CITY OF HOPE
1500 E. DUARTE ROAD
DUARTE, CA 91010

DEPARTMENT OF MEDICAL ONCOLOGY

TITLE: **A Phase I Study of Cytosine Deaminase-Expressing Neural Stem Cells in Combination with Oral 5-Fluorocytosine and Leucovorin for the Treatment of Recurrent High-Grade Gliomas**

CITY OF HOPE PROTOCOL NUMBER: 13401 **VERSION: 10**

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PHASE/TYPE: Phase I

PRINCIPAL INVESTIGATOR: Jana Portnow, MD

COLLABORATING INVESTIGATOR(S): Julie Ressler, MD, Timothy Synold, PharmD, Behnam Badie MD, , Russel Rockne, PhD.

PARTICIPATING CLINICIANS: Mike Chen, MD, PhD., Mihaela Cristea, MD, Rahul Jandial, MD, PhD., Marianna Koczywas, MD., Clarke Anderson, M.D.

STUDY STATISTICIAN: Suzette Blanchard, PhD

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A Phase I Study of Cytosine Deaminase-Expressing Neural Stem Cells in Combination with Oral 5-Fluorocytosine and Leucovorin for the Treatment of Recurrent High-Grade Gliomas

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COLLABORATING INVESTIGATOR(S):

City of Hope
Behnam Badie MD
Massimo D'Apuzzo, MD, PhD
Julie Ressler, MD
Timothy Synold, PharmD

PARTICIPATING CLINICIANS:
Clarke Anderson, MD
Mike Chen, MD, PhD
Mihaela Cristea, MD
Rahul Jandial, MD, PhD
Marianna Koczywas, MD
Robert J. Morgan, MD

STUDY STATISTICIAN:	Suzette Blanchard, PhD

Experimental Design Schema

Dose Escalation Schema

Dose Level	Neural Stem Cells (i.c. on days 1 & 15)	5-FC (mg/kg q 6h p.o. on days 4-10 & 18-24)	Leucovorin (mg q 6h p.o. on days 4-10 & 18-24)	Initial Enrollment	If 1 DLT, additional enrollment
-1[a]	3.75×10^7	28	---	3	3
1	5×10^7	37.5	---	3	3
2	1×10^8	37.5	---	3	3
3	1.5×10^8	37.5	---	3	3
4[b,c]	Highest tolerated dose combination of 5-FC and NSCs		25	3	3

[a]If a DLT is observed in 2 or more patients at dose level 1, then a de-escalation dose**, dose level -1** will be tested. With dose level -1, the doses of NSCs and 5-FC will both be reduced by 25%; however, if the DLT can clearly be attributed only to 5-FC, then only the dose of 5-FC will be reduced by 25% (likewise for the NSCs if the DLT is clearly attributable to only the NSCs) for dose level -1.

[b]If the addition of leucovorin to the highest tolerated dose combination of 5-FC and NSC requires a dose de-escalation, then leucovorin will be discontinued, and the MTD/MFD will be the highest tolerated dose combination of 5-FC and NSCs without leucovorin. A total of 6 patients will be treated at the maximum tolerated dose (MTD), or if the MTD is not reached, at the maximum feasible dose (MFD).

[c]Dose level 4 patients will undergo intracerebral microdialysis to measure NSC-mediated conversion of 5-FC to 5-FU.

Cycle 1[a]

Day 1

- Tumor resection or biopsy is performed.
- HB1.F3.CD NSCs are administered intracranially.
- A Rickham catheter is placed.
- Dose level 4 patients only: a microdialysis catheter is placed.

Days 4-10

- Patients take 5-FC 37.5 mg/kg p.o. q 6h.
- Patients on dose level 4 also take leucovorin 25 mg p.o. q 6h.
- Dose level 4 patients only: serial dialysate and blood samples collected.
- .

Days 15

- HB1.F3.CD NSCs administered intracranially via the Rickham catheter.
- Blood drawn for immunologic correlative studies.

Days 18-24

- Patients take 5-FC 37.5 mg p.o. q 6h.
- Patients on dose level 4 also take leucovorin 25 mg p.o. q 6h.

[a] The length of a treatment cycle is 28 days. After cycle 1, the NSCs will be administered via the Rickham catheter on days 1 and 15 of a cycle. The 5-FC (and leucovorin for dose level 4 patients) will continue to be administered on days 4-10 and 18-24. Blood draws for immunologic correlative studies will be done on days 1 and 15 of cycle 2, and then only on day 15 of subsequent cycles. A brain MRI will be performed after every 2 cycles of treatment.

Protocol Synopsis

Protocol Title:
A Phase I Study of Cytosine Deaminase-Expressing Neural Stem Cells in Combination with Oral 5-Fluorocytosine and Leucovorin for Treatment of Recurrent High-Grade Gliomas
Brief Protocol Title for the Lay Public (if applicable):
A dose escalation study of oral 5-Fluorocytosine (5-FC) and leucovorin in combination with neural stem cells that have been genetically-modified to carry a protein that converts the inactive prodrug, 5-FC, to the chemotherapy agent 5-Fluorouracil (5-FU), in patients with recurrent high grade gliomas.
Study Phase:
Phase I
Participating Sites:
City of Hope
Rationale for this Study:
High-grade gliomas are difficult to treat due to their relative resistance to chemotherapy and radiation, as well as their highly invasive nature. Human neural stem cells (NSCs), modified to express a therapeutic transgene, hold great promise for brain tumor therapy due to their inherent tumor-tropic properties and- potential use as vehicles for delivering chemotherapy directly to infiltrating glioma cells and metastatic tumor cells in the brain. NSCs have the potential to overcome obstacles of drug-delivery that limit current gene therapy strategies to provide an effective anti-tumor response. Data from animal models have demonstrated the safety and efficacy of NSCs for tracking to invasive tumor cells as well as to distant micro-tumor foci and delivering therapeutic gene products to tumor cells. Our recently completed first-in-human pilot study of cytosine deaminase (CD)-expressing NSCs given in combination with oral 5-fluorocytosine (5-FC) showed that one dose of NSCs followed by a 7-day course of 5-FC in recurrent high-grade gliomas patients is safe and feasible. With intracerebral microdialysis, we documented proof-of-concept—that the NSCs convert the prodrug 5-FC to its active metabolite 5-FU in the brain. Results of immunologic correlative studies documented no evidence of NSC immunogenicity after first exposure. Magnetic resonance imaging (MRI) of iron-labeled NSCs showed preliminary evidence of NSCs migrating away from the injection sites. For this phase I clinical trial, we will continue to escalate the dose of the NSCs and now administer repeat cycles of study treatment to patients with recurrent high-grade gliomas. Once dose escalation is completed, leucovorin will be added to the 5-FC, since it is well established that leucovorin can potentiate the efficacy of 5-FU. We hypothesize that repeat cycles of NSCs and 5-FC/leucovorin will be safe and well-tolerated. We will continue to monitor patients for development of immune reactivity against NSCs and measure NSC conversion of 5-FC to 5-FU via intracerebral microdialysis at the maximum tolerated dose (MTD), or if the MTD is not met, at the maximum feasible dose (MFD), of study treatment.
Objectives:
Primary Objectives: **1)** To define the phase II recommended dose of intracerebrally-administered CD-expressing NSCs in combination with oral 5-FC and leucovorin. This will be based on the maximum-tolerated dose (MTD), or if the MTD is not met, the maximum feasible dose and toxicity profile in patients with recurrent high-grade gliomas.

2) To determine the feasibility of treating study patients with more than 1 dose of NSCs followed by 7-day courses of 5-FC and leucovorin.

Secondary Objectives:

1) To assess for possible development of NSC immunogenicity (anti-NSC T cell and/or antibody responses) with repeat doses of NSCs.

2) To characterize the relationship between intracerebral and systemic concentrations of 5-FC and 5-FU at the MTD/MFD level.

3) To describe the clinical benefit (defined as stable disease, partial response, or complete response) of this treatment regimen.

4) To determine, at time of autopsy, the fate of the NSCs.

Study Design:

Based on the encouraging safety data from our first-in-human pilot feasibility study of 1 cycle of intracranially administered CD-expressing NSCs and oral 5-FC in recurrent high-grade glioma patients, we will now perform a phase I clinical trial to define the phase II recommended doses of CD-expressing NSCs given in combination with 5-FC and leucovorin and determine the feasibility of administering repeat cycles of study treatment in patients with recurrent high-grade gliomas. A standard "3 + 3" dose escalation schema will be used, and 4 dose levels of study treatment will be tested. The starting number of NSCs will be 5×10^7, which is a dose that was well-tolerated by patients in the first study. Due to volume limitations for intracerebral administration, the highest dose of NSCs to be tested will be 1.5×10^8. The 5-FC will not be dose-escalated, but leucovorin, which potentiates the efficacy of 5-FU, will be added after dose escalation of the NSCs is completed.

Initially, the NSCs will be injected intracranially on cycle 1 day 1 when study patients undergo tumor resection or biopsy. Afterwards, there will be a waiting period of 3 days to allow time for the NSCs to distribute among foci of tumor. Patients will then take a 7 day course of oral 5-FC (dose level 4 patients will also take leucovorin). Repeat doses of NSCs will be administered through a Rickham catheter/reservoir system placed at the time of surgery. The length of a treatment cycle will be 28 days. NSCs will be given on days 1 and 15 followed by 5-FC and leucovorin (dose level 4 patients) on days 4-10 and 18-24. Brain MRIs will be performed at the end of every even cycle of study treatment to evaluate response.

Immunologic correlative studies will be performed to assess for the possible development of NSC immunogenicity after repeat exposure to the allogeneic NSCs, and we will measure conversion of 5-FC to 5-FU by the NSCs. Once the maximum tolerated dose of the combination therapy is predicted, or, if the maximum tolerated dose is not met, the maximum feasible dose, patients in this cohort will undergo intracerebral microdialysis during the first part of cycle 1 to quantify NSC-mediated conversion of 5-FC to 5-FU at the MTD/MFD level.

Endpoints:

Safety and feasibility: 1) For dose escalation: dose limiting toxicities;
2) Toxicity profile: all attributable toxicities.

Immunogenicity: evaluate patient blood samples for the development of T cell responses and antibodies against the NSCs using TcR Vβ spectratyping, CD 107 degranulation assays, and flow cytometry.

Pharmacokinetics: the following pharmacokinetic variables will be assessed: Tmax, Cmax, AUC,

t ½, and the ratio of the AUC of 5-FU in dialysate to plasma, and the ratio of the AUC of 5-FC in dialysate to plasma.

Determine the fate of the NSCs: NSC persistence

Sample Size:

The expected sample size is 15-18 patients (minimum = 6, maximum = 33, allowing for 3 patients to replace unevaluable/ineligible patients).

Estimated Duration of the Study

30-36 months

Summary of Subject Eligibility Criteria:

Inclusion Criteria:

- Patient with a recurrent high-grade glioma.
- Imaging studies show evidence of recurrent supratentorial tumor(s). The presence of infratentorial tumor is allowed as long as the patient also has supratentorial disease that is amenable to resection or biopsy.
- The patient must be in need of a craniotomy for tumor resection or a stereotactic brain biopsy for the purpose of diagnosis or differentiating between tumor progression versus treatment-induced effects following radiation therapy ± chemotherapy.
- The patient's high grade glioma has progressed after treatment with brain radiation and temozolomide,
- Patient must be at least 18 years old.
- Patient has a Karnofsky Performance Status of ≥ 70%.
- No limit to prior number of therapies.

Exclusion Criteria:

- Patient has anti-HLA antibodies specific for HLA antigens expressed by the HB1.F3.CD.NSCs.
- Patient has not recovered from any toxicity of prior therapies.

Investigational Product Dosage and Administration:

Dose Level	Neural Stem Cells (i.c. on days 1 & 15)	5-FC (mg/kg q 6h p.o. on days 4-10 & 18-24)	Leucovorin (mg q 6h p.o. on days 4-10 & 18-24)	Initial Enrollment	If 1 DLT, additional enrollment
-1[a]	3.75×10^7	28	---	3	3
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[a]If a DLT is observed in 2 or more patients at dose level 1, then a de-escalation dose**, dose level -1** will be tested. With dose level -1, the doses of NSCs and 5-FC will both be reduced by 25%; however, if the DLT can clearly be attributed only to 5-FC, then only the dose of 5-FC will be reduced by 25% (likewise for the NSCs if the DLT is clearly attributable to only the NSCs) for dose level -1.

[b]If the addition of leucovorin to the highest tolerated dose combination of 5-FC and NSC requires a dose de-escalation, then leucovorin will be discontinued and the MTD/MFD will be the highest tolerated dose combination of 5-FC and NSCs without leucovorin. A total of 6 patients will be treated at the maximum tolerated dose (MTD), or if the MTD is not reached, at the maximum feasible dose (MFD).

Dose level 4 patients will undergo intracerebral microdialysis to measure NSC-mediated conversion of 5-FC to 5-FU.

Clinical Observations and Tests to be Performed:

Blood draws for immunologic correlative studies: To assess for possible development of anti-NSC T cell and antibody responses, as well as peripheral persistence of the NSCs, blood samples will be drawn: prior to the start of study treatment, on day 15 of cycle 1 (prior to the 2nd dose of NSCs), days 1 and 15 of cycle 2, and then only on day 15 of subsequent cycles.

Brain MRIs to assess response: At the end of cycle 1 and then after every 2 cycles.

Collection of dialysate and blood samples to determine the neuropharmacokinetics of 5-FC and 5-FU: Only in dose level 4 patients—see section 5.4.

Collection of blood samples to perform RCR testing as part of long-term follow: Prior to surgery, at 3 months, 6 months, 1 year, and annually thereafter.

Statistical Considerations:

Tables will be created to summarize all toxicities and side effects by dose, course, organ, severity (by NCI CTCAE version 4.0) and attribution. Rates and associated 90% confidence limits will be estimated for the DLT rate at the MTD/MFD and the rate of clinical benefit. Descriptive statistics will be provided for study patient demographics.

One secondary objective is to assess the conversion of 5-FC to 5-FU by the NSCs at the MTD/MFD. Pharmacokinetic (PK) data from the patients who undergo intracerebral microdialysis will be summarized using descriptive statistics and graphical methods. The primary PK parameters

of interest will be the Cmax and AUC of 5-FC and 5-FU, measured both in the dialysate samples and in plasma. Leucovorin levels in dialysate samples will likewise be compared to plasma leucovorin levels. The 5-FC and 5-FU PK data will also be compared to the PK data obtained from patients who underwent intracerebral microdialysis in the first study of CD-expressing NSCs (IRB# 08002) to determine if there is a dose effect at the MTD/MFD compared to lower doses of NSCs. All summaries will be exploratory in spirit, with the goal of developing further questions regarding the modulation of therapy, or regarding reasons for efficacy or lack of efficacy. Data from assessing for possible development of NSC immunogenicity with repeat exposure and performing autopsies to determine the fate of the NSCs, will be presented in an exploratory fashion using descriptive statistics and graphical methods.
Sponsor:
City of Hope
Case Report Forms:
Electronic data capture time forms will be used.

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1.0 Goals and Objectives (Scientific Aims)

1.1 Primary Objective:

1.1.1 To define the phase II recommended dose of intracerebrally administered CD-expressing NSCs in combination with oral 5-FC and leucovorin. This will be based on the maximum tolerated doses (MTD), or if the MTD is not met, the maximum feasible dose, and toxicity profile in patients with recurrent high-grade gliomas.

1.1.2 To determine the feasibility of treating study patients with more than 1 dose of NSCs followed by 7-day courses of 5-FC and leucovorin.

1.2 Secondary Objectives:

1.2.1 To assess for possible development of NSC immunogenicity (anti-NSC T cell and/or antibody responses) with repeat doses of NSCs.

1.2.2 To characterize the relationship between intracerebral and systemic concentrations of 5-FC and 5-FU at the MTD/MFD level.

1.2.3 To describe the clinical benefit (defined as stable disease, partial response, or complete response) of this treatment regimen.

1.2.4 To determine, at time of autopsy, the fate of the NSCs.

2.0 Background

2.1 Introduction

High-grade gliomas are difficult to treat due to their relative resistance to chemotherapy and radiation, as well as their highly invasive nature. Human neural stem cells (NSCs), modified to express a therapeutic transgene, hold great promise for brain tumor therapy due to their inherent tumor-tropic properties and- potential use as vehicles for delivering chemotherapy directly to infiltrating glioma cells in the brain. Data from animal models have demonstrated the safety and efficacy of NSCs for tracking to invasive tumor cells as well as to distant micro-tumor foci and delivering therapeutic gene products to tumor cells. Our recently completed first-in-human pilot study of cytosine deaminase (CD)-expressing NSCs given in combination with oral 5-fluorocytosine (5-FC) has shown that treatment with one dose of NSCs followed by a 7-day course of 5-FC in recurrent high-grade glioma patients is safe and feasible.

NSCs can overcome obstacles of drug-delivery that limit current gene therapy strategies and provide an effective anti-tumor response. In this phase I clinical trial we will use the same v-*myc*-immortalized human NSC line (HB1.F3.), retrovirally transduced to express CD (HB1.F3.CD NSCs), (Aboody et al., 2000) as was used in our first-in-human pilot study. This HB1.F3.CD NSC line has been well-characterized, favorably reviewed by the NIH RAC, and established as a Master Cell Bank at the COH Center for Biomedicine & Genetics.

After intracranial administration to patients with recurrent brain tumors, we hypothesize that, based on pre-clinical data, CD-expressing NSCs will distribute throughout the primary tumor site as well as co-localize with infiltrating tumor cells within 3 days of administration. Study patients will then take oral 5-FC and leucovorin for 7 days. The CD-expressing NSCs will locally convert 5-FC to 5-FU (Figure 1), and the 5-FU will diffuse out of the NSCs at tumor sites, thereby generating concentrated cytotoxicity at sites of tumor in the brain. This CD/5-FC prodrug strategy is known to have a large bystander effect. (Huber et al., 1994; Barresi et al., 2003).

Based on the safety data obtained in the pilot feasibility study in patients with recurrent high-grade gliomas who received only round of study treatment, in this phase I study we will assess the safety and feasibility of administering repeat doses of NSCs followed by 7-day course of 5-FC and leucovorin.

After the first dose, the NSCs will be administered through a Rickham catheter via convection enhanced delivery.

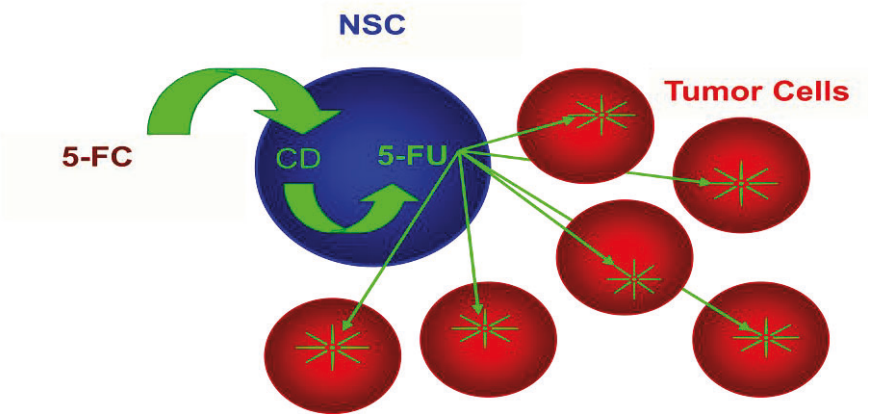


Figure 1. NSCs expressing the enzyme *E. coli* cytosine deaminase migrate to tumor sites. The inactive oral prodrug, 5-FC, crosses the blood-brain barrier and is converted to the active cytotoxic drug 5-FU by CD-expressing NSCs.

2.2 Obstacles to Successful Brain Tumor Therapy

Despite recent advances in molecularly targeted therapies for cancer, brain tumors remain a serious clinical challenge for oncologists. Approximately 22,500 people a year are diagnosed with a malignant primary brain tumor in the United States (CBTRUS 2008). Gliomas account for the majority of these tumors and are virtually incurable. High-grade gliomas are difficult to treat due to their relative resistance to chemotherapy and radiation, as well as their highly invasive nature. Despite recent advances in molecularly targeted therapies for cancer, primary brain tumors, particularly high-grade gliomas, remain a serious clinical challenge for oncologists. A major obstacle to successful pharmacologic treatment of central nervous system (CNS) tumors is the blood-brain barrier (BBB), which prevents most anti-cancer agents from entering the brain. Another key reason for the lack of success in treating gliomas is their diffuse and highly infiltrative nature; no clear border exists between tumor and normal brain. Glioma cells disseminate from the primary site, forming micro-tumor foci throughout the brain the brain that often “hide behind”an intact BBB.

The inability to deliver therapeutic agents to where they are needed likewise inhibits the effectiveness of gene therapy. Cell-mediated vector delivery is limited by the capability of the carrier cell line to migrate through the brain with sufficient biodistribution throughout primary and distant tumor sites. To make a significant impact on the survival of brain tumor patients, new therapies must not only be able to navigate through or around the BBB, but they should also specifically target the invasive tumor cells that escape currently available treatments while minimizing toxicity to normal tissue.

2.3 Human Neural Stem Cells

Because NSCs (murine and human) demonstrate an inherent ability to distribute throughout a tumor mass and can target distant tumor foci, NSCs that are genetically modified to express a therapeutic transgene have the potential to overcome many of the obstacles facing current strategies for brain tumor therapy. Tumor-tropic human NSCs potentially offer a major therapeutic breakthrough for brain tumors, overcoming limitations of currently available therapies by efficient tumor distribution and the ability to deliver a wide range of therapeutic agents directly to invasive tumor cells and distant tumor micro-foci throughout the brain.

NSCs can be engineered to express various therapeutic agents. Several studies in rodent models of orthotopic glioma have demonstrated the ability of intracranially administered NSCs that express a therapeutic transgene, to significantly reduce tumor burden and/or increase long-term survival. Therapeutic agents delivered via NSCs include prodrug activating enzymes (Aboody et al., 2000, 2006; Brown et al., 2003; Danks et al., 2007; Kim et al., 2006a) viral vectors (Herrlinger et al., 2000; Tyler et al., 2009), apoptotic agents (Kim et al., 2005a; Shah et al., 2005), anti-angiogenic agents (Kim et al., 2005b), monoclonal antibodies (Frank et al., 2009), interleukins (Benedetti et al., 2000; Ehtesham et al., 2002; Yuan et al., 2006), and IFN- β (Dickson et al., 2007).

HB1.F3 NSCs were generated from human fetal telencephalon, retrovirally immortalized with the v-*myc* oncogene, cloned and extensively characterized over time and passage for tumor tropism, genetic stability and non-tumorigenicity. (Kim et al., 2002, 2004, 2005a; Jeong et al., 2003; Ryu et al., 2003; Schmidt et al., 2005). Both parental HB1.F3 NSCs and HB1.F3 NSCs modified to express therapeutic transgenes (Aboody et al., 2000) have demonstrated efficacy in pre-clinical models of glioma (Aboody et al., 2013), breast cancer brain metastases (Joo et al., 2009), medulloblastoma (Kim et al., 2006a; Gutova et al., 2012), and metastatic neuroblastoma (Aboody et al., 2006a; Danks et al., 2007). In addition to being used as a vehicle to target therapeutic agents to invasive tumors, this parental HB1.F3 line has also been modified to demonstrate therapeutic efficacy in models of Parkinson's disease (Kim et al., 2004, 2006b; Ryu et al., 2005) Huntington's disease (Kim et al., 2004, 2007; Ryu et al., 2004; Lee et al., 2007), cerebral ischemia (Chu et al., 2003, 2004; Lee et al., 2007a, 2007b) and spinal cord injury (Kim et al., 2007).

2.4 Preclinical Data

2.4.1 Human NSC tumor tropism

Human NSCs possess an inherent ability to distribute throughout a tumor mass and can target distant tumor foci. Aboody et al., 2000 were the first to demonstrate the tumor tropic properties of NSCs with a v-myc–immortalized, clonal murine NSC line (C17.2). When injected either directly into orthotopic experimental glioma or at a distance (contralateral hemisphere, contralateral ventricle, or intravascularly via the tail vein), C17.2 murine NSCs migrated to and efficiently distributed throughout the main tumor mass, as well as localized to satellite tumor foci. These ground-breaking observations have been confirmed for multiple NSC lines and primary pools in many laboratories (reviewed in Aboody et al., 2008).

NSCs can migrate to tumor sites regardless of tumor size, anatomic location, or tissue of origin (reviewed by Aboody et al., 2008). When administered intracranially or intravenously in pre-clinical brain tumor models, NSCs migrate to orthotopic gliomas (Aboody et al., 2000; Herrlinger et al., 2000; Brown et al., 2003;Kim et al., 2005a; Zhao et al., 2008; Ahmed et al., 2011; Thaci et al., 2012), breast cancer brain metastases (Joo 2009), medulloblastoma (Kim et al., 2006a; Shimato et al., 2007, Gutova et al, 2012), and melanoma brain metastases (Aboody et al., 2006a). Migration of HB1.F3.CD NSCs is not affected by the presence of dexamethasone or prior radiation to the brain (Aboody et al., manuscript submitted and under revision with Science Translational Medicine). The ability of NSCs to efficiently cross the BBB provides an attractive advantage in treating CNS tumors.

Transduction with retrovirus, lentivirus, or adenovirus does not affect their migratory potential, as similarly assessed (Najbauer et al., 2008; Aboody et al., 2008; Kendall et al., 2008). Studies with HB1.F3 cells that were adenovirally-transduced with a transgene to express rabbit carboxylesterase(rCE), (HB1.F3.C1) showed that NSCs localized to neuroblastoma metastases in the liver, lung, ovaries and bone marrow. NSCs were rarely found in non-tumor-bearing tissues (lung, liver, and spleen) at 3-4 days post- tail vein injection, and none were detectable in non-tumor-bearing brain, kidney, heart, intestine, or skin (Aboody, et al., 2006c)

2.4.2 Immortalized human NSC lines do not cause secondary tumors

Preclinical data and results of early clinical trials indicate that the most likely toxicity of cell-based gene therapies is the development of secondary malignancies (Pike-Overzet et al., 2007). All of our studies have included tissue assessment for potential NSC-induced tumors. To date, NSCs have not initiated tumors in any of the normal control or tumor model animals, when followed up to 12 months. All published pre-clinical *in vivo* studies using HB1.F3 cells for CNS disease applications have reported no HB1.F3 tumorigenicity, even up to one year following NSC administration (Kim et al., 2004, 2005a, 2005b, 2006a; 2006b, 2007; Lee et al., 2005, 2007a, 2007b; Yasuhara et al., 2006; Danks et al., 2007).

2.4.3 NSC immunogenicity data

Aboody and colleagues (Aboody et al., 2013) investigated whether HB1.F3.CD NSCs induce an immune response from the host, resulting in possible rejection of the NSCs. In an immunocompetent, syngeneic orthotopic glioma model (Gl261 glioma in C57BL/6 mice), although HB1.F3.CD NSCs do elicit a subacute, localized immune response characterized by detectable T-cell infiltration (similar to the reaction generated by other types of cellular injections), viable HB1.F3.CD NSCs persisted for at least 2 weeks. Of note, no immunosuppression was given to the mice, and no global adverse immunogenic responses were observed.

Based on these data, we do not expect the NSCs to be immune-rejected in the first 2–3 weeks after administration, during which time a round of NSCs and a 7 days course of 5-FC/leucovorin will be completed. When NSCs are used as delivery vehicles, they do not need to engraft. They need only survive long enough to mediate the effective therapy. Therefore, although the NSCs may be rejected over time, this would not likely happen before finishing a treatment sequence of 5-FC/leucovorin.

HLA typing results of HB1.F3 cells found that while these NSCs doexpress HLA class I antigens (A*01, A*31, B*07, B*15, C*07), they do not express their HLA class II antigens (DRB1*10, DRB1*13, DQB1*05, DQB1*06, DPB1*02, DPB1*15).. In the presence of tumor, the NSCs do not appear to differentiate, and therefore, they would not express HLA class II antigens. However, other investigators (Kim et al., 2009) have reported low expression levels of HLA Class II antigens by HB1.F3 cells. To be cautious, we will exclude potential study patients who hve antibodies to any of the NSC HLA class I or II antigens listed above. Whether or not the clinical utility of these NSCs will be limited by development of immune responses from repeated exposure to these allogeneic NSCs is unknown (and will be studied in this phase I clinical trial), but preclinical data indicate that the immunogenicity of the HB1.F3.CD NSC line is not prohibitive.

2.4.4 Iron particle cellular MRI for real-time assessment of NSCs biodistribution

Ultrasmall superparamagnetic iron oxide (USPIO) preparations can enable non-invasive, real-time tracking of NSCs by MRI. In previous studies, we demonstrated the effectiveness of MRI to track NSCs labeled with a clinical grade SPIO, ferumoxide (Feridex®) in orthotopic glioma-bearing mice (Thu et al., 2009). Safe use of SPIO MRI contrast agents in patients has been demonstrated for central nervous system tumor visualization and diagnostic MRI purposes (Neuwalt et al., 2007). We have now developed a cell labeling technique that can be safely and effectively used in the clinical setting for MRI tracking of NSCs in the brain. Specifically, we modified a previously developed protocol (Thu et al., 2012) for cell labeling with a clinical grade USPIO, ferumoxytol (Feraheme®), to optimize labeling of the HB1.F3.CD NSCs. We have demonstrated in orthotopic glioma xenograft mouse models that Feraheme-labeled HB1.F3.CD NSCs that also expressed carboxylesterase (HB1.F3.CD.hCE NSCs or Fehe-NSCs) can be detected in a typical 7 Tesla mouse imaging voxel as a hypointense signal (black) following intracranial *(i.c.)* administration (Fig. 2A-D). (Note that HB1.F3.CD and HB1.F3.CD.hCE NSCs are equivalent in terms of viability, proliferation, and migration *in vitro* and *in vivo*). Note that the Fehe-NSCs (blue) distributed within tumor (dark pink) and at invasive tumor edges and foci (Fig. 2E-H), corresponding with hypointense signal by MRI. Tumor cells confirmed by staining with eGFP-DAB (brown, Fig. 2I-L).

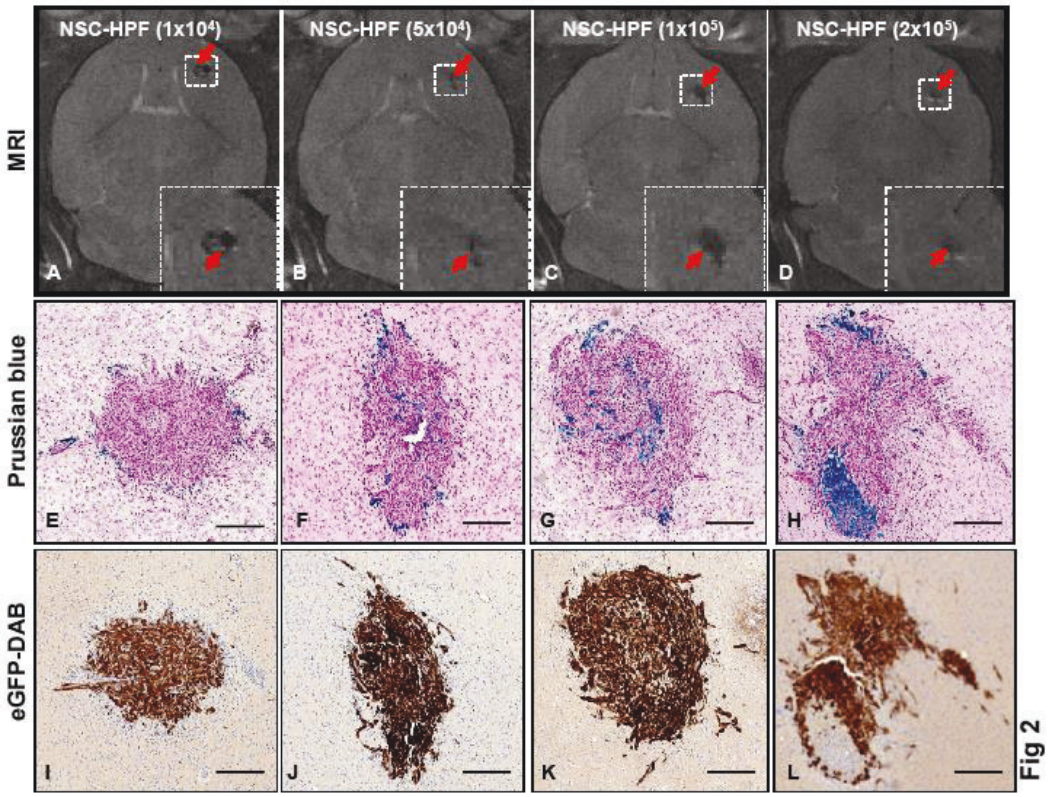


Figure 2. Intracranially injected Feraheme-labeled NSCs migrate and distribute to orthotopic U251 human gliomas in an immunodeficient mouse model. Intracerebral glioma xenografts in mice were established by stereotactic implantation of 2×10^5 U251.eGFP.ffluc cells into the right frontal hemisphere. Three days later, varying numbers of Fehe-NSCs (1×10^4, 5×10^4, 1×10^5, 2×10^5), were injected ipsilaterally, caudal-lateral to the tumor. (**A-D**): T2-weighted MR images of mouse brains were obtained 4 days after NSC administration. Red arrows indicate hypointense (black) signal associated with Fehe-NSCs. Boxed areas are magnified in the image insets. (**E-H**) Prussian blue stained and pararosaniline counterstained mouse brain sections containing tumor and NSCs. Fehe-NSCs that migrated to tumor are stained blue. (**I-L**) Tissue sections adjacent to Prussian blue sections in (E-H) were immunohistochemically stained for eGFP-DAB to identify tumor cells (brown). Sections were counterstained with hematoxylin. Note blue NSCs within tumor nodules and at infiltrative tumor sites. Scale bars, 200 µm.

The iron-labeled cells do not exhibit significant changes in viability, proliferation, and migratory properties, or transgene expression compared to non-labeled cells (Gutova et al, Stem Cells Transl Med, in press, 2013). Preclinical safety/toxicity studies in mice using intracranially injected Feraheme-labeled HB1.F3.CD NSCs, demonstrated no clinical adverse effects, and histopathologic analysis revealed no evidence of neurotoxicity or toxicity to other organs (Gutova et al, Stem Cells Transl Med, in press, 2013).

2.4.5 Efficacy of NSC-mediated therapy in glioma preclinical models

Both parental HB1.F3 NSCs and HB1.F3 NSCs modified to express the therapeutic transgenes, CD and/or rCE, have demonstrated efficacy in pre-clinical models of glioma (Aboody et al., 2000 and unpublished data), medulloblastoma (Kim et al., 2006a; Gutova, et al., 2012) and metastatic neuroblastoma (Aboody et al, 2006c; Danks et al., 2007).

The IND-enabling efficacy study of HB1.F3.CD NSCs and 5-FC (Aboody et al., manuscript submitted and under revision with Science Translational Medicine) was performed using the Master Cell Bank HB1.F3.CD line, and the NSCs were prepared for administration according to the clinical SOP. Ninety-six nude mice with orthotopic U251 glioma were treated with 1 cycle of either CD-expressing NSCs (3 escalating doses, groups 1-3) and 5-FC; CD-expressing NSCs alone (3 escalating doses, groups 4-6); 5-FC alone (group 7) or no treatment (group 8). At the first planned time point of day 30, 32 mice (4 from each group) were sacrificed for analysis. Tumor volumes were measurable in 24 of the 32 mice, 8-20 tissue sections were used for volumetric analysis from each of the 24 mice assessed for therapeutic efficacy.

A statistically significant therapeutic effect was seen at the day 30 time-point with tumor volumes ~3-fold smaller in mice who received HB1.F3.CD NSCs and 5-FC treatment (groups 1-3) compared to mice who did not receive the combination therapy ($p = 0.041$). Representative images of tumors from each group at Day 30 are shown in Figure 3.

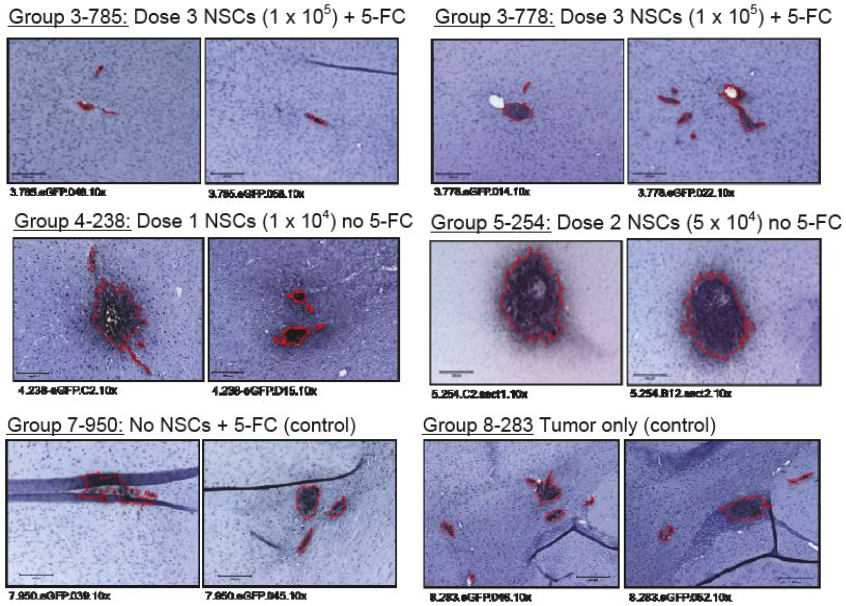


Figure 3. Representative 10x images of brain tissue sections at the 30-day time point comparing examples of tumors from treated (dose 3 NSCs and 5-FC) versus control groups. Male and female adult nude mice bearing frontal lobe U251 glioma received caudal-lateral injection of $1x10^5$, $5x10^5$, or $1x10^6$ NSCs, followed 4 days later by a 7-day course of 5-FC (500 mg/kg i.p. BID). Animals receiving combination of HB1.F3.CD NSCs + 5-FC therapy had 3-fold less tumor volume compared to control animals (Groups 4-6, NSCs only; Group 7, 5-FC only; Group 8, tumor only) ($p = 0.041$). Tumor cells are identified by grey/black cytoplasm following immunocytochemical staining with eGFP antibody–and Peroxidase/DAB-Ni. Tumor area is outlined in red.

2.5 Clinical Data

In our recently completed first-in-human pilot study of these genetically-modified NSCs, patients with recurrent high-grade gliomas were given one dose of CD-expressing NSCs intracranially at the time of tumor resection or biopsy followed by one 7-day course of 5-FC (Portnow et al, 2013a). Two different doses of NSCs were tested (Table 1).

Table 1. Dose escalation schema from the first-in-human pilot feasibility study of CD-expressing NSCs.

Dose Level	Neural Stem Cells	5-FC (mg/kg q 6h x 7 days)	Initial Enrollment	If 1 of Initial 3 has a DLT, Additional Enrollment
1	1×10^7	18.75	3	3
2	1×10^7	37.5	3	3
3	5×10^7	37.5	3	3

2.5.1 Safety data

A total of 18 patients were consented for the study. Three of these patients were determined to be ineligible prior to the start of study treatment due to testing positive for an antibody to one of the NSC HLA antigens, which was one of the study's exclusion criteria. Of the 15 patients who received study treatment, 3 had to be replaced due to being inevaluable for toxicity: 2 died from early progression of disease, and 1 died from a post-surgical complication. This 3rd patient died from an intracerebral bleed occurring in a vessel at the surgical margin. NSCs had not been injected in that area.

All of the patients tolerated the NSCs well. There were no toxicities associated with intra-operative injection of the NSCs into the wall of the tumor resection cavity or tumor biopsy site. Furthermore, no grade 3 or 4 toxicities related to the NSCs occurred. There was 1 dose-limiting toxicity which was felt to possibly be due to 5-FC. A patient on dose level 3 developed transient grade 3 increased ALT and AST. Since elevation of liver function tests is a known side effect of 5-FC, this grade 3 toxicity was attributed as being possibly related to the 5-FC, but the patient also had gallstones, and it was felt that the clinical presentation of his transaminitis was most consistent with an episode of biliary colic. Nonetheless, since an association with 5-FC could not be ruled out, dose level 3 was expanded to treat 3 additional patients. No further hepatic toxicity was observed in those last 3 patients.

With this first-in-human study the FDA would only allow patients to be treated with one round of NSCs and 5-FC, thus clinical benefit was not a primary or secondary objective of the study. The starting dose of NSCs was approximately 60% lower than the human equivalent of the lowest dose tested in safety/efficacy studies in mice. Although in most study patients, tumor growth was seen on the one month follow-up MRI, 1 patient on dose level 3 did not have evidence of progressive disease until 5 months after tumor resection and start of study treatment. He was followed with serial brain MRIs and did not start another chemotherapy regimen until after the MRI at 5 months documented tumor recurrence.

2.5.2 Establishing proof-of-concept

Using the technique of intracerebral microdialysis (please see section 2.8 for details of this technique), we demonstrated proof-of-concept—that the CD-expressing NSCs are converting 5-FC to 5-FU in the brain—by measuring intracerebral levels of 5-FC and 5-FU and comparing them to concentrations in plasma). Intracerebral microdialysis data from all 3 dose levels (Figure 4) document that the NSCs are converting 5-FC to 5-FU in the brain. At the highest dose level of NSCs, the average steady-state concentrations of 5-FU in the brain was 63.9±7.9 nM. The average maximum 5-FU level in brain was 104±88 nM compared to 24±36 nM in plasma, indicating local production of 5-FU in the brain by the NSCs.

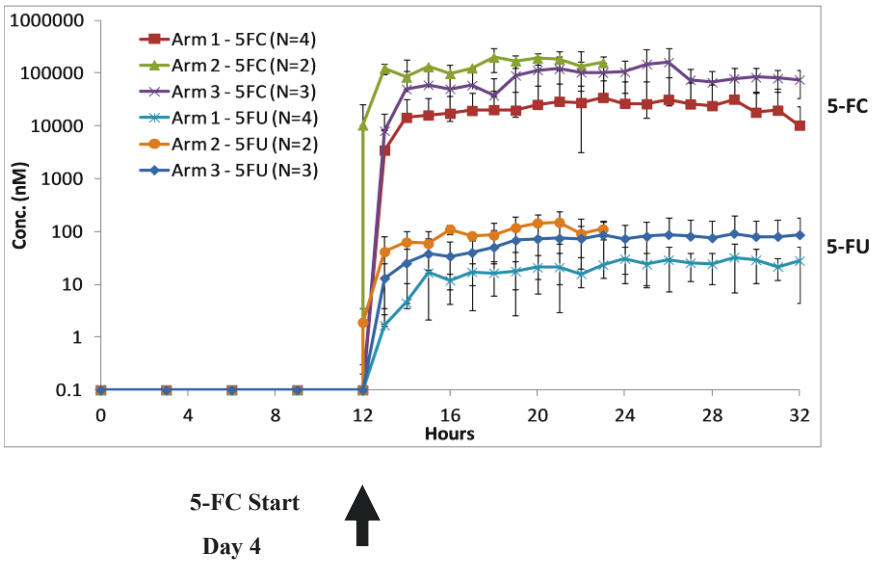


Figure 4. 5-FC and 5-FU Intracerebral Microdialysis data.

Analysis of the plasma samples revealed 5-FC concentrations similar to previously reported values in patients taking 5-FC to treat infections. Brain interstitial 5-FC levels were approximately 20-30% of plasma levels. Plasma 5-FU was detectable in roughly one third of patients studied, but plasma levels were always well below the brain interstitial 5-FU concentrations in these patients. We conclude that 5-FU is produced by enterobacteria in the gut in a subset of patients taking 5-FC; however, the resulting systemic levels of 5-FU cannot explain the levels measured in the brain. Therefore, most, if not all, of the 5-FU measured in brain interstitium must be coming from local production by the CD-transduced neural stem cells. Figure 5 shows documentation that the NSCs continued to convert 5-FC to 5-FU during the entire 5-FC dosing interval.

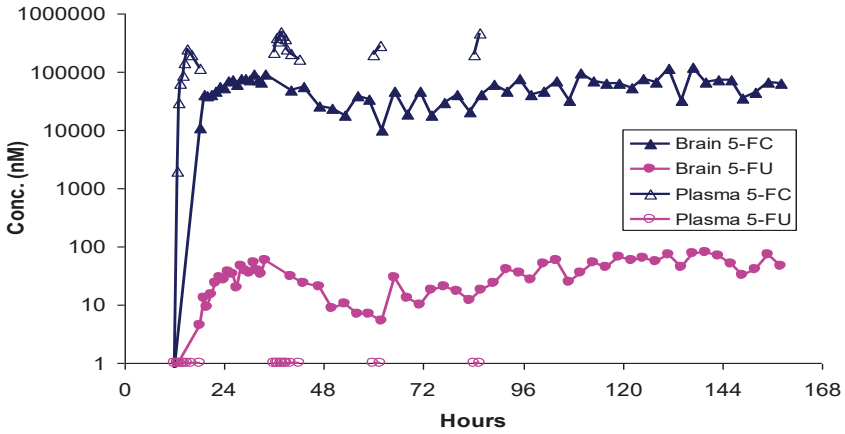


Figure 5. Intracerebral microdialysis data from a study patient demonstrate the ability of the NSCs to convert 5-FC to 5-FU in the brain throughout the 7-day course of oral 5-FC. In this particular patient there were no detectable levels of 5-FU in the plasma, indicating that all of the 5-FU measured in the brain came from NSC conversion of 5-FC.

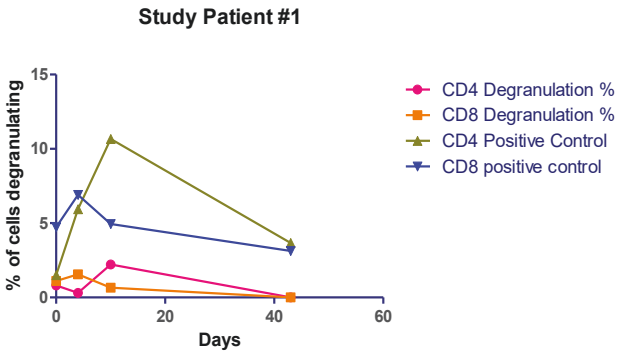
2.5.3 Immunologic correlative studies

2.5.3.1 *No evidence of NSC immunogenicity after first exposure*

2.5.3.1.1 Evaluation of T cell responses

Serial blood samples were obtained from patients prior to surgery and on days 4, 10, 32, and 60 to assess for T cell responses to the NSCs. Peripheral blood mononuclear cells (PBMCs) were evaluated by co-culture with the NSCs followed by flow cytometry assessment of degranulation (CD 107 mobilization). Results from patients in dose levels 1 and 2, as well as the first 4 patients in dose level 3, show no evidence of persistently elevated levels of degranulating T-helper (CD4+) or cytotoxic T lymphocyte (CD8+) responses to the NSCs (figure 6).

A.



B.

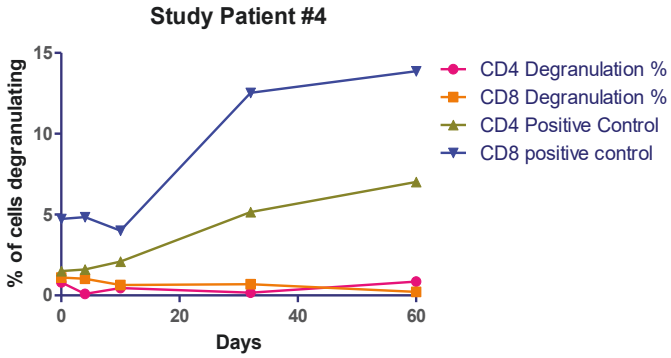


Figure 6. Evaluation of T cell responses: representative patient examples. A) CD4+ and CD8+ T cells from patient #1 fluctuated in the range of 0-2%, beginning and ending low. Natural killer (NK) cells were under 1%. **B)** CD4+ and CD8+ T cells from patient #4 had very low levels of degranulation (<2%) at all time points. NK cells in this patient's samples degranulated at 2-10%, but there was no evidence of increase over time.

2.5.3.1.2 Evaluation of humoral responses

Blood samples were obtained prior to surgery, and on days 32 and 60 to look for evidence of development of anti-NSC antibodies in patients' sera by flow cytometric evaluation of antibody binding to NSCs. Data from patients in dose levels 1 and 2, as well as the first 4 patients in dose level 3, show no detection of anti-NSC antibodies outside the normal range after first exposure (Portnow et al., 2013a).

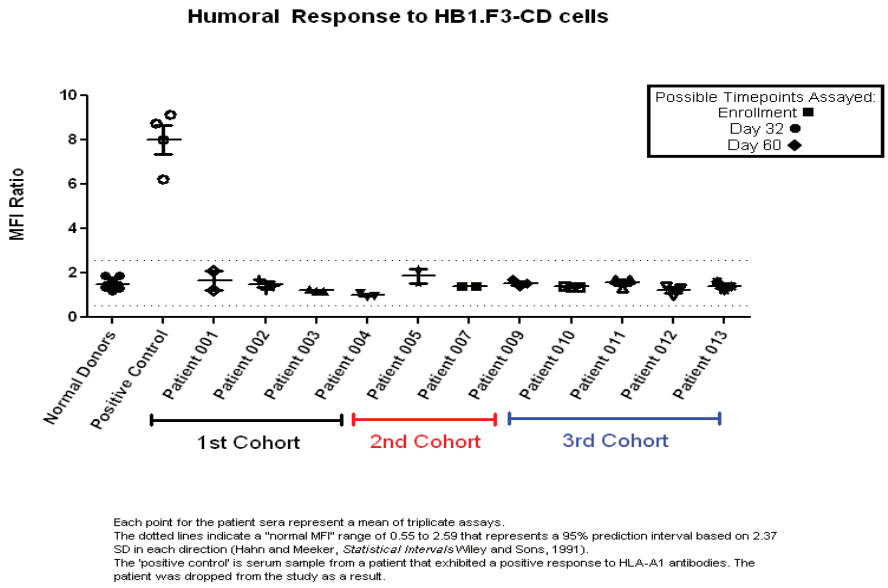


Figure 7. Results of anti-NSC antibody testing. Normal donor sera: n=10. Each data point for normal sera represents the mean of 5 independent experiments. Each data point for study patients' sera represents the mean of triplicate assays. The dotted lines indicate a "normal mean fluorescence intensity (MFI)" range of 0.55 to 2.59 that represents a 95% prediction interval based on a 2.3 standard deviation in each direction (Hahn and Meeker, *Statistical Intervals*, Wiley and Sons, 1991). The "positive control" is the mean of triplicate assays on serum from a patient who was determined to be ineligible for the study due to having an antibody to one of the HLA antigens (A1) on the NSCs.

2.5.3.2 *Evaluation of peripheral persistence of NSCs or replication competent retrovirus (RCR)*

Quantitative polymerase chain reaction (PCR) methods using v-*myc* primers was performed on patients' PBMCs prior to surgery, and on days 4,10, 32, and 60. All patient samples from dose levels 1, 2 and the first 4 patients in dose level 3 have been negative for detection of peripheral persistence of v-*myc* (i.e. NSCs), indicating no evidence of the NSCs traveling and persisting outside of the brain.

Testing for RCR was performed by analyzing patient DNA from whole blood by PCR for RCR-specific sequences prior to surgery, at 3 months, 6 months, and 1 year after study treatment, and then annually thereafter. RCR testing has been negative on all patient samples to date for up to 1 year out.

2.5.4 Assessment of NSC distribution in the human brain

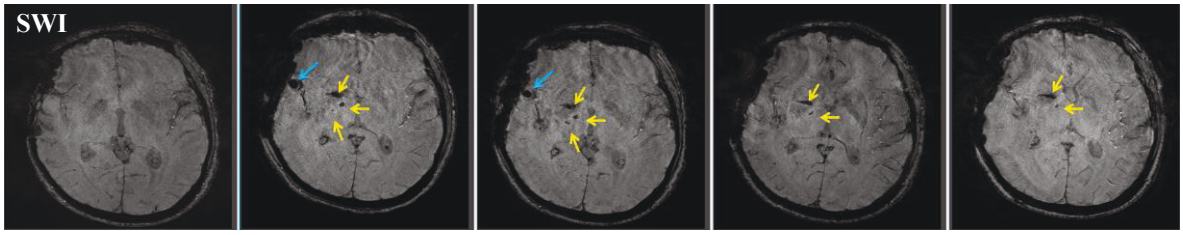
Based on the preclinical safety and imaging data in mice (section 2.4.5) an amendment to the study's IND (#14041) to allow administration of Feraheme-labeled NSCs was approved by the FDA while the pilot feasibility study was finishing dose level 3, and the last 3 patients to enroll in the study received Feraheme-labeled NSCs.

For these patients, the standard MRI brain imaging protocol used at City of Hope was modified by adding susceptibility weighted imaging (SWI) sequences (Figure 8, panel A) for visualizing the ultrasmall superparamagnetic iron oxide (USPIO) in the NSCs. In order to facilitate anatomical correlation, clinical imaging sequences were revised to be performed at a uniform slice thickness of 2mm with the 2nd and 3rd patient instead of the standard 4 mm thickness. The SWI sequences were acquired using the clinical SWI sequence with an echo time (TE) = 20 msec and research SWI multi-echo sequences with TEs ranging from 2.28 to 46.28 msec.. Post-processing methods included automatic alignment, stripping, and evaluation of multiple algorithms for identification and

quantification of USPIO, including use of SWI mapping (SWI-M, Zheng et al., 2013) [Figure 8, panel B].

Image analysis demonstrated a trend of decreasing USPIO susceptibility over serial brain MRIs. Figure 8 shows Feraheme-labeled NSCs detectable on SWI as areas of hypointense signal at the injection sites. Although the current resolution does not enable visualization of smaller amounts of Feraheme-labeled NSCs that may have moved away, signal density at the injection sites did decrease over time. It is unknown whether the continued decrease in signal density is due to NSCs migrating away from the injection sites, macrophages clearing the iron particles and NSCs, or possibly a combination of both. Early changes (i.e., from post-operative days 1 to 10) would likely be too soon to be explained by macrophage clearance (Muldoon et al., 2005). Preliminary post-processing analysis of imaging data from 2 of the 3 patients who received Feraheme-labeled NSCs shows a 20-30% decrease in the volume of NSCs at the injection sites between days 1 to 10.

A.



Pre-op Day 1 Day 3 Day 10 Day 31

B.

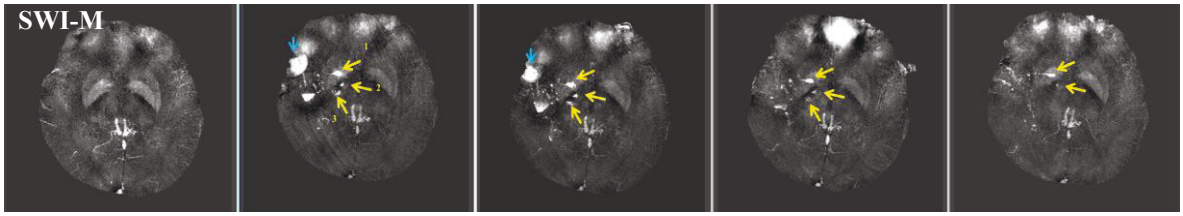


Figure 8. Magnetic resonance images of NSC injection sites that initially contained approximately 5 million USPIO-labeled NSCs each. The serial images demonstrate gradual decrease in the size of signal density during the 31-day period after intracranial administration of the USPIO-labeled NSCs. To facilitate visualization of Feraheme at the injection sites over time, a maximum intensity projection was performed across 5 individual SWI slice images. **Yellow arrows point to 3 NSC injection sites that decrease in intensity over time, indicating migration of viable NSCs away from injection sites.** Blue arrow indicates air bubble resolving over time.

No toxicity from the Feraheme-labeled NSCs has been observed in the 3 study patients. In this next clinical trial of CD-expressing NSCs in combination with 5-FC/leucovorin, we will refine our MRI protocol and optimize post-processing methods to improve our ability to visualize and quantify the Feraheme-labeled NSCs on MRI in order to non-invasively assess NSC migration.

2.5.5 Brain autopsy results

Autopsies were performed on the brains of 2 study patients, showing evidence of NSC migration and no development of secondary tumors.

The first patient was a woman who initially had a glioblastoma in her left occipital lobe, which was treated with resection, radiation and chemotherapy. She then developed recurrent tumor in her right frontal lobe more than 10 years later. She died 44 days after tumor resection and start of study treatment. She received a total of 10 million NSCs divided into 10 injections (1 million cells/100 ul per injection) into the wall of the resection cavity.

Nested PCR for v-*myc* to identify the presence or absence of NSCs was performed on 38 cassettes containing brain tissue from all regions of the brain. Five tissue block samples were positive for v-*myc*. Results showed no evidence of NSCs in the immediate proximity of the resection cavity. The v-*myc* positive areas were distant from the primary injection site and included tissue with scattered infiltrating tumor cells (right frontal parietal lateral and right posterior basal ganglia) and tissue devoid of obvious tumor cells, displaying moderate to marked anoxic ischemic changes (left parietal lateral, left parietal medial and right temporal posterior).

The second autopsy patient was a man who died 79 days after biopsy of tumor in the right parietal lobe and start of study treatment. He received 10 million NSCs in 1 ml, administered into the biopsy site. Detection of NSCs by PCR for v-*myc* was positive in three tissue block samples, 2 of which were located in the contralateral cerebral hemisphere (left frontal lobe/corpus callosum and left occipital lobe). Microscopic evaluation showed diffuse tumor in each of these blocks.

Since the NSCs were derived from female fetal tissue, XY FISH analysis was performed on 5 micron tissue samples from the same brain tissue blocks that were positive for v-*myc* and tumor. Results from the City of Hope Cytogenetics Core showed interspersed cells consistent with female NSCs among male host cells (Figure 9). These data support the conclusion that NSCs migrated away from the injection site to distant tumor foci.

Staining was then done for proliferating cell nuclear antigen (PCNA), a cell division marker. To determine the genotype of PCNA-positive cells in these tissue blocks, PCNA cells were mapped and sequential FISH was performed with XY probes. The slides were then scanned on a BioView Duet Image Analyzer to capture immunohistochemistry (IHC) images, and PCNA-positive cells were selected/mapped for targeted FISH analysis. FISH slides were realigned with IHC brightfield images and comparison images were acquired. One hundred PCNA-positive cells were classified for each block, and all cells were male. There was no evidence of actively dividing female cells in any of the tissue analyzed. These data support the conclusion that the identified remaining NSCs were not dividing and creating secondary tumors.

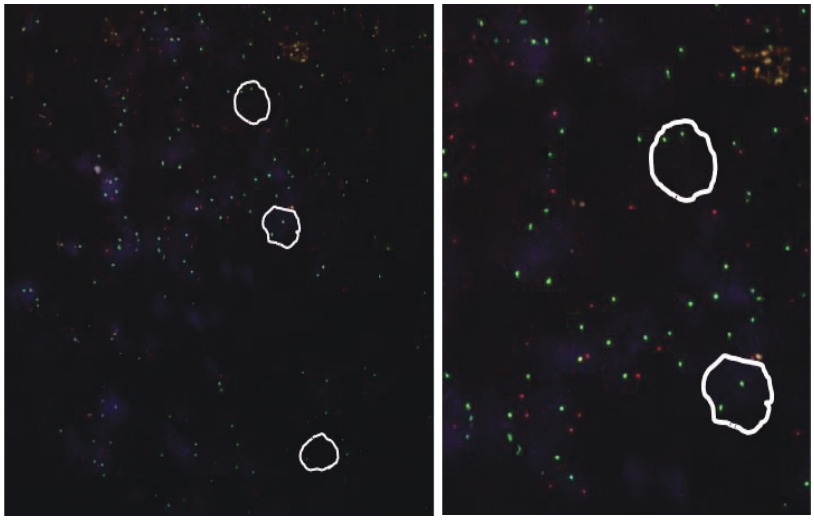


Figure 9. **Selected female cells (circled in white) interspersed among host male tumor cells.** XY FISH analysis (X gene green, Y gene red; 5 micron sections scanned at 63X) suggest the presence of single interspersed female NSCs among the male tumor cells. Individual cells identified by DAPI overlay with red and green channels at 63X.

2.6 Chemotherapy

2.6.1 5-FC

5-FC is primarily used as an antifungal agent and has activity against *Candida*, *Cryptococcus*, and *Aspergillus* species (Vermes et al., 2000a, 2000b). 5-FC is also an inactive prodrug that gets converted to 5-FU by the enzyme CD when it is taken up by those fungi that produce CD. Systemic toxicity from 5-FU is minimized because the host's cells do not convert significant amounts of 5-FC to 5-FU. 5-FC is a small molecule that is not highly protein-bound, and there are data showing that it penetrates well into cerebrospinal fluid (CSF), with CSF levels reaching approximately 80% of serum levels (American Medical Association, 1986). Major side effects of 5-FC include bone marrow suppression and hepatotoxicity, but it is generally well-tolerated in patients who have normal renal function. In this study, 5-FC will be administered at a dose of 37.5 mg/kg every 6 hours for 7 days following each dose of NSCs (i.e. on days 4-10 and 18-24 of every 28 day cycle). This dose is at the high end of range of standard daily dosing when 5-FC is used to treat fungal infections, and this dose was well-tolerated by patients in our first study of CD-expressing NSCs and oral 5-FC.

2.6.2 5-FU

5-FU is classified as an antimetabolite chemotherapeutic agent. It is a synthetic pyrimidine analog that interferes with DNA synthesis by blocking the thymidylate synthase conversion of deoxyuridylic acid to thymidylic acid, resulting in selective killing of surrounding tumor cells that are dividing. It is effective against a variety of solid tumors, including gastrointestinal (GI) cancers and breast cancer.

In contrast to 5-FC, 5-FU does not efficiently cross the BBB. Results of two phase II studies of 5-FU in patients with recurrent primary brain tumors showed 5-FU had only minimal activity in this setting (Stewart et al., 1995; Cascino et al., 1996). Combining data from these studies, among the 44 recurrent glioma patients who were treated with 5-FU and leucovorin, there were only 4 responses. However, *in vitro* cytotoxicity studies showed that glioma cells are as sensitive to 5-FU as GI tumor cells (Miller et al., 2002), with IC_{50}'s in the micromolar range, which is considered the general threshold for 5-FU cytotoxicity. Thus, the lack of efficacy seen in the brain tumor clinical trials of 5-FU is likely due to the limited ability of systemically-administered 5-FU to cross BBB and achieve therapeutic levels in the brain rather than an inherent resistance of gliomas to 5-FU.

A randomized phase II study of 95 patients with newly-diagnosed high-grade gliomas who were treated with locally-delivered 5-FU-releasing microspheres plus radiation versus radiation alone, found an overall survival of 15.2 months in the 5-FU-releasing microspheres plus radiation arm compared with 13.5 months in the control arm of RT alone (Menei et al., 2005). This trend toward improvement in survival with the addition of the locally-delivered 5-FU-releasing microspheres was not statistically significant; however, the study was neither designed nor sufficiently powered to demonstrate a survival difference between the treatment groups. One issue raised by the results of this study is that local delivery of 5-FU may be more effective against brain tumors than systemic administration, and mechanisms for local delivery of 5-FU, e.g., *via* microspheres, implantable polymers, or NSCs, should be investigated further.

The NSC delivery approach that will be studied in this protocol has the advantage of tumor localization, potentially reaching tumor cells that have infiltrated beyond the immediate vicinity of the resection cavity. Following 5-FC administration, HB1.F3.CD NSCs are expected to release 5-FU within close proximity to tumor cells, resulting in a localized concentration of 5-FU that could potentially be higher than that achieved by passive diffusion of 5-FU from microspheres.

2.6.3 Leucovorin (folinic acid)

Leucovorin is a reduced folic acid. It is used in combination with 5-FU in the treatment of cancers of the GI tract because it enhances the effects of 5-FU (Advanced Colorectal Cancer Meta-Analysis Project, 1992) by stabilizing the binding of 5-FU's metabolite (fluorodeoxyuridylic acid) to thymidylate synthase, an enzyme important in DNA repair and replication. Leucovorin readily crosses the blood-CSF barrier (Thyss et al., 1989) and also is used for management of cerebral folate deficiency. There are data in the GI cancer literature showing that the combination of the oral 5-FU prodrug uracil-tegafur and oral leucovorin, given in a dose of 75-90 mg/day or in divided doses, is at least as effective as infusional 5-FU/leucovorin (Lembersky et al., 2006). For this phase I clinical trial, oral leucovorin will be given

along with the 5-FC. Since oral absorption of leucovorin is saturable at doses above 25 mg, study patients will take divided doses of leucovorin: 25 mg every 6 hours with 5-FC for 7 days following each dose of NSCs (i.e. on days 4-10 and 18-24 of every 28 day cycle).

2.7 Use of a Rickham Reservoir/Catheter System for Repeat Intracranial Administration of NSCs

In this next study we will administer repeat infusions of NSCs every 2 weeks using an indwelling Rickham reservoir/catheter system (a Rickham). The catheter will be inserted into the tumor mass at the time of tumor resection or biopsy and connected proximally to a 6 mm diameter reservoir (6 mm diameter).

The Brain Tumor Program at City of Hope has significant experience with this delivery system. In a recently completed immunotherapy study of genetically-modified T cells, we successfully infused repeat intracranial doses of these T cells during a 2 week period via a Rickham placed within tumor tissue (Badie, unpublished data).

In order to determine whether it would be feasible to use a Rickham to deliver repeat doses of NSCs intracranially, an experiment was performed to assess the viability and binding of the HB1.F3.CD NSCs when administered through a Rickham: 9.55 x 10^7 NSCs were suspended in 1.94 ml of artificial CSF (to achieve a concentration equivalent to the highest dose of NSCs that will be administered in the phase I study: 1.5 x 10^8/2.25 mL). The NSCs were then administered at a flow rate of 0.5 ml/hour through extension tubing attached to a Gripper Plus needle that was inserted into the reservoir of a Rickham system and collected over 4 hours followed by a 1 mL flush of artificial CSF over 2 hours.

Table 2 shows that there was no loss of NSCs from binding to tubing, and the viability of the NSCs remained constant over 6 hours of delivery. This experiment demonstrated that our planned system for delivering repeat doses of NSCs intracranially works well, resulting in 100% recovery of the NSCs through the Rickham after flushing, and that NSC viability does not decrease for at least 6 hours after thawing and administering through a Rickham.

Table 2. Summary of HB1.F3.CD NSC counts and viability before and at the end of enhanced delivery.

	A	B	C	AxC
Sample	**Viable cells/mL**	**Viability (%)**	**Total Volume collected (mL)**	**Total Viable Cells**
Pre-CED	4.92E+07	75.3	1.94	**9.55E+07**
1 hr	2.86E+07	75.7	0.57	1.63E+07
2hr	3.29E+07	75.2	1	3.29E+07
3hr	3.42E+07	76.7	1.59	5.44E+07
4hr	3.03E+07	75.8	1.961	5.94E+07
5hr	2.61E+07	78.8	2.478	6.47E+07
6hr	3.31E+07	79.9	2.95	**9.77E+07**

2.8 Intracerebral Microdialysis for Performing a Pharmacodynamic Assessment by Measuring NSC-Mediated Conversion of 5-FU and 5-FC in the Brain

Microdialysis is a technique for continuously analyzing the concentration of a drug or biomolecule in the extracellular fluid (ECF) of body tissues, without significantly disturbing tissue function. This technique, first developed in the 1970s, consists of implanting into a body tissue a catheter that contains a semi-permeable membrane at its tip. The dialysis membrane acts as an artificial capillary, so that when perfusion fluid is slowly, continuously pumped through the microdialysis catheter, diffusion of molecules occurs down their concentration gradients as the ECF equilibrates with the perfusion fluid. The dialysate, i.e. solution that exits the probe, is then collected at regular intervals for analysis. The dialysate will contain a representative proportion of the molecule or drug that is in the ECF. The concentration of drug in the dialysate is not the true concentration in the tissue because full equilibration does not take place across the dialysis membrane when the flow of perfusion fluid is constant. However, the fraction of drug that is recovered in the dialysate is an indirect measurement of the free drug concentration in the interstitium.

Microdialysis has mainly been applied clinically to the study of head trauma (Goodman et al. 1999; Vespa et al., 2003,2005, 2006) subarachnoid hemorrhage (Staub et al., 2000; Sarrafzadeh et al., 2002; Kett-White et al., 2003) and epilepsy (During et al., 1993; Lindberger et al., 2002; Scheyer et al., 1994). In these settings, intracerebral microdialysis catheters are used to monitor metabolic changes (for example, varying levels of lactate, glucose, and glutamic acid) in order to detect possible complications and to evaluate the effects of a therapeutic intervention. Intracerebral microdialysis is also a suitable method for measuring levels of chemotherapy in the brain (De Lange et al., 2000; Blakeley and Portnow, 2010) because microdialysis catheters can serially sample free drug concentrations in the peritumoral cerebral cortex or within the brain tumor itself (Bergenheim et al., 2005; Blakeley et al., 2009).

The Brain Tumor Program at City of Hope has developed expertise in performing intracerebral microdialysis. Having now placed microdialysis catheters in nearly 40 patients with brain tumors (Portnow et al., 2009, 2010, 2013a, 2013b), we have the largest experience in the United States with the applying this technique to perform neuropharmacokinetic and neuropharmacodynamic assessments of chemotherapy agents.

Unique and compelling data about new anticancer drugs can be obtained *in vivo* from patients with brain tumors using intracerebral microdialysis. Unlike studies in which the tumor is removed in order to measure intratumoral drug concentrations at one time point, intracerebral microdialysis can not only determine the time course of changes in drug concentrations in the brain; since tumor is left in place, it also allows observation of the clinical outcomes associated with the drug concentrations measured *in vivo*.

2.8.1 Intracerebral microdialysis catheters

The 70 Brain MD Catheter (M Dialysis, Solna, Sweden) has a semi-permeable membrane with a molecular weight cut-off of 20,000 daltons, and has received 510(k) clearance from the Food and Drug Administration as a Cerebral Tissue Monitoring System for adjunct use in monitoring brain trauma patients at risk for ischemia. This microdialysis catheter is smaller in caliber than the catheters that are typically used for performing ventriculostomies and monitoring intracranial pressure. With the use of image guidance, the catheter can be safely and accurately placed in brain interstitium. Stereotactic placement of intracerebral microdialysis catheters has been shown to be a safe procedure, with bleeding and infectious complication rates similar to those seen with stereotactic biopsy, craniotomy, and other types of intracerebral monitoring (Benjamin et al., 2004). In a study of intracerebral microdialysis catheter use in neurointensive care patients (Poca et al., 2006), 4 of 97 patients (3%) were found to have a small (≤ 1mL) collection of blood around the catheter on follow-up CT scans. None of these bleeds were clinically significant, and no catheter-related infections were reported.

Each catheter has a gold tip that makes it visible on CT scan. Thus, confirmation of correct of the catheters can readily be obtained with a non-contrast CT scan of the brain. These catheters have safely

remained in patients for 1-2 weeks (Kett-White et al., 2003). Sample collections are done while patients are awake and mobile.

2.8.2 *In Vitro* Microdialysis Recovery Data for 5-FU and 5-FC

Prior to performing intracerebral microdialysis in study patients, *in vitro* studies were done to estimate the fractional recovery of 5-FU and 5-FC at a given flow rate. Slower perfusion rates produce a higher fractional recovery (i.e. a concentration of drug in the dialysate that is close to the true interstitial drug concentration), but a longer sample collection interval is needed. The *in vitro* assessments were performed with the same microdialysis equipment that will be used in this clinical trial. A 70 Brain MD Catheter was placed into a reservoir containing a solution with a known concentration 5-FU and 5-FC. The inlet tubing of the catheter was attached to a syringe containing artificial CSF, and the syringe was placed in a variable flow rate pump (107 MD Pump). Artificial CSF perfused through the microdialysis catheter at different flow rates (0.5 – 5 µl/min) and serial samples of dialysate were collected. We also performed an experiment to determine how much 5-FU and 5-FC bind to the tubing of the microdialysis equipment. A solution of known concentrations of 5-FU and 5-FC perfused a 70 Brain MD Catheter, while it sat in a reservoir containing a solution of the same concentrations of 5-FU and 5-FC.

The City of Hope Analytical Pharmacology Core Facility (APCF) developed a quantitative assay for 5-FU and 5-FC, and the concentration of these drugs in the dialysate samples were determined by liquid chromatography/tandem mass spectrometry (LC/MS/MS). At a flow rate of 1 µl/min, the fractional recoveries of 5-FC and 5-FU were 92 ±.2.1% and 91 ± 1.5%, respectively. Binding of either drug to the catheter tubing was negligible (< 5%).

2.9 Overview of Proposed Study

Based on the encouraging safety data from our first-in-human pilot feasibility study of 1 cycle of intracranially administered CD-expressing NSCs and oral 5-FC in patients with recurrent high-grade gliomas, we will now perform a phase I clinical trial to define the phase II recommended doses of CD-expressing NSCs given in combination with 5-FC and leucovorin and determine the feasibility of administering repeat cycles of study treatment in patients with recurrent high-grade gliomas. A standard "3 + 3" dose escalation schema will be used, and 4 dose levels of study treatment will be tested. The starting number of NSCs will be 5×10^7, which is a dose that was well-tolerated by patients in the first study. Due to volume limitations for intracerebral administration, the highest dose of NSCs to be tested will be 1.5×10^8. The 5-FC will not be dose-escalated, but leucovorin, which potentiates the efficacy of 5-FU, will be added after dose escalation of the NSCs is completed.

Initially, the NSCs will be injected intracranially on cycle 1, day 1 when study patients undergo tumor resection or biopsy. Afterwards, there will be a waiting period of 3 days to allow time for the NSCs to distribute among foci of tumor. Patients will then take a 7 day course of oral 5-FC (dose level 4 patients will also take leucovorin). Repeat doses of NSCs will be administered through a Rickham placed at the time of surgery. The length of a treatment cycle will be 28 days. NSCs will be given on days 1 and 15 followed by 5-FC and leucovorin (dose level 4 patients) on days 4-10 and 18-24. Brain MRIs will be performed at the end of every even cycle of study treatment to evaluate response.

Immunologic correlative studies will be performed to assess for the possible development of NSC immunogenicity after repeat exposure to the allogeneic NSCs, and we will measure conversion of 5-FC to 5-FU by the NSCs. Once the maximum tolerated dose (MTD) of the combination therapy is predictedor, if the MTD is not met, the maximum feasible dose (MFD), patients in this cohort will undergo intracerebral microdialysis during the first part of cycle 1 to quantify NSC-mediated conversion of 5-FC to 5-FU at the MTD/MFD level.

3.0 Patient Eligibility

3.1 Inclusion Criteria

3.1.1 Disease Status

3.1.1.1 Patient has had a prior, histologically-confirmed, diagnosis of a grade III or IV glioma (including glioblastoma, anaplastic astrocytoma, gliosarcoma, anaplastic oligodendroglioma, or anaplastic oligoastrocytoma), or has a prior, histologically-confirmed, diagnosis of a grade II glioma and now has radiographic findings consistent with a high-grade glioma (grade III or IV).

3.1.1.2 Imaging studies show evidence of recurrent, supratentorial tumor(s). The presence of infratentorial tumor is allowed as long as the patient also has supratentorial disease that is amenable to resection or biopsy.

3.1.1.3 Patient's high-grade glioma has recurred or progressed after prior treatment with brain radiation and temozolomide.

3.1.2 Age Criteria, Performance Status and Life Expectancy

3.1.2.1 Patient must be at least 18 years old.

3.1.2.2 Patient has a Karnofsky Performance Status of ≥ 70%.

3.1.2.3 Patient has a life expectancy of ≥ 3 months.

3.1.3 Child Bearing Potential

The effects of this treatment on a developing fetus are unknown. Therefore, female patients of childbearing potential and sexually-active male patients must agree to use an effective method of contraception while participating in this study. Women of childbearing potential must have a negative pregnancy test ≤2 weeks prior to registration.

3.1.4 Protocol-Specific Criteria

3.1.4.1 The patient must be in need of a craniotomy for tumor resection or a stereotactic brain biopsy for the purpose of diagnosis or differentiating between tumor progression. versus treatment-induced effects following radiation therapy ± chemotherapy.

3.1.4.2 Based on the neurosurgeon's judgment, there is no anticipated physical connection between the post-resection tumor cavity and the cerebral ventricles.

3.1.4.3 Patient must have adequate bone marrow function (defined as an absolute neutrophil count (ANC) of ≥1500 cells/mm^3 and platelet count ≥100,000 cells/mm^3, adequate liver function with total bilirubin ≤2.0 mg/dl and AST (SGOT) ≤4 times the institutional upper limit of normal, and a serum creatinine ≤ the institutional upper limit of normal.

3.1.5 Prior Therapy

There is no limit to the number of prior therapies.

3.1.6 Informed Consent/Assent

All subjects must have the ability to understand and the willingness to sign a written informed consent.

3.2 Exclusion Criteria

3.2.1 Patient has anti-HLA antibodies specific for HLA antigens expressed by the HB1.F3.CD NSCs.

3.2.2 Patient has not recovered from any toxicity of prior therapies. An interval of

3.2.2.1 At least 6 weeks must have elapsed since taking a nitrosourea-containing chemotherapy regimen.

3.2.2.2 At least 4 weeks since completing a non-nitrosourea-containing cytotoxic chemotherapy regimen (except temozolomide: only an interval of 23 days is required from the last dose administered when patient has been recently treated with the standard temozolomide regimen of daily for 5 days, repeated every 28 days).

3.2.2.3 At least 2 weeks from taking the last dose of a targeted agent.

3.2.2.4 At least 4 weeks from the last dose of bevacizumab.

3.2.3 Patient is unable to undergo an MRI.

3.2.4 Patient is allergic to 5-FC, leucovorin, or 5-FU.

3.2.5 Patient has chronic or active viral infections of the CNS.

3.2.6 Patient has a coagulopathy or bleeding disorder.

3.2.7 Patient has an uncontrolled illness including ongoing or active infection.

3.2.8 Patient is receiving any other investigational agents, or concurrent biological, chemotherapy, or radiation therapy.

3.2.9 Patient has had prior therapy with neural stem cells.

3.2.10 Patient is pregnant or breast feeding. Pregnant women are excluded from this study because 5-FC and leucovorin are agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with 5-FC and leucovorin, breastfeeding should be discontinued if the mother is participating in this study.

3.2.11 Patient has another active malignancy.

3.2.12 Non-compliance

A patient has a serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the safety monitoring requirements and completion of treatment according to this protocol.

3.3 Inclusion Criteria for Proceeding to Treatment with 5-FC

3.3.1 Patient must be tolerating oral intake.

3.3.2 A patient's daily total dose of dexamethasone must be ≤16 mg by day 4.

3.4 Inclusion of Women and Minorities

The study is open anyone regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue approximately 18-21 subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

4.0 Screening and Registration Procedures

4.1 Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. Reference is made to Section 10.0 – Study Calendar.

4.2 Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record.

4.3 Registration Requirements/Process

Registration of participants is to be via completion of the following steps:

4.3.1 Prospective participants must complete the informed consent process, including a signed informed consent, prior to proceeding to study screening.

4.3.2 Screening procedures and windows are detailed in Section 10 and Table 10, Study Activity Calendar.

4.3.3 A study specific eligibility checklist developed by the Clinical Trials Office at the City of Hope will be used to facilitate confirmation that a prospective participant meets all eligibility criteria.

4.3.4 Once all the pre-study requirements have been fulfilled, including a completed eligibility checklist, the study coordinator can register the eligible patient into MIDAS.

4.3.5 Patients failing to meet all protocol eligibility criteria, including informed consent, may not be registered for the trial.

4.4 Dose Level Assignment

Please see section 5.7.

5.0 Treatment Program

5.1 Treatment Overview

Dose Escalation Schema

Dose Level	Neural Stem Cells (i.c. on days 1 & 15)	5-FC (mg/kg q 6h p.o. on days 4-10 & 18-24)	Leucovorin (mg q 6h p.o. on days 4-10 & 18-24)	Initial Enrollment	If 1 DLT, additional enrollment
-1[a]	3.75×10^7	28	---	3	3
1	5×10^7	37.5	---	3	3
2	1×10^8	37.5	---	3	3
3	1.5×10^8	37.5	---	3	3
4[b,c]	Highest tolerated dose combination of 5-FC and NSCs		25	3	3

[a]If a DLT is observed in 2 or more patients at dose level 1, then a de-escalation dose**, dose level -1** will be tested. With dose level -1, the doses of NSCs and 5-FC will both be reduced by 25%; however, if the DLT can clearly be attributed only to 5-FC, then only the dose of 5-FC will be reduced by 25% (likewise for the NSCs if the DLT is clearly attributable to only the NSCs) for dose level -1.

[b]If the addition of leucovorin to the highest tolerated dose combination of 5-FC and NSC requires a dose de-escalation, then leucovorin will be discontinued and the MTD/MFD will be the highest tolerated dose combination of 5-FC and NSCs without leucovorin. A total of 6 patients will be treated at the maximum tolerated dose (MTD), or if the MTD is not reached, at the maximum feasible dose (MFD).

[c]Dose level 4 patients will undergo intracerebral microdialysis to measure NSC-mediated conversion of 5-FC to 5-FU.

Cycle 1[a]

Day 1

- Tumor resection or biopsy is performed.
- HB1.F3.CD NSCs are administered intracranially.
- A Rickham catheter is placed.
- Dose level 4 patients only: a microdialysis catheter is placed.

Days 4-10

- Patients take 5-FC 37.5 mg/kg p.o. q 6h.
- Patients on dose level 4 also take leucovorin 25 mg p.o. q 6h.
- Dose level patients only: serial dialysate and blood samples collected.

Days 15

- HB1.F3.CD NSCs administered intracranially via the Rickham catheter.
- Blood drawn for immunologic correlative studies.

Days 18-24

- Patients take 5-FC 37.5 mg p.o. q 6h.
- Patients on dose level 4 also take leucovorin 25 mg p.o. q 6h.

a The length of a treatment cycle is 28 days. After cycle 1, the NSCs will be administered via the Rickham catheter on days 1 and 15 of a cycle. The 5-FC (and leucovorin for dose level 4 patients) will continue to be administered on days 4-10 and 18-24. Blood draws for immunologic correlative studies will be done on days 1 and 15 of cycle 2, and then only on day 15 of subsequent cycles. A brain MRI will be performed after every 2 cycles of treatment.

5.2 Treatment Plan for Cycle 1

5.2.1 Pre-surgery, post-registration: Pre-surgical planning MRI A pre-surgical planning post-registration MRI may be performed if indicated by the neuro-surgeon.

5.2.2 Cycle 1 Day 1: Intracerebral administration of NSCs during surgery

All study patients must have confirmation of recurrent tumor documented on frozen section at the time of surgery before proceeding with direct intracerebral injection of the NSCs.

The maximum concentration of NSCs is 6.7 x 10^7/ml (or 1 x 10^8/1.5 ml).

5.2.2.1 Patients who undergo a craniotomy for tumor removal:

When the study neurosurgeon has finished resecting tumor, he will directly inject CD-expressing NSCs into the brain tissue of the resection cavity.

Dose level -1 (if a decision is made to dose reduce the NSCs): 3.75 x 10^7 NSC will be suspended in 0.6 ml of 2% human serum albumin (HSA)/artificial CSF (Perfusion Fluid CNS; ref no. P000151, M Dialysis, Solna, Sweden).

Dose level 1: 5 x 10^7 NSCs will be suspended in 0.75 ml of 2% HSA/artificial CSF.

Dose level 2: 1 x 10^8 NSCs will be suspended in 1.5 ml of 2% HSA/artificial CSF.

Dose level 3: 1.5 x 10^8 NSCs will be suspended in 2.25 ml of 2% HSA/artificial CSF.

Dose level 4: As per dose level 3.

The total dose of NSCs will be injected in divided 100-150 µl volumes, as evenly spaced as possible, throughout the wall of the resection cavity; administering them only into areas that are surgically safe and feasible. The NSCs will be injected 2.5 cm deep and tracked up to approximately 1 cm deep, (slowly injecting as the needle is withdrawn, so as to distribute the NSCs from 2.5 cm – 1 cm depth).

After the NSCs have been administered, a 9.5 mm Rickham reservoir attached to an open-ended catheter (a Rickham) will be placed in residual tumor tissue or peritumoral tissue to deliver subsequent doses of NSCs.

A post-operative MRI of the brain will be done on day 2 to serve as a baseline scan with which to compare subsequent MRIs.

5.2.2.2 Patients who undergo a stereotactic brain biopsy:

After performing the tumor biopsy, the study neurosurgeon will place a Rickham into brain tissue through the biopsy track.

Dose level -1 (if a decision is made to dose reduce the NSCs): 3.75 x 10^7 NSCs in 0.6 ml of 2% HSA/artificial CSF will be manually injected in the operating room over 10 minutes through the Rickham, followed by a 0.3 ml artificial CSF flush.

Dose level 1: 5 x 10^7 NSCs in 0.75 ml of 2% HSA/artificial CSF will be manually injected in the operating room over 10 minutes through the Rickham, followed by a 0.3 mL artificial CSF flush.

Dose level 2: 1 x 10^8 NSCs will be administered as follows: 5 x 10^7 NSCs in 0.75 mL of 2% HSA/artificial CSF will be manually injected in the operating room over 10 minutes through the Rickham, followed by a 0.3 mL artificial CSF flush. After the patient is out of Recovery and in a hospital room, the remainder of the dose, 5 x 10^7 NSCs in 0.75 ml 2% HSA/artificial CSF,

will be administered via the Rickham followed by a 1 ml flush of artificial CSF. The rate of the infusion will be 0.5 ml/hr, and the total length of time for infusing the NSCs and the flush will be approximately 2 hours.

Dose level 3 1.5 x 10^8 NSCs will be administered as follows: 6.7 x 10^7 NSCs in 1 ml of 2% HSA/artificial CSF will be manually injected in the operating room over 10 minutes through the Rickham, followed by a 0.3 ml artificial CSF flush.

After the patient is out of Recovery and in a hospital room, the remainder of the dose, 8.3 x 10^7 NSCs in 1.25 mL of 2% HSA/artificial CSF, will be administered through the Rickham followed by a 1 ml flush of artificial CSF. The rate of the infusion will be 0.5 ml/hr, and the total length of time for infusing the NSCs and the flush will be approximately 3 hours.

Dose level 4: As per dose level 3.

A non-contrast CT scan of the brain will be performed after surgery. Patients will have a brain MRI performed on post-operative day 2.

5.2.1.3 Perioperative prophylaxis

Prophylactic anti-convulsants will be administered to study patients. Those who have never had a seizure will continue taking anti-seizure medication for at least 4 weeks after craniotomy or biopsy.

Perioperative antibiotics, corticosteroids, and deep vein thrombosis prophylaxis will be used according to the standard of care for the surgical procedure.

5.2.3 Cycle 1 Day 2: MRI

All patients will undergo an MRI on day 2 (the day after surgery). This MRI will serve as the baseline MRI.

5.2.4 Cycle 1 Days 4-10: Administration of 5-FC (and leucovorin for dose level 4 patients)

If the patient has recovered well from any immediate post-operative effects, is tolerating oral intake, and requiring less than 16 mg/day of dexamethasone by day 4, then s/he will take oral 5-FC, 37.5 mg/kg every 6 hours (dose level 4 and expansion cohort patients will also take oral leucovorin, 25 mg every 6 hours) on days 4-10.

The patient will be discharged from the hospital when medically ready and may proceed with or finish taking the 7-day course of 5-FC (and leucovorin if on dose level 4) as an outpatient.

5.2.4.1 Anti-emetic medication

If nausea develops, prochorperazine 10 mg p.o. every 6 hours (or a similar strength anti-emetic) will be used as needed.

5.2.5 Cycle 1 Day 15: Intracerebral administration of NSCs via the Rickham

Please see section 6.3 for criteria that a patient must meet in order to proceed with a second dose of NSCs on day 15.

The NSCs will be administered in the outpatient setting through the Rickham at a rate of 0.5 ml/hr.

The maximum concentration of NSCs is 6.7 x 10^7/ml (or 1 x 10^8/1.5 ml).

Dose level -1 (if a decision is made to dose reduce the NSCs): 3.75 x 10^7 NSCs will be suspended in 0.6 ml of 2% HSA/artificial CSF, and the infusion tubing will be primed with the NSC suspension before beginning infusion of the NSCs. A 1 ml artificial CSF flush will follow

the infusion of NSCs. The total length of time for infusing the NSCs and flush will be a little over 3 hours.

Dose level 1: 5 x 10^7 NSCs will be suspended in 0.75 ml of 2% HSA/artificial CSF, and the infusion tubing will be primed with the NSC suspension before beginning infusionof the NSCs. A 1 ml artificial CSF flush will follow the infusion of NSCs. The total length of time for infusing the NSCs and flush will be approximately 2 hours.

Dose level 2: 1 x 10^8 NSCs will be suspended in 1.5 ml of 2% HSA/artificial CSF, and the infusion tubing will be primed wth the NSC suspension before beginning infusion of the NSCs. A 1 ml artificial CSF flush will follow the infusion of NSCs. The total length of time for infusing the NSCs and flush will be approximately 3 ½ hours.

Dose level 3:1.5 x 10^8 NSCs will be suspended in 2.25 ml of 2% HSA/artificial CSF, and the infusion tubing will be primed wth the NSC suspension before beginning infusion of the NSCs. A 1 ml artificial CSF flush will follow the infusion of NSCs. The total length of time for infusing the NSCs and flush will be approximatelyl 5 hours.

Dose level 4: As per dose level 3.

5.2.6 Cycle 1 Day 15: Immunologic correlative studies

While data from the first study of these CD-expressing NSCs showed no evidence of NSC immunogenicity after first exposure, since patients in this study will receive more than 1 dose of NSCs, we will monitor for development of immune responses after repeat exposure to the NSCs by taking samples of blood from study patients prior to the start of study treatment, on day 15 of cycle 1, as well as during subsequent treatment cycles (please see section 5.3.1 for further details) to assess for the development of anti-NSC T-cell and/or antibody responses and to look for peripheral persistence of the NSCs (please see section 9.1 for further details).

Additionally, aliquots of serum will be saved from the blood samples drawn for immunologiccorrelative studies prior to start of study treatment and on day 15 of cycle 1 to serve as a baseline comparison if a patient were to develop signs/symptoms of a possible immune response to the NSCs during a subsequent treatment cycle. These serum samples, along with additional serum samples drawn at the time the patient develops clinical findings suspicious for an immune response, will be analyzed via Luminex cytokine panels (please see sections 6.3.3.2, 9.1.4, and 9.1.5.4).

5.2.7 Cycle 1 Days 18-24: Administration of 5-FC (and leucovorin for dose level 4 patients)

Dosing of 5-FC (and possibly leucovorin) as per the dose level the patient is on (see the dose escalation schema table in section 5.1)

Prochorperazine 10 mg p.o. every 6 hours (or a similar strength anti-emetic) will be used as needed.

Study patients will be evaluated by their treating physicians in clinic on day 22 (as per section 10.0 Study Calendar).

5.3 Repeat Cycles of Study Treatment

A treatment cycle is defined as 28 days.

Repeat cycles of study treatment will be administered as long as there is no evidence of progressive disease, and treatment with the NSCs and 5-FC (and leucovorin, as indicated) is well tolerated.

Please see section 6.3 for criteria that the patient must meet in order to or proceed with study treatment on days 1 and 15 of a cycle, as well as instructions for dose modifications if needed with repeat doses of study treatment.

There will be no intra-patient dose escalation of NSCs, 5-FC, or leucovorin.

The NSCs will be administered in the outpatient setting via a Rickham on days 1 and 15 of a cycle, as per section 5.2.4.

5-FC (and leucovorin, as indicated) will be taken by the patient on days 4-10 and days 18-24 as per the dose level the patient is in (see the dose escalation schema table in section 5.1).

Prochorperazine 10 mg p.o every 6 hours (or a similar strength anti-emetic) will be used as needed.

5.3.1 Immunologic correlative studies

In addition to the blood samples drawn prior to the start of study treatment and on cycle 1 day 15, **blood samples will be drawn on days 1 and 15 of cycle 2 and then on day 15 of subsequent cycles** for continued assessment of possible development of anti-NSC T cell and/or antibody responses (please see section 9.1 for further details).

A decision to stop study treatment will be based on development of clinical signs/symptoms of an immune response rather than a particular level of anti-NSC T cells or antibodies (please see section 6.3.3 for further details).

5.3.2 Assessment of response

A brain MRI will be performed at the end of even cycles of study treatment (Day 28 (-3 days) of cycle 2, 4, 6 etc.).

5.4 Intracerebral Microdialysis (Dose Level 4 Patients Only)

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5.4.1 Cycle 1 Day 1: Placement of the microdialysis catheters

Before a catheter is placed into brain it will be flushed with artificial CSF. The catheter will be tunneled subcutaneously and then inserted into the brain tissue adjacent to the tumor resection margin. If feasible, a second catheter will be added. The wound will then be closed in standard fashion, anchoring the catheter to the scalp with suture and sterile dressings.

5.4.1.1 Patients who undergo a craniotomy for tumor removal

Once the study neurosurgeon has finished resecting as much tumor as safely possible, he will insert a 70 Brain MD Catheter (membrane length 10 mm; shaft length 100 mm, ref. no. P000050, M Dialysis, Solna, Sweden) in residual tumor or within 5-15 mm of the resection cavity. If feasible a second catheter will be added to a different geographic location.

The NSCs will then be injected. **At the margin of the catheter location, 2-4 of the 100-150 μl aliquots of NSCs will be injected.** The total dose of NSCs, (1.5×10^8)— will be administered as per section 5.2.2.1.

After the NSCs have been administered, a Rickham will be placed in residual tumor or peritumoral tissue to deliver subsequent doses of NSCs.

5.4.1.2 Patients who undergo a stereotactic brain biopsy

After performing the tumor biopsy, a 70 Brain MD Catheter will be passively introduced into the injection site through the biopsy track. If feasible, a second MD catheter will be added to a different geographic location. The study neurosurgeon will then place the Rickham through the same biopsy track, and the dose of NSCs (1.5×10^8) will then be administered through the Rickham catheter as per section 5.2..2.2

5.4.2 Cycle 1 Day 1: Radiographic confirmation of catheter position

A CT scan without contrast will be obtained as soon as possible post-operatively to determine if the microdialysis catheter was placed correctly.

If the CT scan shows poor positioning of a catheter, such as placement in a ventricle or within the surgical cavity but not in brain tissue, it will be removed.

5.4.3 Cycle 1 Day 1: Microdialysis setup

Once the CT scan confirms correct placement of the microdialysis catheter, the inlet tubing of the catheter will be connected to a 2.5 mL syringe (106 Pump Syringe, ref. no. 8010191, M Dialysis, Solna, Sweden) filled with sterile artificial CSF (Perfusion Fluid CNS). The syringe will be placed in a portable syringe pump (107 MD Pump, ref. no. P000127, M Dialysis, Solna, Sweden). The flow control on the pump will be set at **1µL/min**, and the pump will be turned on. A plastic microvial (Ref. No. P000001, M Dialysis, Solna, Sweden) will be placed in the vial holder at the end of the outlet tubing to collect dialysate. This microvial can hold up to 200 µl of fluid. It will need to be replaced with a new one every 3 hours.

When ready to leave the post-anesthesia care unit, the patient will be transferred to the Intensive Care Unit and stay there as long as the microdialysis catheter remains functional in order to monitor the microdialysis pump and collect the dialysate samples as outlined in sections 5.4.1.4.1 and 9.3.1.

Prophylactic antibiotic use will continue as long as the microdialysis catheter remains in place.

5.4.4 Cycle 1 Days 4-10: Collection of dialysate and blood samples for measurement of intracerebral and plasma levels of 5-FC, leucovorin, and 5-FU.

With patients who undergo intracerebral microdialysis, the first dose of 5-FC/leucovorin will be administered at 6 am on day 4 and continue every 6 hours for the 7 day dosing period.

5.4.4.1 Collection of dialysate samples

On day 4, just prior to the first dose of 5-FC/leucovorin, a new microvial will be placed in the holder of the outlet tubing of the microdialysis catheter. The microvial will be changed to a new one **every 60 minutes for the next 24 hours**, and thereafter the microvial will be switched every 3 hours until the patient has completed the 7 day course of 5-FC/leucovorin. Please see section 9.2.1 for further details about collection of the dialysate samples.

The patient will remain hospitalized for as long as the microdialysis catheter is in place. During the period of dialysate collection, a patient may be mobile within the confines of the collection system. For example, s/he may move from bed to chair or commode and so on.

If the catheter stops functioning and is removed before the patient has completed the 7-day course of 5-FC/leucovorin, then s/he can be discharged from the hospital and finish taking 5-FC/leucovorin as an outpatient.

5.4.4.2 Removal of the microdialysis catheter

Approximately 6 hours after the last dose of 5-FC has been administered, the microdialysis catheter will be removed percutaneously at the bedside. Xylocaine for local anesthesia will be used as needed. The entry site of the catheter will be closed with a suture or steri-strips as necessary and a clean dressing applied.

5.4.4.3 Collection of plasma samples.

During collection of the dialysate samples, blood samples will be drawn in order to compare systemic and intracerebral levels of 5-FC, leucovorin, and 5-FU.

Pharmacokinetic (PK) sampling will be performed before and after 5-FC and leucovorin have reached steady-state.

On days 4 and 5, blood samples will be drawn just prior to the 6 am dose of 5-FC/leucovorin and then every 30 minutes during the first 3 hours after that dose is taken by the patient. Additional blood samples will be obtained 4 hours after the 6 am dose and then just prior to the next dose at noon.

On days 6, 7, and 8, blood samples will be obtained just before the 6 am dose of 5-FC/leucovorin and then 90 minutes later.

Please see section 9.2.2 for further details about collection of the PK blood samples.

If the microdialysis catheter becomes non-functional, then blood draws for plasma PKs will stop.

5.5 Definition of Dose-Limiting Toxicity (DLT)

Toxicities will be graded according to the NCI Common Terminology Criteria for Adverse Events version 4.0. **All patients receiving any number of NSCs will be evaluated for dose limiting toxicity criteria.**

A DLT is defined as an adverse event that:

(a) is related to the administration of NSCs, 5-FC, or leucovorin, or any combination of the agents, with an attribution of possible, probable, or definite, and

(b) occurs during the first treatment cycle (including Cycle 2 Day 1 safety assessments), and

(c) meets any of the following criteria:

Discontinuation toxicities

1. Receives less than 80% of study treatments due to a toxicity related to NSCs, 5-FC, or leucovorin, except CNS toxicities that are present at baseline or attributable to the location of the tumor or the neurosurgery.

Hematological toxicities

1. Grade 4 thrombocytopenia
2. Grade 4 neutropenia lasting > 7 days
3. Grade 4 anemia
4. Febrile neutropenia if ANC < 0.5 x 10^9/L

Nervous system disorders

5. Grade 3 CNS disorder lasting > 7 days that is not present at baseline and is not attributable to the location of the tumor or the neurosurgery.
6. Second occurrence of a grade 3 CNS disorder that is not present at baseline and is not attributable to the location of the tumor or the neurosurgery.
7. Any grade 4 CNS disorder that is not present at baseline and is not attributable to the location of the tumor or the neurosurgery.

Non-hematological, non-nervous system toxicities

8. Grade 3 toxicity despite maximal medical therapy lasting > 7 days.
9. Grade 3 toxicity resulting in study agent discontinuation (permanently removed from study treatment).
10. Grade 4 toxicity, except grade 4 diarrhea that responds to maximal medical therapy within 3 days.

Please note: An allergic reaction to the NSCs is excluded from the DLT definition, because NSC dose reduction is not appropriate in this situation. The possible occurrence of allergic reactions to the NSCs is addressed by the study stopping rules (Section 5.9).

5.6 Study Design

The study will implement a standard 3+3 design. The goal will be to determine the maximum tolerated dose (MTD), which is defined as the highest dose level tested in which fewer than 33% of patients experienced a DLT attributable to the treatment regimen of NSCs with 5-FC and leucovorin, when at least 6 patients were treated at that dose and are evaluable for toxicity. If the MTD has not been reached with 1.5 x 108 NSCs, 37.5 mg/kg q6h 5-FC and 25 mg q6h leucovorin (dose level 3 plus leucovorin), then that dose will be termed the maximum feasible dose (MFD).

5.6.1 Phase I cohorts will be of 3 or 6 participants, as indicated by the dose-escalation criteria.

5.6.2 The dose schedule is provided in the table in section 5.7. The first cohort of patients will start at dose level 1.

5.6.3 Dose escalation will proceed through the identified doses without skipping any dose levels.

5.6.4 ***Enrollment of the first 3 patients to dose levels 1-3will be staggered*** by requiring that each of the first 3 patients be separately observed for 4 weeks (or until the DLT assessment period has ended) before the next patient can start treatment on that dose level.

5.6.5

5.6.6 Dose escalation rules:

5.6.6.1 If 0/3 study participants experiences a DLT, the next cohort of 3 study patients will be treated at the next higher dose level.

5.6.6.2 If 1/3 study participants experiences a DLT, the cohort will be expanded by another 3 study participants who will be treated at the same dose level for a total of 6 study participants.

5.6.6.3 If 1/6 participants experiences a DLT, the next cohort of 3 study participants will be treated at the next higher dose level.

5.6.6.4 If 2 or more participants of either a 3 participant or 6 participant cohort encounter a DLT, then the MTD has been exceeded. If applicable, three (more) participants will be enrolled at the next lower dose level.

5.6.6.5 At least 6 patients will be treated at the MTD (or MFD, if the MTD is not reached).

For a patient to be counted for dose escalation, s/he must receive at least 80% (+/- 5%) of the assigned NSCs on days 1 and 15 and 80% (+/- 5%) of the 5-FC (and leucovorin, as indicated) on days 4-10 and 18-24 during cycle 1 and be followed up to and including cycle 2 day 1 safety assessments or have experienced a DLT.

All study patients enrolled are to be fully followed for toxicity; any study patients who are not evaluable for dose escalation will be replaced. Intra-patient dose escalation is not allowed.

Dose level 4 patients will undergo intracerebral microdialysis, along with collection of blood samples, for pharmacokinetic analysis in order to characterize the relationship between intracerebral and systemic concentrations of 5-FC and 5-FU at the MTD level, as well as to assess intracerebral concentrations of leucovorin.

5.7 Dose Escalation Schema

Dose Level	Neural Stem Cells (i.c. on days 1 & 15)	5-FC (mg/kg q 6h p.o. on days 4-10 & 18-24)	Leucovorin (mg q 6h p.o. on days 4-10 & 18-24)	Initial Enrollment	If 1 DLT, additional enrollment

-1[a]	3.75 x 10^7	28	---	3	3
1	5 x 10^7	37.5	---	3	3
2	1 x 10^8	37.5	---	3	3
3	1.5 x 10^8	37.5	---	3	3
4[b,c]	Highest tolerated dose combination of 5-FC and NSCs		25	3	3

[a]If a DLT is observed in 2 or more patients at dose level 1, then a de-escalation dose**, dose level -1** will be tested. With dose level -1, the doses of NSCs and 5-FC will both be reduced by 25%; however, if the DLT can clearly be attributed only to 5-FC, then only the dose of 5-FC will be reduced by 25% (likewise for the NSCs if the DLT is clearly attributable to only the NSCs) for dose level -1.

[b]If the addition of leucovorin to the highest tolerated dose combination of 5-FC and NSC requires a dose de-escalation, then leucovorin will be discontinued and the MTD/MFD will be the highest tolerated dose combination of 5-FC and NSCs without leucovorin. A total of 6 patients will be treated at the maximum tolerated dose (MTD), or if the MTD is not reached, at the maximum feasible dose (MFD).

[c]Patients on dose level 4 will undergo intracerebral microdialysis to measure NSC-mediated conversion of 5-FC to 5-FU.

5.8 Criteria for Removal from Treatment

5.8.1 At the beginning of cycle 1, if a patient requires more than 16 mg/day of dexamethasone to control cerebral edema by the time s/he is ready to begin treatment with 5-FC (and leucovorin, as indicated), then s/he will be considered neurologically unstable and will not continue with study treatment; however, the patient will still be evaluable for toxicity.

5.8.2 Treatment will be held if a grade III or IV non-hematologic toxicity or a grade IV hematologic toxicity occurs that is at least possibly attributable to NSCs, 5-FC (and leucovorin, as indicated) or the combination. If the adverse event resolves as per criteria in section 6.3, then the patient will be allowed to proceed with the next treatment of NSCs and 5-FC (and leucovorin, as indicated), with dose modifications as outlined in section 6.3. If the adverse event does not resolve within the timeframe specified in section 6.3, or if more than 2 dose reductions are required, then study treatment will be stopped.

5.8.3 Evidence of progressive disease.

5.8.4 Patient needs any treatment not allowed by the protocol.

5.8.5 A patient may always be removed from treatment whenever s/he wishes.

5.8.6 If a patient needs to start full dose anticoagulation while receiving study treatment, s/he will be taken off study.

5.9 Study Stopping Rules

The study will be discontinued if i) the incidence of DLT level toxicities that are probably or definitely attributable to the NSCs and/or 5-FC (and leucovorin) in study patients exceeds 50% in more than 6 patients during cycle one of treatment, or ii) two grade 4 allergic reactions that are probably or definitely related to NSCs. Note death from tumor progression will be scored as a grade 5 toxicity (with very low

level of attribution—unlikely or not related), but only deaths occurring < 30 days from intracerebral administration of NSCs will be considered for the purposes of defining conditions for discontinuation of the study.

5.10 Additional Studies

Please see section 9.0 for further details about the correlative studies that will be performed.

5.11 Long Term Follow-Up

Patients will continue to be followed once they have finished active study treatment. They will be asked to participate in long term follow-up per the guidelines set forth by the FDA's Biological Response Modifiers Advisory Committee (BRMAC) that apply to gene transfer studies. Current recommendations from the BRMAC require a minimum of 15 years of follow-up. This long-term follow-up will consist of annual requests from the patient/patient's primary oncologist for clinical information regarding survival, as well as information pertaining to *de novo* cancer, neurologic, autoimmune, or hematologic disorders, and any adverse event that is at least possibly attributable to this study treatment. RCR testing will be part of the long-term follow-up of study patients and will be performed by analysis of DNA from whole blood by PCR for RCR-specific sequences at the following time points: pre-treatment, 3 months, 6 months, 1 year, and annually thereafter (see section 9.1.6).

6.0 Dose Delays/Modifications for Adverse Events

6.1 Toxicities to be Monitored

The study will use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. A copy of the version 4.0 can be downloaded from: http://evs.nci.nih.gov/ftp1/CTCAE/About.html

6. 2 Expected Toxicities

6.2.1 Surgery toxicities

Bleeding, infection, headache, seizures, cerebral edema, pain, changes in neurologic function.

6.2.2 NSC toxicities

All toxicity studies to date in mice have shown no evidence of organ damage or tumorigenicity due to HB1.F3.CD. NSCs.

In the first-in-human study of this neural stem cell line, no grade 3 or 4 toxicities attributable to the NSCs were observed.

6.2.3 Chemotherapy toxicities

6.2.3.1 5-FC

Per the package insert for systemic 5-FC, the expected toxicities for 5-FC are:

Cardiovascular: Cardiac arrest, myocardial toxicity, ventricular dysfunction.

Ear and labyrinth disorders: vertigo, hearing loss.

Endocrine & metabolic: Hypoglycemia, hypokalemia.

Gastrointestinal: Nausea*, vomiting*, abdominal pain*, diarrhea*, anorexia, dry mouth, duodenal ulcer, gastrointestinal hemorrhage, ulcerative colitis, enterocolitis*.

Hematologic: Anemia, agranulocytosis, aplastic anemia, eosinophilia, leukopenia, pancytopenia, thrombocytopenia, and bone marrow aplasia including fatal cases.

Hepatic: Acute hepatic injury including hepatic necrosis with possible fatal outcome in debilitated patients, hepatic dysfunction, jaundice, bilirubin elevation, increased hepatic enzymes.

Immune system disorders: Allergic reaction.

Nervous system disorders: Ataxia, headache*, paresthesia, parkinsonism, peripheral neuropathy, sedation, convulsions/seizure.

Psychiatric: Confusion*, hallucinations*, psychosis.

Renal and urinary: Azotemia, BUN elevation, crystalluria, renal failure, creatinine elevation.

Respiratory: Respiratory arrest, chest pain, dyspnea.

Skin and subcutaneous tissue disorders: Rash, pruritus, urticaria, photosensitivity, Lyell's syndrome (toxic epidermal necrolysis).

Miscellaneous: Fatigue, weakness, fever/pyrexia.

*An asterisk signifies a common event according to Micromedex (updated July 2014).

6.2.3.2 5-FU

Per the package insert for systemic 5-FU, the expected toxicities for 5-FU are as follows, where the asterisk (*) signifies a common event:

Cardiovascular: myocardial ischemia, angina,

Eye disorders: photophobia, visual changes, lacrimation, lacrimal duct stenosis.

Gastrointestinal: stomatitis*, esophagopharyngitis*, diarrhea*, anorexia*, nausea*, vomiting*, gastrointestinal ulceration and bleeding.

Hematologic: leucopenia* (nadir: days 9-14; recovery by day 30),, pancytopenia, thrombocytopenia, agranulocytosis, anemia.

Immune system disorders: anaphylaxis and generalized allergic reactions.

Nervous system disorders: acute cerebellar syndrome (which may persist following discontinuance of treatment), nystagmus, headache.

Skin and subcutaneous tissue disorders: alopecia*, dermatitis (most commonly pruritic maculopapular rash)*, dry skin; fissuring; photosensitivity, as manifested by erythema or increased pigmentation of the skin; vein pigmentation; palmar-plantar erythrodysesthesia syndrome, as manifested by tingling of the hands and feet followed by pain, erythema and swelling (hand-foot syndrome); nail changes (nail loss)

Psychiatric: disorientation, confusion, euphoria.

Respiratory: epistaxis.

Miscellaneous: thrombophlebitis.

Severe toxicity (such as stomatitis, diarrhea, neutropenia, and possibly neurotoxicity) associated with 5-FU has been attributed to deficiency of dipyrimidine dehydrogenase activity.

6.2.3.3 Leucovorin

As listed in the package insert (updated July 2014) the expected toxicities for oral and parenteral leucovorin are:

Allergic Reactions: anaphylaxis and generalized allergic reactions

Skin and subcutaneous tissue disorders: hives (urticaria) associated with anaphylactoid reactions

Leucovorin with 5-FU:

Leucovorin can enhance the toxicity of 5-fluorouracil. Although the toxicities observed in patients treated with the combination of leucovorin plus 5-fluorouracil are qualitatively similar to those observed in patients treated with 5-fluorouracil alone, gastrointestinal toxicities (particularly stomatitis and diarrhea) are observed more commonly and may be more severe and of prolonged duration in patients treated with the combination.

6.2.4 Microdialysis catheter toxicities

Bleeding along the catheter track, infection localized to or clearly emanating from the catheter. Minor bleeding (less than 1 ml) has been observed in 3% of patients, and there have been no documented microdialysis catheter-related infections.

6.3 Dose Modifications/Delays/Start of Cycle Criteria

6.3.1 General Information

6.3.1.1 The study will use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 to grade toxicities. A copy of the version 4.0 can be downloaded from:

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

6.3.1.2 Whenever treatment is held pending resolution of toxicity to grade 1 or return to baseline, this criterion may also be met if the toxicity resolves to within 1 grade of the baseline value for a pre-existing laboratory abnormality. Baseline values are from screening assessments.

6.3.1.3 Study agents are always held together, regardless of the attribution of the toxicity.

6.3.1.4 NSC or 5-FC dose re-escalation and leucovorin restarting are never permitted in this study.

6.3.1.5 If, for the best interest of the patient, the investigator wants to hold the study agent(s) or dose reduce (including increasing the dose reduction) after an adverse event in a manner not outlined in Table 7.2.4, this is permissible following discussion with and written approval the Principal investigator.*

6.3.1.6 For situations where a participant experiences a toxicity which the treating investigator feels is unlikely related to treatment with either study agent, but which requires hold or reduction of either agent according to Table 7.2.4, maintaining treatment or maintaining treatment dose is allowable per discussion with and written approval by the Principal Investigator.*

6.3.1.7 For holds due to toxicities related to study agent(s), if the participant does not meet criteria to resume treatment within 14 days of the detection of the toxicity, the participant must permanently discontinue study treatment (all agents). However, if the participant is clearly benefiting from the study, the investigator may contact the Principal Investigator to determine if the participant can remain on study and at what dose level. Agreement of the Principal Investigator is to be documented in writing.*

6.3.1.8 For holds for reasons other than treatment related toxicities, such as inclement weather or adverse events unrelated to study agents, if the participant does not meet criteria to resume treatment within 28 days of their last NSC administration, the study agent(s) may be restarted with written approval from the Principal Investigator, as long as there has been no significant evidence of disease progression during the treatment interruption.*

6.3.1.9 Section 6.4 details resuming agent administration following a treatment hold.

*The principal investigator will document assessment of the impact of these determinations on the study design, objectives and endpoints or risk to participants. If any modifications to the treatment plan might affect the study design, objectives, and endpoints, or impact the risk of participants, a single subject exception will be sought from the IRB. If the treating investigator is the principal investigator, the determination and the rationale for the determination will clearly be documented in the medical record.

6.3.2 Start of Cycle Criteria

In order to proceed with the next cycle of study treatment:

- adverse events (except for alopecia) that occurred in the preceding cycle must have improved to a CTCAE v 4.0 grade ≤ 1 (or within 1 grade of starting values for pre-existing laboratory abnormalities),
- ANC must be ≥ 1500 cells/mm^3,
- platelet count ≥ 100,000 cells/mm^3, and

6.3.3 Criteria for First Administration of 5-FC (and leucovorin)

For Cycle 1 Day 4 5-FC (and leucovorin) administration to proceed, the participant must have recovered well from any immediate post-operative effects **and** requires no more than 16 mg/day of dexamethasone. Otherwise the participant will be removed from treatment. Criteria found in Section 6.3 (Dose modifications) also apply.

6.3.4 Dose Modifications

Table 6.3.4 details the criteria for disrupting treatment, dose modification and treatment discontinuation following an adverse event. See section 6.3.1 for general guidelines. The agent listed in Table 6.3.4 indicates the drug to which the toxicity is likely attributed based on previous trials of 5-FC (with leucovorin) and possible anticipated toxicities to the CD-NSCs.

Table 6.3.4 Criteria for Disrupting Treatment, Dose Modification or Discontinuation

Adverse Event	Agent most likely attributed to toxicity	Treatment modification
Immune Response to NSCs (i.e. development of a new neurologic deficit not attributed to the location of brain tumor, fever without localizing source of infection, new or progressive rash, cough etc.)		
Grade 2 allergic reaction to the NSCs. NOTE: a decision to stop study treatment will be based on development of clinical signs/symptoms of an immune response rather than a particular level of anti-NSC T cells or antibodies.	NSCs	**Hold study treatment.** **Initiate immunological testing:** Take 3-5 ml of blood in red top tube, kept upright at room temperature until delivery to CICSL. Store at 4 C after sample clots if unable to deliver sample the same day. Take additional samples (at least one additional sample) every two days. **Initiate pharmacologic management for an allergic reaction:** Restart dexamethasone to a dose of 4 mg twice daily; if patient is already taking dexamethasone, then increase dexamethasone to at least 4 mg twice daily. **Hold study treatment** until sign/symptoms ≤ grade 1, and resume treatment at the same dose, while continuing dexamethasone at a dose determined by the discretion of the treating investigator in consultation with the PI.
Grade 3 or 4 allergic reaction to NSCs NOTE: a decision to stop study treatment will be based on development of clinical signs/symptoms of an immune response rather than a particular level of anti-NSC T cells or antibodies.		**Hold study treatment.** **Initiate immunological testing:** Take 3-5 ml of blood in red top tube, kept upright at room temperature until delivery to CICSL. Store at 4 C after sample clots if unable to deliver sample the same day. Take additional samples (at least one additional sample) every two days. **Initiate pharmacologic management for an allergic reaction:** Restart dexamethasone to a dose of 4 mg twice daily; if patient is already taking dexamethasone, then increase dexamethasone to at least 4 mg twice daily. Continue dexamethasone until event resolves to ≤ Grade 1, and thereafter at a dose determined by treating investigator as clinically indicated. If an immune response to the NSCs is considered at least possible, discontinue study treatment.
Hematological Toxicities		
Thrombocytopenia Grade 1 (75 x 10^9/L -<LLN)	5-FC (with leucovorin)	Hold treatment until recovery to ≥ 100 x 10^9/L. Resume at pre-hold dose.
Thrombocytopenia Grade 2 (50 – <75 x 10^9/L)		Hold treatment until recovery to ≥ 100 x 10^9/L. Resume at pre-hold dose.
Thrombocytopenia Grade 3 (25 – <50 x 10^9/L)		Hold treatment until recovery to ≥ 100 x 10^9/L. *For patients **not** taking leucovorin:* Resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose. *For patients taking leucovorin:* First event: resume treatment at the same dose of NSCs and 5-FC, and permanently discontinue leucovorin. Second event: resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose.

Thrombocytopenia Grade 4 (<25 x 10^9/L)		Hold treatment until recovery to ≥ 100 x 10^9/L. *For patients **not** taking leucovorin:* Resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose. *For patients taking leucovorin:* First event: resume treatment at the same dose of NSCs and 5-FC, and permanently discontinue leucovorin. Second event: resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose.
Neutropenia (ANC) Grade 1 (1.5x 10^9/L -<LLN)	5-FC (with leucovorin)	Maintain treatment.
Neutropenia (ANC) Grade 2 (1.0 – <1.5x 10^9/L)		Maintain treatment.
Neutropenia (ANC) Grade 3 (0.5 – <1.0x 10^9/L)		Hold treatment until recovery to ≤ grade 2 (ANC ≥ 1.0 x 10^9/L). Resume treatment with no dose modifications. Consider administration of GCSF.
Neutropenia (ANC) Grade 4 (<0.5x 10^9/L)		Administer GCSF. Hold treatment until recovery to ≤ grade 2 (ANC ≥ 1.0 x 10^9/L). Resume treatment with no dose modifications. Continue administration of GCSF.
Lymphocytopenia Grade 1-3	5-FC (with leucovorin)	Maintain treatment
Lymphocytopenia Grade 4 (< 0.800 x 10^9/L)		Check CD4 lymphocyte level. If < 0.200 x 10^9/L initiate PCP prophylaxis. Maintain treatment.
Serum Electrolytes		
Serum Electrolytes ≥ Grade 3 Calcium , Potassium, Magnesium, Phosphorous	5-FC (with leucovorin)	Correct electrolyte deficits. Maintain treatment per investigator discretion.
Renal Toxicity Use Cockcroft & Gault formula and actual body weight to determine calculated creatinine clearance		
Calculated creatinine clearance 21.0 ml/min – <51.0 ml/min	5-FC	Increase 5-FC interval to Q12 hours; if leucovorin is being administered, increase leucovorin interval to Q12 hours.
Calculated creatinine clearance 10.0 ml/min – <21.0 ml/min		Increase 5-FC interval to Q24 hours; if leucovorin is being administered, increase leucovorin interval to Q24 hours.
Calculated creatinine clearance <10.0 ml/min		Increase 5-FC interval to Q48 hours; if leucovorin is being administered, increase leucovorin interval to Q48 hours.
Gastrointestinal		
Diarrhea Grade 1 (2-3 stools/day >pretreatment)	5-FC (with leucovorin)	Maintain treatment
Diarrhea Grade 2 (4-6 stools/day > pretreatment)		Hold both study drugs until resolved to ≤ grade 1, then resume treatment at pre-hold dose
Diarrhea Grade 3 (7-9 stools/day > pretreatment) while on optimal anti-diarrhea therapy		Hold study treatment until the diarrhea decreases to ≤ grade 1 *For patients **not** taking leucovorin* Restart treatment with a 25% decrease in 5-FC from the pre-hold dose. *For patients taking leucovorin* First event: resume treatment at the same dose of NSCs and 5-FC, but permanently discontinue leucovorin. Additional events: resume treatment with a 25% dose reduction of 5-FC from the pre-hold dose.

Diarrhea Grade 4 (≥10 stools/day > pretreatment) while on optimal anti-diarrhea therapy		Hold study treatment until the diarrhea decreases to ≤ grade 1 *For patients **not** taking leucovorin* Restart treatment with a 25% decrease in 5-FC from the pre-hold dose. *For patients taking leucovorin* First event: resume treatment at the same dose of NSCs and 5-FC, but permanently discontinue leucovorin. Additional events: resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose.
Vomiting or Nausea Grade 1	5-FC (with leucovorin)	Maintain treatment
Vomiting or Nausea Grade 2		Maintain treatment
Vomiting or Nausea Grade 3, despite maximal medical management		Hold study treatment until resolution to ≤ grade 1 *For patients **not** taking leucovorin* First event: resume treatment at pre-hold dose. Additional events: restart treatment with a 25% decrease in 5-FC from the pre-hold dose. *For patients taking leucovorin* First event: resume treatment at pre-hold dose. Second event: resume treatment at the same dose of NSCs and 5-FC, but permanently discontinue leucovorin. Additional events: resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose.
Vomiting Grade 4, despite maximal medical management		Hold study treatment until resolution to ≤ grade 1 *For patients **not** taking leucovorin* Restart treatment with a 25% decrease in 5-FC from the pre-hold dose. *For patients taking leucovorin* First event: resume treatment at the same dose of NSCs and 5-FC, but permanently discontinue leucovorin. Additional events: resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose.
Nervous System Disorders		
Grade 3 Nervous System Disorder that is <u>not</u> attributable to tumor location or neurosurgery and is considered a least possibly attributable to NSCs, 5-FC, or leucovorin, or any combination and is not an immune response to study agents.	NSCs, 5-FC, leucovorin	Hold treatment until resolution to ≤ grade 1. *First event:* If resolves within 7 days, resume treatment at pre-hold dose. If does not resolve within 7 days, resume treatment with a dose reduction of NSCs and 5-FC by 25% of the pre-hold dose. If the patient is taking leucovorin, discontinue leucovorin.* *Second event:* Resume treatment with dose reduction of NSCs and 5-FC by 25% of the pre-hold dose. If the patient is still taking leucovorin, discontinue leucovorin.* ***If the temporal association implicates NSCs or 5-FC (and leucovorin) rather than both/all agents, dose reductions may apply only to the implicated agent.**

Grade 4 Nervous System Disorder that is <u>not</u> attributable to tumor location or neurosurgery and is considered a least possibly attributable to NSCs, 5-FC, or leucovorin, or any combination and is not an immune response to study agents.		Hold until ≤ grade 1, then perform brain MRI. If objective evidence of clinical benefit, restart treatment with a dose reduction of NSCs and 5-FC by 25% of pre-hold dose, and, if patient is taking leucovorin, discontinue leucovorin.* If no objective evidence of clinical benefit is seen on MRI then, discontinue treatment. ***If the temporal association implicates NSCs or 5-FC (and leucovorin) rather than both/all agents, dose reductions may apply only to the implicated agent.**
Other Unspecified Non-Hematologic Toxicities Considered Related <u>Only</u> to 5-FC (with Leucovorin)		
Grade 1	5-FC (with leucovorin)	Maintain treatment
Grade 2		Maintain treatment
Grade 3		Hold study treatment until resolution to ≤ grade 1 *For patients **not** taking leucovorin* Restart treatment with a 25% decrease in 5-FC from the pre-hold dose. *For patients taking leucovorin* First event: resume treatment at the same dose of NSCs and 5-FC, but permanently discontinue leucovorin. Additional events: resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose.
Grade 4		Hold study treatment until resolution to ≤ grade 1 *For patients **not** taking leucovorin* Restart treatment with a 25% decrease in 5-FC from the pre-hold dose. *For patients taking leucovorin* First event: resume treatment at the same dose of NSCs and 5-FC, but permanently discontinue leucovorin. Additional events: resume treatment with a 25% dose reduction of 5-FC of the pre-hold dose.
Other Unspecified Non-Hem Toxicities Considered Related <u>Only</u> to NSCs		
Grade 1	NSCs	Maintain treatment
Grade 2		Maintain treatment
Grade 3		Hold until ≤ grade 1, then dose reduce NSCs by 25%; resume treatment with same dose of 5-FC and leucovorin.
Grade 4		Hold until ≤ grade 1, then perform brain MRI. If objective evidence of clinical benefit, restart treatment at pre-hold doses of 5-FC and leucovorin and a dose reduction of NSCs by 25% of the pre-hold dose of NSCs. If no objective evidence of clinical benefit is seen on MRI then discontinue treatment.
Other Unspecified Non-Hem Toxicities Considered Related to a Combination of NSCs and 5-FC (with Leucovorin)		
Grade 1	NSCs with 5-FC (and	Maintain treatment
Grade 2		Maintain treatment

Grade 3	leucovorin)	Hold treatment until resolution to ≤ grade 1. *First event:* If resolves within 7 days, resume treatment at pre-hold dose. If does not resolve within 7 days, resume treatment with then dose reduce NSCs and 5-FC by 25% from the pre-hold dose. If the patient is taking leucovorin, discontinue leucovorin. *Second event:* Resume treatment with dose reduction of NSCs and 5-FC by 25% of pre-hold dose. If the patient is taking leucovorin, discontinue leucovorin.
Grade 4		Hold until ≤ grade 1, then perform brain MRI. If objective evidence of clinical benefit, restart treatment with a dose reduction of NSCs and 5-FC by 25% of pre-hold dose, and, if patient is taking leucovorin, discontinue leucovorin. If no objective evidence of clinical benefit is seen on MRI then, discontinue treatment.
Other Unspecified Non-Hem Toxicities Considered UNRELATED to Study Agents		
Other unspecified events of any grade considered unlikely to be related or not related to study agents.	UNRELATED	Maintain treatment with study agents. Interruption of study treatment is permitted if the investigator consults with the Principal Investigator to determine that this is in the best interest of the participant.

6.4 Adjustments to the Treatment Cycle Following an Interruption in Agent Administration

The day count continues despite a hold in agent administration.

Should a hold or delay in treatment occur, evaluations required by the protocol (see Section 5 and 10) will be conducted at the original times during the cycle and safety assessments relevant to the toxicity will be repeated prior to resuming agent administration.

The timing of resumption (whether in the same cycle or as part of the subsequent cycle) depends on where in the cycle agent was held, and when criteria to resume are met. Details are described in the sections that follow.

6.4.1 Holding Day 15 NSCs

If Day 15 NSCs and subsequent 5-FC (with leucovorin) are held, resultant start dates are as follows:

- If participant is able to resume treatment by or on Day 22, he/she will resume study agents as soon as logistically feasible during the same treatment cycle. The cycle length would be extended accordingly, so that 14 days between NSC administrations would be maintained.
- If participant is **not** able to resume treatment by or on Day 22, he/she will continue the current cycle without a second NSC administration and resume study agents at the start of the next treatment cycle. The current cycle would be 28 days in length and the participant will forgo the Day 15 NSCs/ Day 18-24 5-FC (w/ w/o leucovorin).

6.4.2 Holding Any of the 5-FC (with Leucovorin) Administrations After NSC Administration

If the 5-FC (with leucovorin) administration is held after the participant has received the corresponding NSC administration, held doses of 5-FC and leucovorin will may be made up by extending the 5-FC administration for up to one day so that there is a minimum of three days without an agent being administered between 5-FC administration and NSC administration. (Example: Pt receives C2D1 NSCs

and has agents held from C2D3-C2D6; participant will resume 5-FC (with leucovorin) the evening of C2D6 through and including C2D11, and then proceed to Day 15 NSC administration per usual.)

7.0 Data and Safety Monitoring, Unanticipated Problems and Adverse Event Reporting

7.1 Data and Safety Monitoring

This is a Risk Level 4 study, as defined in the "City of Hope Data and Safety Monitoring Plan", http://www.coh.org/dsmc/Pages/forms-and-procedures.aspx involving COH as IND holder and gene therapy.

7.2 Monitoring and Personnel Responsible for Monitoring

The City of Hope Protocol Management Team (PMT) consisting of the PI, collaborating investigators, CRA, protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of the stopping rules for safety.

This study will utilize the Phase I tracking log to monitor data and safety for dose escalation, recording doses administered and resultant adverse events. The tracking log will contain dose levels administered, DLT-defining adverse events, and documentation that the data from a dose level is complete before dose escalation. Those data and safety elements will be reported to the COH DSMC as applicable with the PMT report, which will be submitted quarterly from the anniversary date of activation, as noted in Table 1 below. Protocol specific data collection will include the following items: adverse events per dose level, patient outcomes, and protocol deviations.

A decision to escalate to the next dose level will be made by the PMT only after all patients on the current dose level have been observed through day 28. Enrollment of the first 3 patients to dose levels 1-3 will be staggered by requiring that each of these patients be observed for 28 days before the next patient can begin treatment on a particular dose level.

Table 1: City of Hope PMT Reporting Timelines for the DSMC

Risk Level	Phase	Standard Reporting Requirement
RL 1, RL2, and Compassionate Use Studies	No reports required	
3	I	Every 3 months from activation date, as indicated in MIDAS
3	Pilot, Feasibility, II-IV	Every 6 months from activation date, as indicated in MIDAS
4	Pilot, Feasibility, I-IV	Every 3 months from activation date, as indicated in MIDAS

Data and safety will also be reported to the FDA. All IND safety reports that are submitted to the FDA regarding this protocol will also be submitted to the COH DSMC for review. As required by the FDA, study patients will be followed long term (up to 15 years or until they die; see section 5.11), and the PMT will continue to report data and safety concern to the FDA and DSMC as they arise during this follow-up period.

7.3 Definitions

Adverse event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Unexpected Adverse Event [21 CFR 312.32 (a) – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event

Serious Adverse Event (SAE)* [21 CFR 312.32]** ***is defined as any expected or unexpected adverse event that results in any of the following outcomes:

- Death
- Is life-threatening event (places the subject at immediate risk of death from the event as it occurred);
- Requires in-patient hospitalization (not required as part of the treatment) or prolongation of existing hospitalization;
- A persistent or significant disability/incapacity;
- A congenital anomaly/birth defect
- Secondary Malignancy, or
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasisas of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Unanticipated problem (UP) – Any incident, experience or outcome that **meets all three** of the following criteria:

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

7.4 Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems: Unanticipated problems must be reported to the COH DSMC and IRB **within 5 calendar days** according to definitions and guidelines at http://www.coh.org/hrpp/Pages/hrpp-policies.aspx. Any unanticipated problem that occurs during the study conduct will be reported to the DSMC and IRB by submitting electronically in iRIS (http://iris.coh.org).

Serious Adverse Events - All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to definitions and guidelines at http://www.coh.org/hrpp/Pages/hrpp-policies.aspx and Table 1 below. Those

SAEs that require expedited reporting will be submitted electronically in iRIS (http://iris.coh.org/).

Adverse Events - Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious adverse event or are not unanticipated problems will be reported only at the time of protocol continuation reports (see Table 1 below).

Table 2: City of Hope Adverse Event and Unanticipated Problem Reporting Timelines for the DSMC and IRB

Required Reporting Timelines to DSMC for AE/SAEs
Investigator Initiated Studies

Required Reporting Timeframe to DSMC		
Attribution	**UNEXPECTED**	**EXPECTED**
	Death while on active treatment or within 30 days of last day of treatment	
Possibly, Probably, Definitely	5 calendar days	
Unlikely, Unrelated		
	Death after 30 days of last active treatment/therapy	
Possibly, Probably, Definitely	5 calendar days	No reporting required
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	5 calendar days	10 calendar days
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	10 calendar days	10 calendar days

An event determined by the IRB of record to be an Unanticipated Problem (UP) will be communicated to the Investigator and COH DSMC through the COH IRB Operations Director. The DSMC will review the case and make a determination as to whether the study will be suspended, terminated, amended, or allowed to continue without amendment.

Required Reporting Timeframe to IRB of Record		
Attribution	**UNEXPECTED**	**EXPECTED**
	Death	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual
	Grades 3 and 4 AND meeting the definition of a UP	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual
	Grade 1 and 2 AND meeting the definition of a UP	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual

7.5 ADDITIONAL REPORTING REQUIREMENTS

SAEs meeting the requirements for expedited reporting to the FDA, as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting which can found at:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

The PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the following:

- any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)];

- any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]

- any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

Medical Device Reportable Events

Medical devices that will be used in this study include intracerebral microdialysis catheters and Rickham reservoir/catheter systems.

Medical Device Reportable (MDR) Events are the AEs or problems that the medical device regulation requires to be reported. These events include patient deaths and serious injuries that the medical devices have or may have caused or contributed to, i.e., the devices may have directly caused the events or played a role in the events.

The timely reporting of MDR reportable events is required by the FDA, and will be handled by OIDRA.

National Institutes of Health-Office of Biotechnology Activities (NIH-OBA) and FDA Reporting

Since this study involves gene therapy, and City of Hope holds the IND, adverse events must also be reported to the NIH-OBA.

The PI/designee will submit the adverse event report through the City of Hope electronic reporting system, and then OIDRA will take responsibility for reporting of the adverse event to NIH-OBA.

Follow up of study participants

As required by the FDA, study patients will be followed long term (up to 15 years) and the PI will continue to report data and safety concerns to the FDA, DSMC, and IRB as they arise during this follow-up period.

8.0 Agent Information and Risks

8.1 HB1.F3.CD. NSCs (IND # 14041)

8.1.1 Animal Toxicity Data

All toxicity studies to date in mice have shown no evidence of organ damage or tumorigenicity due to NSCs. Please see sections 2.4.2 and 2.4.3.

8.1.2 Human toxicity data

See sections 2.5.1 and 6.2.2.

8.1.3 Administration

Intracranial administration—either through direct injection into tumor at the time of surgery or via a Rickham.

8.1.4 Supplier

City of Hope

8.2 5-Fluorocytosine (5-FC, Flucytosine, Ancobon; NSC # 103805)

8.2.1 Description

5-FC is classified as an antifungal agent. It is a fluorinated pyrimidine analog structurally related to 5-FU and floxuridine. It is a white to off-white crystalline powder. 5-FC is available in 250 mg and 500 mg capsules. The capsules also contain cornstarch, lactose and talc.

8.2.2 Chemical Name

4-Amino-5-fluoropyrimidin-2(1*H*)-one

Molecular Formula $C_4H_4FN_3O$

Molecular weight 129.09

8.2.3 Human Toxicity

See section 6.2.4.1

8.2.4 Pharmacokinetic Data

5-FC is rapidly and well absorbed. Its oral bioavailability is 78-90%. There is no significant systemic metabolism of 5-FC. More than 90% is excreted by glomerular filtration as unchanged drug. The systemic half-life is 2.5 to 6 hours. The time to peak concentration is 1-2 hours. Distribution: 5-FC distributes widely in body water. Less than 4% is protein-bound. Levels in the cerebrospinal fluid are approximately 80% of serum levels.

8.2.5 Administration

The dose of 5-FC will be given orally every 6 hours for 7 days following each dose of NSCs (i.e. on days 4-10 and 18-24 of every 28 day cycle). The dose will be rounded to the nearest 250 mg. Administer a few capsules at a time over a 15-minute period to minimize nausea and vomiting.

8.2.6 Storage and Stability

Store between 15 and 30 °C in a tight, light-resistant container

8.2.7 Supplier

Commercially available; provided by the study.

8.3 Leucovorin

8.3.1 Description

Leucovorin Calcium Tablets contain either 5 mg or 25 mg leucovorin as the calcium salt of*N*-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-*L*-glutamic acid. This is equivalent to 5.40 mg or 27.01 mg of anhydrous leucovorin calcium (leucovorin calcium (leucovorin calcium (leucovorin calcium tablets) tablets) tablets). In addition each tablet contains the following inactive ingredients: colloidal silicon dioxide, croscarmellose sodium, lactose monohydrate, magnesium stearate, and microcrystalline cellulose. The 25mg tablet also contains D&C yellow no. 10 and FD&C blue no. 1.

8.3.2 Chemical name

N-[4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)-methyl]amino]benzoyl]-*L*-glutamic acid

Molecular formula $C_{20}H_{21}CaN_7O_7$

Molecular weight: 511.51

8.2.8 Human toxicity data see section 6.2.4.2

8.2.9 Pharmacokinetic data

Absorption: Tmax, oral: 1.72 to 2.3 hr; oral bioavailability: 97% (25 mg); 75% (50 mg); 37% (100 mg)
Metabolism: Hepatic and intestinal mucosa: extensive
Elimination Half Life**:** total reduced folates: (oral) 3.5 to 5.7 hr

8.2.10 Administration

Orally once a day for 7 days

8.2.11 Storage and stability

Store at 20° to 25° C (68° to 77° F). Dispense in a tight, light-resistant container. Protect from light and moisture.

8.2.12 Supplier

Commercially available; provided by the study.

8.4 Missed or skipped doses of 5-FC (with Leucovorin)

If the participant misses or skips doses of 5-FC or leucovorin, those doses can be made up.

9.0 Correlative/Special Studies

9.1 Immunological Correlative Studies

In order to assess for possible development of anti-NSC T cell and antibody responses with repeat exposure to the NSCs, blood samples from study patients will be collected for isolation and storage of PBMCs and serum prior to the start of study treatment, on day 15 of cycle 1, days 1 and 15 of cycle 2, and then on day15 of subsequent cycles (please see section 9.1.5 for further details). We will also be assessing study patients for peripheral persistence of the NSCs (section 9.1.5.1) and testing for replication competent retrovirus (RCR) (section 9.1.6).

The following studies, which have been successfully performed in our first neural stem cell study of CD-expressing NSCs and oral 5-FC, will be done by the City of Hope Clinical Immunobiology Correlative Studies Laboratory (COH CICSL).

9.1.1 Assessment of the development of anti-NSC T cell responses

Development of a T cell response to the NSCs will be evaluated by a combination of TcR Vβ spectratyping and CD107 degranulation assays using established laboratory SOP and protocols. Briefly, Vβ spectratyping of patient PBMCs collected prior to and after NSC administration will identify unique clonal populations of T cells that have expanded post-NSC administration. Such expanded populations have the potential to be specific for and to eliminate the administered NSCs. The specificity of the clonally-expanded T cells for the NSCs will be evaluated by 1) incubating pre- and post-infusion PBMC samples with the NSCs, followed by flow cytometric analysis using commercially available monoclonal antibodies specific for the identified clonotypes to determine the candidate T cells and 2) assessing surface CD 107 (a measure of cytotoxicity) on the surface of those T cells.

9.1.2 Determination of development of antibody responses to the NSCs.

An evaluation of humoral responses to NSCs will be performed in the CICSL by flow cytometric evaluation of antibody binding to NSCs. Briefly, aliquots of cultured NSCs will be incubated on ice with cryopreserved patient serum samples that have been heat treated to inactivate complement. Following incubation the NSCs will be washed and bound antibodies detected using a secondary goat anti-human Fc antibody conjugated to FITC. After a second

washing step, the NSCs will be analyzed by CICSL staff using a flow cytometer in the COH Analytical Cytometry Core. Binding of human antibodies to the NSCs will be detected as a shift in the mean fluorescence intensity (MFI) compared to NSCs not treated with human serum. All samples obtained from individual subjects prior to and after therapy will be evaluated in parallel to evaluate possible development of humoral anti-NSC responses over time. The statistical significance of any detected MFI shifts will be evaluated by comparison with data from the NSC binding of a reference panel of normal human sera. The assay will include as a control the parental NSC (not transduced with the CD gene) to evaluate whether antibody responses are directed against the CD gene product.

9.1.3 Evaluation of the peripheral persistence of NSCs using quantitative PCR with *vMyc* primers

An evaluation of the peripheral persistence of NSCs will also be performed in the CICSL by quantitative PCR using established laboratory SOP and primer pairs specific for the *v-myc* gene. Briefly, a standard curve will be generated using DNA isolated from the administered NSCs titrated into DNA from PBMCs (negative for *v-myc*); in parallel reactions, DNA isolated from patient samples will be subjected to amplification. The % NSC DNA (i.e. the % of total nucleated cells in samples that contain *v-myc* DNA) in patient PBMC samples will be determined from the standard curve. A parallel set of amplification reactions using genomic p21 primers (amplifying equivalently from all samples) will be used to normalize across samples.

9.1.4 Assessment of serum cytokine levels in the event that a patient develops a suspected immune response to the NSCs

The Human Cytokine Thirty-Plex Antibody Bead Kit (Invitrogen, Camarillo, CA) will be used to analyze serum samples for 30 cytokines: epidermal growth factor (EGF); eotaxin; basic fibroblast growth factor (FGF-basic); granulocyte colony-stimulating factor (G-CSF); granulocyte macrophage colony-stimulating factor (GM-CSF); hepatocyte growth factor (HGF); interferon alpha (IFN-α); IFN-gamma (γ); interleukin-1 beta (IL-1β); interleukin-1 receptor antagonist (IL-1RA); IL-2; interleukin-2 receptor (IL-2R); IL-4; IL-5; IL-6; IL-7; IL-8; IL-10; IL-12p40/p70; IL-13; IL-15; IL-17; IFN- γ-inducible protein 10 (IP-10); monocyte chemoattractant protein-1 (MCP-1); monokine induced by IFN- γ (MIG); monocyte inflammatory protein-1 alpha (MIP-1α); monocyte inflammatory protein-1 beta (MIP-1β); regulated upon activation, normal T-cell expressed and secreted cytokine (RANTES); tumor necrosis factor (TNF)-α; and vascular endothelial growth factor (VEGF). The assay plate will then be transferred to the Bio-plex HTF Luminex System (Bio Rad Laboratories, Inc., Hercules, CA) instrument for analysis. Cytokine concentrations will be calculated using Bio-plex Manager 3.0 Software with a 5 parameter curve-fitting algorithm applied for standard curve calculations for duplicate samples.

9.1.5 Collection of blood samples for performing the immunological correlative studies

9.1.5.1 To assess for possible development of anti-NSC T cell responses and evaluate for peripheral persistence of the NSCs, approximately 30 ml of blood will be collected from each patient in lavender top tubes (liquid potassium EDTA) prior to the start of study treatment, on day 15 of cycle 1, days 1 and 15 of cycle 2, and then on day 15 of subsequent cycles. The samples will be sent to the City of Hope Clinical Immunobiology Correlative Studies Laboratory and processed, with PBMCs being isolated and stored according to established laboratory SOP.

9.1.5.2 To assess for possible antibody responses, approximately 10 ml of blood will be collected from each patient in red top tubes (free of any anti-coagulant reagent) prior to the start of study treatment, on day 15 of cycle 1, days 1 and 15 of cycle 2, and then day 15 of subsequent cycles. The samples will be sent to the City of Hope Clinical

Immunobiology Correlative Studies Laboratory and processed to yield serum that will be frozen in several aliquots at -80°C.

9.1.5.4 If a patient develops an adverse event that is suspected of being due to an immune response to the NSCs, 3-5 ml of blood will be collected in a red top tube at least twice (separated by approximately 2 days) during the event for analysis of serum cytokine levels via Luminex cytokine panels, as described in section 9.1.4.

9.1.6 Collection of blood samples to perform RCR testing as part of long-term follow

As per section 5.12, prior to surgery, at 3 months, 6 months, 1 year, and annually thereafter, at least 10 ml of blood will be collected from each patient in EDTA treated (lavender top) tubes and sent to CICSL. Samples will be processed with DNA being isolated and stored, according to established laboratory SOP, until RCR testing is performed by PCR.

All blood samples collected for the immunologic correlative studies as per section 9.1.5 and 9.1.6 will be sent to the COH CICSL.

9.2 Measurement of Intracerebral and Plasma Levels of 5-FC, Leucovorin, and 5-FU

In order to characterize the relationship between intracerebral and systemic concentrations of 5-FC and 5-FU at the MTD/MFD of study treatment, all 6 patients in the expansion cohort, who will be treated at the MTD/MFD, will undergo intracerebral microdialysis and collection of blood samples for pharmacokinetic analysis.

9.2.1 Collection of Dialysate samples

9.2.1.1 A microvial can hold up to 200 µl of fluid. It will need to be replaced with a new one every 3 hours (except during the first 24 hours after the 1st doses of 5-FC and leucovorin are taken on day 4, as described in section 5.4.1.4.1).

9.2.1.2 A new syringe containing sterile artificial CSF will be placed in the microdialysis pump daily.

9.2.1.3 A new microvial will be placed in the holder of the catheter's outlet tubing just before the patient takes the 6 am doses of 5-FC (and leucovorin) on day 4. A new microvial will be placed in the holder **every 60 minutes for the next 24 hours**; thereafter microvials will be switched every 3 hours until the patient has completed the 7-day course of 5-FC/leucovorin and the microdialysis catheter is removed.

9.2.1.4 Once the patient has started taking 5-FC/leucovorin, it is important not to flush the catheter unless it becomes clogged, as indicated by dialysate not exiting from the outlet tubing.

While the pump is running, whenever the lid of the microdialysis pump is opened and then closed, the pump automatically goes into a flushing cycle. If the lid of the pump needs to be opened during the 7 day course of 5-FC/leucovorin (eg. in order to place the daily new syringe of artificial CSF in it), then first stop the pump by taking the batteries out, **not** by opening the lid. After the new syringe is in place, close the lid and put the batteries back in. The pump will then resume its flow rate of 1µL/min without going into a flushing cycle.

9.2.1.5 Microvials containing the dialysate samples will be placed on dry ice until they can be moved to an ultralow temperature freezer (≤-70°C) in the Gonda Building, room 1017, where they will be stored until brought to the COH Analytical Pharmacology Core Facility (APCF) for analysis by tandem mass spectrometry (LC-MS/MS).

9.2.2 Collection of blood samples in patients undergoing intracerebral microdialysis

9.2.2.1 Sampling will be performed to define the plasma concentration-time profiles of 5-FC, leucovorin, and 5-FU. On day 4, prior to the patient taking the first doses of 5-FC and leucovorin, a large gauge peripheral catheter (e.g., 19 or 20 gauge angiocath straight set with T-connector, or similar IV access device) will be placed within a vein in the arm of the patient for the collection of pharmacokinetic blood samples. Patency of the sampling catheter should be maintained between blood draws using either a heparin lock (e.g., 10 U/ml in normal saline) or a slow drip of Normal Saline for Injection, USP (e.g., 10 ml/hr).

9.2.2.2 Please see the schedule of blood draws on days 4-8 in section 5.4.1.4.3.

At each sample time point a discard blood volume appropriate for the IV access device must be drawn prior to the sample. Blood (3 ml) will be drawn from a peripheral vein in the patient's arm and collected in plasma tubes containing sodium heparin anticoagulant. Promptly mix the plasma collection tube by gently inverting 6-times and then place it on wet ice, until centrifuged at 1,300 x g for 10 min at 4°C. Samples will be centrifuged for harvesting plasma as soon as possible after collection (within 1 hour). Upon centrifugation, separate the plasma from the blood cells using a pipet and transfer the plasma to an appropriately labeled polypropylene freezer vial. Plasma will be stored frozen at < -70°C until subsequent batch analysis can be performed by the COH APCF via LC-MS/MS.

<u>If the microdialysis catheter becomes non-functional, then blood draws for plasma PKs will stop.</u>

9.43 Brain Tissue Correlates

9.3.1 <u>Determining the fate of the NSCs at time of autopsy</u>

As part of the consenting process, study patients will be asked to give permission for a brain autopsy. The brain autopsy will focus on detecting the presence or absence of NSCs, their location and tumor pathology. Specifically, brain tissue samples will be collected from the center and periphery of the tumor, as well as grossly affected and unaffected areas from distant and contralateral sites. Tissue samples will be formalin fixed and paraffin embedded for sectioning and analysis. When possible, fresh frozen tissue samples will be taken.

NSCs will be detected by PCR methods using specific primers for v-*myc* (only present in the transplanted NSCs). Fluorescence *in situ* hybridization and immunocytochemistry techniques will also be used for the detection and further characterization of the NSCs (including proliferation and differentiation markers).

9.3.1.1 Collecting tumor tissue for future research

If there is resection material available in excess of that needed for standard pathologic studies, this excess material will be used for research purposes.

Excess surgical material will be grown as a dissociated primary cell culture in the Aboody laboratory for NSC migration and toxicity assays under IRB #07074. Additional material will be banked (fixed and frozen) for potential future research under IRB #07074. Such research may include: 1) comparison of gene expression profiles in the tumor and adjacent tissue (if available) prior and after treatment with HB1.F3.CD NSCs/5-FC; 2) presence of immune cells (B-cells, T-cells, macrophages, microglia,

dendritic cells); 3) determination of mitotic index in the tumor by Ki67 immunohistochemistry.

10.0 Study Calendar

	Screening[2]	Cycle 1						Cycle 2[18]			Off Tx Visit[20]	30-Day Post Tx Visit[21]
		Day 1	Day 2	Days 4-10	Day 15	Days 18-24		Day 1 and Day 15	Days 4-10 and 18-24	Day 28[19]		
H&P, Neuro Exam, KPS	X				X	X[17]		X			X	X[22,23]
Informed consent	X[2]											
HLA antibody screening	X[2,3]											
Brain MRI	X[2]	X[7]	X[10]	X						X[19]	X[21]	
Non-contrast brain CT		X[8]										
Toxicity assessment			X		X	X[17]		X			X	X
Collection of blood samples for immunologic correlative studies	X[4,5]				X[4,5,15]			X[4,5, 13,18]			X	
WBC with ANC, hgb, plts	X			X[12]	X	X[17]		X			X	
Serum chemistries and LFTs	X			X[12]	X	X[17]		X			X	
PT/PTT	X											
EKG	X											
CXR	X											
Urine or serum pregnancy test	X											
Tumor resection or biopsy		X										
Placement of a Rickham catheter		X										
Intracerebral administration of CD-expressing NSCs		X[9]			X[16]			X[16]				
Placement of a microdialysis catheter[1]		X										
Administration of 5-FC (and leucovorin, as indicated)				X		X			X			
Collection of Dialysate Samples for Measuring Intracerebral Levels of 5-FC ,LV, and 5-FU[1]				X[13]								
Collection of Blood Samples for 5-FC, LV, and 5-FU Levels[1]				X[14]								
RCR testing	X[6]											

Study tests and procedures may be done +/- 3 days for patient convenience and scheduling. A window period of +/- 1 day is allowed for intracranial administration of NSCs, but there must still be at least an interval of at least

3 days between administration of the NSCs and start of the 7-day course of 5-FC (if a patient receives NSCs 1 day earlier, then it is acceptable to wait 4 days before the patient starts taking the 5-FC.

1 Only dose level 4 patients will undergo intracerebral microdialysis.
2 All screening assessments to be performed within 14 days prior to start of treatment (day of surgery), except for the brain MRI which can be performed within 28 days of registration, and except for signing of the informed consent document and HLA antibody screening, which can be done outside the 14 day window prior to start of study treatment.
3 Blood sample needs to be placed in 1 red top tube and brought at room temperature to the COH Histocompatibility Laboratory.
4 Collect six 6-ml lavender top tubes and send to the COH CICSL.
5 Collect approximately 6 ml of blood in red top tubes and send to the COH CICSL.
6 10 ml of blood will be collected from each patient in EDTA treated (lavender top) tubes and sent to CICSL for RCR testing at the following time points: pre-treatment, 3 months*, 6 months*, 1 year*, and annually thereafter* *+/- 14 days
7 A pre-surgical planning post-registration MRI **may** be performed if indicated by the neuro-surgeon.
8 Only patients who have a microdialysis catheter placed or have only a biopsy performed will undergo a non-contrast CT scan after surgery.
9 At time of surgery
10 A brain MRI will be performed for all patients; this will serve as the baseline MRI.
11
12 On day 3 only.
13 **Place a new microvial in the holder on the outlet tubing every 60 minutes for the first 24 hours** of 5-FC/leucovorin and then every 3 hours until the patient has finished the course of 5-FC/leucovorin.
14 On days 4 and 5, blood samples will be drawn just prior to the 6 am dose of 5-FC/leucovorin and then every 30 minutes during the first 3 hours after that dose is taken by the patient. Additional blood samples will be obtained 4 hours after the 6 am dose and then just prior to the next dose at noon. On days 6, 7, and 8, blood samples will be obtained just before the 6 am dose of 5-FC/leucovorin and then 90 minutes later. If microdialysis catheter becomes non-functional, then blood draws for plasma PKs will stop.
15 Blood samples should be drawn prior to administration of NSCs.
16 Via the Rickham catheter.
17 Day 22 only.
18 Subsequent treatment cycles will be as per cycle 2, except blood samples for **immunologic correlative studies will be collected only on day 15 of a cycle**.
19 End of even cycles only. Day 28 (-3 days). A brain MRI will be performed for all patients.
20 Off treatment assessments to be performed within 7 days after last study agent administration or within 7 days after decision to end treatment. Assessments may continue for ongoing reportable adverse events or events resulting in a dose modification.
21 Imaging to be performed if the prior assessments occurred > 21 days before decision to end treatment and if patient is agreeable to have this procedure. The absence of this MRI with corresponding response assessment will not be considered a protocol deviation.
22 A contact/visit for review of adverse events, KPS, concomitant medication review, and vital status is to be performed at 30 days +/- 2 days after the last dose of study agent is given. The visit may be performed via documented phone conversation with a study nurse or clinician. All participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 30 days of last study agent.
23 KPS and concomitant medications only. Vital signs and Neuro-Physical exam are not required.

11.0 Endpoint Evaluation Criteria/Measurement of Effect

11.1 Response Criteria

The Response Assessment in Neuro-Oncology (RANO) criteria (Wen et al., 2010) will be used to assess response:

Complete Response (CR) Complete disappearance of all enhancing disease (measurable and non-measureable) that is sustained for at least 4 weeks, stable or improved non-enhancing FLAIR/T2 lesions, no new lesions, off corticosteroids (physiologic replacement doses allowed), and neurologically stable or improved.

Partial Response (PR) ≥ 50% decrease of all measurable enhancing lesions, sustained for at least 4 weeks, no progression of non-measurable disease, stable or improved non-enhancing FLAIR/T2 lesions, no new lesions, corticosteroid dose stable or reduced (compared to baseline), and neurologically stable or improved.

Stable Disease (SD) Does not qualify for CR, PR, or PD, stable non-enhancing FLAIR/T2 lesions, stable or reduced corticosteroids (compared to baseline), clinically stable.

Progressive Disease (PD) ≥ 25% increase in enhancing lesions despite stable or increasing steroid dose, increase (significant) in non-enhancing T2/FLAIR lesions that is not attributable to other non-tumor causes, any new lesions, clinical deterioration (not attributable to other non-tumor causes and not due to steroid decrease).

RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1 criteria [36] will be used to assess the response of study patients' systemic disease to eribulin.

12.0 Data Reporting/Protocol Deviations

12.1 Data Reporting

12.1.1 Confidentiality and Storage of Records

Electronic Data Collection will be used for this protocol. The data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

12.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

12.1.3 Data Collection Forms and Submission Schedule

All data will be collected using electronic data collection, stored as indicated in Section 12.1.1, and submitted according to the timelines indicated in Table 12.1.3.

Table 12.1.3 Data Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 7 calendar days of last day of cycle
Adverse Event Report Forms	For cycle 1 only, within 7 calendar days of AE assessment/notification; for all other cycles, within 10 calendar days of AE assessment/notification
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms (concomitant medications, chemistry, hematology, neuro exam, physical exam etc.)	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 calendar days of the protocol defined follow up visit date or call

12.1.3.1 *Eligibility Checklist*

The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and signed by an authorized investigator prior to registering the subject. See Section 4.3 for the registration procedure.

12.2 Protocol Deviations

12.2.1 Deviation Policy

This protocol will be conducted in accordance with COH's "Clinical Research Protocol Deviation Policy" located at http://www.coh.org/dsmc/Documents/Institutional%20Deviation%20Policy.pdf.

Deviations from the written protocol that could increase patient risk or alter protocol integrity require prior IRB approval of a single subject exception (SSE) request. In addition, if contractually obligated, the sponsor must also approve the deviation. IRB pre-approved SSE protocol modifications are considered an amendment to the protocol and not a deviation. The submission of a deviation report is not required.

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from the protocol when necessary so long as such deviation does not threaten patient safety or protocol scientific integrity. Examples include, but are not limited to: a) dose adjustments based on excessive patient weight; b) alteration in treatment schedule due to non-availability of the research participant for treatment; c) laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety. These instances are considered to be deviations from the protocol. A deviation report will be submitted to the DSMC/IRB within five days.

12.2.2 Reporting of Deviations

All deviations will be reported to the COH DSMC within five days. The DSMC will forward to report to the IRB following review.

12.2.3 Resolving Disputes

The COH Investigational Drug Service (IDS) cannot release a research agent that would cause a protocol deviation without approval by the PI. Whenever the protocol is ambiguous on a key point, the IDS should rely on the PI to clarify the issue.

In situations where there is misperception or dispute regarding a protocol deviation among the persons involved in implementing the protocol, it is the responsibility of the PI to resolve the dispute and the PI may consult with the DSMC chair (or designee) to arrive at resolution.

13.0 Statistical Considerations

13.1 Sample Size

The expected sample size is 15-18 patients (minimum=6, maximum= 33, allowing for 3 patients to replace unevaluable/ineligible patients). A sample size of 6 at the MTD will allow us to i) achieve a maximum margin of error for the 90% confidence limits for the DLT rate of 0.35 and ii) see a toxicity with a true rate of 0.25 in approximately 82% of trials.

13.2 Accrual Rate

Given that the first 3 patients entering dose levels 1-3 will enter sequentially after the prior patient completes cycle 1, the study time is mostly determined by cycle length. It is anticipated that this study will complete accrual within approximately 30-36 months.

13.3 Statistical Design and Analysis

13.3.1 Design:

The dose escalation plan is based on a standard 3+3 design and is described in sections 5.6 and 5.7.

13.3.2 Objectives

Primary Objectives:

- To define the phase II recommended dose of intracerebrally administered CD-expressing NSCs in combination with oral 5-FC and leucovorin. This will be based on the maximum tolerated dose (MTD) , or if the MTD is not met, the maximum feasible dose (MFD), and toxicity profile in patients with recurrent high-grade gliomas.
- To determine the feasibility of treating study patients with more than 1 dose of NSCs followed by 7-day courses of 5-FC and leucovorin.

Secondary Objectives:

- To assess for possible development of NSC immunogenicity (anti-NSC T cell and/or antibody responses) with repeat doses of NSCs.
- To characterize the relationship between intracerebral and systemic concentrations of 5-FC and 5-FU at the MTD/MFD level.
- To describe the clinical benefit (defined as stable disease, partial response, or complete response) of this treatment regimen.
- To determine, at time of autopsy, the fate of the NSCs.

13.3.3 Endpoints

Primary Endpoints:

- Safety and feasibility:

- For dose escalation: dose limiting toxicities;
- Toxicity profile: all attributable toxicities.

- Feasibility:
  - Clinically significant allergic reaction to NSCs
  - Mechanical issues with repeat administration of NSCs via Rickham

Secondary Endpoints:

- Immunogenicity: evaluate patient blood samples for the development of T cell responses and antibodies against the NSCs using TcR Vβ spectratyping, CD 107 degranulation assays, and flow cytometry.
- Pharmacokinetics: the following pharmacokinetic variables will be assessed: Tmax, Cmax, AUC, t ½, and the ratio of the AUC of 5-FU in dialysate to plasma, and the ratio of the AUC of 5-FC in dialysate to plasma.
- Tumor response.
- Determine the fate of the NSCs: NSC persistence.

13.3.4 Analysis:

Tables will be created to summarize all toxicities and side effects by dose, course, organ, severity (by NCI CTCAE version 4.0) and attribution. Rates and associated 90% confidence limits will be estimated for the DLT rate at the MTD/MFD and the rate of clinical benefit. Descriptive statistics will be provided for study patient demographics.

Pharmacokinetic (PK) data from the patients who undergo intracerebral microdialysis will be summarized using descriptive statistics and graphical methods. The primary PK parameters of interest will be the Cmax and AUC of 5-FC and 5-FU, measured both in the dialysate samples and in plasma. Leucovorin levels in dialysate samples will likewise be compared to plasma leucovorin levels. The 5-FC and 5-FU PK data will also be compared to the PK data obtained from patients who underwent intracerebral microdialysis in the first study of CD-expressing NSCs (IRB# 08002) to determine if there is a dose effect at the MTD/MFD compared to lower doses of NSCs. All summaries will be exploratory in spirit, with the goal of developing further questions regarding the modulation of therapy, or regarding reasons for efficacy or lack of efficacy.

Data from assessing for possible development of NSC immunogenicity with repeat exposure and performing autopsies to determine the fate of the NSCs, will be presented in an exploratory fashion using descriptive statistics and graphical methods.

14.0 Human Subject Issues

14.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.2 Recruitment of Subjects

Study subjects will be recruited from patients undergoing treatment for their brain tumors at the City of Hope Cancer Center.

14.3 Advertisements

Advertisements to include print, media (radio, television, billboards), telephone scripts, lay summary to be posted on City of Hope's public Clinical Trials On-Line^{SM} website, etc., will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

14.4 Study Location and Performance Sites

This study will be performed at COH.

14.5 Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The study will record individual side effects to study treatment, brain interstitial and plasma levels of 5-FC, leucovorin, and 5-FU, radiologic findings, immunological correlative study results, and autopsy findings (if possible), and these will be linked to the subject's identity using a coded study number. The principal investigator, co-investigators, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results.

14.6 Financial Obligations and Compensation

There will be no increased financial obligations incurred by subjects because of participating in this study. Neither the research participant nor the insurance carrier will be responsible to pay for the research procedures related to this study. For example, the NSCs, 5-FC, leucovorin, and microdialysis equipment will be provided free of charge to the patient. Expenses due to hospital stay in excess of what is standard for the surgical procedure will not be billed to the patient or her/his insurance company. All laboratory tests that will be done solely for research purposes will be covered by the study sponsor. All standard-of-care laboratory tests and neuro-imaging will be billed to the subjects' insurance company. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

14.7 Informed Consent Processes

The Principal Investigator or IRB-approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, s/he will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

Before signing the study consent form, HIPAA authorization form and the Experimental Subject's Bill of Rights, research subjects will undergo an assessment of their comprehension of the study by the Research Subject Advocate. Should sufficient doubt be raised regarding the adequacy of comprehension, further clarifications will be made and the questionnaire repeated until a satisfactory result is obtained. Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of discussion, education and proctoring will be ineligible for enrollment. For those subjects who do comprehend the fundamental aspects of the study, consent will be obtained and documented, followed by eligibility testing. The research team will review the results of eligibility testing and determine if the subject is a candidate for study enrollment.

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Appendix A Karnofsky Performance Status

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

Karnofsky Performance Status
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 Cares for self; unable to carry on normal activity or do active work
KPS 60 Requires occasional assistance, but is 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