Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Hematopoietic Cell Transplantation Provides an Immune-tolerant Platform for Myoblast Transplantation in Dystrophic Dogs

Maura H Parker¹, Christian Kuhr¹, Stephen J Tapscott²,³ and Rainer Storb¹,⁴

¹Program in Transplantation Biology, Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; ²Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; ³Department of Neurology, University of Washington, Seattle, Washington, USA; ⁴Department of Medicine, University of Washington, Seattle, Washington, USA

Duchenne Muscular Dystrophy (DMD) is the most common and severe form of muscular dystrophy in humans. The goal of myogenic stem cell transplant therapy for DMD is to increase dystrophin expression in existing muscle fibers and to provide a source of stem cells for future muscle generation. Although syngeneic myogenic stem cell transplants have been successful in mice, allogeneic transplants of myogenic stem cells were ineffective in several human trials. To determine whether allogeneic muscle progenitor cells can be successfully transplanted in an immune-tolerant recipient, we induced immune tolerance in two DMD-affected (mdx) dogs through hematopoietic cell transplantation (HCT). Injection of freshly isolated muscle-derived cells from the HCT donor into either fully or partially chimeric mdx recipients restored dystrophin expression up to 6.48% of wild-type levels, reduced the number of centrally located nuclei, and improved muscle structure. Dystrophin expression was maintained for at least 24 weeks. Taken together, these data indicate that immune tolerance to donor myoblasts provides an important platform from which to further improve myoblast transplantation, with the goal of restoring dystrophin expression to patients with DMD.

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is the most common and severe form of muscular dystrophy in humans, affecting 1 in 3,500 live births.¹² It is an X-linked recessive disorder caused by mutations in the gene for dystrophin, the largest gene identified in the human genome. Mutations are present at birth, yet symptoms do not manifest themselves until 3–5 years of age. Progressive muscle weakness and wasting forces affected individuals to become wheelchair-bound by 12 years of age, and to succumb to the disease in the third decade of life as a result of respiratory and/or cardiac failure.

Transplantation of myogenic stem cells possesses great potential for long-term repair of dystrophic muscle. Indeed, intramuscular injection of adult satellite cell–derived myoblasts from a normal syngeneic donor into mdx mice results in the formation of dystrophin-positive muscle fibers.³–⁶ However, small-scale human clinical trials reveal that intramuscular injection of donor myoblasts results in transient expression of dystrophin in a small number of recipient muscle fibers.⁷–¹² In the absence of immunosuppression, injection of donor myoblasts triggers cellular immune responses that destroy newly formed donor myotubes.¹³

Immunosuppressive drugs, such as cyclosporine (CSP) and FK506, inhibit calcineurin activity and prevent graft rejection. Yet, patients treated with CSP do not display an increased number of dystrophin-positive myofibers relative to patients receiving placebo.¹⁶ It has been established that CSP alone is not potent enough to prevent graft rejection; additionally, CSP may negatively affect myoblast engraftment, as calcineurin activity is essential for myogenic differentiation in vitro and in vivo.¹⁴,¹⁵ More important, chronic systemic immunosuppression is associated with serious side effects and considerable risk. Taken together, these observations indicate that myogenic stem cell transplant therapy requires an alternative to traditional immunosuppression.

Nonmyeloablative hematopoietic cell transplantation (HCT) results in mixed chimerism, defined as the coexistence of donor and recipient hematopoietic cells.¹⁶ In the clinic, nonmyeloablative transplantation is an outpatient procedure that has been well tolerated in >1,200 patients with malignant and nonmalignant blood disorders.¹⁷ Mixed chimerism in both rodents and large animals has successfully induced tolerance to donor-derived kidney, liver, small bowel, heart, lung, and pancreatic islet cells, without the need for immunosuppression.¹⁸–²¹ Specifically, donor kidneys transplanted into mixed chimeric canine recipients were fully functional and remained intact for at least 5 years, even when only 10% of lymphocytes within the recipient were of donor origin.

Therefore, we sought to determine whether HCT could be used to establish an immune-tolerant, random-bred, large
animal model of DMD for preclinical myogenic stem cell transplantation studies. The canine model of DMD (cxmd) is characterized by a point mutation in the consensus splice acceptor site in intron 6 of the dystrophin gene, introducing a stop codon within the modified reading frame, resulting in a near complete absence of dystrophin protein.\(^{24,29}\) The dystrophic phenotype of the cxmd canine faithfully recapitulates the human disease, making this an ideal model for investigating potential therapies. HCT alone is unable to restore dystrophin expression to cxmd canines.\(^{27}\) Therefore, we specifically asked whether myeloablative and nonmyeloablative HCT in cxmd canines would permit donor-derived myogenic stem cells to stably engraft and restore dystrophin expression.

RESULTS

HCT and muscle-derived cell transplantation protocol

Bone marrow and granulocyte colony-stimulating factor mobilized peripheral blood mononuclear cells were harvested from two normal donors and transplanted into two irradiated leukocyte antigen–identical cxmd recipients (Figure 1a, Materials and Methods). The hematopoietic cells of one cxmd recipient were all donor derived (Table 1; G289; full chimera), whereas the second recipient had a mix of donor and recipient hematopoietic cells (Table 1; G604; mixed chimera). Skeletal muscle–derived mononuclear cells isolated from the same donors were processed for injection immediately after isolation or cultured for 14 days to specifically expand myogenic cells before injection (Figure 1b, Materials and Methods). G289 underwent the myeloablative HCT at 5.5 months of age, followed by muscle-derived cell transplantation at 32 months of age. G604 underwent the nonmyeloablative HCT at 7 months of age, and muscle-derived cell transplantation at 18 months of age.

Injection of donor muscle–derived cells restored dystrophin expression in chimeric cxmd canines

Enzymatic digestion of a skeletal muscle biopsy from the HCT donor released a mixed population of mononuclear cells that included, but was not limited to, satellite cells and fibroblasts. Freshly isolated muscle-derived cells were injected intramuscularly at several sites in the biceps femoris muscle of chimeric recipients. Cryosections from biopsies taken between 4 and 24 weeks after injection were analyzed for dystrophin expression. It was expected that if cells injected into the skeletal muscle of the cxmd chimeric dog were differentiation and fusion competent, then dystrophin expression would be restored.

Indeed, intramuscular injection of 1 × 10^6 fresh donor muscle–derived cells into the fully chimeric cxmd recipient, G289, restored dystrophin expression to muscle fibers in muscle biopsies taken at 5 and 10 weeks after injection (Figure 2a). Dystrophin expression was observed throughout the biopsy, extending ~0.5 cm from the site of injection (data not shown). Similarly, injection of 4.5 × 10^6 freshly isolated donor muscle–derived cells directly into the muscle of the mixed chimeric cxmd canine, G604, resulted in clusters of dystrophin-expressing fibers, grouped near the sites of injection in biopsies at 4, 8, and 24 weeks after injection (Figure 2b). Expression of dystrophin appeared to be maintained over at least 1,000 μm of fiber length, as assessed by examination of serial sections (data not shown).

Quantitation of donor contribution in injected cxmd recipient muscle

The amount of donor-derived wild-type dystrophin messenger RNA (mRNA) was assessed by real-time reverse transcriptase–PCR (RT-PCR) using a probe that specifically recognizes the wild-type dystrophin splice junction and not the abnormal spliced form in cxmd muscle. The level of dystrophin mRNA observed in wild-type donor muscle was arbitrarily set to 100 and resulted in a relative level of dystrophin mRNA of 0.00035% in the fully chimeric cxmd recipient (G289) before muscle-derived cell transplantation. The level of dystrophin in G289 increased significantly to 6.48% (P = 0.0007) 10 weeks after injection of freshly isolated cells.

The relative level of skeletal muscle dystrophin mRNA in G604 was 0.0019% before muscle-derived cell transplantation (Table 2). This increased to 1.29% (P = 0.003) 4 weeks after injection of fresh cells, and remained constant at 24 weeks after injection at 1.32% (P = 0.01). Therefore, in both G289 and G604, the values for dystrophin expression were significantly higher after muscle-derived cell transplantation. Because mononuclear satellite cells and muscle stem cells do not express dystrophin, this indicates that the donor muscle–derived cells differentiated into dystrophin-expressing myotubes.

Genomic DNA (gDNA) isolated from groups of cryosections serially cut from biopsies of injected muscle and analyzed using
variable number of tandem repeat–PCR confirmed the persistence of significant amounts of donor-derived cell DNA at the latest time points of analysis. The fully chimeric cxmd dog, G289, displayed an average of 13.9% donor gDNA before injection, representing donor blood cell nuclei present as the result of HCT (Table 3). Ten weeks after injection of freshly isolated cells, an average of 20.2% of gDNA was donor derived (P = 0.0017), and the highest value of donor contribution to gDNA was 23.1%. The mixed chimeric cxmd dog, G604, displayed an average of 5.0% donor gDNA before injection of muscle-derived cells. Twenty-four weeks after injection of freshly isolated cells, an average of 9.3% was donor derived (P = 0.007), and the highest value of donor contribution to gDNA was 12.3%.

Injection of donor muscle–derived cells did not elicit an immune response in HCT-transplanted recipients

To determine whether injection of donor muscle–derived cells induced an immune response and infiltration of host immune cells in the recipient, cryosections were immunostained
using antibodies directed against CD45, a hematopoietic cell marker; CD3, a T-cell specific marker; and CD14, a monocyte/macrophage-specific marker. Minimal hematopoietic cell infiltration was observed in donor and dystrophic recipient skeletal muscle before transplantation, as evidenced by occasional CD45-expressing cells (Figure 2c—donor and recipient preinjection). There was an absence of CD3-positive T cells (data not shown), yet CD14-positive macrophages were clearly observed in small patches within the recipient DMD-affected muscle before injection (Figure 2d—recipient preinjection), confirming the mild inflammatory process associated with DMD.

At 10 weeks after cell transplantation, a persistent minimal lymphocytic infiltration was observed (Figure 2c—CD45). Careful examination of immunostained cryosections uncovered no CD3-positive cells (data not shown); however, a small region of CD14-positive cells was detected, indicative of monocytes/macrophages, which normally infiltrate degenerating skeletal muscle (Figure 2d—recipient after injection). Therefore, injection of donor muscle–derived cells into cxmd muscle did not induce immune cell infiltration.

Intramuscular injection of donor muscle–derived cells restored muscle structure and reduced regeneration in chimeric cxmd canine

For stem cell therapy to be effective and clinically relevant, function must be restored to skeletal muscle. However, the localized injections used in this study precluded testing for functional improvement. Therefore, we evaluated improvement in muscle structure and stability through histological examination. Hematoxylin and eosin staining of cryosections from the G289 recipient muscle before transplantation revealed a striking loss of muscle structure, considerable variation in muscle fiber size, and increased fatty and connective tissue as compared to normal donor muscle (Figure 3a—donor and recipient preinjection). Injection of freshly isolated muscle–derived cells markedly improved muscle structure at 10 weeks after transplantation and resulted in less connective and fatty tissue infiltration and reduced size variation in fiber diameter when compared to the precell injection biopsy (Figure 3a).

Newly formed skeletal muscle fibers or fibers that have acquired differentiating myocytes as a result of regeneration are characterized by nuclei located in the center of the muscle fiber. In donor wild-type muscle, <1% of muscle nuclei were centrally located. However, ~10% of muscle nuclei in the G289 DMD-affected recipient were centrally located, indicating that a portion of dystrophic muscle was regenerating (Figure 3b). Injection of fresh muscle–derived cells reduced the number of centrally located myonuclei in the G289 DMD-affected recipient muscle to <5%, which was statistically significant as compared to the recipient before injection (P < 0.01). Together, these findings suggested that engraftment of donor muscle–derived cells and restoration of dystrophin expression stabilized cxmd muscle, preventing repeated attempts to regenerate.

Cultured donor muscle–derived cells restore dystrophin expression

Our data demonstrate that direct injection of freshly isolated donor-derived muscle cells engraft without immune rejection in chimeric recipients. However, many muscle stem cell transplant procedures require expansion of cells in culture, which might alter the immunogenic status of the donor cells. Therefore, to determine whether similar engraftment could be obtained by cells expanded in culture, donor muscle–derived cells were cultured before injection into biceps femoris muscle of the chimeric cxmd recipients. We used a standard serial preplating technique to enrich for myoblasts and analyzed cells from preplates (PP2) and 6 (PP6) (Figure 1b). In order to ensure that donor muscle–derived mononuclear cells were myogenic, adherent PP2 and PP6 cells were fixed in proliferation (Supplementary Figure S1a) and differentiation (Supplementary Figure S1b) conditions. Immunostaining with antibodies specific to desmin and Pax7, two markers of myoblasts in culture, established that >90% of proliferating cultured cells were myogenic.

To assess differentiation potential in vitro, PP2 and PP6 cells were cultured until confluent and fixed after an additional 72 hours. The PP2 population differentiated to form multinucleated myotubes that expressed myogenin and myosin heavy chain (MyHC), two markers of myogenic differentiation (Supplementary Figure S1b—PP2). The PP6 population also expressed myogenin and myosin heavy chain, yet remained primarily mononuclear (Supplementary Figure S1b—PP6). Mouse muscle–derived cells cultured in a comparable manner exhibited similar results.28,29

Similar to direct injection of freshly isolated muscle–derived cells, injection of cultured PP6 cells did not induce immune cell infiltration in the injected muscle (data not shown). In addition,
the injection of cultured PP6 cells restored dystrophin expression to skeletal muscle of chimeric cxmd recipients (Figure 4a and b). The level of dystrophin reached a maximum of 1.45% of wild-type levels in the fully chimeric dog, G289, and 0.05% in the mixed chimeric dog, G604 (Table 4). Although these were significantly higher relative to the recipient levels before muscle cell transplantation (P = 0.0026 and P = 0.009, respectively), the maximum levels of wild-type dystrophin was less than that reached after injection of freshly isolated cells (Table 2).

**DISCUSSION**

Finding a clinically relevant means of restoring long-term expression of dystrophin to skeletal muscle has been a continuing challenge in the field of DMD research. Transplantation of normal myoblasts into human patients was unsuccessful because of immune rejection of donor cells. While long-term immunosuppression might lengthen muscle cell survival, it is toxic to many organs, including kidneys, liver, muscle, bone, and the central nervous system, and leaves patients vulnerable to opportunistic infections. In this study, we demonstrated that HCT established immune tolerance in cxmd canines, permitting stable engraftment of donor muscle–derived cells in the absence of pharmacological immunosuppression. Importantly, donor muscle–derived cells restored dystrophin expression for at least 24 weeks following transplantation.

Although dystrophin protein was detected extensively throughout cryosections from muscle biopsies obtained after cell injection, the level of dystrophin transcript was relatively low. Notably, it has been suggested in exon-skipping studies that in-frame dystrophin mRNA needs to achieve only 10% of total dystrophin levels to revert affected muscle to a mild Becker phenotype.30 However, it is possible that the level of mRNA observed may not accurately reflect the level of protein present.31 Although dystrophin protein is easily detected by antibody staining of muscle cryosections, dystrophin is estimated to represent <0.01% of cytoplasmic RNA present in adult skeletal muscle,32 suggesting that the muscle fiber may produce only limiting amounts of dystrophin transcript, yet translate the message efficiently into protein. As such, measuring the level of dystrophin transcript may not accurately represent the level of functional dystrophin protein.

Recent studies of allogeneic transplantation of 10⁷ cultured donor myoblasts into DMD patients using a “high-density injection” protocol resulted in 3.5–26% of dystrophin-positive fibers within the injection site area at 4 weeks after transplantation,33 and in one patient some muscle regions had 34.5% positive fibers 18 months after transplantation.34 This protocol relied on the use of FK506 to prevent immune rejection. In our study, we demonstrate that injection of 10- to 20-fold fewer freshly isolated cells resulted in dystrophin-expressing fibers that encompassed ~30–70% of fibers within the region of injection. Importantly, sustained engraftment did not depend on immunosuppression. This is a crucial distinction, as side effects of FK506 treatment include tremors, hypertension, renal dysfunction, hypomagnesemia, neurologic toxicity, and long-term toxicity to liver and kidneys.

Although only two dogs were included in our study, it is important to note that results from separate injection sites within the same dog consistently showed a similar pattern of dystrophin expression. Furthermore, dystrophin expression was clearly restored in both chimeric dogs. Given that HCT induces tolerance to other organs, such as the kidney, lung, and small intestine, the small number of animals in this study nonetheless represent a definitive demonstration of allogeneic tolerance to donor muscle-derived cells and
provides an important model from which to further improve dystrophin expression after myogenic cell transplantation.

Achieving greater dystrophin expression requires improving donor muscle–derived cell engraftment. Chimeric *cxmd* canines provide a model in which to optimize donor muscle–derived cell isolation methods and test molecules that stimulate donor cell proliferation or reprogram recipient muscle to enhance fusion. More than 40 years of success in translating results from canines to humans in the clinic highlights that HCT is a clinically relevant platform from which to study and improve myoblast transplantation.

**MATERIALS AND METHODS**

**HCT.** The Institutional Animal Care and Use Committee at the Fred Hutchinson Cancer Research Center, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, approved this study. Elevated enclosed runs were used for housing, and dogs were maintained in social groups wherever possible. All dogs were enrolled in a veterinary preventative medicine program that included routine anthelmintics and a standard immunization series against canine distemper, parvovirus, adenovirus type 2, parainfluenza virus, coronavirus, and rabies.

Mixed breed *cxmd* canines were maintained as a colony at the Fred Hutchinson Cancer Research Center, as previously described. Littermates composed of healthy wild-type donors, and *cxmd* recipient dogs were matched by intrafamilial histocompatibility typing using two polymorphic satellite markers—one located in the class I region and one located in the class II region of the major histocompatibility complex. Matching was confirmed in all cases by DRB1 gene sequencing.

The HCT protocol for *cxmd* canines was described previously. The day of HCT was designated as day 0. Donor marrow cells were aspirated 30 days before transplant, under general anesthesia through needles inserted into the humeri and femora, and cryopreserved. Subcutaneous injections of recombinant canine granulocyte colony-stimulating factor (5 mg/kg b.i.d.; gift from Amgen, Thousand Oaks, CA) into the donor from days −5 to −1 were used to mobilize hematopoietic stem cells from the bone marrow to the peripheral blood. Leukaphereses for collection of the bone marrow to the peripheral blood. Leukaphereses for collection of mononuclear cells released from the muscle were performed on day 0 using a percutaneous central dual-lumen catheter and a continuous flow blood separator (Cobe 2997; Cobe BCT, Lakewood, CO).

On day 0, each recipient received total body irradiation at a single dose delivered at 7 cGy/min from a linear accelerator (Varian CLINAC 4, Palo Alto, CA). One recipient received 200 cGy, and the other received 920 cGy. Within 4 hours of total body irradiation, thawed donor bone marrow cells were injected into the recipient dog to be injected, and the fascia was gently opened to reveal the body of the muscle. Nondissolvable sutures were placed directly in the muscle, and five injections of ~50 µl each were administered into the muscle surrounding suture, covering ~1 cm². The suture was sutured and monitored daily during healing.

**Immunostaining.** Cyrosections were cut (8–10 µm) from frozen muscle biopsies using a Leica CM1850 cryostat, and adhered to Superfrost slides (Fisher Scientific, Pittsburgh, PA). Before staining, sections were fixed in acetone at −20°C for 10 minutes, allowed to dry, and washed in PBS. Sections were blocked in blocking buffer (2% goat serum, 1% bovine serum albumin, 0.1% cold fish skin gelatin, 0.05% sodium azide, and 0.01 mol/l PBS) and incubated in primary antibody diluted in primary antibody dilution buffer (1% bovine serum albumin, 0.1% cold fish skin gelatin, 0.05% sodium azide, 0.01 mol/l PBS). Primary antibodies against dystrophin included Dy8/6C5 and Dy4/6D3 (Vector Laboratories, Burlington, CA). Monoclonal antibodies MANDYS102 (7D2), MANDYS107 (4H8), and MANEX1A (4C7), specific for dystrophin, were developed by Glenn Morris and obtained from the Developmental Studies Hybridoma Bank. Monoclonal antibody D33 specific for desmin was purchased from The University of Iowa, Department of Biological Sciences, Iowa City, IA. Other primary antibodies used were anti-CD45 (FHCRC Biologics, Seattle, WA) and anti-CD14 (Carpinteria, CA). Fluorescein isothiocyanate–conjugated anti-mouse secondary antibody (Jackson Immunoresearch, West Grove, PA) was diluted in 1× PBS. Sections were mounted using Vectashield containing 4′,6-diamidino-2-phenylindole.

Cultured cells were fixed in 4% paraformaldehyde and permeabilized with 0.3% Triton X-100. Cells were blocked in 10% goat serum and incubated with primary antibody diluted in primary antibody dilution buffer. Primary antibodies specific for Pax7 (Atsushi Kawakami), myogenin (FD—Woodring E. Wright), and myosin heavy chain (MF—Donald A. Fischman) were obtained from the Developmental Studies Hybridoma Bank. Monoclonal antibody D33 specific for desmin was purchased.
from DAKO (Carpinteria, CA). Fluorescein isothiocyanate–conjugated anti-mouse secondary antibody (Jackson Immunoresearch) was diluted in 1× PBS. Sections were mounted using Vectashield containing 4',6-diamidino-2-phenylindole.

**RNA isolation and real-time RT-PCR.** RNA was isolated from groups of serial cryosections cut from skeletal muscle biopsies. The cryosections were immediately cut, placed in microcentrifuge tubes kept on dry ice, and stored at −80°C until processing. Cryosections were incubated in RNA Later on ice for 1 minute before RNA isolation. RNA was isolated using the RNeasy Mini kit (Qiagen, Valencia, CA) according to the tissue isolation protocol.

Isolated RNA was quantified using Ribogreen (Invitrogen, Carlsbad, CA) and a fluorometer, and 100µg was reverse transcribed into complementary DNA using random hexamers and the High Capacity Complementary DNA Reverse Transcription kit (Applied Biosystems, Foster City, CA), or gene-specific primers and SuperScriptIII (Invitrogen, Carlsbad, CA). The complementary DNA was amplified using the BioRad iQ5 system, with primers and TaqMan probes specific for dystrophin or β-actin as previously described. C values were converted to relative expression levels using the ΔΔCt method.

**ACKNOWLEDGMENTS**

We thank Szczepan Baran for helping with intramuscular injections and biopsies, and Michael Harkey for chimerism analysis. This work was supported by a Research Development Grant from the Muscular Dystrophy Association (MDA4332, M.H.P.), a Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center Grant HD47175, National Institutes of Health grants P01-NS046788, CA15704, and CA78902, Dystrophy Cooperative Research Center Grant HD47175, National Association (MDA4332, M.H.P.), a Senator Paul D. Wellstone Muscular Dystrophy of dogs. Allogeneic transplants of canine peripheral blood stem cells mobilized by G-CSF-135 and Bcl-2 (1991). Storb, R, Raff, R, Deisseroth, K, Grady, G, Appelbaum, F, Schrier, S, et al. (1999). Dose-rate-dependent sparing of the gastrointestinal tract by fractionated total body irradiation in dogs. Int J Radiat Oncol Biol Phys 38: 2437–2449.

**REFERENCES**

1. Chakkalakal, JV, Thompson, J, Parks, R and Jasmin, BJ (2005). Molecular, cellular, and pharmacological therapies for Duchenne/Becker muscular dystrophies (review). FASEB J 19: 880–891.

2. Novak, KJ and Davies, KE (2004). Duchenne muscular dystrophy and dystrophin: pathogenesis and opportunities for treatment (Review). EBio Rep 5: 872–876.

3. Karpati, G, Poulitou, Y, Zubryczka-Gaam, E, Carpenter, S, Ray, PN, Worton, RG et al. (1989). Dystrophin is expressed in mdx skeletal muscle fibers after normal myoblast implantation. Am J Pathol 131: 27–32.

4. Morgan, JE, Hoffman, EP and Partridge, TA (1990). Normal myogenic cells from newborn mice restore normal histology to degenerating muscles of the mdx mouse. J Cell Biol 111: 2437–2449.

5. Partridge, TA, Morgan, JE, Coulton, GR, Hoffman, EP and Kunkel, LM (1989). Conversion of myofibres from dystrophin-negative to -positive by injection of normal myoblasts. Nature 337: 176–179.

6. Huard, J, Labrecque, C, Danseureau, G, Robitaille, I and Tremblay, JP (1991). Dystrophin expression in myotubes formed by the fusion of normal and dystrophic myoblasts. Muscle Nerve 14: 178–182.

7. Gussoni, E, Pavlath, GK, Lancot, AM, Sharma, KR, Miller, RG, Steinman, I et al. (1992). Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. Nature 356: 435–438.

8. Huard, J, Bouchard, J-P, Roy, R, Labrecque, C, Danseureau, G, Lemieux, B et al. (1991). Myoblast transplantation produced dystrophin-positive muscle fibres in a 16-year-old patient with Duchenne muscular dystrophy. Cns Sci (London) 81: 287–288.

9. Huard, J, Bouchard, J-P, Roy, R, Malouin, F, Danseureau, G, Labrecque, C et al. (1992). Human myoblast transplantation: preliminary results of 4 cases. Muscle Nerve 15: 550–560.

10. Mendell, JR, Kisel, JT, Amato, AA, King, W, Signore, L, Prior, TW et al. (1995). Myoblast transfer in the treatment of Duchenne's muscular dystrophy. N Engl J Med 333: 832–838.

11. Miller, RG, Sharma, KR, Pavlath, GK, Gussoni, E, Minhyer, M, Lancot, AM et al. (1997). Myoblast implantation in Duchenne muscular dystrophy: the San Francisco experience. Muscle Nerve 20: 699–708.

12. Tremblay, JP, Malouin, F, Roy, R, Huard, J, Bouchard, JP, Sato, A et al. (1993). Results of a triple blind clinical study of myoblast transplantsations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. Cell Transplant 2: 99–112.

13. Huard, J, Roy, R, Bouchard, JP, Malouin, F, Richards, CL and Tremblay, JP (1992). Human myoblast transplantation between immunohistocompatible donors and recipients produces immune reactions. Transplant Proc 24: 3049–3051.

14. Friday, BB, Horsley, V and Pavlath, GK (2000). Calcineurin activity is required for the initiation of skeletal muscle differentiation. J Cell Biol 149: 657–666.

15. Semanszian, C, Wu, MJ, Ju, YK, Marciniec, T, Yeoh, T, Allen, DG et al. (1999). Skeletal muscle hypertrophy is mediated by a Ca2+-dependent calcineurin signalling pathway. Nature 400: 576–581.