CITY OF HOPE NATIONAL MEDICAL CENTER
1500 E. DUARTE ROAD
DUARTE, CA 91010

DEPARTMENT OF MEDICAL ONCOLOGY

TITLE: A Phase I Study of Intracranially Administered Carboxylesterase-Expressing Neural Stem Cells in Combination with Intravenous Irinotecan in Patients with Recurrent High-Grade Gliomas

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 14108 **Protocol Dated: 08/28/19**

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SITE: Brain
STAGE (If applicable): Recurrent High Grade
MODALITY: Neural Stem Cells/Chemotherapy
TYPE: Phase I
PRINCIPAL INVESTIGATOR: Jana Portnow, M.D.

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Study Statistician: Suzette Blanchard, Ph.D.

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COH Amendment 6	Title Page Dated 07/07/17	Version 06
COH Amendment 7	Protocol Dated 01/30/18	Version 07
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SPONSOR/IND NUMBER: City of Hope/ 16265.

PRINCIPAL INVESTIGATOR: Jana Portnow, MD
Designs protocol, responsible for study conduct and data analysis

COLLABORATING INVESTIGATOR(S): Key individual(s); Assist in study design and development; May be involved in analysis of data	Behnam Badie, MD Massimo D'Apuzzo, MD, PhD Julie Ressler, MD Timothy Synold, Pharm D
PARTICIPATING CLINICIANS: Enrolls and follows eligible patients on study; Not involved in study development or data analysis (Physician or research support staff)	Mike Chen, MD, PhD Rahul Jandial, MD, PhD Mihaela Cristea, MD Marianna Koczywas
STUDY STATISTICIAN:	Suzette Blanchard, PhD
PARTICIPATING SITES:	City of Hope
STUDY SPONSOR AND MONITOR:	City of Hope

Experimental Design Schema

Dose Escalation Schema

NSC Dose Level	Initial Enrollment	If 1 DLT, Addt'l Enrollment	NSC Dose	NSC and Irinotecan[a] Administration Week			
				1	2	3	4
-1	3	3	2.5×10^7	X*		**	
1[b]	3	3	5×10^7	X		**	
2[c]	3	3	5×10^7	X		X	
3	3	3	1×10^8	X		X	
4[d]	3	3	1.5×10^8	X		X	
Expansion Cohort [e]	8		MTD/MFD	X		X	

* "X" denotes treatment weeks for NSCs and irinotecan. NSCs are administered intracranially on Day 1 and 15, followed by intravenousirinotecan, two days later, on Days 3 and 17 of a 28 day cycle.

**Patients on dose levels 1 and -1will only receive 1 dose of NSCs, but they will continue to receive irinotecan every 2 weeks as long as they are tolerating it well, and there is no evidence of tumor progression.

[a]**Dose schedule A, B, and C** will be the NSC dose schema in combination with an irinotecan dose of 180mg/m^2, 150 mg/m^2, or 125 mg/ m^2, respectively. We will start with irinotecan dose schedule A, de-escalating through doses B and C in order as necessary.

b The starting NSC dose level is dose level 1. The first 6 study participants will undergo intracerebral microdialysis to measure levels of irinotecan and NSC-mediated SN-38 production in the brain. Since the dose of NSCs remains the same for dose level 1 and 2, all of these first 6 study patients would receive the same dose of NSCs and irinotecan when undergoing intracerebral microdialysis, even allowing for escalation to dose level 2.

c Starting with dose level 2, a Rickham reservoir/catheter system will be placed at the time of surgery for administering repeat doses of NSCs.

d Six patients will be treated on the maximum tolerated dose(MTD), or, if the MTD is not reached, at the maximum feasible dose (MFD). Since it is anticipated that the MTD/MFD will be 150 million NSCs (dose level 4), the 6 patients who will be treated on dose level 4 will undergo intracerebral microdialysis and be counted as part of the expansion cohort.

e An expansion cohort of 8 patients will be treated at the MTD or MFD to gain more experience with the tolerability of this treatment regimen. These patients will also undergo intracerebral microdialysis to assess how much SN-38 is produced by highest dose of NSCs and evaluate the biologic activity of the NSCs by directly measuring how much the addition of NSCs to treatment with irinotecan increases intracerebral levels of SN-38. As such, 4 patients in the expansion cohort will undergo intracerebral microdialysis after receiving NSCs on the day of surgery followed 2 days later by IV irinotecan, and the other 4 patients in this cohort will not receive NSC administration on the day of surgery. Instead they will be given IV irinotecan 2 days after surgery and dialysate samples will be collected from them to determine how much SN-38 is present in the brain from treatment with

IV irinotecan alone. These 4 patients will then receive their first dose of NSCs through the Rickham on day 15 of cycle 1 followed by irinotecan on day 17.

Simplified Treatment Schema for Cycle 1

Day 1

Tumor resection or biopsy is performed. hCE1m6-NSCs are administered intracranially.^ A Rickham catheter is placed (except in patients on dose levels 1 or -1). The first 6 study patients and those in the expansion cohort: microdialysis catheter(s) is/are placed, and a post-operative CT scan is obtained to confirm correct placement. ^Four participants in the expansion cohort will not receive Day 1 NSCs; they will initiate NSC administration on Day 15.

Day 3

Irinotecan is administered intravenously. Patients undergoing intracerebral microdialysis: serial dialysate and bloodsamples are collected.

Day 15

hCE1m6-NSCs are administered intracranially via the Rickham catheter.* Blood is drawn for immunologic correlative studies.* * Patients in dose level 1 or -1 will not receive NSCs on Day 15, and blood samples for immunologic correlative studies will be drawn on Day 17 when these patients return to clinic to receive irinotecan.

Day 17

Irinotecan is administered intravenously.

Protocol Synopsis

Protocol Title:
A Phase I Study of Intracranially administered Carboxylesterase-Expressing Neural Stem Cells in Combination with Intravenous Irinotecan in Patients with Recurrent High Grade Gliomas.
Brief Protocol Title for the Lay Public (if applicable):
A Phase I study of Neural Stem Cells and Irinotecan in Patients with 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 highly invasive nature and relative resistance to chemotherapy and radiation. Human neural stem cells (NSCs), modified to express a therapeutic transgene, hold great promise for glioma therapy due to their inherent tumor-tropic properties, enabling their use as vehicles for delivering chemotherapy directly to infiltrating glioma cells.This NSC-mediated delivery approach has the potential to overcome obstacles that limited gene therapy efficacy in the past and minimize off target toxicities of current treatment approaches. 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. In our first-in-human pilot study of cytosine deaminase (CD)-expressing NSCs given in combination with oral 5-fluorocytosine (5-FC), we showed that 1 dose of CD-expressing NSCs followed by a 7 day course of 5-FC in recurrent high-grade glioma patients is safe and feasible. With intracerebral microdialysis, we documented proof-of-concept—that the NSCs converted the prodrug 5-FC to its active metabolite 5-FU locally in the brain. Results of immunologic correlative studies documented no findings 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, and autopsy results from 2 study patients showed evidence that NSCs had traveled to distant sites of tumor in the brain. This well characterized, v-*myc*-immortalized, clonal, human fetal NSC line has been further modified by adenoviral transduction to secrete a highly active modified human form (hCE1m6) of carboxylesterase (CE) for efficient conversion of irinotecan to its more potent metabolite, SN-38.We hypothesize that in patients with recurrent high-grade gliomas, CE-secreting NSCs will distribute throughout the primary tumor site as well as co-localize with infiltrating tumor cells within 2 days of administration. Study patients will then be treated with intravenously administered irinotecan. The CE-secreting NSCs will locally convert irinotecan to SN-38, thereby generating concentrated cytotoxicity at sites of tumor in the brain.
Objectives:

Primary Objectives:

To define the recommended phase II dose (RP2D) of intracraniallyadministered hCE1m6-NSCs in combination with intravenous irinotecan in patients with recurrent high grade glioma. The RP2D will be based on the maximum-tolerated dose (MTD), or if the MTD is not reached, the maximum feasible dose (MFD),and the full toxicity profile including toxicities associated with repeat doses of NSCs.

Secondary Objectives:

- To describe the relationship between hCE1m6-NSC dose and SN-38 concentrations in brain interstitium.
- To characterize the relationship between intracerebral and systemic concentrations of irinotecan and SN-38.
- To investigate the biologic activity of hCE1m6 NSCs by comparing SN-38 concentrations in the brain after treatment with hCE1m6 NSCs and irinotecan versus irinotecan alone.
- To assess for possible development of adenovirally transduced NSC immunogenicity after first exposure and with repeat doses of NSCs.
- To describe the clinical benefit (defined as stable disease, partial response, or complete response) in patients who receive treatment with repeat cycles of NSCs and irinotecan.
- To determine, at time of autopsy, the fate of the NSCs.

Study Design:

This phase I clinical trial will define the recommended phase II dosing (RP2D)based on dose-limiting toxicities (DLTs)and the full toxicity profile of the combination of hCE1m6-NSCs and irinotecan using a standard “3 + 3” dose escalation schema in patients with recurrent highgrade gliomas who are undergoing a debulking craniotomy or stereotactic brain biopsy. Except for dose levels 1 and -1, where only 1 dose of NSCs will be given(these patients can continue toreceive treatment with irinotecan every 2 weeks),NSCswill be administered intracranially on days 1 and15, andirinotecan will be given intravenously 2 days later, on days 3 and 17 of a 28 day cycle.With subsequent cohorts of patients, the NSC dose will be increased as tolerated, but the dose of irinotecan will remain the same unless DLTs occur that necessitate lowering the dose of irinotecan.A Rickham reservoir/catheter system will be placed at the time of surgery for delivering repeat doses of NSCs intracranially. MRIs and PET/CT scans of the brain will be performed at least every two months (at the end of every even cycle of study treatment to evaluate response).

Three doses of NSCs will be assessed. The starting dose will be 5 x 10^7NSCs, which was well-tolerated in the first-in-human study of CD-expressing NSCs. Due to volume limitations for intracerebral administration, the highest dose of NSCs to be tested will be 1.5 x 10^8 cells.Study participants will receive repeat cycles of study treatment(dose level 1 and -1 patients will only receive repeat doses of irinotecan) as long as they are tolerating the treatment well, and there is no evidence of progressive disease.

An important secondary objective of this study will be to determine the biologic activity of the hCE1m6-NSCs by measuring concentrations of SN-38 in brain interstitium using intracerebral microdialysis. The first 6 patients to enroll in the study and the last 8 patients in the expansion cohort will undergo microdialysis to collect data regarding intracerebral levels of irinotecan and SN-38 when patients are treated with the lowest and highest NSC doses. Please note, the same dose of NSCs (5 x 10^7) is given to patients on dose levels 1 and 2, so whether the first 6 patients are all treated on dose level 1 or the first 3 dose level 1 and the next 3 on dose level 2, they will all receive the same dose of NSCs.

Once the maximum tolerated doseof the combination therapy has been determined, or, if the maxiumum tolerated dose is not met, the maximum feasible dose,patients in the expansion cohort will undergo intracerebral microdialysis to investigate if treatment with a higher dose of NSCs results in more SN-38 in the brain and to directly determine how much the addition of NSCs to treatment with irinotecan increases

intracerebral levels of SN-38. As such, 4 patients in the expansion cohort will undergo intracerebral microdialysis after receiving NSCs at the time of surgery followed 2 days later by IV irinotecan, but the other 4 patients in this cohort will not receive NSCs on the day of surgery. Instead they will be given IV irinotecan 2 days after surgery, and dialysate samples will be collected from them to determine how much SN-38 is present in the brain from treatment with IV irinotecan alone. These 4 patients will then receive their first dose of NSCs through the Rickham on day 15 of cycle 1 followed by irinotecan on day 17.Another key secondary objective will be to assess for possible development of immune responses to these allogeneic, adenovirally transduced NSCs. Serial blood samples will be collected during treatment cycles to assess for the possible development of T cell and antibody responsesto the NSCs and/or cell surface-expressed adenoviral proteins, and we will follow adenoviral antibody titers.

Other secondary objectives include describing the clinical benefit in patients who receive treatment with repeat cycles of NSCs and irinotecan by obtaining MRIs and PET/CT scans of the brainat least every two months to assess response and, when feasible, determining at time of autopsy the fate of the NSCs.

Endpoints:

Safety and Feasibility: 1) For Dose Escalation: Dose Limiting Toxicities

2) Toxicity Profile: All Attributable Toxicities

Pharmacokinetics: Cmax and AUC of irinotecan and SN-38 in dialysate and plasma.

Immunogenicity: Evaluate patient blood samples for the development of T cell responses and antibodies against the NSCsand/or cell surface-expressed adenoviral proteins using TcR Vβ spectratyping, CD 107 degranulation assays, and flow cytometry. Measure patients' serial adenoviral antibody titers.

Clinical Benefit: Tumor response based on brain MRI and PET/CT scan results.

Determine the Fate of the NSCs: NSC Persistence

Sample Size:

The anticipated sample size is 29patients: minimum=6; maximum=53 (allowing for 42 patients for dose escalation through 5 possible dose levels of NSCs and 3 dose schedules of irinotecan, estimating ~3 patients to replace unevaluable/ineligible patients and an additional 8 patients for the expansion cohort).

Estimated Duration of the Study:

36monthsto complete accrual

Summary of Subject Eligibility Criteria:

Inclusion Criteria

- Patient has a prior, histologically-confirmed, diagnosis of a grade III or IV glioma, 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).
- Imaging studies show evidence of recurrent tumor(s). If a patient is going to be enrolled to dose level two or higher, the patient must have a component of supratentorial disease (so as to enable placement of a Rickham reservoir/catheter) that is amenable to resection or biopsy.

- 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.
- Patient's high-grade glioma has recurred or progressed after prior treatment with brain radiation and temozolomide.
- Patient must be at least 18 years old and less than 70years old.
- Patient has a Karnofsky Performance Status of ≥ 70%.
- No limit to prior number of therapies.

Exclusion Criteria

- Patient is homozygous for the UGT 1A1*28 allele and/or has Gilbert's disease.
- Patient must not be taking any hepatic enzyme-inducing anticonvulsants (phenytoin, carbamazepine, phenobarbital, primidone, oxcarbazepine) for at least 2 weeks prior to enrollment.
- Patients has anti-HLA antibodies specific for HLA class I antigens expressed by the hCE1m6-NSCs.
- Patient is taking flucytosine.

Investigational Product Dosage and Administration:

Dose Escalation Schema

NSC Dose Level	Initial Enrollment	If 1 DLT, Addt'l Enrollment	NSC Dose	NSC and Irinotecan[a] Administration Week 1	2	3	4
-1	3	3	2.5×10^7	X*		**	
1[b]	3	3	5×10^7	X		**	
2[c]	3	3	5×10^7	X		X	
3	3	3	1×10^8	X		X	
4[d]	3	3	1.5×10^8	X		X	
Expansion Cohort [e]	8		MTD/MFD	X		X	

* "X" denotes treatment weeks for NSCs and irinotecan. NSCs are administered on Day 1, and irinotecan, 180 mg/m^2/dose, is administered two days post-NSCs on Day 3 of each treatment week. The length of a treatment cycles is 28 days.

**Patients on dose levels 1 and -1 will only receive 1 dose of NSCs, but they will continue to receive irinotecan every 2 weeks as long as they are tolerating it well, and there is no evidence of tumor progression.

[a]**Dose schedule A, B, and C** will be the NSC dose schema in combination with an irinotecan dose of 180mg/m^2, 150 mg/m^2, or 125 mg/ m^2, respectively. We will start with irinotecan dose schedule A, de-escalating through doses B and C in order as necessary.

b The first 6 study participants will undergo intracerebral microdialysis to measure levels of irinotecan and NSC-mediated SN-38 production in the brain. Since the dose of NSCs remains the same for dose level 1 and 2, and microdialysis will be performed only with the first dose of irinotecan, all of these first 6 study patients would

receive the same dose of NSCs and irinotecan when undergoing intracerebral microdialysis, even allowing for escalation to dose level 2.

c Starting with dose level 2, a Rickham reservoir/catheter system will be placed at the time of surgery for administering repeat doses of NSCs.

d Six patients will be treated on at the maximum tolerated dose (MTD), or, if the MTD is not reached, at the maximum feasible dose (MFD). Since it is anticipated that the MTD/MFD will be 150 million NSCs (dose level 4), the 6 patients who will be treated on dose level 4 will undergo intracerebral microdialysis and be counted as part of the expansion cohort.

e An expansion cohort of 8 patients will be treated at the MTD or MFDto gain more experience with the tolerability of this treatment regimen. These patients will undergo intracerebral microdialysis to assess how much SN-38 is produced by highest dose of NSCs and evaluate the biologic activity of the NSCs by directly measuring how much the addition of NSCs to treatment with irinotecan increases intracerebral levels of SN-38. As such, 4 patients in the expansion cohort will undergo intracerebral microdialysis after receiving NSCs on the day of surgery followed 2 days later by IV irinotecan, and the other 4 patients in this cohort will not receive NSC administration on the day of surgery. Instead they will be given IV irinotecan 2 days after surgery and dialysate samples will be collected from them to determine how much SN-38 is present in the brain from treatment with IV irinotecan alone. These 4 patients will then receive their first dose of NSCs through the Rickham on day 15 of cycle 1 followed by irinotecan on day 17.

Clinical Observations and Tests to be Performed:

Collection of dialysate and blood samples to determine the neuropharmacokinetics of irinotecan and SN-38. *Only in patients who undergo intracerebral microdialysis*—Onday 3 of cycle 1, dialysate samples will be collected every 60 minutes for 24 hours and then every 3 hours for the next 24 hours. Blood samples will be drawn just prior to the start of the irinotecan infusion and at 90 minutes (just prior to the end of the infusion), and then at 30 minutes, 1, 2, 4, 8, 24, and 48 hours after the end of the infusion.

Collection of blood samples for immunologic correlative studies: To assess for possible development of T cell and antibody responses to the NSCs and/or cell surface-expressed adenoviral proteins, adenoviral vector (Ad5) antibody titers and total adenoviral antibody titers, as well as presence of the NSCs and hCE1m6 in the systemic circulation, blood samples will be drawn: prior to surgery, on day 15 of cycle 1 (prior to administering the 2nd dose of NSCs), days 1 and 15 of cycle 2,, and then only on day 15 of subsequent treatment cycles.

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

Brain MRIs and PET/CT scans to assess response: At least every 2 months.

Statistical Considerations:

Tables will be created to summarize all toxicities and side effects by dose, course, organ severity (by NCI CTCAE version4.0), and attribution. Rates and associated 95% Clopper Pearson confidence limits will be estimated for the DLTsand clinical benefit at the RP2D.Descriptive statistics will be provided for study patient demographics. Pharmacokinetic data from patients who undergo intracerebral microdialysis will be summarized using descriptive statistics and graphical methods.The biologic activity of the hCE1m6-NSCs, based on comparison of microdialysis SN38 PK data from patients receiving NSCs + irinotecan vs patient s receiving irinotecan alone will be assessed using a one-sided two-sample t test.Regression analysis will be used to assess the relationship between hCE1m6-NSC dose and SN-38 concentrations in brain interstitium using microdialysis data from the patientstreated with the initial NSC dose (n=6) and from the patients in the expansion cohort treated with the highest NSC dose (n=4).Other secondary objectives include assessing for possible development of NSC immunogenicity after first and repeat exposuresand—when feasible—evaluating the fate of the NSCs at autopsy. These results will be summarized using descriptive statistics and graphical methods.
Sponsor:
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Table of Contents

Tables

List of Abbreviations

Abbreviation	Meaning
AE	Adverse event
Ad5	Adenoviral vector Ad5
AUC	Area under the curve
BBB	Blood brain barrier
CD	Cytosine deaminase
CE	Carboxylesterase
CFR	Code of Federal Regulations
CICSL	Clinical Immunobiology Correlative Studies Laboratory
CNS	Central nervous system
COH	City of Hope
CR	Complete response
CRA	Clinical research associate
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic acid
DSMC	Data Safety Monitoring Committee
FC	Fluorocytosine
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
GCP	Good clinical practice
HLA	Human leukocyte antigen
IB	Investigator Brochure
i.c.	intracranial
ICD	Informed consent document
IDS	Investigational Drug Services
IHC	Immunohistochemistry
IND	Investigational New Drug
IRB	Institutional Review Board
KPS	Karnofsky Performance Status
LC-MS	Liquid chromatography – mass spectrometry
MD	Microdialysis
MFI	Mean fluorescence intensity
MRI	Magnetic resonance imaging
MS	Mass spectrometry
MFD	Maximum feasible dose
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NSC	Neural stem cell
PBMC	Peripheral blood mononuclear cell
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PD	Progressive disease
PI	Principal investigator
PMT	Protocol Monitoring Team
PR	Partial response
RCR	Replication competent retrovirus
RP2D	Recommended phase II doses
SAE	Serious adverse event
SD	Stable disease
SOP	Standing operating procedure
SWI	Susceptibility weighted imaging
TE	Echo time
USPIO	Ultrasmall superparamagnetic iron oxide

1 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

Primary Objectives

To define the recommendedphase II doses (RP2D) of intracranially administered hCE1m6-NSCs in combination with intravenous irinotecan in patients with recurrent high grade glioma. The RP2D will be based on the maximum-tolerated dose (MTD), or if the MTD is not reached, the maximum feasible dose (MFD), and the full toxicity profile including toxicities associated with repeat doses of NSCs.

Secondary Objectives

- To describethe relationship between hCE1m6-NSC dose and SN-38 concentrations in brain interstitium.
- To characterize the relationship between intracerebral and systemic concentrations of irinotecan and SN-38.
- To investigate the biologic activity of hCE1m6 NSCs by comparing SN-38 concentrations in the brain after treatment with hCE1m6-NSCs and irinotecan versus irinotecan alone.
- To assess for possible development of adenovirally transduced NSC immunogenicity after first exposure and with repeat doses of NSCs.
- To describe the clinical benefit (defined as stable disease, partial response, or completeresponse) in patients who receive treatment with repeat cycles of NSCs and irinotecan.
- To determine, at time of autopsy, the fate of the NSCs.

2 BACKGROUND AND RATIONALE

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 glioma therapy due to their inherent tumor-tropic properties and their potential use as vehicles for delivering chemotherapy directly to infiltrating glioma cells. 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.

In our recently completed first-in-human pilot study of the parent NSC line (cytosine deaminase(CD)-expressing NSCs given in combination with oral fluorocytosine (5-FC), we showed that treatment with 1 dose of CD-expressing NSCs followed by a 7 day course of 5-FC was safe and feasible in patients with recurrent high-grade gliomas. With intracerebral microdialysis, we documented proof-of-concept—that the NSCs converted the prodrug 5-FC to its active metabolite 5-FU locally in the brain. Results of immunologic correlative studies documented no findings 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, and autopsy results from 2 study patients showed evidence that NSCs had traveled to distant sites of tumor in the brain.

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 a v-*myc*-immortalized human NSC line (HB1.F3.CD) that has been adenovirally transduced to express a highly active modified human form (hCE1m6) of carboxylesterase (CE) for efficient conversion of the prodrug irinotecan to its more potent metabolite, SN-38. The 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.

We hypothesize that in patients with recurrent high-grade glioma, hCE1m6-NSCs will distribute throughout the primary tumor site as well as co-localize with infiltrating tumor cells within 2 days of administration. Study patients will then be treated with intravenously administered irinotecan. The CE-secreting NSCs will locally convert irinotecan to SN-38 (Figure 1), thereby generating concentrated cytotoxicity at sites of tumor in the brain.

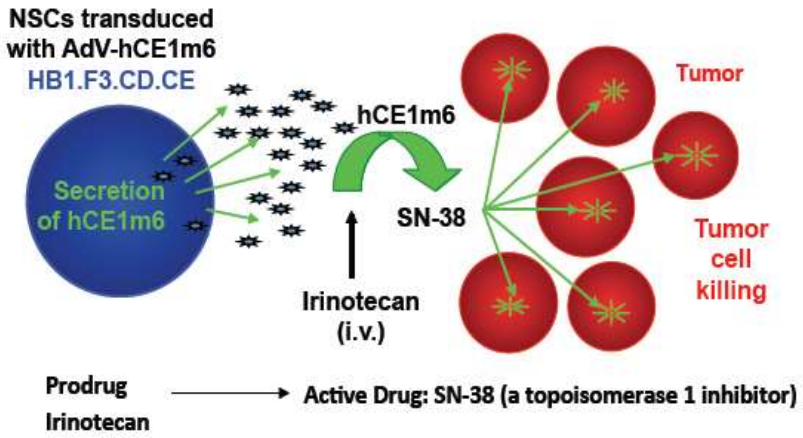


Figure1. Schematic showing NSCs secreting CE enzyme, which converts irinotecan into the more cytotoxic SN-38.

Obstacles to Successful Brain Tumor Therapy

Despite recent advances in molecularly targeted therapies for cancer, brain tumors remain a serious 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. 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 CNS. 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 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 ability 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.

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, 2000 #247}, apoptotic agents {Kim, 2005 #653;Shah, 2005 #716}, anti-angiogenic agents {Kim, 2005 #222}, monoclonal antibodies (Frank et al., 2009), interleukins {Benedetti, 2000 #572;Ehtesham, 2002 #601;Yuan, 2006 #2069}, and IFN- β {Dickson, 2007 #1521}.

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, 2004a; Lee et al., 2007a, 2007b) and spinal cord injury (Kim et al., 2007).

Preclinical Data

2.1.1 Human NSC Tumor Tropism

Human NSCs possess an inherent ability to distribute throughout a tumor mass and can target distant tumor foci. We (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 intravasculary 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, 2000 #557;Kim, 2005 #653}, medulloblastoma {Kim, 2006 #1523;Shimato, 2007 #2970}, and melanoma brain metastases {Aboody, 2006 #2213}. Migration of HB1.F3.CD NSCs is not affected by the presence of dexamethasone or prior radiation to the brain (Aboody, unpublished data). 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, 2007 #13162;Kendall, 2008 #16627;Aboody, 2008 #13268;Mingozzi, 2007 #29278;Muller, 2006 #676;Passier, 2008 #29292;Bhagavati, 2008 #29293}. Studies with HB1.F3 cells that were adenovirally-transduced with a transgene to express rabbit(r) CE, (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 found in non-tumor-bearing brain, kidney, heart, intestine, or skin (Aboody, et al., 2006c).

2.1.2 Immortalized Human NSCLines 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, 2007 #13201}. All of our studies haveincluded 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 1 year following NSC administration {Kim, 2004 #654;Lee, 2007 #1518;Lee, 2007 #1519;Kim, 2007 #1517;Danks, 2007 #1515;Yasuhara, 2006 #1522;Kim, 2006 #1527;Kim, 2006 #1523;Aboody, 2006 #2212;Kim, 2005 #1538;Kim, 2005 #653;Chu, 2005 #1531;Kim, 2004 #654}.

2.1.3 NSCImmunogenicity 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 C57B 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 immuno-rejected in the first 2–3 weeks after administration. When NSCs are used as delivery vehicles, they do not need to engraft. They must only survive long enough to mediate the effective therapy. Therefore, although the NSCs may be immuno-rejected over time, this would not likely happen before the end of a treatment cycle.

HLA typing results of HB1.F3 cells found that while these NSCs do have HLA class I antigens (A*01, A*31, B*07, B*15, C*07), they do not express HLA class II antigens (DRB1*10, DRB1*13, DQB1*05, DQB1*06, DPB1*02, DPB1*15). Other investigators(Kim, 2009) have reported extremely low levels of expression of HLA class II antigens by HB1.F3 cells. In the presence of tumor, the NSCs do not appear to differentiate, and therefore, they would not express HLA class II antigens. Whether or not the clinical utility of these NSCs will be limited by development of immune responses from repeat exposure to these allogeneic NSCs, which have been further modified by adenoviral transduction to also express CE, is unknown (and will be investigated in this phase I study)but preclinical data indicate that the immunogenicity of the HB1.F3.CD NSC line is not prohibitive.

2.1.4 Iron Particle Cellular MRI for Real-Time Assessment of NSC Biodistribution

Ultrasmall superparamagnetic iron oxide (USPIOs) preparations can enable non-invasive, real-time tracking of NSCs by MRI. Inprevious studies, we demonstrated the effectiveness of MRI to track NSCs labeled with 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 (Neuwelt 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 NSCs. We have demonstrated in orthotopic glioma xenograft mouse models that Feraheme-labeled HB1.F3.CD.hCE1m6.NSCs or Fehe-NSCs) can be detected in a typical 7 Tesla mouse imaging voxel as hypointense signal (black)after intracranial (i.c.) administration (Fig. 2A-D). Note that the Fehe-NSCs (blue) distributed within tumor (dark pink) and at invasive tumor edges and foci (Fig. E-H), corresponding with hypointense signal by MRI. Tumor cells confirmed by staining with eGFP-DAB (brown, Fig. I-L).

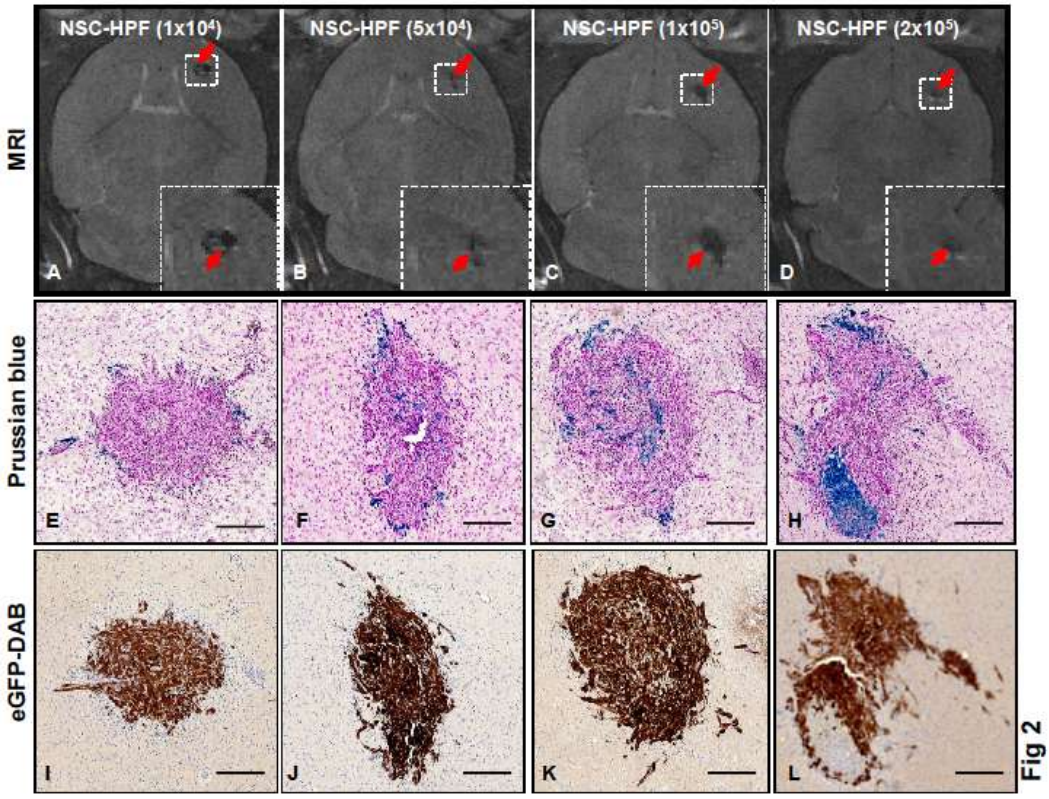


Fig 2

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 human glioma 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 obtained 4 days after NSC administration. Red arrows indicate hypointense (black) signals 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 Trans 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 Trans Med, in press, 2013).

2.1.5 Preclinical Development of hCE1m6, A Modified Form of Human CE

Comparison of rabbit liver CE (rCE) to human CE (hCE1) revealed that the former enzyme more efficiently cleaved irinotecan to SN-38 (Danks, Morton et al. 1999; Bencharit, Morton et al. 2002). The reaction catalyzed by CE requires a relatively anhydrous environment, thus CEs have evolved to accommodate this by having a deep catalytic site, termed the catalytic gorge. Crystallographic studies revealed that for the human isozyme, large substrates such as irinotecan are unable to enter the catalytic gorge due to the lack of flexibility of the amino acids present in nearby loop domains (Bencharit, Morton et al. 2003). Based on these observations and extensive enzymologic studies carried out on CE, a chimera was designed incorporating sequences from the rabbit CE (rCE) enzyme into the human isozyme, hCE1 (Wierdl, Tsurkan et al. 2008). Eight amino acids were substituted in this engineered recombinant hCE which was termed hCE1m6 (Hatfield, Wierdl et al. 2008; Wierdl, Tsurkan et al. 2008). For preclinical development, our group chose to test both the rCE and hCE1m6 in parallel. The CE that performed the best in vitro and in preliminary in vivo microdialysis studies was to be carried forward in more extensive animal studies.

Our data supported the equivalence of hCE1m6 to rCE: (1) both enzymes had comparable enzymatic activity *in vitro*, (2) both equally sensitized glioma cells to irinotecan *in vitro,* (3) the conversion of irinotecan to SN-38 was roughly the same in rat brain when each were expressed by intracranially-administered NSCs and administered intracranially. Given the equivalency of the two enzymes in a variety of studies, the final choice to use hCE1m6 was based primarily on the potential for eliciting an immune response in humans using a lymphocyte degranulation assay. The hCE1m6-expressing NSCscaused less degranulation by CD4+ t cells than those expressing rCE. The difference in the degranulation response was statistically significant at p = 0.028.

The gene therapy agent that will be used in this phase I study is HB1.F3.CD. hCE1m6 NSCs, which will be produced by thawing HB1.F3.CD NSCs from the Master Cell Bank at passage (p)20 and expanding to p27-p28 under standard culture conditions at a seeding density of 2 x 104 cells/cm^2; 30-40 ten layer cell factories will be used. The NSCs will then be transduced with clinical grade hCE1m6 adenovirus (at a multiplicity of infection of 20) from the Master Viral Seed Stock produced at the City of Hope Center for Biomedicine and Genetics and harvested 24 hours later for freeze down in Cryostor 10 at 50 million cells per vial.

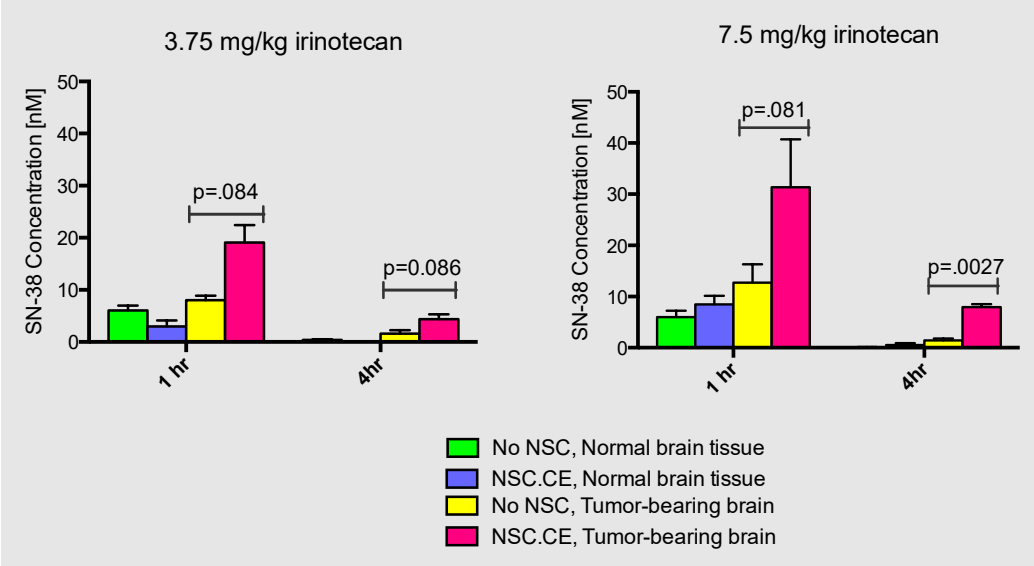
2.1.6 hCE1m6-Expressing-NSCs Increase the Concentration of SN-38 in Tumor-Bearing Brain Tissue

The purpose of this experiment was to determine irinotecan and SN-38 levels at various times afteririnotecan treatment in normal and tumor-bearing brain tissue in the presence and absence of hCE1m6-NSCs.

Es1(e)/SCID mice bearing intracranial U251T human glioma were injected i.c. with either 500,000 hCE1m6-NSC or PBS. Three days later, irinotecan was given at 3.75 mg/kg or 7.5 mg/kg via tail vein injection. Groups of 3 mice each were then sacrificed at 1 or 4 hours post-irinotecan administration, and brains were harvested and quartered. Normal brain tissue was sampled from brain contralateral to where the tumor was engrafted. Normal brain and tumor tissues wereanalyzed by LC-MS/MS to determine irinotecan and SN-38 levels.

We observed levels of irinotecan in the 500 nM range in normal brain and slightly more elevated levels in tumor-bearing tissue, with no observable difference in irinotecan concentrations in the presence or absence of hCE1m6-NSC (data not shown). In the absence of hCE1m6-NSCs, SN-38 in brain would be predicted to come from systemic metabolism of irinotecan in organs such as the liver and gut, but because this compound crosses the blood brain barrier even more poorly than irinotecan, the levels in brain would be expected to be very low, and this was indeed the case (Figure 3). With the addition of hCE1m6-NSC, SN-38 concentrations in tumor tissue were approximately 3-fold higher than in the absence of NSCs. Moreover, there was an irinotecan dose-dependentincrease in SN-38 levels in tumor tissue. In normal brain, we did not observe an increase in SN-38 concentrations with the addition of hCE1m6-NSC likely because the NSCs were administered contralateral to where the sample was taken and remained in the vicinity of the tumor.

Taken together, these findingsunderscore the basic tenet of this technology: CE-expressing NSCs can increase SN-38 locally at the tumor site while limiting exposure to normal tissue. The *in vitro* IC_{50}'s of various glioma cells to SN-38 are in the range of 5 to 100nM. The average intratumoral SN-38 levels at one and four hours after 7.5 mg/kg irinotecan administration in animals that received hCE1m6-NSCs were 31.3 and 7.9 nM, respectively. In contrast, average SN-38 levels in tumor tissue of the animals that did not receive NSCs were 12.7 nM and 1.4 nM at one and four hours, respectively. These results provide *in vivo* proof-of-principleregarding hCE1m6.NSC mediated conversion of irinotecan to SN-38, resulting in exposure of the tumor tissue to cytotoxic concentrations of SN-38.



Figures 3. Es1(e)/scid mice bearing intracranial U251T human gliomas were injected i.c. with either 500,000 HB1.F3.CD.hCE1m6 or PBS. Three days later, the mice were given 3.75 mg/kg or 7.5 mg/kg irinotecan via tail vein injection. Groups of 3 mice each were then sacrificed at 1 hour and 4 hours after irinotecan administration, and tumor-bearing and normal brain tissue were harvested for irinotecan (not shown) and SN-38measurement by LC-MS/MS.

2.1.7 Conversion of Irinotecan to SN-38 is Highest on Day 2 Following hCE1m6-NSCAdministration.

In order to determine the optimal timing of irinotecan administration after injection of NSCs, we performed an experiment where irinotecan was given intravenouslyto tumor-bearing mice either 2, 4,or 7 days afteri.c.

administration of hCE1m6-NSCs. Figure 4 shows the average SN-38 concentrations in tumor tissue one hour after irinotecan was given through the tail vein. These results demonstrate that peak SN-38 production occurs2 days following NSC administration. As in our previous tissue PK experiment, there were no differences in SN-38 levels in normal brain with or without NSCs (data not shown), further demonstrating the tumor specificity of the increased production of SN-38 by the NSCs. The average difference in SN-38 levels in tumor from animals that received NSCs and those that did not was approximately 8-fold higher. The fold difference in tumoral SN-38 levels 4 days after NSC administration dropped to approximately 2-fold, and there was no difference in tumor tissue SN-38 when we waited 7 days between NSC and irinotecan administration. The average SN-38 level in tumor tissue 2 days after NSCs was 111 nM compare to 14 nM without NSCs.Based on these results, for the phase I clinical trial irinotecan will be given 2 days after the NSCs are administered.

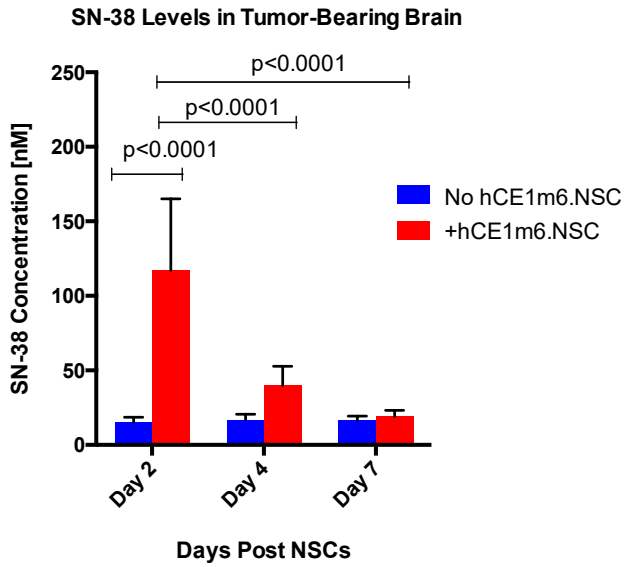


Figure 4. SN-38 concentration in tumor tissue harvested 1 hr post irinotecan administration [7.5 mg/kg] either 2, 4, or 7 days following intracranial injection of hCE1m6-NSCs or harvested at equivalent times but without NSCs (n=6 mice per group). Data were analyzed using 2-Way ANOVA. All possible comparisons were made using Holm-Sidek method to correct for multiple comparisons. SN-38 concentrations were greatest when irinotecan was administered on day 2 in the presence of hCE1m6-NSCs.

2.1.8 Production of SN-38 by hCE1m6-NSCsin Tumor is Irinotecan Dose and Schedule Dependent.

Once the optimal timing of irinotecan dosing relative to NSC administration was determined, we performed an experiment to assess the effect of irinotecan dose and schedule on intratumoral levels of SN-38. For this experiment, irinotecan was given intravenously to tumor-bearing mice at doses of 7.5 mg/kg or 18.75 mg/kg beginning 2 days after i.c. administration of hCE1m6-NSCs. Animals in the group receiving 7.5 mg/kg were given 3 daily irinotecan doses to simulate the multiple daily dosing schedule, while mice in the 18.75 mg/kg group received a single dose to simulate the bolus dosing schedule. Cohorts of mice (n=5) were sacrificed at 1, 4, and 8 hours after either the third dose of 7.5 mg/kg (daily dosing group) or after the single irinotecan dose of 18.75 mg/kg (bolus dosing group). Additional control groups of mice were given a single dose of 18.75 mg/kg in the absence of NSCs. Tumor and contralateral brain tissues were collected for analysis of SN-38 concentrations by LC-MS/MS.

Figure 5 shows the average SN-38 concentrations in tumor tissue over an 8 hour time course following the administration of irinotecan via tail vein. As seen in the figure, the average peak SN-38 level following a dose of 18.75 mg/kg plus NSCs was >10-fold higher than following the same irinotecan dose given without NSCs (92.7 vs 8.6 ng/gm; p=0.018). The $AUC_{0\text{-}8hr}$ of SN-38 after a dose of 18.75 mg/kg was >8-fold higher when given with NSCs

compared to without (286.4 versus 33.8 ng/gm x hr). As in our previous tissue PK experiments, there were no differences in SN-38 levels in normal brain with or without NSCs (data not shown). Average tumoral SN-38 concentrations following the third daily irinotecan dose of 7.5 mg/kg plus NSCs were similar to the concentrations measured following a dose of 18.75 mg/kg given without NSCs. Therefore, the average peak SN-38 level following a dose of 18.75 mg/kg plus NSCs was >7-fold higher than following the third daily dose of 7.5 mg/kg plus NSCs (92.7 vs 12.4 ng/gm; p=0.021). Furthermore, the tumoral $AUC_{0\text{-}8hr}$ of SN-38 after a single dose of 18.75 mg/kg plus NSCs was >6.5-fold higher than after the third dose of 7.5 mg/kg plus NSCs (286.4 versus 41.8 ng/gm x hr).

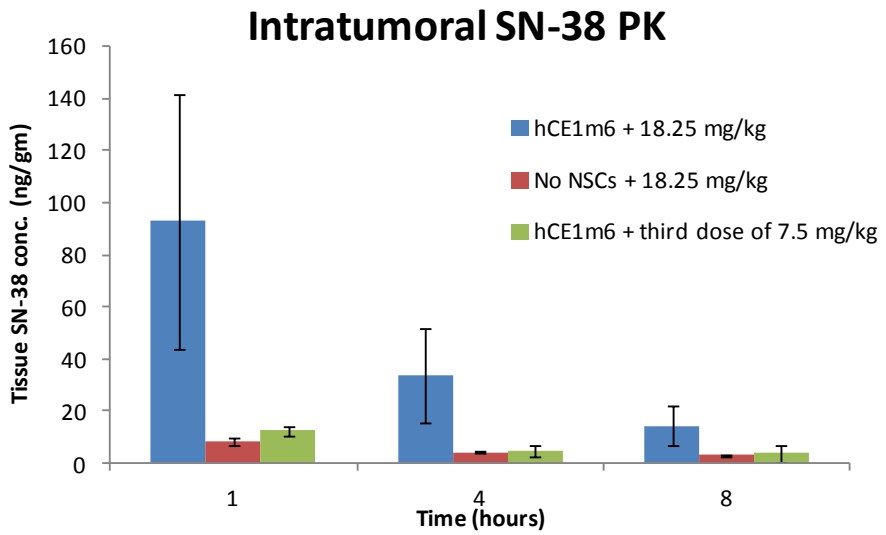


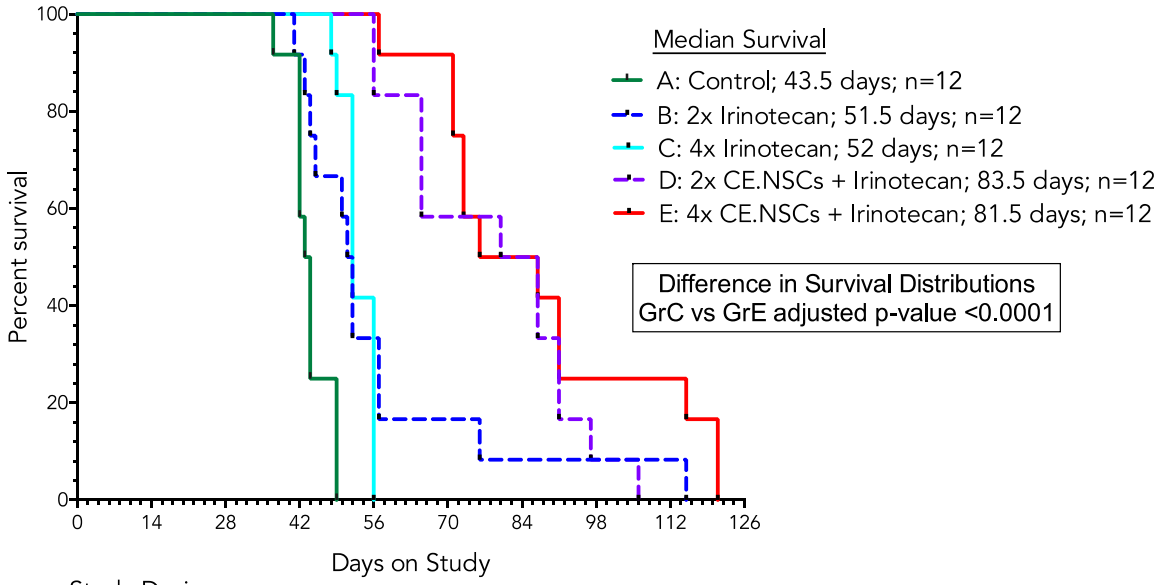
Figure 5. SN-38 concentration in tumor tissue post administration of 18.75 mg/kg plus hCE1m6-NSCs (blue bars), 18.75 mg/kg without NSCs (red bars), or 7.5 mg/kg/day x 3 days (green bars). N=5 for each group.

These results demonstrate that the production SN-38 by hCE1m6-NSCs is highly dependent on the dose and schedule of irinotecan administration. In other words, higher doses of irinotecan result in higher SN-38 peak levels and total exposures (i.e. AUC) specifically in tumor. **Taken together, the results of this experiment and the previous experiment regarding the optimal timing indicate that the maximum benefit of the addition of hCE1m6-NSCs to irinotecan treatment will be obtained by giving the highest irinotecan dose possible within 2 days of NSC administration.**

2.1.9 Efficacy of NSC-Mediated Therapy in Preclinical Models

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., 2006; Gutova, et al., 2012) and metastatic neuroblastoma (Aboody et al, 2006b; Danks et al., 2007).

Results from our long term survival efficacy study in U251 bearing Es1(e)scid mice (Figure 6) demonstrate a statistically significant increase in survival with 2 or 4 weekly treatment rounds of hCE1m6-NSCs (250,000) in combination with irinotecan 37.5 mg/kg (equivalent to the human dose of 125 mg/m^2) as compared to irinotecan alone. Group D receiving two rounds of CE-NSCs + irinotecan treatment had significantly longer survival times than group B receiving two rounds of irinotecan only (p=0.0362). Similarly, group E receiving four rounds of CE-NSCs + irinotecan had significantly longer survival times than group C receiving four rounds of irinotecan only (p<0.0001).



Study Design
Day 0: U251 tumor
Day 7, 14: 250,000 NSCs given to GrA & GrC
Day 7, 14, 21, 28: 250,000 NSCs given to GrB & GrE
Day 9, 16: irinotecan (37.5 mg/kg)
Day 9, 16, 23, 30: irinotecan (37.5 mg/kg)

Figure 6. Survival advantage of mice treated with hCE1m6-NSCs + irinotecan. Five groups of twelve mice bearing orthotopic U251.eGFP.ffluc were left untreated (grp A); treated with 2 or 4 weekly IC injections of hCE1m6-NSCs (grps D and E) followed 2 days later by in IV bolus of irinotecan (37.5 mg/kg/wk) or 2 or 4 weeks of irinotecan alone (grps B and C).

Clinical Data

We recently completed a pilot feasibility study (Portnow et al., 2013a)of the first generation of genetically-modified NSCs (HB1.F3.CD NSCs) and oral 5-fluorocytosine (5-FC), which the NSCs convert to 5-fluorouracil (5-FU). In this first-in-human study, patients with recurrent high-grade glioma 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.

2.1.10 Safety

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 has occurred in those 3 patients, although 2 of them have not yet completed the full toxicity evaluation period.

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.

2.1.11 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 7) 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.

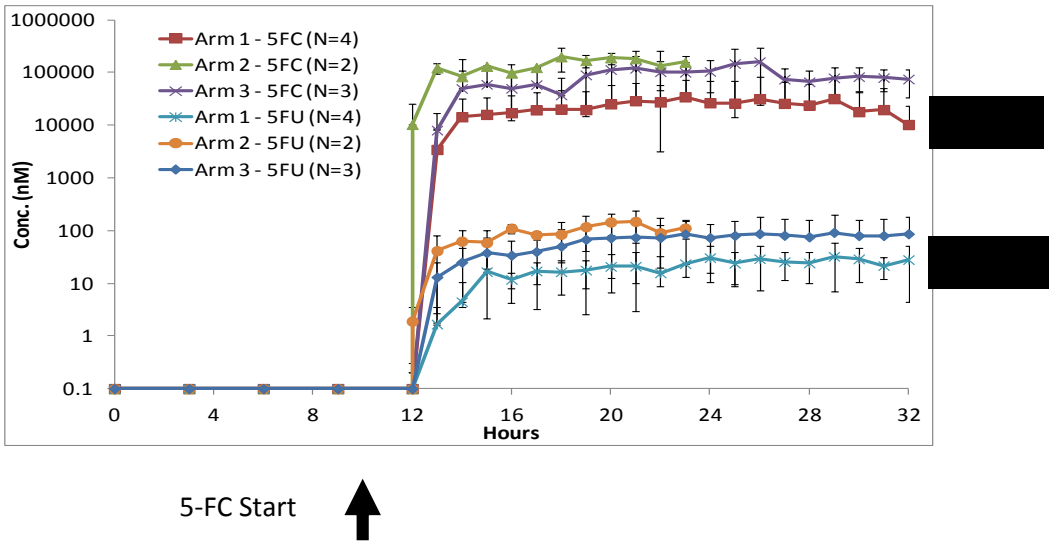


Figure 7. 5-FC and 5-FU Intracerebral Microdialysis Data.

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 8shows documentation that the NSCs continued to convert 5-FC to 5-FU during the entire 5-FC dosing interval.

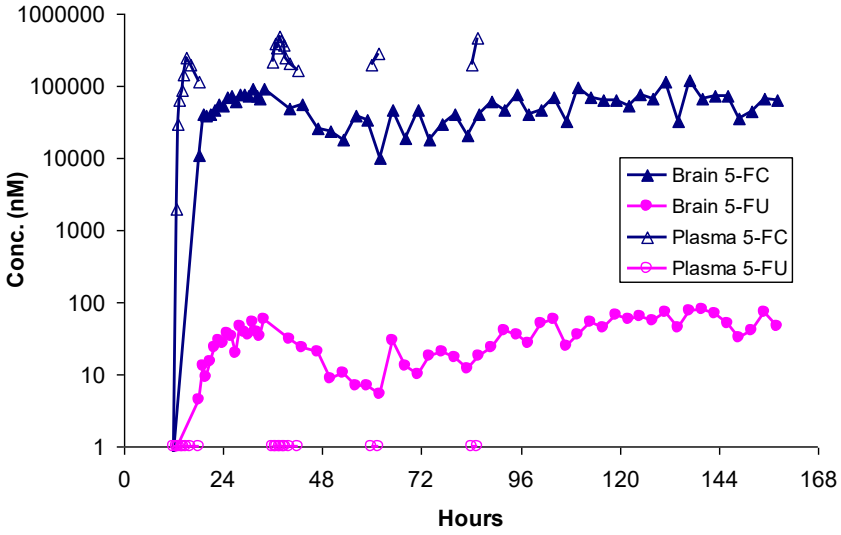


Figure8. Intracerebral microdialysis data from a study patient demonstrate the ability of the NSCs to convert 5-FC to 5-FU in the brain throughout the 7-day course of oral 5-FC. In this particular patient there were no detectable levels of 5-FU in the plasma, indicating that all of the 5-FU measured in the brain came from NSC conversion of 5-FC.

2.1.12 Immunologic Correlative Studies

2.5.3.1Evaluation 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 9).

B.

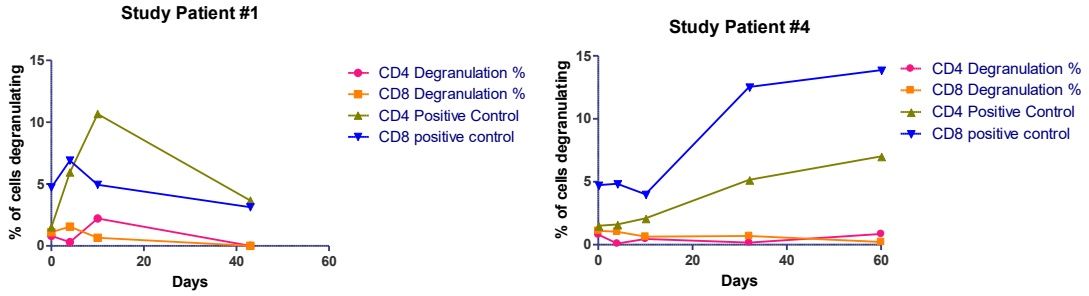


Figure 9 Evaluation of T Cell Responses: Representative Patient Examples. A) CD4+ and CD8+ T cells from patient #1 fluctuated in the range of 0-2%, beginning and ending low. Natural killer (NK) cells were under 1%. **B)** CD4+ and CD8+ T cells from patient #4 had very low levels of degranulation (<2%) at all time points. NK cells in this patient's samples degranulated at 2-10%, but there was no evidence of increase over time.

2.5.3.2Evaluation 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 (figure 10), show no detection of anti-NSC antibodies outside the normal range after first exposure (Portnow et al., 2012).

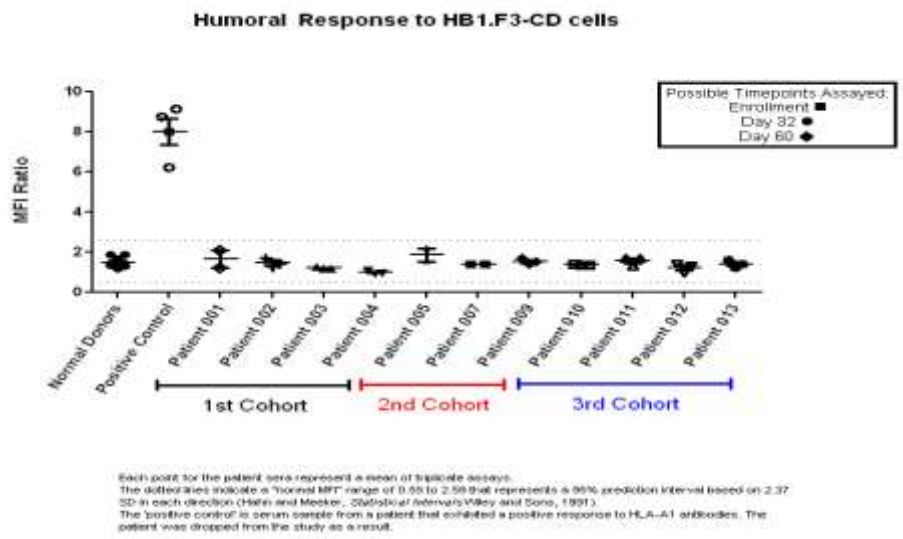


Figure 10. Results of anti-NSC antibody testing. Normal donor sera: n=10. Each data point for normal sera represents the mean of 5 independent experiments. Each data point for study patients' sera represents the mean of triplicate assays. The dotted lines indicate a "normal mean fluorescence intensity (MFI)" range of 0.55 to 2.59 that represents a 95% prediction interval based on a 2.3 standard deviationin 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 .

2.5.3.3 Evaluation of Possible Presenceof NSCs or Replication Competent Retrovirus (RCR) in the Systemic Circulation

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 systemic presence 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.

2.1.13 Assessment of NSCDistribution in the Human Brain

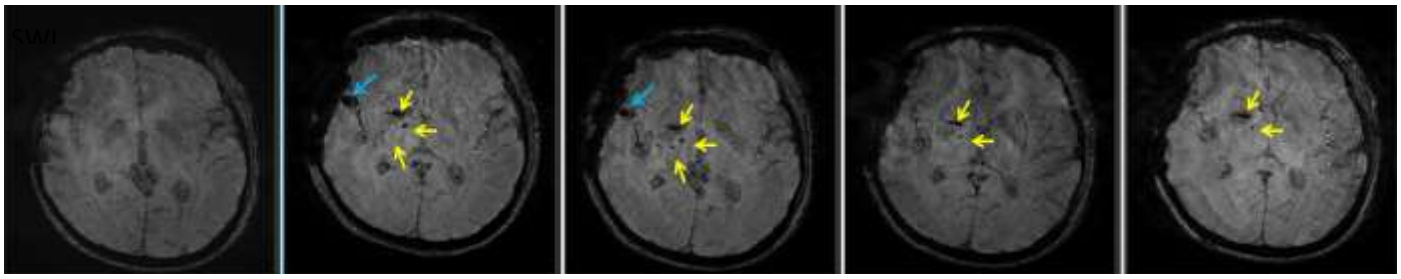
Based on the preclinical safety and imaging data in mice (section 2.3.5) an amendment to the study's IND (#14041) to allow administration of Feraheme-labeled NSCs was approved by the FDA while the pilot feasibility study was finishing dose level 3. As a result the last 3 patients to enroll in the study received Feraheme-labeled NSCs to non-invasively assess NSC migration in the brain by MRI. No toxicity from the Feraheme-labeled NSCs was observed in the 3 study patients.

For these patients, the standard MRI brain imaging protocol used at City of Hope was modified by adding susceptibility weighted imaging (SWI) sequences (Figure 11, panel A)to visualize the ultrasmall superparamagnetic iron oxide (USPIO) in the NSCs. To facilitate anatomical correlation, clinical imaging sequences were revised to be performed at a uniform slice thickness of 2mm, instead of the standard 4 mm thickness, with the 2nd and 3rd patients who received Feraheme-labeled NSCs. 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 included automatic alignment, stripping, and evaluation

of multiple algorithms for identification and quantification of USPIO, including use of SWI quantitative mapping (SWI-M; Zheng et al., 2013) (Figure 11, panel B).

Image analysis demonstrated a trend of decreasing USPIO susceptibility over serial brain MRIs. Feraheme-labeled NSCs were detectable on SWI as areas of hypointense signal at the injection sites (Figure 11). Although the resolution used 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 a combination of both. Early changes in hypointense signal at the injection sites (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 showed 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 10Day 31

B.

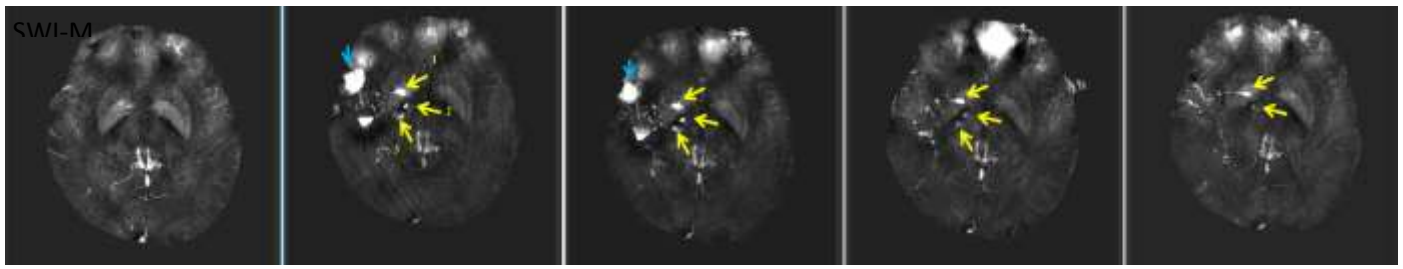


Figure 11. Magnetic resonance images of NSC injection sites that initially contained approximately 5 million USPIO-labeled NSCs each. Serial images of 1 patient demonstrate a gradual decrease in the size of signal density during the 31-day period after intracranial administration of USPIO-labeled NSCs. To facilitate visualization of Feraheme at the injection sites over time, a maximum intensity projection was performed across five 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. A) SWI images; B) SWI-M: quantitative mapping of images in A.

2.1.14 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 (Fig. 12). These data support the conclusion that NSCs migrated away from the injection site to distant tumor foci.

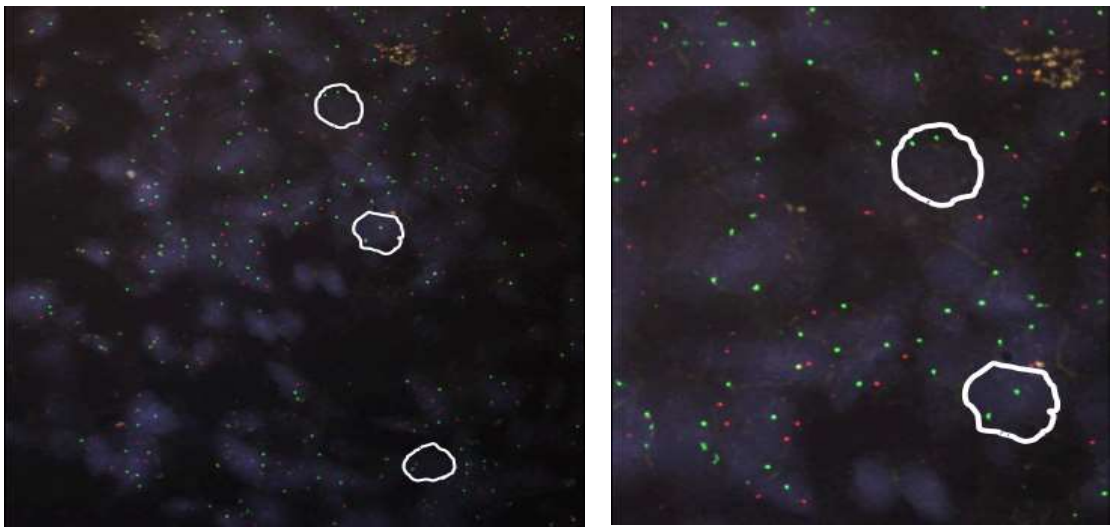


Figure12. **Selected female cells (circled in white) interspersed among host male tumor cells.** XY FISH analysis (X gene green, Y gene red; 5 micron sections scanned at 63X) suggest the presence of single interspersed female NSCs(2 green chromosomes) among the male tumor cells (1 red and 1 green chromosome). Individual cells identified.Second image is a magnification of the first image.

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.

Irinotecan for the Treatment of Gliomas

Irinotecan produces cytotoxicity by inhibiting topoisomerase I, an enzyme required for DNA replication and transcription, ultimately resulting in apoptosis. CE converts irinotecan to its active metabolite, SN-38, which is approximately 100-1,000 times more potent for inhibiting topoisomerase I (Kawato et al., 1991). Irinotecan is

an attractive drug for the treatment of malignant gliomas because its mechanism of action differs from alkylating agents, the only class of drugs that has demonstrated improvement in survival in these patients. Although highly protein-bound, irinotecan can cross the BBB to a certain extent. Using the presence of drug in the CSF as a surrogate for the ability of a drug to penetrate into brain, a study in non-human primates documented that irinotecan has an AUC ratio of CSF to plasma of 14% (Blaney et al., 1998).SN-38 is more highly protein-bound than irinotecan, and only very lowconcentrations of SN-38 have been detected in the brains of rats after intravenous administration of irinotecan (Tim Synold, unpublished data). Combining irinotecan with tumor-tropic CE-secreting NSCshas the potential to significantly increase concentrations of SN-38 in brain tumor via localizedNSC-mediated CE conversion of irinotecan to SN-38.

Irinotecan is approved by the FDA for use in patients with metastatic colorectal cancer,as either a single agent given at a dose of 125 mg/m^2 weekly for 4 weeks followed by a 2 week rest or 350 mg/m^2 once every 3 weeks—orin combination with 5-FU and leucovorin. Irinotecan has also been studied alone (Gilbert et al., 2003; Batchelor et al., 2003, Raymond et al., 2003, Prados et al., 2006) and in combination with other chemotherapy agents (Gruber et al., 2004, Brandes et al., 2004) in patients with recurrent gliomas. Irinotecan has demonstrated only modest activity in these glioma clinical trials, likely due to the poor ability of systemically converted SN-38 to cross into the central nervous system (Blaney et al., 1998). Although not FDA approved for the treatment of gliomas, when irinotecan is use for recurrent glioblastoma, it is commonly given at a dose of 125 mg/m^2 every 2 weeks with bevacizumab (Friedman et al., 2009; Moller et al., 2012). However, when administered with 5-FU and leucovorin as part of the colon cancer regimen FOLFIRI, irinotecan is given at a dose of 180 mg/m^2 every 2 weeks. We have chosen to use this higher dose of irinotecan every two weeks because we want to maximize the amount of prodrug available to the NSCs for conversion to SN-38 at sites of tumor in the brain, and since 180 mg/m^2 of irinotecan is well tolerated in combination therapy, it should also be well tolerated when given as a single agent. If this dose is found to be too toxic, the starting dose will be de-escalated first to 150mg/m^2 every other week, followed by a further dose de-escalation to 125 mg/m^2 every other week if necessary.

Use of a Rickham reservoir/catheter system for repeat intracranial administration of NSCs.

In this study we will slowlyadminister repeat infusions of NSCsthrough an indwelling Rickham reservoir/catheter system (a Rickham). The catheter will be inserted into the tumor mass at the time of resection or biopsy and connected proximally to a Rickham 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 doses of the T cells during a 2 week period via a Rickham catheter 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 hCE1m6-NSCs when administered through a Rickham. hCE1m6-NSCs were thawed and washed 3 times with 2% human serum albumin (HSA)/artificial CSF (25% HSA diluted by Perfusion Fluid CNS for a final HSA concentration of 2%). At the last wash, the total viable cell count was 1.53 x 10^8 cells. The cell pellet was resuspended in 2.23 ml of artificial CSF to give a concentration of 6.67 x 10^7/ml.An aliquot of the cell suspension was taken and counted immediately. The cell count of the aliquot taken up in the syringe was1.45 x 10^8. The cells were administered slowly at a flow rate of 0.5 ml/hr through extension tubing attached to a Gripper Plus needle that was inserted into the reservoir of a Rickham and collected at room temperature over 4 hours into a single tube, followed by a 1 ml flush of 2% HSA/artificial CSF over 2 hours. Samples from the collection tube were taken every hour and immediately counted. At the 6 hour sampling, cells were stored at 4°C for 18 hours. The viability of the hCE1m6-

NSCs at room temperature remained above 90% over 6 hours and above 83% overnight at4°C. The final cell count was 1.27 x 10^8, which represents a cell recovery of 87.6%.

Intracerebral Microdialysis

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; Vespa et al., 2005; Vespa et al., 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; Benjamin et al., 2004) 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; Portnow 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 country with the application of 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*.

2.1.15 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 placement 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.

2.1.16 *In Vitro* Recovery Data for Irinotecan and SN-38

As was done in the pilot feasibility study of CD-expressing NSCs where microdialysis was used to measure intracerebral levels of 5-FC and 5-FU (section 2.5.2), we plan to likewise use microdialysis catheters in this phase I study to measure NSC-mediated hCE1m6 conversion of irinotecan to SN-38 locally in the brain. In preparation for this clinical trial, we performed *in vitro* studies to estimate the recovery of SN-38 and irinotecan with the same microdialysis catheter, pump, and perfusion fluid that will used in the clinical trial. At a flow rate of 1 µl/minute, the *in vitro* fractional recovery of SN-38 and irinotecan was determined to be 60±10% and 108±2%, respectively.

Overview of Study Design and Rationale

Combining irinotecan with tumor-tropic CE-secreting NSCs has the potential to significantly increase concentrations of SN-38, a potent topoisomerase I inhibitor, in brain tumor via localized NSC-mediated CE conversion of irinotecan to SN-38. This phase I clinical trial will define the RP2D based on DLTs of the combination of CE-secreting NSCs and irinotecan using a standard "3 + 3" dose escalation schema in patients with recurrent high grade gliomas who are undergoing a debulking craniotomy or stereotactic brain biopsy. Except for dose level 1 (where only 1 dose of NSCs will be administered, but these patients can continue to receive treatment with irinotecan every 2 weeks), NSCs will be administered intracranially on days 1 and 15, and irinotecan will be given intravenously 2 days later, on days 3 and 17 of a 28 day cycle. With subsequent cohorts of patients, the NSC dose will be increased as tolerated, but the dose of irinotecan will remain the same unless a DLT occurs that necessitates lowering dose of irinotecan. Beginning with dose level two, a Rickham reservoir/catheter system will be placed at the time of surgery for delivering repeat doses of NSCs intracranially. Brain MRI and PET/CT scans will be performed at least every two months (at the end of every second treatment cycle) to evaluate response.

The schedule of dosing is based on pre-clinical data which demonstrates the greatest increase in tumor SN-38 levels is achieved when irinotecan is administered 2 days after NSC administration. Pre-clinical data showing increased SN-38 concentrations in tumor but not normal brain and a survival advantage in mice support maximizing the dose of NSCs and irinotecan administered.

Three different doses of NSCs will be assessed. The starting dose level will be a single administration of 5×10^7 NSCs, which was well-tolerated in the first-in-human study of CD-expressing NSCs. The second dose level will assess the same dose of NSCs given every other week. The third and fourth dose levels will increase the number of NSCs to be administered. Due to volume limitations for intracerebral administration, the highest dose of NSCs to be tested will be 1.5×10^8 cells.

The dose of irinotecan to be administered on this trial will be 180mg/m^2 every other week. This irinotecan dose and schedule is FDA approved for use in colorectal cancer patients as part of the combination chemotherapy regimen FOLFIRI, and is expected to be well tolerated in the glioma population. If this dose is found to be too toxic, the starting dose will be de-escalated first to 150mg/m^2 every other week, followed by a further dose de-escalation to 125 mg/m^2 every other week if necessary.

Study participants will receive repeat cycles of study treatment (dose level 1 and -1 patients will only receive repeat doses of irinotecan) as long as they are tolerating the treatment well, and there is no evidence of progressive disease.

An important secondary objective of this study will be to determine the biologic activity of the hCE1m6-NSCs by measuring concentrations of SN-38 in brain interstitium using intracerebral microdialysis. The first 6 patients to enroll in the study and the last 8 patients in the expansion cohort will undergo microdialysis to collect data regarding intracerebral levels of irinotecan and SN-38 when patients are treated with the lowest and highest NSC doses. Please note, the same dose of NSCs (5×10^7) is given to patients on dose levels 1 and 2, so whether the first 6 patients are all treated on dose level 1 or the first 3 dose level 1 and the next 3 on dose level 2, they will all receive the same dose of NSCs.

Once the maximum tolerated doseof the combination therapy has been determined, or, if the maximum tolerated dose is not reached, the maximum feasible dose,patients in the expansion cohort will undergo intracerebral microdialysis to investigate if treatment with a higher dose of NSCs results in more SN-38 in the brain and to directly determine how much the addition of NSCs to treatment with irinotecan increases intracerebral levels of SN-38. As such, 4 patients in the expansion cohort will undergo intracerebral microdialysis after receiving NSCs at the time of surgery followed 2 days later by IV irinotecan, but the other 4 patients in this cohort will not receive NSCs on the day of surgery. Instead they will be given IV irinotecan 2 days after surgery, and dialysate samples will be collected from them to determine how much SN-38 is present in the brain from treatment with IV irinotecan alone. These 4 patients will then receive their first dose of NSCs through the Rickham on day 15 of cycle 1 followed by irinotecan on day 17. Another key secondary objective will be to assess for possible development of immune responses to these allogeneic, adenovirally transduced NSCs. Serial blood samples will be collected during treatment cycles to assess for the possible development of T cell and antibody responses to the NSCs and/or cell surface-expressed adenoviral proteins, and we will follow adenoviral antibody titers. Other secondary objectives include describing the clinical benefit in patients who receive treatment with repeat cycles of NSCs and irinotecan by obtaining brain MRI and PET/CT scans to assess response after every two cycles and, when feasible, determining at time of autopsy the fate of the NSCs.

3 PARTICIPANT SELECTION

Eligibility Inclusion Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

Informed consent

1. Patient must be able to understand and be willing to sign a written informed consent document.
2. Participant must be willing to comply with study and/or follow-up procedures.

Age Criteria, Performance Status, Life Expectancy and Informed Consent

3. At least 18 years old and less than 70 years old.
4. Karnofsky Performance Status $\geq$ 70% (Appendix A).
5. Life expectancy of $\geq$ 3 months.

Nature of Illness and Treatment History

6. 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).
7. Imaging studies show evidence of recurrent tumor(s).If a patient is going to be enrolled to dose level two or higher, the patient must have a component of supratentorial disease (so as to enable placement of a Rickham reservoir/catheter) that is amenable to resection or biopsy.
8. High-grade glioma has recurred or progressed after prior treatment with brain radiation and temozolomide.
9. Participant 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 $\pm$chemotherapy.
10. Based on the neurosurgeon's judgment, there is no anticipated physical connection between the post-resection tumor cavity and the cerebral ventricles.
11. Neurosurgeon finds the prospective participant is able to undergo neurosurgery.
12. Any number of prior therapies is permitted. From the start of study treatment, the following time periods must have elapsed: 6 weeks from nitrosourea-containing chemotherapy, 4 weeks from non-nitrosourea-containing cytotoxicchemotherapy(except 23 days from last daily dose of temozolomide taken in a 5 of 28 day regimen), and 2 weeks from last dose of a targeted agent (except 4 weeks for bevacizumab). There is no time period requirement for prior radiation therapy.
13. Any clinically significant toxicity from prior therapy must have improved to grade 0 or grade 1.

Clinical, Genetic, and Immunological Labs

14. ANC $\geq$ 1,500 cells/μl, platelets > 100,000 cells/μl
15. Total bilirubin $\leq$ 2.0 mg/dl, AST (SGOT) $\leq$4 times institutional upper limit of normal
16. Serum creatinine $\leq$ 1.5 x the institutional upper limit of normal.

17. Homozygous **negative**for the UGT 1A1*28 allele.
18. Absence anti-HLA antibodies specific for HLA class I antigens expressed by the F3.CD.CE NSCs.
19. Negative serum pregnancy test (women of childbearing potential only)

Child Bearing Potential

20. Agreement by females of childbearing potential and sexually active males to use an effective method of contraception while participating in this study. The effects of study treatment on a developing fetus have the potential for teratogenic or abortifacient effects. Women of childbearing potential must have a negative pregnancy test <2 weeks prior to registration.

Eligibility Exclusion Criteria

Prospective participants who meet any of the following criteria will not be eligible for admission into the study:

Previous therapies

1. Prior therapy with Neural Stem Cells

Concomitant medications

2. Use of CYP3A4 **inducers** including hepatic enzyme-inducing anticonvulsants (phenytoin, fosphenytoin, carbamazepine, phenobarbital, primidone, oxcarbazepine) within 2 weeks prior to start of study treatment (See Table 6.2).
3. Use of **moderate** to **strong** CYP3A4 **inhibitors** within 2 weeks prior to start of study treatment (See Table 6.2).
4. Use of drugs known to **inhibit** UGT1A1, such as Atazanir, Gemfibrozil, Indinavir, or Ketoconazole, within 2 weeks prior to start of study treatment).
5. Co-medication that may interfere with study results; e.g. immuno-suppressive agents other than corticosteroids, such as systemic cyclosporine and tacrilomus. Consult Principal Investigator for questions, including necessary washout period for the specific drug.
6. Flucytosine within 2 weeksprior to start of study treatment.
7. Use of herbal medications.
8. Current use (or planned use during the treatment period) of other investigational agents, or biological, chemotherapy, radiation or other anti-tumor therapy. See Inclusion Criterion 12, for required washout periods from these therapies.

Other illnesses or conditions

9. Patient has known human immunodeficiency virus (HIV) or hepatitis C infection; baselinetesting for HIV or hepatitis C is not required.
10. Prospective participant is unable to undergo an MRI with contrast agent.
11. Known chronic or active viral infections of the CNS.
12. Clinically significant uncontrolled illness
13. Active infection requiring antibiotics.
14. Diagnosis of Gilbert's disease

15. History of allergic reactions attributed to compounds of similar chemical or biologic composition to irinotecan.
16. Known sensitivity to any of the products to be administered during dosing.
17. Any other active malignancy
18. Pregnant women and women who are lactating. Irinotecan is an agent with the potential for teratogenic or abortifacient effects. There is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with irinotecan.
19. 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.

Noncompliance

20. Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

Inclusion of Women and Minorities

The study is open to 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 between 6 and 29subjects, 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 SCREENING AND REGISTRATION PROCEDURES

4.1 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. (See Section 16.7 for additional detail).

Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial, including initiated wash-out for prohibited agents, 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. All screening procedures and their respective windows are detailed in Section 10, Table 10, Study Activity Calendar.

Registration Requirements/Process

Registration of participantsis to be via completion of the following steps:

- Prospective participants must complete the informed consent process, including a signed informed consent, prior to proceeding to study screening.
- Screening procedures and windows are detailed in Section 10 and Table 10, Study Activity Calendar.
- 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.
- Once all the pre-study requirements have been fulfilled, including a completed eligibility checklist, the study coordinator can register the eligible patient into MIDAS.
- Patients failing to meet all protocol eligibility criteria, including informed consent, may not be registered for the trial.

Dose Level Assignment and Assignment for the Expansion Cohort

See Section 5.2 of this protocol document.

Screen Failures and Registered Participants Who Do Not begin Study Treatment

Participants who complete the informed consent process and undergo screening procedures, but do not register to the trial will have their blood samples taken for immunologic and RCR testing destroyed. Similarly, participants who register for the trial but do not begin study treatment will have these blood samples destroyed.

The reason for screen failure or failure to begin treatment for registered participants will be documented.

5 TREATMENT PLAN

This is an open-label Phase I study, with an expansion cohort once the MTD has been established. The schemas thatfollowprovide an introduction of the treatment plan.

Dose Escalation Schema

NSC Dose Level	Initial Enrollment	If 1 DLT, Addt'l Enrollment	NSC Dose	NSC and Irinotecan[a] Administration Week 1	2	3	4
-1	3	3	2.5×10^7	X*		**	
1[b]	3	3	5×10^7	X		**	
2[c]	3	3	5×10^7	X		X	
3	3	3	1×10^8	X		X	
4[d]	3	3	1.5×10^8	X		X	
Expansion Cohort [e]	8		MTD/MFD	X		X	

* "X" denotes treatment weeks for NSCs and irinotecan. NSCs are administered on Day 1, and irinotecan, 180 mg/m^2/dose, is administered two days post-NSCs on Day 3 of each treatment week. The length of a treatment cycles is 28 days.

**Patients on dose levels 1 and -1 will only receive 1 dose of NSCs, but they will continue to receive irinotecan every 2 weeks as long as they are tolerating it well, and there is no evidence of tumor progression.

[a]**Dose schedule A, B, and C** will be the NSC dose schema in combination with an irinotecan dose of 180mg/m^2, 150 mg/m^2, or 125 mg/ m^2, respectively. We will start with irinotecan dose schedule A, de-escalating through doses B and C in order as necessary.

b The first 6 study participants will undergo intracerebral microdialysis to measure levels of irinotecan and NSC-mediated SN-38 production in the brain. Since the dose of NSCs remains the same for dose level 1 and 2, and microdialysis will be performed only with the first dose of irinotecan, all of these first 6 study patients would receive the same dose of NSCs and irinotecan when undergoing intracerebral microdialysis, even allowing for escalation to dose level 2.

c Starting with dose level 2, a Rickham reservoir/catheter system will be placed at the time of surgery for administering repeat doses of NSCs.

d Six patients will be treated on at the maximum tolerated dose (MTD), or, if the MTD is not reached, at the maximum feasible dose (MFD) . Since it is anticipated that the MTD/MFD will be 150 million NSCs (dose level 4), the 6 patients who will be treated on dose level 4 will undergo intracerebral microdialysis and be counted as part of the expansion cohort.

e An expansion cohort of 8 patients will be treated at the MTD or MFD to gain more experience with the tolerability of this treatment regimen. These patients will undergo intracerebral microdialysis to assess how

much SN-38 is produced by highest dose of NSCs and evaluate the biologic activity of the NSCs by directly measuring how much the addition of NSCs to treatment with irinotecan increases intracerebral levels of SN-38. As such, 4 patients in the expansion cohort will undergo intracerebral microdialysis after receiving NSCs on the day of surgery followed 2 days later by IV irinotecan, and the other 4 patients in this cohort will not receive NSC administration on the day of surgery. Instead they will be given IV irinotecan 2 days after surgery and dialysate samples will be collected from them to determine how much SN-38 is present in the brain from treatment with IV irinotecan alone. These 4 patients will then receive their first dose of NSCs through the Rickham on day 15 of cycle 1 followed by irinotecan on day 17.

Simplified Treatment Schema for Cycle 1

Day 1

Tumor resection or biopsy is performed. hCE1m6-NSCs are administered intracranially.^ A Rickham catheter is placed (except in patients on dose level 1or -1). The first 6 study patients and those in the expansion cohort: microdialysis catheter(s) is/are placed, and a post-operative CT scan is obtained to confirm correct placement. ^Four participants in the expansion cohort will not receive Day 1 NSCs; they will initiate NSC administration on Day 15.

Day 3

Irinotecan is administered intravenously. Patients undergoing intracerebral microdialysis: serial dialysate and blood samples are collected.

Day 15

hCE1m6-NSCs are administered intracranially via the Rickham catheter.* Blood is drawn for immunologic correlative studies.* *Patients in dose level 1 or -1 will not receive NSCs on Day 15, and blood samples for immunologic correlative studies will be drawn on Day 17 when these patients return to clinic to receive irinotecan.

Day 17

Irinotecan is administered intravenously.

Study Treatment Schema

Enroll patients with recurrent high grade glioma who are planning to have craniotomy or stereotactic biopsy

3 + 3 stage	•First 3 participants to a dose level will be staggered, whereby each awaits for the previous participant to have completed the 1st cycle. •3+3 dose escalation/de-escalation where at least 6 pts are treated at MTD/MFD[a] for which <33% have DLT
Expansion Cohort	•8 participants to be enrolled at MTD (or MFD if MTD is not reached), all undergoing microdialysis.[a] •To determine impact of NSCs on SN-38 and irinotecan concentrations, 4 will receive first dose of NSCs at Day 15 after microdialysis, to compare with 4 who will receive first dose of NSCs on Day of Surgery.

	Treatment Regimen and Assessment for Response		**Immunological Studies with NSC and virus testing**	**Intracranial and peripheral blood SN-38 and Irinotecan concentrations**	
	All Patients at NSC Dose Levels 1 and -1 **Procedures A-D**[b]	*Patients at NSC Dose Levels 2, 3 & 4* **Procedures E-K**	*All Patients* **Procedures A-K**	*First 6 enrolled, and all 8 in expansion cohort* **Procedures A, C, G, I, K**	
Cycle 1 (28 days)[b]	Day 1: Surgery + NSCs	Day 1: Surgery + NSCs[c]	Pre-surgery: T cells, IgG/M, peripheral NSCs, replication competent retrovirus (RCR)	Day 1: Microdialysis catheters inserted at surgery	
	Day2: Baseline MRI	Day2: Baseline MRI		Day 1: CT post-surgery to confirm catheter placement, catheter set-up	
	Day3: IV Irinotecan	Day3: IV Irinotecan			
		Day 15: NSCs via Rickham catheter	Day 15 pre-tx: T cells, IgG/M, peripheral NSCs	Day 3-5: Microdialysis samples and blood samples pre- and post-irinotecan	
	Day 17: IV Irinotecan	Day 17: IV Irinotecan			
≥ Cycle 2 (28 days)	Day1: IV Irinotecan	Day 1: NSCs via Rickham catheter	Day 1 pre-tx: T cells, IgG/M, peripheral NSCs, (cycle 2 only); RCR (cycle 4 &7)		
		Day3: IV Irinotecan			
	Day 15: IV Irinotecan	Day 15: NSCs via Rickham catheter	Day 15 pre-tx: T cells, IgG/M, peripheral NSCs		
		Day 17: IV Irinotecan			
	Day 28: MRI to assess progression (even cycles only)				
Follow-up	30 days after last tx: assessment of vital status and toxicities		RCR at 3 and 6 months and yearly from the day of surgery		
	Annual medical history				
			Tissue from autopsy, if family consents		

[a] If the maximum tolerated dose (MTD) is not reached, the maximum feasible dose (MFD) will be used.

[b] Procedure letters are used throughout the protocol for operational guidance for the protocol user. **Procedure legend is located in Tables 8.2a/b.**

[c] Dose Levels 1 and -1 (procedures A-D) will have cycle 1 extended to 30 days to maintain 14 days between irinotecan administrations.

[c] Four participants in the expansion cohort (procedure K) will not receive NSCs on Day 1.

[d] Participants undergoing biopsy at NSC dose levels 3 and 4 (biopsy procedures G-J) will have Day 1 NSCs administered during surgery and post-recovery via the Rickham catheter.

Overall Treatment Plan

The following is an overview of the treatment plan:

1. Treatment cycles are defined by Day 1 study agent administration, except for Cycle 1 where Day 1 is defined as day of surgery. After Cycle 1, Day 1 agent administration will be NSCs, or for participants who are no longer receiving NSCs, Day 1 agent administration can be irinotecan.
2. Cycles are planned to be 28 days in duration, however the cycle may be extended for any of the following reasons:
    a. A delay in administering the Day 1 treatment, resulting in an extension of the prior cycle.
    b. A delay in administering the mid-cycle (Day 3, 15, 17) administrations, resulting in an extension of the respective cycle.
    c. Dose level 1 or -1 participants will complete NSC treatmentduring cycle 1, and will start cycle 2 with irinotecan administration. As such, cycle 1 will be extended as needed (e.g. from 28 days to 30 days) to ensure that the cycle 2 day 1 irinotecan dose occurs 14 days after the previous irinotecan dose.
3. All safety assessments and immunology and RCR testing must be performed prior to administration of any study medication, and per the windows defined in Section 10. For planned assessments outside of schedule, written approval must be granted by the Principal Investigator.
4. Start of cycle criteria must be met in order to begin the treatment cycle. See Section 7.2.2.
5. Prior to administering any study agent at any time point, criteria for holding or modification of dose must be reviewed. See Section 7.2.4.
6. Adjustments to the schedule following a hold in study agent(s) are described in Section 7.3.
7. There will be no intra-patient dose escalation of NSCs or irinotecan.
8. Participants who discontinue irinotecan will discontinue all study treatment.
9. If administration of agents must be interrupted, dose reduced or terminated because of unacceptable toxicity or issues with the Rickham catheter, agent dosing will be modifiedaccording to rules described in Section 7. All changes to study agent administration must be recorded.
10. Prior to commencing treatment with NSCs, participants must have confirmed recurrent high grade glioma based on tumor taken during the Day 1 neurosurgery (Section 8.1). If recurrent high grade glioma is not confirmed, participants will be removed from therapy and replaced.
11. Prior to commencing treatment with irinotecan (day 3 of cycle 1) participants must have recovered well, per the neurosurgeon, from any immediate post-operative effects, and the patient must require ≤ 16 mg/day of dexamethasone. Otherwise the participant will be removed from further study agent administration (Section 7.2.3).
12. For participants who receive their initial doses of NSCs and irinotecan, treatment will continue until either evidence of progressive disease, unacceptable toxicity, non-compliance with study follow-up, or withdrawal of consent.

Assigning Treatment and Correlative Study Regimen

A participant's starting NSC dose level, irinotecan dose schedule, correlative study procedures, and NSC regimen will be assigned by the Principal Investigator based on the following criteria:

1. Except where planned in the expansion cohort, participants who do not receive Day 1 NSCs will be replaced.
2. During the 3+3 stage of the trial, any participants who are not evaluable for dose escalation will be replaced.
3. During the 3+3 stage of the trial, the NSC dose level (-1,1,2,3, or 4) and irinotecan dose schedule (A, B, or C) will be determined using criteria defined in Section 5.3.
4. The first six patients enrolled to the trial will have microdialysis catheters inserted and undergo microdialysis and accompanying blood plasma PK studies. If one or more of the six patients does not receive cycle 1 day 3 irinotecan, the patient(s) will be replaced with the next patient(s) enrolled.
5. During the expansion cohort, all eight participants will have microdialysis catheters inserted and undergo microdialysis and accompanying blood plasma PK studies. N.B., since it is anticipated that the MTD/MFD will be 150 million NSCs (dose level 4), the 6 patients who will be treated on dose level 4 will undergo intracerebral microdialysis and be counted as part of the expansion cohort.
6. During the expansion cohort, all 8 participants will follow the NSC dose level and irinotecan dose schedule per the MTD (or if the MTD is not reached, the MFD), however 4 participants will receive their initial NSCadministration on the day of surgery (Day 1), and 4 participants will receive their first NSC administration on Day 15. Participants will be grouped according to surgery type (craniotomy or biopsy). As each group enrolls participants, it will alternate between enrolling the participant to the Day 1 NSC start date and the Day 15 NSC start date. (See Section 5.4 for further explanation).

Based on the above criteria, the Principal Investigator will communicate to the clinical research team and the neurosurgeon the assigned biopsy or craniotomy procedure A-K as detailed in table 8.2a and table 8.2b.

Phase I Study Design – Determining the MTD

The study will implement a standard 3+3 design. The goal will be to determine the maximum tolerated dose (MTD) of NSCs with irinotecan. If the maximum tolerated dose is not reached, then the maximum dose will be the maximum feasible dose (MFD).

5.1.1 Phase I Dose Levels and Schedules for NSCs and Irinotecan

Table 5.3.1 Phase I Dosing for NSCs and Irinotecan

	NSC Dose Level	NSC Number and Administrations			Irinotecan Schedule	Irinotecan Dose *(ongoing Day 3 and Day 17 administration)**
	-1	2.5 x 10^7 NSCs	*one administration only – C1D1*		C	125 mg/ m^2 irinotecan
Initial Cohort	1	5.0 x 10^7 NSCs	*one administration only – C1D1*		B	150 mg/ m^2 irinotecan
	2	5.0 x 10^7 NSCs	*ongoing: Day 1 and Day15*	*Initial Cohort*	A	180 mg/ m^2 irinotecan
	3	1.0 x 10^8 NSCs	*ongoing: Day 1 and Day15*			
	4	1.5 x 10^8 NSCs	*ongoing: Day 1 and Day15*			

*Dose level 1 and -1 participants will only receive one dose of NSCs, but will continue to receive irinotecan. As such, starting with cycle 2, their irinotecan administrations will be on Day 1 and Day 15 instead of Day 3 and Day 17.

5.1.2 Phase I Cohorts and Dose Escalation and De-escalation Criteria

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

Enrollment of the first 3 patients to a dose level will 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.

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

To be counted for dose escalation, a study patient must receive at least 80% of the assigned NSCs and 80% of the irinotecan for a particular dose level and be followed for 4 weeks **OR** have experienced a DLT as defined in Section 5.3.3.

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

The NSC dose levels and irinotecan dose schedulesare provided in Table 5.3.1. The first cohort will start at NSC dose level 1 and irinotecan schedule A.

The following dose escalation rules will be used, using the DLT definition as provided in Section 5.3.2:

- If 0/3 study participants experiences a DLT, the next cohort of 3 study patients will be treated at the next higher dose level.
- 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.
- If 1/6 participants experiences a DLT, the next cohort of 3 study participants will be treated at the next higher dose level.
- 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.
- At least 6 patients will be treated at the MTD (or MFD, if the MTD is not reached).

If the 3+3 design rules indicate a need for de-escalation, the followingwill apply:

- If all DLTs in a dose level areattributed uniquely to irinotecan,then only the irinotecan schedule will de-escalate.
- If all DLTs in a dose level are attributed uniquely to NSCs, and are not attributed to 'NSCs with irinotecan', nor to irinotecan alone, then only the NSC dose level will de-escalate.
- If all the DLTs in a dose level are attributed to a combination of NSCs and irinotecan, then the PMT will evaluate the details of each toxicity to determine whether the NSC dose level or the irinotecan dose schedule should be de-escalated.
- If the situation occurs when one DLT in a dose level is attributed to irinotecan alone and another is attributed to NSCs alone, then the PMT will evaluate the specific details of these toxicities and make a decision as to whether the dose of NSCs or irinotecan should be de-escalated.

5.1.3 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, irinotecanor the combination of NSCs with irinotecan, 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 or irinotecan, except CNS toxicities that are present at baseline and 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 5.6).

5.1.4 Definition of Maximum Tolerated Dose and Maximum Feasible Dose

The MTD/MFD will be based on the assessment of DLTs as defined in Section 5.3.3. The MTD is the dose levelat which 0/6 or 1/6 participants experience a DLT with the next higher dosehaving at least 2/3 or 2/6 participants encountering a DLT. If the MTD has not been reached with NSC dose level 4 and irinotecan schedule A, then that dose level will be termed the maximum feasible dose (MFD).

Treatment Plan: Expansion Cohort

After the MTD/MFD of the combination therapy is defined, an expansion cohort of 8 patients will be treated to gain more experience regarding the tolerability of study treatment at the MTD/MFD and to assess the biological activity of the hCE1m6-NSCs using intracerebral microdialysis. Since it is anticipated that the MTD/MFD will be 150 million NSCs (dose level 4), the 6 patients who will be treated on dose level 4 will undergo intracerebral microdialysis and be counted as part of the expansion cohort.

Four patients in the expansion cohort will undergo intracerebral microdialysis after receiving NSCs at the time of surgery followed 2 days later by IV irinotecan, but the other 4 patients in this cohort will not receive NSCs on the day of surgery. Instead they will be given IV irinotecan 2 days after surgery, and dialysate samples will be collected from them to determine how much SN-38 is present in the brain from treatment with IV irinotecan alone. These 4 patients will then receive their first dose of NSCs through the Rickham on day 15 of cycle 1 followed by irinotecan on day 17.

To determine who will receive their initial NSCs administration on the day of surgery, and who will receive their first NSC administration on Day 15 via the Rickham catheter, participants will be stratified according to surgery type (craniotomy or biopsy), and,as each group enrolls participants, it will alternate between enrolling the participant to the Day 1 NSC start date and the Day 15 NSC start date. For example, the first craniotomy participant will be assigned Day 1 NSC start date, the second craniotomy participant will be assigned Day 15 NSC start date, the third will be assigned Day 1 NSC start date, the fourth will be assigned Day 15 NSC start date, and so on as applicable. The same alternate enrollment scheme will apply to participants undergoing the biopsy procedure. The rationale for this plan is to ensure to the degree possible an even distribution of start dates for each surgery group which should increase the ability for direct comparison. There is no requirement for specific number of biopsy or craniotomy participants in the expansion cohort.

If the DLT rate in the expansion cohortexceeds 34%, the toxicity data will be reviewed.

Criteria for Removal from Participation or Treatment

For participants who begin treatment, duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may begin and continue until or unless one of the following criteria applies:

- Pathology review of the frozen section obtained at the time of surgery does not confirm recurrent high grade glioma (Section 8.1). Such participants will not continue on to receive any study agents, and all study procedures will end.

- Participant does not meet the criteria for initiating irinotecan dosing following surgery (Section 7.2.3). During cycle 1, if a participant requires more than 16 mg/day of dexamethasone to control cerebral edema at the time s/he is ready to begin treatment with irinotecan, then s/he will be considered neurologically unstable and will not continue with study treatment; however, the patient will still be evaluable for toxicity.
- Evidence of disease progression.
- Unacceptable adverse event(s) (see Section 7.2).
- Intercurrent illness that prevents further administration of treatment.
- Participant needs a treatment not allowed by the protocol.
- A participant may always be removed from treatment whenever s/he wishes.
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

Participants will be removed from study treatment when any one of the criteria listed above applies. The reason for study treatment removal and the date the participant was removed must be documented. Alternative care options will be discussed with the participant.

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 or NSCs and irinotecan in study patients exceeds 50% in more than 6 patients, 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), and only deaths occurring < 30 days from intracerebral administration of NSCs will be considered for the purposes of defining conditions for discontinuation of the study.

Subject 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 other anti-cancer therapy, progression of disease, survival, as well as information pertaining to *de novo* cancer, neurologic, autoimmune and hematologic disorders. In addition, unexpected medical problems including information on hospitalizations and medications will be collected. Alternatively, participants may be seen at the COH clinic to obtain this information.

Testing for replication competent retrovirus (RCR) 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.

6 CONCOMITANT MEDICATIONS, SUPPORTIVE CARE, AND INCREASED MONITORING FOLLOWING TOXICITIES

6.1 Prophylaxis, Supportive Treatment and Increased Monitoring

Unless expressly indicated elsewhere in this section, increased monitoring, prophylaxis, and ancillary treatment after any toxicity is left to the clinical judgment of the investigator and must be compliant with Section 6.2 Concomitant Medications.

6.1.1 **REQUIRED** Peri- and post-operative Prophylaxis

Levetiracetem (Keppra)—or another anticonvulsant that does not cause significant induction of hepatic metabolic enzymes, such as lacosamide or valproic acid—will be administered to all study patients. Those who have never had a seizure will start taking levetiracetam within 1 week prior to surgery and continue taking the anti-seizure medication for 4 weeks after craniotomy or biopsy.

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

For participants with microdialysis catheters, prophylactic antibiotic use will continue as long as the microdialysis catheter(s) remains in place.

6.1.2 **REQUIRED** Catheter Infection Prophylaxis and Monitoring for Infection

Also, to monitor for possible catheter-related infections, an attempt to aspirate fluid from the Rickham reservoir will be done prior to administering a dose of hCE1m6-NSCs. The fluid will be sent for culture, as well as routine cell count, glucose and protein levels, depending on the amount of fluid obtained.

A 14 day culture for bacteria and a 28 day culture for fungus will be started on every final NSC product. The NSC product will be administered to the study patient before the results of the sterility testing are known. If these culture results were to become positive for bacteria, or fungus, then an Infectious Diseases consultation would be obtained for recommendations regarding appropriate treatment of the isolate that grows out.

6.1.3 **REQUIRED** Nausea Prophylaxis, Participant Education, and Treatment

Participants will be pre-medicated with ondansetron 16 mg p.o. at least 30 minutes prior toeach infusion of irinotecan.

Additional use of anti-emetics is at the discretion of the investigator.

6.1.4 **REQUIRED** Diarrhea Treatment and Participant Education

Diarrhea is a common side effect of irinotecan, and aggressive management with anti-diarrheal agents is necessary. All study patients will be counseled about the importance of early recognition and treatment of diarrhea.

Orders will be written for standard use of atropine (to prevent early onset diarrhea) and loperamide (for management of later diarrhea) as needed. Prophylactic use of atropine to prevent early onset diarrhea accompanied by cholinergic symptoms is left to investigator discretion.

Each patient should be instructed to have loperamide readily available and to begin treatment for diarrhea at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normally expected for the patient. Loperamide 4 mg should be taken at the first loose stool or more frequent

than usual bowel movements, followed by 2 mg as needed, no more frequently than every 4 hours not to exceed a total of 16 mg in 24 hours. If the diarrhea persists (> grade 1)despite use of loperamide, then s/he should start taking diphenoxylate/atropine (Lomotil) 2 tablets every 6 hours.

6.1.5 **REQUIRED** Increased Monitoring following Initial Indications of Possible Immune Response to the NSCs

If a patient starts exhibiting signs/symptoms concerning for a possible immune response to the NSCs (such as development of a new neurologic deficit that cannot be attributed to the location of the patient's brain tumor, fever without a localizing source of infection, or rash not likely due to a concomitant medication), then **serum samples will be collected from the patient as soon as possible, and if feasible without putting the participant at increased risk, prior to administration of supportive care. A second sample, and additional samples if possible, should be taken approximately every 2 days to determine response to supportive care for the immune response.**The patient's stored serum samples from prior to start of study treatment, will alsobe analyzed for the same cytokine panel. See Section 8.6 for blood collection information.

In particular, we would be looking for changes in levels of IL-1RA, IL-6, IFN-γ, IP-10, MIP-1β, MCP-1, IL-2RA, IL-8, IL-10, MIP-1α, G-CSF, IL-15, IL-7, IL-2, TNF-α, IL- 1β**.** Findings consistent with the presence of a "cytokine storm" would add strength to the conclusion that the patient is experiencing an immune reaction to the NSCs. See Section 12.2.5 for additional information regarding the cytokine panel.

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.

See Section 6.1.5 for required supportive care.

6.1.6 **REQUIRED**Management ofPossible Immune Response to the NSCs

Participants who develop a grade 2 or higher Immune Response to NSCs, will take dexamethasone to a maximum dose of 4 mg twice daily until symptoms resolve to ≤ Grade 1. For participants continuing to receive NSCs, dexamethasone should continue at a dose determined by the treating investigator and per consultation with the principal investigator. For participants no longer receiving NSC administration may continue dexamethasone at the discretion of the treating investigator and per consultation with the principal investigator.

6.1.7 Prophylaxis and management of cholinergic irinotecan-related syndrome

Patients may experience an acute syndrome of lacrimation, diaphoresis,abdominal cramping, and diarrhea (early diarrhea) during or shortly after irinotecanadministration; this syndrome is thought to be cholinergically mediated. Prophylactic ortherapeutic administration of atropine should be considered in patients experiencing cholinergic symptoms.

6.1.8 **REQUIRED**Serum electrolyte abnormalities ≥ CTCAEv3 grade 3

If serum chemistries ≥ CTCAEv4 grade 3 or greater:

Patient's potassium, calcium, phosphorous or magnesium should be immediately supplemented or otherwise corrected following the availability of those laboratory results, in order to minimize the time patients have abnormal values.

More frequent testing should be done if clinically indicated, e.g. patient has had prior low values, patient is experiencing gastro-intestinal toxicities or is taking medications that can result in lowering of their potassium, magnesium, phosphorus or calcium levels.

6.2 **Concomitant Medications**

Participants must be instructed not to take any additional medications (including over-the-counter products) during the trial without prior consultation with the investigator. All medications taken within 30 days of screening should be recorded. If concomitant therapy must be added or changed, including over-the-counter medications or alternative therapies, the reason and name of the agent/therapy should be recorded.

In general, the use of any concomitant medication/therapies deemed necessary for the care of the participant are allowed, including drugs given prophylactically (e.g. antiemetics) with the following exceptions:

- No other investigational therapy should be given to patients
- No anticancer agents other than the study medications administered as part of this studyprotocol should be given to patients. If such agents are required for a patient then the patientmust first be withdrawn from the study.
- CYP3A4 Inducers and Moderate to Strong Inhibitors: Because concomitant use of CYP3A4 hepatic-enyzme inducing anticonvulsants can result in decreased blood levels of irinotecan, the following anticonvulsants are not allowed while the patient is participating in treatment phase of this study: phenytoin (Dilantin), fosphenytoin (Cerebyx), carbamazepine (Tegretol), phenobarbital (Luminal, Solfoton), primidone (Mysoline), and oxcarbazepine (Trileptal).

  Additionally, patients must not take other medications that are moderate to strong CYP3A4 inhibitors or strong inducers (Table 6.2) while undergoing study treatment.

Table 6.2 CYP3A4 Inhibitors and Inducers

Strong CYP3A4 Inhibitors (≥5-fold increase in AUC)	Moderate CYP3A4 Inhibitors (≥2-but < 5-fold increase in AUC)	Strong CYP3A4 Inducers
Atazanavir	Amprenavir	Aminoglutethimide
Clarithromycin	Aprepitant	Bexarotene
Indinavir	Diltiazem	Bosentan
Itraconazole	Erythromycin	Carbamazepine
Ketoconazole	Fluconazole	Efavirenz
Nefazodone	Fosamprenavir	Fosphenytoin
Nelfinavir	Grapefruit juice	Griseofulvin
Ritonavir	Pomegranate juice	Modafinil
Saquinavir	Verapamil	Nafcillin
Telithromycin		Nevirapine
Suboxone		Oxcarbazepine
		Phenobarbital
		Phenytoin
		Primidone
		Rifabutin
		Rifampin
		Rifapentine
		St. John’s wort

If over the course of study treatment the patient receives any of these drugs, efforts should be made to discontinue the use of inhibitors or potent inducers as soon as possible. Such patients may continue studytreatment only at the discretion of the principal investigator. Patients who cannot be discontinued from use of a prohibited CYP3A4 inducer or inhibitor must be removed from the treatment phase of the study.

- Use of flucytosine (5-FC) during study treatment is also not allowed. These NSCs also have the ability to convert 5-FC to 5-fluorouracil, but the safety of the combination of 5-FC and irinotecan in brain tumor patients being treated with these NSCs is unknown at this time.
- Use of drugs known to **inhibit** UGT1A1, such as Atazanir, Gemfibrozil, Indinavir, or Ketoconazole, are prohibited while undergoing study treatment as they are likely to increase SN-38 levels and increase overall toxicity to irinotecan.
- Co-medication that may interfere with study results; e.g. immuno-suppressive agents other than corticosteroids, such as systemic cyclosporine and tacrilomus are prohibited during the treatment phase of the study.
- Use of herbal medications may have unknown interactions with the metabolism of the study agents, and therefore are prohibited from use during the treatment phase of the trial.

7 EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Anticipated Toxicities

NOTE: See Section 6.1 for required and recommended prophylaxis and management.

7.1.1 Neurosurgery Toxicities

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

7.1.2 NSCToxicities

All toxicity studies to date in mice have shown no evidence of organ damage or tumorigenicity due to hCEm16-NSCs.

In the first-in-human study of HB1.F3.CD.NSCs (i.e., the parent cell line that was adenovirally transduced to create the hCE1m6-NSCs being studied in this clinical trial), no grade 3 or 4 toxicities attributable to the NSCwere observed.

Immune responses to other cellular or gene therapy agents have occurred and may occur to hCE1m6-NSCs.

7.1.3 Microdialysis Catheter Toxicities

Bleeding along the catheter tract, infection localized to or clearly emanating from the catheter. Minor bleeding (less than 1 mL) has been observed in 3% of patients (Poca et al., 2006), and there have been no documented microdialysis catheter-related infections.

7.1.4 Irinotecan Toxicities

Common:

Neutropenia and/or late diarrhea (occurring more than 8 hours after irinotecan administration) are common dose-modifying toxicities.

Other commonly observed adverse events includenausea and vomiting, anorexia, weight loss, abdominal cramping, constipation, alopecia, asthenia,lymphocytopenia, anemia, fever, and dehydration as a result of diarrhea and/or vomiting.

Patients may experience an acute syndrome of lacrimation, diaphoresis,vasodilation (flushing), abdominal cramping, and diarrhea (early diarrhea) during or shortly after irinotecanadministration; this syndrome is thought to be cholinergically mediated.

Less common: mucositis, colitis (sometimes with gastrointestinal bleeding), skin irritation at infusion site, headache, dizziness, confusion, somnolence, flatulence, dyspepsia, rash, minor infection

Rare, but serious: pulmonary toxicity (manifested as shortness of breath, non-productive cough, and transient infiltrates on chest x-ray), elevated hepatic enzymes, increased serum creatinine, thrombocytopenia, infusion reaction, neutropenic infection, febrile neutropenia

Dose Modifications/Delays/Start of Cycle Criteria

7.1.5 General Information

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

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.

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

4. NSC or irinotecan dose re-escalation is never permitted in this study.

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. 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.*

7. For holds due to toxicities related to study agent(s), if the participant does not meet criteria to resume treatment within 28 days of their last NSC administration (or irinotecan for Dose Level 1/-1 participants after they have completed cycle 1), the participant must permanently discontinue study treatment (both 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.*

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.*

9. Section 7.3 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 by 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.

7.1.6 Start of Cycle Criteria

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

a) 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),

b) ANC must be ≥ 1500 cells/mm^3,

c) platelet count ≥ 100,000 cells/mm^3, and

d) treatment-related diarrhea has resolved.

For participants who received two administrations of NSCs with irinotecan (or irinotecan only for participants in dose level 1/-1 ≥ cycle 2) in the previous treatment cycle, a delay of up to 14 days in starting the next cycle is allowed in order for the patient to have time to recover from toxicities of the prior cycle.

7.1.7 Criteria for First Administration of Irinotecan

For Cycle 1 Day 3 irinotecan administration, 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 7.2.4 also apply.

7.1.8 Dose Modifications

Table 7.2.4 details the criteria for disrupting treatment, dose modification and treatment discontinuation following an adverse event. See section 7.2.1 for general guidelines. The agent listed in Table 7.2.4 indicates the drug to which the toxicity is likely attributed based on previous trials to irinotecan and possible anticipated toxicities to the hCE1m6-NSCs. The investigator will determine attribution of all toxicities.

Table 7.2.4 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 supportive care:** 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 supportive care:** 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)	Irinotecan	Maintain treatment
Thrombocytopenia Grade 2 (50 – <75 x 10^9/L)		Maintain treatment
Thrombocytopenia Grade 3 (25 – <50 x 10^9/L)		Hold treatment until recovery to ≤ grade 2 (plts ≥ 50 x 10^9/L).
Thrombocytopenia Grade 4 (<25 x 10^9/L)		Hold treatment until recovery to ≤ grade 1 (plts ≥ 75 x 10^9/L). Resume treatment with an irinotecan dose reduction of 30 mg/m^2.
Neutropenia (ANC) Grade 1 (1.5x 10^9/L -<LLN)	Irinotecan	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 -<LLN)		Hold treatment until recovery to ≤ grade 2 (ANC ≥ 1.0 x 10^9/L). *First occurrence*: Resume treatment with no dose modifications. *Each occurrence thereafter*: Resume treatment with an irinotecan dose reduction of 30 mg/m^2.
Neutropenia (ANC) Grade 4 (<0.5x 10^9/L -<LLN)		Hold treatment until recovery to ≤ grade 2 (ANC ≥ 1.0 x 10^9/L). Resume treatment with an irinotecan dose reduction of 30 mg/m^2.
Febrile neutropenia ≥ Grade 3 (ANC< 1.0x 10^9/L, fever > 38.5°C)	Irinotecan	Hold treatment until recovery to ≤ grade 1 (ANC ≥ 1.5 x 10^9/L). Resume treatment with an irinotecan dose reduction of 30 mg/m^2.
Serum Electrolytes		
Serum Electrolytes ≥ Grade 3 Calcium , Potassium, Magnesium,Phosphorous	Irinotecan (via diarrhea)	Provide supportive care per Section 6.7. Maintain treatment per investigator discretion.
Gastrointestinal		
Diarrhea Grade 1 (2-3 stools/day >pretreatment)	Irinotecan	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)		Event present while on optimal anti-diarrheal therapy: Hold study treatment until the diarrhea decreases to ≤ grade 1, and then restart treatment with the dose of irinotecan reduced by 30 mg/m^2
Diarrhea Grade 4 (≥10 stools/day > pretreatment)		Event present while on optimal anti-diarrheal therapy: Hold study treatment until the diarrhea decreases to ≤ grade 1, and then restart treatment with the dose of irinotecan reduced by 60 mg/m^2.
Vomiting or Nausea Grade 1	Irinotecan	Maintain treatment
Vomiting or Nausea Grade 2		Maintain treatment
Vomiting or Nausea Grade 3, despite maximal medical management		*1st event present when on optimal anti-nausea therapy:* Hold until resolved to ≤ grade 1, then resume treatment at pre-hold dose *≥ 2nd event present when on optimal anti-nausea therapy*: Hold until resolved to ≤ grade 1, then resume with an irinotecan decrease of 30 mg/m^2 dose reduction
Vomiting Grade 4		Event present while on optimal antiemetic therapy: Hold study treatment until the vomiting decreases to ≤ grade 1, and then restart treatment with the dose of irinotecan reduced by 30 mg/m^2.
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 with or without irinotecan, and is not an immune response to study agents.	NSCs with or without irinotecan	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 by 25% and dose reduce irinotecan* by 30 mg/m^2. *Second event:* Resume treatment with then dose reduce NSCs by 25% and dose reduce irinotecan* by 30 mg/m^2. *If the event occurred after recent administration with NSCs but before administration with irinotecan, dose reductions indicated only apply to NSCs. Otherwise both agents will be dose reduced.
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 with or without irinotecan, and is not an immune response to study agents.	NSCs with or without irinotecan	Hold until ≤ grade 1, then perform brain MRI and PET/CT scans. If objective evidence of clinical benefit, continue tx* with dose reduce NSCs by 25% and dose reduce irinotecan by 30 mg/m^2. If no objective evidence of clinical benefit, discontinue treatment. *If the event occurred after recent administration with NSCs but before administration with irinotecan, dose reductions indicated only apply to NSCs. Otherwise both agents will be dose reduced.
Other unspecified Non-Hem Toxicities considered Related <u>only</u> to Irinotecan		
Grade 1	Irinotecan	Maintain treatment
Grade 2		Maintain treatment
Grade 3		Hold treatment until resolution to ≤ grade 1, then dose reduce irinotecan by 30 mg/m^2.
Grade 4		Hold treatment until resolution to ≤ grade 1, then dose reduce irinotecan by 60 mg/m^2.

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% and dose reduce irinotecan by 30 mg/m^2.
Grade 4		Hold until ≤ grade 1, then perform brain MRI and PET/CT scans. If objective evidence of clinical benefit, continue treatment with dose reduce NSCs by 25%. If no objective evidence of clinical benefit, discontinue treatment.
Other unspecified Non-Hem Toxicities considered Related NSCs with irinotecan		
Grade 1	NSCs with irinotecan	Maintain treatment
Grade 2		Maintain treatment
Grade 3		Hold until ≤ grade 1, then dose reduce NSCs by 25% and dose reduce irinotecan by 30 mg/m^2.
Grade 4		Hold until ≤ grade 1, then perform brain MRI and PET/CT scans. If objective evidence of clinical benefit, continue tx with dose reduce NSCs by 25% and dose reduce irinotecan by 30 mg/m^2. If no objective evidence of clinical benefit, 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.

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 10) will be conducted at the original times during the cycle and safety assessments (clinical laboratory tests, neuro-exam, physical exam, vitals, KPS) will be repeated prior to resuming agent administration. For instance, if the participant is unable to take Day 15 NSCs, he/she will still have all procedures performed on Day 15, but will have safety assessments performed again prior to taking NSCs later in the cycle.

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.

7.1.9 Holding Day 15 NSCs with Day 17 irinotecan

If Day 15 NSCs with Day 17 irinotecan 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 **<u>not</u>** able to resume treatment by or on Day 22, he/she will continue the current cycle without a second Day 15 NSC 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 15/17 NSCs/irinotecan.

7.1.10 HoldingDay 17 irinotecan after administering Day 15 NSCs

If Day 17 irinotecan is held, and the participant has already received Day 15 NSCs, resultant start dates are as follows:

- If participant is able to resume treatment by or on Day 24, he/she will resume irinotecan. The cycle length will be extended so that 14 days between irinotecan administrations (the second administration of the current cycle and the first administration of the subsequent cycle) would be maintained.

- If participant is not able to resume treatment by or on Day 24, he/she will continue the current cycle without a second irinotecan 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 Day 17 irinotecan.

7.1.11 HoldingDay 3 irinotecan after administering Day 1 NSCs

If Day 3 irinotecan is held, and the participant has already received Day 1 NSCs, resultant start times are as follows:

- If participant is able to resume treatment by or on Day 10, he/she will resume irinotecan. The subsequent NSC administration will be timed such that 14 days between irinotecan administrations will be maintained. The total cycle length will be maintained accordingly

- If participant is not able to resume treatment by or on Day 10, he/she will continue the current cycle without a 'Day 3'irinotecan administration and resume NSC with irinotecan administration on Day 15 and Day 17, respectively, of the treatment cycle. Assuming there is no delay is starting the subsequent treatment cycle, the current cycle would 28 days in length and the participant will forgo Day 3 irinotecan.

7.1.12 HoldingDay 15 irinotecan – Dose Level 1/-1 for ≥ cycle 2

If Day 15 irinotecan is held, resultant start dates are as follows:

- If participant is able to resume treatment by or on Day 26, he/she will resume irinotecan. The cycle length will be extended so that 14 days between irinotecan administrations (the second administration of the current cycle and the first administration of the subsequent cycle) would be maintained.

- If a participant is not able to resume treatment by or on Day 26, he/she will end the current treatment cycle and resume irinotecan as part of the next treatment cycle. The current cycle may be cut short to avoid further delay in agent administration.

8 STUDY PROCEDURES INCLUDING AGENT ADMINISTRATION

All required study procedures, their timepoints and corresponding windows are detailed in Section 10 and Table 10 Study Activity Calendar. The Principal Investigator will clarify to the participant's treating investigators and clinical research team which study procedures (microdialysis, expansion cohort omission NSC start date) and which NSC dose level and irinotecan dose schedule the participant will undergo, when the participant begins the trial.

Procedures performed by the clinical research team that require additional clarification beyond that provided in Table10 Study Activity Calendar, are detailed in this section.

Pathology Review on Real-Time Frozen Section

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.

Confirmation of recurrent high-grade glioma by pathological frozen section of a tissue sample taken from a site different from the initial tumor presentation requires the simple identification of high grade glioma features (nuclear atypia, increase in cellularity, significant proliferative activity).

In suspected recurrences at the site of initial presentation or in areas subjected to radiation treatment previously, a higher threshold will be used for diagnosing recurrent tumor. In order to distinguish patients who have"quiescent" paucicellular residual tumor foci (who would thus be ineligible to proceed with administering NSCs) from those with true tumor recurrences,

one or more of the following findings should be present for confirmation of recurrent HGG:

- neoplastic cells with high nuclear to cytoplasmic ratio (with the exception of giant cell glioblastoma)
- high mitotic activity
- pseudopalisading necrosis
- micro-endothelial proliferation
- infiltrated parenchyma devoid of radiation changes

Surgery, Catheter Placement, and NSC Administration at the time of Surgery

The Principal Investigator will communicate the procedure (A-K) to the neurosurgeon.

Table 8.2a Craniotomy Procedure

Dose Level	**Craniotomy Procedure**	**Microdialysis Catheters**	Number of NSCs	Final volume	**Rickham**
-1	**A**	Microdialysis Catheters	$2.5x\ 10^7$	0.375 mL	-
-1	**B**	-	$2.5x\ 10^7$	0.375 mL	-
1	**C**	Microdialysis Catheters	$5\ x\ 10^7$	0.75 mL	-
1	**D**	-	$5\ x\ 10^7$	0.75 mL	-
2	**E**	Microdialysis Catheters	$5\ x\ 10^7$	0.75 mL	Rickham
2	**F**	-	$5\ x\ 10^7$	0.75 mL	Rickham
3	**G**	Microdialysis Catheters	$1\ x\ 10^8$	1.5 mL	Rickham
3	**H**	-	$1\ x\ 10^8$	1.5 mL	Rickham
4	**I**	Microdialysis Catheters	$1.5\ x\ 10^8$	2.25 mL	Rickham
4	**J**	-	$1.5\ x\ 10^8$	2.25 mL	Rickham
-	**K**	Microdialysis Catheters	N/A	N/A	Rickham

Table 8.2b Biopsy Procedure

Dose Level	**Biopsy Procedure**	Number of NSCs	Total volume	**Rickham**	**NSC # and volume administered during surgery** ***over 10 minutes***	**Microdialysis Catheters**	**NSC # and volume administered post recovery, duration of administration[a]**
-1	**A**	$2.5x\ 10^7$	0.375 mL	-	2.5×10^7 (.375 mL)	Microdialysis Catheters	-
-1	**B**	$2.5x\ 10^7$	0.375 mL	-	2.5×10^7 (.375 mL)	-	-
1	**C**	5×10^7	0.75 mL	-	5×10^7 (.75 mL)	Microdialysis Catheters	-
1	**D**	5×10^7	0.75 mL	-	5×10^7 (.75 mL)	-	-
2	**E**	5×10^7	0.75 mL	Rickham	5×10^7 (.75 mL)	Microdialysis Catheters	-
2	**F**	5×10^7	0.75 mL	Rickham	5×10^7 (.75 mL)	-	-
3	**G**	1×10^8	1.5 mL	Rickham	5×10^7 (.75 mL)	Microdialysis Catheters	5×10^7 (.75 mL) - 1.5hrs
3	**H**	1×10^8	1.5 mL	Rickham	5×10^7 (.75 mL)	-	5×10^7 (.75 mL) - 1.5hrs
4	**I**	1.5×10^8	2.25 mL	Rickham	6.7×10^7(1 mL)	Microdialysis Catheters	8.3×10^7 (1.25mL) – 2.5 hrs
4	**J**	1.5×10^8	2.25 mL	Rickham	6.7×10^7(1 mL)	-	8.3×10^7 (1.25mL) – 2.5 hrs
-	**K**	N/A	N/A	Rickham		Microdialysis Catheters	-

[a]Post-recovery NSC administration is detailed in Section 8.5.2.

8.1.1 Craniotomy

The following procedures are listed in the order they would occur, if they are applicable:

2. Pathology Real-Time Review for Recurrent High Grade Glioma – all participants

   Pathologist criteria detailed in Section 8.1.

   ****Participants who do not have confirmation of recurrent tumor via the pathology review of frozen sections will not proceed with study procedures.

After resecting as much tumor as safely possible, and after confirmation of recurrent high grade tumor, and at the study neurosurgeon's discretion:

3. Microdialysis Catheter – A, C, E, G, I, K

   - Before placing catheter(s) into brain, flush with sterile artificial CSF
   - Tunnel catheter subcutaneously, then insert into residual tumor or peritumoral tissue.
   - Insert 1 to 2 catheters

4. Injection of NSCs – A-J

   - Cells will arrive in the operating room already diluted into the final volume of 2% HSA/artificial CSF (per Table 8.2a). The cells are to remain at room temperature. Sterile technique will be used in the handling of cells. Refer to Table 8.2a for the number and volume of NSCs.
   - For procedures A,C,E,G,I (MD catheter present), inject between two to four 100-150 µL suspensions of NSCs at the margins of the catheter(s). The remaining number of NSCs will be injected in divided 100-150 µL volumesas evenly spaced as possible throughout the wall of the resection cavity, administering them only into areas that are surgically safe and feasible.
   - For procedures B,D,F,H,J(no MD catheter), inject the NSC volumes as evenly spaced as possible throughout the wall of the resection cavity, administering them only into areas that are surgically safe and feasible.
   - TheNSCs 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).

5. Placement of Rickham – E-K

   - After the NSCs are injected, the study neurosurgeon will place a 9.5mm Rickham in residual tumor tissue which will be used to deliver subsequent doses of NSCs.

6. Wound Closure–all participants

   - The wound will then be closed in standard fashion, anchoring the catheter(s) to the scalp with suture and sterile dressings.

8.1.2 Biopsy

The following procedures are listed in the order they would occur, if they are applicable:

1. Pathology Real-Time Review for Recurrent High Grade Glioma – all participants

   Pathologist criteria detailed in Section 8.1.

   ****Participants who do not have confirmation of recurrent tumor via the pathology review of frozen sections will not proceed with study procedures.

After resecting as much tumor as safely possible, and after confirmation of recurrent high grade tumor, and at the study neurosurgeon's discretion:

2. Placement of Rickham – E-K

   - Before the NSCs are injected, the study neurosurgeon will place a 9.5mm Rickham in residual tumor tissue which will be used to deliver subsequent doses of NSCs.

3. Injection of NSCs – A-J

   - Cells will arrive in the operating room already diluted into the appropriate volume of 2% HSA/artificial CSF (per Table 8.2b). The cells are to remain at room temperature. Sterile technique will be used in the handling of cells.
   - For procedures A-D (no Rickham),the study neurosurgeon will slowly inject the cells over 10 minutes along the original biopsy track, followed by a 0.3 mL 2% HSA/artificial CSF flush as the biopsy needle is slowly being withdrawn over one minute to minimize reflux of the NSCs.
   - For procedures E-J, the neurosurgeon will be manually inject the cells over 10 minutes through the Rickham, followed by a 0.3 mL 2% HSA/artificial CSF flush as the biopsy needle is slowly being withdrawn over one minute to minimize reflux of the NSCs.

4. Microdialysis Catheter – A, C, E, G, I, K

   - Before placing catheters into brain, flush with sterile artificial CSF
   - For procedures A and C (no Rickham), a 70 Brain MD Catheter will be passively introduced into the injection site through the needle track. When feasible, a second microdialysis catheter will be inserted into a geographically different part of the tumor, as per the next bullet point.
   - If a Rickham catheter or first MD catheter are in the biopsy site, tunnel an MD catheter subcutaneously, then insert into residual tumor or peritumoral tissue.
   - Insert 1 to 2 MD catheters

5. Wound Closure - all participants

   - The wound will then be closed in standard fashion, anchoring the catheter(s) to the scalp with suture and sterile dressings.

8.1.3 Peri- and post-operative Prophylaxis

Levetiracetem (Keppra)—or another anticonvulsant that does not cause significant induction of hepatic metabolic enzymes, such as lacosamide or valproic acid—will be administered to all study patients. Those who

have never had a seizure will start taking levetiracetam within 1 week prior to surgery and continue taking the anti-seizure medication for 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.

For participants with microdialysis catheters, prophylactic antibiotic use will continue as long as the microdialysis catheter(s) remains in place. Furthermore, these participants will be transferred to the Intensive Care Unit (ICU) after they leave the post-anesthesia care unit. These participants will stay in the ICU as long as the microdialysis catheter(s) remain in place. Microdialysis catheter(s) will be removed ahead of schedule if they stop functioning.

Study patients will be discharged from the hospital when medically ready, except for participants with microdialysis catheters who will remain in the ICU for as long as the microdialysis catheter(s) is/are in place.

Microdialysis Catheter- Related Procedures (Post-Surgery)

Procedures A, C, E, G, I, K.

8.1.4 Confirmation of Microdialysis Catheter Position

A CT scan without contrast will be obtained as soon as possible post-operatively to determine if the microdialysis catheter(s) was/were 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.

For participants who will undergo post-recovery NSC administration though the Rickham catheter (biopsy procedures G and I), the CT scan and microdialysis set up is to precede NSC administration.

8.1.5 Materials needed for microdialysis set up, maintenance and dialysis

For each microdialysis catheter, the following materials will be used:

- One 2.5mL syringe (106 Pump Syringe, ref. no. 8010191, M Dialysis, Solna, Sweden) filled with sterile artificial CSF (Perfusion Fluid CNS) for each day the MD catheter is in place.
- 1 portable syringe pump (107 MD Pump, ref. no. P000127, M Dialysis, Solna, Sweden).
- Roughly sixty microvials (Ref. No. P000001, M Dialysis, Solna, Sweden), pre-silanized and acidified by the City of Hope Analytical Pharmacology Core Facility.
- Dry ice for the collection of all microvials removed from the catheter.

8.1.6 Microdialysis Setup, Maintenance, and Collection of Dialysate

Once the CT scan confirms correct placement of the microdialysis catheter(s), the microdialysis tubing and pump will be set up as follows (and for biopsy procedures G and I, will occur prior to post-recovery NSC administration):

- Inlet tubing of each catheter will be connected to a 2.5 mL syringe filled with sterile artificial CSF.
- The syringe will then be placed in a portable syringe pump.
- The flow control on the pump will be set at 1µL/min and the pump will be turned on.
- A plastic microvial 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 until the dose of irinotecan is administered** (see Section 8.3.5).
- When replacing the microvials, compare volumes of dialysate in the microvial to a control microvial to check for clogging. Should clogging occur, as indicated by dialysate not exiting from the outlet tubing,

open the lid with the pump still on. The pump will automatically go into a flushing cycle. If the clog does not clear, the microdialysis set up and catheter are to be removed. PK blood samples will not proceed once there is no longer a functional catheter in place.

- A new syringe containing sterile artificial CSF will be placed in microdialysis pump daily, as follows:
    - First stop the pump by taking the batteries out, **not** by opening the lid. (If the lid is opened while the batteries are still in, the pump will automatically go into a flushing cycle).
    - Open the lid and replace the syringe.
    - Once 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.
- Once treatment with irinotecan has started, it is important **not** to flush the catheter unless it becomes clogged.
- All microvials containing dialysate will be placed on dry ice until they can be moved to an ultralow temperature freezer (≤-70°C) where they will be stored until shipped to the City of Hope Analytical Pharmacology Core Facility.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.
- For patients with more than one intracerebral microdialysis catheter, it is important to consistently distinguish the catheter source.

8.1.7 Set up for Blood Sample Collection and Procedures for Blood Sample Collection and Processing

Sampling will be performed to define the plasma concentration-time profiles of irinotecan and SN-38. On day 3, prior to administering irinotecan, 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).

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. Polypropylene freezer vials containing 10 µL 3N HCL will be provided. Plasma will be stored frozen at <-70°C until sent to the City of Hope Analytical Pharmacology Core Facility.

8.1.8 Blood and Dialysate Collection Timepoints

Dialysate microvials will be collected as the microvials are changed every three hours from the time of microdialysis set to irinotecan administration.

On day 3, just prior to the start of the irinotecan infusion, a new microvial will be placed in the holder(s) of the outlet tubing of the microdialysis catheter(s). The microvials will be changed to new ones **every 60 minutes for the next 24 hours**, and then the microvial(s) will be switched every 3 hours to new ones for the subsequent 24 hours.

On day 3, blood samples will be drawn just prior to the start of the irinotecan infusion and at 90 minutes (just prior to the end of the infusion), and then at 30 minutes, 1, 2, 4, 8, 24, and 48 hours after the end of the infusion.

While it is important to collect samples as close to hour as possible, it will not be a deviation if the samples are not taken at the specified time, so long as the actual time of collection is recorded.

The time of each sample collection is to be recorded.

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

8.1.9 Removal of the Microdialysis Catheter

After microdialysis procedures have finished (approximately 48 hours after irinotecan administration), the microdialysis catheter will be removed percutaneously at the bedside by neurosurgery personnel. 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.

Following the removal of the Microdialysis Catheter, the patient may leave the ICU, per the discretion of the neurosurgeon.

Administration of Irinotecan

Please see section 7.2 for criteria that a patient must meet in order to proceed with irinotecan administration.

8.1.10 Nausea and Diarrhea Prophylaxis

Patients will be pre-medicated with ondansetron 16 mg p.o. at least 30 minutes prior to the infusion of irinotecan.

Orders will also be written for standard use of atropine (to prevent early onset diarrhea) and loperamide (for management of later diarrhea) as needed.

See Section 6 for additional details regarding ancillary treatments and prophylaxis.

8.1.11 Cycle 1 Day 3 Irinotecan Special Considerations

For Cycle 1 Day 3 irinotecan administration, 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 (Section 7.2.3).

In addition, for participants undergoing microdialysis (Procedures A, C, E, G, I, K), the cycle 1 day 3 administration of irinotecan is accompanied by various samples before and after irinotecan administration. See section 8.3.3-5 for details.

8.1.12 Intravenous Administration of Irinotecan

Irinotecan will be diluted with D5W to a total volume of 500 ml and infused intravenously over 90 minutes. Start and end time of the administration are to be recorded.

Intracerebral Administration of NSCs via the Rickham

8.1.13 Post-recovery NSC administration on day of surgery

NSCs will be brought from the Aboody Laboratory to the outpatient clinic at room temperature and already resuspended at the indicated volume in 2% HSA/articial CSF.

The NSCs followed by a flush will be administered post-recovery following surgery, in the hospital room.

Table 8.5.1 Post-recovery NSC administration on day of surgery

Dose Level	**Biopsy** Procedure	Number of NSCs	Volume	Duration of NSC administration (rate of 0.5 ml/hr)
3	G-H	5.0×10^7	0.75 mL	1.5 hrs
4	I-J	8.3×10^7	1.25 mL	2.5 hrs
Flush 2% HSA/artificial CSF			1.0 mL	2.0 hrs

Method of Administration

- Prime the infusion tubing with the NSC suspension before beginning a slow infusion of the NSCs at 0.5 mL/hr.
- Follow with a 1 mL 2% HSA artificial CSF flush at a rate of 0.5ml/hr.

8.1.14 NSC administration via Rickham (not including day of surgery)

Please see section 7.2 for criteria that a patient must meet in order to proceed with NSC repeat administration.

NSCs will be brought from the Aboody Laboratory to the outpatient clinic at room temperature and already resuspended at the indicated volume in 2% HSA/articial CSF.

If a participant has a dose reduction of NSC's the NSCs will be resuspended to a concentration of 6.7×10^7NSCs/mL, and infused at a rate of 0.5mL/hr.

The NSCs followed by a flush will be administered in the outpatient setting.

Table 8.5.2 Repeat NSC administration via Rickham

Dose Level	Procedure	Number of NSCs	Volume	Duration of NSC administration (rate of 0.5 ml/hr)
2	E-F	5.0×10^7	0.75 mL	1.5 hrs
3	G-H	1.0×10^8	1.5 mL	3.0 hrs
4	I-J	1.5×10^8	2.25 mL	4.5 hrs
Flush 2% HSA/artificial CSF			1.0 mL	2.0 hrs

Method of Administration

- Prime the infusion tubing with the NSC suspension before beginning a slow infusion of the NSCs at 0.5 mL/hr.
- Follow with a 1 mL flush of 2%HSA/artificial CSF at a rate of 0.5ml/hr.

Samples for Performing the Immunological Studies and RCR Testing

All assays to be performed by CICSL and Clinical Pathology are detailed in Section 12.2.

8.1.15 Immunological Studies- Correlative Studies

Notify the CICSL prior to sample collection. Notification should be at least 24 hours in advance (the earlier the better) in the form of Outlook calendar notifications by e-mail to vtran@coh.org. CICSL would prefer samples to be delivered before 4 pm each day.

To assess for possible development of T cell responses to the adenovirally transduced NSCs and to evaluate for systemic presence of the NSCs and/or hCE1m6, approximately **30 ml** of blood will be collected in lavender top tubes (liquid potassium EDTA). The samples will be placedon wet ice and delivered to City of Hope's CICSL for processing.

To assess for possible antibody responses to the NSCs and/or adenoviral proteins, approximately **10 ml** of blood will be collected in red top tubes (free of any anti-coagulant reagent). Samples should be kept in an upright position at room temperature for 1 – 2 hours before processing to allow blood to clot. If processing cannot be done the same day, samples should be kept in an upright position at 4°C and processed within 24 hours. Centrifuge the sample at 1000 x g for 15 minutes at 4°C. Remove the serum without disrupting the blood at the bottom of the tube. Serum samples will be aliquoted and distributed to CICSL and Clinical Pathology.

8.1.16 Immunological Studies – Suspected NSC Immune Response

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 (free of any anti-coagulant reagent) at least twice (separated by approximately 2 days) during the event for analysis of serum cytokine levels via Luminex cytokine panels. Samples should be kept in an upright position at room temperature for 1 – 2 hours to allow blood to clot. Take samples to CICSL for processing. If unable to take to CICSL the same day, samples should be kept in an upright position at 4°C until delivery the next day. For the first sample, communicate to CICSL that the pre-treatment sample is also to be assayed to provide a baseline for comparison.

8.1.17 RCR Testing

For RCR testing, at least **10 ml** of blood will be collected from each patient in EDTA treated (lavender top) tubes maintained at room temperature with occasional gentle agitationand sent to City of Hope's CICSL.

9 AGENT INFORMATION AND RISKS

hCE1m6 - NSCs (IND # 16265)

9.1.1 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.

9.1.2 Human Toxicity Data

See sections 2.5 and 7.1.2.

9.1.1 Handling

Following thawing and resuspension in the Karen Aboody Laboratory, cells are to be maintained at room temperature until administration. Sterile technique is to be used in the handling of cells.

9.1.2 Administration

Intracranial administration---either through direct injection into tumor at the time of surgery, or via a Rickham that is placed in tumor tissue.

9.1.3 Supplier

City of Hope –Karen Aboody Laboratory will provide NSCs, already thawed and resuspended (per SOP) for administration. To order cells, call COH extension 63613 or email Marianne Metz at mmetz@coh.org or her designee.

Irinotecan

Irinotecan (CAMPOSTAR) is an FDA approved chemotherapy agent. Please refer to the Package Insert for additional details not provided in this section.

9.1.4 Description

Irinotecan hydrochloride trihydrate {CPT-11, (4S)-4, 11-diethyl-4-hydroxy-9-{(4-piperidinopiperidino) carbonyloxy}-IH-pyroano{3',4':6,7}indolzino{1,2-b}quino line-3, 14(4H,12H)dione hydrochloride trihydrate} is a topoisomerase I inhibitor.

9.1.5 Human Toxicity

See section 7.1.4.

9.1.6 Formulation

Irinotecan is supplied in three forms: 2 mL vials containing 40 mg of drug, 5mL vials containing 100 mg of drug, and 15 mL vials containing 300 mg of drug. The drug is supplied in brown vials and appears as apale-yellow-to-yellow crystalline powder and pale yellow transparent solution whenreconstituted.

9.1.7 Storage and Stability

Irinotecan vials must be stored in a cool, dry place, protected from lightin a locked cabinet accessible only to authorized individuals. It is stable to the expiration date on its label. Irinotecan is stable for at least 24 hours in glass bottles or plastic bags after reconstitution with D5W.

9.1.8 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent.

9.1.9 Administration

Irinotecan will be diluted with D5W to a total volume of 500 ml and infused intravenously over 90 minutes. Nothing else should be added to the bag.

9.1.10 Supplier

City of Hope will purchase irinotecan formulated for human use from a commercial source, but separate supplies for this investigational protocol will be accounted for by the Investigational Drug Service.

10 STUDY CALENDAR

Table10, Study Activity Calendar, describes required procedures and provides references where additional information is necessary. All procedures may increase in frequency if clinically indicated or oriented following a toxicity/adverse event. See Section 7.3 for adjustments to the study calendar following a delay of study agent administration.

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 2 days between administration of the NSCs and irinotecan.

During the treatment cycle, **on days of agent administration, all safety assessments must be performed and reviewed prior to administration of any study agent**.

Table 10 Study Activity Calendar

	Screening[b]	Cycle 1[a]										Cycle 2+					End of Tx[g]	30-Day Post Tx[h]	Long Term Follow-Up, Autopsy[i]
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 8	Day 10	Day 15	Day 17	Day 22	Day 1[c,d]	Day 3	Day 15[e]	Day 17	Day 28[f]			
Informed consent[j]	X																		
Background/medical history [k]	X																		
Inclusion/Exclusion criteria[l]	X																		
Histology confirmation	X[m]	X[n]																	
Vitals, KPS, neuro &physical exams[o]	X		X	X					X[p]			X		X			X	X[q]	
Adverse event assessment [r]	X		X	X			X		X[p]		X	X		X			X	X	
Concomitant meds review [s]	X		X	X					X[p]			X		X			X	X	
HLA antibody screening [u]	X																		
UGT1A1 polymorphism[v]	X																		
Immunological studies [w]	X[x]								X[p]			X[y]		X			X		
RCR testing[z]	X[x]																		
Coagulation: PT/INR, PTT	X																		
CBC with ANC[aa]	X			X			X		X[p]			X[c]		X[c]			X		
Comprehensive Metabolic Panel[bb]	X			X					X[p]			X[c]					X		
Serum Pregnancy Test [cc]	X																		
Chest X-ray and EKG	X																		
Imaging – MRI	X[dd]	X[ee]	X[ff]													X[ff]	X[hh]		
Imaging – PET/CT scan of the brain		X[gg]														X[gg]	X[hh]		
Response Assessment[ii]																X	X[ii]		
Neurosurgery		X[jj]				X[kk]													
Administration of hCE1m6 - NSCs		X[ll]							X[mm]			X[mm]		X[mm]					
Non-contrast Brain CT		X																	
Dialysate, blood pK collection[oo]				X	X	X													
IV administration of irinotecan[pp]				X[rr]						X		X[ss]	X[tt]	X[ss]	X[tt]				
Post-Study Tx Medical History [uu]																			X
Tissue from autopsy [vv]																			X

a. For patients on dose level 1 /-1, Cycle 1 will be a minimum of 30 days to ensure that 14 days have passed between irinotecan doses.

b. To be performed within 14 days from planned date of surgery, except for MRI (footnote ee) to be performed within 28 days of registration.

c. Laboratory evaluations to be performed and results reviewed within 2 days prior to agent administration. Laboratory evaluations may be performed at a clinic local to the participant.

d. 'Start of Cycle' criteria are found in Section 7.2.2. All assessments to occur prior to administration of NSCs (or irinotecan for dose levels 1/-1).

e. All assessments to occur prior to administration of NSCs (or irinotecan for dose levels 1/-1).

f. Imaging (brain MRI and PET/CT scans) should at least be done at the end of every 2 cycles (Day 28 -3days).Imaging can be done more frequently than after every 2 cycles to closely follow up on changes of unknown significance seen on a prior brain MRI or PET/CT scan.

g. End of 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.

h. 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 study agent is given. This 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.

i. Long-term follow-up to occur until death for updated medical history, RCR testing, and tissue at autopsy. Required elements occur at 3 months (+/-14 days), 6 months (+/-14 days) and annually from start of study treatment, and upon patient death. See footnotes z, rr, andss for details.

j. Performed by MD attending only. Informed consent process to be fully documented.

k. Background/medical history – to include review of treatment history for glioma, any ongoing medical conditions and medical history pertaining to eligibility on study and involvement during study.

l. Inclusion/exclusion criteria. Source documentation providing investigator's confirmation that patient has met all eligibility criteria must be available prior to registration. In addition, source documentation by neurosurgeon that (a) a physical connection between the post-resection tumor cavity and cerebral ventricles is not anticipated, (b) a gross total resection is not anticipated, (c) the prospective participant is able to undergo neurosurgery, and (d) documentation that patient "needs a biopsy/craniotomy" is needed.

m. Histology confirmation for to be performed by COH pathologist.

n. Real-time histology confirmation to occur during surgery on fresh frozen section. See section 8.1 for pathology criteria. If confirmation of high grade glioma not obtained, all further study procedures will cease.

o. Vital signs: Weight, heart rate, blood pressure, respiration rate, temp. Height required only at baseline. KPS not required at Day 2 and 3. See Appendix A for KPS scale.

p. Participants no longer receiving NSCs (e.g. Dose Levels 1 and -1) may have their Day 15 assessments performed on Day 17, prior to irinotecan dosing.

q. KPS and vital status only. Vital signs and Neuro-Physical exam are not required.

r. Adverse events experienced by participants will be collected and recorded from signing of informed consent document up to 30 days of the last dose of study medication. The period for collection and recording of AEs is extended for participants with ongoing "reportable" adverse events that are related to study agent. Adverse event reporting begins for events that occur on Day 1 (day of neurosurgery).

s. The only concomitant medication that needs to be documented in the case report form is whether the patient is taking dexamethasone (or another corticosteroid), and if so, what the dose is.

t. For HLA testing, collect blood in1 red top tube. Maintain at room temperature. Bring samples to COH Histocompatibility Laboratory.

u. Participants who have already undergone UGT1A1 genotyping may use those results and do not the procedure repeated.

v. For immunological correlative studies:notify CICSL by e-mail to vtran@coh.org at least 24 hours in advance of sampling. Collect **six** 6-ml lavender top (K-EDTA) tubes, keep on wet ice, and send to COH CICSL; collect approximately **10 ml** of blood in red top tubes, keep upright at room temperature and process as per Section 8.6.1 before sending to the COH CICSL and Clinical Pathology.On days where study agent is administered, samples are always to be taken prior to agent administration.

w. If participant is removed (or withdraws) from the trial prior to receiving study agent, the samples are to be discarded.

x. Cycle 2 only.

y. For RCR testing, collect **two** 6-ml lavender top (K-EDTA) tubes (or a volume of 10ml) maintained at room temperature with gentle agitation ~hourlyand send to CICSL.On days where study agent is administered, samples are always to be taken prior to agent administration. Notify CICSL by e-mail to vtran@coh.org in advance of sampling,

z. RCR testing is required for all patients at 3 months* and 6 months*and annually* thereafter post start of study treatment, whether patient is on treatment on in long term follow-up. For patients still on treatment, assuming there are no delays in treatment, this means RCR testing is to be performed at C4D1 and C7D1. Take RCR testing at most appropriate clinic visit for patients still on study treatment. For patients in long term follow-up, samples may be obtained via local physician and shipped to COH for testing. (*+/-14 days)

aa. CBC with ANC: hemoglobin, hematocrit, platelets, total WBC and neutrophils

bb. Comprehensive metabolic panel: Sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, calcium, glucose, albumin, total bilirubin, alkaline phosphatase, total protein, ALG/SGPT and AST/SGOT.

cc. Serum pregnancy test only for women of child bearing potential.

dd. A contrast, non-contrast MRI is to be performed for screening within 28 days from registration. (Day 2 post-surgical MRI will serve as the baseline MRI).

ee. A pre-surgical planning post-registration MRI **may** be performed if indicated by the neuro-surgeon.

ff. A contrast, non-contrast MRIwill be performed for all patients at least every two months.

gg. A PET/CT scan will be performed within 7 days after surgery to serve as the baseline scan, and then a PET/CT scan will performed whenever possible at the time follow up brain MRIs are done.

hh. Imaging to be performed if the prior assessments occurred > 21 days before decision to end treatment, if patient is agreeable to have this procedure. The absence of this MRI and/or PET/CT scan with corresponding response assessment will not be considered a protocol deviation.

ii. Response assessment using RANO criteria, at time of imaging and as indicated clinically. Dexamethasone dose to be integrated into response determination. See Section 13 for RANO criteria.

jj. Neurosurgery will include real-time pathology review and may include Rickham Catheter and/or Microdialysis Catheter placement and/or NSC administration. See section 8.2 for details.

kk. For participants undergoing microdialysis (procedures A, C, E, G, I, K) only, microdialysis catheter is to be removed following completion of microdialysis by the neurosurgical team. See section 8.3.6for all relevant information.

ll. On day of surgery, NSC administration will occur either fully during surgical procedure (craniotomy procedure A-J, biopsy procedure A-F), or will occur both duringthe surgical procedure **and** following the surgical procedure via the Rickham (biopsy procedures G-J only). Half of the participants in the expansion cohort will not receive Day 1 NSCs (procedure K).

mm. Dose level 2, 3, 4 (procedures E-J) participants only. Via Rickham catheter. See Section 8.5 for procedure.

nn. For participants undergoing Microdialysis only (procedures A, C, E, G, I, K). Post surgical non-contrast CT used to confirm appropriate placement of catheter. See section 8.3.1 for details.

oo. For participants undergoing Microdialysis only (procedures A, C, E, G, I, K). Sampling of blood and dialysate to begin prior Day 3 irinotecan administration and up to 48 hours after irinotecan administration. For the blood pKs, a venous catheter is required. See Section 8.3.2 to 8.3.5 for details.

pp. Irinotecan administration is detailed in Section8.4, including required prophylaxis, observation periods and documentation for required elements

qq. (This has been deleted)

rr. In order to receive Day 3 irinotecan, participant must have recovered well, per the neurosurgeon (documented in clinical record), from any immediate post-operative effects, and the patient must require ≤ 16mg/day of dexamethasone. Otherwise the participant will be removed from all study treatments.

ss. Dose level 1 and -1(procedures A-D) participants will have irinotecan administration on Day 1 and Day 15. NOTE: it is imperative that Cycle 1 is extended as needed (e.g. from 28 days to 30 days) to ensure that the Day 1 irinotecan dose occurs 14 days after the previous irinotecan dose.

tt. Dose level 2, 3 and 4 (procedures E-J) participants only

uu. Post-Study Treatment Medical History will consist of annual requests from the patient/patient's primary oncologist for clinical information regarding other anti-cancer therapy, progression of disease, survival, as well as information pertaining to de novo cancer, neurologic, autoimmune and hematologic disorders. In addition, unexpected medical problems including information on hospitalizations and medications will be collected. To occur annually (+/-14 days) from the start of study treatment. Alternatively, participants may come into the clinic to provide the previous year's medical history.

vv. Efforts will be made to obtain tissue at the time of autopsy.

11 DATA AND SAFETY MONITORING, UNANTICIPATED PROBLEMS AND ADVERSE EVENT REPORTING

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 gene therapy, and City of Hope holds the study's IND**.**

Monitoring and Personnel Responsible for Monitoring

The 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 use a 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 3 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 for 4 weeks. Enrollment of the first 3 patients to a dose level will be staggered by requiring that each of the first 3 patients be separately observed for 4 weeks before the next patient can start treatment on that dose level.

Reporting of data and safety to the DSMC will occur on a quarterly basis using the PMT report until the last study patient has been observed for 4 weeks.

Table 11.2 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 DSMC for review. As required by the FDA, study patients will be followed long term (up to 15 years or until death; see section 5.16), and the PMT will continue to report data and safety concern to the FDA, City of Hope DSMC, and IRB as they arise during this follow-up period.

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.

UnexpectedAdverse 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.

ExpectedAdverse 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 experiences (places the subject at immediate risk of death from the event as it occurred);
- Unplanned hospitalization equal or greater than 24 hours)) 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 dyscrasias 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 agents, 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.

Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems: Unanticipated problems must be reported to the COH DSMC and IRB **within 5 calendar days** accordingto 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 OR are not unanticipated problems will be reported only in the continuation reports and PMT reports (see Table 11.4 below).

Table 11.4City 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

Required Reporting Timeframe to IRB of Record		
Attribution	UNEXPECTED	EXPECTED
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

Additional Reporting Requirements: FDA and National Institutes of Health – Office of Biotechnology Activities (NIH-OBA) Reporting

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

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 and NIH-OBA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA and NIH-OBA in accordance with the following:

- Any unexpected fatal or life threatening adverse experience associated with use of the agent 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 agent 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)]

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.

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.

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.

12 CORRELATIVE/SPECIAL STUDIES

Intracerebral microdialysis

In order to gather data regarding intracerebral levels of irinotecan and SN-38 when brain tumor patients are treated with CE-secreting NSCs and irinotecan, microdialysis catheters will be used to obtain samples of brain interstitial fluid, as described in sections 8.3, from the first 6 study patients and 8 patients treated at the MTD/MFD level in the expansion cohort. During dialysate collection, blood samples will also be drawn in order to compare systemic and intracerebral levels of irinotecan and SN-38 as described in sections 8.3.

Analysis will be performed by the City of Hope Analytical Pharmacology Core Facility.

Immunological Correlative Studies

In order to 1) assess for possible development of T cell and antibody responses to the NSCs and/or cell surface-expressed adenoviral proteins, 2) measure adenoviral vector (Ad5) antibody titers and total adenoviral antibody titers, and 3) look for systemic presence of the NSCs and/or hCE1m6, samples of study patients' blood will be collected for isolation and storage of PBMCs and serum prior to surgery, on day 15 of cycle 1, days 1 and 15 of cycle 2, and on day 15 of subsequent treatment cycles. We will also test for replication competent retrovirus (RCR) (section 12.2.6).

The following studies, which were successfully performed in the first-in-human neural stem cell study of HB1.F3.CD NSCs and oral 5-FC, will be done by the City of Hope Clinical Immunobiology Correlative Studies Laboratory (CICSL), with the exception of the total adenoviral antibody titers, which will be performed by City of Hope Clinical Pathology:

12.1.1 Assessment of the Development of Anti-NSC T Cell Responses

Development of a T cell response to the adenovirally transduced 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.

12.1.2 Determination of Development of Antibody Responses to the Adenoviral Vector and/or NSCs.

We will assess for development of antibody responses to the NSCs and/or cell surface-expressed adenoviral proteins.

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 any bound antibodies will be detected using a secondary goat anti-human Fc antibody conjugated to FITC. After a second washing step, the NSCs will be analyzed by flow cytometry. 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 controls the parental NSC (not transduced with the CD gene) and the CD-expressing NSCs that have not been transduced with adenovirus to evaluate whether antibody responses are directed against the CD, CE, or adenoviral gene products.

If evidence of an immune responses are observed in patients, then we will assess for expression of the 72Kd DNA binding protein, which is specific to the adenoviral vector that was used to transduce the NSCs, and evaluate anti-hCE1m6 antibody titers to try to understand whether the immune response is to the NSCs themselves or the result of adenoviral transduction of the NSCs.

12.1.3 Measurement of Adenoviral Antibody Titers

Serum adenoviral vector (Ad5) IgG and IgM antibody titers as well as total adenoviral IgG and IgM antibody titers will be assessed at baseline and then at the same time points when blood is collected for investigating the presence of T cell and antibody responses to the adenovirally transduced NSCs.

These blood samples will be sent to CICSL (adenoviral vector [Ad5] antibody titers) and Clinical Pathology (total adenoviral antibody titers) for processing and determining of adenoviral antibody titers by complement fixation testing and ELISA.

12.1.4 Evaluation of Possible Presence of NSCs and/or hCE1m6 in the Systemic Circulation Using QPCR

An evaluation of possible presence of NSCs and/or hCE1m6 in the systemic circulation will be performed in the CICSL by quantitative PCR using established laboratory SOP and primer pairs specific for the *vMyc* gene and CD. For example, a standard curve will be generated using DNA isolated from the administered NSCs titrated into DNA from PBMCs (negative for *vMyc*); 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 *vMyc* 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.

12.1.5 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.

12.1.6 Replication Competent Retrovirus (RCR) Testing

The City of Hope CICSL will process samples, with DNA being isolated and stored, according to established laboratory SOP, until RCR testing is performed by PCR.Samples will be taken at 3 months, 6 months, and annually thereafter until their passing or withdrawal from follow-up.

Brain Tissue Correlates

12.1.7 Determining the Fate of the NSCs at the time of Autopsy

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 be used for the detection and further characterization of the NSCs (including proliferation and differentiation markers). We will also look for the presence of hCE1m6 in brain tissue samples.

12.1.8 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 City of Hope IRB#07047. Additional material will be banked (fixed and frozen) for potential future research under IRB #07047. 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.CE/irinotecan; 2) presence of immune cells (B-cells, T-cells, macrophages, microglia, dendritic cells); 3) determination of mitotic index in the tumor by Ki67 immunohistochemistry.

13 ENDPOINT EVALUATION CRITERIA/MEASUREMENT OF EFFECT

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).

14 DATA REPORTING/PROTOCOL DEVIATIONS

Data Reporting

14.1.1 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.

14.1.2 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.

14.1.3 Data Collection Forms and Submission Schedule

All data will be collected using electronic data collection, stored as indicated in Section 14.1.1, and will be submitted according to the timelines indicated in Table 14.1.3.

Table 14.1.3 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 10 calendar days of treatment administration
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

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.

Protocol Deviations

14.1.4 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.

14.1.5 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.

14.1.6 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.

15 STATISTICAL CONSIDERATIONS

Sample Size

The anticipated sample size is 29patients: minimum=6; maximum= 53(allowing for 42 patients for dose escalation through 5 possible dose levels of NSCs and 3 dose schedules of irinotecan, estimating ~3 patients to replace unevaluable/ineligible patients and an additional 8 patients for the expansion cohort).A sample size of 6 at the MTD/MFD will allow us to i) achieve a maximum margin of error for a 95% confidence interval for the DLT rate of 0.38 and ii) detect a 0.25 toxicity rate in approximately 82% of trials. The additional 8 patients in the expansion cohort, for a total of 14 patients treated at the MTD/MFD, will allow us to i) achieve a maximum margin of error for a 95% confidence interval for the DLT rate of 0.27 and ii) detect a 0.15toxicity rate in approximately 90% of trials.

An important purpose of the expansion cohort is to obtain information about the biologic activity of the hCE1m6-NSCs using intracerebral microdialysis. Sample size calculations based on preclinical results for the AUC from 0 to 20 hours (nMxhr) for SN38in rats (the combination therapy group mean= 7, sd=4.4; irinotecan alone mean=1.8, sd=1.2) implementing a natural log scale (mean difference 1.36 and sd=0.66) indicate 4 participants per group could provided 80% power todetermine if the addition of NSCs to treatment with irinotecan increases intracerebral concentrations of SN-38, using a one-sided two-sample t test with a 0.05 level of significance. As we expect our patient data to be more variable than the preclinical rat data we consider these results to be exploratory.

Accrual Rate

Given that the first 3 patients entering a cohort until the MTD/MFD is determined 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 36 months.

Statistical Design and Analysis

15.1.1 Design:

The dose escalation plan is based on a standard 3+3 design and is described in section 5.3.

15.1.2 Primary Objectives

To define the recommended phase II dose (RP2D) of intracranially administered hCE1m6-NSCs in combination with intravenous irinotecan in patients with recurrent high grade glioma. The RP2D will be based on the maximum-tolerated dose (MTD), or if the MTD is not reached, the maximum feasible dose (MFD), and the full toxicity profile including toxicities associated with repeat doses of NSCs.

15.1.3 Secondary Objectives

- To describe the relationship between hCE1m6-NSC dose and SN-38 concentrations in brain interstitium.
- To characterize the relationship between intracerebral and systemic concentrations of irinotecan and SN-38.
- To investigate the biologic activity of hCE1m6 NSCs by comparing SN-38 concentrations in the brain after treatment with hCE1m6-NSCs and irinotecan versus irinotecan alone.
- To assess for possible development of adenovirally transduced NSC immunogenicity after first exposure and with repeat doses of NSCs.

- To describe the clinical benefit (defined as stable disease, partial response, or complete response) in patients who receive treatment with repeat cycles of NSCs and irinotecan.
- To determine, at time of autopsy, the fate of the NSCs.

15.1.4 Primary Endpoints:

- Safety and Feasibility**:**
  - For Dose Escalation: Dose Limiting Toxicities
  - Toxicity Profile: All Attributable Toxicities

15.1.5 Secondary Endpoints:

- Pharmacokinetics:Cmax and AUC of irinotecan and SN-38 in dialysate and plasma.
- 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. Measure patients' serial adenoviral antibody titers.
- Clinical benefit: Tumor response based on brain MRI and PET/CT scans results
- Determine the Fate of the NSCs**:** NSC persistence

15.1.6 Analysis Plan:

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 95% Clopper Pearson confidence limits will be estimated for DLTs and clinical benefit at the RP2D. Descriptive statistics will be provided for study patient demographics.

Pharmacokinetic data from patients who undergo intracerebral microdialysis will be summarized using descriptive statistics and graphical methods. The biologic activity of the hCE1m6-NSCs, based on comparison of microdialysis SN38 PK data from patients receiving NSCs + irinotecan vs patients receiving irinotecan alone, will be assessed using a one-sided two-sample t test.Regression analysis will be used to assess the relationship between hCE1m6-NSC dose and SN-38 concentrations in brain interstitium using microdialysis data from the patientstreated with the initial NSC dose (n=6) and from the patients in the expansion cohort treated with the highest NSC dose (n=4).Other secondary objectives include assessing for possible development of NSC immunogenicity after first and repeat exposures, and—when feasible—evaluating the fate of the NSCs at autopsy. These results will be summarized using descriptive statistics and graphical methods.

16 HUMAN SUBJECT ISSUES

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.

Recruitment of Subjects

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

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.

Study Location and Performance Sites

This study will be performed at City of Hope.

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 irinotecan and SN-38, 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.

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, irinotecan, 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 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.

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, Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

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