Neural stem cell (NSC)-mediated conversion of 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU) in recurrent glioma patients: A proof of concept.

Jana Portnow MD; Timothy W Synold PhD; Simon F Lacey; Massimo D'Apuzzo; Paul H Frankel; Mike Yue Chen MD; Karen S Aboody MD; Behnam Badie MD

City Of Hope National Medical Center, Duarte, CA

Introduction

Human NSCs are inherently tumor-tropic, making them an attractive drug delivery vehicle. This first-in-human Human NSCs are inherently tumor-tropic, making them an attractive drug delivery vehicle. This first-in-human phase I study is assessing the safety and feasibility of using genetically-modified NSCs for tumor selective enzyme/prodrug therapy. An immortalized, clonal NSC line was retrovirally-transduced to stably express cytosine deaminase (CD), which converts the prodrug 5-FC to 5-FU, producing chemotherapy locally at sites of tumor in the brain while minimizing systemic toxicities.

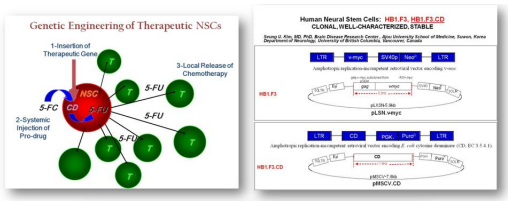
Methods

Patients with recurrent high-grade gliomas undergo intracranial administration of NSCs during resection or biopsy of tumor. Four days later, 5-FC is administered orally every 6 hours for 7 days. Patients receive only 1 round of NSCs and 5-FC. This study uses a standard 3 +3 dose escalation schema for increasing the doses of NSCs and 5-FC. A microdialysis catheter is placed at the time of surgery to measure intracerebral levels of 5-FC and 5-FU, and serial blood samples are obtained to measure systemic concentrations of these drugs. Peripheral blood mononuclear cells are collected on days 4, 10, 32, and 60 for analysis by flow cytometry-based antibody binding assays and CD4/CD8 degranulation assays to assess NSC immunogenicity.

Results

Accrual to dose levels 1 and 2 has been completed. Thus far, there have been no DLTs. Microdialysis data demonstrate the presence of 5-FU in the brain throughout the entire 5-FC dosing interval. Average steady state concentrations of 5-FU and 5-FC in brain are 23.4 nM and 24.5 mM, respectively in cohort 1 (n=4) and 106.7 nM and 153.3 mM, respectively in cohort 2 (n=2). Analysis of plasma samples to date have shown high levels of 5-FC, but no detectable 5-FU in circulation. Anti-NSC antibody and T-cell responses have not been detected.

NSC Pilot Feasibility Clinical Trial

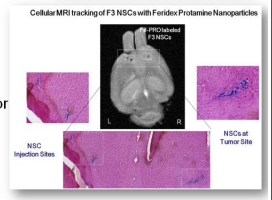


Timeline	Q1	Q2	Q3	Q4
2007	Pre-IND meeting with FDA			NIH RAC Presentation
2008		Master Cell Bank Established	CPRMC, IRB, and IBC Approval	MCB Release Testing
2009		IND Submission	Placed on Clinical Hold	
2010		Off Clinical Hold	Study Open to Accrual	First Patient Enrolled

NSCs as Vehicles for Tumor-Targeted Drug Delivery

Neural Stem Cells are Tumor- Tropic

A strategy that can
- "circumvent" blood-brain barrier
- Potentially provide more concentrated, local cytotoxic effect
- Decrease toxicity to normal tissues
- Potential use as a platform for further therapeutic strategies and for other types of cancer



Study Design

NSC Pilot Feasibility Study

- Primary Objective:
  To determine the safety and feasibility of intracerebral administration of genetically-modified NSCs (HB1.F3.CD) in combination with oral 5-FC in patients with recurrent high-grade gliomas.
- Secondary Objectives:
  - Demonstrate proof of concept
    - Measure intracerebral levels of 5FC and 5FU via microdialysis
    - (GF-IuMS)
  - Assess immunogenicity of the NSCs
  - Gain a preliminary understanding of the imaging characteristics of NSCs
    - MRI,MRS, perfusion/permeability sequences
  - Study the intracerebral distribution of the NSCs
  - Determine the fate of the NSCs at autopsy

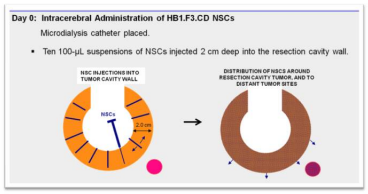
Dose Escalation Schema

Dose Level	Neural Stem Cell Dose	5FC (mg/kg q 6h x 7 days)	Initial Enrollment	Additional Enrollment if 1 of initial 3 has a DLT,
1	1×10^7	18.75	3	3
2	1×10^7	37.5	3	3
3	5×10^7	37.5	3	3
4	1×10^8	37.5	3	3

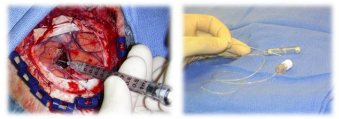
Eligibility Criteria

- Age ≥ 13, Karnofsky Performance Score ≥ 70%.
- Prior diagnosis or radiographic findings consistent with a grade III or IV glioma.
- Imaging shows evidence of recurrent, supratentorial tumor(s).
- Progressive disease after prior chemoradiation
- Resectable or unresectable tumor*
- No anticipated physical connection between the post-resection cavity and the cerebral ventricles.
- Patient must not have any anti-HLA antibodies specific for HLA antigens expressed by the NSCs.

Treatment Schema



Characterize the relationship between intracerebral and systemic concentrations of 5-FC and 5-FU with increasing NSC dose using intracerebral microdialysis



Safety and In Vivo Efficacy

Dose levels 1 and 2 completed

11 patients screened: 2 pts were ineligible due to testing positive for antibodies against HLA class I antigens on the NSCs.

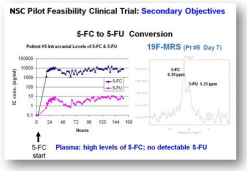
9 pts treated: 4 on dose level 1; 5 on dose level 2
3 pts did not complete the toxicity evaluation period and were replaced for the purpose of dose escalation
- 2 pts died of progressive disease on days 31 & 44
- 1 pt died of a post-op complication (intracerebral hemorrhage) on day 5 (HTN)

Treatment with NSCs and 5-FC was well-tolerated except for 1 episode of grade 3 thrombocytopenia occurring in a heavily pre-treated patient

No radiographic responses (1 pt had stable dz on day 32 MRI)

NSC Pilot Feasibility Clinical Trial: Secondary Objectives

5-FC to 5-FU Conversion



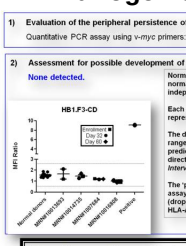
Brain Steady-State 5-FC/5-FU Concentrations

Patient	Arm	5-FC (uM)	5-FU (nM)
1	1	4.9	8.8
2	1	10.5	35.9
3	1	29.6	10.9
4	1	53.0	37.8
	Avg =	24.5	23.4
5	2	138.2	83.9
9	2	168.4	129.4
	Avg =	153.3	106.7

Immunogenicity of NSC

1) Evaluation of the peripheral persistence of NSCs
Quantitative PCR assay using *v-myc* primers: **All patient samples are negative.**

2) Assessment for possible development of antibody responses to NSCs
None detected.



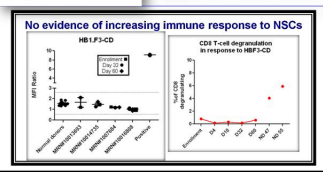
Normal donor sera n=10. Each data point for normal donor sera represents the mean of 5 independent experiments.

Each point for the #08002 study sera represents mean of triplicate assays.

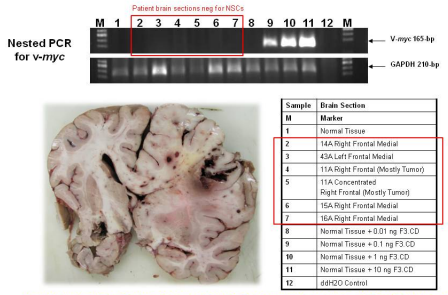
The dotted lines indicate a "normal MFI" range of 0.55 to 2.59 that represent a 95% prediction interval based on 2.37 SD in each direction (Hahn and Meeker, *Statistical Intervals*, Wiley and Sons, 1991).

The 'positive control' is the mean of triplicate assays on serum from MRNM#10014905 (dropped from study due to antibodies to HLA-A1).

No evidence of increasing immune response to NSCs



NSC Fate at Autopsy (Patient #1)



Sample	Brain Section
M	Marker
1	Normal Tissue
2	14A Right Frontal Medial
3	43A Left Frontal Medial
4	11A Right Frontal (Mostly Tumor)
5	11A Concentrated Right Frontal (Mostly Tumor)
6	15A Right Frontal Medial
7	16A Right Frontal Medial
8	Normal Tissue + 0.01 ng F3.CD
9	Normal Tissue + 0.1 ng F3.CD
10	Normal Tissue + 1 ng F3.CD
11	Normal Tissue + 10 ng F3.CD
12	ddH2O Control

No evidence of NSCs (as detected by PCR for v-*myc*) in brain at Day 44 post-surgery

Conclusions

Although accrual is ongoing, dialysate and plasma data from the first 2 patient cohorts demonstrate the proof-of-concept that the NSCs are converting 5-FC to 5-FU locally in the brain. Furthermore, levels of 5-FU in the brain increase in a 5-FC dose-dependent manner. No immune responses to these allogeneic NSCs have occurred after first exposure.

Clinical Team
Jana Portnow (PI)
Behnam Badie
Mike Chen

Funding
CIRM
NCI

Manufacturing of NSCs
Karen Aboody, Marianne Metz, Tien Vo, Josip Najbauer, Alex Annala, Patricia Huang, Yasmine Shad, David HSU, Misty Shakeley, Margarita Gutova, Derek Kong, Amira Ahmed, Kelsey Herrmann, Soraya Aramburo, Rachel Namba, Yelena Abramyants, Mary Danks, Wei Dang, Ray Merchant, Nancy Gonzalez, Christine Knoblauch, Matthew Luo

Office of IND Development and Regulatory Affairs
Larry Couture
Suenell Broyer
Catherine Matsumoto
Rachel Magnusson