Chapter

Cell Therapy for Muscular Dystrophy

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Abstract

Muscular dystrophy is a major unmet medical need associated with an inevitable progressive muscle damage and loss of function. Currently, treatment is only symptomatic and supportive. This chapter focuses on cell therapy as a potential treatment approach for muscular dystrophy. Mechanism of action of cell therapy and its ability to alter disease pathology have been discussed. A review of preclinical and clinical studies has been presented with the advantages and shortcomings of various cell types. Rationale for our treatment protocol and experience of treating muscular dystrophy patients has been discussed. Our published results have shown the efficacy of the intrathecal and intramuscular administration of autologous bone marrow mononuclear cells in different types of muscular dystrophy patients. The scores on outcome measures such as 6-minute walk distance, North star ambulatory assessment, Brooke and Vignose scale, Functional independence measure, and manual muscle testing either improved or were maintained suggestive of slowing down disease progression. Efficacy and safety of the treatment was also studied using comparative MRI-MSK and EMG showing decreased fatty infiltration in various muscles post-cellular therapy. Thus, it was found that autologous BMMNC transplantation is a safe and effective treatment option and improves the quality of life of MD patients.

Keywords: muscular dystrophy, stem cells, cell therapy, autologous, bone marrow mononuclear cells

1. Introduction

The term 'muscular dystrophy' first used by Erb (1891), is used for a heterogeneous group of disorders that are hereditary in nature and are characterized by primary involvement of muscles and a tendency for progressive muscle weakness and wasting [1]. They are inherited as X-linked, autosomal dominant, or recessive disease. Over 30 variants of muscular dystrophy (MD) have been identified using genetic or histochemical testing [2, 3]. Most commonly found types of MDs are Duchenne Muscular Dystrophy

(DMD), Becker Muscular Dystrophy (BMD), Limb Girdle Muscular Dystrophy (LGMD), Emery-Dreifuss Muscular Dystrophy (EDMD), Fascioscapulohumeral Muscular Dystrophy (FSHMD), Oculopharyngeal Muscular Dystrophy (OPMD), and Congenital Muscular Dystrophy (CMD). The overall worldwide prevalence of combined MDs is estimated to be 3.6 per 100,000 individuals [4].

Although the onset of symptoms and the rate at which disease progresses is variable and depends mainly on the gene mutation [5], MD is associated with an inevitable progressive muscle damage and loss of function. Loss of ambulation, contractures and deformities are common. Scoliosis is frequently seen in wheelchair-dependent patients. Although the disorder primarily affects skeletal muscles, structural and functional abnormalities are also known to be seen in cardiac muscle, smooth muscle, and the brain [6–8]. Impairment of respiratory function due to weakness of the respiratory muscles is frequent. Cardiac involvement is a feature commonly seen in DMD, BMD, myotonic dystrophy, LGMD, EDMD and CMD. Functional involvement of the brain has also been seen in CMD, myotonic dystrophy, DMD and in some LGMD variants. About one-third boys with DMD have co-morbid mental retardation and other behavioral or psychiatric comorbidities [9, 10].

Currently, treatment is mainly symptomatic and supportive. Though corticosteroids delay loss of function, it is ineffective in stopping progression in MDs. Steroids only reduce inflammation and long-term use is associated with side effects including stunted growth, cataracts, and osteoporosis [11]. MDs represent a major unmet medical need and are associated with progressive disability causing economic and personal burden. Many of the MDs result from mutations in the genes encoding components of the dystrophin-glycoprotein complex (DGC) [12] that links the extracellular matrix of muscle fiber with the F-actin cytoskeleton. The DGC plays an important role in providing mechanical support to the plasma membrane during muscle fiber contraction and is thought to protect muscle fibers from contraction induced damage [13, 14]. Its disruption leads to altered mechanical and signaling functions resulting in increased entry of calcium, immune cell infiltration, progressive muscle wasting, necrosis, and membrane fragility (**Figure 1**) [15, 16]. Gene therapy using viral and non-viral vectors can be a promising treatment option for MD but shows adverse immune responses to vectors raising concerns regarding safety of the treatment [17]. Antisense oligonucleotide (ASO) mediated exon skipping therapy is also a treatment option for MD which targets removal of introns from pre-mRNA to give functional proteins. Currently ASOs for mutations in dystrophin gene amenable to exons 51,53 and 45 are approved by Food and Drug Administration (FDA) which showed the resumption of dystrophin production in MD patients. But the expense of ASOs, its off-target effects and its delivery are current concerns regarding their use [18].

Although the initial cause of muscle damage is genetic in origin, there is now increasing evidence of stem cell dysfunction in MD [19–21], as a contributing factor to the progression of the disease. Blau et al. reported a defect in the proliferative capacity of resident stem cells in DMD [19]. Also, there is increased inflammatory responses in MD patients that disrupt muscle homeostasis and inhibit muscle repair and regeneration [22–24]. It is observed that in DMD and several other forms of MD, the regenerated muscles are prone to degeneration, producing repeated cycles of degeneration and regeneration. As a result, the resident stem cell population is either exhausted or loses the potential to mediate repair leading to progressive replacement of muscle tissue with adipose and fibrotic tissue [25]. Therefore, gene therapy alone

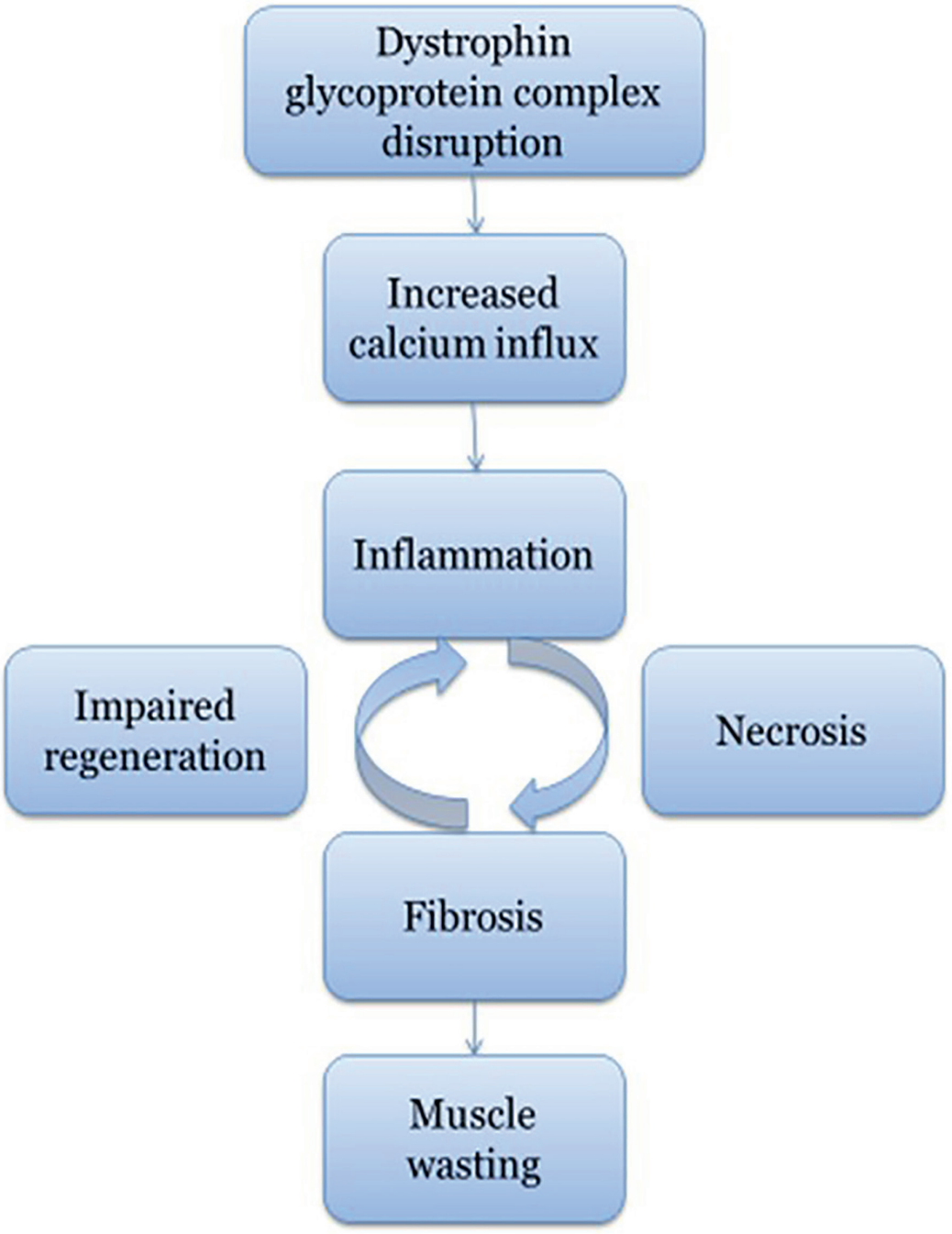

Figure 1.
Sequelae of DGC disruption in MD.

is inadequate as it cannot replenish the stem cell pool and even where applicable and available clinically, needs to be combined with treatment approaches that replenish the stem cell pool.

The objective of any effective treatment lies in restoring dystrophin expression in muscle fibers and in promoting regeneration of muscle fibers. While dystrophin restoration can be achieved by gene therapy or cell therapy or combination of the two, regeneration of muscle can be achieved through cell therapy only and it, therefore, represents an essential treatment approach for MDs.

2. Pathophysiology of MD

As described earlier, the core pathophysiology of MD (**Figure 2**) is genetic mutations resulting in altered expression of proteins in DGC and stem cell dysfunction. DGC is the essential component of the cell wall; its alteration leads to increased cell damage even with minimal contractile stress. Muscle damage is repaired by resident muscle stem cells also known as satellite cells. Progressive damage of the muscle is repaired with continuous satellite cell mediated regeneration process which leads to depletion in the satellite cells pool. Due to which there is an increased muscle damage leading to adipose tissue infiltration causing muscle weakness as shown in **Figure 3** [26]. Increased damage also increases the immune cell infiltration resulting in increased inflammation [27]. Inflammation accelerates cell apoptosis and necrosis in the damaged areas [28]. Also, in MD the muscle function is affected because of impaired blood supply to the muscle leading to chronic ischemia. The DGC is also involved in the formation of synaptic connectivity, abnormal DGC leads to impaired neurotransmission and abnormally formed

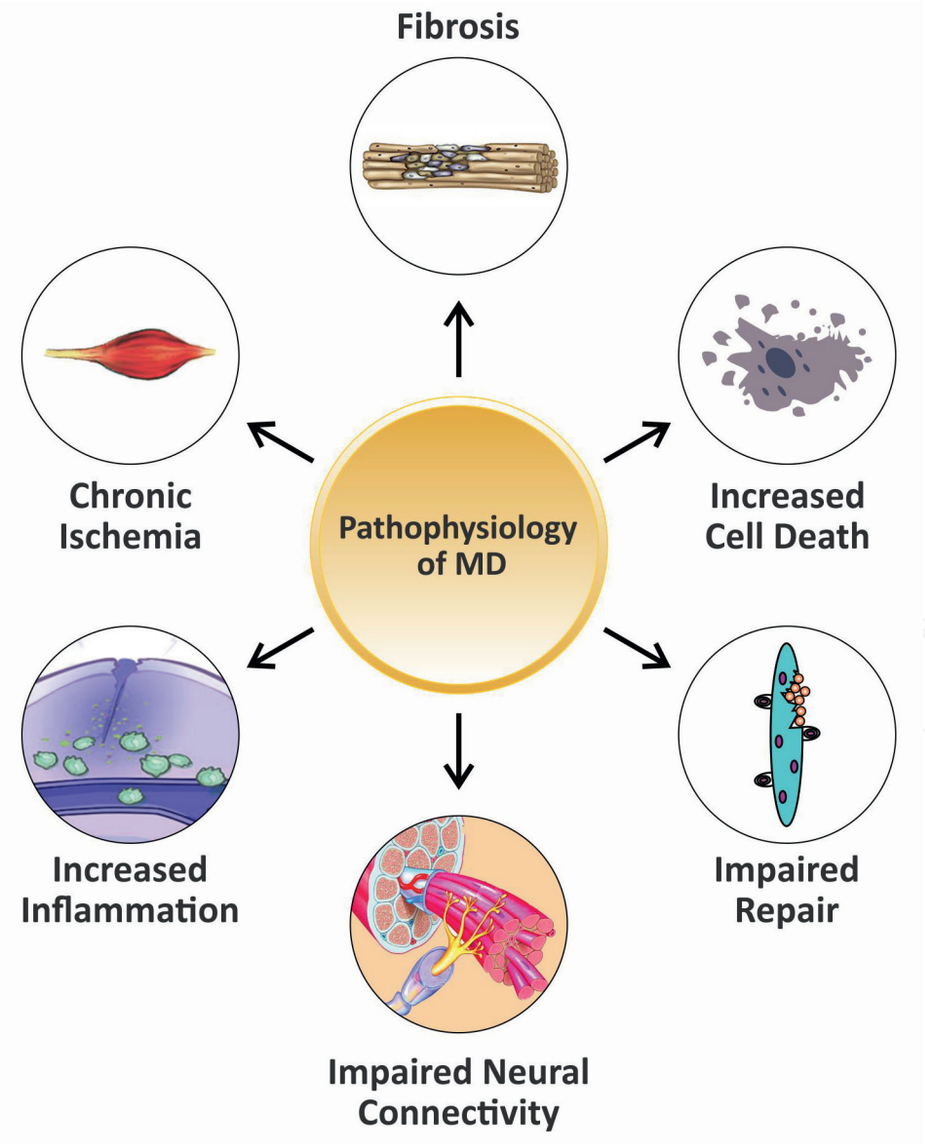

Figure 2.
Pathophysiology of MD.

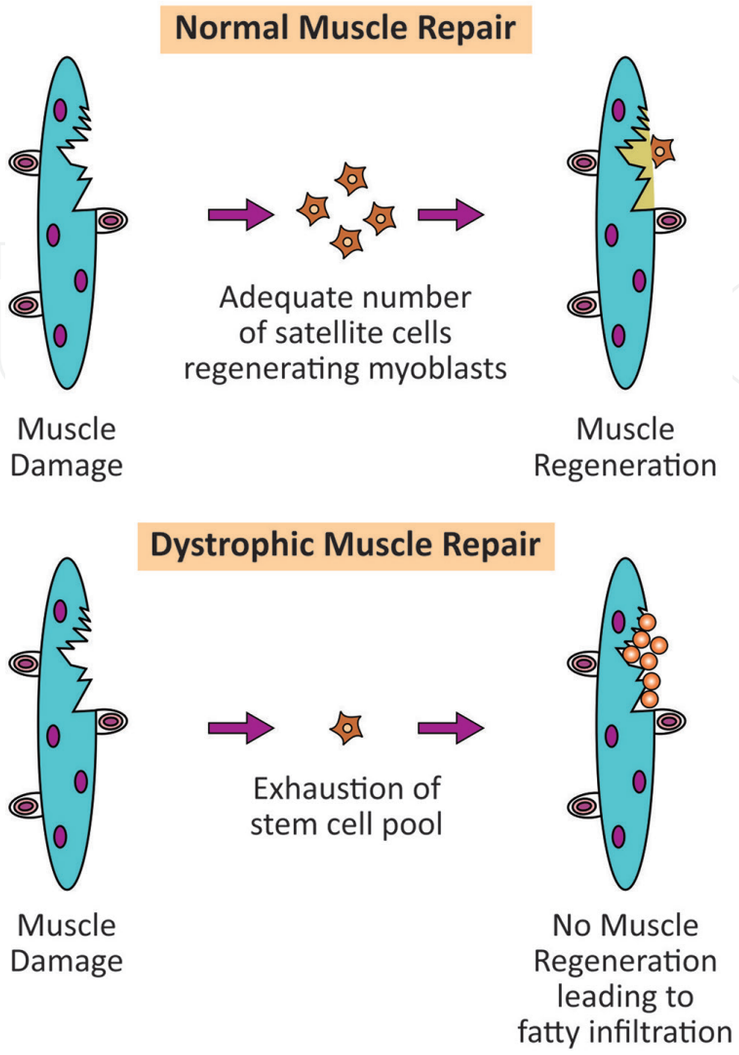

Figure 3.
Normal muscle repair having adequate number of satellite cells versus dystrophic muscle repair demonstrating exhaustion of stem cells.

neural junctions increasing muscle wasting [29, 30]. Hence, the pathophysiology of MD is Multifaceted having multiple contributing factors.

3. Role of stem cells in MD

Stem cells have the ability of self-renewal and migration to the site of damage/injury and carry out repair and restoration processes. They divide and differentiate to replace the damaged and dead cells [31]. In Vitro experiments have shown that stem cells restore dystrophin expression in duchenne-skeletal muscle cells [32]. Stem cells halt further damage by exerting paracrine mechanisms and stimulate endogenous cells to carry out repair processes. Stem cells secrete various cytokines and chemokines exerting anti-inflammatory, anti-apoptotic, angiogenic and immune-modulatory effects [33]. Effectiveness of cell therapy depends on various factors such as number of cells, route of delivery, myogenic potential, migration and homing capabilities of cells, type of MD and extent of muscle damage. Cell therapy can be a promising long-term solution for MD [34].

4. Understanding stem cells

Stem cells are unique undifferentiated cells characterized by their ability for self-renewal and for differentiation into specialized cell lineages [35].

4.1 Classification of stem cells

4.1.1 Based on potency

Stem cells can be classified according to their ability to differentiate as (**Figure 4**):

- *Totipotent stem cells* that can differentiate to form any tissue including entire organism;
- *Pluripotent stem cells* that can differentiate to all tissue types but not to an entire organism;
- *Multipotent stem cells* that can differentiate into multiple cell types but within one organ system only;

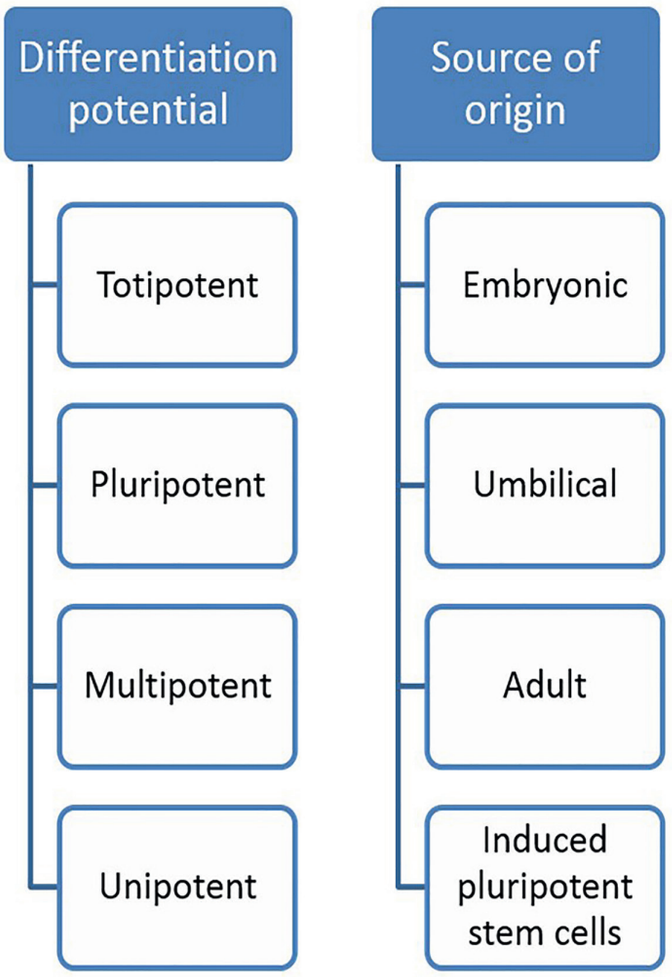

Figure 4.
Classification of stem cells.

- *Unipotent stem cells* are highly specialized stem cells that can differentiate into one cell type committed to a single lineage [36].

4.1.2 Based on their source

Depending on the source, they can be differentiated (**Figure 4**) into

- Embryonic stem cells (ESCs) are pluripotent stem cells derived from inner cell mass of blastocysts.
- Umbilical cord stem cells can be easily derived from umbilical cord without any risk to the donor.
- Adult stem cells are multipotent cells and can be derived from bone marrow, adipose tissue etc.
- Induced pluripotent stem cells (iPSCs) are produced by reprogramming differentiated multipotent adult stem cells to the pluripotent state that can differentiate into any cell of the organism.

Human ESCs are faced with ethical considerations and are also associated with a risk of tumorigenicity whereas umbilical cord stem cells and adult stem cells do not possess ethical concerns.

Cells procured from the patients themselves are autologous cells. Stem cells acquired from donors are allogenic cells. Autologous cells are safer than allogenic cell as they do not show immune rejection while proper HLA typing of donor and recipient is required in case of allogenic cells.

5. Cell therapy as a therapeutic strategy for MD

5.1 Preclinical evidence of effectiveness of cell therapy in MD

5.1.1 Induced pluripotent stem cells

Therapeutic use of ESCs is restricted due to immune rejection and ethical concerns. These concerns have however been overcome partly by Yamanaka et al. by showing that iPSCs can be generated from somatic cells [37]. Pre-clinical studies have shown the ability of myoblasts and mesenchymal stem cells (MSCs) derived from iPSCs to fuse with mature muscle fibers [38, 39] and improve muscle function in dystrophic mice [40].

5.1.2 Stem cells of muscle tissues

Another type of cell that is Myoblast cells, naturally residing in the muscles, are the primary skeletal muscle stem cells responsible for maintenance of resident stem cell pool by self-renewal and repair of adult skeletal muscle and were the source of stem cells in the earliest cell-based therapies for treating MDs [41]. Pre-clinical studies demonstrated that myoblasts can be expanded in vitro [42, 43] and are able to regenerate muscle [44–46]. Stem cells other than myoblasts that are present within

muscle and possess myogenic potential include muscle derived stem cells [47–49], mesoangioblasts [50–52], muscle derived stem cells, pericytes, CD 133+ stem cells and muscle side population cells that can contribute to muscle regeneration [53]. Transplantation of allogenic muscle derived stem cells contributed to muscle repair, dystrophin expression, satellite cell replenishment and clinical efficacy in MD dogs [54]. Recently, mesoangioblasts, pericytes and CD 133+ have demonstrated promise as stem cell source in treatment of MDs due to their ability to contribute to muscle regeneration and because they can be delivered systemically. Also, mesoangioblasts can reconstitute the satellite cell pool [55]. The ability of mesoangioblasts to contribute to muscle regeneration and to restore muscle structure and function has been tested in the mouse model of LGMD [56, 57] and in the dog model of DMD [58]. Tedesco et al. demonstrated expression of normal dystrophin in muscle fibers and production of functional muscle fibers in mice model of DMD following intramuscular injection of genetically corrected mesoangioblasts [55]. However, the disadvantage of these cell types is their limited ability to colonize in the muscles due to incomplete adhesion and extravasation of these cells [52, 56]. Pericytes are known to be developmentally derived from mesoangioblasts [50, 59], possess myogenic potential and have shown to promote muscle regeneration in dystrophic mice following intra-arterial delivery [59, 60]. Though promising, further research of the role of mesoangioblasts and pericytes in muscle regeneration are required. Torrente et al. demonstrated that CD 133+ cells contributed to muscle regeneration in dystrophic mice [61]. These cells can migrate through blood vessel walls [62]. Intramuscular and intra-arterial application of genetically corrected CD 133+ cells resulted in significant improvement in muscle function and dystrophin expression in dystrophic mice [63]. Laumonier et al. showed that Pax7+/MyoD− muscle reserve stem cells of human origin in immunodeficient mice intramuscularly promoted lacerated muscle regeneration [64].

5.1.3 Bone marrow derived stem cells

Furthermore, Hematopoietic stem cells (HSCs) and MSCs are the two main cell types that can be isolated from the adult bone marrow. MSCs are multipotent stem cells and possess the ability to differentiate into myoblasts [65]. Additionally, MSCs can induce an anti-inflammatory effect and anti-apoptosis through paracrine function. These cells can be easily isolated from the bone marrow, are relatively safe and have minimal tumorigenicity. Ferrari et al. demonstrated that marrow derived stem cells can migrate to areas of degeneration and participate in muscle regeneration [66]. Intravenous delivery of bone marrow stem cells in the mouse model of DMD, donor derived nuclei were incorporated into muscle with partial restoration of dystrophin expression [67]. Maeda et al. showed intraperitoneal transplantation of bone marrow derived MSCs in DKO (dystrophin-utrophin double-knockout) mice increased satellite cells in mice, improved their locomotion, reduced kyphosis, and increased their longevity [68].

5.1.4 Wharton jelly derived stem cells

Park et al. demonstrated that intravenous transplantation of human Wharton jelly derived MSC regenerated muscles, reduced apoptosis, and fibrosis in mdx mice model [69].

5.2 Clinical evidence

Only myoblasts, bone marrow derived stem cells and to a lesser extent umbilical cord-MSCs, muscle derived CD 133+ and cardiosphere derived stem cells (CDCs) have been investigated in humans.

Though safety and dystrophin production were seen in a case of DMD following myoblast transplantation [70], dystrophin production was seen in some but not all the later studies [71–80]. Although clinical benefit was observed in many studies, clinical use of these cells is hindered by several disadvantages. They cannot be delivered to all the muscles via systemic route, expansion in culture reduces their regenerative capacity [81], allogenic transplantation requires immunosuppression, and these cells have poor dispersion after intramuscular injection [82]. Also, these cells fail to participate in long term regeneration [83] and rapidly die in the first 72 hours after transplantation [84]. These problems have been tried to be resolved [85], but interest in use of these cells in treatment of MD has waned.

There is an ongoing Phase I/II clinical trial using systemic transfer of allogenic muscle derived mesoangioblasts from HLA identical donors in DMD [86]. Autologous transplantation of muscle derived CD 133+ cells in 8 boys with DMD was found to be safe and demonstrated an increased capillary per muscle fiber ratio with a switch from slow to fast myosin-positive myofibers [87]. 82 patients with progressive MD received double transplantation of autologous bone marrow derived MSCs and umbilical cord MSCs [88]. Treatment efficacy was evident in 68 of the 82 patients with no adverse event during the treatment course. Intravenous transplantation of allogenic cord blood cells in a patient with DMD resulted in improved function in standing, walking, and turning over with a mild graft versus host disease (GVHD) [89]. In another study, umbilical cord derived MSCs intravenously and intramuscularly in 11 DMD patients caused stabilization of muscle power as compared with control group and demonstrated safety without inducing GVHD [90]. A total of 15 studies demonstrated the benefits of intrathecal and intramuscular autologous bone marrow mononuclear cell transplantation in MD [91–105]. Dai et al. demonstrated four times administration of allogenic Wharton jelly-derived MSCs via intra-arterial and intramuscular routes in 9 DMD patients resulted in improved pulmonary function and increased dystrophin level with no adverse effects [106]. Another study revealed that intramuscular transplantation of fetal progenitor cells in 22 DMD patients improved their muscle activity, gait quality and reduced pseudohypertrophy [107]. The safety and efficacy of intravenous administration of human allogenic CDCs was studied by Mcdonald et al. in 26 late stage-DMD patients in a multicentre, randomized, double-blind, placebo-controlled, phase 2 clinical trial. The patients showed improved cardiac function and structure with maintained upper limb function with no major adverse effects [108].

6. Our experience of autologous bone marrow mononuclear cell therapy in MD treatment

6.1 Our treatment protocol

After careful review of available literature and evidence, we have a protocol that we follow for stem cell transplantation at NeuroGen Brain and Spine Institute.

6.1.1 Patient selection

The patient's selection is based on the World Medical Association's Helsinki declaration of Ethical Principles for medical research involving human subjects. The protocol was reviewed by the Institutional Ethics Committee (IEC) which is registered with the Central Drugs Standard Control Organization (CDSCO).

6.1.2 Pre-intervention evaluation

Before cell therapy intervention, the patients undergo a comprehensive evaluation consisting of neurological examination as well as evaluation on various outcome measures such as 6-minute walk distance, North star ambulatory assessment, Brooke and Vignose scale, Functional independence measure and manual muscle testing (MMT). Motor points of the patients are also identified and plotted for the weak muscles by experienced physiotherapists in which BMMNCs are to be injected.

6.1.3 Transplantation of BMMNCs

Transplantation of autologous bone marrow mononuclear cells intrathecally and intramuscularly is done in 3 steps (**Figure 5**). The protocol includes 300mcg granulocyte colony-stimulating factor (GCSF) administration subcutaneously 48 hours and 24 hours before cell therapy to enhance mobilization of cells [109].80–120 ml of bone marrow is aspirated from the anterior superior iliac spine. Mononuclear cells are then separated using the density gradient method. The separated cells are checked for viability under microscope using trypan blue and CD34+ cells are identified using fluorescence-activated cell sorting (FACS) analysis. Separated cells are then transplanted intrathecally and intramuscularly. The cells are diluted in the patient's own cerebrospinal fluid and divided into two parts. One part is transplanted intrathecally by lumbar puncture at the level between 4th and 5th lumbar vertebrae and the other part is further divided and injected intramuscularly at the motor point of muscles that are weak and of functional importance. Motor point is the point at which the innervating nerve enters a muscle, and it has the highest density of myoneural junctions. The

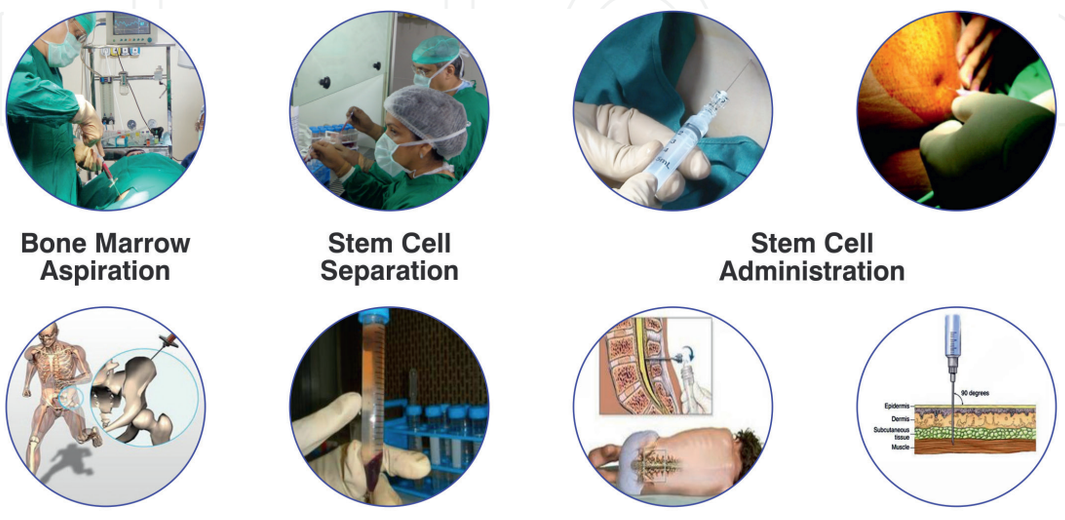

Figure 5.
3 step cell transplantation 1. Bone marrow aspiration; 2. Stem cell separation; 3. Intrathecal and intramuscular injection of cell.

identification of motor points is made by using electrical stimulation. A motor point can be identified as a superficial point overlying a muscle that exhibits a contraction at lowest level of stimulation (faradic stimulation with pulse duration of 1 millisecond).

6.1.4 Neurorehabilitation

The transplantation is followed with an individualized rehabilitation program incorporating physiotherapy, occupational therapy, speech therapy, aquatic therapy, psychological counseling, and dietary and nutritional advice. Patients are closely monitored for post procedure adverse events during their hospital stay. They are advised to continue the rehabilitation program at home preferably under professional supervision after discharge.

6.1.5 GCSF administration

After cell therapy, patients are also given one GCSF injection per month for the next 6 month after cell therapy that mobilizes the stem cells and improves muscle strength in MD patients [110, 111].

6.1.6 Follow up and adverse event monitoring

Patients are monitored for short term adverse reactions during their 4 days hospital stay. Patients are also advised to have regular follow-up at 3 months and 6 months, followed by yearly follow-up. During each follow-up, the patients undergo complete neurological assessment and are monitored for any long-term adverse effects.

6.2 Rationale for the protocol

6.2.1 Autologous bone marrow mononuclear cells

Although autologous bone marrow mononuclear cells carry the genetic abnormality in patients of MD, they have shown the potential to alter disease pathology and thereby disease progression. Also, autologous cell transplantation is not faced with the risk of immune rejection and therefore does not need immunosuppression. Bone marrow derived cells are easy to isolate, are excluded from ethical concerns, can be easily accessed, and transplanted [112], sufficient number of cells can be obtained by minimally invasive procedure and are marked by no immunogenicity and tumorigenicity [113, 114]. Pre-clinical studies have shown these cells to possess neurogenic [115] and myogenic potential [68, 116]. Also, they can migrate to the site of muscle degeneration; repopulate the satellite cell pool facilitating muscle regeneration [68, 116] and can survive in the injected muscles for long periods of time [83] promoting long term regeneration. These cells constitute a combination of cells including MSCs, hematopoietic cells, monocytes. Macrophages, stromal cells, very-small embryonic like stem cells, progenitor cells, hemangioblasts, endothelial progenitor cells and tissue-committed stem cells [117]. These cells are known to exert therapeutic benefits mainly through paracrine effects (**Figure 6**). They secrete a broad spectrum of cytokines and growth factors that exert anti-inflammation and immunosuppression, inhibition of apoptosis, homing of endogenous satellite cells, angiogenesis, and regulation of metabolic pathways [118, 119]. In MD, muscle fiber degeneration is followed by an invasion of inflammatory cells such as macrophages and T-lymphocytes [120]. The latter play

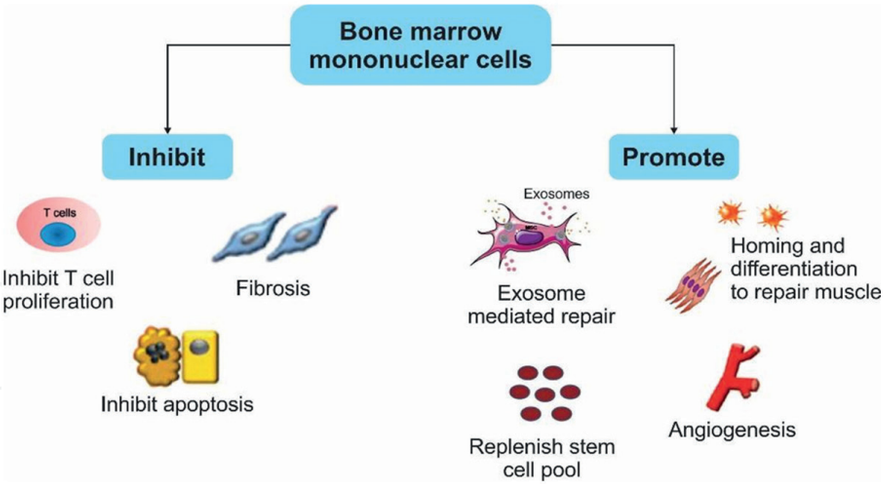

Figure 6.
Postulated mechanism of action of bone marrow mononuclear cells in MD.

a role in fibrosis which further hinders the ability of muscle fibers to regenerate. The anti-inflammatory effect of MSCs may provide protection from damage caused by T-lymphocytes [121]. Also, membrane derived vesicles, called as exosomes, arising from these cells may promote transcript transfer from the stem cells to the injured cells, causing injured cells to re-enter cell cycle further facilitating muscle repair [122, 123]. Though the ideal cell types for transplantation in MD continues to remain elusive, autologous bone marrow mononuclear cells are an attractive interim treatment solution with a potential to slow disease progression (**Figures 7** and **8**).

6.2.2 Intrathecal and intramuscular delivery of cells

Occurrence of co-morbid intellectual disability and cognitive impairment in patients with DMD and BMD is suggestive of nervous system involvement in MDs [9, 10]. Dastur and Razzak found an overlap of pathological changes in muscle biopsy specimens of

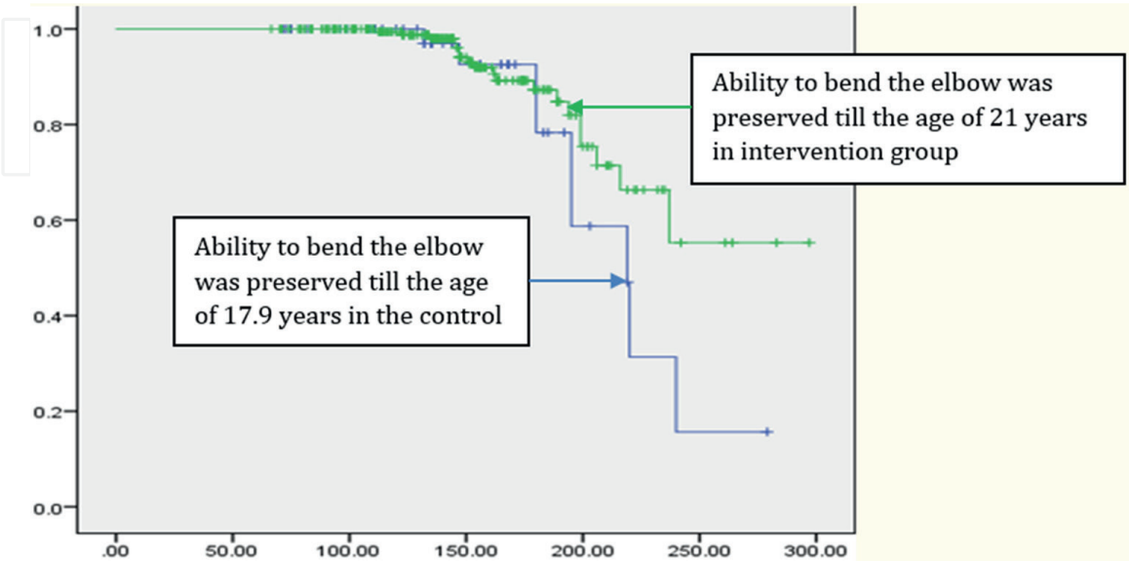

Figure 7.
Kaplan Meier graph showing comparison of the estimated time till loss of ability to lift the hand to mouth or deteriorate to the score of 5 on Brooke scale in intervention and control group.

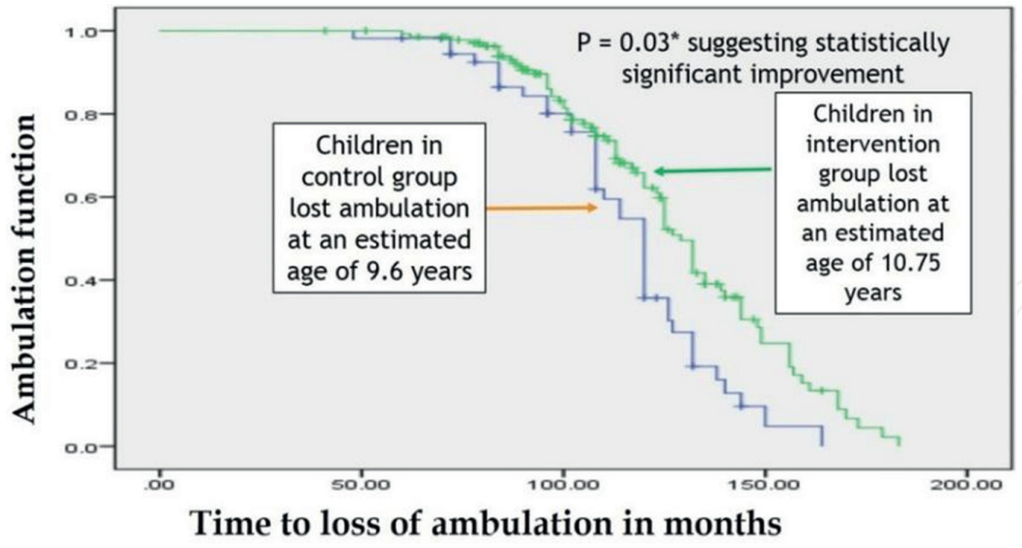

Figure 8.
Kaplan Meier graph showing comparison of the estimated time taken till loss of ambulation in intervention and control group.

patients with MD and patients with anterior horn cell disorders [124]. Histological study of the muscle biopsy specimens revealed small, atrophied muscle fibers in one fourth of the MD patients, suggesting a possible denervation and involvement of the neural systems in MD. These findings support intrathecal administration of stem cells and in our experience; it facilitates nerve repair and tightening of neuromuscular junction. Although intra-arterial and intravenous administration of stem cells is feasible because most of the skeletal muscles in the body are affected, only a small fraction of cells reaches the muscle tissue due to significant filtration of cells into the lungs, kidneys, and spleen [125, 126]. Moreover, the DGC is abundant at the neuromuscular junction and plays a role in neuromuscular homeostasis. Defects in DGC as in MD impair neuromuscular transmission and cause motor end plate abnormalities. Injecting the cells directly at motor points ensures repair of the nerve, muscle and myoneural synapse [30].

6.2.3 Combination of rehabilitation with cell therapy

Studies have investigated rehabilitation as a method to optimize cell therapy. Treadmill running following systemic transplantation of bone marrow derived MSCs in mouse models enhanced the contribution of donor cells in muscle fiber regeneration [127]. Endurance exercise results in an increase in blood levels of cytokines and an increase in bone marrow derived progenitor cells in humans. In response to exercise, there is an increase in secretion of vascular endothelial growth factor (VEGF) which is known to stimulate angiogenesis and satellite cell proliferation [128] and migration [129]. Intramuscular injection cannot be given in all the muscles and can only be given in selective muscles based on strength and accessibility. Widespread distribution and recruitment of circulatory stem cells is important which may be achieved through exercise. Long term, low-intensity, and low-load weight-bearing exercise programs may cause a shift in type II fibers to type I muscle fibers which are less susceptible to degeneration in MD [130]. Exercise increases expression of utrophin which is a homolog of dystrophin and can increase dystrophic muscle function [131]. Exercise also helps improve respiratory and cardiac function which is frequently affected in MD.

6.2.4 GCSF injection after cell therapy

A preclinical study showed higher numbers of normal muscle fibers and reduced inflammation in mdx mice treated with GCSF than the untreated mice [132]. A study in mdx mice model also demonstrated that GCSF has positive effect on the satellite cell proliferation and helps in muscle fiber regeneration [133]. It was also observed that periodic GCSF administration induces mobilization of stem cells including cells having proangiogenic potential such as endothelial progenitor cells and monocytes in MD patients [110]. The mobilized monocytes are recruited at the site of ischemia which in turn stimulate angiogenesis via paracrine mechanisms [134]. A prospective, non-randomized clinical trial demonstrated that repeated GCSF injections were safe and resulted in increased muscle strength and ambulation in 19 MD patients of age ranging from 5 to 15 [111].

6.2.5 Musculoskeletal magnetic resonance imaging

Skeletal muscle histopathology is a widely used tool in monitoring disease progression in MD cases; however, it is invasive, painful, gives limited information and might not be representative of the entire muscle [135]. In contrast, musculoskeletal MRI is a noninvasive technique and free of ionizing radiation. MRI-MSK provides information about all aspects of muscle structure and function and gives high resolution images of soft tissues and muscle fatty infiltrations [136]. MRI-MSK is sensitive to disease progression in MD and comparative MRI can serve as a biomarker for disease progression and to assess treatment efficacy [91–105, 137].

The efficacy of autologous bone marrow mononuclear cells in muscular dystrophy patients was studied and MRI-MSK was used as an outcome measure. After cell therapy patients showed increased muscle fibers in vastus medialis and lateralis (**Figure 9**), semitendinosus (**Figure 10**), tibialis (**Figure 11**), gastrocnemius (**Figures 12** and **13**), peroneus longus and brevis (**Figures 14** and **15**), triceps (**Figure 16**) and soleus (**Figure 17**) muscles.

6.3 Published results

A total of 15 studies (3 Cohort studies and 12 case reports) have been published that demonstrated the efficacy of autologous bone marrow mononuclear cell transplantation followed by rehabilitation in MD [91–105].

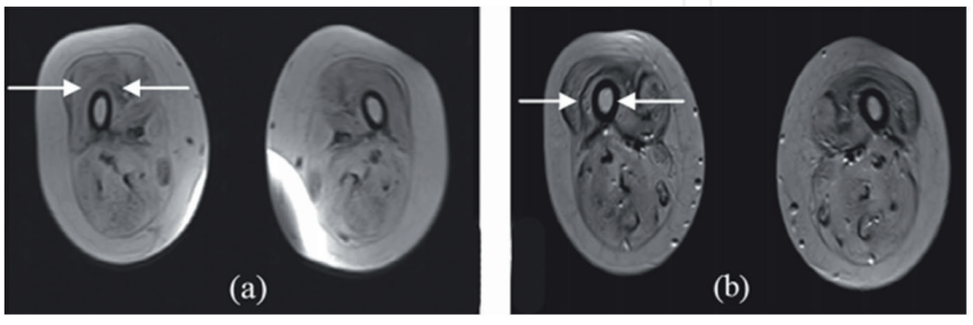

Figure 9.
(a) MRI scan of vastus medialis and lateralis muscles (arrows) pre-cell therapy. (b) MRI scan of vastus medialis and lateralis muscles (arrows) post- cell therapy showing muscle regeneration.

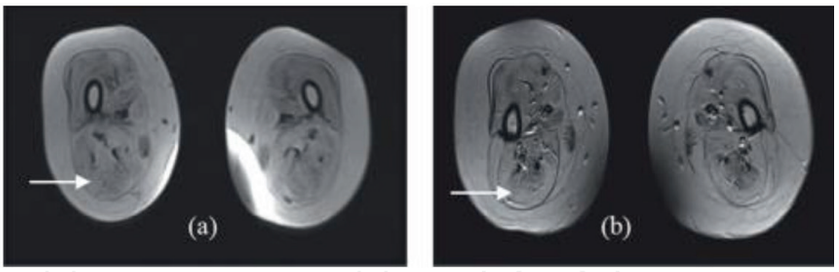

Figure 10.
(a) MRI scan of semitendinosus muscles (arrow) pre-cell therapy. (b) MRI scan of semitendinosus muscles (arrow) post-cell therapy showing muscle regeneration.

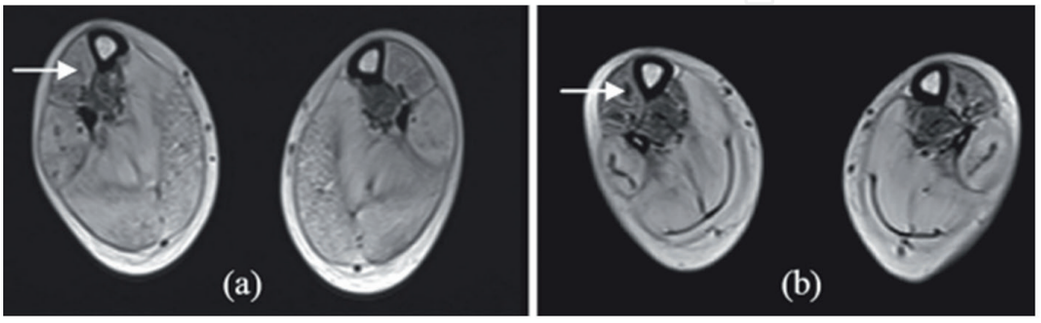

Figure 11.
(a) MRI scan of tibialis anterior (arrow) muscles pre-cell therapy. (b) MRI scan of tibialis anterior (arrow) muscles post-cell therapy showing muscle regeneration.

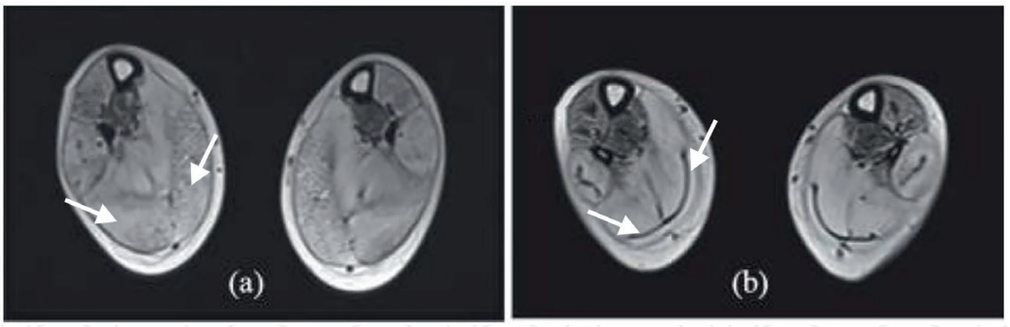

Figure 12.
(a) MRI scan of medial and lateral head of gastrocnemius muscle (arrow) muscles pre-cell therapy. (b) MRI scan of medial and lateral head of gastrocnemius muscle (arrow) muscles post-cell therapy showing muscle regeneration.

6.3.1 Results of published cohort studies

In 2012, a clinical study demonstrating the effect of bone marrow mononuclear cell transplantation in neurological and neuromuscular disorders in the pediatric population was published [97]. At a mean follow up of 15 ± 1 months post transplantation, 37 of the total38 patients with MD showed improvement in muscle strength (**Figure 18**). 73% showed improved trunk strength, 42% patients showed improvement in upper limb function and 71% patients showed improvement in lower limb function (**Figure 19**). Comparative musculoskeletal magnetic resonance imaging (MRI-MSK) done post intervention in two of the patients revealed decrease in fatty

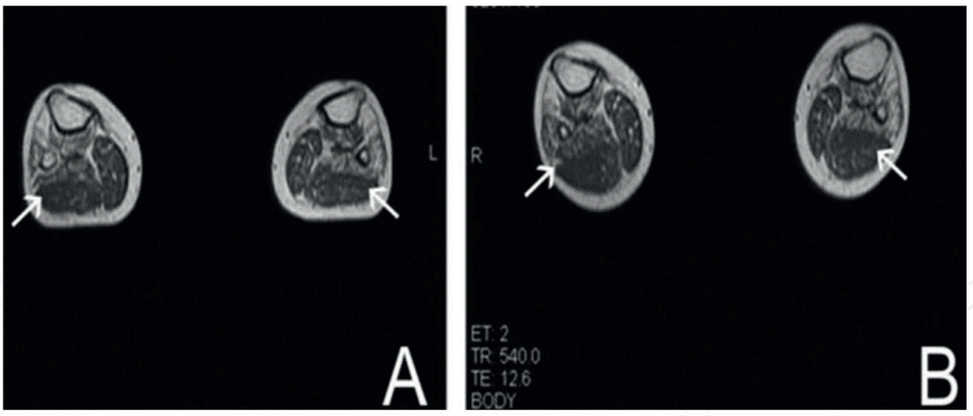

Figure 13.
(A) MRI scan of lateral head of gastrocnemius (arrow) pre-cell therapy. (B) MRI scan of lateral head of gastrocnemius (arrow) post-cell therapy showing muscle regeneration.

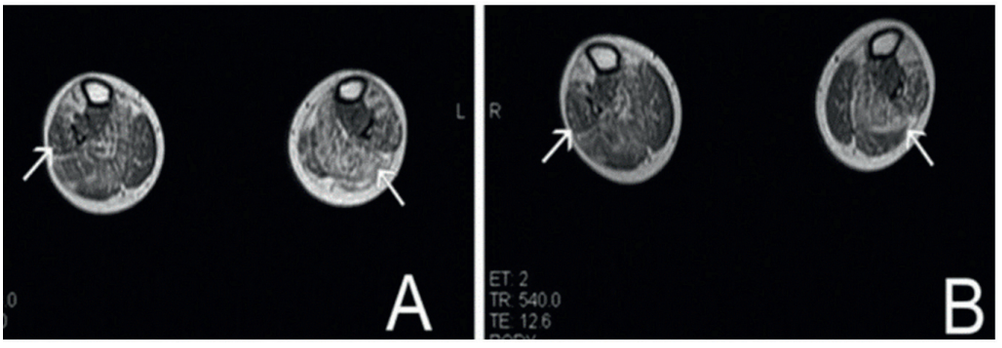

Figure 14.
(A) MRI scan showing peroneus longus and brevis (arrow) pre-cell therapy. (B) MRI scan showing peroneus longus and brevis (arrow) post cell therapy demonstrating muscle regeneration.

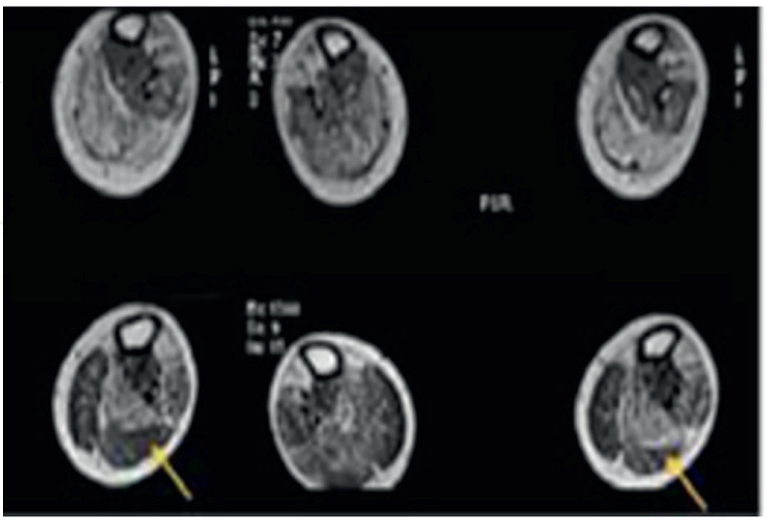

Figure 15.
Upper row. T1 weighted axial MRI images of peroneus longus and brevis, before cell therapy; lower row: T1 weighted axial MRI images of peroneus longus and brevis, 6 months post cell therapy showing increased isointense areas suggesting muscle regeneration.

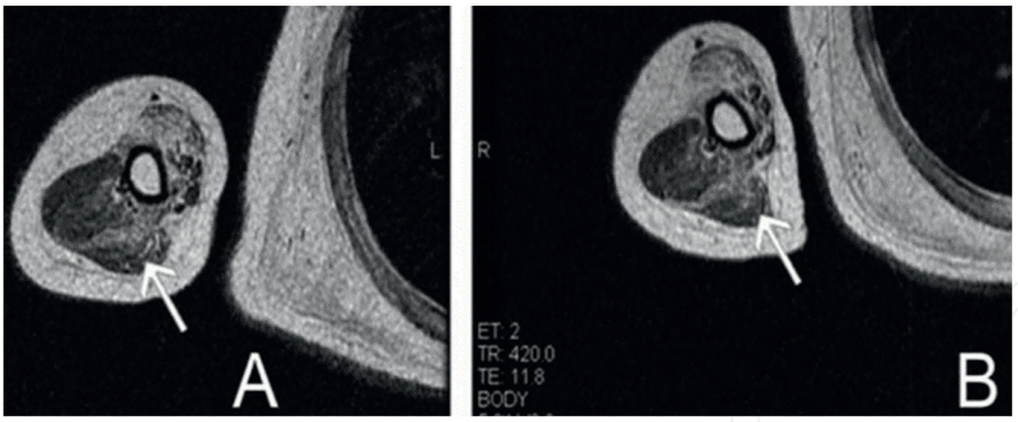

Figure 16.
(A) MRI scan of long, medial, and lateral head of triceps (arrow) pre-cell therapy. (B) MRI scan of long, medial, and lateral head of triceps (arrow) post-cell therapy showing muscle regeneration.

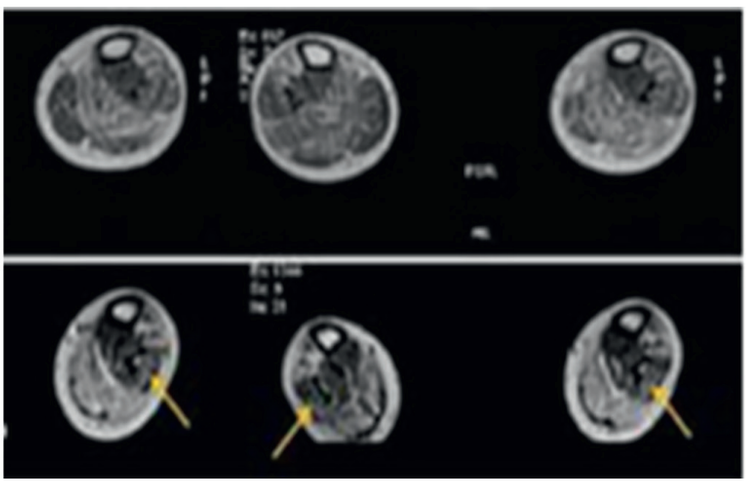

Figure 17.
Upper row: T1 weighted axial MRI images of gastrocnemius and soleus, before cell therapy. Lower row: T1 weighted axial MRI images gastrocnemius and soleus, 6 months after cell therapy showing increased isointense areas suggesting muscle regeneration.

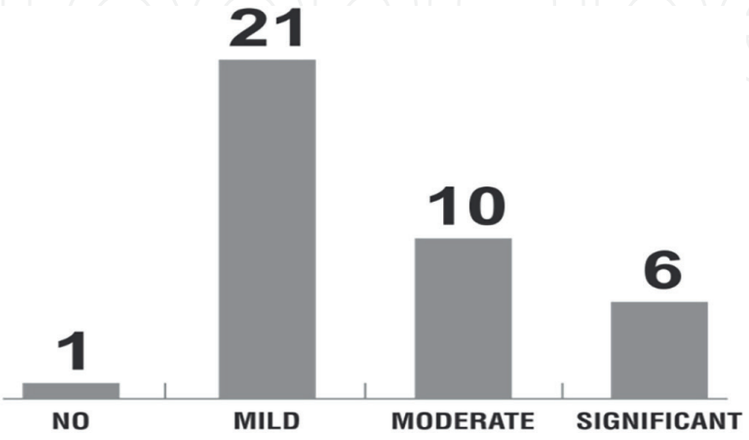

Figure 18.
Graph showing improvement in patients with MD post cell therapy.

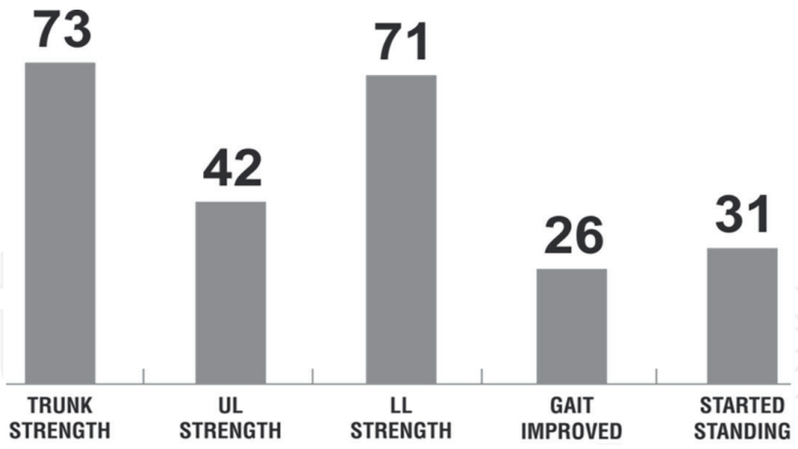

Figure 19.
Graph representing symptom-wise improvements in MD patients post cell therapy.

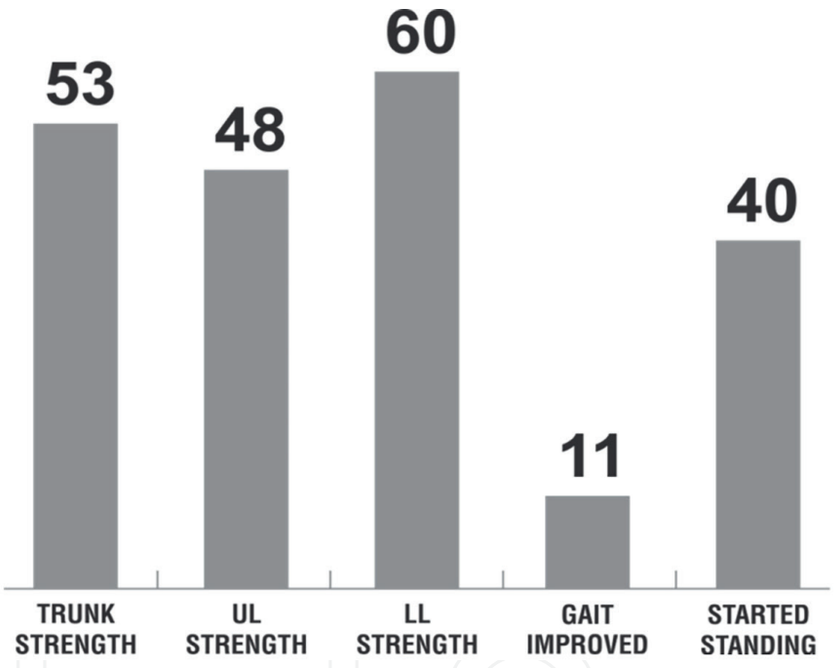

Figure 20.
Graph showing symptom wise improvements in muscular dystrophy patients after stem cell therapy. Number of patients showing improvements with respect to trunk strength, upper limb (UL) strength, lower limb (LL) strength, gait, and standing are shown.

infiltration with minimal muscle regeneration seen mostly in the muscles that had received cells (**Figures 9–12**). Improved muscle electrical activity was noted in 3 patients on comparative EMG done post intervention.

In 2013 an open label study that included 150 patients with MD was published [91]. On a mean follow up of 12 ± 1 months, 86.67% cases showed strength improvement with 53% patients showed improvement in trunk strength and 60% patients improving in lower limb strength (**Figure 20**). Improvements were seen on EMG in 9 cases and 6 cases showed improvement on MRI-MSK (**Figures 13**, **14** and **16**).

A longitudinal study of 65 LGMD patients was published in 2015 [98]. Depending on the number of transplants, the patients were divided into 3 groups. Group 1 included patients that underwent single transplantation, group 2 included patients that underwent 2 transplantations and group 3 included patients that

underwent 3 transplantations. 97% of patients displayed improved function on FIM scale in group 1. Statistically significant strength improvements were noted 6 months post transplantation. In group 2, 96% of the patients displayed improved function on FIM scale. Statistically significant strength improvements were noted 6 months post intervention. In group 3, of the 4 patients, 1 patient deteriorated in FIM score, 2 patients improved and in 1 patient, the FIM score was maintained. Most patients had maintained muscle strength. The patient who showed deterioration in FIM score also showed deterioration in muscle strength. This patient had come for the cell therapy at an advanced stage of the disease, where the muscle strength was minimal, and he was completely dependent for all his activities of daily living.

6.3.2 Results of published case reports

Though at a variable rate, progressive skeletal muscle weakness is consistent with all the MD variants. The improvements after cellular therapy was measured on various outcome measures which are as follows-

a. 6-minute walk test

Improvement was seen in the case of BMD in the 6-minute walk test. Maintenance and even improvement of function on the 6-minute walk test, over a follow up period of 1 year, was reported in two individual cases of DMD post intervention [94, 101]. Improvement also observed in a case of LGMD patient (94a). Considering the progressive nature of the disease, the natural decline shown by MD ambulant patients is 22.7 meters in the first year and 64.7 meters in the second year [138] which is slowed down because of cellular therapy.

b. North star ambulatory Assessment (NSAA)

Improvement was observed in DMD and BMD patients in North star ambulatory Assessment scale [92, 101, 102]. In a published case report, a DMD patient of 10 years of age showed a maintained score on NSAA after 13 months of follow up [93] which shows positive effects of cellular therapy as there is continuous decrease in the scores of NSAA in DMD patients after 7 years of age [138].

c. Brooke and Vignos Scales

The improvements are also observed in Brooke and Vignos scales that measure the strength of upper and lower extremity respectively. The improvement in ambulation and gait contributed toward a positive shift on Brooke and Vignos Scales in MD patients [91, 97]. Improved scores also observed in a case of DMD [103]. The scores are maintained on two cases of BMD which shows the efficacy of SCT considering the progressive nature of the disease.

d. Functional Independence Measure

The improved functional independence measure (FIM) score is observed after cellular therapy in most of the BMD, DMD and LGMD patients and is evident by improved quality of life in those patients [91, 93, 97–99, 102, 103]. In two BMD and one LGMD patient the score was maintained demonstrating the halted disease progression [92, 95, 100].

e. Manual muscle Testing (MMT)

The efficacy of cellular therapy was also assessed by MMT score. There are improvements observed in MMT grading which is attributed to improved muscle strength in many DMD, BMD and LGMD patients [91, 95, 96, 98–104]. In a DMD patient, though the grading did not change but the control and quality of movement had improved, grip strength and pinch strength also showed minimal changes on both sides [100]. This indicates alteration in the disease progression, as the natural course of the disease shows reduction of muscular strength by 0.3 MMT units/year and 3.9% reduction in muscle strength every year [103].

f. MRI-MSK

Comparative MRI-MSK was done to study the efficacy of cellular therapy. The comparative MRI-MSK findings showed no increase in the fatty infiltration in BMD, DMD and LGMD patients [96, 99, 101–103, 105]. BMD is associated with progressive increase in fatty infiltration of muscle tissue and the comparison of MRI scans of children with DMD also suggests a 5% increase of fatty infiltration every year [139]. Thus, no significant increase in the fatty infiltration shows the effectiveness of cellular therapy in halting the disease progression. Decrease in fatty infiltration was reported in a case of BMD in bilateral peroneus longus and brevis muscle fibers (**Figures 14** and **15**) 6 months post intervention [100].

g. Electromyography (EMG)

Comparative EMG-NCV (EMG-nerve conduction velocity) showed no increase in the dystrophic changes of the muscles, suggesting maintained muscle integrity suggesting altered disease progression [99]. In a DMD patient, EMG studies showed improvement in the recruitment of the vastus medialis muscle, which is a key muscle in patellar stability and knee stability while walking which was functionally reflected as the ability to stand and walk independently, maintained over 3 years [103]. A decrease in extramyocellular lipid (EMCL) resonance peak was seen in a case of LGMD [96]. The EMCL quantifies fat content in a diseased muscle. Improvement in electrical activity of muscle on EMG also observed in 9 patients post cellular therapy [91].

6.3.3 Adverse events

All these preliminary studies demonstrated safety of cell therapy using intrathecal and intramuscular transplantation of autologous bone marrow mononuclear cells. These studies encountered no procedure related major complications. 3 studies reported minor adverse events which included headache, nausea, vomiting, backache, and pain at injection site [91, 97, 98]. These were self-limiting and resolved within a week.

7. Unpublished results

7.1 DMD

A total of 296 patients with DMD underwent autologous transplantation of bone marrow mononuclear cells by intrathecal and intramuscular routes followed

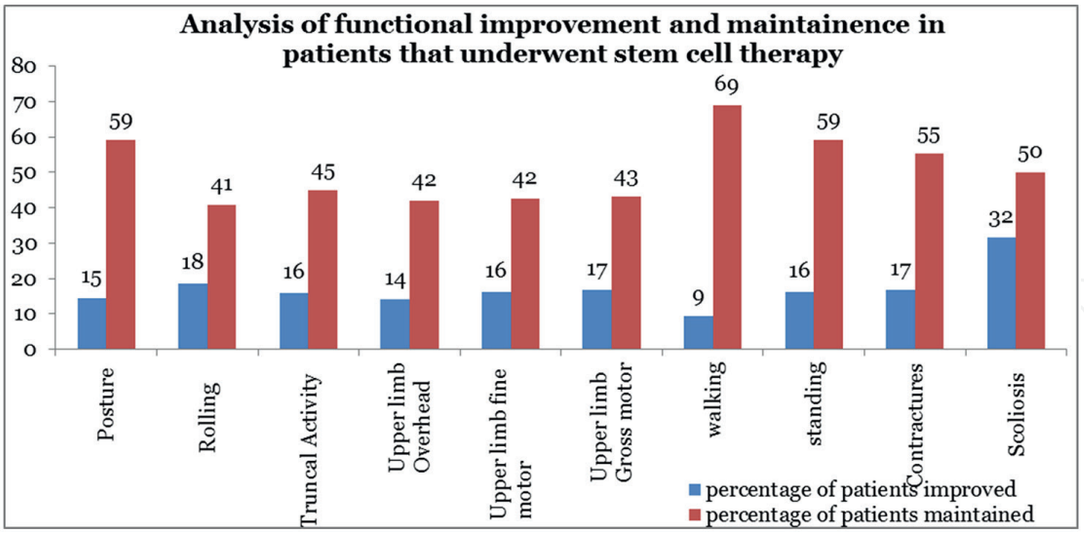

Figure 21.
Graph showing percentage analysis of symptoms in DMD patients post cell therapy.

by rehabilitation. There were no major side effects. 5.4% patients experienced minor procedural adverse events which included spinal headache, fever, nausea and vomiting, pain at aspiration site, backache, neck pain, pain in lower limbs and loose motions. These were self-limiting and resolved within a week with medications. 55 patients that only received standard treatment for DMD formed the control group.

On a follow-up period ranging from 6 months to 78 months (median 18 months), 76% patients showed symptomatic and functional maintenance and/or improvements (**Figure 21**). The natural course of DMD being progressive, symptomatic, or functional maintenance or improvement were considered together.

7.1.1 Effect of cell therapy on muscle strength

Difference between muscle strength on MMT in individual muscle groups at the first and last assessment at mean follow up duration of 12.2 ± 8.6 months was analyzed using Wilcoxon signed rank test. Except for hip extensors, knee extensors and shoulder flexors, all muscle groups had maintained muscle strength. Also, there was a statistically significant increase in muscle strength post intervention in the wrist flexors. Overall muscle strength (%MRC) was calculated for each patient. Difference between %MRC at the first and last assessment was analyzed using the Wilcoxon signed rank test (**Table 1**). We found a statistically insignificant decline in %MRC of 1.04% in patients aged 5–13 years, over mean follow up of 12.2 ± 8.6 months. This was lower than the annual decline of 3.9% in natural controls [140]. There was a statistically insignificant increase of 0.92% in %MRC in patients aged 14 years or more. A 2% annual decline in %MRC in this age group has been reported in previous literature [141].

7.1.2 Comparison of functional decline between treatment and control group

On comparing the predicted age at which patients could not lift their hand to the mouth and lost their ability to self-feed (Brooke score declining to 5) using Kaplan-Meier analysis, patients that received cell therapy reached score 5 at age 21 years while patients that did not receive cell therapy reached the score at age 18 years (**Figure 7**

Variables	MMT score		% MRC		Significance
	Before intervention	After intervention	Before intervention	After intervention	
Mean Score (5–13 years age group)	9.15	8.98	57.19%	56.15%	p = 0.1031
Mean Score (>14 years age group)	5.87	6.01	36.67%	37.59%	p = 0.0750

Table 1.
Results of analysis of overall manual strength (%MRC) before and after intervention in the age group 5–13 years and > 14 years at a mean follow up of 12 months (p < 0.05 was considered statistically significant).

	Control Group(months)	Intervention Group (months)	Statistical significance
Total no. of patients	55	139	—
Estimated time to loss of ambulation in months	117	130	0.006*
Estimated time to reach score of 5 on Brooke scale	216	252	0.150
Estimated time of survival	260	297	0.173

Table 2.
*Results of Kaplan Meier analysis in intervention and control group (*indicates statistically significant difference between the groups).*

and **Table 2**). This difference of 36 months was clinically meaningful however not statistically significant (p = 0.150).

Comparing the predicted age to loss of ambulation using Kaplan-Meier analysis, the predicted time to loss of ambulation was 130 months in the cell therapy group and 117 months in the control group (**Figure 8** and **Table 2**). There was a statistically significant (p < 0.05) increase in time to loss of ambulation by 13 months in the cell therapy group.

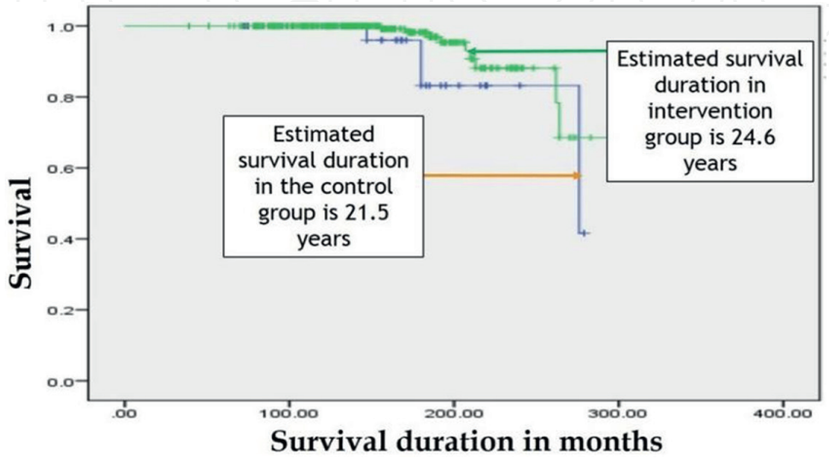

Figure 22.
Kaplan Meier graph showing comparison of estimated survival duration of the intervention and control group.

On comparing the survival duration using Kaplan-Meier analysis, the estimated survival duration of patients that received cell therapy was 297 months, while that of the control group was 260 months (**Figure 22**). Though statistically insignificant ($p = 0.173$), there was an increase in estimated survival duration by 37 months in the cell therapy group.

8. Summary of effects of SCT in MD

MD is considered as stem cell disease as symptoms are visible only after depletion of the stem cell pool. Therefore, replenishment of the stem cell pool is necessary to ameliorate the symptoms. This can be achieved by stem cell therapy which is found to be a safe and effective treatment strategy for MD. SCT delays the progression of the disease, improves functionality and quality of life of MD patients. SCT in combination with rehabilitation and GCSF administration gives better results. The effects of SCT may be further enhanced by other integrative therapies such as hyperbaric oxygen therapy (HBOT), ozone therapy and deep tissue mobilization (DTM). These therapies may help to fasten the process of regeneration and repair because of their therapeutic effects.

9. Integrative therapies in MD

9.1 Hyperbaric oxygen therapy (HBOT)

The pathophysiology of MD patients includes vascular dysfunction, altered angiogenesis, hypoxia, inflammation, and ischemia [142]. Studies have shown that HBOT enhances healing of wounds, ischemia, and inflammations [143]. It also reduces hypoxia and increases angiogenesis by inducing secretion of VEGF (Vascular endothelial growth factor) and bFGF (basic fibroblast growth factor) [144]. Thereby, HBOT is a promising therapy to ameliorate the condition of MD individuals. A study revealed that HBOT promotes muscle regeneration and satellite cell proliferation in mouse skeletal muscles injury models [145]. Thom et al. demonstrated that exposure to HBOT rapidly mobilizes stem progenitor cells in humans and mice [146]. Thus, HBOT in combination with stem cell therapy can show better therapeutic effects. HBOT stimulates neurogenesis and synaptogenesis, thereby improving motor functions and cognitive functions [147]. Leitman et al. demonstrated that HBOT also improves myocardial functions which are profoundly affected in MD patients [148].

9.2 Ozone therapy

Ozone therapy enhances tissue oxygenation and improves cellular metabolism. It also reduces oxidative stress and inflammation [149]. Preclinical study has shown that dystrophin deficient mdx mice have increased reactive oxygen species (ROS) levels in their heart which play a critical role in the development of dilated cardiomyopathy and inflammation of heart [150]. Also, Myofiber necrosis in MD patients is closely associated with increased inflammation and ROS levels [151]. The anti-inflammatory and antioxidant properties of ozone therapy may help in limiting disease progression by reducing oxidative stress and inflammation in MD patients. Ozone therapy also helps in mobilization of stem cells and homing of stem cells toward injured/ischemic sites, thus, aid in tissue repair [152]. This evidence suggests that stem cell therapy in combination with ozone therapy may show better therapeutic efficacy in MD patients.

9.3 Deep tissue mobilization (DTM)

Soft tissue mobilization helps in removing scars and soft tissue regeneration after injury [153]. Massage therapy reduces inflammation and promotes angiogenesis in the injured tissues after muscle damage [154]. In 20 DMD patients, calf massage showed increase in calf and hamstring muscle length and reduction in calf muscle stiffness [155]. Thereby, DTM is a beneficial option in cases of MD as they have increased inflammation [151]. An increased level of profibrotic factor TGFβ1 is observed in muscles of DMD patients [156] which is reduced by brief stretching of tissue beyond the habitual range [157]. A preclinical study demonstrated that massage therapy in 32 rats have increased satellite cell number in their gastrocnemius muscle [158]. This finding suggests that DTM along with stem cell therapy is a beneficial option for the treatment of MD.

10. Future directions and conclusion

Clearly, the initiation of disease pathology is due to genetic defect, but progression is due to satellite cell exhaustion and loss of regenerative capacity of satellite cells. Replenishing satellite cell pool and enhancing regenerative capacity of muscle fibers is essential for any treatment to be effective. Cell therapy represents a potential treatment strategy for MD. Goals that need to be fulfilled include delivery of normal protein to affected muscle fibers, effective fusion of donor cells to affected muscle fibers, delivery of large numbers of stem cells, repopulating of the resident stem cell pool, long term survival of stem cells to facilitate long term muscle repair and homeostasis. All these goals may not be met using one cell type and may require a combination of cell therapies. Future studies could explore systemic delivery of cells along with transplantation of different types of autologous/allogeneic cells in larger numbers intramuscularly and/or with intrathecal administration. Studies could also combine the genetic therapies with stem cell replacement to identify the most effective cure for DMD. Larger randomized studies to determine the ideal cell combination, route of delivery and cell dosage are recommended. Studies can also explore the effects of cellular therapy in combination with integrative therapies such as HBOT, ozone therapy and DTM.

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