Concise Review: Mesoangioblast and Mesenchymal Stem Cell Therapy for Muscular Dystrophy: Progress, Challenges, and Future Directions

SUZANNE E. BERRY

Key Words. Mesoangioblast • Mesenchymal stem cells • Muscular dystrophy • Therapy • Stem cell therapy • Review

ABSTRACT

Mesenchymal stem cells (MSCs) and mesoangioblasts (MABs) are multipotent cells that differentiate into specialized cells of mesodermal origin, including skeletal muscle cells. Because of their potential to differentiate into the skeletal muscle lineage, these multipotent cells have been tested for their capacity to participate in regeneration of damaged skeletal muscle in animal models of muscular dystrophy. MSCs and MABs infiltrate dystrophic muscle from the circulation, engraft into host fibers, and bring with them proteins that replace the functions of those missing or truncated. The potential for systemic delivery of these cells increases the feasibility of stem cell therapy for the large numbers of affected skeletal muscles in patients with muscular dystrophy. The present review focused on the results of preclinical studies with MSCs and MABs in animal models of muscular dystrophy. The goals of the present report were to (a) summarize recent results, (b) compare the efficacy of MSCs and MABs derived from different tissues in restoration of protein expression and/or improvement in muscle function, and (c) discuss future directions for translating these discoveries to the clinic. In addition, although systemic delivery of MABs and MSCs is of great importance for reaching dystrophic muscles, the potential concerns related to this method of stem cell transplantation are discussed.

MUSCULAR DYSTROPHY AND THE RATIONALE FOR STEM CELL THERAPY FOR MUSCULAR DYSTROPHY

The muscular dystrophies are genetic disorders of the muscles characterized by progressive muscle degeneration resulting in muscle weakness. The present review focused on cell therapy for two of the most common forms of muscular dystrophy, Duchenne muscular dystrophy (DMD) and limb-girdle muscular dystrophy (LGMD). DMD is a progressive, fatal muscle-wasting disorder affecting 1 in 3,500 males born, resulting from mutations in the dystrophin gene [1]. LGMD is a descriptive term applied to muscular dystrophies with a common pattern of weakness in the scapular, pelvic girdle, and trunk muscles that result from a mutation in up to 31 different loci in the genome, including genes encoding calpain 3, dysferlin, α-, β-, δ-, or γ-sarcoglycan, desmin, caveolin 3, and many others [2–4]. The LGMD incidence varies by region, but some prevalence rates have been as high as 5–15 per 100,000 individuals [4]. Current treatments of LGMD and DMD address the symptoms rather than the cause, although many promising strategies are emerging to replace the mutant gene or to facilitate skipping of the mutant exons to allow production of a partially functional dystrophin protein [5–13]. Detailed summaries of emerging therapies for these disorders are given in recent comprehensive reviews [14–16], as the present review focuses on stem cell therapy for DMD and LGMD.

The rationale for using stem cells as vehicles for gene therapy in muscular dystrophy is based on the regenerative nature of skeletal muscle, involving the repair of damaged muscle fibers and generation of new myofibers from specialized endogenous stem cells known as satellite cells. This process can be exploited for stem cell therapy of dystrophic muscle by transplanting exogenous myogenic stem cells with a wild-type copy of the mutated gene into the dystrophic muscle. The cells will generate new myofibers or fuse with existing fibers in the muscle, restoring expression of the absent muscle protein. Initial efforts at stem cell therapy for dystrophic muscle used myoblasts, the progeny of satellite cells. Positive results in an animal model for DMD [17] led to clinical trials in patients with DMD. However, the trials did not result in improvements in muscle strength, and dystrophin expression was not restored in most patients [18, 19] or was detected only at low levels [20–23]. The lack of success was attributed to an immune reaction against the donor cells [21, 24–26], a low proliferative
capacity, and limited migration of myoblasts through the muscle tissue [27]. Thus, subsequent studies using myoblasts for cell therapy for dystrophic muscles have used immune suppression, larger numbers of cells, and injection of cells into many sites in each affected muscle ("high-density injection protocols") [28, 29] (reviewed in [30]). With these modifications, a phase I clinical trial administering myoblasts to patients with DMD yielded 3.5%–26% of all myofibers containing detectable wild-type dystrophin. The results lasted up to 18 months in at least 1 patient [29], although the expression varied among the patients [28]. However, wild-type dystrophin was limited primarily to the injection tracks in the muscle [28]. Because of the limited migration of myoblasts, numerous injections throughout each muscle, such as the "high-density injection" protocols reported by Skuk et al. [28, 29], are required for myoblast therapy to dystrophic muscle. Such protocols are not feasible for muscular dystrophies with large numbers of affected muscles. However, it might be feasible to use myoblast therapy to treat muscular dystrophies with fewer, small affected muscles, such as oculopharyngeal muscular dystrophy (OPMD) and facioscapulohumeral muscular dystrophy. In support of this possibility, a phase I/IIa trial of patients with OPMD was conducted [31]. They reported improvement in the quality of life for all patients (n = 12), a cell dose-dependent improvement in swallowing, no subsequent decline in swallowing after initial myotomy and cell transplantation, and no adverse events [31]. Subsequent efforts have also focused on identifying other stem cell populations for regenerating dystrophic muscle, primarily myogenic stem cells that participate directly in muscle repair, although a recent study also demonstrated the importance of other cells such as regulatory T cells that indirectly influence this process in damaged muscle [32]. Ideally, myogenic stem cells for directly regenerating muscle would be easy to isolate and expand in culture, infiltrate muscles from the circulation, migrate through the tissue, and engraft and regenerate muscle, restoring the missing protein.

**Mesenchymal Stem Cells for Repair of Dystrophic Skeletal Muscle**

Mesenchymal stem cells (MSCs) are self-renewing, multipotent cells with a perivascular position in bone marrow, a distinct marker profile, and the capacity to differentiate into bone, cartilage, adipocytes, fibroblasts, and adventitial reticular cells in vivo [reviewed in 33]. Cells have been isolated from other connective tissues that express a similar surface marker profile and can be induced to express markers of adipocytes, chondrocytes, and osteocytes in vitro, which are also referred to as MSCs. This is in agreement with the criteria proposed by the Society for Cell Therapy for defining MSCs [34]. Debate has been ongoing regarding the use of the term MSCs, because many cells referred to as MSCs have not been shown to self-renew or have not been studied stringently in vivo to establish their capacity to generate a complete heterotopic bone or to reconstitute in vivo the tissue from which they were derived [33, 35–37]. The present report reviews preclinical studies of cell therapy in animal models of muscular dystrophy in which the investigators have identified the therapeutic cell population as MSCs, regardless of the tissue of origin or whether the cells demonstrated the capacity to self-renew or reconstitute their tissue of origin.

MSCs derived from umbilical cord (ucMSCs) can be induced to express myosin and desmin and fuse into myotubes in vitro. Human ucMSCs were transplanted systemically into Swiss Jim Lambert (SJL/J) mice [38, 39] (a murine model for LGMD containing a mutation in the dysferlin gene), the laminin α2 dystrophia muscularis 2 Jackson (Lama2dy-2J) mice [40] (a murine model for congenital muscular dystrophy), and Golden Retriever muscular dystrophy (GRMD) dogs [39, 41] (a canine model for DMD with a naturally occurring mutation in the gene encoding dystrophin). In addition, canine ucMSCs were isolated and transplanted into dog leukocyte antigen-histocompatible GRMD littermates [39]. In all three studies, ucMSCs infiltrated dystrophic muscle from the circulation, detected by polymerase chain reaction for human DNA, immunohistochemistry for human antigens, or the presence of Y chromosome-specific sequences in recipient females. Functional benefit was observed in both SJL/J and Lama2dy-2J mice. Despite this, neither human nor canine ucMSCs expressed muscle proteins [38–41], indicating that ucMSCs lack myogenic potential in vivo in animal models for muscular dystrophy. However, forced expression of MyoD in ucMSCs enhanced skeletal muscle differentiation in vitro [42] and might also be useful to stimulate their myogenic differentiation in vivo, because this approach has been used to improve skeletal muscle differentiation of MSCs from synovium and orthopedic surgery remnants in vitro and in vivo [43, 44].

In contrast to the results with ucMSCs, evidence has shown that murine, canine, and human bone marrow MSCs (BM-MSCs) can differentiate into muscle cells in dystrophic muscle. Dystrophin was detected in 6%–11% of muscle fibers in the mdx mouse, a murine model for DMD [45–47] after transplantation of murine BM-MSCs transduced with a human microdystrophin gene [45, 47] or human BM-MSCs [46]. However, only one of these studies demonstrated conclusively that the dystrophin-positive myofibers detected originated from the donor cells by examining the percentage of dystrophin-positive fibers that expressed human microdystrophin [46]. This distinction is relevant, because the low number of dystrophin-positive myofibers detected might have been naturally arising “revertant” fibers, which are detected at low levels in dystrophic muscle, rather than fibers generated by donor MSCs. In another study, clusters of small-caliber de novo myofibers were observed in the skeletal muscle of Beagle-based canine X-linked muscular dystrophy dogs with a mutation in the dystrophin gene after transplantation of canine MSCs transduced with luciferase [48]. The small luciferase-positive fibers expressed developmental myosin heavy chain but rarely expressed dystrophin by 12 weeks after cell transplantation [48]. The numbers of luciferase-positive myofibers with or without dystrophin were not provided. Murine BM-MSCs transduced with human microdystrophin also resulted in satellite cells that expressed human microdystrophin in the muscles of recipient mdx mice [45]. This finding is similar to that from a previous report in which human and rat green fluorescent protein (GFP)-positive BM-MSCs generated GFP-positive Pax7+ satellite cells in murine skeletal muscle that subsequently generated GFP-positive myofibers after cardiotoxin damage [49]. BM-MSCs, therefore, could have the potential to generate new, dystrophin-expressing myofibers and contribute to the satellite cell niche in dystrophic muscle; however, they would do so at low rates, which is likely the reason for the lack of functional improvement [46, 47].

MSCs derived from human adipose tissue will infiltrate damaged and dystrophic muscles from the circulation and engraft in higher numbers than BM-MSCs. MSCs isolated from adipose

©AlphaMed Press 2015
tissue of young human donors without DMD restored dystrophin expression in more than 50% of the fibers in mdx mice after intramuscular transplantation, and the myofibers persisted for at least 6 months, without immune cell infiltration [50]. The investigators detected human nuclei in approximately 70% of dystrophin-positive myofibers, using a probe specific to human centromeres, and reported dystrophin expression in approximately 1% of uninjected contralateral myofibers, likely representing revertant fibers [50]. MSCs isolated from adult human adipose tissue also restored dystrophin in mdx muscle, although cardiotoxin was used to enhance the muscle damage [51]. The number of dystrophin-positive myofibers was not reported; however, human β2-microglobulin was present in most, supporting the assertion that the donor cells were responsible for the dystrophin expression detected [51]. MSCs from adult adipose tissue restored expression of the dysferlin gene to 14% expression detected [51].

MSCs from adult adipose tissue will not only generate new muscle fibers, but also give rise to dystrophin-positive myofibers in the recipient [52]. Dystrophin protein was also detected in another study in 11% of myofibers in the GRMD dog model for LGMD [52]. Because of poor immunostaining for dysferlin, the investigators also examined expression of human dystrophin. They detected it in the forelimb and hind limb muscles by Western blotting and in approximately 50% of myofibers by immunofluorescence using an antibody specific to human dystrophin, indicating that the donor cells differentiated into myofibers in the dystrophic muscle of the recipient [52]. Dystrophin protein was also detected in another study in 11%–19% of myofibers in the GRMD dog model for DMD by immunofluorescent microscopy and Western blotting, using antibodies specific for human dystrophin [53]. Adult human adipose-derived MSCs also gave rise to human β2-microglobulin-positive cells in mdx muscle. These cells expressed Pax7, a marker of satellite cells required for regeneration of damaged muscle fibers, and participated in regeneration after additional cardiotoxin-induced damage [51]. Collectively, these reports have demonstrated that adipose MSCs might be good candidates for cell therapy for muscular dystrophy, because they are easy to isolate, will "home" to dystrophic muscle and engraft, and will not only generate new muscle fibers, but also give rise to satellite cells.

**Mesoangioblasts for Regeneration of Dystrophic Muscle**

Mesoangioblasts (MABs) are vessel-derived stem cells originally discovered and isolated from the dorsal aorta of quail and murine embryos [54]. They express markers consistent with both an endothelial and a perithelial origin, self-renew, and contribute to the smooth muscle layer of small and large arteries and pericytes in the capillaries of skeletal muscle after transplantation into chick embryos. Unexpectedly, they were also found to contribute to skeletal and cardiac muscle, bone, hyaline cartilage, and blood [54]. Because of their potential to differentiate into skeletal muscle in vitro and in vivo, MABs were tested for their capacity to regenerate damaged skeletal muscle and restore protein expression in a variety of animal models for muscular dystrophy. Clonally derived populations of MABs from embryonic dorsal aorta, including murine D16 MABs [54], homed to and engrafted in dystrophic muscle after arterial delivery in α-sarcoglycan (α-SG)-null mice, a model for LGMD. D16 MABs restored α-SG expression in greater than 20% of the muscle fibers in 4 different muscles downstream from the site of injection by 24 weeks after 3 consecutive cell injections [55]. This was accompanied by a decrease in damaged muscle fibers and fibrosis and the ability to run for 3 minutes on a rotarod compared with the untreated mice, which were unable to run for longer than 1 minute. Transplantation of allogeneic, H2-mismatched mesoangioblasts, combined with immunosuppression, also restored α-SG expression to the muscle fibers in α-SG-null mice to levels comparable to syngeneic D16 MABs [56]. Subsequent studies demonstrated that D16 mesoangioblasts expressed the receptor for advanced glycation end products on their surface and that it was responsible, in part, for their homing to damaged muscles [57], although homing and engraftment could be increased significantly by pretreating D16 MABs with cytokines or nitric oxide donors or overexpressing the α4 integrin [58, 59]. Using a combination of these approaches, Galvez et al. detected α-SG expression in more than 90% of muscle fibers in the tibialis anterior muscle of α-SG-null mice 4 months after a single arterial injection of D16 cells, resulting in protein restoration to approximately 60% of wild-type levels [58].

Mesoangioblasts were derived independently by our research group from the aorta (aorta-derived mesoangioblasts [ADMs]) of juvenile C57Bl/6 mice [60]. ADMs engrafted into muscle of dystrophin- and utrophin-deficient double knockout (mdx/utrn−/−) mice, a phenotypic model for DMD [61, 62], formed de novo muscle fibers and restored dystrophin expression to approximately 50% of levels in the gastrocnemius from age-matched wild-type mice [60]. This correlated with a 50-fold reduction in damaged fibers compared with the contralateral, sham-injected gastrocnemius. ADMs also migrated into the adjacent tibialis anterior and contralateral gastrocnemius muscle, demonstrating their potential to home to damaged muscles through the circulation, similar to observations in mdx mice [63]. ADMs gave rise to satellite cells, smooth muscle cells in vessels, and, surprisingly, Schwann cells in peripheral nerve bundles [60], correlating with increased numbers of peripheral nerve bundles in the ADM-transplanted gastrocnemius [64] and suggesting that the donor cells had the capacity to repair multiple tissues in severely damaged muscle. Embryonic MABs and ADMs display many of the characteristics ideal for treatment of dystrophic muscle, with the exception of ease of isolation. The isolation of cells from the human aorta would be challenging for clinical translation. The collection of cells from aorta punches generated during by-pass surgery is a potential source for isolation of mesoangioblasts from the adult human aorta; however, subsequent work has demonstrated that a similar population of cells can be isolated from skeletal muscle, which could be obtained from muscle biopsies of patients with DMD or HLA-matched donors for therapy.

Cells have been isolated from bone marrow [57], the atria and ventricles of the heart [65], and skeletal muscle [66–68] with a morphology similar to MABs from the embryonic dorsal aorta. These cells are also referred to as MABs [66, 69–71] or pericyte-derived cells [67, 68] and are thought to originate from embryonic MABs in the dorsal aorta [67, 68]. Skeletal muscle MABs are derived from explant cultures of muscle tissue, followed by selection of cells with a small, round, refractile appearance for clonal expansion [67, 68]. Many of these cells express alkaline phosphatase (AP), a marker on pericytes in skeletal and cardiac muscle [65, 67], indicating they might be pericytes, although some skeletal muscle-derived MABs with myogenic potential do not express AP [69]. Skeletal muscle-derived MABs have a marker profile distinct from that of aorta-derived MABs and do not self-renew. They adopt a large, flat morphology at approximately 20–22 population doublings and undergo senescence [67–70, 72]. In contrast, MABs isolated from embryonic dorsal aorta will self-renew and acquire an unlimited lifespan [54], and MABs from postnatal aorta...
were maintained in culture for more than 50 population doublings [54, 55, 60, 64]. Skeletal muscle-derived MABs will spontaneously differentiate into skeletal myoblasts in culture in horse serum or low serum, in contrast to aorta-derived MABs, which require coculture with skeletal myoblasts [67, 69].

MABs from skeletal muscle infiltrate damaged muscle from the circulation, engraft, and restore absent proteins in animal models of muscular dystrophy. Skeletal muscle-derived MABs restored dysferlin expression in 9%–12% of skeletal myofibers of dysferlin-deficient mice and in 10%–70% of muscle fibers in the GRMD after arterial delivery [66, 69]. Skeletal muscle-derived MABs also restored α-SG expression in the α-SG-null mouse and dystrophin expression in up to 10% of skeletal muscle fibers in the scid-mdx mouse after intramuscular injection [71, 72]. To examine the potential for genetic correction of MABs for homologous cell therapy, MABs from mdx mice and GRMD dogs were genetically corrected and transplanted into mdx mice or GRMD dogs, respectively. Genetically corrected murine MABs restored dystrophin expression in 5%–10% of muscle fibers in mdx mice, resulting in functional improvement [71]. Genetically corrected canine MABs restored dystrophin expression in 5%–50% of skeletal muscle fibers of GRMD dogs with arterial injection [66]. However, genetically corrected human iPSC-derived MAB-like cells (HIDEMSs) from patients with LGMD only restored α-SG to 2% of skeletal myofibers in α-SG-null mice after intramuscular injection [73], suggesting that autologous therapy with genetically corrected HIDEMSs could be challenging. In summary, the large body of research generated for mesoangioblasts from different tissues and species, transplanted into multiple animal models for muscular dystrophy, provides a strong rationale for translating mesoangioblast therapy to the clinic and for refining the methods of delivery, genetic correction, and treatment regimens.

**Mesoangioblasts for Cell Therapy of Dystrophic Cardiac Muscle**

Although nearly 100% of patients with DMD develop cardiomyopathy [74–78], it has been undertreated [79], and consensus is lacking regarding effective treatments. In contrast to adult skeletal muscle, adult cardiac muscle does not appear to be highly regenerative in nature, although recent studies have shown that new cardiomyocytes are generated in low numbers throughout life in the adult heart during homeostasis and after damage to the myocardium [80–84]. With the hope of generating new cardiomyocytes in the damaged human heart, multiple studies have been conducted of patients after myocardial infarction. A recent meta-analysis of these trials indicated that stem cells have provided modest, but frequently transient, functional benefit [85–87], which has largely been attributed to indirect effects of the cells, such as production of trophic factors. Damage in the dystrophin-deficient heart is diffuse and develops over time, in contrast to the acute, focal damage of an infarct. Thus, stem cells should be transplanted before extensive fibrosis and inflammatory cell infiltration in the dystrophic heart, and the outcomes of cell therapy might, therefore, differ from those in the infarcted heart. However, very few studies have been conducted to examine the potential of stem cells to generate new cardiomyocytes in the dystrophic heart. We transplanted ADMs, which differentiate into cardiac-like cells in vitro [88] and in mdx heart [63], into the heart of mdx/utrn−/− mice [88]. We previously reported that mdx/utrn−/− mice develop dilated cardiomyopathy similar to that of patients with DMD [89]. Although ADMs prevented ventricular dilation and declining function [88], dystrophin expression was not restored. ADMs might have provided functional benefit by inducing angiogenesis, because increased CD31 expression [88] and capillary density (S. Berry, unpublished data) were observed with ADM transplantation. ADMs also induced division of an endogenous population of cardiac stem cells, and nestin-expressing cardiomyocytes were detected surrounding clusters of damaged cardiomyocytes and macrophages in the ADM-transplanted heart, indicative of newly formed cardiomyocytes [88]. Therefore, although ADMs do not restore dystrophin in the heart, they might activate endogenous cardiac stem cells to generate new cardiac myocytes in the dystrophic myocardium. These data suggest new potential therapeutic targets for treating cardiomyopathy in the dystrophin-deficient heart, including nestin-positive cardiac stem cells.

**Systemic Delivery of Stem Cells**

Mesoangioblasts and MSCs infiltrate damaged muscle from the circulation. Therefore, systemic delivery of these cells could result in their engraftment in a large number of affected muscles with minimal invasiveness. However, systemic delivery poses additional hurdles. Without modification or pretreatment, most MSCs and MABs will become trapped in filter organs. More than 30% of MABs from the dorsal aorta were detected in the liver, lungs, and spleen 24 hours after arterial injection [58] and were still present in similar numbers 8 months later [58]. As many as 90% of MABs from human skeletal muscles were localized in filter organs of a mouse model for DMD 24 hours after arterial delivery [67]. Similarly, systemically delivered MSCs from rat bone marrow, human bone marrow, umbilical cord, and synovial tissue, and swine adipose tissue localized to filter organs [90–93]. About 90% of human BM and UCSCs localized to the lungs after intravenous injection in mice, and human synovial MSCs remained in the lungs of mice up to 6 months after intravenous delivery [91]. Trapping of large numbers of systemically delivered stem cells in filter organs decreases the number of donor cells available for engrafting into the dystrophic muscle. To address this problem, aorta-derived MABs can be modified to improve homing to damaged muscle [58], extravasation into damaged muscle [94], and survival after muscle infiltration [59]. Other reports have focused on increasing clearance of donor stem cells from filter organs. Kerkela et al. recently reported that using pronase, rather than trypsin, to detach MSCs before transplantation reduced donor cell entrapment by fivefold from the lungs of mice and pigs and enhanced homing of donor cells to an area of inflammation [95]. In addition, the cell surface markers CD49d and CD49f have been linked to the homing of donor cells to an area of inflammation [99]. These two reports suggest that transiently altering the stem cell surface might decrease trapping in filter organs; however, additional studies are needed to establish whether such a strategy would alter or decrease the potential of MSCs or MABs to infiltrate and regenerate dystrophic muscle.

Another concern regarding the arterial delivery of stem cells is the potential for interruption of blood flow through the artery or the microvasculature downstream of the site of injection. In particular, the large size of MSCs could be problematic. Delivery of MSCs via the iliac artery resulted in clusters of donor cells in the microvessels of the cremaster muscle that obstructed the blood flow through the microcirculation and resulted in death of most trapped cells [97]. In a separate study, MSC injection into
the abdominal aorta also disrupted the microcirculation in cre-
master muscles, with MSCs becoming trapped in the capillaries
in the muscle. Furthermore, the donor cells caused pulmonary
embolism and subsequent death in up to 40% of mice [98]. MSCs
delivered into the porcine coronary artery also decreased arterial
blood flow, resulting in myocardial injury, although increased
numbers of donor MSCs were detected in the heart with arterial
delivery compared with other methods [99]. In agreement with
these observations, injection of MSCs into the coronary artery
of healthy dogs resulted in changes in the ECG parameters char-
acteristic of myocardial ischemia, despite normal behavior
and activity in the dogs. The changes correlated directly with
the number of transplanted cells. After euthanasia, multiple
lesions consistent with subacute microinfarction were observed
in the heart [100]. No studies have examined disruption of blood
flow after transplantation of mesoangioblasts after arterial deliv-
ery, although heparin was injected with mesoangioblasts in
one study to reduce the risk of embolism [66]. Collectively, these
data indicate that arterial delivery of stem cells might disrupt ar-
terial and/or microvascular blood flow, potentially leading to is-
chemia and tissue damage and the loss of donor myogenic
stem cells.

FUTURE DIRECTIONS

To build on the extensive body of promising research regarding
mesoangioblast and mesenchymal stem cell therapy for muscular
dystrophy, one area worthy of additional study is establishing
protocols for isolation and culture of MSCs and mesoangioblasts
that minimize in vitro manipulation and do not require the use of
animal products. The current protocols for isolation of mesoan-
gioblasts and some populations of MSCs discussed in the present
review include subjective and descriptive analysis, requiring ex-
plant culture, the selection of cells by appearance, extensive time
in culture in vitro, and the use of animal products such as fetal bo-
vine serum for culture of human MSCs and MABs [40, 47, 52, 53,
67, 68]. These protocols do not lend themselves to large-scale
preparations for clinical trials, and it is difficult to use them to re-
producingly isolate the same potent cell population from different
samples of tissue or independent laboratories. Moreover, ex-
tended culturing of stem cells outside of the niche might result
in changes in their differentiation potential and marker profile
and increased regulation for their use in the clinic.

A second topic to address in future studies is examining the
effect of systemically delivered donor cells in off-
target tissues, such as filter organs. For example, it will be impor-
tant to establish whether prolonged accumulation of stem cells
in the lungs will lead to inflammation, fibrosis, or impaired gas
exchange.

A third topic relevant to the translation of cell therapy for
DMD to the clinic is whether or how corticosteroid therapy will
affect the activity of therapeutic stem cells. Agreement has been
reached among physicians and scientists that corticosteroids pre-
serve muscle strength, prolong ambulation, and decrease scolio-
sis in patients with DMD [101]. A recent report also demonstrated
prolonged survival with steroid treatment [102]. Corticosteroids
are, therefore, currently the standard of care for patients with
DMD [79] and will likely be present in most patients with DMD
before, during, and after potential transplantation of stem cells
for clinical trials or treatment. The mechanism by which
corticosteroids benefit patients with DMD is not known but might
involve anti-inflammatory and immunosuppressive effects [103]
and stimulation of myogenic repair [104]. These activities could
affect the homing and regenerative activities of systemically de-
ivered MABs and MSCs in DMD skeletal muscle.

A fourth consideration, when using MSCs or mesoangioblasts
as vehicles for gene therapy in muscular dystrophy, is the gene
dosage. Donor cells will likely constitute only a portion of the total
number of nuclei in regenerating skeletal myofibers; therefore,
overproduction of the missing protein by donor cells would en-
hance protein restoration and perhaps functional improvement
or recovery. An example of a potential strategy for overexpres-
sion of full-length, fully functional proteins by donor cells would
be to use stem cells carrying human artificial chromosomes con-
taining multiple cDNAs encoding the missing protein.

A fifth area of interest for future studies is the potential to use
iPSCs derived from patients with muscular dystrophy or matched
donors to derive MSCs for treatment of muscular dystrophy. iPSCs
have been directed to differentiate into MSCs that promoted re-
generation of vessels and muscle tissue in ischemically damaged
skeletal muscle [105]. In a separate study, iPSCs were successfully
derived from patients with DMD and Becker muscular dystrophy
[106]. These data suggest the possibility of generating MSCs from
patient-specific iPSCs from patients with DMD or from immuno-
logically matched donors for cell therapy for muscular dystrophy
that might decrease the rejection of the donor cells.

Another area important for future study will be to examine
whether stem cells, including MABs derived from skeletal or car-
diac muscle or MSCs, will generate new cardiac muscle cells and
restore dystrophin expression in dystrophic myocardium. We ob-
served a division of endogenous cardiac stem cells and small,
nestin-expressing cardiomyocytes surrounding clusters of dam-
aged muscle cells and macrophages in the dystrophin-deficient
heart near the end stage of disease in mdx/utrn−/− mice [107].
These observations suggest that repair or regeneration occurs
during disease progression in the dystrophic heart. Additional
study is needed to establish whether cardiac regeneration is sim-
lar to skeletal muscle regeneration and whether what is known
about skeletal muscle regeneration can be exploited to improve
repair of the dystrophic myocardium.

CONCLUSION

The progress in stem cell therapy for muscular dystrophy has been
rapid, with many promising results on which to build. Based on
the early successes in preclinical studies, a clinical trial is under-
way to transplant skeletal muscle-derived MABs into the circula-
tion of patients with DMD. We await the results from the trial and
will continue to focus on methods to improve the regeneration
and function of dystrophic muscle.

ACKNOWLEDGMENTS

I thank Dr. David Miller for his thoughtful comments. I apologize
to those whose work has been omitted owing to space limitations.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The author indicated no potential conflicts of interest.
96 MAB and MSC Therapy for Muscular Dystrophy

negative to -positive by injection of normal myo-
blasts. Nature 1989;337:176–179.
19 Karpati G, Ajdukovic D, Arnold D et al. Myoblast transfer in Duchenne muscular dys-
trophy. Ann Neurol 1993;34:8–17.
20 Morandi L, Berinasco P, Gebbia M et al. Lack of mRNA and dystrophin expression in
DMD patients three months after myoblast transfer. Neuromuscul Disord 1995;5:291–295.
21 Gussoni E, Pavlath GK, Lanctot AM et al. Normal dystrophin transcripts detected in Du-
chenne muscular dystrophy patients after myo-
blast transplantation. Nature 1992;356:435–438.
22 Tremblay JP, Malouin F, Roy R et al. Results of a triple blind clinical study of myo-
blast transplantsations without immunosup-
pressive treatment in young boys with Duchenne muscular dystrophy. Cell Transplant
1993;2:99–112.
23 Mendell JR, Kissel JT, Amato AA et al. Myoblast transfer in the treatment of Duch-
enne’s muscular dystrophy. N Engl J Med 1993;333:832–838.
24 Miller RG, Sharma KR, Pavlath GK et al. Myoblast implantation in Duchenne muscular dys-
trophy: The San Francisco study. Muscle Nerve 1997;20:469–478.
25 Roy R, Tremblay JP, Huard J et al. Anti-
body formation after myoblast transplantation in Duchenne-dystrophic patients, donor HLA
compatible. Transplant Proc 1993;25:995–997.
26 Huard J, Rouleau R, Bouchard JP et al. Human myoblast transplantation between immunohis-
tocompatible donors and recipients produces immune reactions. Transplant Proc 1992;24:
3049–3051.
27 Partridge T. Myoblast transplantation. Neuromuscul Disord 2002;12(suppl 1):53–56.
28 Skud D, Goulet M, Roy B et al. Dystrophin expression in muscles of Duchenne muscular
dystrophy patients after high-density injections of normal myogenic cells. J Neuropathol Exp
Neurol 2006;65:371–386.
29 Skud D, Goulet M, Roy B et al. First test of a “high-density injection” protocol for myo-
genic cell transplantation throughout large volumes of muscles in a Duchenne muscular dys-
trophy patient: Eighteen months follow-up.
Neuromuscul Disord 2007;17:38–46.
30 Briggs D, Morgan JE. Recent progress in satellite cell/myoblast engraftment—
Relevance for therapy. FEBS J 2013;280:
4281–4293.
31 Périsé S, Trollet C, Moully V et al. Autolo-
gous myoblast transplantation for ocularpha-
yngeal muscular dystrophy: A phase I/IIa clinical study. Mol Ther 2014;22:219–225.
32 Burzyn D, Kuswanto W, Kolodin D et al. A special population of regulatory T cells poten-
tiates muscle repair. Cell 2013;155:1282–1295.
33 Bianco P, Cao X, Frenette PS et al. The meaning, the sense and the significance: Trans-
lation the science of mesenchymal stem cells in-
to medicine. Nat Med 2013;19:35–42.
34 Dominici M, Le Blanc K, Mueller J et al. Minimal criteria for defining multipotent mes-
enchymal stromal cells: The International So-
ciety for Cellular Therapy position statement.
Cytotherapy 2006;8:315–317.
35 Pinhey DG, Galipeau J, Krampera M et al. MScS: Science and trials. Nat Med 2013;
19:812.
36 Pittenger MF. MScS: Science and trials.
Nat Med 2013;19:811.
37 Fibbe WE, Dazzi F, LeBlanc K. MScS: Sci-
ence and trials. Nat Med 2013;19:812–813.
38 Vieira NM, Zuconi E, Bueno CR et al. Hu-
man multipotent mesenchymal stromal cells from different sources show different in vivo po-
tential to differentiate into muscle cells when injected in dystrophic mice. Stem Cell Rev
2010;6:560–566.
39 Zuconi E, Vieira NM, Bueno CR et al. Pre-
clinical studies with umbilical cord mesenchy-
mal stromal cells in different animal models for muscular dystrophy. J Biomed Biotechnol
2011;2011:175251.
40 Secco M, Bueno CRr, Vieira NM et al. Sys-
temic delivery of human mesenchymal stromal cells combined with IGF-1 enhances muscle
functional recovery in LAMDA2/2 dystrophic mice. Stem Cell Rev 2013;9:93–109.
41 Zuconi E, Vieira NM, Bueno DF et al. Mesenchymal stem cells derived from canine umbilical cord vein—A novel source for cell
therapy studies. Stem Cells Dev 2010;19:
3902.
42 Kocaefe C, Balci D, Hayta BB et al. Reprog-
ramming of human umbilical cord stromal mesenchymal stem cells for myogenic differen-
tiation and muscle repair. Stem Cell Rev 2010;6:
512–522.
43 Meng J, Adkin CF, Arechavala-Gomeza V
et al. The contribution of human synovial stem
cells to skeletal muscle regeneration. Neuro-
muscul Disord 2010;20:6–15.
44 Gonçalves MA, Swidens J, Holkers M et al. Genetic complementation of human mus-
cle cells via directed stem cell fusion. Mol Ther
2008;16:741–748.
45 Feng SW, Chen F, Cao J et al. Restoration of muscle fibers and satellite cells after isogenic
MSC transplantation with microdystrophin
gene delivery. Biochem Biophys Res Commun
2012;419:1–6.
46 Gang Ej, Darabi R, Bosnakovski D et al. Engraftment of mesenchymal stem cells into dystrophic-deficient mice is not accompanied
by functional recovery. Exp Cell Res 2009;315:
2624–2636.
47 Xiong F, Xu Y, Zheng H et al. Microdystro-
phin delivery in dystrophic-deficient (mdx)
mouse by genetically-corrected syngeneic MSCs transplantation. Transplant Proc 2010;42:
2731–2739.
48 Nitahara-Kasahara Y, Hayashita-Kinoh H,
Ohshima-Hosoyama S et al. Long-term engraft-
ment of multipotent mesenchymal stromal cells that differentiate to form myogenic cells in
dogs with Duchenne muscular dystrophy. Mol Ther 2012;20:168–177.
49 Dezawa M, Ishikawa H, Itokazu Y et al. Bone marrow stromal cells generate muscle cells and repair muscle degeneration. Science
2005;309:314–317.
50 Rodrigue AM, Pisani D, Dechesne CA et al. Transplantation of a multipotent cell pop-
ulation from human adipose tissue induces dys-
trophin expression in the immunocompetent
mdx mouse. J Exp Med 2005;201:1397–1405.
51 Liu Y, Yan X, Sun Z et al. Flik-1+ adipose-
derived mesenchymal stem cells differentiate
into skeletal muscle satellite cells and amelio-
rate muscular dystrophy in mdx mice. Stem Cells Dev 2007;16:695–706.
myogenic precursors distinct from satellite cells. Nat Cell Biol 2007;9:255–267.
68 Tonlorenzi R, Dvellaeve A, Schnapp E et al. Isolation and characterization of mesoangioblasts from mouse, dog, and human tissues. Curr Protoc Stem Cell Biol 2007;3:28B.1.28:1.29
69 Diaz-Manera J, Touvier T, Dvellaeve A et al. Partial dysferlin reconstitution by adult murine mesoangioblasts is sufficient for full functional recovery in a murine model of dysferlinopathy. Cell Death Dis 2010;1:e61.
70 Morosetti R, Giglio T, Broccoli A et al. Mesoangioblasts from facioscapulohumeral muscular dystrophy display in vivo a variable myogenic ability predictable by their in vitro behavior. Cell Transplant 2011;20:1299–1313.
71 Tedesco FS, Hoshiya H, D’Antona G et al. Stem cell-mediated transfer of a human artificial chromosome ameliorates muscular dystrophy. Sci Transl Med 2011;3:96ra78.
72 Pessina P, Conti V, Tonlorenzi R et al. Necdin enhances muscle reconstitution of dystrophic muscle by vessel-associated progenitors, by promoting cell survival and myogenic differentiation. Cell Death Differ 2012;19:827–838.
73 Tedesco FS, Gerli MF, Perani L et al. Transplantation of genetically corrected human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy. Sci Transl Med 2012;4:140ra89.
74 Eagle M, Baudouin SV, Chandler C et al. Survival in Duchenne muscular dystrophy: Improved survival in life expectancy since 1967 and the impact of home nocturnal ventilation. Neuromusc Disord 2002;12:926–929.
75 Eagle M, Bourke J, Bullock R et al. Managing Duchenne muscular dystrophy—the additive effect of surgical spine and home nocturnal ventilation in improving survival. Neuromuscul Disord 2007;17:470–475.
76 Bushby K, Muntoni F, Bourke JP. 107th CINRG Duchenne Natural History Study demonstrate the effectiveness of spinal surgery and nocturnal ventilation in improving survival. Neuromuscul Disord 2002;12:926–929.
77 Black BA, Bion J, Wilding P et al. Partial dysferlin reconstitution by adult murine mesoangioblasts in mdx/utrn-/- mice improves survival and reduces limb-muscle fatty infiltration. Dev Med Child Neurol 2006;48:163.
78 Spurney C, Shimizu R, Hache LP et al. CIRNCG Duchenne Natural History Study demonstrates insufficient diagnosis and treatment of cardiomyopathy in Duchenne muscular dystrophy. Muscle Nerve 2014;50:258–268.
79 Bergmann O, Bhardwaj RD, Bernard S et al. Time-dependent migration of systemically delivered bone marrow mesenchymal stem cells to the infarcted heart. Cell Transplant 2010;19:219–230.
80 De Bari C, Dell’Accio F, Vandenabeele F et al. Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane. J Cell Biol 2003;160:909–918.
81 Barbash IM, Chouraqui P, Baron J et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocar- dium: Feasibility, cell migration, and body distribution. Circulation 2003;108:863–868.
82 Van Bortel LM, Hsu D, Oon JJ et al. Intracoronary and retrograde coronary venous myocardial delivery of adipose-derived stem cells in swine infarction lead to transient myocardial trapping with predominant pulmonary redistribution. Catheter Cardiovasc Interv 2014;83:E17–E25.
83 Giannotta M, Benedetti S, Tedesco FS et al. Targeting endothelial junctional adhesion molecule-α (EPCAM)/Rap-1 axis as a novel strategy to increase stem cell engraftment in dystrophic muscles. EMBO Mol Med 2014;6:239–258.
84 Kerkelä E, Hakkakariainen T, Mäkelä T et al. Transient proteolytic modification of mesenchymal stromal cells increases lung clearance rate and targeting to injured tissue. STEM CELLS TRANSLATIONAL MEDICINE 2013;2:510–520.
85 Nystedt J, Anderson H, Tikkanen J et al. Cell surface structures influence lung clearance rate of systemically infused mesenchymal stromal cells. STEM CELLS 2013;31:317–326.
86 Toma C, Wagner WR, Bowry S et al. Fate of culture-expanded mesenchymal stem cells in the microvasculature: In vivo observations of cell kinetics. Circ Res 2009;104:398–402.
87 Furlani D, Ugurlucan M, Ong L et al. Is the intravascular administration of mesenchymal stem cells safe? Mesenchymal stem cells and...
intravital microscopy. Microvasc Res 2009;77:370–376.

99 Freyman T, Polin G, Osman H et al. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. Eur Heart J 2006;27:1114–1122.

100 Vulliet PR, Greeley M, Halloran SM et al. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. Lancet 2004;363:783–784.

101 Bushby K, Muntoni F, Urtizberea A et al. Report on the 124th ENMC International Workshop. Treatment of Duchenne muscular dystrophy: defining the gold standards of management in the use of corticosteroids. 2-4 April 2004, Naarden, The Netherlands. Neuromuscul Disord 2004;14:526–534.

102 Schram G, Fournier A, Leduc H et al. All-cause mortality and cardiovascular outcomes with prophylactic steroid therapy in Duchenne muscular dystrophy. J Am Coll Cardiol 2013;61:948–954.

103 Kissel JT, Burrow KL, Rammohan KW et al. Mononuclear cell analysis of muscle biopsies in prednisone-treated and untreated Duchenne muscular dystrophy. Neurology 1991;41:667–672.

104 Anderson JE, Weber M, Vargas C. Deflazacort increases laminin expression and myogenic repair, and induces early persistent functional gain in mdx mouse muscular dystrophy. Cell Transplant 2000;9:551–564.

105 Lian Q, Zhang Y, Zhang J et al. Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. Circulation 2010;121:1113–1123.

106 Park IH, Arora N, Hsu H et al. Disease-specific induced pluripotent stem cells. Cell 2008;134:877–886.

107 Berry SE, Andruszkiewicz P, Chun JL et al. Nestin expression in end-stage disease in dystrophin-deficient heart: Implications for regeneration from endogenous cardiac stem cells. STEM CELLS TRANSLATIONAL MEDICINE 2013;2:848–861.