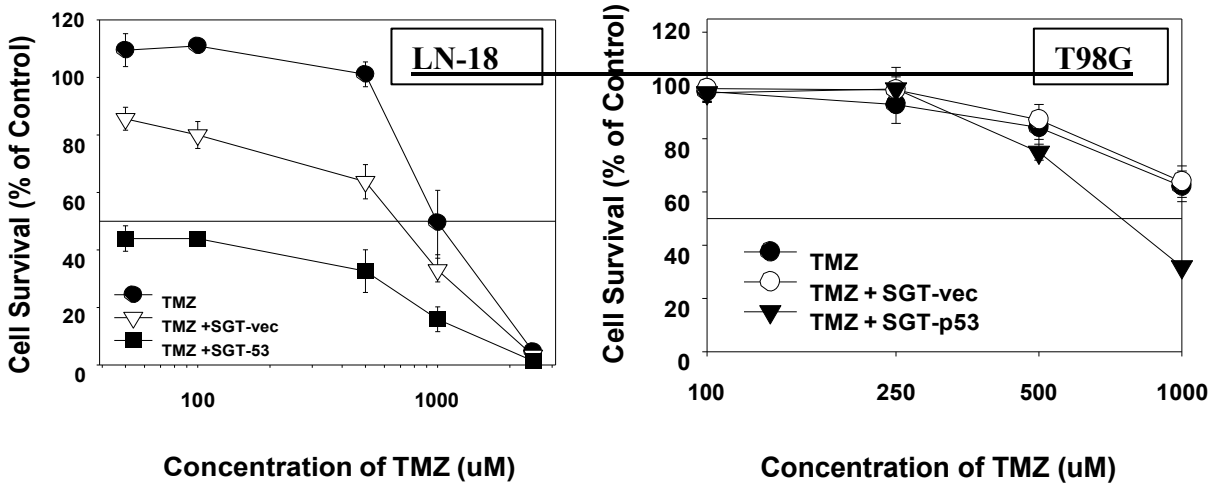
Figure 1 **Figure 2**



treated with TMZ alone, or the combination of TMZ plus SGT-53. As a control, cells were also treated with the combination of TMZ and the scL nanodelivery system carrying the same vector used to construct the pSCMVp53 plasmid, but without the p53 insert (SGT-vec). The cells were plated at 2×10^3 per well in a 96-well plate and treated 24 hours later with SGT-53 or SGT-vec. 24 hours post-transfection, the TMZ was added in increasing concentrations. The XTT assay was performed 72 h after the addition of the TMZ to the wells and the IC_{50} values (the concentration yielding 50% growth inhibition) determined. As these two cell lines are known to be sensitive to TMZ, it was not unexpected that there was some response to TMZ alone. However, as shown in Figures 1 and 2 when compared to TMZ alone, there is a significant increase in sensitization to TMZ in both cell lines when the cells are transfected with wtp53 delivered by the scL nanodelivery system. Minimal to no sensitization (U87 and U251, respectively) was observed with the complex carrying the empty vector, demonstrating that the effect is due to the p53 and not the delivery system.

However, as only a subset of brain tumor patients respond to TMZ it was more critical to assess the ability of SGT-53 to sensitize TMZ resistant tumors to this chemotherapeutic agent. Thus, to assess the ability of scL delivered wtp53 to sensitize TMZ resistant brain tumor cells to this first-line chemotherapeutic agent, human brain tumor derived LN-18 and T98G cells were treated with TMZ alone, or the combination of TMZ plus SGT-53. As a control, cells were also treated with the combination of TMZ

Figure 3 **Figure 4**



and SGT-vec. The cells were plated at 2×10^3 per well in a 96-well plate and treated 24 hours later with SGT-53 or SGT-vec. 24 hours post-transfection, TMZ was added in increasing concentrations. The XTT assay was performed 24 or 72h after the addition of TMZ (T98G and LN-18, respectively) to the wells and the IC_{50} values determined. As shown in Figure 3, after transfection with SGT-53 the TMZ resistant LN-18 cells are now responding to even very low doses of TMZ. More than 50 % of the cells are killed at a dose of TMZ as low as ~50uM compared to TMZ alone where no significant cell death is observed until a dose of ~1000uM.

Although the T98G cells were not as responsive to TMZ after SGT-53 treatment as LN-18, these highly TMZ resistant cells are also sensitized to the killing effects of this drug by SGT-53 (Figure 4). The cells treated with SGT-53 prior to exposure to TMZ have an IC_{50} of 700uM while those receiving TMZ only do not reach IC_{50} even at a concentration of 1000uM. Extrapolating this curve, the IC_{50} for TMZ alone would be at least 2000uM. As above, there is minimal or no effect on the response of the cells to TMZ after transfection with the control SGT-vec indicating that the response in these resistant cell lines is due to the presence of wtp53.

4.3.2 *In vivo* studies

As in the *in vitro* studies above, the *in vivo* experiments described below employed both TMZ responsive and TMZ resistant models of IC brain tumors. In Sections 4.3.2.1 to 4.3.2.5. tumors were induced by the IC inoculation of TMZ responsive cell line U87MG. In Sections 4.3.2.6. to 4.3.2.8. the TMZ resistant cell line T98G was used to induce IC tumors in the animals.

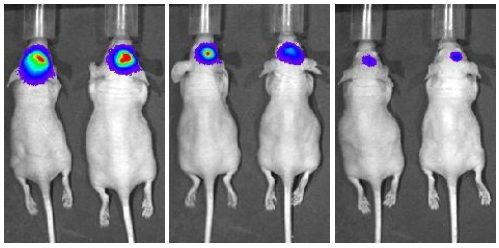
4.3.2.1 Systemic Treatment with SGT-53 Sensitizes an Orthotopic (Intracranial) Mouse Model of Human Brain Cancer (U87) to TMZ

The ultimate test of clinical potential is the demonstration of efficacy in an intracranial mouse model of brain cancer. To this end we initially performed a small proof-of-principle *in vivo* efficacy study to examine the ability of i.v. administered SGT-p53 to sensitize orthotopic brain tumors to TMZ. U87MG-Luc xenograft brain tumors were induced in nude mice by intracranially inoculating $5x10^5$ U87MG-luc cells. This cell line, obtained from Caliper Life Sciences, has been modified to stably express the Luciferase gene. 10 days post-inoculation,

tumor-bearing animals were i.v. tail vein injected with TMZ alone (5.0 mg/kg) or TMZ in combination with SGT-53 (30 ug DNA/mouse). As a control, one group received only the empty, unliganded liposome (vehicle). All i.v. injections were administered 2X/week for 2.5 weeks (total 5 injections). To assess tumor response, bioluminescence imaging (BLI) was performed using IVIS® Imaging System's Xenogen. The bioluminescence intensity of the brain tumors, a measure of tumor size/growth, was compared between groups using Xenogen Living Image® software. The results are shown in Fig 5 and Table 1.

There was a significant difference in tumor response between the three groups. The intense bioluminescence signal in the mice that received only the Liposome (vehicle) indicates that the brain tumors grew significantly and were not affected by Liposome. U87 cells are known to be sensitive to TMZ. Thus, it is not unexpected that there was some response in these animals to TMZ, as shown by the decreased signal compared to the control mice. However, there was a significantly more pronounced tumor growth inhibition in the mice that received the combination of SGT-53 and TMZ. In these animals only a small area of very weak bioluminescence was evident post-treatment. This difference was quantified based upon the measured intensity as shown in Table 1. The combination of SGT-53 (SGT-53) and TMZ resulted in a *27 Fold decrease* in signal intensity compared to the vehicle control. Moreover, this intensity was more than *7 Fold lower* than that with TMZ alone.

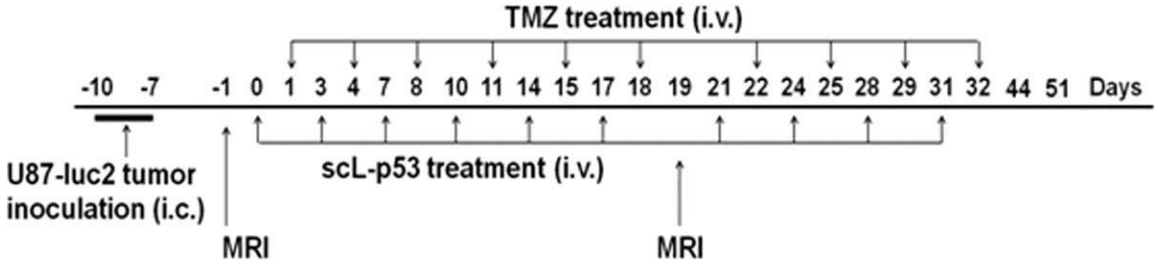
Figure 5



	Radiance (x10^6 p/s/cm^2/sr)
Vehicle	4.07 ± 0.34
TMZ	1.11 ± 0.49
TMZ + SGT-53	0.15 ± 0.01

Thus, i.v. treatment with SGT-53 can significantly sensitize orthotopic brain tumors to first-line chemotherapeutic agent TMZ. These results, along with the fact that the scL nanocomplex can cross the BBB, and that SGT-53 sensitized *in vitro* TMZ resistant cell lines (see Figures 3amd 4 above) supports potential of this combinatorial approach as a more effective clinical treatment modality for TMZ resistant GBM and other hard to treat tumors in the brain, and potentially at other sites in the body.

4.3.2.2 Tumor Regression in an Intracranial (IC) Mouse Model of Brain Cancer (U87) Induced by Systemic Treatment with the Combination of SGT-53 plus TMZ



A second larger experiment was performed to examine tumor growth inhibition induced by the sensitization of IC brain tumors to TMZ by systemic administration of SGT-53. U87MG-Luc xenograft brain tumors were induced in nude mice by intracranially inoculating $5x10^5$ U87MG-luc cells. This cell line, obtained from Caliper Life Sciences, has been modified to stably express the Luciferase gene. 10 days post-inoculation, tumor-bearing animals were i.v. tail vein injected with TMZ alone (5.0 mg/kg/injection, equivalent to 15 mg/m^2), SGT-53 alone (30 ug DNA/mouse/injection) or TMZ in combination with SGT-53. As a control, one group received PBS (vehicle). The TMZ and SGT-53 were i.v. administered on alternating days as shown in the above schematic. All i.v. injections were administered 2X/week to a total of 10 injections. To assess tumor response, bioluminescence imaging (BLI) was performed using IVIS® Imaging System's Xenogen. Figure 6 is a comparison of *in vivo* anti-tumor efficacy of the various groups using representative animals repeatedly imaged over time. Bioluminescence signals which correlate to tumor size are shown in a color map. Red color: the stronger signal, Violet color: the weaker signal. The bioluminescence intensity of the brain tumors in all of the mice, a measure of tumor size/growth, was compared between groups using Xenogen Living Image® software and is plotted over time in Figure 7. The arrowed bar indicates the duration of treatment (Last treatment = Day 24). While TMZ alone and SGT-53 alone had some minimal effect on IC tumor growth during treatment, the tumors in both groups rapidly increased in size after the end of treatment. In contrast, the tumors in the group of mice that received the combination of SGT-53 and TMZ displayed not only tumor growth inhibition, but tumor regression during treatment. More significantly, this regression continued for more than 20 days after treatment had ended.

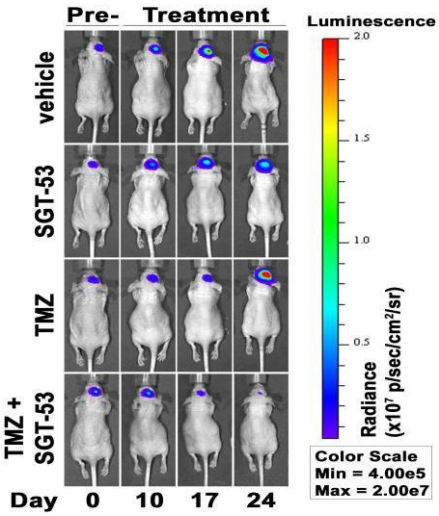
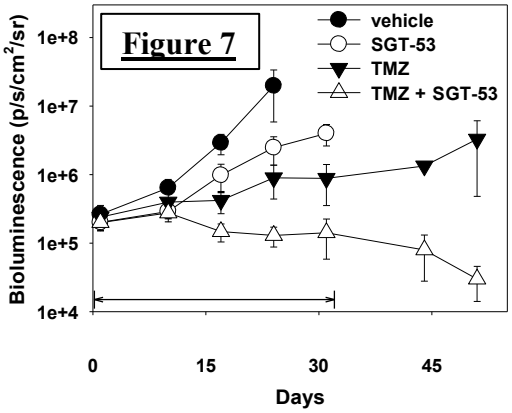
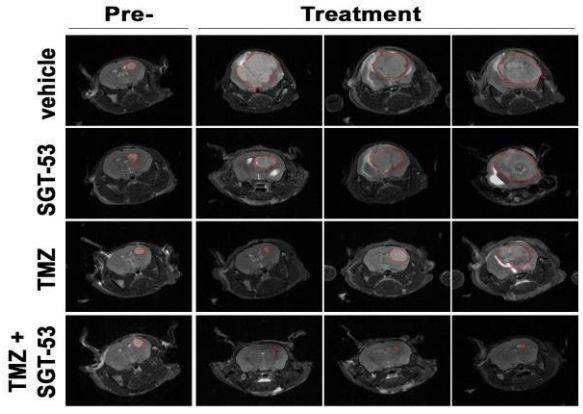


Figure 6



To confirm the bioluminescence findings, the mice were also imaged by MRI, without contrast agent, before and after 3 weeks of



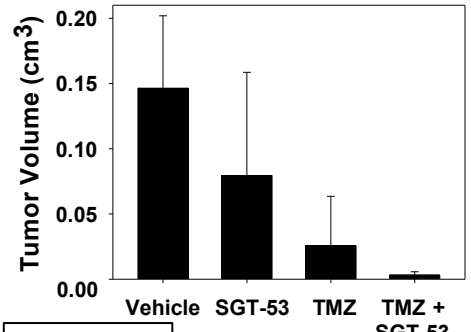


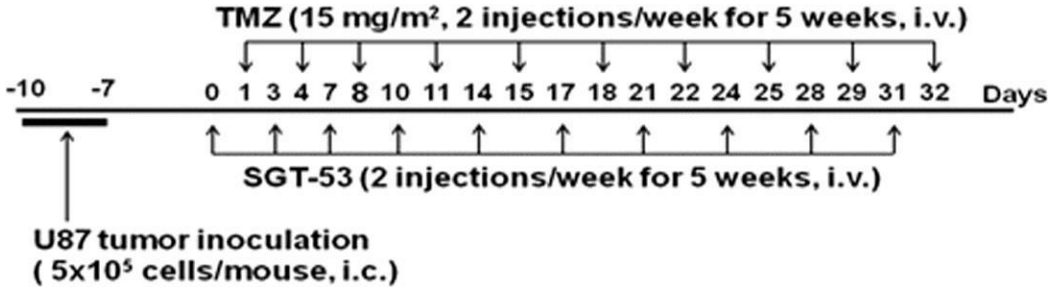
Figure 8

treatment. The tumor regression observed in the combination treatment group by bioluminescence was also observed here. In Figure 8 the red lines indicate the glioblastoma tumors. It is evident that instead of increasing in size post treatment as is evident in the single agent treatment groups, any residual tumor is barely detectable in the animals that received both SGT-53 and TMZ.

The volume of the tumors detected by MRI in Figure 8A was calculated after 3 weeks of treatment and is shown in Figure 8B. This graphic representation clearly demonstrates the increased efficacy of the combination of SGT-53 and TMZ in this animal model. Confirming the data obtained in the initial proof-of-principle study, this experiment demonstrates that the scL-delivered wtp53 can sensitize GBM tumors to TMZ leading to significant tumor response (regression) not just tumor growth inhibition.

4.3.2.3 Increased Survival of Mice Bearing Intracranial U87 GBM Tumors After Systemic Treatment with the Combination of SGT-53 Plus TMZ

As the above experiments demonstrated significant tumor responses, including regression, post-treatment we wanted to assess the effect of this combination treatment on survival. U87MG-Luc xenograft brain tumors were induced in nude mice as described above. 10 days post-inoculation, tumor-bearing animals were i.v. tail vein injected with TMZ alone, SGT-53 alone (30 ug DNA/mouse/injection) or the combination of SGT-53 and TMZ. The doses of TMZ and SGT-53 employed here were identical to those used in the experiment described above (4.3.2.2.): 15 mg/m^2/mouse/injection and 30 ug DNA/mouse/injection, respectively. As a control, one group received PBS (vehicle). All i.v. injections were administered 2X/week to a total of 10 injections. The TMZ and SGT-53 were i.v. administered on alternating days as shown in the schematic below. The animals were monitored 2-3 times/wk



and euthanized when moribund. The results, analyzed by Kaplan–Meier method, are shown in Figure 9. The arrowed lines indicate the duration of treatment. Although SGT-53 alone and TMZ alone was able to prolong survival for a period of time in this TMZ responsive cell line, all of the mice succumbed to their tumor by ~day 60. The Median Survival with SGT-53 alone was 30 days, a 15% increase in survival relative to the untreated mice. The Median Survival in the mice treated with TMZ only was somewhat longer at 45 days which was a 73% increase in survival relative to the untreated mice. However, the survival time was extended considerably with the combination of SGT-53 and TMZ. In these animals 20% of the mice survived beyond 100 days. Thus, the %

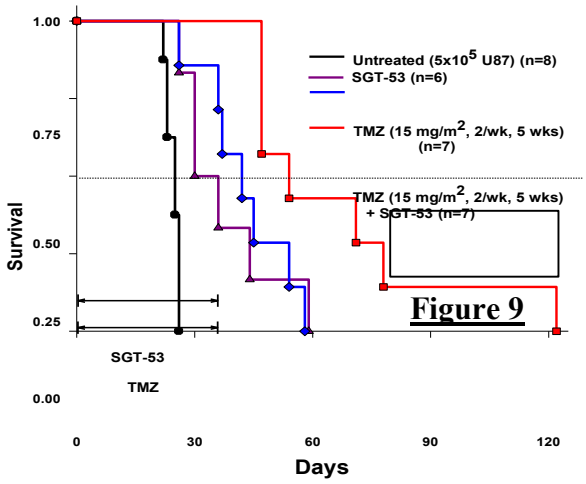
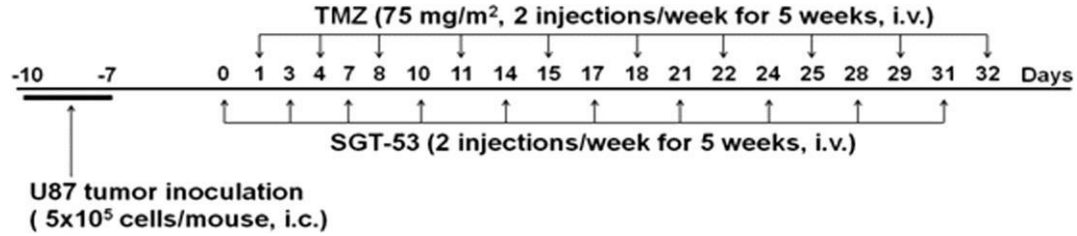


Figure 9

survival prolongation for mice receiving this combination regimen 115% that of the untreated mice. Thus, the combination of SGT-53 and TMZ at the doses used not only results tumor growth inhibition/regression, but in a significant increase in long term survival, the goal of any cancer treatment.

A second survival experiment was performed which followed this same treatment schedule but with a higher dose of TMZ to see if the survival benefit could be further increased. U87 IC tumors were induced as above. 10 days post-inoculation, tumor-bearing animals were i.v. tail vein injected with TMZ alone, SGT-53 alone (30 ug DNA /mouse/injection) or the combination of SGT-53 and TMZ. While the dose of SGT-53 remained unchanged, the dose of TMZ was increased to 75 mg/m^2/injection. As control, one group received PBS (vehicle). All i.v. injections were administered 2X/week to a total of 10 injections. The TMZ and SGT-53 were i.v. administered on alternating days as shown in the schematic below. The animals were monitored 2-3 times/week and euthanized when moribund.



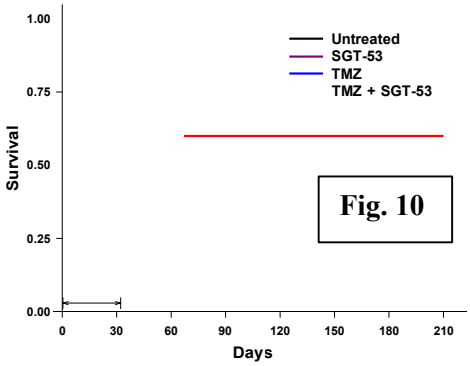


Fig. 10

The results, analyzed by Kaplan–Meier method, are shown in Figure 10 and Table 2. The arrowed bar indicates the duration of treatment. As expected since the dose of SGT-53 was not changed, the mice that received SGT-53 alone all died or were euthanized by 60 days post-inoculation, a finding similar to the results shown in Figure 9. Although this higher dose of TMZ alone was able to prolong survival for an extended period of time in this experiment compared to the previous one with the lower dose of TMZ, all of the mice here also succumbed to their tumor by ~day 155, with a Median Survival of 112 days. More significantly, when the mice were treated with both SGT-53 and TMZ, their life-span was significantly extended. In these animals 60% of the mice were still surviving at day 210 which is more than 6 after the end of treatment. Therefore, the % survival prolongation for mice receiving this combination regimen was >740 % greater than that of the untreated mice, 500% that of SGT-53 alone and almost twice that of TMZ alone. Thus, when administered in combination with the SGT-534 nanocomplex, increasing the dose of TMZ would significantly increase survival.

Table 2

Treatment	n	Median Survival (Days)	Survival Prolongation (%) *	Log Rank p-value
Untreated	4	25	-	-
SGT-53	5	35	40	0.0117
TMZ	5	112	348	0.0088
TMZ +	5	>210	>740	0.58

4.3.2.4 Enhanced Apoptosis in Intracranial U87 Brain Tumors by the Combination of SGT-53 and TMZ

Tumor suppressor p53 is known to play a role in the apoptotic (programmed cell death) pathway. To begin to evaluate the mechanism responsible for this increase in tumor cell response and increase in animal survival, the level of Apoptosis induced in U87MG-luc2 brain tumors after various treatments was determined using Annexin V-FITC and Flow Cytometry. U87MG-luc2 brain tumors were induced as described above. 10 days post inoculation of the cells, the mice were treated with either TMZ alone (15mg/m^2 /mouse/injection, 2 injections per week), SGT-53 alone (30 ug DNA per injection per mouse, 2 injections per week) or the combination of SGT-53 and TMZ. Each animal received a total of 3 injections after which the animals were euthanized, and the tumors weighed. A single cell population was subsequently isolated from the tumors and subjected to the Annexin V assay. As shown in Figure 11a, there is a major difference in the weight of the tumors from the mice that received the combination of SGT-53 and TMZ even after just three injections when compared to those that receive single agent treatment. Although SGT-53 and TMZ alone both somewhat inhibited tumor growth compared to the untreated controls (a 50-60 % decrease in tumor weight), this response was significantly greater after the combination treatment. In these mice there was an almost 90% decrease in the weight of the tumor compared to the untreated controls. In addition, this response was least 1.5 fold greater than that observed with either single agent treatment. More importantly, these findings regarding tumor weight correlated with

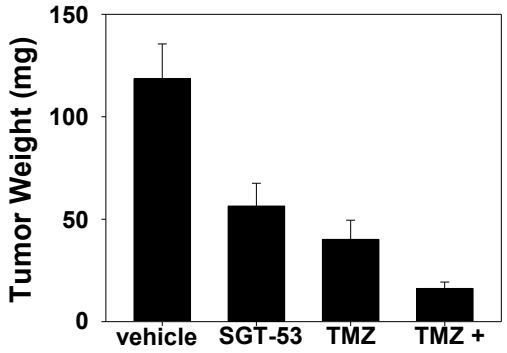


Figure 11a

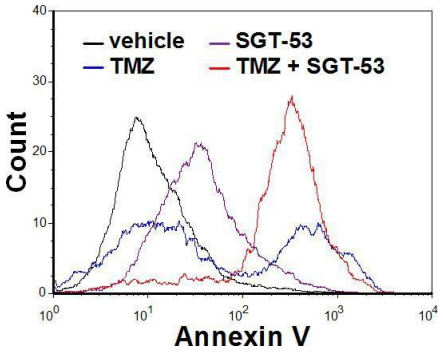


Figure 11b

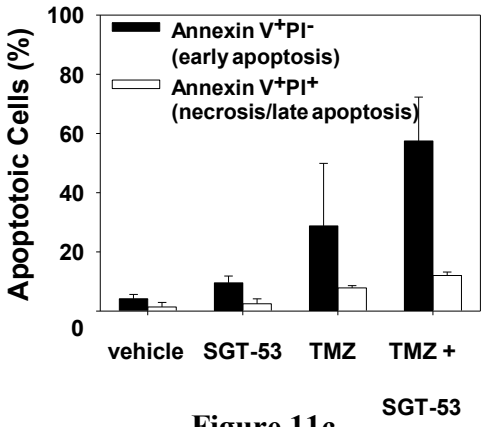


Figure 11c

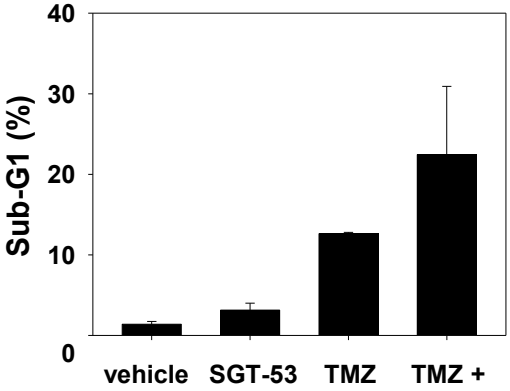
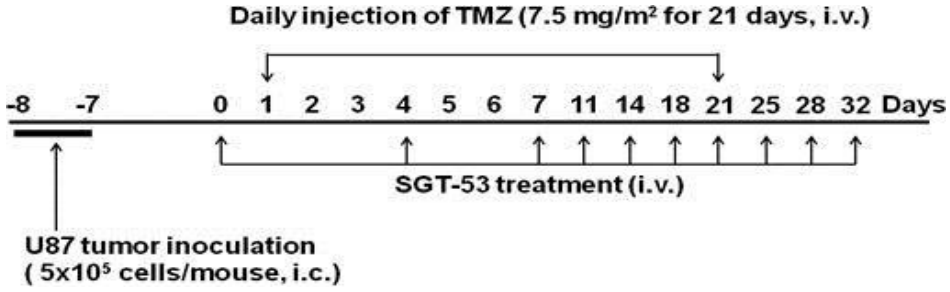


Figure 11d

apoptosis in the tumors. There was a significant increase in the percent of the tumors cells in apoptosis after treatment with the combination of SGT-53 and TMZ compared to either treatment alone when assessed by flow cytometry (Figure 11b), by staining with Annexin V (Figure 11c) and by assessment of the percent of cell in the sub G1 phase of the cell cycle (Figure 11d). Thus, these results indicate that systemic administration and uptake of SGT-53 increases the response of IC tumors to chemotherapeutic agent TMZ through enhancement of the apoptotic pathway.

4.3.2.5. Increased Survival of Mice Bearing Intracranial U87 GBM Tumors After Systemic Treatment with a 21 Day Cycle of TMZ Administered in Combination of SGT-53

Due to the potential toxicities associated with TMZ we began to explore the possibilities of using lower doses of TMZ in combination with SGT-53 by extending the time frame of TMZ administration. As it has been reported that human clinical trials have been performed wherein TMZ is administered daily for 21 days, we chose this time frame for testing here.



U87MG-Luc xenograft brain tumors were induced in nude mice as described above. 7 days post-inoculation, tumor-bearing animals were i.v. tail vein injected with TMZ alone, SGT-53 alone (30 ug DNA/mouse/injection) or the combination of SGT-53 and TMZ. TMZ was administered at a dose of 7.5 mg/m^2/mouse/injection. As a control, one group received PBS (vehicle). SGT-53 i.v. injections were administered 2X/week to a total of 10 injections. TMZ was i.v. administered daily for 21 days on Days 1 through 21 as shown in the above schematic. The animals were monitored 2-3 times/week and euthanized when moribund. The results, analyzed by Kaplan–Meier method, are shown in Figure 12. The duration of treatment is indicated by the arrowed lines. As expected since the dose of SGT-53 was the same as employed in previous experiments, the mice that received SGT-53 alone all died or were euthanized by day 32 post-inoculation, a finding similar to the results shown in Figures 9 and 10. Based upon the survival data it appears that this 21 day administration of a lower dose of TMZ alone was able to prolong survival in this experiment. However, all of the mice treated with TMZ only succumbed to their tumor by ~day 60, with a Median Survival of 45 days, compared to a Median Survival of only 18 days for the untreated animals. More significantly, here once again, when the mice were treated

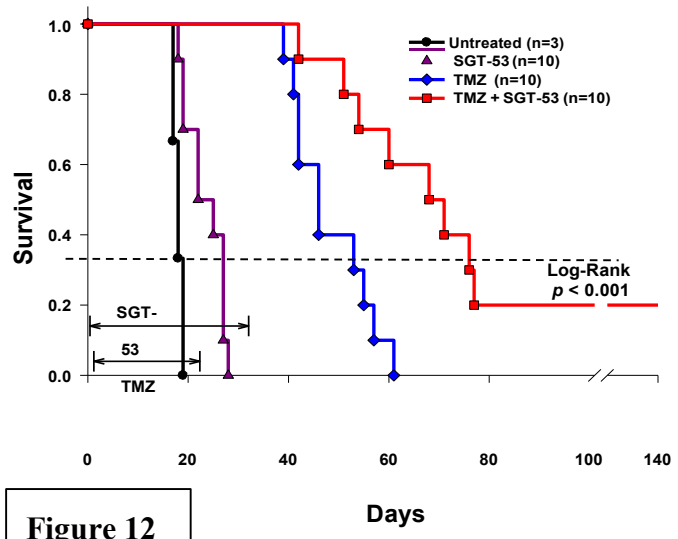


Figure 12

with the combination of SGT-53 and TMZ, their life-span was considerably extended. In these animals the Median survival was ~70 days, 290% longer than that of the untreated mice and 80% longer than those that received TMZ only. Moreover, 20% of the mice were still surviving beyond 140 days, which is more than 100 days after the end of treatment. The difference between the groups of mice that received only TMZ and those that received the combination treatment was found to be statistically significant (Log-Rank $p<0.001$). Thus, this treatment of 21 day low dose TMZ, when administered in combination with SGT-53, has the potential to be an effective clinical regimen with the possibility of reduced TMZ side effects.

4.3.2.6. Treatment of *TMZ Resistant* Intracranial T98 G GBM Tumors with SGT-53 Downmodulates MGMT Expression *In Vivo*

The primary mechanism of resistance to TMZ is overexpression of O^6-methylguanine-DNA-methyl transferase (MGMT), which repairs TMZ-induced DNA lesions by removing the O^6-guanine adducts. Thus, a means to downmodulate MGMT activity would enhance the therapeutic effect of TMZ. A number of reports have indicated that increasing wtp53 expression could downregulate expression of MGMT and increase the sensitivity of tumor cells to alkylating agents such as TMZ. We investigated if uptake of SGT-53 had an effect on the level of MGMT expression *in vivo*. An experiment to analyze the duration of scL-mediated p53 expression and MGMT downmodulation was performed in mice bearing TMZ-resistant T98G human glioblastoma intracranial xenograft tumors. IC T98G tumors were induced in athymic nude mice by intracranially inoculation of T98G human glioblastoma cell line ($5.0x10^5$ cells per animal). Ten days after the inoculation, animals were imaged by MRI. Immediately after imaging, a single IV injection of SGT-53 (30 ug DNA/injection) was administered to each animal. At 6, 16, 24, 48, and 72 hours following the injection, the mice were euthanized, tumors harvested, and protein extracted. The control group did not receive SGT-53 treatment. Forty micrograms of total protein was electrophoretically fractionated using a Nu-PAGE Precast 4-12 % gradient gel, transferred to nitrocellulose membrane, and probed for expression of p53, MGMT, cleaved PARP (a marker of apoptosis), and GAPDH by Western blot analysis. The signal was detected by ECL reagent. As shown in Figure 13, at 6 and 16 hours after the single SGT-53 i.v. injection, an increase in the level of p53 protein is evident as compared to the untreated animal indicating the presence of the exogenous p53. The transient expression of p53 peaks at 48 hours post-injection, then this signal decreases back to a level close to that of the untreated control by 72 hours.

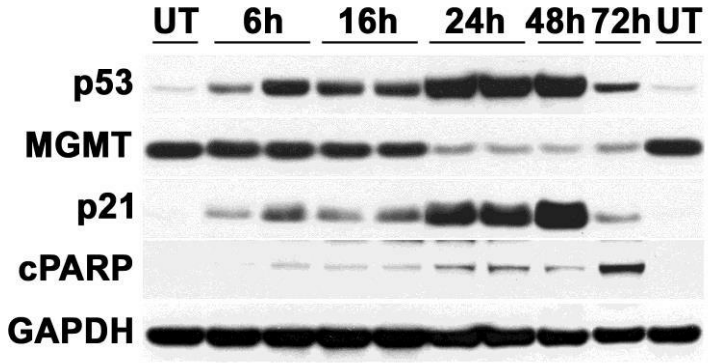


Figure 13

Importantly, the opposite was true for the expression of MGMT. There was a significant decrease in the expression of MGMT observed at 24 hours, which lasted as long as 72 hours after the injection. The timing for the observed decrease in MGMT signal is consistent with the mechanism of action of p53 in sensitizing cells to TMZ by interfering with DNA repair mechanisms. Expression of cleaved PARP, an indicator of apoptosis, was detected as early as 6 hours and showed maximum expression 72 hours after the injection. Consistent expression of GAPDH protein demonstrated equal protein loading. Thus, there is a correlation between expression of the exogenous p53 (delivered through administration of SGT-53) and the down

modulation of MGMT in these TMZ resistant tumors.

4.4. Clinical Data with SGT-53

4.4.1. Phase 1 trial of SGT-53 monotherapy in patients with advanced solid tumors

A total of 11 humans have been infused with the TfRscFv-liposome-DNA complex, i.e. SGT-53, at doses of 0.6, 1.2, 2.4 or 3.6 mg DNA/infusion administered IV over 1 to 2 hours twice weekly for 5 weeks. There was no intra-patient dose escalation. These 11 patients were both male and female and ranged in age from 37 to 75 years of age. They suffered from a range of cancer types, including metastatic adenocarcinoma of the colon, metastatic medullary carcinoma of the thyroid, metastatic colorectal cancer, adenoid cystic carcinoma, metastatic thymoma, metastatic nasopharyngeal carcinoma, metastatic cervical cancer, metastatic and progressive vaginal cancer and leiomyosarcoma.

70% of the patients displayed stable disease after a single cycle of SGT-53. Significant tumor necrosis was evident in two of these subjects, one who received the lowest dose of 0.6 mg DNA/infusion and one who received the 2.4 mg DNA/infusion dose. A report of the findings from this trial have recently been published (32).

An MTD was not reached. The study was ended as biologically relevant doses were reached The Adverse events experienced by these 11 subjects were 95% were Grade 1 and 2 and only 5% were either Grade 3 or Grade 4. These toxicities are outlined in the table in section 7.2b, and they included:

- Grade 1: fatigue, fever, hypotension, neutropenia, thrombocytopenia, leucopenia, headache, chills, tachycardia, pleural effusion, cough, dizziness, anorexia.

- Grade 2: fatigue, dehydration, diarrhea, hypotension, fever, anemia, leucopenia, elevated AST/ALT, headache, neutropenia.

- Grade 3: A single patient experienced fatigue correlating with massive tumor necrosis

- Grade 4: Three events were reported, all of which were classified as Unrelated or Unlikely to be related to SGT-53.

There were four Serious Adverse Events reported during the trial. Of these, three were classified as Unrelated or Unlikely to be related to the SGT-53 study drug. One was classified as only possibly related. Overall SGT-53 was found to have a good safety profile at potentially therapeutic doses.

No MTD was reached in the Phase I trial which included doses up to 3.6mg DNA/infusion which is the dose at which the presence of exogenous normal (wt) p53 DNA has been demonstrated in tumor tissue.

Of note, 8 out of 11 patients experienced grade 1 to 2 hypotension.

However, due to the evidence that i.v. liposome-DNA complexes do induce an inflammatory response and the observation of induction of fever in the first subject to be infused with SGT-53, each trial participant was premedicated before every infusion with acetominophen, a combination of histamine H1 and H2 blockers (e.g. Benadryl and Pepcid), and dexamethasone as described below in Section 9.3.1.

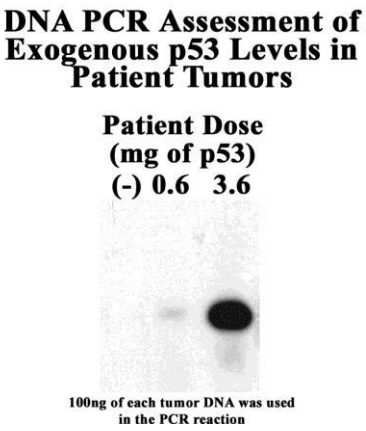


Figure 16

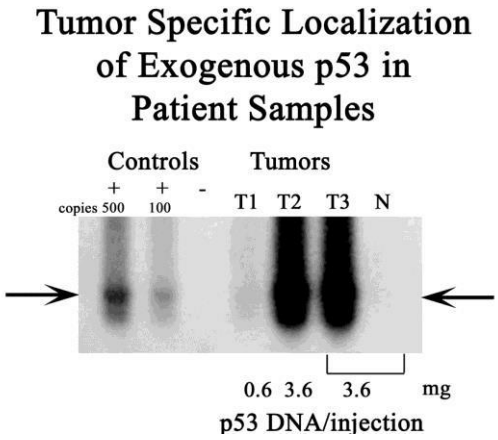


Figure 17

4.4.1.1.Presence of Transgene (Exogenous p53) in Patients' Tumors

A critical requirement for gene therapy to be successful is the demonstration that the transgene delivered by SGT-53 (exogenous wtp53 DNA) was present in the tumors of treated subjects. Moreover, the transgene should not be detected in the normal tissue. Thus to assess tumor specific delivery of SGT-53, we performed DNA PCR to determine if we could detect the exogenous p53 gene delivered by the SGT-53 complex in the tumors. We amplified a 700bp fragment using one primer in the vector and one primer in the p53 insert. Using this approach, we could specifically amplify only the exogenous p53 transgene. Tumor biopsies were obtained from subjects after the end of treatment with the lowest dose (0.6mg/infusion) (T1) and with the 3.6mg/infusion dose (T2). As shown above in Figure 16, the amplified 700bp fragment of the exogenous p53 gene was clearly present in both tumors. More importantly, the transgene was present in a dose dependent manner as would be expected with tumor-targeted delivery. With a third subject who had also received the 3.6mg/infusion dose (T3), in addition to the tumor biopsy, a biopsy sample was obtained from normal skin. Figure 17 demonstrates that while there is strong PCR signal in the tumor tissue, indicating a significant presence of the exogenous p53 in the tumor of this individual, no p53 could be detected in the normal skin sample. These results indicate the tumor-targeting ability systemically administered SGT-53 in patients.

4.4.2. Phase 1b trial of SGT-53 in combination with docetaxel

Currently, there is a Phase Ib study ongoing at the Mary Crowley Cancer Research Center, Dallas Texas to test the safety of SGT-53 in combination with docetaxel. This Phase Ib is an open-label, range finding trial with a set dose of SGT-53 at 2.4mg DNA/infusion and escalating doses of docetaxel as shown in the Schedule for Phase Ib below. Eight subjects have received treatment to date. These subjects were male (3) and female (5) ranging in age from 33 to 77

years of age and had been diagnosed primarily with metastatic grade 3 or 4 pancreatic, endometrial, ovarian, anal, angiosarcoma, NSCLC or esophageal cancers. Only the subject with angiosarcoma was categorized as grade Ia. Three of the subjects demonstrated significant tumor shrinkage by RECIST, one of which demonstrated a 47% , one a 51% and the third a 79% decrease in tumor size. This subject is receiving a second round of treatment. An additional subject demonstrated significant tumor shrinkage based upon photographic evidence.

SCHEDULE FOR PHASE 1b

Weeks	Days[1]					
	1	2	3	4	5	
1	SGT53[2]		Doc[3]	SGT53		Cycle 1
2	SGT53					
3	SGT53					
4	SGT53		Doc[4]	SGT53		Cycle 2
5	SGT53					
6	SGT53					
7	SGT53		Doc[5]	SGT53		Cycle 3

[1]In case of holiday or other logistic issue, schedule will allow for weeks 1, 4, and 7 treatments to be delivered on days 1, 4 and 5 or on days 2, 4 and 5. Weeks 2, 3, 5 and 6 treatments may, for the same circumstances be administered on day 2.
[2]SGT53 = SGT-53 2.4mg per infusion
[3]Doc = Starting dose-Docetaxel 40 mg/m^2
[4]Doc = For cohort 1, cycle 2 intra-patient dose escalation of docetaxel to 60 mg/m^2 if no DLT occurred in cycle 1.
[5]Doc = Same dose at cycle 2 if no DLT; if DLT, may repeat cycle 1 dose (40 mg/m^2) as per PI.

The number and dose of infusions of SGT-53 and Docetaxel is shown in the Table below:

Infusions Phase Ib

Patient ID	# of Infusions of SGT-53	DoseSGT-53 /Infusion	# of Infusions of Docetaxel	Dose Docetaxel/Infusion
02-018 [a]	1	2.4 mg	1	40 mg/m^2
02-019[b]	7	2.4 mg	2	40/60 mg/m^2
02-020	9	2.4 mg	3	40/60/60 mg/m^2
02-023	10	2.4 mg	3	40/60/60 mg/m^2
02-024[c]	2	2.4 mg	1	75 mg/m^2
02-025	10	2.4 mg	3	75/75/75 mg/m^2
02-026	10	2.4 mg	3	75/60/60 mg/m^2

[a] Early termination due to Disease Progression

[b] Subject withdrew voluntarily from study
[c] Early Termination due to only possibly related syncopal episode

Drug related grade 1, 2 AEs occurring in ≥ 5% of patients are shown below. These primarily consisted of transient fever, chills and hypotension

Adverse Events

Grade 1 & 2 AE's			
Pt ID	**Possible**	**Relation to Study Drug** **Probable**	**Definite**
02-018	Fever, Chills, Emesis	None	None
02-019	Intermittent fatigue,	None	None
02-020	Intermittent diarrhea, Intermittent anemia	Chills, Headache	None
02-023	None	None	Hypotension, Fever
02-024	Hypotension Elevated ALT Elevated AST Hypocalcemia		
02-025	None	None	None
02-026	Intermittent hypotension Intermittent dehydration	None	None

Combination therapy in Phase 1b has been relatively well tolerated to date. Five SAEs have been recorded, two of which were in one subject. All were categorized as unrelated to the administration of SGT-53. Two were related to disease progression, one was related to docetaxel administration and two were related to possible small bowel obstruction.

4.5. Study Design Rationale

4.5.1 Medical background

To date, there is no consensus on the standard of care for relapsed/recurrent glioblastoma. Prior to 2009, patients were offered older approved chemotherapies such as carmustine or lomustine, TMZ at different regimens, or even no further therapeutic intervention. In 2009, Avastin® (bevacizumab), the humanized monoclonal antibody to human VEGF, was approved by FDA as a single agent for adult patients with progressive disease following prior therapy on the basis of objective response rate, but has not (to date) demonstrated an effect on survival. Different regimens are commonly employed by clinicians in an effort to prolong life and/or provide other clinical benefits for patients with recurrent GBM but there is also no consensus in this clinical

setting. The prognosis for patients with relapsed/progressive disease remains very poor (median survival about 6 months).

A Phase 1 trial of SGT-53 monotherapy has been completed in patients with advanced solid tumors as described above. Tolerance of the agent was acceptable with no SAEs related to SGT-53 reported. No MTD was established. Ten doses of SGT-53 were administered over 5 weeks. The combination therapy of SGT-53 (10 doses) and docetaxel in the ongoing Phase 1b has been relatively well tolerated to date and responses have been observed. The dose for this Phase II trial will be 3.6 mg DNA per infusion. This is the highest dose administered in the Phase Ia trial.

This trial is a single arm study wherein SGT-53, at a dose of 3.6 mg DNA per infusion, will be administered by iv infusion. Patients will receive SGT-53 and Temozolomide in 28 day cycles. Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycle of SGT-53/Temozolomide therapy at investigator's discretion. The primary objective is to evaluate the 6 month PFS in patients treated with SGT-53 in combination with Temozolomide.

Surgical resection for tumor analysis before the commencement of SGT-53/Temozolomide treatment is an optional procedure. For these patients, SGT-53 will be administered twice during the week prior to surgical resection of the tumor. 14-21 days post-surgical resection, patients will receive SGT-53 and Temozolomide in 28 day cycles. These patients who respond to treatment (at least stable disease by RANO) may receive three additional cycle of SGT-53/Temozolomide therapy at investigator's discretion. The primary objective of this surgical resection is to assess nanoparticle tumor delivery by analysis of the presence of exogenous wt p53 in the tumor.

All patients enrolled in the study will be evaluated for PFS, PFS-6 and OS.

4.5.2. Patient Population

The target study population is subjects with confirmed glioblastoma or gliosarcoma who have proven tumor recurrence or progression.

This study will enroll males or females aged 18 years or older with recurrent GBM or GS who may benefit from receiving SGT in combination with a cyclical TMZ. Eligible patients must have histological confirmation of GBM or GS and a diagnosis of recurrent disease with an interval of at least 3 months after radiotherapy plus TMZ. For patients who have had surgery, there must be an interval of at least 14-21 days post operatively and having recovered from the effects of surgery for the recurrent disease before initiation of cyclical treatment.

4.6 Study Design

Based on efficacy and overall safety demonstrated in preclinical studies and the first-in-man Phase Ia and Phase Ib study in combination with docetaxel, SynerGene Therapeutics, Inc. proposes to perform a Phase II clinical study of the combination SGT-53 and temozolomide in subjects with confirmed glioblastoma who have proven tumor recurrence or progression. The goals of the proposed clinical study are to determine the anti-tumor activity of the combination

of SGT-53 and temozolomide by RANO and by evaluating the 6 month PFS; PFS and, OS and by evaluating the safety of the combination of SGT-53 and temozolomide.

The goals of the optional surgical resection are to demonstrate the nanoparticle delivery of systemically administered SGT-53 to tumor in GBM patients and to assess induction of apoptosis by SGT-53 in the tumor. Participation in the optional surgical resection will be at the discretion of the subject and investigator.

Based on the pre-clinical intra-tumoral half-life, in Phase Ia SGT-53 was given as a twice-weekly intravenous infusion over 1.5 – 2 hours, depending on dose level. The time of infusion can be extended in the event of infusion reactions (see Section 9.3.2); however infusion of the study agent must be completed as soon as possible, but no longer than eight hours after dilution in dextrose.

Below is the schedule of treatment:

This is a single arm study wherein SGT-53, at a dose of 3.6 mg DNA per infusion, will be administered. Patients will receive SGT-53 every 3rd or 4th day in 28 day cycles (total of 6 infusions) starting on Day 1 (cycle 1), Day 29 (cycle2) and Day 57 (cycle 3). Temozolomide will be administered by mouth daily on days 9-13 of each cycle at 150mg/m2 (cycle 1), and 200mg/m2 as tolerated for cycle 2 and beyond provided the patient meets the criteria for non-hematological toxicity, ANC and platelet counts (as given in 9.7.2). In those patients who had a surgical resection, the 1st cycle of SGT-53 and TMZ will start 14-21 days post operatively and having recovered from the effects of surgery.

Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycle of SGT-53/Temozolomide therapy at investigator's discretion. Alternatively, they may continue on Temozolomide alone at investigator's discretion.

If optional surgical resection will be performed, SGT-53 will be administered on day -3 and day -1 prior to surgery. Surgical resection of recurrent or progressive tumor is Day 0. 14-21 days post operatively and having recovered from the effects of surgery the patients will then start cyclical TMZ with SGT as described above. A maximum of 6 patients will be have this optional testing. Once the presence of exogenous wtp53 from SGT-53 is demonstrated in the resected tumor of 3 patients, the option for surgical resection for tumor analysis will no longer be offered.

4.6.1. Dose Selection:

In the Phase 1a study of SGT-53 as a single agent the nanocomplex was administered twice weekly for five consecutive weeks. No patient received more than 10 infusions in a single cycle. Two cycles of 10 infusions were administered to one patient.

In various clinical trials Temozolomide has been administered daily for 5 days in one 28 day period. To optimize effectiveness of the potential chemosensitization of SGT53, SGT53

treatment will be given three times prior to the start of temozolomide treatment. Pre-clinical studies have shown that the wtp53 tumor suppressor gene delivered by the SGT-53 nanoparticle functions to sensitize tumors to the chemotherapeutic agent, making them more responsive to the drug. Thus, it is critical that p53 be expressed when temozolomide is administered in order to have the benefit of the SGT-53. Using the proposed schedules, SGT-53 is being expressed at the start of temozolomide treatment. For patients who undergo optional surgical resection for tumor analysis, two doses of SGT-53 will be administered in the week prior to surgical resection of the tumor to demonstrate the presence of exogenous wtp53 in the tumor to validate SGT-53 crossing the BBB and targeting the tumor.

4.6.2. Treatment Duration:

Patients will receive a total of 3 cycles of TMZ in combination with SGT. Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycles of SGT-53/Temozolomide therapy at investigator's discretion. Alternatively, they may continue on Temozolomide alone at investigator's discretion.

Withdrawal from the study will be allowed at any point due to unacceptable toxicity or withdrawal for other reasons (eg patient withdrawal of consent, or protocol violation etc), evidence of disease progression as defined in Section 9.5. All patients who received treatment with SGT-53 must be followed-up for 30 days after discontinuation of study drug. The 30-day follow-up visit should include a physical examination, vital signs, concomitant medications, assessment of AEs/SAEs and Urine analysis. Blood samples will be taken for: Hematology (CBC with differential, platelet count), Chemistries (BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein, glucose, LDH), PT/PTT and D-dimer.

Patients who permanently discontinue study drug(s) for reasons other than complete response, progression, or death should also have visits approximately every 2 months for the first year that should include a MRI, physical examination, vital signs, and recording of concomitant medications for glioblastoma.

Patients who are withdrawn from study treatment due to a complete response will have the 30-day follow-up visit. Further follow-up visits every 2 months will include a physical/neurological exam, concomitant medications, vital signs and a MRI.

A telephone contact to check on vital status and any additional treatment for glioblastoma will occur yearly.

4.6.3. Endpoints:

4.6.3.1 Primary Endpoints

PFS-6 using the RANO criteria (see Section 18.2) will be used as primary endpoints.

4.6.3.2. Secondary Endpoints

- Safety of the combination of SGT-53 and Temozolomide.
- PFS, OS
- Anti-tumor activity of the combination of SGT-53 and Temozolomide based upon the RANO criteria.
- The induction of apoptosis by SGT-53 in the tumor (optional procedure).
- Nanoparticle delivery (optional procedure)

4.6.4. Statistical hypothesis and sample size:

A total of 26 patients will be enrolled. A maximum of 6 patients will be enrolled to participate in the optional surgical resection for tumor analysis.

Patients who have received at least one dose of study drug will be considered evaluable for safety and toxicity endpoints. Patients who require a dose interruption or reduction during the initial cycle will remain evaluable for toxicities if the reduction or interruption is due to a DLT. Patients who do not complete one cycle of treatment but do not have a DLT may be replaced as described in more detail in Section 9.6.

The primary endpoint for the trial is PFS6. Progression-free survival will be defined as the time from study enrollment until the time of first occurrence of disease progression, relapse, or death due to disease.

The analysis of the biological correlate data in patients with surgical resection for tumor analysis has the overall goal of providing an increased understanding of the ability of SGT-53 to cross the Blood-brain Barrier and be delivered into tumor cells in the brain. As such, the objective of the tumor analysis is qualitative rather than quantitative. It should be noted that in the Phase I dose escalation trial of SGT-53, the presence of the exogenous wtp53 delivered by SGT-53 was found to be present in the tumors in a DNA dose dependent manner. Moreover, even the lowest dose of SGT-53 (0.6mg DNA/infusion) was not only found to be present in the tumors of the patients, but to have some anti-tumor effect. A DNA dose 6-fold higher (3.6mg DNA/infusion) is being used in this trial.

Reverse transcription PCR to determine transgene expression is not applicable for these studies because it would not be able to unequivocally establish that the p53 protein detected was from the exogenous transgene. In contrast, since we have designed primers that included part of the plasmid vector backbone, only the exogenous DNA is amplified. This DNA PCR approach was used to detect the presence of the transgene in the tumors in the Phase I trial (See Figures 16 and 17 in Section 4.4.1.1) and will be used for these studies. The presence of at least 30 copies of exogenous p53 in the tumors sample will be considered positive evidence of drug penetration.

All of the patients in the trial will receive the same dosing of the combination of SGT-53 and TMZ (6 doses of SGT-53 and 5 doses of TMZ every 4 weeks for three 4 week cycles) and thus patients with or without surgical resection for tumor analysis will be used for the Simon two stage calculation. Even for the patients who undergo the optional surgical resection for tumor analysis PFS6 is the primary endpoint. No TMZ will be administered during the time these patients receive the two doses of SGT-53 prior to the resection. Exogenous p53 expression is transient. The patients who receive surgery will not begin treatment for 2-3 weeks post-surgery. Previous studies have demonstrated that there is no indication of the exogenous wtp53, even by DNA PCR, after this length of time. Thus, there will be no additional residual DNA present from the two pre-surgery doses of SGT-53 by the time the combination therapy being evaluated is initiated.

According to the Simon Minimax design, our study, with its sample size of n = 26, has a 10% probability of rejecting (a) the hypothesis of a PFS6 15% (P0) (the historical PFS6 rate) and a 70% probability of accepting (1-b) the hypothesis of a PFS-6 of 30% (P1). If two, or fewer of the first 18 patients were progression free at 6 months, PFS-6 would be considered <15% and the study terminated. Otherwise, the study would be completed, the accrual target being 26 patients. If six or fewer of the 26 patients were progression-free at 6 months, then no further investigation of the treatment regimen would be considered warranted. PFS6 and secondary endpoints progression free survival and overall survival will be evaluated using the Kaplan-Meier product-limit survival curve methodology.

4.6.5. **Study Objectives**:

Primary:

- To evaluate the 6 month PFS in patients treated with SGT-53 in combination with Temozolomide.

Secondary:

- To assess safety of the combination of SGT-53 and Temozolomide.
- To evaluate progression free survival (PFS) and OS in patients in patients with recurrent or progressive glioblastoma or gliosarcoma treated with SGT-53 in combination with Temozolomide.
- To determine the anti-tumor activity of the combination of SGT-53 and Temozolomide based upon the RANO criteria in patients with recurrent or progressive GBM tumors.
- To assess nanoparticle delivery to tumor site by analysis of the presence of exogenous wt p53 in the tumor (optional procedure).
- To assess induction of apoptosis by SGT-53 in the tumor by flow cytometry or histology (optional procedure).

5. INVESTIGATIONAL PLAN

This is a prospective open label single arm Phase II study to assess the efficacy of nanoparticle delivery to tumor site of exogenous wt p53. This trial will also evaluate the safety, tolerability,

toxicity and efficacy of SGT-53 (given in combination with temozolomide) on PFS and OS in adult patients with recurrent Glioblastoma or gliosarcoma.

The study population (26 patients) will be registered through and treated at: MD Anderson Cancer Center, Texas, USA and China Medical University Hospital, Taichung, Taiwan, based on Inclusion/Exclusion Criteria (see Sections 6.1. and 6.2.)

Although an MTD for SGT-53 was not reached in the previous Phase Ia trial, the highest dose tested was 3.6mg of DNA. This is the dose of SGT-53 that will be employed in this trial. Below is the schedule of treatment:

Cycles 1-3 (1 Cycle is 28 days)

- Day 1: SGT infusion
- Day 4: SGT infusion
- Day 8: SGT infusion
- Days 9-13-: TMZ (150mg/m2 (cycle 1) increasing to 200mg/m2 (from cycle 2 onwards) as tolerated, provided the patient meets the criteria for non-hematological toxicity, ANC and platelet counts (see 9.7.2)
- Day 11: SGT infusion
- Day 15: SGT infusion
- Day 18: SGT infusion
- Days 19-28: No treatment

Cycles 4-6: Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycles of SGT-53/Temozolomide therapy at investigator's discretion. Alternatively, they may continue on Temozolomide alone at investigator's discretion.

When the Patient has Undergone Optional Surgical Resection For Tumor Analysis (4-6 patients only):

- SGT infusion on days -3 and -1
- Surgery on Day 0
- 14-21 days post operatively and having recovered from the effects of surgery the patients then start 28 day cycles of treatment as described above: SGT-53 will be administered every 3rd or 4th day in a 28 day cycle (total of 6 infusions)] starting on Day 1 (cycle 1), Day 29 (cycle2) and Day 57 (cycle 3). Day 1(cycle 1) will be the day of the first infusion of SGT-53 post-surgery. Temozolomide will be administered by mouth daily on days 9-13 of each cycle at 150mg/m2 (cycle 1), and 200mg/m2 as tolerated for cycle 2 and beyond provided the patient meets the criteria for non-hematological toxicity, ANC and platelet counts (see 9.7.2)..
- Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycles of SGT-53/Temozolomide therapy at investigator's discretion. Alternatively, they may continue on Temozolomide alone at investigator's discretion.

Post Treatment safety follow up:

All patients who received treatment with SGT-53 must be followed-up for 30 days after discontinuation of study drug. The 30-day follow-up visit should include a physical examination, vital signs, concomitant medications and assessment of AEs/SAEs and Urine analysis. Blood samples will be taken for: Hematology (CBC with differential, platelet count), Chemistries (BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein, glucose, LDH), PT/PTT, and D-dimer. Patients who permanently discontinue study drug(s) for reasons other than complete response, progression, or death should also have visits approximately every 2 months for the first year that should include a MRI, physical examination, vital signs, and recording of concomitant medications for glioblastoma.

Patients who are withdrawn from study treatment due to a complete response will have the 30-day follow-up visit as described above. Further follow-up visits every 2 months will include only a physical/neurological exam, concomitant medications, vital signs and a MRI.

A telephone contact to check on vital status and any additional treatment for glioblastoma will occur yearly.

6. STUDY POPULATION:

The study population is subjects at least 18 years of age with histologically confirmed glioblastoma or gliosarcoma who have proven tumor recurrence or progression.

6.1. Inclusion Criteria:

Eligible patients must meet all of the following inclusion criteria:

1. Signed informed consent prior to any study-mandated procedure.

2. Histologically confirmed glioblastoma or gliosarcoma in first, second or third relapse. A pathology report constitutes adequate documentation of histology for study inclusion. Subjects with an initial diagnosis of a lower grade glioma are eligible if a subsequent biopsy is determined to be glioblastoma. The amount of prior systemic therapy for this population is, nevertheless, restricted to three regimens.

3. Radiographic demonstration of disease progression following prior therapy (RANO criteria)

4. Measurable disease on MRI performed within 14 days prior to registration. Baseline MRIs for subjects who underwent salvage surgery after first or second relapse must be obtained ≥ 4 weeks after the procedure.

5. Male or female patients ≥ 18 years of age.

6. Recurrent disease with an:

    - interval of ≥ 3 months following radiotherapy + temozolomide;

- interval of ≥ 14 days between end of surgery and start of protocol therapy for patients who have undergone surgery for recurrent disease.

7. Patients who tolerated previous administration with temozolomide, i.e.,during adjuvant Temozolomide, no dose reductions below the starting dose were required due to treatment-related toxicities

8. Recovery from the effects of prior therapy, including the following:

   - Four weeks from cytotoxic agents (3 weeks from procarbazine, 2 weeks from vincristine)
   - 6 weeks from nitrosoureas (CCNU, BCNU)
   - Four weeks from any investigational agent
   - One week from non-cytotoxic agents
   - 12 weeks from radiotherapy to minimize the potential for MRI changes related to radiation necrosis that might be misdiagnosed as progression of disease, or 4 weeks if a new lesion, relative to the pre-radiation MRI, develops that is outside the primary radiation field

9. Karnofsky performance status ≥ 60% (see Section 18.3. for details).

10. Complete blood count (CBC)/differential at screening (within 7 days of study initiation), with adequate bone marrow function as defined by: absolute neutrophil count (ANC) ≥ 1,500/μL; platelets ≥ 100,000/μL; hemoglobin ≥ 10 g/dL.

11. If patient is receiving steroids, must be on stable or decreasing steroid dose within 5 days prior to treatment initiation with SGT-53.

12. Patients must be willing to forego other cytotoxic and non-cytotoxic drug or radiation therapy against the tumor while enrolled in the study.

13. Women of childbearing potential must have a negative serum beta-HCG pregnancy test documented within 3 days prior to study initiation.

14. Women of childbearing potential must agree to use two reliable methods of contraception from screening and up to 30 days after discontinuation of study treatment (refer to Section 11.15 for further details).

    •Reliable methods of contraception include intrauterine devices (IUD) or intrauterine systems (IUS), tubal sterilization, and barrier methods (male condom, diaphragm, or cervical cap). A female partner's vasectomy still requires 1 additional method of contraception. Abstinence, the rhythm method, or contraception by the other partner alone, will not be considered as a reliable methods of contraception.

15. Males not naturally or surgically sterile, who have a female partner of childbearing potential, must agree to use two reliable methods of contraception from screening and up to 30 days after discontinuation of study treatment (refer to Section 11.15. for further details).

16. Acceptable liver function (obtained within 7 days prior to registration):

    - Bilirubin ≤ 1.5 times upper limit of normal (CTCAE Grade 1 baseline); Total bilirubin ≤3xULN with direct bilirubin within normal range in patients with well documented Gilbert's syndrome
    - AST (SGOT), ALT (SGPT) ≤ 2.5 x ULN (CTCAE Grade 1 baseline)
    - Serum creatinine ≤ 1.5 X ULN (CTCAE Grade 1 baseline)

17. Acceptable blood sugar control (based on labs obtained within 7 days prior to registration): Fasting glucose value < 160 mg/dL (CTCAE Grade 1 baseline)

18. Urinalysis (obtained within 7 days prior to registration): No clinically significant abnormalities.

19. PT and PTT ≤ 1.5 X ULN after correction of nutritional deficiencies that may contribute to prolonged PT/PTT (obtained within 7 days prior to registration).

20. Have recovered from any previous therapy side effects or toxicities (to ≤ grade 1, except alopecia) prior to initiating protocol study infusions.

21. Organ function characterized by ≤ Grade 1 scores defined by CTCAE v4.03 unless, at the discretion of the investigator, the condition is not deemed to cause unacceptable risk to the patient. If deemed not to cause unacceptable risk to the patient, organ function of grade 2 is acceptable.

6.2. Exclusion Criteria

Eligible patients must not meet any of the following exclusion criteria:

1. Histology other than astrocytoma grade IV (GBM or gliosarcoma).

2. Tumor foci detected below the tentorium or beyond the cranial vault.

3. Glioblastoma or gliosarcoma disease with leptomeningeal spread.

4. Patients with a history of any other cancer, unless in complete remission, and off all therapy for that disease for a minimum of 5 years (except history of basal or squamous cell skin cancers that are completely excised/cured at time of enrollment).

5. Patients with serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) > 2.5 X the upper limit of normal (ULN) and bilirubin >1.5 ULN, (unless there is a medical justification for the elevation of bilirubin and ALT, AST).

6. Moderate to severe hepatic impairment, i.e., Child-Pugh Class B/C.

7. Positive results from HIV serology testing, if any available.

8. Supine systolic blood pressure < 100 mmHg or supine diastolic blood pressure < 50 mmHg at screening and baseline, or documented medical history of orthostatic hypotension.

9. Renal insufficiency (estimated creatinine clearance < 50 mL/min) or serum creatinine >1.5 X ULN at screening.

10. Females who are pregnant or lactating or plan to become pregnant during the course of this study.

11. Substance or alcohol abuse or dependence, within 12 months prior to screening.

12. Prior chemotherapy for recurrent GBM with nitrosourea compounds including Gliadel® (carmustine) wafers or bevacizumab.

13. Prior focal radiotherapy (stereotactic radiotherapy or Gamma Knife) within 3 months of screening.

14. Planned treatment, or treatment with any investigational drug within 4 weeks prior to screening.

15. Severe, active co-morbidity, including: Cardiac disease – congestive heart failure class III/IV NYHA; unstable angina or new onset angina (began within the last 3 months) or acute or chronic myocardial infarction within the past 6 months; cardiac ventricular arrhythmias requiring anti-arrhythmic therapy; cerebrovascular accident within 6 months prior to enrollment; acute bacterial or fungal infection requiring intravenous antibiotics at the time of screening; chronic hepatitis B or C infection; chronic obstructive pulmonary disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of screening; hematological and bone marrow diseases. Severe malabsorption (defined as > 15% unintentional loss of body weight in the last 6 months prior to study enrollment), Major medical illnesses or psychiatric impairments that, in the investigator's opinion, will prevent administration or completion of protocol therapy.

16. Patients who are currently taking Coumadin or Coumadin derivatives other than to maintain patency of venous access lines.

17. Requiring renal dialysis

18. Receiving hematopoietic growth factors

19. Have significant baseline neuropathies (> grade 2 based upon CTCAE v 4.03)

20. Had prior exposure to gene vector delivery products within 6 months

21. Any condition that prevents compliance with the protocol or adherence to therapy.

22. Active, uncontrolled bacterial, viral, or fungal infections, requiring systemic therapy.

23. Treated with antibiotics for infection within one week prior to study entry.

24. Fever (> 38.1ºC)

25. Have diastolic blood pressure of > 90 mm Hg resting at baseline despite medication. (Acceptable if on hypertensive medication and diastolic blood pressure is < 90 mm Hg.).

26. Serious nonmalignant disease (e.g., hydronephrosis, liver failure, or other conditions) that could compromise protocol objectives in the opinion of the Investigator and/or the Sponsor.

27. Enrollment in a concomitant clinical study

28. Have a history of hypersensitivity reaction (such as urticaria, allergic reaction including anaphylaxis, toxic epidermal necrolysis, and Stevens-Johnson syndrome) to any of the components of Temozolomide

29. Have a history of hypersensitivity to dacarbazine (DTIC)

6.2.1. Concomitant Medications:

Allowed concomitant medications

Concomitant medications permitted during all study periods include:

- Anti-epileptics

- Oral corticosteroids, provided that the dose is stable for at least 5 days prior to each contrast-enhanced brain MRI performed to assess tumor progression. Any change in the dose of corticosteroids must be recorded in the Case Report Form (CRF).

- Other concomitant medications: therapies considered necessary for the well-being of the patient may be given at the discretion of the treating physicians for both prophylaxis and treatment (e.g., anti-emetic).

All concomitant medications must be recorded in the CRF.

Prohibited concomitant medications

Concomitant medications prohibited during all study periods include:

- Concurrent treatment with bevacizumab or nitrosourea, including Gliadel® wafers.

- Cytotoxic chemotherapy other than TMZ.

- Any investigational drug other than the study drugs.

6.2.2. Concomitant Procedures:

If whilst on protocol, a patient needs surgery for tumor progression or brain surgery for other causes e.g. hydrocephalus, bleeding, the patient can be continued on the study at the discretion of the investigator.

7. STUDY DRUGS:

7.1. Temozolomide (Temodar®) (NDC 0085-1366)

a. DESCRIPTION

4-Methyl-5-oxo-2,3,4,6,8-pentazabicyclo[4.3.0]nona-2,7,9-triene-9-carboxamide (Temozolomide) is a white-to-off-white crystalline powder with a molecular weight of 194.1508

Mechanism of Action: Temozolomide is an imidazotetrazine derivative Temozolomide is not directly active but undergoes rapid nonenzymatic conversion at physiologic pH to the reactive compound 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC). The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O^6 and N^7 positions of guanine.

b. TOXICOLOGY

Human Toxicology:

In patients with newly diagnosed Glioblastoma Multiforme, during the concomitant phase (temozolomide+radiotherapy), adverse reactions including thrombocytopenia, nausea, vomiting, anorexia, and constipation were more frequent in the temozolomide+RT arm. The incidence of other adverse reactions was comparable in the two arms. The most common adverse reactions across the cumulative temozolomide experience were alopecia, nausea, vomiting, anorexia, headache, and constipation. Forty-nine percent (49%) of patients treated with temozolomide reported one or more severe or life-threatening reactions, most commonly fatigue (13%), convulsions (6%), headache (5%), and thrombocytopenia (5%). Overall, the pattern of reactions during the maintenance phase was consistent with the known safety profile of temozolomide.

Myelosuppression (neutropenia and thrombocytopenia), which is a known dose-limiting toxicity for most cytotoxic agents, including temozolomide, was observed. When laboratory abnormalities and adverse reactions were combined, Grade 3 or Grade 4 neutrophil abnormalities including neutropenic reactions were observed in 8% of the patients, and Grade 3 or Grade 4 platelet abnormalities, including thrombocytopenic reactions, were observed in 14% of the patients treated with temozolomide.

In patients with refractory anaplastic astrocytoma,the most frequently occurring adverse reactions were nausea, vomiting, headache, and fatigue. The adverse reactions were usually NCI Common Toxicity Criteria (CTC) Grade 1 or 2 (mild to moderate in severity) and were self-limiting, with nausea and vomiting readily controlled with antiemetics. The incidence of severe nausea and vomiting (CTC Grade 3 or 4) was 10% and 6%, respectively. Myelosuppression (thrombocytopenia and neutropenia) was the dose limiting adverse reaction. It usually occurred within the first few cycles of therapy and was not cumulative. Myelosuppression occurred late in the treatment cycle and returned to normal, on average, within 14 days of nadir counts. The median nadirs occurred at 26 days for platelets (range: 21-40 days) and 28 days for neutrophils (range: 1-44 days). Only 14% (22/158) of patients had a neutrophil nadir and 20% (32/158) of patients had a platelet nadir, which may have delayed the start of the next cycle. Less than 10%

of patients required hospitalization, blood transfusion, or discontinuation of therapy due to myelosuppression.

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

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

Contraindication:
Temozolomide is contraindicated in patients who have a history of hypersensitivity reaction (such as urticaria, allergic reaction including anaphylaxis, toxic epidermal necrolysis, and Stevens-Johnson syndrome) to any of its components. TEMODAR is also contraindicated in patients who have a history of hypersensitivity to DTIC, since both drugs are metabolized to 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC).

Pregnancy and Lactation: Temozolomide can cause fetal harm when administered to a pregnant woman. Male and female patients who take temozolomide should use effective birth control. Female patients and female partners of male patients should avoid becoming pregnant while taking temozolomide. Administration of temozolomide to rats and rabbits during organogenesis at 0.38 and 0.75 times the maximum recommended human dose (75 and 150 mg/m2), respectively, caused numerous fetal malformations of the external organs, soft tissues, and skeleton in both species. There is no data on temozolomide administration during human pregnancy, and it is not currently known if metabolites are excreted in human milk. However, many drugs are excreted in human milk, and there is a potential for adverse effects in nursing infants. Therefore, the use of temozolomide should be avoided in pregnant or nursing women because of the potential hazard to the fetus or infant.

c. PHARMACOLOGY

Kinetics: Absorption: Temozolomide is rapidly and completely absorbed after oral administration with a peak plasma concentration (Cmax) achieved in a median Tmax of 1 hour. Food reduces the rate and extent of temozolomide absorption. Mean peak plasma concentration and AUC decreased by 32% and 9%, respectively, and median Tmax increased by 2-fold (from 1-2.25 hours) when temozolomide was administered after a modified high-fat breakfast. A pharmacokinetic study comparing oral and intravenous temozolomide in 19 patients with primary CNS malignancies showed that 150 mg/m^2 TEMODAR for injection administered over 90 minutes is bioequivalent to 150 mg/m^2 TEMODAR oral capsules with respect to both Cmax and AUC of temozolomide and MTIC. Following a single 90-minute intravenous infusion of 150 mg/m^2, the geometric mean Cmax values for temozolomide and MTIC were 7.3 mcg/mL and 276 ng/mL, respectively. Following a single oral dose of 150 mg/m^2, the geometric mean

Cmax values for temozolomide and MTIC were 7.5 mcg/mL and 282 ng/mL, respectively. Following a single 90-minute intravenous infusion of 150 mg/m^2, the geometric mean AUC values for temozolomide and MTIC were 24.6 mcg·hr/mL and 891 ng·hr/mL, respectively. Following a single oral dose of 150 mg/m^2, the geometric mean AUC values for temozolomide and MTIC were 23.4 mcg·hr/mL and 864 ng·hr/mL, respectively.

Distribution: Temozolomide has a mean apparent volume of distribution of 0.4 L/kg (%CV=13%). It is weakly bound to human plasma proteins; the mean percent bound of drug-related total radioactivity is 15%. Metabolism and Elimination: Temozolomide is spontaneously hydrolyzed at physiologic pH to the active species, MTIC and to temozolomide acid metabolite. MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC), which is known to be an intermediate in purine and nucleic acid biosynthesis, and to methylhydrazine, which is believed to be the active alkylating species. Cytochrome P450 enzymes play only a minor role in the metabolism of temozolomide and MTIC. Relative to the AUC of temozolomide, the exposure to MTIC and AIC is 2.4% and 23%, respectively.

Excretion: About 38% of the administered temozolomide total radioactive dose is recovered over 7 days: 37.7% in urine and 0.8% in feces. The majority of the recovery of radioactivity in urine is unchanged temozolomide (5.6%), AIC (12%), temozolomide acid metabolite (2.3%), and unidentified polar metabolite(s) (17%). Overall clearance of temozolomide is about 5.5 L/hr/m^2. Temozolomide is rapidly eliminated, with a mean elimination half-life of 1.8 hours, and exhibits linear kinetics over the therapeutic dosing range of 75 to 250 mg/m^2/day.

Formulation: 100mg capsules of temozolomide are supplied as a white capsule with a pink cap . The capsules contain temozolomide and inactive ingredients lactose anhydrous, colloidal silicon dioxide, sodium starch glycolate, tartaric acid, and stearic acid in the form of a white powder. The body of the capsules are made of gelatin and are opaque white. The cap is also made of gelatin, and the colors vary based on the dosage strength.

Storage and Stability: Store temozolomide Capsules at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F) .

Administration: Oral

Handling Precautions: Care should be exercised in the handling and preparation of TEMODAR. Vials and capsules should not be opened. If vials or capsules are accidentally opened or damaged, rigorous precautions should be taken with the contents to avoid inhalation or contact with the skin or mucous membranes. The use of gloves and safety glasses is recommended to avoid exposure in case of breakage of the vial or capsules. Procedures for proper handling and disposal of anticancer drugs should be considered. (1)

d. SUPPLIER

Temozolomide is commercially available as Temodar® for the treatment of adult patients with newly diagnosed glioblastoma multiforme concomitantly with radiotherapy and then as

maintenance treatment. Temodar® is manufactured by Merck&Co., Inc., Whitehouse Station, New Jersey, USA. Generic drug may also be used with prior approval from sponsor.

7.2.SGT-53

a. DESCRIPTION

The study agent, SGT-53, consists of a DOTAP/DOPE liposome encapsulating a plasmid containing a cDNA encoding the normal human wild type p53 gene, with a TfRscFv targeting entity decorating the surface of the liposome A final concentration of 10% sucrose is included in the complex as an excipient. Each of the components is prepared under cGMP conditions at the following facilities:

TfRscFv – TfRscFv single chain antibody cell paste and inclusion bodies were manufactured at the Waisman Clinical BioManufacturing Facility, Madison, WI.
TfRscFv single chain antibody was isolated from the inclusion bodies by Advanced Bioscience Laboratories, Inc., Kensington, MD

- Liposome – Walter Reed Army Institute for Research, Silver Spring, MD, USA
- DNA – Aldevron, Fargo, ND, USA

Mechanism of Action: The therapeutic agent in SGT-53 is a DNA sequence encoding normal human p53. The p53 gene is a vital tumor suppressor gene in humans. Numerous human tumors, including brain cancers possess a loss or mutation of the wild type p53 (wtp53) gene. In addition to playing a crucial role in cell cycle control, the p53 protein is a critical component in two of the pathways involved in regulating tumor cell growth: cell death (apoptosis) and the regulation of angiogenesis. Due to its role in the apoptotic pathway, restoration of functional p53 in tumor cells permits the cells to enter apoptosis when treated with conventional chemotherapeutic agents. This results in sensitization of the tumors to the killing effects of chemotherapy and more effective tumor cell death.

b. TOXICOLOGY

Human Toxicology:

No MTD reached when used as a single agent in Phase Ia trial. No Grade 4 AEs related to SGT-53 occurred in the Phase Ia Trial. The Table below list the Grades 1-3 AEs that were observed in the Phase Ia single agent train and the Phase Ib combination trial of SGT-53 and docetaxel to date.

Phase Ia **Grade 1, 2, & 3 AE's**									
Pt ID	**Relation to Study Drug**								
	Grade 1			**Grade 2**			**Grade 3**		
	Possible	**Probable**	**Definite**	**Possible**	**Probable**	**Definite**	**Possible**	**Probable**	**Definite**
02-003	None	None	None	None	Intermittent Diarrhea, Intermittent Dehydration, Hypotension	None	None	None	None
02-006	None	Chills, Hypotension, Headache, Tachycardia, Fever	Fever	Fatigue	Fever	None	None	None	None
02-007	Fatigue, Fever, Neutropenia, Chills	Fever, Tumor Necrosis in Mouth	None	Fatigue, Dehydration, Phlebetis IV site L	Neutropenia, Tumor Necrosis in Mouth	None	Fatigue*	None	None
02-008	None	Hypotension	None	None	Hypotension	None	None	None	None
02-010	None	None	Hypotension	Intermittent Diarrhea	None	None	None	None	None

02-011	Anorexia, Fatigue, Tachycardia, Dizziness, Oral Ulcer, Lightheadedness, Elevated D-Dimer	Fever, Hypotension	Fever, Hypotension,	Anemia, Fatigue, Headache	Fever, Leukopenia, Elevated AST, Elevated ALT, Neutropenia	Fever	None	None	None
02-012	Fatigue	Hypotension	None	Anemia, Elevated LDH	None	None	None	None	None
02-013	Anorexia, Nausea	None	None	None	Anemia	Chills/Rigor, Fever, Hypotension, Stomatitis	None	None	None
02-014	Intermittent Diarrhea	None	Chills/Rigor	Diarrhea	None	None	None	None	None
02-015	Nausea, Hypomagnesemia, Flushed, Wheezing, Intermittent Nausea, Transient Shortness of Breath	None	Chills/Rigor, Nausea	Diffuse Patchy Infiltrates, Wheezing, Fluid Overload, Tachycardia, Dyspepsia, Neutropenia, Chest Pain	Fatigue	Fever, Fatigue, Hypotension	None	None	None

02-016	Fatigue, Weakness, Blurred Vision, Flushing, Headache, Mild Nausea, Dizziness, Insomnia, Lightheadedness, Chest Tightness, Dyspnea on Exertion, Choking Feeling, Weight Loss	None	None	Diarrhea, Nausea, Depression, Malaise, Nervousness, Increased Shortness of Breath	None	None	None	None	None

* - Not a serious adverse event

Phase Ib Grade 1, 2, & 3 AE's									
Pt ID	**Relation to Study Drug**								
	Grade 1			**Grade 2**			**Grade 3**		
	Possible	**Probable**	**Definite**	**Possible**	**Probable**	**Definite**	**Possible**	**Probable**	**Definite**
02-018	**SGT-53** Nausea, Vomiting, Fever **Docetaxel** Fever, Coating on the tongue	**SGT-53** Chills **Docetaxel** Fever	None	**SGT-53** Hypotension, Fatigue **Docetaxel** Dehydration, Intractable nausea, Intractable vomiting Fatigue	None	None	**SGT-53** Neutropenia	**Docetaxel** Neutropenia	None
02-019	**SGT-53** Intermittent fatigue **Docetaxel** Intermittent fatigue	None	None	None	None	None	None	None	None
02-020	**SGT-53** Diarrhea, Anorexia, Anemia, Weakness **Docetaxel**	**SGT-53** Chills, Headache,	None	**SGT-53** Diarrhea, Anemia, Dehydration **Docetaxel** Anemia, Dehydration	None	None	None	None	None

	Anorexia, Anemia, Weakness								
02-023	**Docetaxel** Thrush, Thrombocyto-penia	None	**SGT-53** Fever, Hypo-tension	**Docetaxel** Thrombocytopenia	None	None	None	None	None
02-024	**p53** Elevated ALT, Hypocalcemia, Elevated AST,	None	None	**p53** Elevated AST, Hypotension	None	None	**p53** Syncopal episode	None	None
02-025	None	None	None	None	None	None	None	None	None
02-026	None	None	None	**p53** Intermittent hypotension, dehydration	None	None	None	None	None

Pregnancy and Lactation: No Data Available

c. PHARMACOLOGY

Kinetics: No Data Available

Formulation:

Each of the components is prepared under cGMP conditions at the following facilities:

- TfRscFv – Walter Reed Army Institute for Research, Silver Spring, MD, USA
- Liposome – Walter Reed Army Institute for Research (WRAIR), Silver Spring, MD, USA
- DNA – Aldevron, Fargo, ND, USA

The cGMP grade sucrose is obtained in crystalline form. A sterile 70% aqueous solution for use in the complex was prepared under cGMP conditions at the WRAIR, Silver Spring MD as part of the process for production of the SGT-53 complex.

The SGT-53 complex was prepared under cGMP conditions at WRAIR, Silver Spring MD. The components were mixed in a set order and at a specific ratio of TfRscFv: Liposome:DNA as per SOP supplied by SynerGene Therapeutics, Inc to WRAIR. The resulting SGT-53 complex (at a dose of 0.6mg DNA/vial) was vialed, lyophilized and stored at -20ºC at WRAIR. This batch was tested and qualified for human use. These vials are shipped in batches of 50 - 100 vials to the Investigational Pharmacy of clinical trial site on dry ice and stored at the Investigational Pharmacy at the clinical trial site at 2-8ºC . They are reconstituted in the same facility with WFI using the procedure provided by SynerGene Therapeutics, Inc.

Storage and Stability:

The doses are given as mg of wtp53 plasmid DNA. The complex is prepared at a specific ratio of 1 ug of DNA to a specific amount of TfRscFv and a specific amount of liposome. Therefore, as the amount of DNA increases, the amount of TfRscFv and liposome in the complex increases proportionally. The complex will then be mixed with 5% Dextrose for infusion. The final volume of the complex/5% Dextrose per infusion will be ~200 ml and will be infused intravenously over 90-120 minutes.

The complex diluted in 5% Dextrose (at ratios of 1:2 to 1:5) is stable for at least eight hours at 20ºC once prepared. Infusion of the complex should be initiated within one hour of preparation and the infusion completed within eight hours of dilution of study agent in dextrose.

The investigator will be responsible for dispensing and accounting of all components of the complex provided by the sponsor. Records of document control numbers and dates received will be kept on a Drug Inventory Form provided by the sponsor. Under no

circumstances will the investigator supply study drug to other investigators, allow study drug to be used other than directed by this protocol, or destroy or dispose of study drug in any other manner without prior authorization from the sponsor.

Any residual clinical trial materials must be returned to the clinical site's investigational drug pharmacy for disposition. All unopened or partially used vials of the SGT-53 components will be returned to SynerGene Therapeutics, Inc. at the end of the study.

Reconstitution:

With sterile WFI as directed by Sponsor supplied SOP

Administration: Intravenous.

Handling Precautions:

None to date.

d. SUPPLIER

SGT-53 is not commercially available. It is provided free of charge by the Sponsor

SynerGene Therapeutics, Inc
9812 Falls Road, Suite 114
Potomac MD 20854
Tel: 301-706-1509
Email: clinicaltrial@synergeneus.com

8. STUDY ENDPOINTS:

8.1. Safety Endpoint Definitions

Patients will be evaluated and adverse events (AEs) assessed according to the Study Calendar (Section 18.1) using Common Toxicity Criteria 4.0 (CTC 4.03, 2010). For definition of DLT see Section 9.7.

8.2. Efficacy Endpoints

To evaluate the PFS, 6 month PFS, OS in patients treated with SGT-53 in combination with Temozolomide. These parameters will be evaluated using RANO Response Criteria (Response Assessment in Neuro-Oncology). All patients who have measureable disease according to RANO, received at least one dose of SGT-53, and have had disease re-evaluated will be included in the analyses of secondary endpoints.

Optional surgical resection for tumor analysis:

DNA PCR will be employed to determine the presence of the nanoparticle delivered exogenous wtp53 gene in the tumor sample obtained during surgical resection. A maximum of 6 patients will be have this optional testing. Detection of exogenous wtp53 in the tumors from at least 3 patients will be considered a positive outcome. If the presence of the exogenous wtp53 is detected in at least 3 of the first 4 tumors assessed, the option for surgical resection for tumor analysis will no longer be offered.

9. TREATMENT PLAN:

Cycles 1-3 (1 Cycle is 28 days)

- Day 1: SGT infusion
- Day 4: SGT infusion
- Day 8: SGT infusion
- Days 9-13: TMZ (150mg/m2 increasing to 200mg/m2 as tolerated, provided the patient meets the criteria for non-hematological toxicity, ANC and platelet counts)
- Day 11: SGT infusion
- Day 15: SGT infusion
- Day 18: SGT infusion
- Days 19-28: No treatment

In those patients who had a surgical resection, the 1st cycle of SGT-53 and TMZ will start 14-21 days post operatively and having recovered from the effects of surgery.

Cycles 4-6: Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycles of SGT-53/Temozolomide therapy at investigator's discretion. Alternatively, they may continue on Temozolomide alone at investigator's discretion.

Optional surgical resection for tumor analysis (Maximum of 6 Patients):

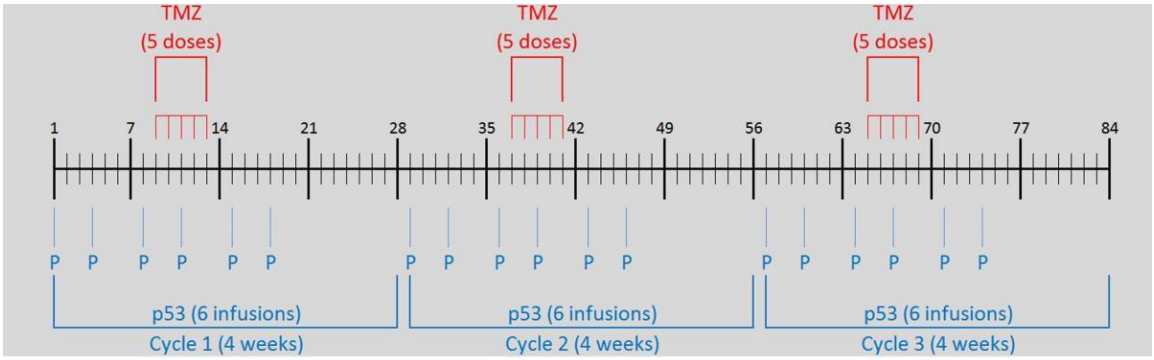
- SGT infusion on days -3 and -1
- Surgery on Day 0
- 14- 21 days post operatively and having recovered from the effects of surgery, the patients will then start cyclical TMZ with SGT-53 as described above: SGT-53 will be administered every 3rd or 4th day in a 28 day cycle (total of 6 infusions)] starting on Day 1 (cycle 1), Day 29 (cycle2) and Day 57 (cycle 3). The day of the first post-surgical infusion of SGT-53 will be Day 1 (cycle 1). Temozolomide will be administered by mouth daily on days 9-13 of each cycle at 150mg/m2 (cycle 1), and 200mg/m2 as tolerated for cycle 2 and beyond provided the patient meets the criteria for non-hematological toxicity, ANC and platelet counts (see 9.7.2).
- Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycle of SGT-53/Temozolomide therapy at investigator's discretion. Alternatively, they may continue on Temozolomide alone at investigator's discretion.

9.1. Dosing Plan:

Administration of SGT-53 and Temozolomide therapy

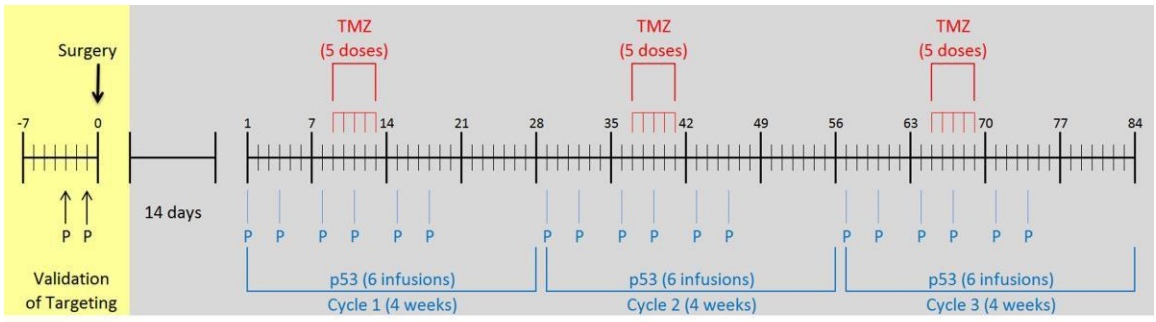
Agent	Dose	Route
Temozolomide	150 mg/m^2 (starting dose)	Oral
SGT-53	3.6mg DNA per infusion	IV over 90-120 minutes

9.1.1. Treatment Schedules



P: SGT-53 treatment, IV, 3.6 mg DNA, twice/week for 3 weeks.
T: TMZ treatment, oral, 150 mg/m² daily, for 5 days in Cycle 1; 200 mg/m² daily for 5 days in Cycles 2 and 3.

With Optional Surgical Resection for Tumor Analysis



P: SGT-53 treatment, IV, 3.6 mg DNA, twice/week for 3 weeks.
T: TMZ treatment, oral, 150 mg/m² daily, for 5 days in Cycle 1; 200 mg/m² daily for 5 days in Cycles 2 and 3.

In case of holiday or other logistic issue, schedule will allow for SGT-53 treatments to be delivered on Monday and Thursday, or Tuesday and Friday of each week. If necessary, other schedules may be permitted with the approval of the Sponsor and PI.

9.2. Premedication

Pre-medication will follow emerging principles for liposomal agents, consisting of allergic prophylaxis and close monitoring for bronchospasm (i.e., grade 3 hypersensitivity reaction) and other signs of acute hypersensitivity (≥ grade 2 hypersensitivity). Specifically, for each infusion of SGT-53, patients will receive dexamethasone 8 mg i.v. 1 hour +/-5 minutes prior to dosing, and a combination of histamine H1 and H2 blockers (e.g. Benadryl 25 mg and Pepcid 20 mg, both i.v.) 30 minutes +/- 15 minutes and indocin 25 mg, or an equivalent NSAID, p.o. approximately 30 +/- 5 minutes prior to receiving SGT-53. All patients will also receive acetaminophen 650 mg p.o. just prior to SGT-53 administration as prophylaxis for pyretic reactions. Generic drugs may be used with prior approval from sponsor.

Premedications for temozolomide will be at investigator discretion, according to institutional practices. Antiemetic(s) suitable for controlling mild to moderate nausea and vomiting should be given prior to chemotherapy.

9.3. SGT-53 Infusion

Once the study drug is prepared, the investigational pharmacy will immediately deliver the study drug to the outpatient clinic for administration to the study participant. A final volume of ~200 ml of SGT-53 in 5% Dextrose will be infused over 90-120 minutes intravenously via a newly inserted central venous line or following flushing of an intact central venous line. Infusion must be completed as soon as possible, but no later than within eight hours of dilution of SGT-53 in dextrose.

If infusion reactions occur, acetaminophen, diphenhydramine, steroids or other medications may be given for symptom control as needed. Although prolonged use of systemic corticosteroids to prevent allergic reactions is discouraged, intermittent use of corticosteroids is permitted. Use of corticosteroids (dose, name, start/off dates) should also be documented in CRFs

9.3.1. Medications and Observations During and After Infusions of SGT-53

Four hours after completion of each infusion of SGT-53, subjects will receive acetominophen, 650 mg p.o. In addition, Demerol, 12.5 mg i.v.p.b. will be available for prn use (up to two doses) as needed for rigors. All infusions will be given where epinephrine (1:1000) 0.3-0.5 mL s.q. or i.m. (or epinephrine (1:10,000) 3-5 mL i.v.). is IMMEDIATELY available, along with dexamethasone 20 mg i.v. for prn use for suspected acute hypersensitivity reactions, including hypotension or bronchospasm. Insulin will be available for use for subjects with elevation in blood glucose levels.

Subjects will have vital sign (pulse, blood pressure and temperature) monitoring every 15 ± 5 minutes during infusion and every 30 ± 5 minutes for two hours after each infusion, and will be monitored closely for wheezing, change in blood pressure or signs of hypoperfusion such as cold, clammy skin, cyanosis or tachycardia. If any of these signs occur, infusion will be stopped. All patients will continue to have an i.v. fluid (at a rate

of at least 100 mL/hr) during this two hour post-infusion observation period. A 12-lead ECG will be performed weekly (2 hours post-infusion) during the first 2 weeks of cycle 1,and at the End Of Treatment (EOT) visit. If these patients receive additional rounds of SGT-53/Temozolomide a 12-lead ECG will only be performed as necessary and at the EOT visit.

9.3.2. **SGT-53 Infusion Reactions**

Infusion reactions are defined as any adverse event that occurs during the infusion of SGT-53 or during the two hours following completion of the infusion. Hypersensitivity reactions are those events defined by NCI criteria, e.g., bronchospasm, dyspnea, etc.

If infusion reactions occur during the infusion of SGT-53, the infusion will be discontinued and appropriate medical care administered. SGT-53 is to be administered only by physicians or nurses with training and experience with the administration of chemotherapeutic agents. All medications and interventions used to treat infusion reactions will be recorded and reviewed by the DSMB at the routine scheduled meetings (see Section 14).

The SGT-53 infusion may resume at a slower infusion rate if the symptoms resolve within two hours. If the infusion recurs at the same grade or higher, the infusion will be terminated and no further infusions of SGT-53 administered to that patient.

If a hypotensive reaction (BP < 90 systolic) occurs during infusion of SGT-53, infusion should be discontinued and not resumed during that visit. SGT-53 infusions may resume if the symptoms of hypotension have resolved by the time of the next scheduled infusion. Should this occur during SGT-53 infusions and not resolve within 24 hours the patient will not receive any further treatments (neither Temozolomide nor SGT-53) that week. The treatments will begin again the following Monday, repeating the interrupted week.

Should a second acute hypotensive reaction (BP < 90 systolic) occur during infusion of SGT-53 the patient will be removed from protocol and be replaced.

In the event that an infusion reaction consists of respiratory wheeze, including symptomatic bronchospasm or any grade 3 or 4 hypersensitivity reaction, the infusion is to be permanently discontinued and the patient replaced.

If infusion reactions occur, acetaminophen, diphenhydramine, steroids or other medications may be given for symptom control as needed. Although prolonged use of systemic corticosteroids to prevent allergic reactions is discouraged, intermittent use of corticosteroids is permitted. Use of corticosteroids (dose, name, start/off dates) should also be documented in CRFs

9.3.3. **Observation for First SGT-53 Infusion**

For the first infusion of SGT-53, subjects will be monitored and observed for 23 hours from the time of admittance to the clinic. Subjects will receive the infusion in the

outpatient clinic and be monitored there until infusion is completed and for 2 to 8 hours after the completion of the infusion. After the initial period of 2-8 hours, the patient will be admitted and monitored as an inpatient for the remainder of the 23 hour observation period. Alternatively, patients can be admitted directly to the hospital prior to receiving the first infusion of SGT-53 and to be monitored and observed there for, at least, 24 hours from the time of admittance.

Additionally a 12-lead ECG will be performed 2 hours and 23 hours after the first infusion of SGT-53.

During the observation period, subjects will have continued i.v. access with maintenance of a 20 gauge catheter for rapid fluid management if necessary. The IV access rate should be ≥ 100 mL/hour during the observation period.

The patient will continue to receive i.v. fluid at ≥ 100 mL/hr during the observation period after the initial infusion and vital signs (blood pressure, pulse, temperature and respiration) will be obtained every 15 + 5 minutes during infusion, every 30 + 5 minutes for the first two hours after the infusion and every 1 hour ± 15 minutes during hours 3 to 8 post-infusion, and thereafter every 2 hours ± 15 minutes for the remainder of the observation period. Patients will be monitored closely for wheezing, change in blood pressure or signs of hypoperfusion such as cold, clammy skin, cyanosis or tachycardia.

Four hours after completion of the first infusion of SGT-53, subjects will receive acetaminophen, 650 mg p.o. The patient will receive acetaminophen 650 mg po alternating with Indocin 25 mg, or an equivalent NSAID, po every 4 hours during the observation period.

During the hospitalization, Demerol 12.5 mg i.v.p.b. must be available as needed for rigors. Epinephrine (1:1000 0.3-0.5 mL s.q. or i.m.) or epinephrine [(1:10,000) 3-5 mL i.v.] must also be IMMEDIATELY available, along with dexamethasone 20 mg i.v. for prn use for suspected acute hypersensitivity reactions, including hypotension or bronchospasm. Insulin will be available for use for subjects with elevation in blood glucose levels.

The next day the subjects will be discharged if medically stable. If at the end of the observation period, the subject has not resolved any SGT-53 related toxicities to ≤ grade 1, the subject will be observed until the time at which the managing physician deems the patient medically stable for discharge.

If patient is receiving a second course of treatment, the 23 or 24hour observation period and 12-lead ECG are not required.

9.3.4. **Outpatient Clinic for Subsequent SGT-53 Infusions**

For subsequent infusions, subjects will be infused with SGT-53 in the outpatient infusion unit of in one of the outpatient ambulatory treatment centers at the clinical trial sites. The treatment centers are staffed with medical personnel and equipment to treat emergency and non-emergency events and is highly experienced with phase I and II trials. Subjects

will be monitored for two hours, as in Section 9.3.1., and will be discharged only if medically stable. The subject can be monitored for up to 8 hours in the clinic prior to discharged if medically stable. If necessary, the subject can be admitted for 23 hour observation as described for the first infusion (Section 9.3.3.).

9.4. Study drug discontinuation and study withdrawal:

Every reasonable effort should be made to maintain patient compliance and participation in the study. However, all patients are free to withdraw from participation in this study at any time, for any reason, and without prejudice.

A patient will be considered as withdrawn from the study if, and only if, he/she withdraws consent, or is lost to follow-up after unsuccessful attempts by the investigator to contact the patient.

The Investigator may terminate a patient from the study for administrative reasons, or in the investigator's opinion, to protect the patient's best interest. Reasons may include the following:

- Patient withdraws consent and refuses to continue procedures/observations.
- If at any time, continued participation in the study is determined not to be in the best interest of the patient.
- Development of a severe (grade 4) or intolerable adverse event that does not resolve within a 14 day time-frame.
- Development of a significant intercurrent illness.
- Deterioration in patient's disease status such that chemotherapy is no longer indicated.
- Termination of the study by the Sponsor. Patient's care will not be affected in the event of premature termination of this study.
- At any time during study in patients with Progressive Disease as defined below, as determined by Investigator (see Section 18.2.)

If a patient is withdrawn before completing the study, the reason for withdrawal will be entered on the appropriate CRF. Whenever possible and reasonable, the evaluations that were to be conducted at the completion of the study and radiographic tumor measurement should be performed at the time of premature discontinuation.

For grade 2 events of increased bilirubin, AST, ALT, dyspnea, or DIC, the dose of SGT-53 can be held for up to two weeks. If the grade 2 event resolves within two weeks, then infusion of SGT-53 can resume at the same dose per calendar at incident week + 1-2 weeks (i.e., treatment would be made up and moved 1-2 weeks later).

For non-hematological events other than increased bilirubin, AST, ALT, dyspnea, or DIC, participants who develop a > or = to grade 2 toxicity (except for alopecia, or fever, chills, hypotension that respond to treatment) or grade 3 fatigue will discontinue twice-

weekly infusions until the toxicity level reduces to at least grade 1. Infusions will be held for one week intervals, and infusions that are withheld will be made-up (i.e. moved 1-2 weeks later). If the toxicity does not resolve within two weeks, study drug will be discontinued.

In the event that a patient is removed from the study for reasons other than toxicity, they can be replaced at the same dose level.

Patients who go off-treatment after receiving any infusions of SGT-53 will be considered eligible for evaluation of toxicity. Patients withdrawing consent prior to initiation of therapy will be replaced at the same dose level.

9.4.1. Patient follow up after study discontinuation:

All patients who received treatment with SGT-53 must be followed-up for 30 days after discontinuation of study drug. The 30-day follow-up visit should include a physical examination, vital signs, concomitant medications and assessment of AEs/SAEs and Urine analysis. Blood samples will be taken for: Hematology (CBC with differential, platelet count), Chemistries (BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein, glucose, LDH), PT/PTT, and D-dimer. Patients who permanently discontinue study drug(s) for reasons other than complete response, progression, or death should also have visits approximately every 2 months for the first year that should include a MRI, physical examination, vital signs, and recording of concomitant medications for glioblastoma.

Patients who are withdrawn from study treatment due to a complete response will have the 30-day follow-up visit as described above. Further follow-up visits every 2 months will include only a physical/neurological exam, concomitant medications, vital signs and a MRI.

A telephone contact to check on vital status and any additional treatment for glioblastoma will occur at yearly.

9.5. Replacement policy

Patient replacement will be decided by the Safety Monitoring Committee.

9.6. Dose Modification

Reduction/interruption of dosing for adverse events may take place at any time. Below are guidelines for dose modification for drug-related toxicities as well as guidelines for their management. These parameters are only a guide and are not intended to supersede the clinical judgment of the treating physician. All adjustments should be made in consultation with the Medical Monitor and the Sponsor.

All dose modifications should be based on the worst preceding toxicity. If study treatment is being held due to toxicity, scheduled visits and all assessments should continue to occur except the dosing of the study drug.

9.7. Dose Modification Plans:

9.7.1. SGT-53 and/or Temozolomide

Table 9.7.1. reviews management of **non-hematologic** toxicities as they pertain to both study agents SGT-53 and/or Temozolomide.

Toxicity	Grade 1	Grade 2	Grade 3	Grade 4
Hyperbilirubin-emia	Continue both at same dose	Hold both until grade 1 or less in 2 weeks	Discontinue TMZ, hold SGT-53 until grade 1 or less (up to 2 weeks)*	Discontinue TMZ, hold SGT-53 until grade 1 or less (up to 2 weeks)*
AST &/or ALT	Continue both at same dose	Hold both until grade 1 or less in 2 weeks	Discontinue TMZ, hold SGT-53 until grade 1 or less (up to 2 weeks)*	Discontinue TMZ, hold SGT-53 until grade 1 or less (up to 2 weeks)*
Dyspnea not associated with infusion	Continue both at same dose	Hold both until grade 1 or less in 2 weeks	Discontinue TMZ, hold SGT-53 until grade 1 or less (up to 2 weeks)*	Discontinue TMZ, hold SGT-53 until grade 1 or less (up to 2 weeks)*
DIC	Continue both at same dose	Hold both until grade 1 or less in 2 weeks	Discontinue TMZ, hold SGT-53 until grade 1 or less (up to 2 weeks)*	Discontinue TMZ, hold SGT-53 until grade 1 or less (up to 2 weeks)*
Fatigue	Continue both at same dose	Continue both at same dose	Hold both until grade 1 or less in 2 weeks	Discontinue both
Infusion reaction	May rechallenge after 1st event, if resolves in 2 h**	May rechallenge after 1st event, if resolves in 2 h**	Discontinue inciting agent**	
Hypotension during infusion	See Section 9.3.2.	See Section 9.3.2.	See Section 9.3.2.	

Other non-hemetoxicity* **	Continue both at same dose	Hold both until grade 1 or less in 2 weeks	Hold both until grade 1 or less in 2 weeks	Discontinue both

*If toxicity reduces to grade 1 or less in 2 weeks, patient may continue SGT-53 until end of cycle at discretion of investigator.

**Note that infusion reactions are discussed in detail in Section 9.3.2.

***Other non-heme toxicity refers to any non-heme toxicity at least possibly related to SGT-53, or temozolomide with the exception of alopecia, inadequately treated nausea, vomiting, diarrhea, or fever, chills or hypotension which respond promptly to treatment and lasts less than 24 hours.

For grade 2 events of increased bilirubin, AST, ALT, dyspnea, or DIC, the dose of SGT-53 and Temozolomide can be held for up to two weeks. If the grade 2 event reduces at least to grade 1 within two weeks, then infusion of SGT-53 and Temozolomide can resume at the same dose per calendar at incident week + 1-2 weeks (i.e., treatment would be made up and moved 1-2 weeks later).

For non-hematological events other than increased bilirubin, AST, ALT, dyspnea, or DIC, participants who develop a > or = grade 2 toxicity (except for alopecia, fever, chills or hypotension that respond to treatment) or grade 3 fatigue will discontinue twice-weekly infusions until the toxicity level reduces to at least grade 1. Infusions will be held for one week intervals, and infusions that are withheld will be made-up (i.e. moved 1-2 weeks later). If the toxicity does not resolve within two weeks, study drug will be discontinued.

A minimum of 2-week rest period will be required if there is grade III or greater non-hematologic toxicity related to SGT-53/Temozolomide that cannot be controlled with supportive medications/measures. After the minimum 2-week rest period and the toxicity has resolved to a grade II or better, the patient will resume taking the drug at a reduced dose level as shown in Table 9.7.2b below.

Subjects who receive less than four of twelve of the intended drug infusions, due to experiencing these grade 2 events that have not resolved as described above, and that are determined by the PI to be at least probably related to SGT-53, will be considered to have experienced a DLT.

Patients forced to discontinue temozolomide due to a temozolomide-related toxicity (e.g., grade 3, 4 hypersensitivity) will be removed from the study.

9.7.2. Temozolomide

Table 9.7.2a. reviews management of hematologic toxicities associated with Temozolomide treatment. Note that there are no dose adjustments for anemia.

	Grade 1	Grade 2	Grade 3 or Grade 4
Neutropenia	< LLN to 1500/mm^3 Proceed at current dose	<1500 to 1000/mm^3 Proceed at current dose	Grade 3: <1000 to 500/mm^3 Grade 4: <500/mm^3 If resolves to grade ≤ 2 within 14 days, rechallenge at same dose of SGT-53, and reduce dose of TMZ in increments after each event according to Table 9.7.2b below, i.e. in Cycle 1reduce to 100 mg/m^2 after the first event, in Cycle 2 reduce to 150 mg/m2 after the first event, if recurs reduce to 100 mg/m^2 etc If recurs at lowest dose of TMZ (100 mg/m^2 or less), withdraw from study. If at any time it does not resolve within 14 days, withdraw from study.
Thrombo-cytopenia	<LLN to 75,000/mm^3 Proceed at current dose	<75,000 to 50,000/mm^3 Proceed at current dose	Grade 3: <50,000 to 25,000/mm^3 Grade 4: <25,000/mm^3 If resolves to grade ≤ 2 within 14 days, rechallenge at same dose of SGT-53, and reduce dose of TMZ in increments after each event according to Table 9.7.2b below, i.e.in Cycle 1 reduce to 100 mg/m^2 after the first event, in Cycle 2 reduce to 150 mg/m^2 after the first event, if recurs reduce to 100 mg/m^2 etc If recurs at lowest dose of TMZ (100 mg/m^2 or less), withdraw from study. If at any time it does not resolve within 14 days, withdraw from study.

Table 9.7.2.b Dose de-escalation for TMZ:

Treatment Cycle	**Agent**	**Schedule**	**Level**	**Level**	**Level**
			0 (Target Dose)	-1	-2
Cycle 1	TMZ	Days 9-13	150 mg/m^2	100mg/m^2	
Cycle 2 onwards		Days 9-13	200 mg/m^2	150 mg/m^2	100 mg/m^2

In the event of grade III or higher toxicity associated with TMZ after cycle 1, treatment will be interrupted and patient monitored for resolution of toxicity, with subsequent re-initiation of treatment. TMZ dosing will resume after complete resolution of toxicities to Grade II or less.

The dose of Temozolomide will only be escalated to 200 mg/m2 if the patient meets the following criteria: If the CTC nonhematologic toxicity for Cycle 1 is $\leq$ Grade 2 (except for alopecia, nausea, and vomiting, diarrhea, or fever, chills or hypotension which respond promptly to treatment and lasts less than 24 hours), absolute neutrophil count (ANC) is $\geq$ 1,500/mm^3, and the platelet count is $\geq$ 100,000/mm^3. The dose remains at 200 mg/m2 per day for the first 5 days of each subsequent cycle except if toxicity occurs. If the dose was not escalated at Cycle 2, escalation should not be done in subsequent cycles.

If more than a 2 week delay is required to meet hematologic parameters for treatment, the patient must be removed from study and replaced.

9.7.3. Dose de-escalation for SGT-53:

Permitted dose modifications for SGT-53

Investigators should follow the guidelines described in Table 9.7.1. above and Table 9.7.3. below for the modification of SGT-53 treatment unless there is an urgent need for action due to consideration of patient safety.

If the patient requires a dose interruption of >42 days from the previous dose, then the patient must be discontinued from study treatment. Within the 42-day dose delay, if the toxicity has resolved, there is no evidence of true progressive disease, and the patient has not started any other antineoplastic therapy, the patient may resume study treatment. For patients who undergo dose interruptions (delays), if the same toxicity returns after re-initiation of treatment, regardless of duration, the second re-initiation must resume at a lower dose (see Table 9.7.3. below). Only two dose reductions will be allowed. If further reduction is needed, the patient must be discontinued from study treatment. Patients who discontinue the treatment due to an adverse event or an abnormal laboratory value must be followed until resolution or stabilization of the event. All dose changes or interruptions must be recorded in the dosage administration record CRF, as appropriate.

Table 9.7.3. Recommended Dose modifications and dose delays for suspected SGT-53 treatment related hematologic toxicities

Recommended dose modifications	
Worst toxicity CTCAE grade at any time during treatment	
Hematologic:	
Neutropenia (ANC)	
Grade 1 (ANC<LLN-1500/mm^3)	Maintain dose
Grade 2 (ANC<1500-1000/mm^3)	Maintain dose

Grade 3 or 4 (ANC< 1000-500/mm3 or <500/mm3)	Hold dose until resolved to ≤ grade 1 If resolved in ≤ 14 days, then maintain dose level

	If resolved >14 days, then decrease dose of SGT-53 to 2.4 mg DNA/infusion. If occurs at 2.4mg DNA dose then decrease dose of SGT-53 to 1.2 mg DNA/infusion. If occurs at this second reduced dose of SGT-53 withdraw from study.
Thrombocytopenia	
Grade 1 (PLT<LLN-75,000/mm^3)	Maintain dose
Grade 2 (PLT<75,000-50,000/mm^3)	Maintain dose
Grade 3or 4 (PLT<50,000-25,000/mm^3 or <25,000/mm^3)	Hold dose until resolved to ≤ grade 1 If resolved in ≤ 14 days, then maintain dose level If resolved >14 days, then decrease dose of SGT-53 to 2.4 mg DNA/infusion. If occurs at 2.4mg DNA dose then decrease dose of SGT-53 to 1.2 mg DNA/infusion. If occurs at this second reduced dose of SGT-53 withdraw from study.

10. STUDY ASSESSMENTS:

10.1. Pretreatment Evaluation

Pre-study assessments will be performed according to the Study Calendar (Section 18.1.) before study registration and include:

Within 7 days of registration:

- Radiographic tumor measurements
- Complete medical history
- Concomitant medication assessment
- Baseline Review of Systems and AE documentation
- Physical examination (all body systems)
- Vital signs (temperature, blood pressure, pulse rate, and respiratory rate)
- KPS Performance Status

- 12-lead electrocardiogram
- Limited Echocardiogram with the results reviewed by a cardiologist
- Serum chemistry including: sodium, chloride, carbon dioxide, potassium, calcium, phosphorous, magnesium, BUN, creatinine, blood sugar (random), albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin (total, direct), and total protein.
- Coagulation parameters
- CBC with differential and platelet counts
- Urinalysis

Within 3 days of initiation of treatment:

- Pregnancy test (for nonsterile women of childbirth potential)

10.1.1. Laboratory Certification and Normal Values

Before initiation of the study, the laboratory certification for all local laboratories to be used in the study must be provided to the Sponsor. Normal ranges of all laboratory parameters required during the study must be provided by the Investigator.

10.2. Evaluation During Study

The starting dose of SGT-53 will be 3.6 mg of DNA. If necessary, dose de-escalation for SGT-53 will proceed according to the dose de-escalation scheme in Table 9.7.3 above. Assessment of DLTs will be made based on the first cycle (4 weeks of treatment). At the end of the first cycle, if no DLT are observed, additional patients may be enrolled and treated simultaneously. If the first patient experiences a DLT in the first cycle (4 weeks), then only one additional patient will be enrolled and evaluated for DLT. If the second patient does not experience DLT in the first cycle (4 weeks), then additional patients may be enrolled simultaneously.

10.2.1. Patients with Optional Surgical Resection for Tumor Analysis:

Beginning 5 days prior to scheduled surgical resection of their recurrent glioblastoma, patients will initiate 3.6mg DNA dosing/infusion with SGT. Dosing will continue through the day before surgery.

- One portion of the surgical section will be saline rinsed and flash frozen for measurement of exogenous wt p53 in tumor tissue, one portion will be immediately formalin-fixed for evaluation of PD biomarkers of activity of SGT-53 including but not limited to Annexin V and TUNEL staining. In addition, one portion of fresh tissue will be used for flow cytometry and/or immunohistochemistry analysis to determine the level of apoptosis in Cancer Stem Cells (CSC) and bulk tumor cells. Any remaining tumor tissue from an earlier procedure will be collected and stored in a research tissue bank at Georgetown University or the clinical site.

Following surgery, an MRI scan should be done no later than 72 hours from the time of surgery.

Following recovery from surgical resection (POD ~14-21), patients will restart SGT-53 and TMZ on the morning of Cycle 1 Day 1 (C1D1). Each cycle will consist of 28 days. Patients will return to the clinic for twice weekly (every 3rd or 4th day) infusions of SGT-53 for three weeks (see treatment schedule diagram in section 9.1.1). Temozolomide will be taken daily by mouth daily on days 9-13 of each cycle. During week 4 of each cycle the patient will not receive any treatments. Patients will return to the clinic on Day 29 (C2D1) and Day 57 (C3D1) for evaluation. If the drug has been well tolerated, continued dosing will be permitted even if they appear to be progressing.

Patients will be monitored for response and disease progression with MRI brain scans every 2 cycles (8 weeks) and/or at the end of cycle 3, or earlier if progressive disease is suspected.

All patients who participate in this study will continue treatment with SGT-53 and TMZ after surgery. The following conditions should be satisfied before initiation of treatment with SGT:

1) They have recovered from the effects of surgery including adequate wound healing.

2) A brain MRI should be done no later than 72 hours in the immediate post-operative period to best assess the extent of residual measurable disease post-operatively.

3) The patient has followed the study requirements for birth control and pregnancy testing since the initial pre-operative doses of SGT and TMZ.

4) Post-operatively, patients must have adequate bone marrow function (absolute neutrophil count > 1,500/μL and platelet count of >100,000/μL), normal coagulation profile (PT/PTT), adequate liver function (SGPT, SGOT, and alkaline phosphatase <2.5 times upper limit of normal and bilirubin < 1.5 X upper limit of normal), adequate renal function (BUN or creatinine <1.5 times upper limit of institutional normal within 7 days prior to starting therapy.

5) The patient continues to meet all the eligibility criteria of the study outlined above.

10.2.2. <u>Patients Not undergoing Surgical Resection</u>:

Patients who have had surgery for recurrent glioblastoma will follow the guidelines listed above for patients with optional surgical resection for tumor analysis.

If no surgery has been performed, patients will start SGT-53 and TMZ on the morning of Cycle 1 Day 1 (C1D1). Each cycle will consist of 28 days. Patients will return to the clinic for twice weekly (every 3rd or 4th day) infusions of SGT-53 for three weeks (see treatment schedule diagram in section 9.1.1). Temozolomide will be taken daily by mouth daily on days 9-13 of each cycle. During week 4 of each cycle the patient will not

receive any treatments. Patients will return to the clinic on Day 29 (C2D1) and Day 57 (C3D1) for evaluation. If the drug has been well tolerated continued dosing will be permitted even if they appear to be progressing.

Patients will be monitored for response and disease progression with MRI brain scans every 2 cycles (8 weeks) and/or at the end of cycle 3 or earlier if progressive disease is suspected.

10.2.3. <u>All Patients</u>:
Evaluations done at each clinic visit are detailed in Section 18.1.

Patients will return to clinic at least weekly for assessments (until the last week of protocol therapy). Refer to the Study Calendar, Appendix 18, for evaluations to be completed during each clinic visit. Each clinic visit will include: assessment of vital signs (blood pressure, pulse and temperature). The following will be performed only on a weekly basis (usually on the first visit of each week) unless clinically significant values were obtained at the previous visit: physical exam; assessment of the ECOG/ Karnofsky Performance Status; assessment of concomitant medications; assessment of adverse events; completion of a serum chemistry panel; completion of a CBC with differential. At the beginning of every cycle a urinalysis panel will be completed. A 12-lead ECG and echocardiogram will be performed at baseline. Additional ECGs/ echocardiograms should be obtained on any patient who demonstrates signs or symptoms of myocardial degeneration. Additionally a 12-lead ECG will be performed 2 hours and 24 hours after the first infusion of SGT-53. A 12-lead ECG will be performed weekly (2 hours post-infusion) during the first 2 weeks of cycle 1, and at the End Of Treatment (EOT) visit. If these patients receive additional rounds of SGT-53/Temozolomide a 12-lead ECG will only be performed as necessary and at the EOT visit.

The first two subjects enrolled in this trial under the current protocol will be monitored for PT/PTT, D-dimer and LDH at baseline/screening; in cycle 1 ~20 hours after the first infusion (during the 23 observation period), and weekly during cycle 1, and once in two weeks during cycles 2 and 3 and at the exit visit. If these patients receive additional rounds of SGT-53/Temozolomide PT/PTT, D-dimer and LDH will be monitored once in two weeks during cycle 1 and monthly in cycle 2 and beyond.

Subsequent subjects will be monitored for PT/PTT, D-dimer and LDH at:

(i) baseline/screening;
(ii) in cycle 1 (a) ~20 hours after the first infusion (during the 23 observation period)
(iii) at the exit visit.

Patients who are responding to treatment (at least stable disease by RANO) may receive three additional cycle of SGT-53/temozolomide therapy at investigator's discretion. Alternatively, they may continue on Temozolomide alone at investigator's discretion.

10.2.4. End of Treatment Evaluations (Day 85, at the end of Cycle 6 if the patient received cycles 4-6, or for premature withdrawal/study drug discontinuation)

Once a patient has completed study treatment, including premature withdrawal/study drug discontinuation, the patient should complete an End of Treatment Visit. During this visit the following assessments will be completed:

- Radiographic tumor measurement
- Complete medical history
- Physical examination (all body systems)
- Karnofsky Performance Status
- Vital signs (temperature, blood pressure, pulse rate, and respiratory rate)
- Concomitant medication assessment
- Serum chemistry including: sodium, chloride, carbon dioxide, potassium, calcium, phosphorous, magnesium, BUN, creatinine, blood sugar (random), albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin (total, direct), and total protein.
- CBC with differential and platelet counts
- 12-lead electrocardiogram
- Limited Echocardiogram, only if needed
- Urinalysis
- Assessment of adverse events

10.3. Follow-up

If the patient was removed from the Study for toxicity, the patient should be followed with weekly clinic visit and laboratory studies as appropriate, at clinical trial site or the patient's local MD, until the AE has resolved to baseline or is documented as irreversible.

All patients who received treatment with SGT-53 must be followed-up for 30 days after discontinuation of study drug. The 30-day follow-up visit should include a physical examination, vital signs, concomitant medications and assessment of AEs/SAEs and Urine analysis. Blood samples will be taken for: Hematology (CBC with differential, platelet count), Chemistries (BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein, glucose), PT/PTT, D-dimer, LDH.

Patients who permanently discontinue study drug(s) for reasons other than complete response, progression, or death should also have visits approximately every 2 months for the first year that should include a MRI, physical examination, vital signs, and recording of concomitant medications for glioblastoma.

Patients who are withdrawn from study treatment due to a complete response will have the 30-day follow-up visit as described above and in Sections 5 and 9.4.1. Further follow-up visits every 2 months will include only a physical/neurological exam, concomitant medications, vital signs and an MRI.

10.3.1. Long Term Follow-up

The FDA currently recommends that safety data from participants participating in studies evaluating gene transfer vectors be collected for 15 years (post gene transfer). All participants receiving a dose of SGT-53 will continue to be followed after the completion of all study related procedures to meet the requirements of 15 years post gene transfer follow-up.

All participants upon completion or exit from the study will be presented with written instructions on how to contact the investigator if they experience any serious adverse event that they consider possibly related to study agent or study participation. All participants will be instructed to notify the investigator of a change of address or contact information.

Participants will be contacted on an annual basis by the Principal Investigator and requested to provide information concerning any cancer, neurological, autoimmune, or hematological disorder that occurred or worsened since last contact. The participants will also be requested to provide information concerning any unexpected medical problems including hospitalizations or use of medications. Information will be collected via questionnaire, either by mail or by telephone. The investigator will submit these follow-up data to the sponsor.

The sponsor will submit all information collected during long-term follow-up in annual reports to the FDA. All participants participating in the study will receive an explanation of these procedures at the time of Informed Consent

10.4. Efficacy Assessments

10.4.1. Progression-Free Survival

The percentage of patients who are alive and progression-free clinically and radiologically 6 and 12 months after starting treatment will be evaluated using the Kaplan-Meier method. Progression is defined by any of the following using the RANO criteria:

a) >25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids*.

b) Significant increase in T2/FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy *not caused by co-morbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects)

c) Any new lesion

d) Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose

e) Failure to return for evaluation as a result of death or deteriorating condition

f) Clear progression of non-measurable disease

Note: All measurable and non-measurable lesions must be assessed using the same techniques as at baseline.

*Stable doses of corticosteroids include patients not on corticosteroids.

10.4.2. Tumor response rate:

The percentage of patients with a complete or partial response 6 and 12 months after starting treatment will be evaluated using the RANO criteria:

Complete response (CR). Requires all of the following

a) complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks

b) no new lesions

c) stable or improved non-enhancing (T2/FLAIR) lesions

d) patients must be off corticosteroids (or on physiologic replacement doses only)

e) stable or improved clinically

Note: Patients with non-measurable disease only cannot have a complete response; the best response possible is stable disease.

Partial response (PR). Requires all of the following:

a) >50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks

b) No progression of non-measurable disease

c) No new lesions

d) Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan

e) The corticosteroid dose at the time of the scan evaluation should be no greater than the dose at the time of baseline scan

f) Improved or stable clinically

Note: Patients with non-measurable disease only cannot have a partial response; the best response possible is stable disease.

Stable disease (SD). Requires all of the following:

a) Does not qualify for complete response, partial response, or progression

b) Stable non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

10.4.3. Time to progression

The time to progression is defined as the time from treatment initiation until a contrast-enhanced MRI documents a progression of the disease or until neurological deterioration (if a brain MRI cannot be performed due to the patient's debilitation). Clinical deterioration and worsening of symptoms, in the absence of a MRI must be considered by the investigator to be linked to disease progression and not any other cause(s). Patients without progression or neurological deterioration at EOS will be censored at their last date without progression or neurological deterioration.

10.4.4. Overall survival

Overall survival is defined as the time from treatment initiation to death or to time last seen alive. The overall survival rate will be estimated by the proportion of patients who are alive at EOS and 12 months after starting treatment using the Kaplan-Meier method.

10.5. Safety Endpoint Definitions

Patients will be evaluated and adverse events (AEs) assessed according to the Study Calendar (Section 18.1.) using Common Toxicity Criteria 4.03 (CTC 4.03, 2010). For definition of DLT see Section 9.7.

11. ADVERSE EVENTS

Spontaneous reports of adverse events to the investigator by patients, and toxicities encountered during the study will be evaluated for severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (June 2010) [NCI 2010] and recorded at study visits. The investigator and site staff should have access to the CTCAE version 4.03 by hard copy or on the internet.

Information about all adverse events, whether reported by the patient, discovered by the investigator upon questioning the patient, or detected from a physical examination, lab result or other means, will be collected and recorded in the CRF as an AE or on an SAE form (as applicable) and will be followed as appropriate.

11.1. Definition of Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure.

Medical conditions or diseases present prior to starting study treatment are only considered AEs if they worsen after starting study treatment. After the ICF is signed and prior to the start of study treatment, only serious adverse events are reported if related to a study mandated procedure.

Adverse events include:

- Exacerbation of a pre-existing disease.
- Increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Disease or medical condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Lack of efficacy in the acute treatment of a life-threatening disease.
- Events considered by the investigator to be related to study-mandated procedures.
- Abnormal assessments, e.g., change on physical examination, ECG findings, if they represent a clinically significant finding that was not present at baseline or worsened during the course of the study.
- Laboratory test abnormalities if they represent a clinically significant finding, symptomatic or not, which was not present at baseline or worsened during the course

of the study or led to dose reduction, interruption or permanent discontinuation of study drug.

Adverse events do not include:

- Medical or surgical procedure, e.g., surgery, endoscopy, tooth extraction, transfusion. However, the event leading to the procedure is an AE, with one exception. Patients who participate in the optional surgical resection for tumor analysis are pre-treated with SGT-53 prior to surgery; therefore, this event is recorded as medical history and is not required to be reported as an AE. However medical complications related to this surgery must be reported as AE. For all other events, the event leading to the procedure is reported as an AE, and if the event is serious, the procedure must be described in the serious adverse event narrative.

- Pre-existing disease or medical condition that does not worsen.

- Situations in which an adverse change did not occur, e.g., hospitalizations for cosmetic elective surgery or for social and/or convenience reasons.

- Planned Hospitalization during the 23 hour observation period after the first infusion

- Overdose of SGT-53, TMZ or concomitant medication without any signs or symptoms. Any overdose of SGT-53 or TMZ must be noted in the patient's Study Drug Log. In the event of an overdose with TMZ, a hematological evaluation should be performed.

Patients will be evaluated for AEs for the first 28 days of therapy. Adverse events will be reported at all subsequent study visits (i.e. prior to each infusion of SGT-53 or at the end of each cycle of treatment).

The Safety Monitoring Committee will convene every 3 months, or more frequently, if deemed necessary, to review safety data.

A treatment-emergent AE is further defined in this protocol as any AE temporally associated with the use of study drug (from study drug initiation until 30 days after study drug discontinuation), whether or not considered related to the study drug.

11.1.1. Definition of Serious Adverse Events

An SAE is any untoward medical occurrence that at any dose has the following characteristics:

- Results in death
- Is life-threatening
- Requires unscheduled inpatient hospitalization or prolongation of existing hospitalization

- Results in permanent (persistent) disability/incapacity
- Is a congenital anomaly
- Is an important medical event

Medical and scientific judgment should be exercised in deciding whether it is appropriate to consider other situations serious, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

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

An elective hospital admission to treat a condition present before exposure to the test drug, or a hospital admission for a diagnostic evaluation of an AE, does not qualify the condition or event as an SAE.

A newly diagnosed pregnancy in a patient that has received a study drug is not considered an SAE unless it is suspected that the test article interacted with a contraceptive method and led to the pregnancy. A congenital anomaly in an infant born to a mother who was exposed to the study drug during pregnancy is an SAE.

11.2. Relationship of Adverse Event to Investigational Product

The assessment of the relationship of an adverse event to the administration of study drug is a clinical decision based on all available information at the time of the completion of the case report form.

The relationship to study drug therapy should be assessed using the following definitions:

Not Related: Evidence exists that the AE has an etiology other than the study drug (eg, pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).

Possibly/Probably Related: A temporal relationship exists between the event onset and administration of the study drug. It cannot be readily explained by the patient's clinical state, intercurrent illness or concomitant therapies. In case of cessation or reduction of the dose, the event abates or resolves and reappears upon rechallenge. It should be emphasized that ineffective treatment should not be considered as causally related in the context of AE reporting.

11.2.1. Relationship to study drugs

Each adverse event must be assessed by the investigator as to whether or not there is a reasonable possibility of causal relationship to the study drugs, and will be reported as either related or unrelated to SGT-53 and/or TMZ. Therefore, the causal relationship of each AE, based on the judgment of the Investigator with respect to SGT-53 and TMZ must be reported.

Related to study drug(s)

This category applies to any AE (whether serious or not) that appears to have a reasonable possibility of causal relationship to the use of SGT-53 and/or TMZ (i.e., a relationship cannot be ruled out). Guidelines to determine whether an event might be considered related include (but are not limited to) the following:

- The event occurred in close temporal relationship to study drug administration.
- The event abated (diminished) or disappeared when treatment with the study drug was down-titrated, interrupted, or discontinued.
- The event reoccurred when treatment was reintroduced.
- Environmental factors such as clinical state, and other treatments, or other reasons not related to SGT-53, Temozolomide, or both, such as intercurrent illness, could equally have caused the event.

Unrelated to study drug(s)

This category applies to any AE (whether serious or not) that does not appear to have a reasonable relationship to the use of SGT-53 and/or TMZ (see above guidelines).

11.3. Severity of adverse events

The severity of AEs will be classified according to the CTCAE version 4.03 (June 2010) [NCI 2010]. Grades 1 through 5 refer to the severity of the AE, which are based on general guidelines described below. Not all grades are appropriate for each AE. Therefore, the CTCAE may list some AEs with less than five options for grade selection.

Grade 1

- Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2

- Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL) which refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money etc.

Grade 3

- Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL which refer to bathing, dressing and undressing, feeding self, using the toilet, taking medication, and not bedridden.

Grade 4

- Life-threatening consequences; urgent intervention indicated.

Grade 5

- Death related to AE

11.4. Reporting of adverse events

All AEs occurring after study drug initiation and up to 30 days after study drug discontinuation must be recorded in the CRF.

Any AE reported with a toxicity level of grade 4 or grade 5 indicate a level of seriousness that must be reported as a serious adverse event (refer to Section 11.1.1. for definition of SAEs). The Investigator must consider if AEs reported with a toxicity level of grade 3 meet at least one of the criteria for seriousness (refer to Section 11.1.1.) and should be reported as an SAE.

11.5. Follow-up of adverse events

Adverse events that are ongoing after study drug discontinuation for a given patient must be followed until 30 days after study drug discontinuation, or until resolution or stabilization, or until the event is otherwise explained.

11.6. Adverse events of special interest

Temozolomide is associated with a ≥ 10% incidence of grade 3 and grade 4 hematological abnormalities [Temodar® Prescribing Information]. If ANC or platelet levels reach thresholds of <1,000/μL or <50,000/μL respectively, the dose of TMZ may be lowered or interrupted (see Section 9.7.). Time to recovery from grade 3 and grade 4 hematological events for these selected AEs will be evaluated.

SGT-53 is associated with a ≥ 10% incidence of grade 1 and grade 2 fever and hypotension after the first infusion, as well as grade 1 and grade chills and mild nausea. Due to the known occurrence of fever/hypotension after the first infusion, a 23 hour

observation period for the first infusion is planned and described in the protocol (Section 9.3.3.).

11.7.Definition of Dose Limiting Toxicity (DLT)

- See Section 9.4.

11.8. Definition of Start Date, Stop Date and Duration

The definitions of AE start/stop dates and duration will be as follows:

Start Date:	The date at which the AE is first noted
Stop Date:	The date at which the AE is known to be resolved. If it is not known to have stopped, then indicate "ongoing."
Duration:	A time in days

11.9. Action(s) Taken

Action(s) taken may consist of the following:

None:	No actions taken
Discontinued test article:	Test article was permanently discontinued because of the AE
Change test article:	Test article was given at a lower dose, at a longer interval between doses, or was temporarily withheld because of the AE
Treatment:	Specified medication (to be listed on the concomitant medication chart) has been used as a countermeasure
Others:	Other actions, such as an operative procedure were required because of the AE

11.10. Definition of Outcome at the Time of Last Observation

The outcome at the time of last observation will be classified as:

- Resolved
- Resolved with Sequelae
- Ongoing
- Death
- Unknown

Death should only be entered as the outcome for an AE when the patient's death is probably related to the AE (Note: the causal relationship of the AE to the test article is

not to be considered in making this decision). If more than 1 AE is possibly related to the patient's death, the outcome of death should be indicated for each such AE.

11.11. Treatment period

All SAEs, regardless of causal relationship, must be reported, including those related to study-mandated procedures. Those SAEs occurring during study drug administration, i.e., between study drug initiation and 30 days after study drug discontinuation, are defined as treatment-emergent SAEs.

Treatment-emergent SAEs are reported on the Drug Safety SAE form and also on an AE CRF page. These events are entered into both the Drug Safety and Clinical Trial Databases. As grade 4 and grade 5 events are by definition within seriousness criteria, within the clinical database, events reported as grade 4 and grade 5 should have a corresponding SAE documented on the AE page and a corresponding SAE form completed (i.e., SAE documented in the Argus Drug Safety Database). The investigator must consider if adverse events reported with a toxicity level of grade 3 meet at least one of the criterion for seriousness [Section 11.1.1] and should be reported as an SAE. The toxicity grade of an event reported on the AE CRF page must be entered in the narrative section of the Drug Safety SAE form.

11.12. Documentation of Adverse Events

The Investigator will monitor and/or ask about or evaluate AEs using non leading questions at each visit or evaluation. The occurrence of all AEs will be documented in the CRF with the following information, where appropriate:

- AE name or term
- When the AE first occurred (start date)
- When the AE stopped (stop date), (or an indication of “ongoing”)
- How long the AE persisted (optional)
- Severity of the AE
- Seriousness
- Actions taken
- Outcome
- Investigator opinion regarding the relationship of AE to the study drug(s)

Adverse events should be documented from the time informed consent is obtained. The AEs must be documented as soon and as completely as possible on the “Adverse Event

Report Form" pages in the CRF. Follow-up information must be entered as soon as available. The causality assessment must be assigned by the Investigator.

Corrections to most AE details may be made, but must be dated and signed according to ICH rules. (Exceptions: Changes in severity and/or seriousness; see below). If a suspected diagnosis has been ruled out, the Investigator or a delegated member of the research team may change the AE term, but should add a comment stating the original suspected diagnosis as well as reasons for change.

A clinically relevant worsening of an AE (e.g. relevant change in severity, seriousness) must result in a new entry. The original entry remains unresolved and is given an end date reflecting the date of the worsening and a comment must be entered stating that the AE is continuing with a changed severity/seriousness (eg, "continues as event name with onset date and new severity/seriousness"). The onset date of the new entry is also the date of worsening. The onset date of a SAE is the time at which the event fulfills a criterion for seriousness.

Adverse events that occur during the study should be treated using established standards of care in order to protect the life and health of the subject. If such treatment constitutes a violation of study entry criteria, as determined by the Investigator, the subject should be withdrawn from the study and the reason must be documented in the CRF.

11.13. Follow-up of Patients With an Adverse Event

The Investigator will follow patients with AEs, even if the patient was withdrawn from the study due to the AE, until the adverse event has resolved:

- the patient has returned to baseline state of health,
- the patient is lost to follow-up,
- the event is otherwise explained,
- the Investigator does not expect any further improvement or worsening of the adverse event.

11.14. SAEs after the 30-day follow-up period

New SAEs occurring at any time after the 30-day follow-up period after study drug discontinuation should be reported to SynerGene within 24 hours of the investigator's knowledge of the event, if considered causally related to previous exposure to study medication by the investigator. These SAEs are only entered in the drug safety database, and hence will not affect study closure.

11.15. Pregnancy

11.15.1. Teratogenicity

Due to the unknown potential for teratogenicity of SGT-53 and the known effects of TMZ on impairment of fertility, as well as the carcinogenic and mutagenic potential of TMZ, females of childbearing potential and males must take appropriate precautions from screening until 30 days after discontinuation of SGT-53 and TMZ, to prevent pregnancy in female patients and female partners of male patients. Women of childbearing potential must agree to use two reliable methods of contraception from screening and up to 30 days after discontinuation of study treatment.

- Women of childbearing potential with negative serum and urine pre-treatment pregnancy tests are allowed in the study if they consistently and correctly use two reliable methods of contraception at the same time. Reliable methods of contraception include intrauterine devices (IUD) or intrauterine systems (IUS), tubal sterilization, and barrier methods (male condom, diaphragm, or cervical cap). A female partner's vasectomy still requires one additional method of contraception. Abstinence, the rhythm method, or contraception by the other partner alone, will not be considered as reliable methods of contraception.

A woman is considered to be of childbearing potential unless she meets at least one of the following criteria:

- previous bilateral salpingo-oophorectomy or hysterectomy
- premature ovarian failure confirmed by a specialist gynaecologist
- pre-pubescence, XY genotype, Turner syndrome, uterine agenesis
- Age > 50 years and not treated with any kind of hormone replacement therapy for at least 2 years prior to screening, and with amenorrhea for at least 24 consecutive months prior to screening. An assessment of serum follicle stimulating hormone showing a level of > 40 IU/L at screening may be used to exclude childbearing potential, based on the discretion of the investigator.

Males who are not naturally or surgically sterile who have a female partner of childbearing potential must agree to use two reliable methods of contraception from screening and up to 30 days after discontinuation of study treatment (e.g., one male contraceptive method and another reliable method used by a male's female partner).

If a female patient becomes pregnant, or if pregnancy is suspected, the study drugs must be immediately withheld until the result of a serum pregnancy test is available. If the pregnancy is confirmed, the patient must be discontinued from the study, and the sponsor notified within 24 hours. The investigator must counsel the female patient and discuss the risks of the pregnancy and the possible effects on the fetus.

11.15.2. Reporting of pregnancy

Any pregnancy occurring during study drug administration with SGT-53macitentan or TMZ or up to 30 days after study drug discontinuation must be reported to the SynerGene within 24 hours of the investigator's knowledge of the event.

Pregnancies must be reported on the Actelion Pregnancy form, which is faxed to SynerGene, and as an AE in the eCRF if it occurred during the treatment period or 30-day follow-up period.

11.15.3. Follow-up of pregnancy

Any pregnancy must be followed to its conclusion and its outcome must be reported to SynerGene.

Such follow-up information will only be entered in the Drug Safety database, and hence will not affect study closure.

11.16. Notification of Sponsor of Serious Adverse Events

The Investigator must report all SAEs promptly to SynerGene within 24 hours of first becoming aware of the event, sooner if possible. If an SAE occurs, the site should notify the designated SynerGene individual(s) by telephone. The primary contact is:

Chris Poki Leung, Ph.D.,
Clinical Research Coordinator
SynerGene Therapeutics, Inc.
9812 Falls Road, Suite 114
Potomac MD 20854
Tel: 301-706-1509
Email: clinicaltrial@synergeneus.com
The secondary phone number for this contact is 301-802-8639

The Investigator must follow up the initial telephone notification within 48 hours of first becoming aware of the event by providing a written report by facsimile or electronic mail describing the SAE to SynerGene. This report will be accomplished by completing a Medwatch 3500A form which is available on the FDA website at http://www.fda.gov, and Sponsor supplied forms.

At the time of first notification of an SAE, the following information should be provided by the site to the SynerGene contact person, if available:

- Patient's study number and initials
- Patient's date of birth
- Patient's gender
- Date of first dose of test article
- Date of last dose of test article, if applicable
- Adverse event term
- Time and date of occurrence of the event

- A brief description of the event, outcome to date and any actions taken
- The seriousness criteria(on) that were met
- Concomitant medication at onset of the event
- Relevant past history information
- Relevant laboratory test findings
- Investigator's opinion of the relationship to test article (refer to Section 11.6.4.7)

Any missing or additional relevant information concerning the SAE should be provided in a written follow-up report as soon as possible, but no later than 14 days after the first report. Additional written follow-up reports will be sent as necessary. The last report will be the final report after the resolution of the SAE.

The Investigator is required to comply with applicable regulations regarding the notification of his/her IRB or Ethics Committee.

The research site should also report the serious adverse event (SAE) by completing the study SAE case report forms and submitting to the Sponsor (SGT). Reports should be faxed or e-mailed to SynerGene and the Data Safety Monitoring Committee for review.

The following items should be included at the time of forwarding the SAE notification to SynerGene and to the IRB as per institutional guidelines.

- (i) SAE cover sheet – within case report form packet

- (ii) Completed Sponsor supplied forms and MedWatch Form FDA 3500A – available online at (www.fda.gov/medwatch/getforms.htm)

- (iii) Any de-identified supporting clinical documents serving as source document of the SAE as deemed important by the site or as requested by the coordinating center.

11.17. Reporting Adverse Events to the Competent Authorities

The sponsor will be responsible for reporting AEs to the FDA as described in 21 CFR Section 312.32 (IND Safety Reports) and to other competent authorities according to local regulations.

In addition, the Investigator is required by FDA regulations to notify the IRB promptly of all unexpected SAEs occurring at the Investigator's study site. The Investigator is also required by FDA regulations to forward to the IRB all IND Safety Reports received from the sponsor.

Counseling for Women of Reproductive Age

All women of reproductive age who participate in the study should be counseled on the need to practice adequate birth control and on the importance of avoiding pregnancy during study participation. Women should be instructed to contact the Investigator or study staff immediately if pregnancy occurs or is suspected.

12. REGULATORY CONSIDERATIONS

12.1.Ethics

This study will be conducted in compliance with the Declaration of Helsinki and its amendments, the International Conference on Harmonisation (ICH) principles of Good Clinical Practice (GCP; including archiving of essential study documents), and all US FDA regulations (for US sites).

A properly constituted, valid Institutional Review Board (IRB) must review and approve the protocol, the Investigator's informed consent document, and related patient information and recruitment materials before the start of the study.

It is the responsibility of the Investigator to ensure that written informed consent is obtained from the patient before any activity or procedure is undertaken that is not part of routine care.

Refer to the following regulations if additional clarity is needed:

12.2.FDA Regulations

Refer to the following US Code of Federal Regulations (CFR):

- FDA Regulations 21 CFR, Parts 50.20 – 50.27
  Subpart B – Informed Consent of Human Subjects
- FDA Regulations 21 CFR, Parts 56.107 – 56.115
  Part 56 – Institutional Review Boards
  Subpart B – Organization and Personnel
  Subpart C – IRB Functions and Operations
  Subpart D – Records and Reports
- FDA Regulations 21 CFR, Parts 312.50 – 312.70
  Subpart D – Responsibilities of Sponsors and Investigators

12.3. Good Clinical Practice Guidelines

ICH GCP guidelines can be found at the following URL:

- http://www.fda.gov/RegulatoryInformation/Guidances/UCM122049

12.4.Finance and Insurance

Details on finance and insurance will be outlined in separate agreements between the Institutions, Investigator and CRO and/or between the Investigator and the Sponsor, as appropriate.

13. DATA QUALITY ASSURANCE

Steps to assure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the Investigator and associated personnel prior to the study, and periodic monitoring visits by SynerGene and/or its representative. Data will be reviewed for accuracy and completeness during and after on-site monitoring visits and any discrepancies will be resolved with the Investigator or designees as appropriate.

13.1. Monitoring Procedures

The study monitor(s) will conduct site visits to the study facilities to monitor the study or via remote access. The Investigator agrees to allow these monitors and other authorized SynerGene personnel access to the clinical supplies dispensing and storage area and to study documentation for the above-mentioned purpose and agrees to assist the monitors in their activities, if requested. Requests by regulatory agencies to inspect the study sites may be made. The Investigator agrees to allow inspectors from regulatory agencies to review records and to assist the inspectors in their duties, if requested.

Source documents are the physician's or hospital's subject records maintained at the study site. In most cases, the source documents will be the physician's or hospital's subject chart. In some cases, the source documents may be electronic. In both cases, the information captured on the CRF must match the information in the chart or electronic source document. Periodically, the monitor or SynerGene representative will visit the study site for the purpose of directly comparing the data in the CRF with the source. The Investigator agrees to make source documents (hard copy or electronic) available for this purpose.

It is the Investigator's responsibility to ensure accurate completion of the CRFs.

13.2. Data Management (Collection)

Data entry into the research database will be audited by the Principal Investigator at 100% of study participants. Summary reports will be prepared for monthly review by the P.I. and the research nurse. The study will be reviewed by the Department of Neuro-Oncology, every 6 months while it is open for accrual. Full datasets of patient results generated under the protocol will be provided to Sponsor promptly after completion of each cycle of treatment for each patient.

14. DATA SAFETY MONITORING BOARD

The study will be independently reviewed by a Data Safety Monitoring Board (DSMB).

The DSMB will be comprised of three to four physicians not associated with the UT M.D. Anderson Cancer Center, and who are not investigators on the study. The DSMB shall receive a monthly report from the investigators to review toxicities (potential toxicities include infusion reactions, hypersensitivity reactions, fever, flushing, anaphylaxis and elevation in hepatic enzymes). In addition, the DSMB will request follow up results of participants with particular emphasis placed on significant adverse events. The DSMB will keep reports of these meeting which will be forwarded to the Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) at the M.D. Anderson Cancer Center. The DSMB reports will include toxicities and will be submitted to the IRB. The

DSMB has the authority to halt accrual or further dose escalations and may audit the source documents to confirm or clarify any details of the trial results.

Furthermore the adequacy of the data and safety monitoring plan will be reviewed by the IBC and IRB on a quarterly basis. All Severe Adverse Events are required to be reported to the IRB. Based on the occurrence and severity of the Severe Adverse Events, the IRB retains the authority to close the study to further accrual pending more detailed reporting and/or modifications to further reduce risk to trial participants and maximize the safety of the participating subjects.

DSMB recommendations should be based not only upon results from the study but also upon data available to the DSMB from other studies. It is the responsibility of the PI, John DeGroot, MD, to ensure that the DSMB is kept apprised of non-confidential results from related studies that become available. It is the responsibility of the DSMB to determine the extent to which this information is relevant to its decisions related to the specific study being monitored.

A written copy of the DSMB recommendations will be given to the clinical study PI, John DeGroot, M.D., and chairs of the IBC and IRB. If the DSMB recommends a study change for subject safety or efficacy reasons, or that the study is to be closed early due to slow accrual, the study PI must act to implement the change as expeditiously as possible. In the unlikely event that the trial PI does not concur with the DSMB, then the UT M.D. Anderson Cancer Center IRB Chair must be informed of the reason for the disagreement. The trial PI, DSMB Chair, and UT M.D. Anderson Cancer Center IRB Chair will be responsible for reaching a mutually acceptable decision about the study. Confidentiality must be preserved during these discussions. However, in some cases, relevant data may be shared with other selected study investigators and staff to assist in reaching a mutually acceptable decision.

If a recommendation is made to change the study for reasons other than subject safety or efficacy, or for slow accrual, the DSMB will provide a rationale in writing to the sponsor for its decision.

15. GENERAL CONSIDERATIONS

15.1. Discontinuation of the Study

SynerGene reserves the right to discontinue this study for safety or administrative reasons at any time.

15.2. Regular Study Termination

The end of this study is defined as the date of the last visit of the last patient (last patient out or last patient last visit) participating in the study. Within 90 days of the end of the clinical study, SynerGene or designee will notify the IRB and regulatory authorities about the regular termination of the study as required.

15.3. Premature Study Termination

The study may be terminated prematurely for any reason and at any time by SynerGene, an IRB/IEC, or a Regulatory Authority. A decision to prematurely terminate the study is binding to all investigators of all study sites.

If the study is terminated prematurely, all investigators have to inform their patients and take care of appropriate follow-up and further treatment of the patients to ensure protection of the patients' interests.

15.4. Changes to the Protocol

This protocol cannot be altered or changed except through a formal protocol amendment, which requires the written approval of the Sponsor. The protocol amendment must be signed by the Investigator and approved by the IRB or IEC before implementation. Protocol amendments will be filed with the appropriate regulatory agency(s) having jurisdiction over the conduct of the study.

15.5. Protocol Deviations for Emergency or Adverse Event

An Investigator shall notify the Sponsor-Investigator, the CTN and the reviewing IRB of any deviation from the investigational plan to protect the life or physical well being of a patient in an emergency. Such notice shall be given as soon as possible, but in no event later than two working days after the emergency occurred. Except in such an emergency, prior approval by the Principal Investigator and Sponsor is required for changes or deviations from a plan, and if these changes or deviations affect the scientific soundness of the plan or the rights, safety, or welfare of patients, notification of the FDA and IRB in accordance with government regulations is also required.

15.6. Source Documents

All information recorded in the CRF (electronic or hard copy) must be supported by corresponding source documentation. Examples of acceptable source documentation include, but are not limited to, hospital records, clinic and office charts, laboratory notes, and recorded data from automated instruments, memoranda, and pharmacy dispensing records. All source documents will be secured and maintained by the study site. Monitoring access must be provided for proper quality assurance as outlined in Section 11 Quality Assurance.

15.7. Use of Information and Publication

All information concerning SGT-53, SynerGene's operations, patent applications, formulas, manufacturing processes, basic scientific data and formulation information supplied by SynerGene or the CRO to the Investigator and not previously published, is considered confidential and remains the sole property of SynerGene. Case Report Forms also remain the property of SynerGene. The Investigator agrees to use this information for purposes of study execution through finalization only.

The information developed in this study will be used by SynerGene in connection with the continued development of SGT-53 and thus may be disclosed as required to other clinical investigators or government regulatory agencies.

Publication or other public presentation of SGT-53 data resulting from this study will be done in collaboration with the Sponsor and requires prior review and written approval of SynerGene. Abstracts, manuscripts and presentation materials should be provided to SynerGene for review ≥ 30 days prior to the relevant submission deadline.

16. FINAL CLINICAL STUDY REPORT

SynerGene will retain ownership of the data.

The final clinical study report (CSR) will be written within 6 months of completion of the clinical part of the study as a collaboration between the PI at the M D Anderson Cancer Center and the Sponsor. This report will include a summary of the study results based on a statistical evaluation and clinical assessment.

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18. APPENDIX

18.1 Schedule of Assessments

18.1.1. Patient WITHOUT Surgical Resection for Tumor Analysis

TYPE OF EVALUATION	Baseline Screening	Cycle 1																						
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23-D28
Informed Consent	X																							
Physical Examination[1]	X	X							X							X							X	
Symptom-oriented Exam[1]					X							X							X					
ECG[2]	X	X	X						X															
Limited ECHO	X																							
Vital Signs	X	X			X				X			X				X			X				X	
Performance Status	X	X			X				X			X				X			X				X	
Medical History[3]	X	X			X				X			X				X			X				X	
Hematology[4,9]	X	X							X							X							X	
PT/PTT, D-dimer, [5]	X		X						X							X							X	
Chemistries[6,9]	X	X							X							X							X	
Urine Analysis	X																							
Serum Pregnancy Test	X																							
MRI Scan [7]	X																							
SGT-53 Administration[8]		X			X				X			X				X			X					
Temozolomide Administration										X	X	X	X	X										

[1] Physical examination will be done on 1st visit of each week. A symptom-oriented examination will be done on the intervening study visits only if necessary.

[2] ECG will be performed at baseline; 2 hours and 24 hours after the 1st SGT-53 infusion; weekly (2 hours post-infusion) during the first 2 weeks of Cycle 1 and at the EOT visit when the patient goes off study. If these patients receive additional rounds of SGT-53/Temozolomide a 12-lead ECG will only be performed as necessary and the EOT visit when the patient goes off study.

[3] Medical History includes review of concomitant medications and adverse events.

[4] Hematology values include complete white count (CBC) with differential and platelet count.

[5] PT/PTT and D-dimer for the first two subjects: at baseline/screening; ~20 hours post-infusion (during the 23 hour observation period after the first SGT-53 infusion), and thereafter once a week during Cycle 1, once in two weeks during Cycles 2 and 3; and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.
PT/ PTT and D-dimer for subsequent subjects: at baseline/screening; at Cycle 1, ~20 hours post-infusion 1 (during the 23 observation period); and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.

[6] Chemistries include: BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total and direct bilirubin, total protein, glucose and LDH..

[7] Tumor size will be assessed by MRI at baseline, every 2 cycles (8 weeks) and/or at the end of cycle 3 to determine if the patient is to continue on study.

[8] Prior to each infusion of SGT-53, subjects will receive the following premedication: dexamethasone (i.v.), a combination of histamine H1 and H2 blockers (i.v.), indocin (p.o.) and acetominophen (p.o.).

[9] Hematology and Full panel chemistries will be completed at each visit only if they were clinically significant at the previous visit.

TYPE OF EVALUATION	Cycle 2																						
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23-D28
Informed Consent																							
Physical Examination[1]	X							X							X							X	
Symptom-oriented Exam[1]				X							X							X					
ECG[2]																							
limited ECHO																							
Vital Signs	X			X				X			X				X			X				X	
Performance Status	X			X				X			X				X			X				X	
Medical History[3]	X			X				X			X				X			X				X	
Hematology[4,9]	X							X							X							X	
PT/PTT, D-dimer, [5]	X														X								
Chemistries[6,9]	X							X							X							X	
Urine Analysis	X																						
Serum Pregnancy Test																							
MRI Scan[7]																							X
SGT-53 Administration[8]	X			X				X			X				X			X					
Temozolomide Administration									X	X	X	X	X										

[1] Physical examination will be done on 1st visit of each week.

[2] ECG will be performed at baseline; 2 hours and 24 hours after the 1st SGT-53 infusion; weekly (2 hours post-infusion) during the first 2 weeks of Cycle 1 and at the EOT visit when the patient goes off study. If these patients receive additional rounds of SGT-53/Temozolomide a 12-lead ECG will only be performed as necessary and the EOT visit when the patient goes off study..

[3] Medical History includes review of concomitant medications and adverse events.

[4] Hematology values include complete white count (CBC) with differential and platelet count.

[5] PT/PTT and D-dimer for the first two subjects: at baseline/screening; ~20 hours post-infusion (during the 23 hour observation period after the first SGT-53 infusion), and thereafter once a week during Cycle 1, once in two weeks during Cycles 2 and 3; and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.
PT/ PTT and D-dimer for subsequent subjects: at baseline/screening; at Cycle 1, ~20 hours post-infusion 1 (during the 23 observation period); and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.

[6] Chemistries include: BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total and direct bilirubin, total protein, glucose and LDH.

[7] Tumor size will be assessed by MRI at baseline, every 2 cycles (8 weeks) and/or at the end of cycle 3 to determine if the patient is to continue on study

[8] Prior to each infusion of SGT-53, subjects will receive the following premedication: dexamethasone (i.v.), a combination of histamine H1 and H2 blockers (i.v.), indocin (p.o.) and acetominophen (p.o.).

[9] Hematology and Full panel chemistries will be completed at each visit only if they were clinically significant at the previous visit.

TYPE OF EVALUATION	Cycle 3																								
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23-D28	EOT	30 Day Follow-up[11]
Informed Consent																									
Physical Examination[1]	X							X							X							X		X	X
Symptom-oriented Exam[1]				X							X							X							
ECG[2]																								X	
limited ECHO [7]																								X	
Vital Signs	X			X				X			X				X			X				X		X	X
Performance Status	X			X				X			X				X			X				X		X	
Medical History[3]	X			X				X			X				X			X				X		X	X
Hematology[4, 10]	X							X							X							X		X	X
PT/PTT, D-dimer, [5]	X														X									X	X
Chemistries[6, 10]	X							X							X							X		X	X
Urine Analysis	X																							X	X
Serum Pregnancy Test																									
MRI Scan[8]																							X		
SGT-53 Administration[9]	X			X				X			X				X			X							
Temozolomide Administration									X	X	X	X	X												

[1] Physical examination will be done on 1st visit of each week. A symptom-oriented examination will be done on the intervening study visits only if necessary.
[2] ECG will be performed at baseline; 2 hours and 24 hours after the 1st SGT-53 infusion; weekly (2 hours post-infusion) during the first 2 weeks of Cycle 1 and at the EOT visit when the patient goes off study. If these patients receive additional rounds of SGT-53/Temozolomide a 12-lead ECG will only be performed as necessary and the EOT visit when the patient goes off study..

[3] Medical History includes review of concomitant medications and adverse events.

[4] Hematology values include complete white count (CBC) with differential and platelet count.
[5] PT/PTT and D-dimer for the first two subjects: at baseline/screening; ~20 hours post-infusion (during the 23 hour observation period after the first SGT-53 infusion), and thereafter once a week during Cycle 1, once in two weeks during Cycles 2 and 3; and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined. PT/ PTT and D-dimer for subsequent subjects: at baseline/screening; at Cycle 1, ~20 hours post-infusion 1 (during the 23 observation period); and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.

[6] Chemistries include: BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total and direct bilirubin, total protein, glucose and LDH..

[7] A limited ECHO will only be performed if needed..

[8] Tumor size will be assessed by MRI at baseline, every 2 cycles (8 weeks) and/or at the end of cycle 3 to determine if the patient is to continue on study
[9] Prior to each infusion of SGT-53, subjects will receive the following premedication: dexamethasone (i.v.), a combination of histamine H1 and H2 blockers (i.v.), indocin (p.o.) and acetominophen (p.o.).
[10] Hematology and Full panel chemistries will be completed at each visit only if they were clinically significant at the previous visit.
[11] The 30 day and long-term follow-ups will be performed as described in Section 10.3

18.1.2. **Patients Who Undergo Surgical Resection for Tumor Analysis**

TYPE OF EVALUATION	Baseline Screen	Pre- Surgery			**Cycle 1**																						
		D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D 18	D 19	D 20	D 21	D 22	D23-D28
Informed Consent	X																										
Physical Examination[1]	X	X			X							X							X							X	
Symptom-oriented Exam[1]				X				X							X							X					
ECG[2]	X	X	X		X							X															
Limited ECHO	X																										
Vital Signs	X	X		X	X			X				X			X				X			X				X	
Performance Status	X	X		X	X			X				X			X				X			X				X	
Medical History[3]	X	X		X	X			X				X			X				X			X				X	
Hematology[4, 9]	X	X			X							X							X							X	
PT/PTT, D-dimer[5]	X		X		X							X							X							X	
Chemistries[6, 9]	X				X							X							X							X	
Urine Analysis	X				X																						
Serum Pregnancy Test	X																										
MRI Scan[7]	X																										
SGT-53 Administration[9]		X		X	X			X				X			X				X			X					
TMZ Administration													X	X	X	X	X											

[1] Physical examination will be done on 1st visit of each week. A symptom-oriented examination will be done on the intervening study visits only if necessary. .

[2] ECG will be performed at baseline; 2 hours and 24 hours after the 1st SGT-53 infusion; weekly (2 hours post-infusion) during the first 2 weeks of Cycle 1 and at the EOT visit when the patient goes off study. If these patients receive additional rounds of SGT-53/Temozolomide a 12-lead ECG will only be performed as necessary and the EOT visit when the patient goes off study.

[3] Medical History includes review of concomitant medications and adverse events.

[4] Hematology values include complete white count (CBC) with differential and platelet count.

[5] PT/PTT, and D-dimer for the first two subjects: at baseline/screening; ~20 hours post-infusion (during the 23 hour observation period after the first SGT-53 infusion), and thereafter once a week during Cycle 1, once in two weeks during Cycles 2 and 3; and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.

PT/ PTT, D-dimer and LDH for subsequent subjects: at baseline/screening; at Cycle 1, ~20 hours post-infusion 1 (during the 23 observation period after the first infusion); and at the exit visit. When blood chemistries are not being assessed on this same day, LDH will also be determined.

[6] Chemistries include: BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total and direct bilirubin, total protein, glucose and LDH.

[7] Tumor size will be assessed by MRI at baseline, no later than 72 hours post-surgery, every 2 cycles (8 weeks) and/or at the end of cycle 3 to determine if the patient is to continue on study

[8] Prior to each infusion of SGT-53, subjects will receive the following premedication: dexamethasone (i.v.), a combination of histamine H1 and H2 blockers (i.v.), indocin (p.o.) and acetominophen (p.o.).

[9] Hematology and Full panel chemistries will be completed at each visit only if they were clinically significant at the previous visit.

TYPE OF EVALUATION	Cycle 2																						
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23-D28
Informed Consent																							
Physical Examination[1]	X							X							X							X	
Symptom-oriented Exam[1]				X							X							X					
ECG[2]																							
Limited ECHO																							
Vital Signs	X			X				X			X				X			X				X	
Performance Status	X			X				X			X				X			X				X	
Medical History[3]	X			X				X			X				X			X				X	
Hematology[4, 9]	X							X							X							X	
PT/PTT, D-dimer, [5]	X														X								
Chemistries[6, 9]	X							X							X							X	
Urine Analysis	X																						
Serum Pregnancy Test																							
MRI Scan[7]																							X
SGT-53 Administration[8]	X			X				X			X				X			X					
Temozolomide Administration									X	X	X	X	X										

[1] Physical examination will be done on 1st visit of each week. A symptom-oriented examination will be done on the intervening study visits only if necessary.

[2] ECG will be performed at baseline; 2 hours and 24 hours after the 1st SGT-53 infusion; weekly (2 hours post-infusion) during the first 2 weeks of Cycle 1 and at the EOT visit when the patient goes off study. If these patients receive additional rounds of SGT-53/Temozolomide a 12-lead ECG will only be performed as necessary and the EOT visit when the patient goes off study.

[3] Medical History includes review of concomitant medications and adverse events.

[4] Hematology values include complete white count (CBC) with differential and platelet count.

[5] PT/PTT and D-dimer for the first two subjects: at baseline/screening; ~20 hours post-infusion (during the 23 hour observation period after the first SGT-53 infusion), and thereafter once a week during Cycle 1, once in two weeks during Cycles 2 and 3; and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.

PT/ PTT and D-dimer for subsequent subjects: at baseline/screening; at Cycle 1, ~20 hours post-infusion 1 (during the 23 observation period); and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.

[6] Chemistries include: BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total and direct bilirubin, total protein, glucose and LDH.

[7] Tumor size will be assessed by MRI at baseline, no later than 72 hours post-surgery, every 2 cycles (8 weeks) and/or at the end of cycle 3 to determine if the patient is to continue on study

[8] Prior to each infusion of SGT-53, subjects will receive the following premedication: dexamethasone (i.v.), a combination of histamine H1 and H2 blockers (i.v.), indocin (p.o.) and acetominophen (p.o.).

[9] Hematology and Full panel chemistries will be completed at each visit only if they were clinically significant at the previous visit.

TYPE OF EVALUATION	Cycle 3																								
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23-D28	EOT	30 Day Follow-up[11]
Informed Consent																									
Physical Examination[1]	X							X							X							X		X	X
Symptom-oriented Exam[1]				X							X							X							
ECG[2]																								X	
Limited ECHO[7]																								X	
Vital Signs	X			X				X			X				X			X				X		X	X
Performance Status	X			X				X			X				X			X				X		X	
Medical History[3]	X			X				X			X				X			X				X		X	X
Hematology[4, 10]	X							X							X							X		X	X
PT/PTT, D-dimer, [5]	X														X									X	X
Chemistries[6, 10]	X							X							X							X		X	X
Urine Analysis	X																							X	X
Serum Pregnancy Test																									
MRI Scan[8]																							X		
SGT-53 Administration[9]	X			X				X			X				X			X							
Temozolomide Administration									X	X	X	X	X												

[1] Physical examination will be done on 1st visit of each week. A symptom-oriented examination will be done on the intervening study visits only if necessary..

[2] ECG will be performed at baseline; 2 hours and 24 hours after the 1st SGT-53 infusion; weekly (2 hours post-infusion) during the first 2 weeks of Cycle 1 and at the EOT visit when the patient goes off study. If these patients receive additional rounds of SGT-53/Temozolomide a 12-lead ECG will only be performed as necessary and the EOT visit when the patient goes off study.

[3] Medical History includes review of concomitant medications and adverse events.

[4] Hematology values include complete white count (CBC) with differential and platelet count.

[5] PT/PTT and D-dimer for the first two subjects: at baseline/screening; ~20 hours post-infusion (during the 23 hour observation period after the first SGT-53 infusion), and thereafter once a week during Cycle 1, once in two weeks during Cycles 2 and 3; and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.

PT/ PTT and D-dimer for subsequent subjects: at baseline/screening; at Cycle 1, ~20 hours post-infusion 1 (during the 23 observation period); and at the exit visit. When blood chemistries are not being assessed on this same day LDH will also be determined.

[6] Chemistries include: BUN, Cr, Na^+, K^+, CO_2, Cl, Mg^{++}, Ca^{++}, phosphorus, albumin, alkaline phosphatase, ALT, AST, total and direct bilirubin, total protein, glucose and LDH.

[7] A limited ECHO will only be performed if needed..

[8] Tumor size will be assessed by MRI at baseline, no later than 72 hours post-surgery, every 2 cycles (8 weeks) and/or at the end of cycle 3 to determine if the patient is to continue on study

[9] Prior to each infusion of SGT-53, subjects will receive the following premedication: dexamethasone (i.v.), a combination of histamine H1 and H2 blockers (i.v.), indocin (p.o.) and acetominophen (p.o.).

[10] Hematology and Full panel chemistries will be completed at each visit only if they were clinically significant at the previous visit.

[11] The 30 day and long-term follow-ups will be performed as described in Section 10.3

18.2. Summary of the RANO Response Criteria

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

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.

*Progression occurs when this criterion is present.

†Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration

18.3. Karnofsky Performance Status Scale

Patient performance status will be graded according to the following scale:

KPS 100 Normal; no complaints; no evidence of disease

KPS 90 Able to carry on normal activity; minor signs or symptoms of disease

KPS 80 Normal activity with effort some sign or symptoms of disease

KPS 70 Care for self; unable to carry on normal activity or do active work

KPS 60 Requires occasional assistance, but able to care for most personal needs

KPS 50 Requires considerable assistance and frequent medical care

KPS 40 Disabled; requires special care and assistance

KPS 30 Severely disabled; hospitalization is indicated, although death not imminent

KPS 20 Very sick; hospitalization necessary; active support treatment is necessary

KPS 10 Moribund; fatal processes progressing rapidly

KPS 0 Dead