Glial Induction of Human Bone Marrow Stromal Cells:
A fast & safe glial progenitor cell-based therapy for congenital myelin disorders of the central nervous system

Guy Lam[1, 2], Graham Ka-Hon Shea[2], Lap Kei Wu[1], Maximillian Li Tak Sui[1], Kwok Chun Hei[1], Yvonne Wong Cheuk Yin[1], Alex Yat Ping Tsui[1] , Daisy Kwok Yan Shum[1,3,4] and Ying Shing Chan[1,3,4]

School of Biomedical Sciences[1], Departments of Orthopaedics and Traumatology[2], Li Ka Shing Faculty of Medicine, Research Centre of Heart, Brain, Hormone & Healthy Aging[3], and State Key Laboratory of Brain and Cognitive Science[4], The University of Hong Kong

KEYWORDS

Glial Progenitor Cells, Bone Marrow Stromal Cells, Stem Cell Therapy, Remyelination

ABSTRACT/ SUMMARY

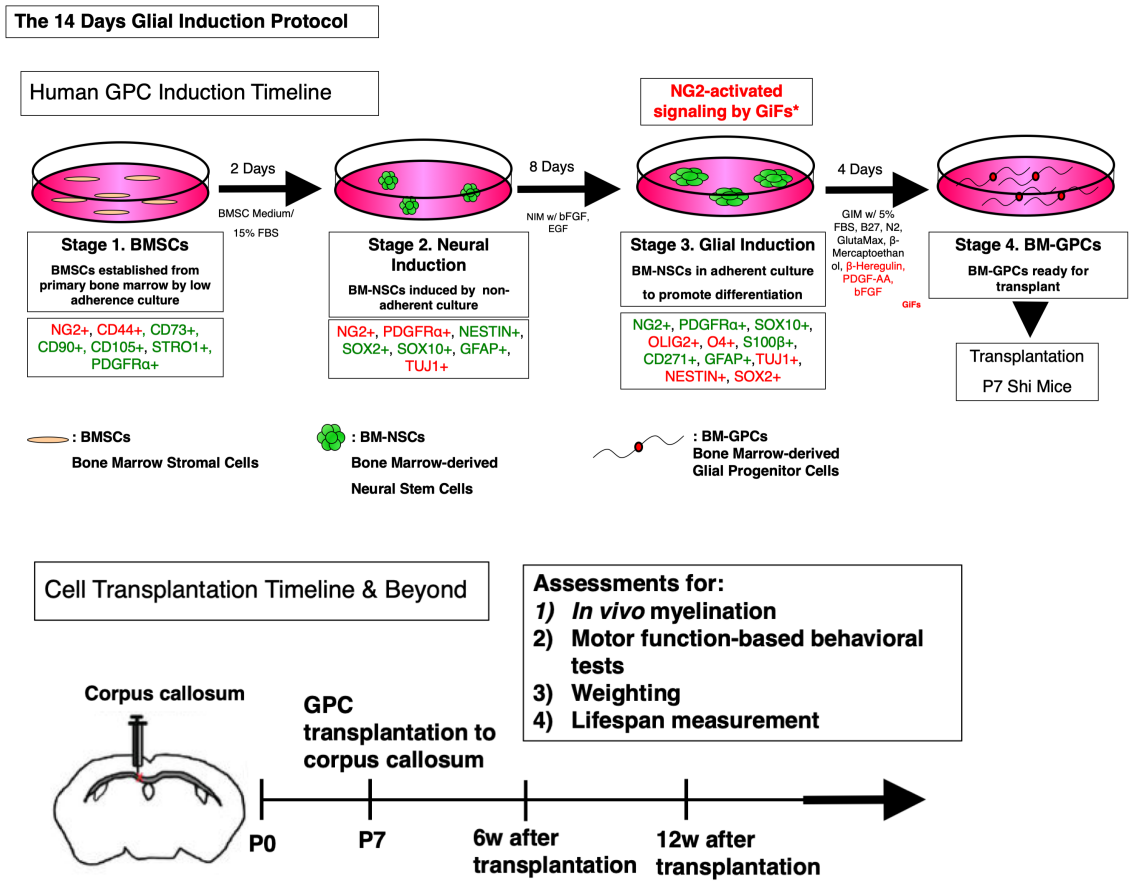
Oligodendrocytes (OLs) are the only myelinating glia in the central nervous system (CNS). In congenital myelin disorders, OL dysfunction or death results in loss of myelin. This causes progressive and irreversible impairment to motor and cognitive functions, and is amongst the most disabling neurological disorder.

Neonatal engraftment by glial progenitor cells (GPCs) allows the robust myelination of congenitally dysmyelinated brain, thereby preserving brain function and quality of life of patients. However, endogenous sources of glial progenitors are hard to obtain without causing secondary injury, while use of exogenous sources such as embryonic stem cells and induced-pluripotent stem cells face considerable ethical and safety issues.

To circumvent such hurdles, we asked whether $NG2^+$ cells in the bone marrow could be a potential cell source for GPCs. We successfully generated glial progenitor cells (GPCs) from human bone marrow stromal cells (hBMSCs) from 3 donors using a 14-day induction protocol. The generated hBMSC-GPCs were highly enriched in OPC marker expression, including OLIG2, PDGFRα, NG2, SOX10 and O4, and showed efficient differentiation into myelinogenic oligodendrocytes when transplanted into postnatal day 7 (P7) myelin-deficient shiverer mice. Remyelination of the shiverer mouse brain significantly extended lifespan and improved motor function.

The novel induction protocol described here provides a method for fast, simple and effective glial therapy for myelin disorders, overcoming existent hurdles of cell source restriction and time frame requirement.

Graphical Abstract



Highlights

- Human bone marrow stromal cells (BMSCs) can be programmed to myelinating glia (GPCs, glial progenitor cells), via a novel 14-day *in vitro* induction protocol
- Transplantation of these hBM-GPCs robustly remyelinated myelin deficient shiverer mice.
- hBM-GPC transplant significantly extended lifespan, increased body weight and improved motor function

INTRODUCTION

The significance of myelination

Ensheathment of axons by myelin in the white matter provides electrical insulation for the fast and effective transmission of nerve impulses, as well as protection for axons against inflammatory and oxidative insults.[1] (Bankston et al., 2013)

In the Central Nervous System (CNS), oligodendrocytes (OL) are responsible for myelinating axons.[2] (Baumann et al., 2001) Myelin disorders due to OL dysfunction or death, resulting in a loss of myelin. Myelin disorders are amongst the most disabling and prevalent of neurological conditions. Myelin disorders can be classified into (1) myelinoclastic diseases and (2) demyelinating leukodystrophic diseases [3] (Love, 2016) and (3) demyelination due to insult, such as radiation therapy to the brain or exposure to OL-toxins such as ethidium bromide. Myelin disorders cause a progressive and irreversible impairment in cognitive function as well as motor and executive impairment due to the depletion of the oligodendrocyte precursor pool and subsequent demyelination. [4] (Piao et al., 2015)

Demyelination, cell replacement therapy and application

Rapid remyelination of demyelinated axons is crucial for preservation of axon connectivity and the function of neural circuitries, since demyelinated axons not only have lower conductance, they are vulnerable to degenerative processes.

The ability to harness and commit stem cells to specific neural fates with a high reproducibility has opened the doorway to effective cell replacement therapies. (Tabar and Studer, 2014)[7] Various cell-based strategies have been established for repair of demyelinated lesions in both the brain and spinal cord (Ben-Hur and Goldman, 2008; Franklin and ffrench-Constant, 2008).[5, 6]

Congenital genetic disorders such as myelin basic protein defect-associated dysmyelination, where endogenous glial cells and their progenitors are not available/ fail to perform effective myelination (Franklin and ffrench-Constant, 2008) [6], are compelling targets for cell replacement strategies. Aside from overt OL loss and failure to form myelin (such as in leukodystrophies and inflammatory demyelinations), several neurodegenerative and psychiatric disorders may be linked to astrocytic and oligodendrocytic pathology causally as well. (Goldman et al., 2017) [8] The relative contribution of glial dysfunction to these disorders may allow corresponding treatment by the transplantation of allogeneic glial progenitor cells (GPCs), the precursors to both astroglia and myelin-producing OLs.

Glial Progenitor Cells and replacement strategies

I) *In Vivo* Role of GPCs

Human neuron-glial antigen 2 (NG2)-positive glial progenitor cells (GPCs) arising from neural stem cells of the ventricular subependyma. (Nishiyama et al., 2009; Goldman et al., 2012) [9,10], are found throughout the brain in both gray and white matter (Roy et al., 1999) [11]. *In vitro* and *in vivo* studies have demonstrated that human GPCs are bipotent in nature until the last division, generating both major macroglial

phenotypes, astrocytes and OLs, in a context-dependent fashion (Windrem et al., 2004) [12]. As the principal source of new OLs, GPCs are responsible for translational remyelination in the demyelinated adult CNS (Tripathi et al., 2010; Zawadzka et al., 2010) [13,14].

II) Translational application

As such, GPCs can concurrently restore myelin, while addressing the astrocytic function disorders related to white matter disease. (Goldman et al., 2017) [8]

Since harvesting primary GPCs from neural tissue induces secondary damage, recent studies on GPC transplant employed human (1) embryonic stem cells, (2) fetal and adult brain tissues and (3) induced pluripotent stem cells (iPSCs) as the source of GPCs. (Dietrich et al., 2002; Roy et al., 1999; Windrem et al., 2004; Hu et al., 2009b; Izrael et al., 2007b; Keirstead et al., 2005) [15,11,12,16,17,18]. GPCs thus derived were shown to mitigate both congenital (Sim et al., 2011; Windrem et al., 2004; Windrem et al., 2008; Duncan et al., 2009; Hu et al., 2009, Wang et al., 2013; Douvaras et al. 2014; Yang et al. 2013) [19, 12, 20,16, 21, 22, 23] and adult (Windrem et al., 2002) [24] CNS demyelination. However, use of ESCs and iPSCs face significant ethical and safety concerns (Sugarman et al., 2008; Saha et al., 2009) [26, 27].

Additionally, while existing GPC derivation protocols have been proven to yield functional myelinating glia, they remain complex and protracted. To the best of our knowledge, the fastest protocol requires at least 28 days and viral media to generate GPCs (Ehrlich et al., 2017) [28], while others usually require months. (Piao et al., 2015; Wong et al., 2013) [4, 21] This limits therapeutic usage for adult demyelination. It highlights the importance and benefit of developing a fast and robust method for obtaining GPCs from a safe and readily available cell source to meet the demands of clinical application.

Our approach on cell replacement therapy

Adult bone marrow stromal cells (BMSCs) represent a potential source of neural progenitors for autologous transplantation devoid of the previous concerns (Tomita et al., 2006; Chen et al., 2006) [29, 30]. In this regard, we aimed to convert BMSCs into GPCs with a faster, non-viral protocol of higher yield.

Our Findings

Human BMSCs from 3 traumatically injured patients were used generate glial progenitor cells (GPCs) in a 14-day GPC induction protocol, achieved via Neuron-Glial Antigen 2 (NG2) activation. A high percentage of the generated cell population express OPC markers (OLIG2, PDGFRα, NG2, SOX10 and O4), supporting the potential of BMSCs to be programmed into myelinating glia. Neonatal engraftment of GPCs derived using our protocol into myelin-deficient Shiverer mice resulted in remyelination of axons in the brain. GPC transplant ameliorated sensory-motor deficits and extended the lifespan of these mice.

This study thus provides a novel induction protocol and a new viable human cell source for prospectively autologous, fast, simple and clinically safe glial therapy to

treat both congenital and acquired myelin disorder, overcoming existing hurdles of cell source restriction and time frame requirement.

RESULTS

Human BMSCs efficiently programmed to glial cell fate in 14 days

The first question we asked, with reference to current limitations of OPC therapies, is whether mesenchymal stem cells (MSCs) could be directed into glial fate within a short timeframe for clinical application.

To this end, we developed a 3-stage protocol to generate early myelinating glial progenitor cells within 14 days (detailed in Supplemental Methods and schematized in Figure 1**A**), comparing to past protocols that require 110-150 days (Wang et al., 2012) [21].

Human bone marrow stromal cells (hBMSCs) harvested from 3 donors were used as the starting source for GPC generation. All three bone marrow samples were harvested from the tibia of patients that suffered from bone fracture across a great age variety (Age: 11, 59, 82 Years) with no other disease diagnosed.

A population of mesenchymal stem cells (MSCs), as indicated by immunoreactivity for $CD90^+$/ 105^+/ 44^+/ 73^+/ STRO-1^+/ $NG2^+$/ $PDGFRA^+$, was first isolated from the sample by selective adherent culture (Figure 1**B**). These cells were then harvested and expanded in suspension induction culture for neurospheres. After 8 days of neural induction, intermediate neural stem cells from neurospheres were highly enriched in neural stem cell markers NESTIN, SOX2 (Figure 1**C**) and GFAP.

8-day old neurospheres were then returned to adherent culture for glial induction. At the 4th day of the glial induction, $NG2^+$/ $PDGFR\alpha^+$/ $SOX10^+$/ $OLIG2^+$/ $O4^+$ was highly expressed in the cell population, with suppressed NESTIN and SOX2 (neural stem cell markers) expression, across all three patients.

The generated glial progenitor cells (GPCs) possess a tripotential fate to be differentiated into astrocytes, oligodendrocytes (OLs) or neurons, shown by the simultaneous expression of both astrocyte, OPC and neural stem cell markers within the same population (Figure 1**D**).

Derived hBM-GPC population highly expressed OPC markers

To quantify myelinogenic glial progenitor purity within our obtained GPC culture, flow cytometry analysis was conducted to characterize the derived cell population.

Undifferentiated hBMSCs of the human samples consistently expressed MSC markers to a high extent (>90%) (Figure 2**A**), as follows: CD44: 93.6%, CD73: 99.8%, CD90: 99.9%, CD105: 99.6%, STRO1: 93.3%, Alpha SMA: 99.7%. NG2 & PDGFRa was also expressed up to 82.1% & 64.7% (n= 3, 16,000-19,000 gated events per sample). This is in line with immnostaining data reported in Figure 1**B**.

Generated hBM-GPC population highly expressed OPC markers: 98.3% NG2+, 95.5% PDGFRα+, 95.1% O4+, 91% OLIG2+, 91.6% SOX10+ (Figure 2**B,** n=3, labelled respectively in Red, Blue & Orange, with ~20,000 gated events per sample). This also corroborated immunostaining data and suggested potency of our derived cells in generating OLs. The majority of the generated GPCs remained as progenitors as shown by the low expression of the mature OL marker myelin basic protein (MBP). Notably, the cells retained neural stem cell (TUJ1: 72.5%), astrocyte (GFAP: 89.9%) and PNS glial (S100β: 81.7%, CD271: 98.6%) potency. With respect to the overall high purity of cells by marker selection, no specific cell sorting was required.

(Whole Transcriptome Sequencing to be supplemented here upon completion)

Neonatally transplanted hBM-GPCs functionally myelinated the brain *in vivo*

Given the high purity of GPCs generated, as suggested by marker expression, we asked if the cells could indeed generate mature myelinating OLs in vivo. To address this question, we transplanted the cells into newborn homozygous shiverer mice at postnatal day 7 (P7), taking advantage of immune tolerance in the early postnatal stage. Neonatal pups were transplanted bilaterally in the corpus callosum (1mm next to the central cleavage of the corpus callosum, 1.5mm deep into cortex) and the hindbrain with a total of 90,000 cells, with reference to previous studies (Windrem et al., 2008; Wang et al., 2013) [20, 21] At week 12, the mice were sacrificed for analysis of donor cell localization, density, myelination patterns and myelination efficiency.

hBM-GPCs generated from all 3 donors were able to myelinate the shiver brain 11 weeks post-transplant, as shown by confocal images of week 12 shiverer mice brain coronal sections stained for MBP (Figure 3**B**). Compared to previous studies (Windrem et al., 2008; Wang et al., 2013) 20, 21, our hBM-GPCs did not demonstrate extensive post-transplantation lateral migration. The cells also demonstrated limited caudal migration along the striatal tract (Figure 3**B**, cells labeled by human nuclei antibody), as indicated by its localization at the upper striatum.

Quantitative analysis of 3D renderings (Imaris) revealed a mean density of 638 cells at the corpus callosum per 20 µm thick of section. Local myelination (MBP, green) was observed at the subcortical region (Figure 3**C**), as well as at the upper striatum (Figure 3**D**, **E**). The relatively high proportions of axons myelinated reflects the myelination efficiency. (*Number to be supplemented, further histology studies are required to identify the proportion of $CFSE^+$/ MBP^+ cells along $TUJ1^+$ axons.*)

hBM-GPCs-derived oligodendrocytes ensheathed axons *in vivo* in shiverer brain

To ascertain that the observed MBP signal was resultant from formation of compact myelin, ultrathin sections of corpus callosum and upper striatum tissue were obtained from 12-week old shiverer mice for electron microscopy.

Compact myelin around axons were abundant in corpus callosum tissue from hBM-GPC–transplanted mice, which were absent in untreated shiverer controls.

The upper striatum featured rings of immaturely myelinated axons (Figure 4**A**) and at the callosa, numerous compact myelin sheaths ensheathing central axons at the corpus

callosum were observed (Figure 4**B**). The tissue was myelinated at a density up to 56 axons/ 500um^2.

Due to limited migration featured, our myelination, especially at the distal ends of the striatum, are not as dense and compact as previous studies (Windrem et al., 2008; Wang et al., 2013) [20, 21].

Neonatally transplanted hBM-GPCs rescue motor function, body weight and extend shiverer lifespan through remyelination

We finally asked if the myelination of corpus callosum and striatal tissue could improve symptoms resultant from congenial loss of myelin. Body weight and motor function were assessed in all the mice at week 6 and week 12. The lifespan of mice with and without hBM-GPC transplantation was also recorded. (Detailed in Supplemental Methods)

Cell-transplanted (treatment group) animals were significantly heavier than controls at 12 weeks. a timepoint featuring completion of murine maturational growth, shiverer mouse functional decline, arrest and death. (Figure 5$\mathbf{A_2}$) A 13.98% increase (27.48g to 20.89g) for the week 12 male group and 14.5% increase (23.92g to 20.89g) for the female group was observed. At week 6, a timepoint corresponding to adolescence, no significant difference in body weight was found. (Figure 5$\mathbf{A_1}$)

Treated animals had significant improvements in accelerating rotarod motor behavioral test at week 6 compared to controls (Figure 5$\mathbf{B_1}$), with the mean latency of fall increased to 27.96 seconds versus 17.24 seconds in controls (a 62.84% improvement) on an accelerating profile. Treated animals demonstrated even greater improvements at week 12 (Figure 5$\mathbf{B_2}$), from 14.35 seconds to 25.88 seconds, a total of 80.34% in the motor behavioral test. In the balance beam behavioral test, treated animals were faster when traversing the beam (6.8 seconds with 13.61% increase at week 6; 5 seconds with 13.31% increase at week 12), yet due to a high standard deviation the demonstrated is insignificant (Figure 5**C**).

Treated animals also manifested significantly extended mean lifespan up to 130 days when compared with the control mean lifespan of 101 days (Figure 5**D**), giving a significant increase of 27.21%.

In conclusion, there is a significant increase/ improvement observed in: 1) body weight, 2) motor function and 3) lifespan in mice that received hBM-GPC transplantation.

DISCUSSION

In this study, we established a novel glial induction protocol, employing the unexploited differentiation potential of human bone marrow stromal cells (hBMSCs), to rapidly and efficiently generate population of glial progenitors cells (GPCs). These bone marrow-derived glial progenitor cells (BM-GPCs) are enriched in oligodendrocyte progenitor cell (OPC) markers and capable of differentiating into

mature oligodendrocytes in vivo and myelinate CNS axons of shiverer mice Apart from histological evidence, we further provided behavioural evidence that hBM-GPCs indeed preserved function of brain circuits in mice that would otherwise suffer from significant motor deficits.
hBMSCs have long been adopted in cell replacement strategies, being characterized as a safe and autologous source when compared to the ethically disputable embryonic stem cells (ESCs) and unstable induced pluripotent stem cells (iPSCs). We established the potential of BMSCs to be programmed into myelinating glia and shortened the induction timeframe to 14 days. This is a significant improvement from current protocols for GPC production which require at least 28 days (Ehrlich et al., 2017) [28]. As short time frame enhances the translational significance of our protocol for clinical use as therapy for various myelin disorders.

3 patient samples of great diversity in age were all successfully derived into hBM-GPCs without modification to the protocol. This suggests that our protocol is versatile and repeatable, provided that good quality of red bone marrow (~2-3 cm^3) is used.

NG2 signaling and glial fate commitment

NG2, fully known as Neuron-Glial Antigen 2 (NG2), is a hallmark protein of oligodendrocyte progenitor cells (OPCs) (Polito et al. 2005) [34]. NG2 is involved in cell proliferation and growth; survival, angiogenesis and cell migration. (Ampofo, et al. 2017) [35] It also has an interesting role to play on the fate of neuron-glial synapses during cell proliferation and differentiation.

NG2, is an integral membrane proteoglycan (Chondroitin Sulphate Proteoglycan 4/ CSPG4). We reasoned that NG2 is at the hub for controlling OL fate and maintaining OPC identity. Serving as the binding site for PDGF-AA (Tillet et al. 1997, Goretzki et al. 1999) [36,37], NG2 is well poised to trigger critical signaling for OPC fate commitment (Baron et al. 2000) [38]. While its juxtamembrane domain was reported to interact with bFGF (Tillet et al. 1997, Goretzki et al. 1999) [36,37], which is key to keeping OPC from differentiating into mature OLs.

Yet with NG2 discovered 20 years ago, few translational studies have addressed or exploited its potential. This glial induction protocol probes the feasibility of activating PDGF and bFGF signaling through Neuron-Glial Antigen 2 (NG2). A simple growth factor-only approach was used in our protocol (detailed in Supplemental Methods), in contrast to previous studies that required transcription factor promoting viral constructs. (Ehrlich et al., 2017) [28] Since NG2 is also expressed in MSCs and pericytes, the success of this protocol raises the question whether all $NG2^+$ cells are glial-inducible.

In previous studies (Wang et al., 2013) [21], OPCs derived from other cell source with different protocols were low in purity and yield (4-12%) and required follow-up fluorescence activated cell sorting (FACS) before transplantation. Most recent studies endeavored to optimize the yield (Ehrlich et al., 2017) [28], yet only a purity of 65-70% had been reached. Our protocol improves the purity significantly, generating a cell population with over 90% expression of OPC markers ($OLIG2^+$, $PDGFR\alpha^+$, $NG2^+$, $SOX10^+$ and $O4^+$).

In light of the *in vivo* myelination performed by neonatally engrafted hBM-GPCs, we

investigated 1) whether hBM-GPCs exhibit similar migration throughout the major white matter tracts as its IPSC-or ESC-derived counterparts and; 2) the potency of hBM-GPCs in rescuing the functional deficits of congenitally myelin-deficient mice models.

We found that the cell migration was much lower than that of IPSC and ESC-derived sources (Wang et al., 2013) [21], with only limited migration to the upper striatal region, while most of the engrafted cells aggregated at the upper callosal region. Such migration properties could be related to the cell source or the stage of the cells, as more naïve cells (without a biased differentiation tendency) possess a better capacity to differentiate and proliferate (implicated by the total number of labeled cells to transplanted cells), yet requiring a longer time for fate-commitment (implicated in the time required for differentiation). Our hBM-GPCs may interact with naked axons by SHP-1 and juxtacrine ErbB signaling [41, 42], which promotes its rapid differentiation into mature OLs and hence facilitate myelination.

Despite the limited areas remyelinated by hBM-GPCs in shiverer mice, we nonetheless observed a significant rescue of the motor function along with extension of the lifespan of shiverer mice up to 27%. We suggest this could be due to 1) the concentrated output of the cells and/ or 2) the critical motor coordinating role of the corpus callosum. Though these questions await further tests, results demonstrate the potential of using hBM-GPCs as therapy for myelin disorders. We also adopted a lower transplantation cell count (~90,000 cells per mice) in our study and enhanced output could be expected with an increase in the quantity of hBM-GPCs engrafted. No evidence of tumorgenesis was observed in mice engrafted with hBM-GPCs up to 5 months. This suggests hBM-GPCs generated using our protocol were fate committed and safe for translation.

The study demonstrated the utility of hBMSCs as a feasible and effective source of GPCs for deriving myelinogenic oligodendrocytes. Clinical significance is further enhanced by short induction time and high purity achieved using this protocol, along with its robustness in deriving GPCs irrespective of donor age. A variety of disease settings, including congenital (e.g. myelin genetic disorders: Pelizaeus-Merzbacher Disease) and acquired myelin disorders (e.g. traumatic demyelination and multiple sclerosis) may benefit from hBM-GPC transplant.

Figure 1

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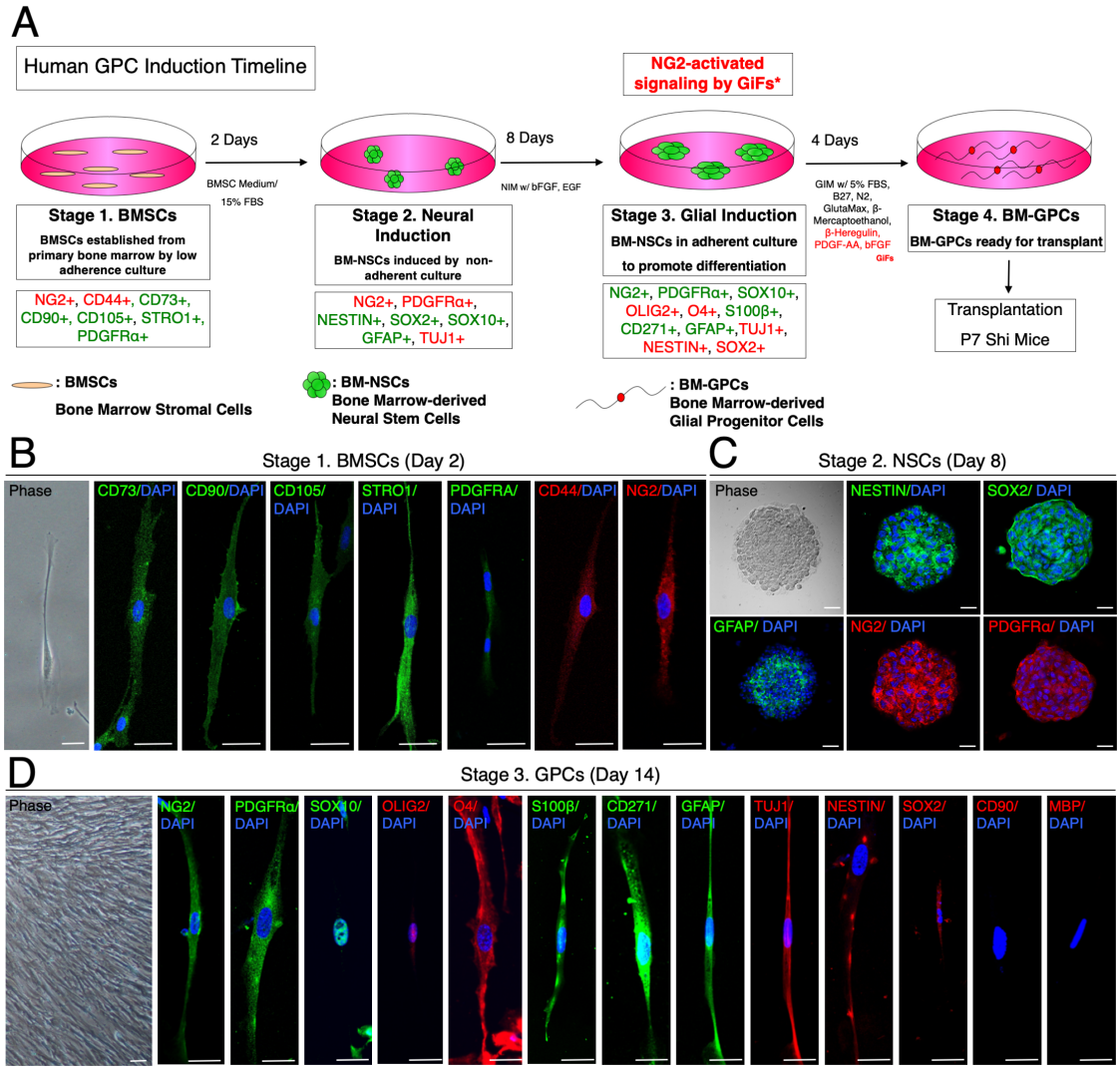

Figure 1. Direction of hBMSCs into GPC fate within 14 days

(**A**) A schematic protocol for directed differentiation of hBMSCs into GPCs in 14 days. BMSCs from human patients (hBMSCs) were purified and established *in vitro* from Day 0-2. Spheres of neural stem cells (NS; neurospheres) were differentiated from hBMSCs (Stage 1) from Day 3-10 in neural induction medium (NIM; refer to Methods) with bFGF and EGF. From Day 11-14, neurospheres were then pushed towards glial fate induction to become GPCs in glial induction medium (GIM; refer to Methods) featuring critical Glial induction Factors (GiFs: β-Heregulin, PDGF-AA, bFGF).

(**B**) Immunocytochemistry of Stage 1, undifferentiated hBMSCs. MSC markers CD90+/ CD105+/ CD44+/CD73+/ STRO1+ were expressed. NG2, PDGFRA were also expressed.

(**C**) By Stage 2, the spheres expressed neural stem cell markers, being NESTIN+/ SOX2+/ GFAP+/STRO1+. NG2, PDGFRA was also expressed.

(**D**) By Stage 3, markers of early myelinogenic glial progenitors, including: NG2/ PDGFRα/ OLIG2/ SOX10/ O4 were highly expressed. Neural stem cell markers: SOX2/ NESTIN/ TUJ1 were also present. Astrocyte marker GFAP and PNS glial markers: CD271/ S100β were also expressed. MSC marker CD90 & mature OL marker: MBP were not expressed, indicating the early myelinogenic fate of the derived cell.

Scale: **B, C, D**: 25 µm.

Figure 2

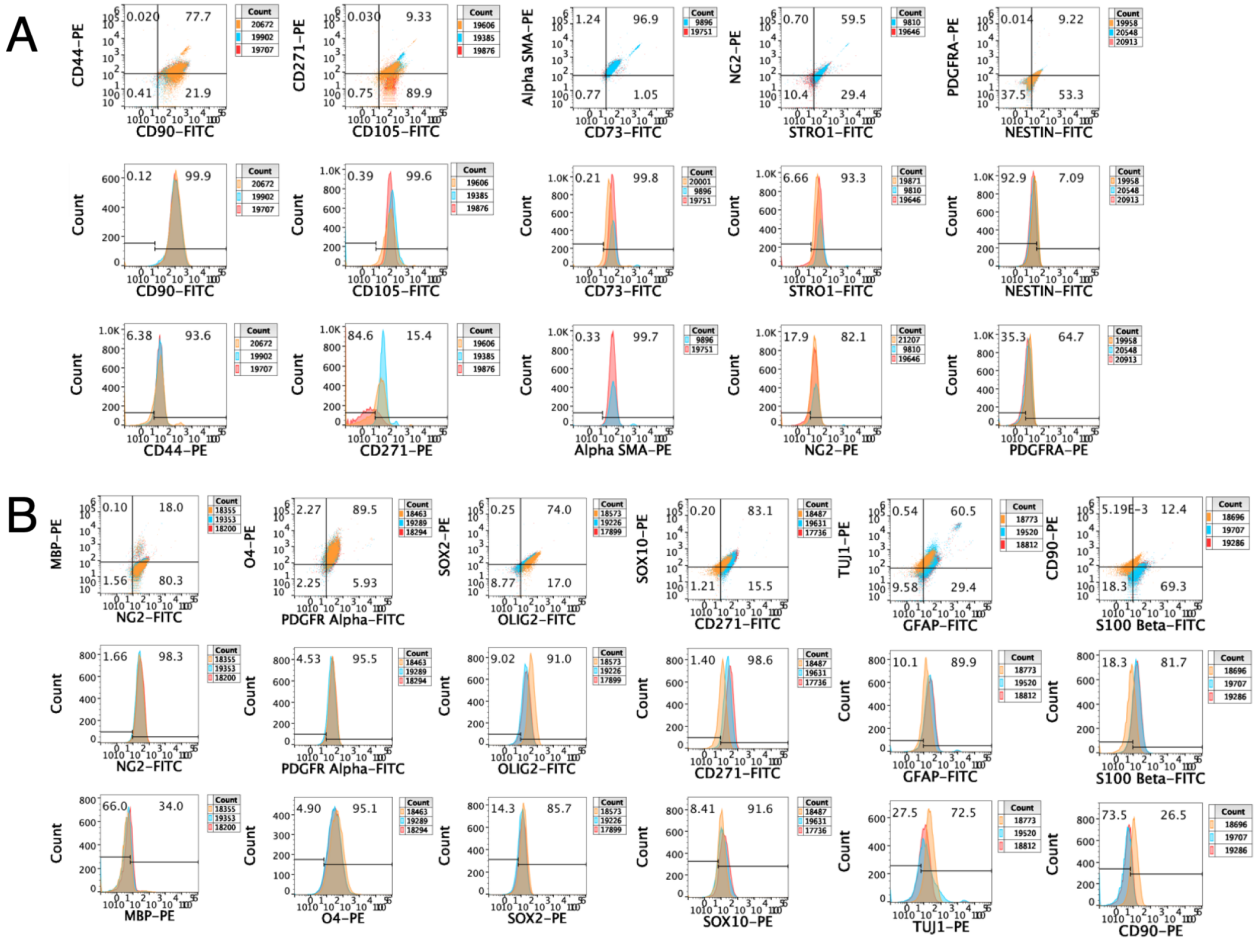

Figure 2. Induced GPCs feature high myelinogenic glial maturation potential
(A) Undifferentiated hBMSC population (n=3, labelled respectively in Red, Blue & Orange, with ~20,000 gated events per sample) was characterized with flow cytometry. BMSC markers CD44, CD73, CD90, CD105, STRO1, Alpha SMA were consistently expressed in more than 90% of cells; NG2 & PDGFRa was also expressed up to 82.1% & 64.7%.
(**B**) Generated hBM-GPC population (n=3, labelled respectively in Red, Blue & Orange, with ~20,000 gated events per sample) was characterized with flow cytometry analysis. Over 90% of the population expressed OPC markers NG2, PDGFRα, O4 and OLIG2 in concordance with immunocytochemistry data presented in Figure 1, suggesting their potency to generate OLs. Neural stem cell, astrocyte and PNS glial potency was also suggested by the expression of TUJ1, GFAP, CD271 and S100β.

Figure 3

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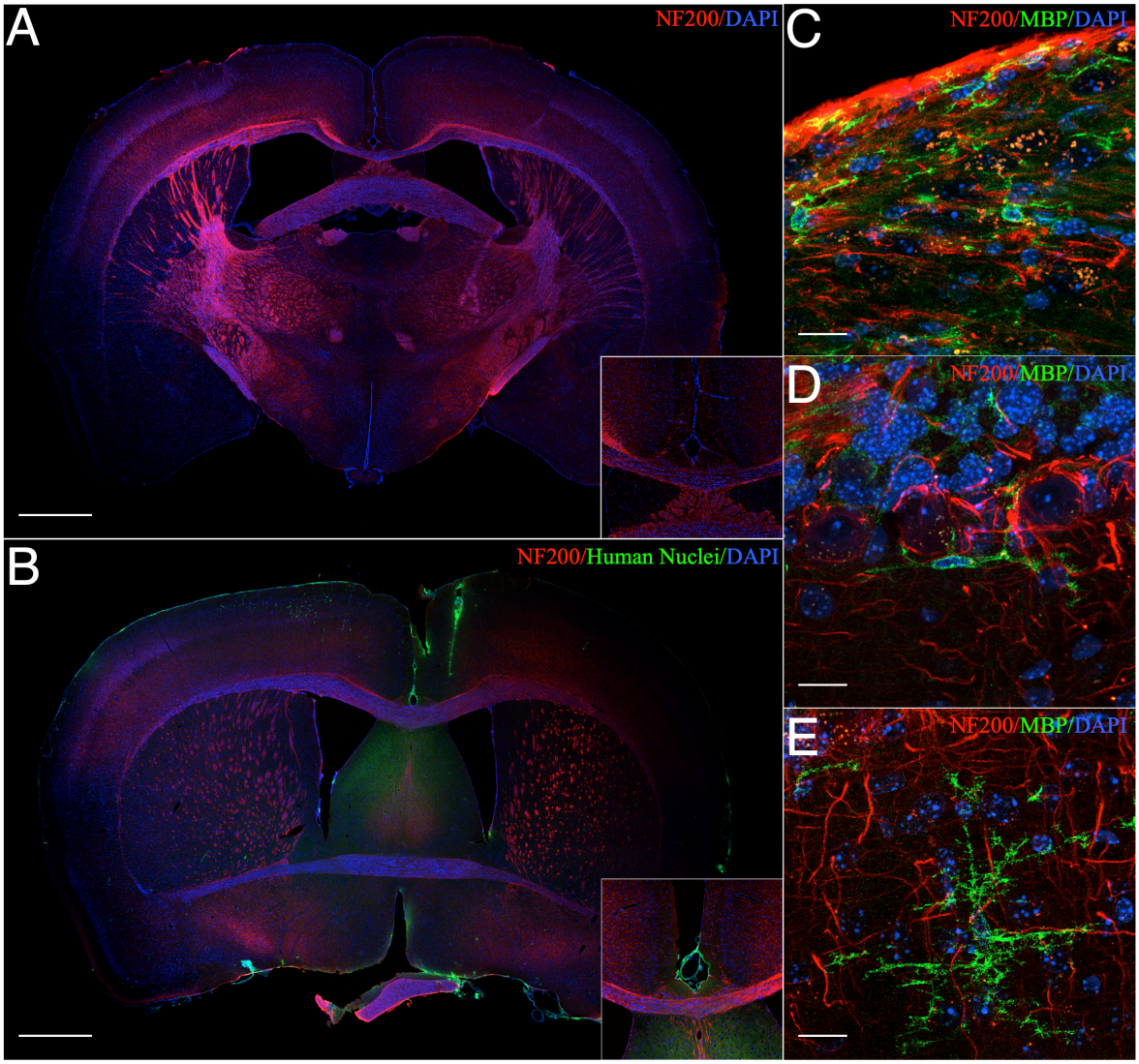

Figure 3. Neonatally transplanted hBM-GPCs myelinate *in vivo*

Confocal images of coronal sections of the shiverer mice brain harvested at week 12.
(**A**) Histology featuring the coronal mid-brain section of a control shiverer mouse.
(**B**) Coronal mid-brain section of a shiverer mouse injected with hBM-GPCs. hBM-GPCs, marked by human nuclei (green), transplanted next to the corpus callosum demonstrated limited caudal migration along the striatal tract. No lateral cortical migration was observed.
(**C**) Local myelination (MBP, green) was observed at the subcortical region.
(**D, E**) Local myelination (MBP, green) was observed at the upper striatum.
Scale: **A, B**: 1 mm; **C, D, E**: 100 µm.

Figure 4

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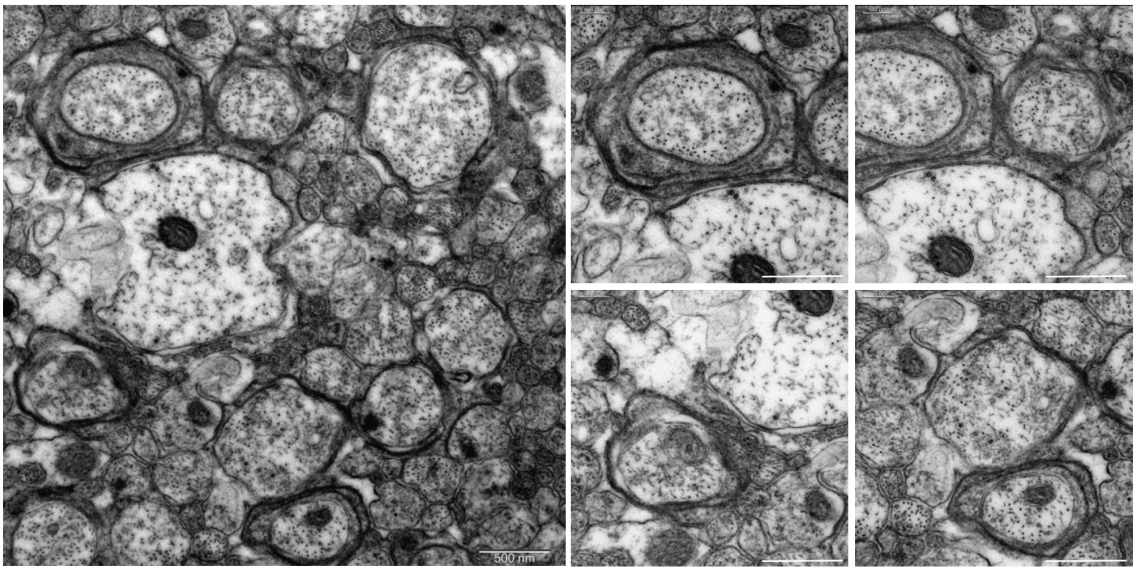
A



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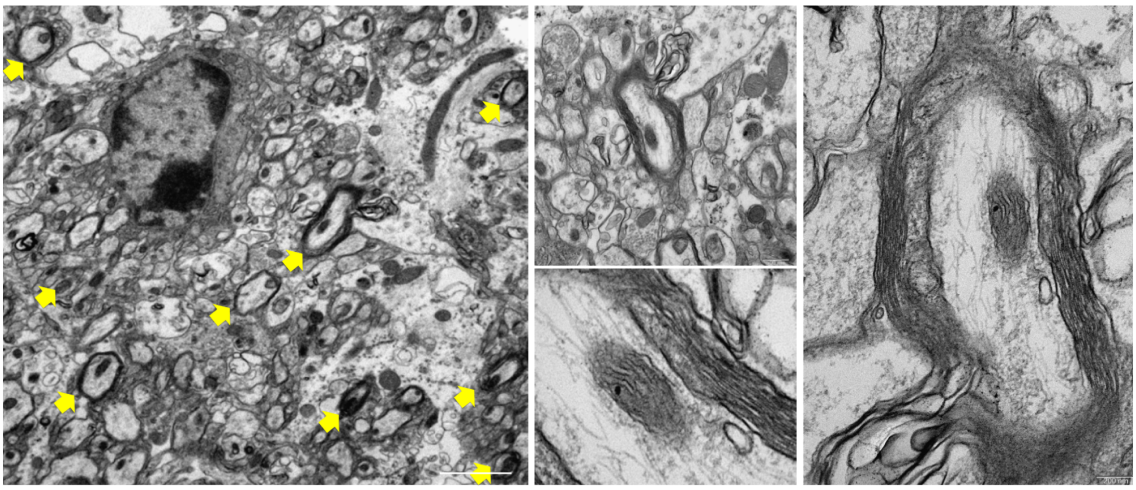

Figure 4. hBM-GPCs ensheathed axons in myelin ambient to the transplantation site

Representative electron microscopy images of sagittal sections of shiverer mice brain harvested at week 12.

(**A**) Immaturely myelinated axons with only one ring of myelin were found in the upper striatum.

(**B**) Multiple compact myelin sheaths (yellow arrows) ensheathing central axons were observed in the corpus callosum. (9 Compactly Myelinated axons per 196um2/ 2200x field)

Scale: **A**: 500 nm; **B1**: 2 um, **B2**: 500 nm**, B3**: 100 nm**, B3**: 100 nm, **B4**: 200 nm.

Figure 5

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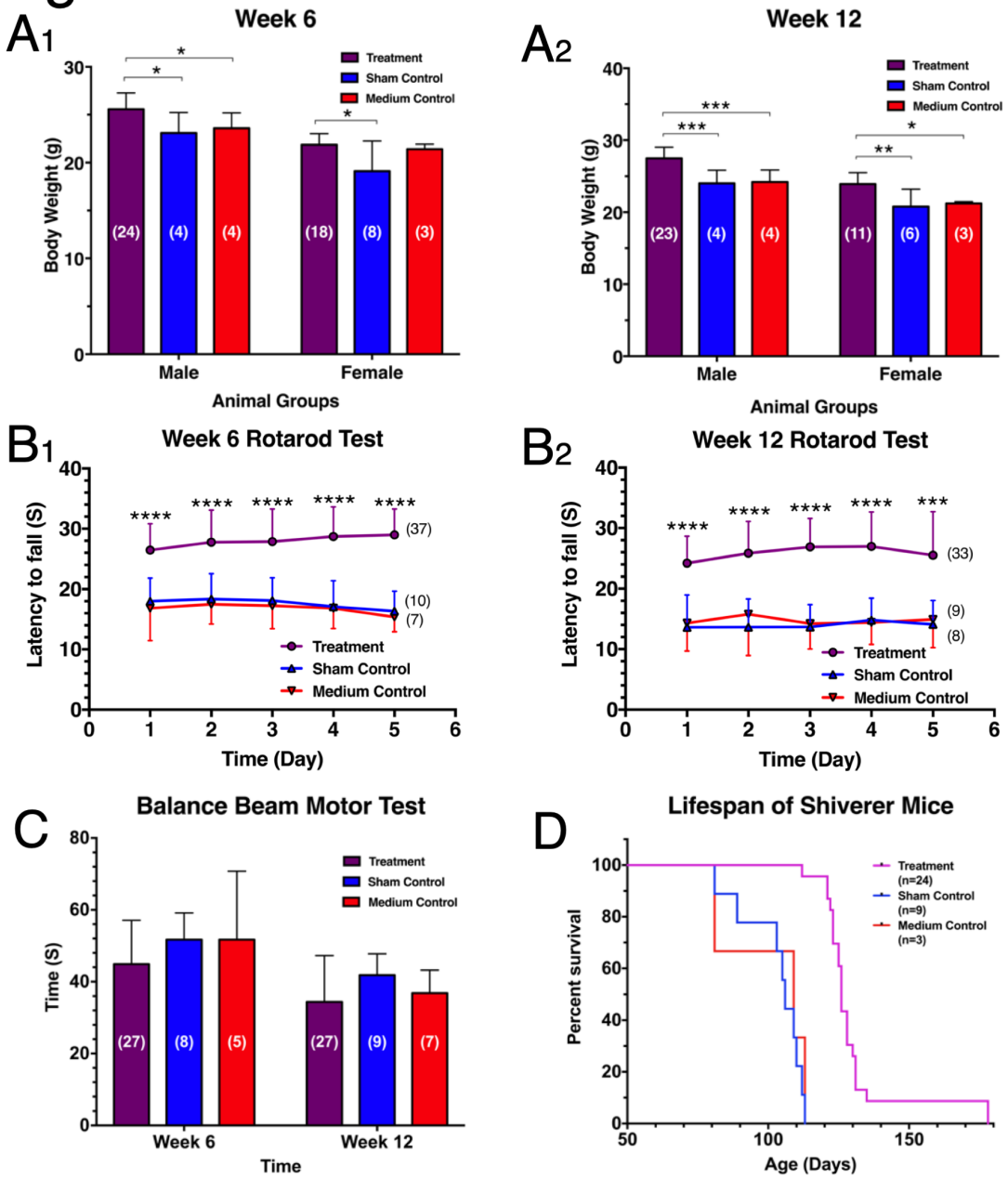

Figure 5. Neonatally transplanted hBM-GPCs rescue motor function, body weight and extend shiverer lifespan through remyelination

(**A₁**) hBM-GPC-transplanted (treatment group) shiverer mice were significantly heavier than age-matched controls at adolescent stage (week 6, total number of mice = 61 at this time point) &

(**A₂**) adult stage (week 12, mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ two-way ANOVA. Total number of mice = 51 at this time point).

(**B₁**) hBM-GPC-transplanted shiverer mice demonstrated significant better performance in accelerating rotarod motor behavioral test at week 6 &

(**B₂**) week 12 compared to age-matched controls.

(**C**) hBM-GPC-transplanted shiverer mice performed slightly better that age-matched controls in balance beam behavioral test at week 12, but the improvement was statistically insignificant.

(**D**) hBM-GPC transplantation significantly extended mean lifespan of shiverer mice to 130 days compared to 100 days of control mice.

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REFERENCES

1. Andrew N. Bankston, Mariana D. Mandler, and Yue Feng. (2013). Oligodendroglia and neurotrophic factors in neurodegeneration. Neurosci Bull. 2013 Apr; 29(2): 216–228. Published online 2013 Apr 5. doi: 10.1007/s12264-013-1321-3
2. Baumann, Nicole; Pham-Dinh, Danielle (2001). "Biology of Oligodendrocyte and Myelin in the Mammalian Central Nervous System". Physiological Reviews. 81 (2): 871–927. ISSN 0031-9333. PMID 11274346.
3. Bugiani M, Boor I, Powers JM, Scheper GC, van der Knaap MS. (2010). Leukoencephalopathy with vanishing white matter: A review. J Neuropathol Exp Neurol 2010; 69(10): 987-996.
Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitors Remyelinate the Brain and Rescue Behavioral Deficits following Radiation
4. Piao J, Major T, Auyeung G, Policarpio E, Menon J, Droms L, Gutin P, Uryu K, Tchieu J, Soulet D, Tabar V. (2015). Human embryonic stem cell-derived oligodendrocyte precursors remyelinated the brain and rescued behavioural deficits following radiation. Cell Stem Cell 2015; 16(2): 198-210.
5. Ben-Hur T, Goldman SA. (2008) Prospects of cell therapy for disorders of myelin. Ann N Y Acad Sci. 2008 Oct;1142:218-49. doi: 10.1196/annals.1444.014.
6. Robin J.M. Franklin and Steven A. Goldman. (2015) Glia Disease and Repair—Remyelination. Cold Spring Harb Perspect Biol. 2015 Jul; 7(7): a020594.doi: [10.1101/cshperspect.a020594]
7. Tabar V, Studer L. (2014) Pluripotent stem cells in regenerative medicine: challenges and recent progress. Nat Rev Genet. 2014 Feb;15(2):82-92. doi: 10.1038/nrg3563.
8. Goldman SA. Progenitor cell-based treatment of glial disease. (2017). Prog Brain Res. 2017;231:165-189. doi: 10.1016/bs.pbr.2017.02.010. Epub 2017 Apr 13.
9. Nishiyama, A.; Komitova, M.; Suzuki, R.; Zhu, X. (2009). "Polydendrocytes (NG2 cells): Multifunctional cells with lineage plasticity". Nature Reviews Neuroscience. 10 (1): 9–22. doi:10.1038/nrn2495. PMID 19096367
10. Goldman SA, Nedergaard M, Windrem MS (2012). Glial Progenitor Cell–Based Treatment and Modeling of Neurological Disease. Science New Series, Vol. 338, No. 6106 (26 October 2012), pp. 491-495.
11. Roy NS, Wang S, Harrison-Restelli C, benraiss A, Fraser FA, Gravel M et al (1999). Identification, isolation, and promoter-defined separation of mitotic oligodendrocyte progenitor cells from the adult human subcortical white matter. J Neurosci 19: 9986–9995.
12. Windrem MS, Nunes MC, Rashbaum WK, Schwartz TH, Goodman RA, McKhann G, Roy NS, Goldman SA. (2004) Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nature Medicine. 2004;10:93–97.
13. Tripathi R.B., Rivers L.E., Young K.M., Jamen F., Richardson W.D. (2010) NG2 glia generate new oligodendrocytes but few astrocytes in a murine experimental autoimmune encephalomyelitis model of demyelinating disease. J. Neurosci. 2010;30:16383–16390.
14. Zawadzka M., Rivers L.E., Fancy S.P.J., Zhao C., Tripathi R., Jamen F., Young K., Goncharevich A., Pohl H., Rizzi M. (2010) CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. Cell Stem Cell. 2010;6:578–590.
15. Dietrich J, Noble M, Mayer-Proschel M. (2002) Characterization of A2B5+ glial precursor cells from cryopreserved human fetal brain progenitor cells. Glia. 2002;40:65–77.
16. Hu BY, Du ZW, Zhang SC. (2009) Differentiation of human oligodendrocytes from pluripotent stem cells. Nat Protoc. 2009b; 4:1614–1622.
17. Izrael M, Zhang P, Kaufman R, Shinder V, Ella R, Amit M, Itskovitz-Eldor J, Chebath J, Revel M. (2007) Human oligodendrocytes derived from embryonic stem cells: Effect of noggin on phenotypic differentiation in vitro and on myelination in vivo. Mol Cell Neurosci. 2007a;34:310–323.
18. Keirstead HS, Nistor G, Bernal G, Totoiu M, Cloutier F, Sharp K, Steward O. (2005) Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci. 2005; 25:4694–4705
19. Sim FJ, McClain CR, Schanz SJ, Protack TL, Windrem MS, Goldman SA. (2011) CD140a identifies a population of highly myelinogenic, migration-competent and efficiently engrafting human oligodendrocyte progenitor cells. Nat Biotechnol. 2011; 29:934–941.

20. Windrem MS, Schanz SJ, Guo M, Tian GF, Washco V, Stanwood N, Rasband M, Roy NS, Nedergaard M, Havton LA, et al. (2008) Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. Cell Stem Cell. 2008;2:553–565.
21. Wang S, Bates J, Li X, Schanz S, Chandler-Militello D, Levine C, Maherali N, Hochedlinger K, Windrem M, Goldman SA. (2013) Human iPSC-derived oligodendrocyte precursor cells can myelinate and rescue a mouse model of congenital hypomyelination. Cell Stem Cell 2013; 12(2): 252-264.
22. Douvaras P, Wang J, Zimmer M, Hanchuk S, O'Bara MA, Sadiq S, Sim FJ, Goldman J, Fossati V. (2014) Efficient generation of myelinating oligodendrocytes from primary progressive multiple sclerosis patients by induced pluripotent stem cells. Stem Cell Rep 2014; 3(2): 250-259.
23. Yang, N., Zuchero, J.B., Ahlenius, H., Marro, S., Ng, Y.H., Vierbuchen, T., Hawkins, J.S., Geissler, R., Barres, B.A., and Wernig, M. (2013). Generation of oligodendroglial cells by direct lineage conversion. Nat Biotechnol 31, 434–439.
24. Windrem MS, Roy NS, Wang J, Nunes M, Benraiss A, Goodman R, McKhann GM, Goldman SA. (2002) Progenitor cells derived from the adult human subcortical white matter disperse and differentiate as oligodendrocytes within demyelinated lesions of the rat brain. J Neurosci Res. 2002;69:966–975.
25. Nave KA, Werner HB. (2014) Myelination of the nervous system: Mechanisms and functions. Annu Rev Cell Dev Biol 2014; 30: 503-533.
26. Sugarman J. (2008) Human stem cell ethics: beyond the embryo. Cell Stem Cell 2008; 2(6), 529-533.
27. Saha K, Jaenisch R. (2009) Technical challenges in using human induced pluripotent stem cells to model disease. Cell Stem Cell 2009; 5(6): 584-595.
28. Ehrlich M, Mozafari S, Glatza M, Starost L, Velychko S, Hallmann AL, Cui QL, Schambach A, Kim KP, Bachelin C, Marteyn A, Hargus G, Johnson RM, Antel J, Sterneckert J, Zaehres H, Schöler HR, Baron-Van Evercooren A, Kuhlmann T. (2017) Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors.
29. Tomita M, Mori T, Maruyama K, Zahir T, Ward M, Umezawa A, Young MJ. (2006) A comparison of neural differentiation and retinal transplantation with bone marrow-derived cells and retinal progenitor cells. Stem Cells 2006; 24(10): 2270-2278.
30. Chen Y, Teng FYH, Tang BL. (2006) Coaxing bone marrow stromal mesenchymal stem cells towards neuronal differentiation: progress and uncertainties. Cell Mol Life Sci 2006; 63(14): 1649-1657.
31. Shea GK, Tsui AYP, Chan YS, Shum DKY. (2010) Bone marrow-derived Schwann cells achieve fate commitment – a prerequisite for remyelination therapy. Exp Neurol 2010; 224(2): 448-458.
32. Mung KL, Tsui AYP, Tai EW, Chan YS, Shum DKY, Shea GK. (2016) Rapid and efficient generation of neural progenitors from adult bone marrow stromal cells by hypoxic preconditioning. Stem Cell Res Ther 2016; 7: 146.
33. Tsui AYP (2013) Derivation of oligodendrocyte precursor cells from adult bone marrow stromal cells.
34. Polito A, Reynolds R. (2005) NG2-expressing cells as oligodendrocyte progenitors in the normal and demyelinated adult central nervous system. J Anat. 2005 Dec; 207(6):707-16.
35. Ampofo E, Schmitt BM, Menger MD and Matthias W. (2017) Laschke The regulatory mechanisms of NG2/CSPG4 expression. Cellular & Molecular Biology LettersAn International Journal201722:4 https://doi.org/10.1186/s11658-017-0035-3
36. Tillet E, Gential B, Garrone R, Stallcup WB. (1997) NG2 proteoglycan mediates beta1 integrin-independent cell adhesion and spreading on collagen VI. J Cell Biochem. 2002;86(4):726-36.
37. Goretzki L, Burg MA, Grako KA, Stallcup WB. (1999) High-affinity binding of basic fibroblast growth factor and platelet-derived growth factor-AA to the core protein of the NG2 proteoglycan. J Biol Chem. 1999 Jun 11;274 (24):16831-7.
38. Qi Y, Cai J, Wu Y, Wu R, Lee J, Fu H, Rao M, Sussel L, Rubenstein J, Qiu M. (2001) Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor. 2001;128:2723–2733.
39. Zhou Q, Wang S, Anderson DJ. (2000) Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. 2000;25:331–343
40. Y Liang et al. (2013) The propensity for tumorigenesis in human induced pluripotent stem cells is related with genomic instability.
41. Kuo E, Park DK, Tzvetanova ID, Leiton CV, Cho BS, Colognato H. (2010) Tyrosine phosphatases Shp1 and Shp2 have unique and opposing roles in oligodendrocyte development. J Neurochem. 2010 Apr;113(1):200-12. doi: 10.1111/j.1471-4159.2010.06596.x. Epub 2010 Feb 2.

42. Makinodan M, Rosen KM, Ito S, Corfas G. (2012) A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science. 2012 Sep 14;337(6100):1357-60. doi: 10.1126/science.1220845.