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Mesenchymal Stem Cells for Trinucleotide Repeat Disorders

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Keywords

human mesenchymal stem cells; neurite outgrowth; neurodegenerative diseases; hypoxia; tissue repair; Huntington's disease; clinical trials; BDNF

1. INTRODUCTION

1a. Overview

There is interest in the use of Mesenchymal stem cells/marrow stromal cells (MSCs) to treat neurodegenerative trinucleotide repeat disorders, in particular those that are fatal and difficult to treat, such as Huntington's disease (HD). MSCs present a promising tool for cell therapy, and are currently being tested in FDA-approved Phase I to III clinical trials for many disorders. They have been extensively tested and proven effective in pre-clinical studies for neurodegenerative disorders. Studies have examined the potential benefits from the innate trophic support from MSC or from augmented growth factor support, such as delivering brain-derived neurotrophic factor (BDNF) or glial-derived neurotrophic factor (GDNF) into the brain to support injured neurons, using genetically engineered MSC as the delivery vehicles. Proposed regenerative approaches to neurological diseases using MSC include cell therapies in which cells are delivered via intracerebral or intrathecal injection. Upon transplantation, MSC in the brain promote endogenous neuronal growth, encourage synaptic connection from damaged neurons, decrease apoptosis, reduce levels of free radicals and regulate inflammation, primarily through paracrine actions. MSC transplanted into the brain have been shown to promote functional recovery by producing trophic factors that induce survival and regeneration of host neurons. Clinical trials for MSC injection into the central nervous system to treat Amyotrophic Lateral Sclerosis (ALS), traumatic brain injury and stroke are currently ongoing. The current protocols are provided in support of applying MSC-based cellular therapies to the treatment of trinucleotide repeat disorders.

1b. Mesenchymal stem cells for trinucleotide repeat disorders

There is currently much interest in the use of MSC to treat neurodegenerative diseases, in particular those like Huntington's disease that are fatal and difficult to treat, through providing neurotrophic factors to encourage repair and potentially new growth of neurons from neural progenitor cells. Proposed regenerative approaches include delivery via

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intracerebral or intrathecal injection or even infusion via an intranasal route¹. Therapies will capitalize upon the innate trophic support from MSC or on augmented growth factor support, such as delivering brain-derived neurotrophic factor (BDNF) or glial-derived neurotrophic factor (GDNF) into the brain to support injured neurons, using genetically engineered MSC as the delivery vehicles.

Upon transplantation, MSC in the brain promote endogenous neuronal growth, decrease apoptosis, and regulate inflammation, primarily through the use of secreted factors. MSC can mediate modification of the damaged tissue microenvironment to enhance endogenous neural regeneration and protection. MSC transplanted at sites of nerve injury have been shown to promote functional recovery by producing trophic factors that induce survival and regeneration of host neurons². Transplantation of human bone marrow stem cells into the brain of immunodeficient mice markedly increased the proliferation of endogenous neural stem cells³. In an experimental allergic encephalomyelitis model of multiple sclerosis, rodents that received an intraventricular infusion of mesenchymal stem cells were found to have almost twice the number of axons as control animals.⁴ Although candidate molecules are under investigation, further detailed studies are needed to carefully define the factors responsible for the MSC-mediated induction of proliferation in resident neural stem cells, in order to best capitalize upon this type of therapy for the repair of neurodegenerative diseases.

1c. Neural growth factor delivery by MSCs

An interesting development candidate on which we and others are working for consideration of treatment of the trinucleotide repeat disorder Huntington's disease (HD) is allogeneic human MSC engineered to secrete BDNF as a neurotrophic strategy to enhance neural survival and regeneration. BDNF levels are very low in mice and humans with HD. In rodent models, BDNF has been shown to ameliorate symptoms and to extend survival, and therefore BDNF therapy is a leading candidate for use in treating HD. Striatal neurons depend on BDNF levels for function and survival.⁵ In the later stages of HD, available BDNF levels plummet since the mutant protein prevents production at the RNA level.^{6, 7} This reduction of BDNF levels affects the onset and severity of the disease in HD mice.⁵ Up-regulation of BDNF in the brains of transgenic rodent models of HD has shown amelioration of the disease phenotype (reviewed by Zuccato et al⁸). Due to its pro-survival effects in striatal neuropathology, BDNF is a leading candidate for neuroprotective therapies in HD (reviewed by Alberch⁹).

In transgenic mouse models there is a significant reduction in behavioral phenotype following administration of MSC/BDNF.¹⁰ The Dunbar laboratory has shown that MSCs, especially those engineered to over-express BDNF, have significant ameliorative effects on disease progression in a transgenic mouse model of HD.¹⁰ These studies have demonstrated that MSCs engineered to produce BDNF and implanted into the striata significantly increased time to fall in rotorod testing, demonstrating an increased coordination and reduction in movement disorders in treated mice, over the 13 months of the study. The MSC/BDNF also significantly reduced limb clasping, a hallmark behavioral defect in HD mice, over the same time period.¹⁰

A major impediment for the clinical use of neurotrophic factors is their inability to cross the blood-brain barrier in therapeutic amounts. In the quest to begin translating promising studies such as those published by Dey et al¹⁰ to the clinic, we have shown biosafety of intracranial administration of MSCs in long term rodent and non-human primate models (MS in preparation, 2011). Our group uses human MSC as safe and long-lived delivery factories for cytokines and growth factors. We are currently evaluating the safety and

efficacy of MSC engineered to secrete BDNF in peripheral tissues and in the brain to enhance neurorestorative capacity in HD.

The use of MSC to deliver factors, both through their own innate responses and through engineering, has benefits over direct protein administration, because transplanted MSCs can provide sustained and long-term delivery of factors at supraphysiological levels, as we and others have shown over the past two decades.^{11–17} Using immune deficient mouse models, we have recovered human MSC from numerous organs, including the brain, at timepoints from 1–18 months post-transplantation, with continued expression of the gene product.^{11, 12, 14, 16, 18–20} We also performed a decade-long biosafety study to demonstrate that genetically engineered human MSC are safe and do not cause adverse events in vivo.²¹ Efforts from our laboratory and others are currently evaluating the effects of BDNF expression from human MSC implanted into the striata of immunocompromised HD mice. Data from our lab and others provide support for the potential for MSCs to safely deliver augmented neurotrophic support in the central nervous system.

The potential benefit to risk ratio and in particular safety are always the foremost considerations by the Food and Drug Administration. This review has covered the potential benefits of MSC-based therapies for the treatment of neurodegenerative diseases. We and many others have documented biosafety of MSC therapies, which are now in phase III trials for some indications, and further safety and efficacy data are being collected by numerous groups. Extending MSC-based therapies to neurodegenerative diseases, in particular those for which there are currently no effective treatments, such as Huntington's disease, would have a high potential benefit to risk ratio. The need for safe and effective cellular therapies to treat Huntington's disease is great, since current therapies only target symptoms and there are currently no drugs or other treatments that effectively delay the relentless loss of striatal volume in affected patients.

2. METHODS

2A. Materials

Reagents

- Fetal Bovine Serum (heat inactivated @ 56° C for 30 mins.) Sscreened and certified lot from FDA approved vendor (BSE, virus, bacteria, and endotoxin free)
- Dulbecco's Modified Eagle Media (DMEM), store at 2–8° C, protect from light
- Dulbecco's Phosphate Buffered Saline (without calcium or magnesium) (DPBS), store @ 15–30° C, Sterile for cell culture
- Recombinant Trypsin (animal component free)
- Trypan Blue, 0.5% in 100ml saline. Store at room temperature in small aliquot.
- 100X (200mM) L-Glutamine or GlutaMAX store at 2–8° C
- Ficoll-Paque at room temperature
- 70 % Ethanol
- 10% Bleach

Supplies and Equipment

- 15ml sterile conical centrifuge tubes
- 50ml sterile conical centrifuge tubes

- Serological pipettes, sterile, individually wrapped: 1ml, 2ml, 5ml, 10ml, 25ml
- 2ml cryovials
- 75 cm² (T 75) Tissue culture flasks with filter caps
- 225 cm² (T 225) Tissue culture flasks with filter caps
- 0.22 µm Bottle-top filter flasks, 500ml
- 1ml microcentrifuge tubes
- Hemacytometer
- 20ul Pipettor
- Pipette tips for 20ul pipettor
- Inverted Microscope
- Centrifuge with GS-6R Rotor
- 37° C, 5% CO₂ Incubator

2B. Media

Dexter's Original Medium for Stromal cells/MSC (DOM)—350 ml Iscove's Modified Dulbecco's Medium (IMDM)

75 ml heat- inactivated (HI) horse serum*

75 ml HI Fetal calf serum*

5 ml L-glutamine (200 mM stock)

2.5 ml Pen/Strep (stock = 10,000 U/ml penicillin and 10,000 ug/ml streptomycin)

500 ul 2-ME (10⁻¹ M stock)

500 ul hydrocortisone (10⁻³ M stock)

Dulbecco's Modified Eagles medium with 10% fetal calf serum and high glucose (D10HG)—450 ml Dulbecco's Modified Eagles medium with high glucose

50 ml heat- inactivated (HI) Fetal calf serum*

5 ml L-glutamine (200 mM stock)

2.5 ml Pen/Strep (stock = 10,000 U/ml penicillin and 10,000 ug/ml streptomycin)

2C. Obtaining And Plating Human Bone Marrow-Derived Msc

2C.1. Marrow Filtration Screens—The screens used to filter marrow during harvest are the richest source of mesenchymal stem cells, as we have described^{11, 18}. Many small bony spicules packed with stroma (as well as hematopoietic stem and progenitor cells) will get lodged in the screen, and can be easily removed by flushing. The cells from one harvest screen, from a normal donor, should be split between 4 × T-75 vent-cap flasks in 15 mls of stromal medium (section 3) per flask. The cells will then be expanded, as described below (section 3). Filter vent-cap flasks are used for long-term culture, despite their greater cost as compared to standard screw-caps, because the risks for air-borne fungal spore contamination

are high for cultures which can be grown for 1–2 months. The tightly closed, gas permeable filter vent caps reduce the risk of cross-contamination between flasks.

Many transplant programs are now using G-CSF mobilized peripheral blood as a stem cell source in lieu of bone marrow. Unfortunately, MSC are not found in appreciable levels in G-CSF mobilized blood. The use of newer mobilization agents, such as the CXCR4 antagonist AMD3100 (Mozobil)²², might provide better MSC mobilization than the standard regimens. Until then, researchers at those institutions can purchase whole marrow, or even purified and cryopreserved MSC, from commercial sources, or might consider beginning a normal donor program for marrow donation to be used for research. In lieu of flushing harvest screens, when whole marrow is available from these sources, the investigator should proceed as directed below.

2C.2. Bone marrow aspirates—If the richest source of MSC- bone marrow harvest screens- is not available, whole aspirated bone marrow can be used as a source of mesenchymal stem cells. Spicules from unseparated BM will be present in the aspirate, and can be collected by gravity sedimentation. The liquid marrow is then removed to another tube for additional processing. It is advisable to perform at least one red cell lysis and wash before plating, if using this method. The washing technique is described. The spicules from a 10 ml aspirate should then be plated in T-75 vent-cap flasks in 15 mls of stromal medium (Dexter's original medium = DOM, section 2), which is the richest medium and rapidly forces contaminating hematopoietic cells into erythroid and monocytic differentiation. A simpler medium can also be used, as described in section 2. If the aspirate providing the spicules is larger, the number of flasks should be scaled up accordingly. If the BM sample must be ficolled for other studies, and the MSC investigator is salvaging spicules, use the techniques described in the section below.

2C.3. Spicules from RBC pellet of ficoll layer in marrow aspirate processing—The ficolled “buffy coat” is not a rich source of MSC, but is what many investigators have to work with. The MSC are far more rare in the aspirated marrow fraction than in spicules from the harvest screens. They are even rarer if the sample is first ficolled (approximately 1×10^6 /ml in the “buffy coat” or mononuclear fraction. If whole marrow aspirates are to be used, an optimal strategy is to use the mononuclear fraction, and also to recover the spicules from the bottom of the 50 ml ficoll tubes, since the small pieces of bone will fall through the density gradient.

For ficolling, first mix an equal volume of whole marrow and $1 \times$ phosphate buffered saline and then gently layer 25 mls over an equal volume of ficoll-paque in a 50 ml conical tube. Centrifuge the cells at 2000 rpm for 15 minutes. Once approximately 15 mls of the serum layer has been removed and discarded, buffy coat cells can be collected in another 10–15 mls, washed, and plated as described below. Then there are 5–10 mls of packed red blood cells and bony spicules left in the bottom of the tube. PBS should then be added up to a volume of 50 mls. Allow the tubes to settle upright for 3 minutes, without centrifugation. Remove 40 mls PBS and RBC, then repeat the washing step: add PBS, let the spicules settle out, and remove RBC/PBS down to the final 10 mls. At this point, the red blood cells are sufficiently diluted out to allow plating of the spicules. Add another 40 mls PBS, centrifuge, remove fluid down to the last ml, and plate as described below.

2D. Expanding human MSC

2D.1. Plating MSC (initial seeding)—If total (RBC lysed) or ficolled marrow is used, to expand mesenchymal stem cells, cells are plated at a concentration of 5×10^6 mononuclear

cells per ml in 75 cm² flasks, in 15–20 mls total volume. Optimally, plate the cells in Dexter's original medium (DOM), which is prepared as shown in the Materials section.

Preparation of spicules for plating is described above, in section 2. Spicules obtained from one harvest screen should be divided between four T-75 flasks containing 15 mls of DOM or D10HG each. Spicules obtained from the RBC pellet resulting from 10–15 mls of ficolled marrow can be plated in one T-75 flask, in 15 mls of medium. Cells are then expanded and transduced as described below.

2D.2. MSC expansion—Following the initial seeding, described in section 3, the MSC are allowed to adhere to the flasks overnight. The next morning, non-adherent cells can be gently flushed from the flasks and replated in a second flask, in the same medium. The initial flask is refed fresh medium. DOM is the richest medium for MSC expansion without differentiation, and the horse serum rapidly forces contaminating hematopoietic cells into erythroid and monocytic differentiation, so that hematopoietic stem cells will not contaminate the stromal layer after 3 passages. A minimal medium, D10HG, which contains only fetal calf serum, can also be used, but hematopoietic stem cells will survive happily on the stromal layer in this medium. MSC have not yet been expanded efficiently without the use of fetal calf serum, and it is imperative to screen the serum for optimal MSC growth without differentiation, when using either medium.

MSC colonies begin to develop as the cells expand out of the marrow spicules (Figure 1). There are many other cells in the culture at this point. However, as the MSC grow and expand, the other cells differentiate out and /or can be removed. In the fetal calf/horse serum mixture (DOM), the developing erythroid cells become non-adherent and are easily flushed away as the MSC layer develops and is expanded. Alternately, a depletion step can be done at passage 2–3, to remove Glycophorin A+ cells. Early monocytes can also be removed by flushing, but mature macrophages are tightly adherent to the tissue culture flask and cannot be removed, even with trypsin. Therefore, the MSC can be taken to a new flask, while leaving the macrophages behind to be discarded.

Alternately, the cells can be collected using an EDTA-based cell dissociation buffer (rather than trypsin, which cleaves away many cell surface proteins), and then a FACS-based depletion can be done to remove CD45+ cells, including CD14+ monocyte/macrophages, from the developing MSC monolayer.

If not using FACS to fractionate MSC subpopulations based on cell surface proteins, it is best to use trypsinization (trypsin-EDTA), to dissociate sub-confluent monolayers of primary mesenchymal stem cells from the flask. For general maintenance and expansion, the cells should be “split” no more than 1:10 when they reach 70–80% confluency (Figure 2).

MSCs are not usually transduced or used for other studies until passage #3 or 4. At this point (Figure 2), most hematopoietic cells will have been eliminated, except for mature macrophages which typically will comprise less than 1% of the culture.

The cells should be used for transduction, experiments, or transplantation between passage 3–6 for optimal results. By passage ten, they can begin to differentiate and become senescent. Since the primary MSC cultures are not immortalized, they do have a finite lifespan, and by later passage, they begin to slow down in growth and to become larger and more differentiated.

3. DETAILED METHODS

3A. MSC isolation and expansion

1. Plate ficolled (buffy coat) marrow in D10HG (Table 1B) at a concentration of 5×10^6 cells per ml, in 75 cm² flasks. Put the flasks into the incubator, at 37 degrees Celsius, with 5% CO₂. Alternately, plate spicules from harvest screens or from the pellet of the mononuclear fraction, as described in section 2.
2. 12–24 hours after plating, remove the nonadherent fraction, which contains primarily hematopoietic cells. Flush the adherent layer with PBS to remove as many hematopoietic cells as possible, and add the flushings to the collected nonadherent cell fraction. The nonadherent hematopoietic cells can be cryopreserved for later use if desired: the spicules are a rich source of hematopoietic, as well as mesenchymal stem cells. Refeed each adherent layer 15 mls of DOM (reagents section), for expansion of mesenchymal stem cells.
3. When the mesenchymal stem cells reach 70–80% confluency (70–80% of the plastic flask surface covered, figure 2), split them by trypsinization. Remove the medium from the flask, and discard. Rinse the flask with 15 mls 1xPBS and discard. Add 2 mls trypsin/EDTA solution, and tip the flask back and forth gently, to completely coat the adherent layer. Remove excess trypsin, leaving approximately 500 uls in the flask.
4. Incubate for 10–15 minutes at 37°C. Pick up the flask and turn it to coat all surfaces every 3–4 minutes during the trypsinization process. Mesenchymal stem cells will be readily trypsinized if they are in subconfluent monolayers. If they are allowed to become confluent, they form a 3-dimensional tissue with excessive buildup of collagen and other extracellular matrix molecules between the layers of cells. The collagen layers are harder for the trypsin to digest than the adhesion foci with which the cells adhere to the plastic flask. The result is a useless sheet, or large chunks, of cells which will quickly deplete nutrients from the medium, and will necrose in the center. If the mesenchymal stem cells were healthy and subconfluent prior to trypsinization, a single cell suspension will result.
5. To neutralize the trypsin, resuspend the cells from each flask in 45 mls DOM or other serum-containing medium. Transfer 15 mls each to three new flasks. Discard the original flask, which will contain firmly adherent macrophages.
6. Grow the mesenchymal stem cells until they reach 80% confluency, once again. Repeat steps 4–6. Grow up and repeat, to generate a “passage 3” layer. The monolayer should be a smooth, homogeneous mesenchymal stem cell population. The cells will be rather “chunky”, not spindle shaped as will happen in straight FCS without the addition of horse serum. If the monolayer has numerous phase-bright macrophage contaminants, perform a CD45+ cell depletion using magnetic beads or FACS, or repeat steps 4–6. The resultant monolayer will be completely CD45-negative, due to the loss of hematopoietic cells. No phase-bright cells will be seen adhering to the MSC monolayer (Figure 2).
7. When the mesenchymal stem monolayer has the correct appearance (Figure 2), collect the cells from one 80% confluent flask containing passage 3–6 mesenchymal stem cells by trypsinization. Re-plate each flask so that it is split 1:6 for viral supernatant addition, as described in the sections below. It is imperative that the MSC will not become confluent during the transduction procedure, but will remain in rapid growth. Contact inhibition in adherent cells, such as MSC, increases intracellular levels of the CDK inhibitor p27, which halts cell cycle^{23–25}.

Target cells must traverse cell cycle to allow integration of retroviral vectors, and must be at least metabolically active for effective lentiviral vector transduction and integration^{26, 27}.

4. MSC transduction using retroviral and lentiviral vectors

For retroviral transduction, add supernatant from MoMuLV- based retroviral vectors with protamine sulfate (final concentration = 4 ug/ml) four times, over a 48 hour period. Protamine sulfate is a polycationic compound which neutralizes the negatively charged retroviral particles and cell surfaces. Add it only once every 24 hours, or it will be toxic. This should result in 20–40% of the flask being transduced, due to the rapid division of the MSC. The cells must be subconfluent when each aliquot of supernatant is added. Confluent cells are contact inhibited and will not divide to allow retroviral vector integration.

VSV-G pseudotyped lentiviral vector supernatant can be added once or twice at an MOI of 10–100, without the need for protamine sulfate. Select the cells according to the selectable marker included in the chosen vector (if using G418 to select for the neo gene, the best concentration is 0.75 mg/ml active drug), or use as partially - transduced monolayers. Transduced MSC are excellent vehicles from which to secrete proteins, as we have described^{11, 16–18, 28}.

While the methodologies for transducing MSC are relatively simple, since in log phase the cells are rapidly dividing and incorporate vector very easily, in comparison to hematopoietic stem cells^{29–31}, several cautions do exist for transduction and for reliably assessing the success of the MSC transduction. Although lentiviral vectors can enter non-dividing cells, MSC monolayers should still be subconfluent prior to transduction, or the VSV-G envelope can cause cell fusion, resulting in multinucleate cells which appear overnight in the culture.

5. Notes

An important caution in interpretation of transduction is that MSC can and do “share” proteins with neighboring cells, through junction formation or other as-yet-unknown mechanisms. For this reason, we have described that MSC (marrow stromal cells) must be plated at subconfluency for selective agents such as G418 to work effectively^{11, 18}. This is also reflected in the fact that fluorescent markers such as eGFP can be shared between cells. Transduced cells dropped into a confluent plate of non-transduced MSC can cause a green “halo” to be seen in neighboring cells, although it is not as bright in intensity as seen in the cell that is expressing the transgene. For this reason, caution should be exercised when interpreting immunofluorescence or FACS data from partially transduced MSC cultures. If stringent parameters are set for the highly expressing cells, those that contain transgene product assimilated using the “bystander effect” will not be included. The propensity for MSC to share proteins is, however, a factor in making them excellent vehicles for delivering enzyme products or other transgenes to cells in a deficient animal or in an injured tissue.

References

1. Danielyan L, Schafer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, Klopfer T, et al. Intranasal delivery of cells to the brain. *European journal of cell biology*. 2009; 88(6):315–24. [PubMed: 19324456]
2. Crigler L, Robey RC, Asawachaicharn A, Gaupp D, Phinney DG. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neurogenesis. *Experimental neurology*. 2006; 198(1):54–64. [PubMed: 16336965]

3. Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. *Proc Natl Acad Sci U S A*. 2005; 102(50):18171–6. [PubMed: 16330757]
4. Kassir I, Grigoriadis N, Gowda-Kurkalli B, Mizrahi-Kol R, Ben-Hur T, Slavin S, et al. Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. *Archives of neurology*. 2008; 65(6):753–61. [PubMed: 18541795]
5. Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martin-Ibanez R, Munoz MT, et al. Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. *J Neurosci*. 2004; 24(35):7727–39. [PubMed: 15342740]
6. Her LS, Goldstein LS. Enhanced sensitivity of striatal neurons to axonal transport defects induced by mutant huntingtin. *J Neurosci*. 2008; 28(50):13662–72. [PubMed: 19074039]
7. Wu LL, Fan Y, Li S, Li XJ, Zhou XF. Huntingtin associated protein-1 interacts with proBDNF and mediates its transport and release. *J Biol Chem*. 2009
8. Zuccato C, Cattaneo E. Brain-derived neurotrophic factor in neurodegenerative diseases. *Nature reviews*. 2009; 5(6):311–22.
9. Alberch J, Perez-Navarro E, Canals JM. Neurotrophic factors in Huntington's disease. *Progress in brain research*. 2004; 146:195–229. [PubMed: 14699966]
10. Dey ND, Bombard MC, Roland BP, Davidson S, Lu M, Rossignol J, et al. Genetically-Engineered Mesenchymal Stem Cells Reduce Behavioral Deficits in the YAC 128 Mouse Model of Huntington's Disease. *Behavioural brain research*. 2010 in press.
11. Dao MA, Pepper KA, Nolta JA. Long-term cytokine production from engineered primary human stromal cells influences human hematopoiesis in an in vivo xenograft model. *Stem Cells*. 1997; 15(6):443–54. [PubMed: 9402657]
12. Meyerrose TE, Roberts M, Ohlemiller KK, Vogler CA, Wirthlin L, Nolta JA, et al. Lentiviral-transduced human mesenchymal stem cells persistently express therapeutic levels of enzyme in a xenotransplantation model of human disease. *Stem Cells*. 2008; 26(7):1713–22. [PubMed: 18436861]
13. Nolta, J. *Genetic Engineering of Mesenchymal Stem Cells*. Kluwer; 2006.
14. Nolta JA, Dao MA, Wells S, Smogorzewska EM, Kohn DB. Transduction of pluripotent human hematopoietic stem cells demonstrated by clonal analysis after engraftment in immune-deficient mice. *Proc Natl Acad Sci U S A*. 1996; 93(6):2414–9. [PubMed: 8637888]
15. Nolta JA, Kohn DB. Comparison of the effects of growth factors on retroviral vector-mediated gene transfer and the proliferative status of human hematopoietic progenitor cells. *Hum Gene Ther*. 1990; 1(3):257–68. [PubMed: 2081193]
16. Tsark E, Dao M, Wang X, Weinberg K, Nolta J. IL-7 enhances the responsiveness of human T cells that develop in the bone marrow of athymic mice. *J Immunol*. 2001; 166(1):170–81. [PubMed: 11123290]
17. Wang X, Ge S, McNamara G, Hao QL, Crooks GM, Nolta JA. Albumin expressing hepatocyte-like cells develop in the livers of immune-deficient mice transmitted with highly purified human hematopoietic stem cells. *Blood*. 2003; 101(10):4201–4208. [PubMed: 12560238]
18. Nolta JA, Hanley MB, Kohn DB. Sustained human hematopoiesis in immunodeficient mice by cotransplantation of marrow stroma expressing human interleukin-3: analysis of gene transduction of long-lived progenitors. *Blood*. 1994; 83(10):3041–51. [PubMed: 7514050]
19. Meyerrose T, De Ugarte D, Hofling A, Herrbrich PE, Cordonnier TD, Shultz LD, et al. In vivo distribution of human adipose-derived mesenchymal stem cells in novel xenotransplantation models. *Stem Cells*. 2007; 25(1):220–7. [PubMed: 16960135]
20. Meyerrose, T.; Rosova, I.; Dao, M.; Herrbrich, P.; Bauer, G.; Nolta, J. Establishment and transduction of primary human stromal/ mesenchymal stem cell monolayers. Vol. vol. Chapter 2. Kluwer Academic Publishers: Dordrecht; the Netherlands: 2006.
21. Bauer G, Dao MA, Case SS, Meyerrose T, Wirthlin L, Zhou P, et al. In vivo biosafety model to assess the risk of adverse events from retroviral and lentiviral vectors. *Mol Ther*. 2008; 16(7): 1308–15. [PubMed: 18461052]

22. Devine SM, Flomenberg N, Vesole DH, Liesveld J, Weisdorf D, Badel K, et al. Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. *J Clin Oncol*. 2004; 22(6):1095–102. [PubMed: 15020611]
23. Polyak K, Kato JY, Solomon MJ, Sherr CJ, Massague J, Roberts JM, et al. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to cell cycle arrest. *Genes Dev*. 1994; 8(1):9–22. [PubMed: 8288131]
24. Kato A, Takahashi H, Takahashi Y, Matsushime H. Contact inhibition-induced inactivation of the cyclin D-dependent kinase in rat fibroblast cell line, 3Y1. *Leukemia*. 1997; 11(Suppl 3):361–2. [PubMed: 9209390]
25. Fuse T, Tanikawa M, Nakanishi M, Ikeda K, Tada T, Inagaki H, et al. p27Kip1 expression by contact inhibition as a prognostic index of human glioma. *J Neurochem*. 2000; 74(4):1393–9. [PubMed: 10737594]
26. Miller DG, Adam MA, Miller AD. Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection [published erratum appears in *Mol Cell Biol* 1992 Jan; 12(1):433]. *Mol Cell Biol*. 1990; 10(8):4239–42. [PubMed: 2370865]
27. Sutton RE, Reitsma MJ, Uchida N, Brown PO. Transduction of human progenitor hematopoietic stem cells by human immunodeficiency virus type 1-based vectors is cell cycle dependent. *J Virol*. 1999; 73(5):3649–60. [PubMed: 10196257]
28. Dao MA, Nolte JA. Inclusion of IL-3 during retrovirally-mediated transduction on stromal support does not increase the extent of gene transfer into long-term engrafting human hematopoietic progenitors. *Cytokines Cell Mol Ther*. 1997; 3(2):81–9. [PubMed: 9287247]
29. Dao MA, Hwa J, Nolte JA. Molecular mechanism of transforming growth factor beta-mediated cell-cycle modulation in primary human CD34(+) progenitors. *Blood*. 2002; 99(2):499–506. [PubMed: 11781230]
30. Dao MA, Taylor N, Nolte JA. Reduction in levels of the cyclin-dependent kinase inhibitor p27(kip-1) coupled with transforming growth factor beta neutralization induces cell-cycle entry and increases retroviral transduction of primitive human hematopoietic cells. *Proc Natl Acad Sci U S A*. 1998; 95(22):13006–11. [PubMed: 9789031]
31. Dao M, Nolte J. Molecular control of cell cycle progression in primary human hematopoietic stem cells: methods to increase levels of retroviral-mediated transduction. *Leukemia*. 1999; 13(10):1473–80. [PubMed: 10516745]

Figure 1.
Human MSC beginning to grow out of a bony spicule obtained by gravity sedimentation from a normal donor bone marrow aspirate - day 3 after plating.

Figure 2. Expanded human MSC at 75 % confluency. This passage 3 culture was grown out of bone marrow spicules as described. The cells have a fairly uniform myofibroblastic appearance.