

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/282661459>

Intranasal Delivery of Therapeutic Neural Stem Cells to Target Intracerebral Glioma

Article in *Neuro-Oncology* · April 2015

DOI: 10.1093/neuonc/nov204.29

CITATION

1

READS

272

13 authors, including:



Margarita Gutova

City of Hope National Medical Center

60 PUBLICATIONS 1,036 CITATIONS

[SEE PROFILE](#)



Diana Oganessian

Azusa Pacific University

9 PUBLICATIONS 26 CITATIONS

[SEE PROFILE](#)



Lusine Danielyan

University of Tuebingen

28 PUBLICATIONS 756 CITATIONS

[SEE PROFILE](#)



William H. Frey 2nd

HealthPartners Institute

182 PUBLICATIONS 9,109 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



nanotechnology in targeted MRI contrast agents; Non-invasive determination of electrical conductivity of human brain tissues [View project](#)



Our Current Clinical Trials [View project](#)

Intranasal Delivery of Therapeutic Neural Stem Cells to Target Intracerebral Glioma

Margarita Gutova^{1*}, Davit Shahmalyan¹, Diana Oganessian¹, Yelena Abramyan¹, Lusine Danielyan², William H. Frey II³, Vazgen Khankaldyan⁴, Joseph Najbauer^{1, #}, Irina V. Balyasnikova⁵, Rex A. Moats⁴, Maciej S. Lesniak⁵, Michael E. Barish¹, and Karen S. Aboody^{1*}

¹Department of Neurosciences, Beckman Research Institute of City of Hope, Duarte, California, 91010, USA

²Department of Clinical Pharmacology, University Hospital of Tübingen, D-72076 Tübingen, Germany

³Neurosciences Research, HealthPartners Institute for Education and Research, St. Paul, Minnesota 55101, USA

⁴Department of Radiology, Children's Hospital of Los Angeles, Keck School of Medicine, University of Southern California, Los Angeles, California 90027, USA

⁵Brain Tumor Center, University of Chicago, Chicago, Illinois 60637, USA

***Corresponding authors:** Margarita Gutova, MD, Department of Neurosciences, Beckman Research Institute of City of Hope 1500 E. Duarte Rd., Duarte, CA 91010, USA, Tel: (626)-3598111 x65637; Fax: 626-471-7371; E-mail: mgutova@coh.org

Karen S. Aboody, MD, Department of Neurosciences, Beckman Research Institute of City of Hope 1500 E. Duarte Rd., Duarte, CA 91010, USA, Tel: (626) 471-7177; Fax: (626) 301-8857; E-mail: [HYPERLINK "mailto:kaboody@coh.org"](mailto:HYPERLINKmailto:kaboody@coh.org) kaboody@coh.org

[#]Current address of JN: Department of Immunology and Biotechnology, University of Pécs Medical School, Pécs, Hungary

Citation: Gutova M, Shahmalyan B, Oganessian D, Abramyan Y, Danielyan L, et al. (2015) Intranasal Delivery of Therapeutic Neural Stem Cells to Target Intracerebral Glioma. *Enliven: J Stem Cells Regen Med* 1(1):007.

Copyright: © 2015 Dr. Margarita Gutova. This is an Open Access article published and distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Received Date: 13th February 2015

Accepted Date: 09th April 2015

Published Date: 15th April 2015

Abstract

Despite aggressive multimodal therapy and advances in imaging, surgical and radiation techniques, high-grade gliomas remain incurable, with patient survival often measured in months. Treatment failure is largely attributable to the invasive nature of glioma cells, ineffective delivery of chemotherapeutic agents across the blood-brain barrier, and dose-limiting systemic toxicities. Neural stem cells (NSCs) have inherent tumor-tropic properties that can be exploited for targeted delivery of anti-cancer agents. However, current intracranial and intravenous injection approaches for administering NSCs are not optimal, especially for repeat administrations, because the methods are invasive and may lead to complications. We hypothesized that intranasal administration of NSCs would circumvent these challenges. In this study, we evaluated the biodistribution of NSCs administered intranasally to severely immunodeficient esterase-deficient Esle mice bearing orthotopic xenografts of U251.eGFP.fluc human gliomas. Histological imaging and 3D reconstruction revealed that NSCs specifically localized to tumor sites, but not to non-tumor areas of the brain. Importantly, mice treated with intranasally administered NSCs that were genetically modified to express carboxylesterase, a prodrug-activating enzyme, in combination with CPT-11 showed increased survival and reduced tumor growth. These results support further development of intranasal delivery of NSCs as a new approach to treating glioma and possibly other invasive brain tumors.

Keywords: Glioma; Brain tumor; Neural stem cells; Intranasal delivery; Targeted therapy; Enzyme/prodrug; Carboxylesterase; Irinotecan; CPT-11

Introduction

Stem cell therapies have shown much promise in preclinical animal models for the treatment of central nervous system (CNS) diseases and brain tumors [1]. The major challenge in developing such therapies has been effective delivery of therapeutic agents and cells to injury sites within the brain [2-4]. Many studies have shown that neural stem cells (NSCs) that are intracranially injected into the brain localize specifically to areas of CNS pathology [5-8]. However, intracranial injection is invasive and impractical for repeated dosing. Although intravenously administered NSCs have the potential to cross the blood-brain barrier (BBB) and localize to areas of

damaged brain tissue, including tumors, this approach may lead to off-target systemic effects, as well as immune reactions toward allogeneic NSCs. Thus, although encouraging results have been obtained, current methods of NSC administration are imperfect and there is an urgent need for new methods of stem cell delivery. Intranasal administration has already been used to deliver proteins, growth factors, hormones and small molecules to the brain to treat neurological disorders [9] and has been adapted for the specific use of intranasal insulin to treat Alzheimer's disease [10]. In addition, intranasally administered mesenchymal stem cells (MSCs)

can bypass the BBB to target the CNS, and their migration to CNS sites is enhanced by pretreatment with intranasal hyaluronidase [11]. Several research groups have used intranasal delivery of MSCs to successfully target and treat CNS damage in animal models of neonatal ischemia [12], Parkinson's disease [13], ischemic stroke [14] and neonatal brain damage [15,16]. A recent study in an orthotopic mouse glioma model showed that intranasally administered MSCs that express TNF-related apoptosis-inducing ligand rapidly reach tumor sites and induce a cytotoxic effect *in vivo* [17]. Similarly, intranasally administered neural stem/progenitor cells have been reported to target intracerebral malignant gliomas in mice [18].

In the current study, we examined the migration of an established therapeutic clonal human NSC line (HB1.F3.CD) to human glioma tumors implanted into the frontal lobe of immunodeficient mice after intranasal administration. This HB1.F3.CD NSC line demonstrated safety in a first-in-human safety/feasibility study when used in combination with 5-fluorocytosine (5-FC) in patients with recurrent glioblastoma (clinical trial ID # NCT01172964). In the studies reported here, this same NSC line was further modified to express a modified human carboxylesterase (hCE1m6), which converts the CPT-11 (irinotecan) prodrug to the potent topoisomerase I inhibitor, SN-38 [20]. Following intranasal administration, these CE-expressing NSCs (NSC.CE) migrated specifically to tumor sites within the brain in two different immunodeficient mouse models and induced a cytotoxic effect when given in combination with CPT-11 in plasma esterase-deficient (*Esle*) SCID mice. These results suggest that NSCs can be intranasally administered and used to target anti-cancer agents to intracranial tumors, following further optimization. Overall, intranasal delivery of NSCs may offer a more efficient and less invasive method of implementing stem cell therapies in many CNS diseases, which could significantly improve and expand stem cell-based therapeutic applications.

Materials and Methods

Neural Stem Cell Culture

HB1.F3.CD NSCs derived from a clinical equivalent research lot (City of Hope cGMP Facility) were thawed and cultured in T-175 tissue culture flasks in DMEM (Invitrogen, 10313-021; Carlsbad, CA), supplemented with 10% heat-inactivated FBS (Gemini, GBP-100500, West Sacramento, CA) and 2 mM L-glutamine (Invitrogen, 25030-081), and incubated at 37°C, 6% CO₂ for 72 h prior to labeling with superparamagnetic iron oxide (SPIO) nanoparticles (Molday ION Rhodamine BTM; CL-50Q02-6A-50; BioPhysics Assay Laboratory, Inc., Worcester, MA). Control unlabeled HB1.F3.CD cells were cultured under the same conditions as the labeled cells. HB1.F3.CD cells (passage 25) were labeled with Molday ION as described previously (Cromer Berman SM et al. [21]) For therapeutic efficacy studies, HB1.F3.CD cells were adenovirally transduced to express a humanized form of carboxylesterase (hCE1m6) (Wierdl M et al. [20]).

Animal Models of Glioma Tumors

Beige nude XID and *Esle* esterase deficient SCID mice were used to investigate the effect of various immunodeficient hosts on NSC migration. The severely immunodeficient beige nude XID strain was ideal for this study because it allowed us to test the stand-alone viability of intranasally delivered NSCs with minimal interference from the immune system. The beige nude XID strain lacks key immunological components such as B cells, macrophages, and lymphokine activated killer (LAK) cells. For therapeutic efficacy studies we used the less immunodeficient *Esle* SCID mice, which lack B cells and T cells, similar to the beige nude XID strain, but possess NK cells, LAK cells, and macrophages.

Biodistribution Studies

To establish glioma tumor xenografts, U251.eGFP.ffluc human glioma cells (1×10^5 cells/2 μ l) were implanted stereotactically (2 mm right of bregma, 1 mm rostral caudal and 2.5-3 mm deep) into the frontal lobe of mice. Fourteen days after tumor cell implantation, NSCs labeled with Molday ION Rhodamine BTM were administered intranasally (6×10^5 cells) in a total volume of 12 μ l PBS (n= 8 mice). Two immunodeficient mouse hosts were used: beige nude XID mice and *Esle* esterase deficient SCID mice (n=4 for each model). Intranasal administration of NSC.CEs was performed as previously described [13,18]. Briefly, NSCs (5×10^4 cells/1 μ l) were applied to each nostril in doses of 3 μ l with 2 min intervals after hyaluronidase treatment (100 units/mouse) (Sigma-Aldrich; H3757). Four mice were euthanized 24 h post-NSC administration, and 4 mice were euthanized 4 days after NSC administration. Brains were fresh frozen or embedded in paraffin. Serial 10- μ m horizontal sections were made from the brains and processed for H&E staining, Prussian blue staining, and fluorescent imaging. Prussian blue staining was performed using the Accustain iron stain kit (Sigma-Aldrich, St. Louis, MO; HT20-1KT) according to the manufacturer's protocol. Mouse peripheral organs (lungs, liver and spleen) and spinal cords were also collected, tissue was digested using proteinase K, and DNA was extracted and amplified by PCR using primers for *v-myc* to test for the presence of NSCs in non-tumor organs. The brain of a mouse injected intranasally with NSCs that were not labeled with Molday ION Rhodamine BTM was used for PCR analysis. Because the left hemisphere was not implanted with tumor, it was used as a control for the right frontal lobe, which had the implanted tumor. The GAPDH gene was used as a DNA loading control. DNA purified from HB1.F3.CD cells was used as a positive control for PCR amplification of the *v-myc* gene (replicon size 170 bp). Pure water was used as a negative control. PCR products were analyzed by agarose gel electrophoresis and staining of DNA bands with ethidium bromide. Therapeutic efficacy studies were performed using the same U251.eGFP.ffluc glioma xenograft model in *Esle* esterase deficient SCID mice. Briefly, on day 1 mice received intracranial injections of U251.eGFP.ffluc glioma cells (1.5×10^5 cells/2 μ l). Tumor growth was monitored by Xenogen imaging. All mice developed tumors were then intranasally administered NSC.CE cells on days 7 and 14 after tumor cells implantation. A bolus intravenous injection of CPT-11 (37.5 mg/kg) was given 4 days after NSC.CE injection (i.e., days 11 and 18). After two rounds of NSC.CE/CPT-11 treatment, mice were followed for long-term survival. Animals were monitored daily, to perform euthanasia on any animals showing objective signs of dying. The signs include: any obvious prolonged illness including such signs as lethargy, decreased fecal production, bleeding, dehydration, difficulty breathing, central nervous system disturbances, or chronic diarrhea or constipation, weight loss (> 20% body weight); scruffy hair coat, hunched posture, hypo or hyperthermia, impaired ambulatory movement, inability to remain upright. Animal meeting any of these criteria were euthanized, and the date was recorded as their death.

3D Reconstruction of Tumors and Brain Tissue

Three-dimensional (3D) reconstruction was performed using Reconstruct (version 1.1.0.1) software (Dr. Kristen M. Harris et al., SynapseWeb, Laboratory of Synapse Structure and Function, Austin, TX, USA; <http://synapses.clm.utexas.edu>). For each tumor, 7-11 serial brain sections (10 μm each) separated by 200 μm were imported into the Reconstruct program and aligned manually. Tumor alignment and NSC quantification were done on merged fluorescent image slides; auto-tracing of the tumor was done on eGFP and red-blue merged slides. NSC cells were identified as red (Molday ION Rhodamine BTM -labeled), and DAPI staining was performed to visualize mouse brain tissue. To produce a 3D image, structures of interest were separated based on color (Prussian blue label for NSCs) and cell density (eGFP, tumor areas). Volumes containing these cells and structures were determined using Reconstruct.

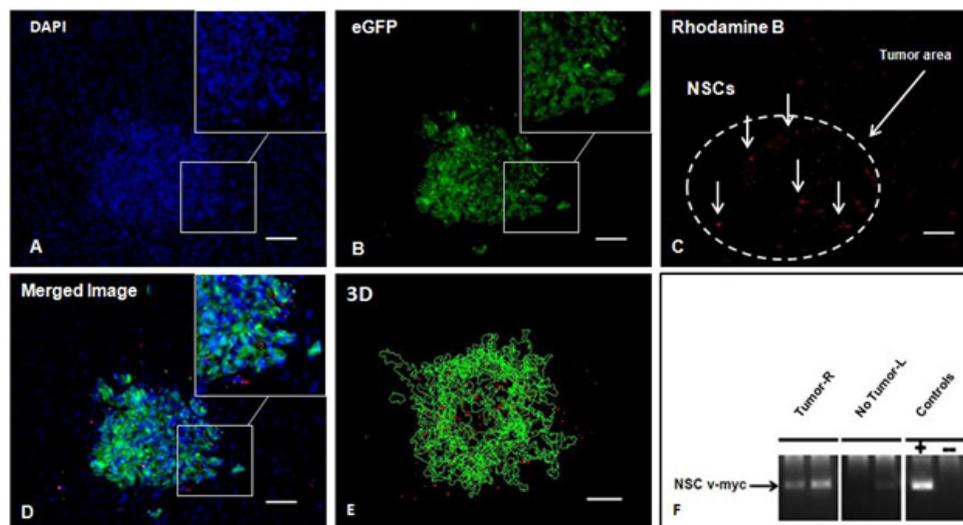
Results

NSCs Migrate to Sites of Intracranial Glioma in Beige Nude XID Mice

All studies used the established NSC line HB1.F3.CD, which is a *v-myc*-immortalized, human clonal NSC line that has been genetically modified to stably express *E. coli* cytosine deaminase (CD) [5]. We have extensively characterized these NSCs as being genetically and functionally stable, non-tumorigenic, and minimally immunogenic [21]. The HB1.F3.CD line is established as a master cell bank at City of Hope, which should accelerate translation of promising findings toward clinical use.

First, we sought to determine if intranasally administered NSCs maintained their inherent tumortropism *in vivo*. HB1.F3.CD cells were iron-labeled with Molday ION Rhodamine B or feraheme to allow for detection by both Prussian blue staining and fluorescent visualization of NSCs in histological sections [20]. U251.eGFP.fluc human glioma cells (1×10^5) were injected into the frontal lobe of beige nude XID mice using stereotactic coordinates as previously described ($n=4$) [21]. Fourteen days after tumor implantation, HB1.F3.CD cells were administered intranasally. Mice were euthanized one or four days after NSC administration. NSCs were found to migrate and distribute selectively to tumor sites, but were not detected in non-tumor areas of the brain (Figure 1A-1E). The presence of NSCs at the tumor site was determined by histological analysis of the brain sections. Two brains from the mice euthanized on day 1, were cryosectioned and stained with DAPI to visualize cell nuclei (Figure 1A). eGFP fluorescence was used to visualize the eGFP-expressing U251 tumor cells (Figure 1B). Molday ION Rhodamine B-labeled NSCs were detected (red) at tumor sites after NSCs injection by fluorescence imaging (Figure 1C), merged image (Figure 1D) and 3-dimensional (3D) reconstruction of serial histological sections for each brain were performed (Figure 1E). The brain of one mouse euthanized on day 4 post-NSC injection was analyzed by PCR to detect *v-myc* transcripts at the tumor sites; *v-myc* is a specific marker for the HB1.F3.CD cells (Figure 1F). PCR analysis using *v-myc* specific primers (replicon size 170 bp) showed that *v-myc* DNA was present in the right hemisphere, where tumor cells were implanted. However, *v-myc* was not detected in the left hemisphere, where tumor cells were not present (Figure 1F). Quantitative results showing the numbers of NSCs at the tumor site for each mouse are summarized in (Table 1).

Figure 1. Intranasally administered NSCs migrate to sites of brain tumor in an immunodeficient mouse model.



Severely immunodeficient beige nude XID mice (4 weeks old) were implanted intracranially with U251.eGFP.fluc human glioma cells. After 14 days, NSCs (6×10^5) labeled with Molday ION Rhodamine B were administered intranasally. Biodistribution of the NSCs was examined 24 h after administration and on day 4. Axial view of brain sections from the frontal lobe and containing tumor tissue (A) stained with DAPI (blue), (B) imaged for eGFP (green) to identify tumor cells, (C) imaged for Molday ION Rhodamine B (red) to identify NSCs. Vertical arrows indicate NSCs that migrated to the tumor site. Dashed white line outlines the tumor area. (D) Merged image. (E) Three-dimensional (3D) reconstruction of tumor and NSCs. (F) Nested PCR analysis for the *v-myc* gene (replicon size 170 bp) to determine the presence of HB1.F3.CD cells at the tumor site (right frontal lobe). No Tumor: left frontal lobe (no tumor); Controls: DNA purified from HB1.F3.CD cells (+) or no DNA added (-). Scale bars: 100 μm .

Table 1. Quantification of NSC numbers per brain using 3D reconstructions

	I	II	III	IV	
Object	Number of NSCs	Number of brain sections	Brain sections per tumor	Total NSCs per brain	Mice
M01 day 1	170	9	180	3400	Beige nude XID
M04 day 1	116	8	160	2320	Beige nude XID
M07 day 1	209	11	220	4180	<i>Es1^e</i> SCID
M09 day 4	371	7	140	7420	<i>Es1^e</i> SCID

I Number of NSCs quantified per brain sections used for 3D

II Number of brain sections (within the tumor) that were used for 3D reconstruction

III Total number of brain sections overlapping the tumor

IV Estimated total number of NSCs per brain: (I x III)/II

*M01, M04, M07, M09 – mice used for analysis

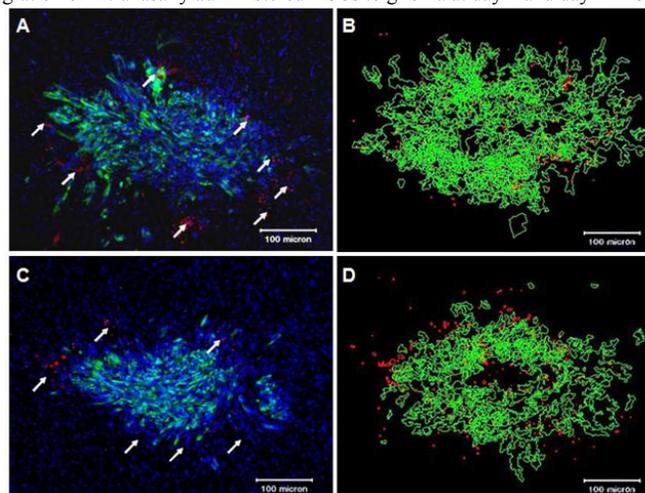
Migration of HB1.F3.CD Cells to Orthotopic Glioma Xenografts in *Es1^e* SCID Mice

Plasma esterase deficient *Es1^e* SCID mice (n=4) were used to more closely mimic human levels of CE, so human equivalent doses of CPT-11 could be used in therapeutic studies by residual immunological reactivity, we used *Es1^e* SCID mice (n=4). Although these mice are immunodeficient, they have some ability to launch an immune response. As early as 24 h after intranasal injection, HB1.F3.CD cells were identified in and around U251.eGFP.fffuc tumors in this model (Figure 2A). NSCs were not detected in the opposite hemisphere of the brain, which had not been implanted with tumor cells. Analysis of NSC migration four days after NSC administration revealed increased NSC accumulation at the tumor site (Figure 2C), when compared to brains of mice euthanized one day after injection of NSCs (Figure 2A and Table 1).

We then used 3D reconstruction of fluorescent images of axial (horizontal) brain sections to quantitatively analyze the number of NSCs at the tumor sites (Figure 2B, 2D). Using images obtained from H&E and Prussian blue staining,

we determined the brain area where the tumor was present. Brain sections (every 10th section) were selected for DAPI staining, fluorescent imaging and 3D analysis (Figure 2B, 2D). Fluorescent images (7-10 brain sections/mouse) were used for 3D analysis. Imaging was performed using blue, green and red channels to detect DAPI stained nuclei, tumor cells (U251.eGFP.fffuc green) or NSCs (Molday ION Rhodamine-labeled, red), respectively. Tumor alignment and quantification were done on DAPI-stained slides; auto tracing of the tumor was done on images using the green channel, and tracing of NSCs on red/blue merged sections (Figure 2B and 2D; tumor pseudo-colored green and NSCs red). The number of NSCs was quantified in each brain section, and these values were multiplied by the number of sections. The total number of NSCs per brain was approximated based on the number of unstained intervening sections (Table 1). The same approach was used to estimate the numbers of NSCs present at the tumor sites in each mouse.

Figure 2. Migration of intranasally administered NSCs to glioma at day 1 and day 4 in SCID mice



(A, C) SCID mice (*Es1^e*) were implanted intracranially with U251.eGFP.fffuc cells (1×10^5 cells). Fifteen days after implantation of U251.eGFP.fffuc cells, NSCs (Molday-labeled HB1.F3.CDs, 6×10^5) were administered intranasally. Biodistribution of the NSCs was evaluated one (A) or four days (C) after NSC injection. White arrows in A and C indicate NSCs (red), tumor green and mouse brain blue (DAPI labeled). Scale bars: 100 μ m. 3D tumor reconstruction of brain and NSCs that migrated to the tumor site one (B) or four days (D) after intranasal administration of NSCs.

Detection of HB1.F3.CD NSCs in Peripheral Organs by Nested PCR

Nested PCR was used to detect the *v-myc* gene sequence (a marker for HB1.F3.CD NSCs) in the organs of mice that received intranasally administered NSCs (n=8). This approach could detect *vmyc* in 0.01 ng of genomic DNA purified from HB1.F3.CD NSCs. In our previous studies, we have estimated that nested PCR analysis of *v-myc* is sensitive enough to detect 10-20 NSCs per 500 ng of mouse brain tissue DNA [21]. Nested PCR failed to detect NSCs in the spinal cord as well, which was not surprising because no abnormalities were detected in this tissue by pathologic analysis. This approach did detect NSCs in tumor areas. NSCs were occasionally detected in the lungs of

mice (e.g., left lungs in two mice, one out of four duplicate samples were positive) and may have been associated with pulmonary passage of intranasally administered NSCs on days 1 and 4 after NSCs injection (Supplementary Table 1, number of dashes indicate the duplicate samples). All PCR reactions were repeated 3 times (n=8). Further, PCR analysis of blood samples did not detect *v-myc* DNA in samples obtained 24-48 h after intranasal NSC administration, suggesting that NSCs were cleared from the bloodstream (data not shown). Therefore, we concluded that NSC distribution in the brain was specific to the U215 tumor areas after intranasal administration.

Supplementary Table 1. PCR analysis of the organs for the presence of *v-myc* DNA

Sample ID	M01	M03	M04	M05	M06	M07	M08	M09
Liver	----	----	--	--	--	--	--	--
Lung #1	----	----	--	--	--	--	--	--
Lung #2	+---	+---	--	--	--	--	--	--
Spleen	----	----	--	--	--	--	--	--
Kidney #1	----	----	--	--	--	--	--	--
Kidney #2	----	----	--	--	--	--	--	--
Spinal Cord	----	----	--	--	--	--	--	--

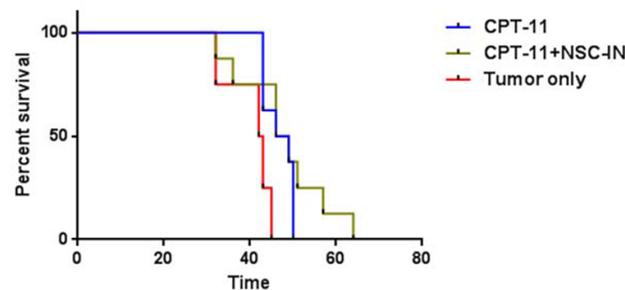
*M01-M09 mice used for analysis

Intranasal Administration of NSC.CEs in Combination with CPT-11 in Mouse Models of Glioma

We performed long-term survival studies using the U251 xenograft model to determine the effect of combination therapy with intranasally administered NSC.CE cells and CPT-11. NSC.CE cells (5×10^5) were administered intranasally to U251 tumor-bearing mice (n=8) on day 7 after tumor implantation, and the first dose of CPT-11 (37.5 mg/kg) was given on day 11 of the study as a bolus dose. The treatment with both NSC.CE cells and CPT-11 was repeated once the following week. Control groups included: 1) U251 tumor-bearing, no treatment (n=4); and 2) U251 tumorbearing, treatment with

CPT-11 only (CPT-11 treatment was given on the same schedule as for mice that received NSC.CE + CPT-11) (n=8) (Figure 3). Statistical analysis of long-term survival data (using Log-rank Mantel-Cox test) showed that survival curves were significantly different for untreated vs. treated groups (p=0.02). However, median survival of mice treated with CPT-11 only and NSC.CE + CPT-11 was not statistically significant (median survival 47.5 days); median survival for the untreated group was 42.5 days. Further studies will be needed to optimize the treatment regimen for NSC-mediated CE/CPT-11 therapy.

Figure 3. Long-term survival study of tumor-bearing mice treated with CPT-11 or NSC+CPT-11)



We performed long-term survival studies using the U251 xenograft model to determine the effect of combination therapy with intranasally administered NSC.CE cells and CPT-11. NSC.CE cells (5×10^5) were administered intranasally to U251 tumor-bearing mice (n=8) on day 7 after tumor implantation, and the first dose of CPT-11 (37.5 mg/kg) was given on day 11 of the study as a bolus dose. The treatment with both NSC.CE cells and CPT-11 was repeated once the following week. Control groups included: 1) U251 tumor-bearing, no treatment (n=4); and 2) U251 tumorbearing, treatment with CPT-11 only (CPT-11 treatment was given on the same schedule as for mice that received NSC.CE + CPT-11) (n=8) (Figure 3). Statistical analysis of long-term survival data (using Log-rank Mantel-Cox test) showed that survival curves were significantly different for untreated vs. treated groups (p=0.02). However, median survival of mice treated with CPT-11 only and NSC.CE + CPT-11 was not statistically significant (median survival 47.5 days); median survival for the untreated group was 42.5 days. Further studies will be needed to optimize the treatment regimen for NSC-mediated CE/CPT-11 therapy.

Discussion

In this study we provide evidence that, similar to biomolecules and MSCs, NSCs can use the intranasal pathway to gain access to the brain and to target brain tumors. Specifically, we have shown by fluorescent imaging, as well as PCR analysis, that after intranasally administered NSCs can migrate to glioma xenographs in the frontal lobe of mice. We have also demonstrated the potential for therapeutic efficacy of intranasally administered NSC.CEs followed by CPT-11. Intranasal administration of therapeutic NSCs provides several advantages over conventional methods, including intravenous and intracranial injections [22-24], and overcomes the inherent weaknesses of these methods. For example, intracranial administration involves invasive surgery of the brain and a significant proportion of stem cells are lost at the site of injection, resulting in an overall low rate of engraftment and survival of administered stem cells. Intranasal delivery of NSCs may also be superior to intravenous delivery methods because the host immune system is not exposed to the NSCs. The brain is considered an immunologically privileged organ, the CNS immune responses are generally primed in the periphery, and the access of circulating immune cells to the CNS is tightly controlled by the BBB [25]. Thus intranasal administration could minimize the likelihood of an immune response to repeated rounds of such therapeutic cells. Thus, current drawbacks to intracranial and intravenous delivery of NSCs may be partially overcome by using intranasal delivery. The HB1.F3.CD NSC line has been characterized for genetic stability, cellular identity, therapeutic gene expression, and tumor targeting ability [5,26-27]. We have also determined that HB1.F3.CD NSCs express HLA class I antigens but do not express detectable levels of class II antigens, indicating that they are unlikely to elicit a CD4+ helper T or CD8+ cytotoxic T cell response [21]. We have confirmed the feasibility of using intranasal delivery of therapeutic NSCs to target brain tumors. Prussian blue staining of iron nanoparticle-labeled NSCs, Molday ION Rhodamine B fluorescence imaging and PCR analysis confirmed that intranasally administered NSCs targeted sites of glioma (Figures 1 and 2). Further, we have provided evidence that after intranasal administration, NSCs migrate specifically to sites of glioma in the brain and do not appear to be present in non-tumor areas of the brain or other organs. Our data also suggest that intranasal delivery can potentially serve as a practical method of delivering therapeutic NSCs to the brain. Of note, the extended survival of glioma-bearing mice treated with intranasally administered NSC.CE cells in combination with CPT-11 as compared to control groups, though modest, warrants further studies to refine and optimize. Testing the intranasal NSC.CE + CPT-11 therapy in combination with other therapies to achieve a synergistic therapeutic effect is also of interest. We are further refining the intranasal delivery method, and are examining the clearance of NSCs through the circulation when they are injected intravenously. Intranasal delivery of NSCs represents a novel and promising tool for delivering NSCs to brain tumors for therapeutic approaches, such as enzyme/prodrug therapy of brain tumors [26]. If developed further, the intranasal delivery strategy could likely eventually be used to treat glioma patients and other CNS disease patients in a minimally invasive and highly effective manner.

Acknowledgments

The authors acknowledge the technical expertise and advice of Sofia Loera of the Pathology Core supported by the National Cancer Institute of the National Institutes of Health award (P30CA33572) and the editorial assistance of Dr. Keely L. Walker (City of Hope). This work was supported by funding from the California Institute of Regenerative Medicine (DR1-01421), NIH/National Institute of Neurological Disorders and Stroke (U01 NS069997), Alex's Lemonade Stand Foundation and Pediatric Cancer Research Foundation, Altschul Foundation.

Disclosure of Potential Conflicts of Interest

K.S.A. is a director and chief scientific officer of Thera Biologics, Inc., a clinical stage biopharmaceutical company focused on the development of stem cell-mediated cancer therapies.

References

- 1 Aboudy K, Capela A, Niazi N, Stern JH, Temple S (2011) Translating stem cell studies to the clinic for CNS repair: current state of the art and the need for a Rosetta Stone. *Neuron* 70: 597-613.
- 2 Miller RH, Bai L (2012) Translating stem cell therapies to the clinic. *Neurosci Lett* 519: 87-92.
- 3 Ming GL, Brüstle O, Muotri A, Studer L, Wernig M, et al. (2011) Cellular reprogramming: recent advances in modeling neurological diseases. *J Neurosci* 31: 16070-16075.
- 4 Noble M, Davies JE, Mayer-Pröschel M, Pröschel C, Davies SJ (2011) Precursor cell biology and the development of astrocyte transplantation therapies: lessons from spinal cord injury. *Neurotherapeutics* 8: 677-693.
- 5 Kim SU (2004) Human neural stem cells genetically modified for brain repair in neurological disorders. *Neuropathology* 24: 159-171.
- 6 Daniela F, Vescovi AL, Bottai D (2007) The stem cells as a potential treatment for neurodegeneration. *Methods Mol Biol* 399: 199-213.
- 7 Vescovi A, Gritti A, Cossu G, Galli R (2002) Neural stem cells: plasticity and their transdifferentiation potential. *Cells Tissues Organs* 171: 64-76.
- 8 Hawro T, Saluja R, Weller K, Altrichter S, Metz M, et al. (2014) Interleukin-31 does not induce immediate itch in atopic dermatitis patients and healthy controls after skin challenge. *Allergy* 69: 113-117.
- 9 Dhuria SV, Hanson LR, Frey WH 2nd (2010) Intranasal delivery to the central nervous system: mechanisms and experimental considerations. *J Pharm Sci* 99: 1654-1673.
- 10 Schiöth HB, Craft S, Brooks SJ, Frey WH 2nd, Benedict C (2012) Brain insulin signaling and Alzheimer's disease: current evidence and future directions. *Mol Neurobiol* 46: 4-10.
- 11 Danielyan L, Schäfer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, et al. (2009) Intranasal delivery of cells to the brain. *Eur J Cell Biol* 88: 315-324.
- 12 van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ (2010) Nasal administration of stem cells: a promising novel route to treat neonatal ischemic brain damage. *Pediatr Res* 68: 419-422.
- 13 Danielyan L, Schäfer R, von Ameln-Mayerhofer A, Bernhard F, Verleysdonk S, et al. (2011) Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of Parkinson disease. *Rejuvenation Res* 14: 3-16.

- 14 Wu H, Li J, Zhang Q, Yan X, Guo L, et al. (2012) A novel small Odorranalectin-bearing cubosomes: preparation, brain delivery and pharmacodynamic study on amyloid-beta₂₅₋₃₅-treated rats following intranasal administration. *Eur J Pharm Biopharm* 80: 368-378.
- 15 Donega V, van Velthoven CT, Nijboer CH, Kavelaars A, Heijnen CJ (2013) The endogenous regenerative capacity of the damaged newborn brain: boosting neurogenesis with mesenchymal stem cell treatment. *J Cereb Blood Flow Metab* 33: 625-634.
- 16 Donega V, Nijboer CH, van Tilborg G, Dijkhuizen RM, Kavelaars A, et al. (2014) Intranasally administered mesenchymal stem cells promote a regenerative niche for repair of neonatal ischemic brain injury. *Exp Neurol* 261: 53-64.
- 17 Balyasnikova IV, Prasol MS, Ferguson SD, Han Y, Ahmed AU, et al. (2014) Intranasal delivery of mesenchymal stem cells significantly extends survival of irradiated mice with experimental brain tumors. *Mol Ther* 22: 140-148.
- 18 Reitz M, Demestre M, Sedlacik J, Meissner H, Fiehler J, et al. (2012) Intranasal delivery of neural stem/progenitor cells: a noninvasive passage to target intracerebral glioma. *Stem Cells Transl Med* 1: 866-873.
- 19 Wierdl M, Tsurkan L, Hyatt JL, Edwards CC, Hatfield MJ, et al. (2008) An improved human carboxylesterase for enzyme/prodrug therapy with CPT-11. *Cancer Gene Ther* 15: 183-192.
- 20 Cromer Berman SM, Kshitiz, Wang CJ, Orukari I, Levchenko A, et al. (2012) Cell motility of neural stem cells is reduced after SPIO-labeling, which is mitigated after exocytosis. *Magn Reson Med* 69: 255-262.
- 21 Aboody KS, Najbauer J, Metz MZ, D'Apuzzo M, Gutova M, et al. (2013) Neural stem cell-mediated enzyme/prodrug therapy for glioma: preclinical studies. *Sci Transl Med* 8: 184ra159.
- 22 Campbell C, Morimoto BH, Nenciu D, Fox AW (2012) Drug development of intranasally delivered peptides. *Ther Deliv* 3: 557-568.
- 23 Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, et al. (2011) Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Mol Ther* 19: 1769-1779.
- 24 Shingaki T, Inoue D, Furubayashi T, Sakane T, Katsumi H, et al. (2010) Transnasal Delivery of Methotrexate to Brain Tumors in Rats: A New Strategy for Brain Tumor Chemotherapy. *Mol Pharm* 7: 1561-1568.
- 25 Serwer LP, James CD (2012) Challenges in drug delivery to tumors of the central nervous system: an overview of pharmacological and surgical considerations. *Adv Drug Deliv Rev* 64: 590-597.
- 26 Ransohoff RM, Engelhardt B (2012) The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat Rev Immunol* 12: 623-635.
- 27 Aboody KS, Najbauer J, Danks MK (2008) Stem and progenitor cell-mediated tumor selective gene therapy. *Gene Ther* 15: 739-752.
- 28 Gutova M, Shackleford GM, Khankaldyyan V, Herrmann KA, Shi XH, et al. (2013) Neural stem cell-mediated CE/CPT-11 enzyme/prodrug therapy in transgenic mouse model of intracerebellar medulloblastoma. *Gene Ther* 20: 143-150.

Submit your manuscript at
<http://enlivenarchive.org/submit-manuscript.php>

New initiative of Enliven Archive

Apart from providing HTML, PDF versions; we also provide **video version** and deposit the videos in about 15 freely accessible social network sites that promote videos which in turn will aid in rapid circulation of articles published with us.