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Mesenchymal Stem Cells as Anti-inflammatories: Implications for Treatment of Duchenne Muscular Dystrophy

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Abstract

Duchenne muscular dystrophy (DMD) is a lethal X-linked musculodegenerative condition consisting of an underlying genetic defect whose manifestation is augmented by inflammatory mechanisms. Previous treatment approaches using gene replacement, exon-skipping or allogeneic cell therapy have been relatively unsuccessful. The only intervention to mediate improvement in survival, albeit minor, is glucocorticoid treatment. Given this modality appears to function via suppression of underlying inflammation; we focus this review on the inflammatory response as a target for mesenchymal stem cell (MSC) therapy. In contrast to other cell based therapies attempted in DMD, MSC have the advantages of: a) ability to fuse with and genetically complement dystrophic muscle; b) possess anti-inflammatory activities; and c) produce trophic factors that may augment activity of endogenous repair cells. We conclude by describing one practical scenario of stem cell therapy for DMD.

Introduction

Duchenne Muscular Dystrophy (DMD) is a lethal X-linked genetic disorder caused by a deficient dystrophin production. Mutations in the *DMD* gene, or duplications/deletions of its exons appears to be the underlying defect [1]. Dystrophin is a critical component of the dystrophin glycoprotein complex (DGC), which is involved in stabilizing interactions between the sarcolemma, the cytoskeleton, and the extracellular matrix of skeletal and cardiac muscles [2]. A consequence of the DGC inefficiency is the enhanced rate of myofibre damage and subsequent death during muscle contraction. Although satellite cells compensate for muscle fiber loss in the early stages of disease [3], eventually these progenitors become exhausted as witnessed by shorter telomere length and inability to generate new muscle [4]. In the MDX mouse model of DMD, embryonic loss of myocyte progenitors has been described, thus further predisposing for poor compensatory myogenesis [5]. As a result of high demands for myogenesis and poor compensatory mechanisms, fibrous and fatty connective tissue eventually overtake the functional myofibres both in animal models and in the clinical situation. Contributing to this process are inflammatory cell infiltration, cytokine production and complement activation [6, 7]. These changes culminate in progressive muscle wasting, with majority of patients being wheelchair-bound in their early teens. Patients succumb to cardiac/respiratory failure in their twenties, although rare cases of survival into the thirties has been reported [8].

With exception of corticosteroids, which have limited activity and cause numerous adverse effects [9], therapeutic interventions in DMD have had little, if any success. Current areas of investigation include replacement gene therapy with dystrophin [10], induction of exon-skipping by antisense or siRNA to correct the open reading frame of mutated DMD genes [11], and transfer of myoblast or other putative progenitor cells [12-14]. Tremblay's group has been successful at restoration of dystrophin expression using allogeneic myoblasts under the cover of immune suppression, however significant functional benefits have not been reported [15-17].

Development of therapeutic approaches may require understanding not only of the genetic defect and associated cellular pathology, but also the contribution of the host response to the damaged myocytes which appears to perpetuate the deterioration. Accordingly, a brief description of the inflammatory associated changes in DMD is described below.

DMD is Associated with Chronic Inflammation

Muscle degeneration associated with DMD seems to be a multifactorial process in which numerous types of intervention may be envisioned. Although induction of dystrophin expression is paramount to cure, it appears that inflammatory events secondary to myocyte dystrophin mutation also play a major role in disease progression. Intense exercise in wild-type muscles is associated with transient inflammation [18], which is part of a homeostatic process. In contrast, DMD patients are believed to have a prolonged inflammatory milieu subsequent to muscular strain, which appears to

contribute to muscle deterioration [19]. Clinically, DMD onset and progression are known to be associated with upregulation of inflammatory genes [20, 21], which has been confirmed by microarray studies in the MDX mouse model of DMD [22]. It is known that the inflammatory-associated transcription factor NF-kB is upregulated in muscles of both animal models and patients with DMD and that its inhibition in the MDX model results in therapeutic benefit by decreasing macrophage infiltration and permitting a higher level of myogenesis [23]. At the protein level, inflammatory mediators such as TNF-alpha have been detected at elevated systemic levels as compared to healthy controls [24]. In fact, inhibition of TNF-alpha with clinically-used agents such as Etanercept or Remicade has been demonstrated to diminish muscle deterioration in the MDX mouse [25, 26]. It is therefore conceivable that soluble inflammatory factors contribute to progression of degeneration by direct inhibition of muscle function [27], as well as elicitation of immunological cells to area of muscle damage [28].

The possibility that local inflammation is occurring as muscle damage progresses is confirmed at a cellular level by observations of immune cell infiltration. For example, monocytic infiltration occurs with such selectivity to degenerating muscles that these cells have been proposed as vectors for delivery of gene therapy [29]. In that study it was demonstrated that labeled monocytes selectively infiltrated areas of acute muscle damage induced by local freezing injury. In the MDX model, which in contrast to the freezing injury model, has a more chronic muscle deterioration, monocyte migration to the area of myofiber damage was also observed. Another study using the same model showed that the initial period of muscle destruction, which occurs at about 4 weeks of age, is associated with macrophage infiltration directly adjacent to areas of necrosis. A causal relationship was proposed given that inhibition of macrophage nitric oxide resulted in reduction of necrosis [30].

An important question is, why would inflammatory macrophages enter the muscle? Is it as a result of necrotic/apoptotic tissue already present, or a chemotactic signal secreted by the injured muscles, or a combination? The cytokines IL-6, MCP-1, and IP-10 where identified as potential mediators [30]. In the diaphragm of the MDX mouse, which is one of the muscles most injured due to repeated physical activity, MIP-1 alpha and RANTES are expressed by the muscle itself [31]. Furthermore, other studies have confirmed expression of these, and also the monocyte-chemattractant CCL6 in dystrophic limb muscle, thus suggesting upregulation of chemokine synthesis may be a systemic occurrence in DMD [28]. Actual transmigration of monocytes may be mediated by VCAM-1 expression on the endothelium, which has previously been shown to attract CD133 positive stem cells into exercised dystrophic muscle [32], but is also a known ligand for leukocyte expressed VLA-4.

Further involved in the self-perpetuating inflammatory cascade is the renin-angiotensin system which increases the fibrotic cytokine TGF-b [33], and upregulation of TNF-alpha which is directly toxic to myocytes [34, 35]. Furthermore, the active production of these inflammatory factors by infiltrating macrophages has been shown to play a large role in disease progression. M1 macrophages have been demonstrated to directly kill myocytes in vitro, whereas healing of muscles is associated with M2 macrophages, thus

manipulation of this overall inflammatory state may be a potential area of intervention [30].

Another component of the inflammatory process is fibrosis. The increased fibrotic state of muscles in DMD is associated with upregulated expression of MMP inhibitors such as TIMP1 and 2 in patients [36]. Modification of the MMP/TIMP ratio by administration of MMP overexpressing cells has yielded therapeutic benefit in the mdx model, which were associated with increased neovascularization [37]. In fact, altered blood vessels were cited as a possible cause of DMD in historical literature [38].

Modulation of Inflammation is Beneficial in DMD

The only clinical intervention appearing to have positive, albeit, mild effects is corticosteroid therapy, which has been shown in numerous trials to inhibit long-term muscle deterioration [9], and even induce short term functional improvement [39, 40]. Efficacy of this approach seems to be associated with inhibition of ongoing inflammatory reactions that contribute to muscle degeneration. Here we will discuss some effects of inhibiting inflammatory reactions in the context of DMD.

In addition to their well-studied immunological functions, the macrophage plays a significant role in tissue remodeling. For example, macrophages are critical for angiogenesis, tissue regeneration, and reduction of fibrosis [41]. In the context of DMD macrophages play both a reparative and destructive role depending on context. Broadly speaking there are two types of macrophages distinguished based on cytokine production and arginine metabolism. M1 macrophages are primarily antiangiogenic, characterized by high levels of nitric oxide production, and possess cytotoxic activity, whereas M2 macrophages generally are anti-inflammatory, support angiogenesis, and associated with tissue repair [42]. This concept has been demonstrated in situations such as cancer, in which M2 tumor infiltrating macrophages play an important role in neovascularization and immune evasion [43]. In contrast, stimulation of M1 macrophages has been shown to inhibit tumor growth [44]. This dual ability of macrophages to promote either damage or healing has been observed in other biological systems, for example, administration of M1 macrophages accelerates adriamycin-induced kidney failure whereas M2 macrophages are protective [45].

In the context of DMD, M1-like macrophages are found infiltrating the dystrophic muscle, and inhibition of this phenotype through blockade of the NF-kB pathway results in amelioration of disease [23]. Another method of altering the M1 to M2 macrophage state is through exposure to the cytokine IL-10. Treatment of macrophages with this cytokine reduces ability to cause muscle damage and augments regenerative activity through alteration of arginine metabolism to reduce nitric oxide production and augment polyamine synthesis [30]. The dual role of macrophages is further supported by studies in which macrophage conditioned media, in absence of inflammatory stimuli, was capable of eliciting ex vivo myoblast expansion [46].

Modulation of other innate immune components has been successful at altering disease progression. For example, neutrophils are associated with progression of pathology, and interventions such as blockade of osteopontin result in disease inhibition and reduction of neutrophil infiltration [47]. Direct depletion of neutrophils using antibodies has been demonstrated to significantly reduce pathology in the MDX model [35]. Mast cells have also been demonstrated to be involved in muscular deterioration in that daily cromolyn injections to prevent mast cell degranulation results in reduction of basal and exercise-induced myofibre necrosis [48].

T cell immunity is also known to contribute to DMD progression. Suggesting this possibility at a clinical level, Kissel et al found that in a double-blind trial of prednisone significant decreases in lymphocytic infiltrates in muscle biopsies were observed in the treated but not control patients [49]. These clinical observations have also been described in animal studies where immune suppressants such as cyclosporine A, which targets T cells, have been demonstrated to reduce progression of pathology [50]. T cells are believed to be associated with stimulation of TGF-b and augmentation of fibrosis. For example, it was demonstrated that depletion of T and B cells results in reduction of myocytic damage in SCID mice that have been bred onto the MDX background [51]. Studies in which thymic tissue was transplanted into T cell deficient MDX mice confirmed the critical role of T cells in fibrosis [52]. Dystrophic muscles express upregulate expression of MHC I [53, 54], which may be the result of local inflammatory cell activation. There is some evidence of a direct autoimmune component in DMD in that IgG anti-muscle antibodies, indicating class-switching had occurred [55]. In fact, some studies suggest that muscular inflammation may be transferred into naïve recipients by administration of T cells from dystrophic mice together with muscle extracts [56]. These data would suggest the T cell compartment not only acts as a passive response to dystrophic injury but may play a more substantial role. Mechanistically, T cells appear to mediate muscle damage through secretion of osteopontin [47], which promotes fibrosis, as well as direct perforin-mediated cytotoxicity [57].

Cellular Therapy of DMD

Before describing how MSC therapy may beneficially affect DMD progression by concurrent inhibition of inflammation and dystrophin positive muscle regeneration, we will discuss previous work in cell therapy for this condition. Initial attempts at cellular therapy for DMD have focused on administration of muscle precursor cells, particularly allogeneic, dystrophin-expressing myoblasts that have been expanded in culture. One of the original descriptions of this therapy was published in 1991 in which 3 DMD patients where injected with 8 million allogeneic myoplasts into the extensor digitorum brevis (EDB) muscle under the cover of cyclosporine. Mild increase in tension in the injected but not contralateral control muscles was observed as well as expression of dystrophin and reduction in microscopic characteristics of DMD pathology [58]. The same group expanded on the approach in a Phase II trial of 21 patients administered 5 billion myoblasts in 48 intramuscular injections into 22 major muscles. Patients were treated using cyclosporine to prevent rejection. Thirteen of the patients were assessed for improvements in muscle strength, of 69 muscle groups (knee extensors, knee flexors,

plantar flexors) tested. As compared to pre-treatment, three months after cell administration 43% of patients showed a mean increase in strength of 41.3% +/- 5.9, 38% of patients did not exhibit changes and 19% had diminished muscle capacity of 23.4% +/- 3.1. Not adverse effects were associated with administration [13].

Increasing frequency of cell administration was performed in a 12 patient study involved 6 monthly injections of 110 million fraternal or paternal derived myoblasts into the biceps brachii muscles of one arm with the contralateral arm serving as a control, with half of the patients receiving cyclosporine and the other half placebo. Six months after the last injection one patient expressed dystrophin in 10.3 percent of muscle fibers, while 3 other patients had < 1% and the remaining 8 were negative. No increase in muscle strength was observed [14].

Given the undesirable effects of systemic immune suppression, Tremblay et al attempted myoblast transplant in 5 patients without cyclosporine. Administration of myoblasts was performed into one biceps brachii with the opposite biceps brachii as a control. No increase in isometric force was observed during the 2-18 months period post cell injection. In the biceps brachii of both sides 6 mo after the transplantation, less than 1.5% of dystrophin-positive fibers were detected. Interestingly, complement-fixing antibodies were identified in all patients post-injection that had ability to lyse myotubes. The authors concluded that immune suppression was necessary for allogeneic myoblast transplants [59].

Several other studies have been conducted using a variety of dosing regimens. Karpati et al used cyclophosphamide immune suppression in 8 DMD patients receiving a dose of 55 million cells in the biceps. No functional improvement or dystrophin expression was reported [60]. Law et al reported a 32 patient study using 48 injections of a total of five billion myoblasts were transferred into 22 major muscles in both lower limbs, Nine months after cell transplant 60% of the 60 ankle plantar flexors (AF), examined showed an average increase of 50% in force; 28% showed no change; and only 12% showed a mean decrease in force of 29% when compared to the function of the same muscles before transplantation of cells. Unfortunately, when the results of all muscle groups tested were compared, there was no change in force at 3, 6, or 9 months post transplant [61]. Miller et al. administered 100 million myoblasts in the anterior tibial muscle of one leg and placebo in the other leg of 10 patients. Muscle force increased in both legs, which the authors attributed to cyclosporine effects. Of the 10 patients, myoblast survival and dystrophin mRNA expression was observed in 3 patients after 1 month and only in 1 patient after 6 months [62]. Relatively, the major advancement in allogeneic myoblast transplant came from the group of Tremblay which developed a high density injection methodology in which cells are injected at a distance of 1 to 2 mm from each other. Using this approach temporarily higher increases in the percentage of myofibers expressing donor's dystrophin was seen in comparison to other protocols, with expression varying from 3.5% to 26% [16]. A more recent study by the same group demonstrated actually an increase in the number of donor-derived dystrophin being expressed in the myofibers, with 27.5% at 1 month after transplant and 34.5% 18 months. Unfortunately significant improvement in strength was not reported [15].

Mesenchymal Stem Cell Therapy Preventing Inflammation and Accelerating Healing

As seen from the above discussion, one of the major problems with allogeneic myoblasts therapy is associated with immune rejection. In fact, the process of rejection may actually be involved in acceleration of dystrophic progression due to increased inflammation during the rejection process. Mesenchymal stem cells (MSC) are conventionally defined as adherent, non-hematopoietic cells expressing markers such as CD90, CD105, and CD73, and being negative for CD14, CD34, and CD45. While originally identified in the bone marrow [63], MSC have been extracted from numerous tissues including adipose [64], heart [65] , Wharton's Jelly [66], dental pulp [67], peripheral blood [68], cord blood [69], menstrual blood [70-72], and more recently fallopian tube [73].

One of the major properties of MSC is ability to differentiate into various tissues. The traditional, or "orthodox" differentiation properties of MSC are their ability to become adipocytes, chondrocytes, and osteocytes in vitro after treatment with induction agents [74]. Non-orthodox differentiation into other tissues, for example, cells resembling neurons [75, 76], muscles [77], hepatocytes [78] and pancreatic islets [79], has also been reported. There is some evidence that MSC may differentiate selectively into tissues that have been injured. For example, Tao et al systemically administered MSC clones into immune deficient mice subsequent to carbon tetrachloride hepatic injury. Differentiation into albumin-expressing hepatocyte-like cells was observed [80]. Similar specific differentiation of non-induced MSC into injured tissue has been demonstrated in post myocardial infarct models [81, 82], in stroke [83], kidney damage [84], pulmonary fibrosis [85], and bone fractures [86]. Several chemokine signals appear to be associated with MSC migration to injured tissue. Stromal Derived Factor (SDF)-1 seems to be a ubiquitous MSC chemoattractant associated with a plethora of diverse tissue injuries ranging from noise induced auditory spiral ligament damage in the cochlea of the ear [87], to burn injury [88, 89], to bone fractures [90]. Most commonly studied is the critical role of SDF-1 stimulation of stem cell homing to areas of hypoxia. In many injury situations such as myocardial infarction or stroke, SDF-1 has been demonstrated to be associated with mobilization of stem cells into the periphery and homing to the site of injury [91, 92]. Thus the ability of MSC to differentiate into various injured tissue, including muscle, as well as ability to complement dystrophin deficiency [93], makes them an attractive therapeutic candidate for DMD.

The use of mesenchymal stem cells as inhibitors of inflammation is conceptually appealing. In the bone marrow it has been speculated that one of their main functions is the protection of hematopoietic precursor from inflammatory damage [94]. This potent activity of MSC is best exemplified in an experiment where these cells were capable of inhibiting one of the most potent inflammatory processes, septic shock. The investigators demonstrated that administration of bone marrow derived MSC was capable of increasing survival in the lethal cecal-puncture ligation murine model through modulation of macrophage activity [95]. More inhibition of chronic inflammatory processes such as models of autoimmune arthritis [96, 97], diabetes [98, 99], multiple sclerosis [100, 101],

and lupus [102], has been well documented by syngeneic, or in some cases allogeneic MSC.

Mechanistically, MSC play multifactorial roles in terms of controlling inflammation. They have ability to selectively home towards damaged tissue via expression of receptors for SDF-1, lysophosphatidic acid [103], and CCL2 [104]. Conceptually, once homed to the area of tissue damage, they act to regulate inflammatory associated biological processes such as: a) suppressing macrophage activation [105, 106]; b) inhibiting Th17 generation [104]; c) inhibit Th1 cell generation [107]; d) suppressing NK and T cytotoxic cell function [108]; e) stimulating generation of Th2 cells [109]; f) inducing generation of Treg cells [110] and g) eliciting suppression of DC maturation [105, 111]. Mechanistically, MSC suppress various immune functions through: a) release of immune suppressive cytokines such as IL-10 [112, 113], TGF-beta [114], and LIF [115]; b) express the T and NK inhibitory enzyme indolamine 2,3 deoxygenase [116]; c) produce soluble HLA-G [117]; and d) express contact-dependent inhibitory molecules such as PD-1L. Importantly, these immune modulatory properties seem to be inducible preferentially in the presence of an active immune response [117-120].

Given these anti-inflammatory properties, MSC have been used in numerous clinical applications with various degrees of success. Despite the recent failure of Osiris's two Phase III clinical trials in graft versus host disease (GVHD) [121], several academic Phase I and II trials of MSC demonstrated efficacy in this indication [122-127], it may be that differences in MSC culture protocols may have contributed to discordant results. Other studies have used MSC in treatment of osteogenesis imperfecta [128], Hurler syndrome, metachromatic leukodystrophy [129], amyotrophic lateral sclerosis [130], SLE [131], liver failure [132], perianal fistula [133], and acceleration of hematopoietic stem cell engraftment [134-136].

Therapeutic effects of MSC are believed to occur not only by direct differentiation into injured tissue but also by production of paracrine factors that inhibit apoptosis, stimulate endogenous cell proliferation, and/or activate tissue resident stem cells in the site of injury. For example, MSC exert renoprotective effects in a model of cisplatin-induced kidney failure primarily through secretion of insulin like growth factor (IGF)-1, which prevents apoptosis of proximal tubular epithelial cells [137]. IGF-1 has also been demonstrated to play a critical role in MSC amelioration of post-myocardial infarct damage [138]. Keratinocyte growth factor (KGF)-1, has been demonstrated to be responsible for MSC-mediated protection of endotoxin induced pulmonary injury [139].

Mesenchymal Stem Cell Therapy of DMD

Given the regenerative and anti-inflammatory effects of MSC, several studies have used this population in animal models of DMD. Early studies transplanted bone marrow or fetal liver ROSA cells into MDX mice in utero at day 14 of pregnancy [140]. Engrafted donor cells were found in multiple sections from hindlimb skeletal muscles, diaphragms, and hearts from both stem cell sources. No alteration in muscle function was made, however the study demonstrated feasibility of restoring functional muscle cells from a

stem cell source. A subsequent study administered human fetal derived MSC into MDX recipients in utero at days 14-16 of pregnancy. A similar distribution of cells into the major muscles was observed, however degree of chimerism was minor (less than 1 %) [141]. An flk-1 positive adipose derived mesenchymal stem cell population was demonstrated to selectively home to necrotic muscle fibers in MDX mice, with some demonstration of dystrophin regeneration. The authors did not show functional improvement, however did show muscle neovascularization, which they believe may have been associated with muscle remodeling [77]. Administration of rat bone marrow MSC intravenously into irradiated MDX mice lead to improvement in serum chemistry as judged by decreased serum creatine kinase (CK) and centrally nucleated fiber (CNF) [142]. More therapeutically relevant would be the administration of allogenic MSC in an intramuscular manner. Using the delta-sarcoglycan-deficient dystrophic hamster model intramuscular injections of human and pig derived MSC were performed. No upregulation of inflammatory cytokines, serum markers, or intramuscular NF-kB was observed. Reduction in muscular oxidative stress and entry of myocytes into cell cycle was reported. Additionally, the MSC remained viable intramuscularly and where associated with new muscle fibers, as well as neocapillary formation [143]. This study supports at least the safety aspects of administration of mismatched MSC into dystrophic muscles. Unfortunately, the majority of studies using MSC in animal models did not report significant, if any, upregulation in muscle contractile force [144].

Case Report of Stem Cell Therapy for DMD

An alternative approach towards DMD treatment involves combination of MSC with other possibly therapeutic cells, as well as utilization of MSC types that may have differential properties than conventional bone marrow derived cells. An MSC-like cell, the endometrial regenerative cell (ERC) has been demonstrated to express higher levels of matrix metaloproteinases (MMPs) and angiogenic activity as compared with other MSC [70, 145]. Distinct myogenic proclivity, as well as ability to induce dystrophin expression has been reported using cells similar to ERC [146, 147]. One-year safety follow-up has been published with allogeneic ERC administered intravenously and intrathecally [148]. Synergy has been reported in animal and pilot cases between hematopoietic and mesenchymal stem cells [149, 150].

We report a case study of a 23 year old male diagnosed with DMD at age 3, who manifested a progressive decrease in muscular strength and became wheelchair bound at age of 12. Supportive treatment with intermittent courses of prednisone, pain management, along with physical therapy was provided. Frequent respiratory infections secondary to a poor respiratory effort with decrease clearance of the secretions were managed with standard antibiotic therapy. On August 5-14th, 2008, the patient was treated with a combination of ERC and CD34 umbilical cord blood, mixed lymphocyte reaction-matched positive cells, subsequently on November 25-28, the patient received another course of therapy including placental matrix derived mesenchymal stem cells (Table 1). Cells were prepared and administered as previously described by us [148, 149]. No adverse events were associated with the stem cell infusion. A significant increase in muscle strength occurred in all the muscle groups, and was accompanied by an increase

in the functional capacity of the patient. Thus, a pre-transplantation strength of 2-2.5/5 in the neck, shoulder, upper, and lower extremities began to improve after each of the two stem cell administrations, and reached a final 4/5 level 1 month after second transplantation treatment. The increments in muscle strength after the two stem cell administration appeared to be additive, with most benefit recorded after the second. Upper extremity improvement in strength evolved from the incapacity to lift against gravity before the transplantation towards the ability to lift 2 lbs weights after the procedure. Trunk balance and strength were also markedly improved. The patient gained 20 lbs, along with an increased general activity level. The frequency of respiratory infections decreased from 3-4/year before stem cell therapy to none. The inspiratory effort improved from -32 to -40 cm H_20. A muscle biopsy taken in January 2009 demonstrated normal (>50%, normal = 50-100% expression of normal-molecular size) levels of muscular dystrophin. The improvement in muscular strength, clinical respiratory function, and general level of activity are maintained to date.

To our knowledge this is the first report of profound dystrophin expression occurring in a non-ambulatory DMD patient after ERC treatment. One question that arises is whether cell therapy in this case can achieve a level of selectivity for injured muscles. It is known that CD34 cells express VLA-4, which is the ligand for VCAM-1, whose expression is elevated in dystrophic muscles [32]. Furthermore, CD34 chemokines such as MIP-1 alpha and RANTES expression is found in dystrophic muscle [31]. It may therefore be possible that local intramuscular MSC be able to add chemotactic/trophic support for the intravenously administered CD34. In fact, it is reported that mesoangioblasts, which reside within the CD34 population, as well as cord blood CD34 cells have had positive activity on DMD in animal models [151-153], although this appears to be short-lived. If mesoangioblasts are the main contributors to de novo myogenesis, it may be possible that intramuscular ERC, or alternatively MSC, administration may provide a more suitable environment for muscle regeneration. Muscle-derived CD133 mesoangioblast-like cells have already been used clinically with mild degree of success [12], it may be promising to explore combinations of these, or perhaps even myoblasts, with ERC/MSC in order to augment therapeutic efficacy by suppressing local inflammation.

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TABLE I

Stem Cell Administration Scheme				
Date	**CD34+ IV**	**ERC IM**	**ERC IV**	**MSC IV**
First Cycle				
Aug 5		12 million		
Aug 6		12 million		
Aug 7		12 million		
Aug 11		12 million		
Aug 12		12 million		
Aug 13	3 million			
Aug 14	3 million			
Second Cycle				
Nov 25		8 million		
Nov 26	1.5 million	20 million	6 million	
Nov 27	1.5 million			3 million
Nov 28		28 million		