



## Concise Review: Neural Stem Cell-Mediated Targeted Cancer Therapies

RACHAEL MOONEY <sup>a,b</sup> MOHAMED HAMMAD,<sup>a</sup> JENNIFER BATALLA-COVELLO,<sup>a,b</sup>  
ASMA ABDUL MAJID,<sup>a</sup> KAREN S. ABOODY <sup>a</sup>

**Key Words.** Cellular therapy • Chemotaxis • Chemotherapy • Clinical translation • Gene delivery systems in vivo or in vitro • Glioma • Progenitor cells • Viral persistence

<sup>a</sup>Department of Developmental and Stem Cell Biology, Beckman Research Institute, City of Hope Duarte, California, USA, <sup>b</sup>Irell and Manella Graduate, School of Biological Sciences, Beckman Research Institute, City of Hope Duarte, California, USA

Correspondence: Karen S. Aboody, M.D., Department of Developmental and Stem Cell Biology, Beckman Research Institute of City of Hope, 1500 East Duarte Ave., Duarte, California 91010, USA. Telephone: 626-218-8177; e-mail: kaboody@coh.org

Received January 3, 2018; revised March 28, 2018; accepted for publication April 24, 2018; first published August 21, 2018.

<http://dx.doi.org/10.1002/sctm.18-0003>

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### ABSTRACT

Cancer is one of the leading causes of morbidity and mortality worldwide, with 1,688,780 new cancer cases and 600,920 cancer deaths projected to occur in 2017 in the U.S. alone. Conventional cancer treatments including surgical, chemo-, and radiation therapies can be effective, but are often limited by tumor invasion, off-target toxicities, and acquired resistance. To improve clinical outcomes and decrease toxic side effects, more targeted, tumor-specific therapies are being developed. Delivering anticancer payloads using tumor-tropic cells can greatly increase therapeutic distribution to tumor sites, while sparing non-tumor tissues therefore minimizing toxic side effects. Neural stem cells (NSCs) are tumor-tropic cells that can pass through normal organs quickly, localize to invasive and metastatic tumor foci throughout the body, and cross the blood-brain barrier to reach tumors in the brain. This review focuses on the potential use of NSCs as vehicles to deliver various anticancer payloads selectively to tumor sites. The use of NSCs in cancer treatment has been studied most extensively in the brain, but the findings are applicable to other metastatic solid tumors, which will be described in this review. Strategies include NSC-mediated enzyme/prodrug gene therapy, oncolytic virotherapy, and delivery of antibodies, nanoparticles, and extracellular vesicles containing oligonucleotides. Preclinical discovery and translational studies, as well as early clinical trials, will be discussed. *STEM CELLS TRANSLATIONAL MEDICINE* 2018;7:740–747

### SIGNIFICANCE STATEMENT

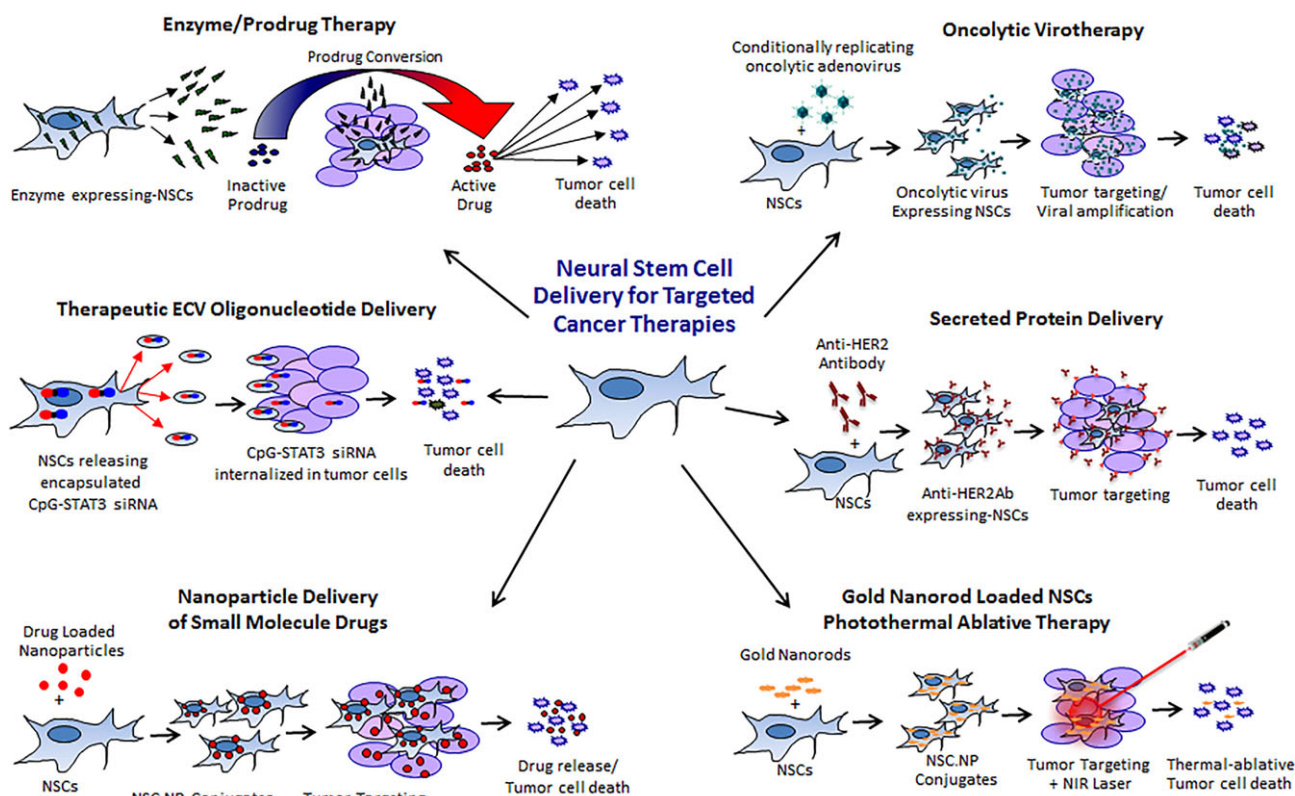
Cancer is a leading cause of death worldwide despite significant advances in biomedical research. Current cancer treatments are hindered by a lack of tumor specificity, which can lower their efficacy and cause undesirable side effects. Tumor-specific penetration of freely delivered therapeutic agents is difficult to achieve, but stem cell-mediated delivery can enable these agents to penetrate tumors, increasing exposure of the tumor while sparing non-tumor tissues. In this review, we briefly describe the tumor tropism of neural stem cells and their use as therapeutic vehicles, providing representative examples of this state-of-the-art approach in cancer therapy. Outlooks on novel therapeutic strategies and the probable direction of stem cell-mediated cancer therapy in the near future are also discussed.

### INTRODUCTION

There are more than 400,000 drugs that can eliminate tumor cells in vitro; however, cancer is still a leading cause of death (8.8 million deaths globally in 2015) [1, 2] in part due to our inability to selectively deliver adequate drug doses to residual tumor cells after surgery and radiation. Developing alternative strategies to selectively deliver sufficient amounts of anticancer agents to tumors is of utmost importance. One promising biological approach is to use live,

migratory stem cells. Many types of stem cells exhibit inherent pathotropism toward tissue damage and inflammatory conditions, including tumors.

Although the precise molecular mechanisms underlying the tumor-directed migration of stem cells are still being elucidated, tumors can be characterized as “wounds that never heal,” continuously releasing chemoattractants that recruit stem cells to the site of injury [3]. These include cytokines (e.g., monocyte chemoattractant protein-1 [4], stem cell factor [5], and



**Figure 1.** Engineering NSCs to deliver targeted anticancer payloads. Tumor-tropic NSCs can be engineered to deliver anticancer agents selectively to tumor foci. Approaches explored include enzyme/prodrug gene therapy, oncolytic virotherapy, therapeutic protein delivery (antibody delivery shown as example), sustained-release or stimuli-responsive nanoparticle delivery, and extracellular vesicle oligonucleotide delivery. Abbreviation: NSC, neural stem cell.

hepatocyte growth factor [6]), pro-angiogenic growth factors (e.g., vascular endothelial growth factor [VEGF] [7]), hypoxia-inducible factors [8], extracellular matrix [9], and other inflammatory mediators [10]. Stem cells express surface receptors responsive to these tumor-derived factors (e.g., CCL2 [4], c-Kit [5], c-Met [6], VEGF receptors [7], and hypoxia-responsive receptors [8]), which trigger their migration toward the tumor.

Tumor-directed migration of various stem cell types was demonstrated initially in preclinical models of primary brain tumors [11, 12] and secondary brain metastases [13–15]. An increasing number of preclinical studies have also demonstrated stem cell tropism to metastatic solid tumors outside the central nervous system (CNS), including ovarian [16, 17], pancreatic [18], lung [19], and breast cancers [19], as well as melanoma [20] and neuroblastoma [21]. Routes of delivery have included intracerebral, intraventricular, intravenous, intranasal, and intraperitoneal [22, 23]. Arming stem cells with antitumor agents *ex vivo* not only promises a higher degree of tumor specificity but also may increase the half-lives of stem cell-loaded therapeutics [24].

Stem cells at various stages of development have demonstrated tumor-tropic properties, but most preclinical and clinical stem cell-mediated approaches have used either trans-differentiated, immortalized, or freshly isolated multipotent progenitor cells (hematopoietic, mesenchymal, and neural stem cells [NSCs]) in an effort to avoid the potential tumorigenicity of pluripotent cell sources [25]. Hematopoietic stem cells have been used to locally produce prodrug-converting enzymes in breast, lung, and brain cancer settings [26], but

have otherwise not been widely used for targeting of solid tumors due to their ability to promote tumor angiogenesis [27]. Mesenchymal stem cells (MSCs) and NSCs have been more extensively investigated in the solid tumor setting. Efforts to use MSCs for tumor-specific delivery of therapeutics are reviewed elsewhere [28, 29]. In this concise review, we cover the preclinical and clinical use of NSCs as targeted delivery vehicles for anticancer agents. We also provide perspective on our translation of early NSC therapies to help guide future development efforts.

#### PRECLINICAL DEVELOPMENT OF NSCs AS THERAPEUTIC VEHICLES

Over the past 20 years, extensive research efforts have investigated the potential of NSC-mediated therapeutic delivery. Tumor-tropic NSCs are amenable to *ex vivo* manipulations that enable them to deliver a variety of anticancer agents selectively to tumor foci (Fig. 1). The advantages of NSC-mediated delivery of these cancer therapies include more effective and selective delivery to and distribution throughout tumor foci, minimal immunogenicity, and limited off-target effects, thereby resulting in lower toxicity to normal tissues. The efficacy of various NSC-delivered anticancer agents has been demonstrated in preclinical tumor models, summarized in Table 1, showing much clinical promise [30]. The different therapeutic cargo explored to date is discussed in the following section.

**Table 1.** NSC-mediated cancer therapy: Preclinical in vivo studies

Anticancer agent	Mechanism	Tumor model	NSC route of administration	Species: NSCs/ tumor/host	Tumor location	Reference
HSV-TK/GCV	GCV-triphosphate cytotoxicity	Breast cancer to brain metastases	Intracerebral, intra-arterial	Mouse/human/ mouse	Metastatic	[31, 32]
5-FU	Inhibits TS	Glioma	Intratumoral	Human/human/ mouse	Orthotopic brain	[11]
5-FU	Inhibits TS	Breast cancer	Intratumoral	Human/human/ mouse	Orthotopic mammary fat pad	[33]
5-FU	Inhibits TS	Medulloblastoma	Intratumoral	Human/human/ mouse	Orthotopic	[34]
SN-38	Inhibits topoisomerase	Medulloblastoma	Intratumoral	Human/human/ mouse	Orthotopic cerebellar	[12]
SN-38	Inhibits topoisomerase	Neuroblastoma	Intravenous	Human/human/ mouse	Metastatic	[21]
SN-38	Inhibits topoisomerase	Breast	Intravenous	Human/human/ mouse	Orthotopic	[10]
SN-38	Inhibits topoisomerase	Pancreas	Intravenous	Human/human/ mouse	Ectopic flank	[18]
SN-38	Inhibits topoisomerase	Lung	Intravenous	Human/human/ mouse	Ectopic flank	[19]
CRAAd-S-pk7 virus	Oncolysis	Glioma	Intratumoral	Human/human/ mouse	Orthotopic brain	[35]
Trastuzumab	Inhibits HER2	Breast to brain metastases	Intravenous	Human/human/ mouse	Orthotopic brain	[36]
TRAIL	Induces apoptosis	Glioma	Intravenous	Human/human/ mouse	Orthotopic brain	[37]
Osteoprogenin	Inhibits osteoclast-mediated bone resorption	Neuroblastoma bone metastases	Intravenous	Human/human/ mouse	Metastatic	[38]
Thrombospondin-1	Antiangiogenic	Glioma	Intracerebral	Human/human/ mouse	Orthotopic brain	[39]
PEX	Blood vessel effector	Glioma	Intracranial	Human/human/ mouse	Orthotopic brain	[40]
IL-4	Immune-stimulatory	Glioma	Intracerebral	Mouse/mouse/ mouse	Orthotopic brain	[37]
IL-12	Immune-stimulatory	Glioma	Intracerebral	Mouse/mouse/ mouse	Orthotopic brain	[24]
IL-23	Immune-stimulatory	Glioma	Intracerebral	Mouse/mouse/ mouse	Orthotopic brain	[41]
Iron/iron oxide magnetic nanoparticle + AMF	Physical disruption	Melanoma	Intravenous	Mouse/mouse/ mouse	Orthotopic flank	[20]
Gold nanorods + NIR	Thermal ablation	Breast, bladder cancer	Intratumoral	Human/human/ mouse	Ectopic flank	[42]
Docetaxel-loaded NPs	Inhibits microtubules	Breast cancer	Intratumoral	Human/human/ mouse	Orthotopic breast	[43]
Cisplatin-loaded NPs	DNA damage	Ovarian cancer	Intraperitoneal	Human/human/ mouse	Orthotopic ovarian	[16]
Doxorubicin-loaded NPs	DNA intercalation	Glioma	Intracerebral	Human/human/ mouse	Orthotopic brain	[44]

Abbreviations: 5-FU, 5-fluorouracil; AMF, alternating magnetic field; GCV, ganciclovir; HER2, human epidermal growth factor receptor 2; HSV-TK, herpes simplex virus thymidine kinase type 1; IL-12, interleukin-12; IL-23, interleukin-23; IL-4, interleukin-4; NIR, near infrared; NPs, nanoparticles; NSC, neural stem cells; PEX, hemopexin C domain autolytic fragment of matrix metalloproteinase-2; SN-38, irinotecan; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TS, thymidylate synthase.

### NSC-Mediated Enzyme/Prodrug Therapy

Neural stem cells can deliver prodrug-activating enzymes throughout tumor foci to convert inactive prodrugs into tumor-toxic effector drugs. Various cells have been engineered to enhance the efficacy of five of the more than 50 different enzyme–prodrug combinations developed during the past two decades [28]. Once generated, the effector drugs can initiate a “bystander effect,” affecting multiple surrounding tumor cells through diffusion, intercellular gap junctions, or endocytosis of apoptotic bodies released from dying cells. Genetically engineering cells to express prodrug-converting enzymes also provides a critical safety switch that can eliminate the cells after their therapeutic effect has been actualized [45]. Our laboratory has performed preclinical efficacy and safety/toxicity studies for two different modifications of a *v-myc* immortalized clonal NSC line (HB1.F3.C1) [11, 21]. In both cases, the NSCs were engineered to express prodrug-converting enzymes for tumor-localized chemotherapy production following intracerebral administration for recurrent high-grade glioma patients. Preclinical efficacy and safety/toxicity studies enabled successful Investigational New Drug (IND) applications to the U.S. Food and Drug Administration (FDA). First, the NSCs were retrovirally transduced to stably express *Escherichia coli* cytosine deaminase (HB1.F3.CD21; CD-NSCs), which converts the prodrug 5-fluorocytosine (5-FC) to the active chemotherapeutic 5-fluorouracil (5-FU) [11]. These same NSCs were further modified to secrete a modified human carboxylesterase (hCE1m6; CE-NSCs), which converts the prodrug irinotecan (IRN; CPT-11) to its active metabolite SN-38, a potent topoisomerase I inhibitor [46].

### NSC-Mediated Oncolytic Virotherapy

Oncolytic viruses can induce death of cancer cells regardless whether the cells are resistant to radio- or chemotherapy, and can stimulate immune system recognition of cancer cells as a result of exposure of tumor antigens after lysis. Although clinical trials to date have demonstrated the safety of oncolytic viruses, the efficacy of this approach has been limited by delivery hurdles such as rapid immune system inactivation of viruses, poor viral penetration of tumors, and the inability of the viruses to reach invasive foci that are separated from the main tumor mass by normal tissue [47, 48]. In collaboration with Dr. Lesniak’s group at the University of Chicago, we engineered our CD-NSC line to deliver a conditionally replication-competent adenovirus (CRAd-Survivin-pk7) that proliferates specifically in cells that overexpress *survivin*, a protein highly expressed in glioma cells (upregulated by radiation) but not in normal differentiated cells. Once the NSCs seed the virus into the invasive glioma sites, the virus continues to reproduce in tumor cells until normal tissue is reached and the effect ceases, resulting in reduced tumor burden and prolonged survival of mice bearing patient-derived glioma xenografts [49–51]. The minimal immunogenicity of the NSCs permits them to improve viral delivery and should enable repeat administrations.

### NSC-Mediated Therapeutic Protein Secretion

Neural stem cells can be transduced with integrating vectors so that they can stably release anticancer proteins, overcoming the short half-lives of conventional delivery regimens. To date,

several therapeutic proteins have been successfully engineered into NSCs, which have demonstrated anticancer effects when secreted in various preclinical carcinoma models.

**Growth Factor-Antagonists.** We modified our CD-NSC line to stably secrete a full-length anti-HER2 antibody (HER2Ab), which is functionally equivalent to trastuzumab [52]. Preclinical *in vivo* experiments using HER2Ab-overexpressing NSCs in a breast cancer brain metastasis mouse model demonstrated that intracerebral injection of HER2Ab-NSCs significantly improved survival [36]. The CD-NSC line was also modified to stably secrete osteoprogenin, which can reduce osteolysis in bone tumors. Preclinical *in vivo* experiments in a neuroblastoma mouse model demonstrated a decrease in bone disease and slowed overall disease progression [38].

### Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) binds to death receptors preferentially overexpressed in cancer cells and induces apoptosis via activation of caspases. Shah et al. generated a secretable version of TRAIL that can be efficiently secreted from NSCs and used to induce apoptosis in glioma cell lines both *in vivo* and *in vitro* [53].

**Interleukins.** Interleukins exert antitumor effects via direct tumoricidal effects or positive modulation of the endogenous immune system. NSC-mediated delivery of interleukins (IL-4, IL-12, and IL-23) [24, 37, 41] has been used to improve anti-glioma immune surveillance by activating cytotoxic lymphocytes and natural killer cells.

**Antiangiogenic Proteins.** Neural stem cells are particularly attracted to angiogenic regions of tumors with high-VEGF expression. NSCs engineered to provide sustained on-site delivery of secretable antiangiogenic thrombospondin (TSP-1) to tumor vasculature reduced tumor vessel density, inhibited tumor progression, and increased survival in glioma-bearing mice [39]. Consistent with these findings, NSCs engineered to secrete the hemopexin (PEX) fragment of matrix-metalloprotease II caused a 44.8% decrease in tumor angiogenesis and a 90% reduction in tumor volume [40].

### NSC-Mediated Nanoparticle Delivery

NSCs are appealing for use as nanoparticle (NP) carriers to overcome suboptimal penetration, distribution, and retention of therapeutic NPs. NSCs maintain their tumor tropism when transporting either surface-bound or internalized NPs. To date, two NSC-NP treatment strategies for tumors have been explored, although efficacy convincing enough to pursue a clinical trial has not yet been achieved.

**NSC-NP Conjugates for Small-Molecule Drug Delivery.** Drug-loaded NPs can be conjugated to the surface of NSCs or internalized before migrating to invasive tumor sites. The NSC-NP platform offers potential for the selective release (triggered or sustained) of anticancer drugs at tumor sites. NSCs can distribute NPs to brain tumor foci in mice when injected adjacent to the tumor, into the contralateral hemisphere, or into the tail vein [44, 54]. Furthermore, the NPs can be up to ~fivefold larger than those typically used for cancer therapies, which translates into a >100-fold increase in the loading potential of



**Table 2.** Cell-mediated cancer therapy: Clinical trials

Anticancer agent	Mechanism	Tumor targeted	Route of administration	Tumor-tropic cell	Clinicaltrials.gov ID	Reference
5-FU	Inhibits TS	Recurrent high-grade glioma	Intracerebral	HB1.F3.CD21	NCT01172964	[56]
5-FU	Inhibits TS	Recurrent high-grade glioma	Intracerebral	HB1.F3.CD21	NCT02015819	[56]
SN-38	Inhibits topoisomerase	Recurrent high-grade glioma	Intracerebral	HB1.F3.CD21.hCE1m6	NCT02192359	[46]
HSV-TK + GCV	GCV-triphosphate cytotoxicity	Recurrent high-grade glioma	Intracerebral	Nonmigratory fibroblasts	NCT00001328	[57]
CRAd-S-pk7 virus	Oncolysis	Newly diagnosed glioma	Intracerebral	HB1.F3.CD21.CRAd-S-pk7	NCT03072134	[51]

Abbreviations: 5-FU, 5-fluorouracil; GCV, ganciclovir; HSV-TK, herpes simplex virus thymidine kinase type 1; SN-38, irinotecan; TS, thymidylate synthase.

a typical small molecular weight chemotherapeutics. The utility of this approach has also been investigated in preclinical orthotopic breast [43] and ovarian cancer settings [16].

**NSC-NP Conjugates for Physical Tumor Destruction/Photothermal Ablative Therapy.** Loading NSCs with inert, metallic, stimuli-responsive NPs affords clinicians the ability to trigger the destruction of drug-resistant tumors on demand. NSCs loaded with gold nanorods result in well-distributed, tumor-localized antennae that can convert near infrared (near infrared [NIR]) light into heat capable of “burning” tumor tissue while avoiding collateral damage to surrounding healthy tissue [42]. In cases where the tumor lies beyond the penetration limits of NIR light, NSCs can alternatively be loaded with iron oxide discs. When exposed to an alternating magnetic field, the discs induce physical disruption of the tumor [20].

#### NSC-Secreted Exosomes as Carriers for Oligonucleotide Therapeutics

Oligonucleotide therapeutics (ONTs) can overcome the limitations of small-molecule inhibitors of many molecular targets, such as oncogenic transcription factors. However, short circulatory half-life and intratumoral penetration are still major hurdles in the clinical application of ONTs. NSCs secrete exosomes, which may enable the transfer of endogenous microRNAs, antigenic peptides, and perhaps even synthetic ONTs [55]. Our preliminary studies suggest that NSCs can rapidly internalize exosome-encapsulated ONTs and retain them for  $\leq 3$  days (unpublished data). We are still investigating whether NSC-mediated ONT delivery improves access to glioma and tumor-associated myeloid cells and thereby reduces tumor burden.

#### Summary of Preclinical Progress

At present, the most successful NSC-mediated approaches that have advanced to the clinic involve cargo that exhibits a substantial bystander or amplification effect (i.e., prodrug-converting enzymes and oncolytic viruses). NSCs can also potentially improve the efficacy of other therapeutic protein- or NP-based cargo while minimizing off-target toxicities. Further development will focus on optimizing the number and distribution of NSCs throughout loco-regional and systemic solid tumor metastases. Furthermore, the majority of preclinical studies to date have involved immunodeficient xenograft models. Given the emerging importance of eliciting an

antitumor immune response for effective treatments, studies in syngeneic and/or humanized mouse models will be important.

#### CLINICAL DEVELOPMENT OF NSCs AS THERAPEUTIC VEHICLES

Despite these challenges, several academic institutions have begun the translational process for NSC-mediated anticancer therapies. Table 2 lists both ongoing and complete clinical trials using cells to deliver therapeutic cargo to tumors. The lessons learned from these clinical trials are discussed in the following section, “Impact on Disease Treatment.” All human trials were performed with informed consent and were preceded by approval by both local institutional review board approval and the U.S. Department of Health and Human Services, U.S. Food and Drug Administration.

#### First-in-Human Fibroblast-Mediated Enzyme/Prodrug Therapy for Glioblastoma

This trial (1992–2010) was the first clinical attempt to treat malignant tumors in human beings by in vivo genetic manipulation of the tumor’s genome. GBM tumors were directly injected with nonmigratory fibroblasts engineered to deliver a retrovirus that incorporated herpes-thymidine kinase to tumor cells (NCT00001328). This trial underscored the need for migratory cells to distribute the therapeutic payload to tumor satellites.

#### First-in-Human NSC-Mediated Enzyme/Prodrug Therapy for GBM

A first-in-human pilot safety/feasibility study was completed in 2013 to assess the safety of using genetically modified allogeneic NSCs for tumor-selective enzyme/prodrug therapy (NCT01172964). Fifteen patients with recurrent GBMs received an intracerebral dose of CD-NSCs at the time of resection or biopsy, followed by a 7-day course of oral 5-FU. Intracerebral microdialysis results demonstrated safety, non-immunogenicity, and proof-of-concept for brain tumor-localized conversion of 5-FU to 5-FU by CD-NSCs [56]. Brain autopsy data revealed that NSCs migrated to distant tumor sites and were non-tumorigenic, demonstrating safety and proof-of-concept regarding the ability of NSCs to target tumor foci in the brain and locally produce chemotherapy. A phase I dose-escalation, multiple-treatment round study of CD-NSCs in combination

with 5-FC and folinic acid (Leucovorin) to determine phase II recommended dose was completed in 2017 (NCT02015819).

### Second-Generation NSC-Mediated Enzyme/Prodrug Therapy for GBM and Neuroblastoma

A phase I clinical trial for recurrent GBM patients is ongoing, similar to the previous protocol, using intracerebrally administered hCE1m6-NSCs combined with intravenous IRN (NCT02192359). The primary objective of this dose-escalation, multi-treatment round study is to demonstrate safety and define the phase II NSC recommended doses. Secondary objectives include pharmacokinetics, immunogenicity, and cell-fate correlative studies. Intravenous administration of hCE1m6-NSCs in combination with IRN and temozolomide (TMZ) is planned for a phase I clinical trial for pediatric patients with metastatic neuroblastoma in 2018. This will be the first clinical trial in which therapeutic NSCs are administered intravenously for treatment of solid tumor metastases. The primary objectives are to demonstrate safety and define the phase II recommended dose.

### First-in-Human NSC-Mediated Oncoviral Therapy for GBM

A first-in-human trial of CRAd-Survivn-pk7-loaded NSCs in combination with TMZ and radiation therapy is currently being conducted at Northwestern University and City of Hope in newly diagnosed GBM patients (NCT03072134). The primary objectives of this dose-escalation trial are to establish safety and to determine the maximum tolerated CRAd-Survivn-pk7-loaded NSC dose for phase II trials.

### Summary of Clinical Progress

The results of glioma clinical trials to date with the allogeneic, clonal HB1.F3.CD21 NSC line have demonstrated safety, even when multiple-treatment rounds are administered into the brain. The initial study, in which one round of treatment was given, demonstrated non-immunogenicity and proof-of-principle for brain tumor-localized NSC-mediated prodrug conversion and long-distance migration to tumor microsatellites. A first-in-human trial for metastatic neuroblastoma, in which NSCs will be administered intravenously, is pending. Once safety of intravenous NSC administration is established, the challenge will be to use therapeutic cargo that is potent and selective enough to more significantly reduce and eliminate tumor burden.

### IMPACT ON DISEASE TREATMENT

With sufficient development, NSC-mediated therapies can revolutionize the way cancer patients are treated and significantly improve their quality of life during and after treatments. So far, the use of allogeneic, immortalized NSCs has provided a safe, economical, and reliable way to translate this platform technology into the clinic. Immortalized karyotypically normal, v-myc immortalized NSCs have shown chromosomal and functional stability over multiple passages and good manufacturing practice scale-up, and have demonstrated clinical safety and non-tumorigenicity. Although allogeneic NSCs have no major histocompatibility complex (MHC) class 2 expression and low MHC class 1 expression, in early trials we are screening out patients with the same MHC class 1 expression as the

allogeneic NSC line. Careful monitoring of potential immune responses after each dose in multiple treatment round studies is also important. In vitro allorecognition of NSCs by peripheral blood lymphocytes has been reported [58]. In one study in which NSCs were injected into immunocompetent cotton rat and hamster models, the NSCs became vulnerable to immune-mediated clearance within 3 days post-transplantation [50]. One possible alternative is the use of patient-derived fibroblasts that have been transdifferentiated into tumor-homing NSCs [59]. This autologous approach could eliminate complications of immunosuppressive regimens and also prolong therapeutic NSC persistence to increase treatment durability [59]. Much work is still needed to realize the full potential of NSC-mediated cancer treatments.

Ongoing development work for the glioma trials is focused on improving NSC administration to optimize tumor coverage. We estimate that each intratumoral administration results in up to 25%–50% tumor coverage [30], suggesting a need to develop repeat administration strategies, alternative administration routes, or a self-amplifying therapeutic payload. Ongoing investigations are assessing the relative effectiveness of intraventricular [60] and intranasal [22] administration. Alternatively, we are considering the use of a hydrogel delivery matrix placed in the resection cavity to protect and increase viability of NSCs implanted, and maximize the NSC/tumor interface [53]. Until NSC administration is optimized, the best clinical efficacy will likely be observed using cargo with the largest bystander effect, or self-amplifying replication-competent oncolytic viruses. While initial trials have focused on local and loco-regional disease settings, they will soon expand into the peripheral setting with the upcoming neuroblastoma trial. Pulmonary bypass may limit the type of therapeutic cargo that can effectively be delivered intravenously to gene-based therapies, which hold little risk of off-target deposition given that administered NSCs only remain viable if they have reached the tumor site.

While the intended role of tumor-tropic NSCs is to deliver and/or produce a therapeutic within the tumor, NSCs are complex biological machines that may have additional unintended effects on tumor progression (positive or negative) if they persist or replicate within the tumor environment for an extended period of time. The duration of NSC persistence within the tumor depends on several factors, including post-transplantation survival efficiency, immune recognition, and tumorigenicity of the NSCs. Like most other cell therapies, post-transplantation survival efficiency of NSCs is currently low (<10%), so new efforts are underway to equip them with viability-promoting antioxidants [61], antiapoptotic transgenes [62], or support matrices [53].

As the duration of therapeutic NSC persistence increases, it will be important to gain more insight into how NSCs impact the immune microenvironment within the tumor. The immunosuppressive properties of NSCs have been reported in animal models of experimental autoimmune encephalomyelitis, in which NSCs secrete molecules that inhibit the proliferation and activation of T-cells, antigen-presenting cells, and microglia, and can even induce apoptosis in blood-borne, CNS-infiltrating, pro-inflammatory T helper type 1 cells [63]. However, the net impact that NSCs have on the tumor immune microenvironment remains to be elucidated. Early clinical studies using tumor-tropic NSCs within tumor settings were performed without collecting complete immune profiles, and the majority of preclinical

therapeutic efficacy studies have been evaluated in immunodeficient xenograft models. It has been reported that NSCs delivering oncolytic viral payloads can release pro-inflammatory immunomodulators, IL-6, and tumor necrosis factor  $\alpha$ , which are associated with triggering an innate immune response, but NSCs also release the immunosuppressive cytokine IL-10 [49]. NSCs can also decrease oncolytic virus-mediated astroglial activation within nude mouse brains, but no measurable effects with regard to macrophage infiltration have been observed [49]. And no effects of the NSCs on adaptive immune cells within the context of a tumor model have yet been reported. If NSCs are determined to have an overall immunosuppressive effect within the tumor, it may be necessary to engineer them with immune-stimulating cytokines (e.g., IL-4, IL-12, and TRAIL) or secretable nucleotide sequences.

Tumorigenicity of tumor-tropic NSCs has not yet been a problem, despite it being a substantial regulatory concern when the first-in-human allogeneic NSC-mediated CD/5-FC trial for recurrent glioma (NCT01172964) was initiated in 2010. Concerns arose after donor-derived tumors developed in settings in which NSCs were intended to engraft and provide sustained therapeutic benefit (i.e., a preclinical peripheral nerve injury model [64] and a clinical case of ataxia telangiectasia in which the NSC source was not adequately defined [65]). Through these studies, it became apparent that each NSC source (clone or pool) must be characterized for genetic and functional stability over time and passage, as well as tumortropism. Recent clinical results using the allogeneic clonal HB1.F3.CD NSC line, which is extensively characterized to be chromosomally stable, have demonstrated non-tumorigenicity [11, 56].

Future work will focus on developing more effective methodologies to image NSC biodistribution within patients. In addition, NSC-mediated cancer therapy is capable of synchronizing the delivery of multiple anticancer agents, which will likely afford more robust therapeutic efficacy against dynamic

and heterogeneous tumors that acquire resistance to single-agent treatment approaches. Improved efficacy of combinatorial MSC-mediated therapies has been confirmed [26], but this approach has yet to be explored fully using NSC delivery vehicles.

#### ACKNOWLEDGEMENTS

Financial support was provided by the Ben & Catherine Ivy Foundation, the Rosalinde and Arthur Gilbert Foundation, the Alvarez Charitable Foundation, the Anthony F. and Susan M. Markel Foundation, STOP Cancer, City of Hope, H.N. & Frances Berger Foundation, Norman & Melinda Payson Fellowship, and NIH-NCI grants R01 CA198076, R01 FD004816, R43 CA86768, R44 CA8678, and P30 CA03357 NIH-NINDS U01NS082328. K.S.A. is a director and officer of TheraBiologics Inc.

#### AUTHOR CONTRIBUTIONS

R.M.: collection, assembly, and interpretation of references; manuscript organization and writing. M.H.: collection of references, manuscript organization. J.B.-C.: collection of references, manuscript editing. A.A.M.: figure preparation. K.S.A.: collection, assembly, and interpretation of references; manuscript organization and writing.

#### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

R.M. discloses patent holder and research funding. K.A. discloses employment/Advisory role/stock ownership with CSO, TheraBiologics, Inc.; intellectual property with City of Hope and Harvard Patents. All other authors disclosure no potential conflict of interest.

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