Chapter 2

Stem Cells as a Therapeutic Modality in Muscular Dystrophy

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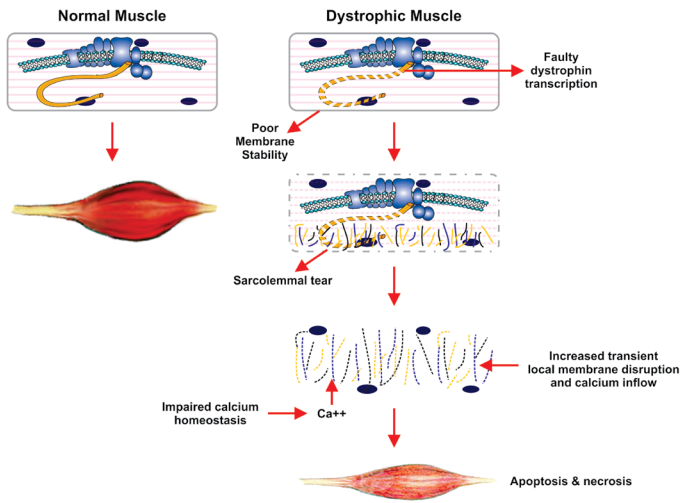
Muscular Dystrophy: A Stem Cell Isease

Muscular dystrophies are heterogenous neuromuscular disorders caused by a genetic abnormality that leads to abnormal transcription of cytoskeletal proteins. The cytoskeletal proteins form dystrophin glycoprotein complex (DGC) essential for sarcolemmal integrity [1]. Easy disruption of sarcolemma leads to cell death and progressive muscle loss observed in muscular dystrophy. Although it is caused by a core genetic abnormality the disease is progressive due to lack of sufficient stem cells to replace the degenerated cells (Figure 1). Animal models suggest that it is caused by exhaustion of stem cell pool. It is therefore highlighted by some of the prominent scientists that muscular dystrophy is a stem cell disease and stem cell replacement is essential for its treatment [2]. An insightful statement from former postdoctoral fellow Jason Pomerantz, MD, now an assistant professor at the University of California, San Francisco, explains the need for stem cells as a treatment for stem cell therapy. He quotes,

"If a treatment (for muscular dystrophy) does not replenish the stem cell compartment, it will be likely fail; it would be like pushing the gas pedal to the floor when there is no reserve."

Therefore, treatment approaches for muscular dystrophy must incorporate stem cell replacement!!



Dystrophic Muscles in DMD

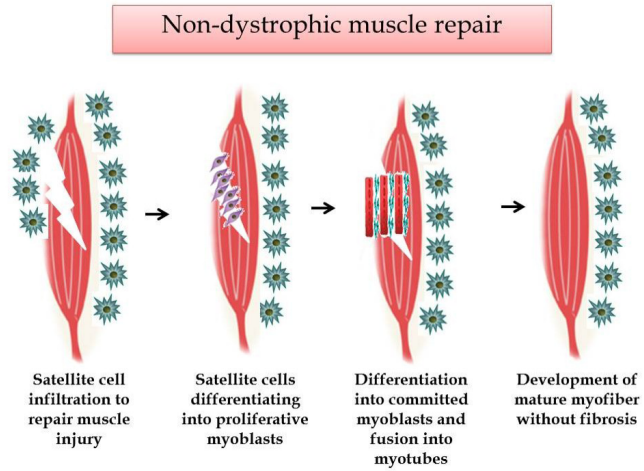


Figure (1a)

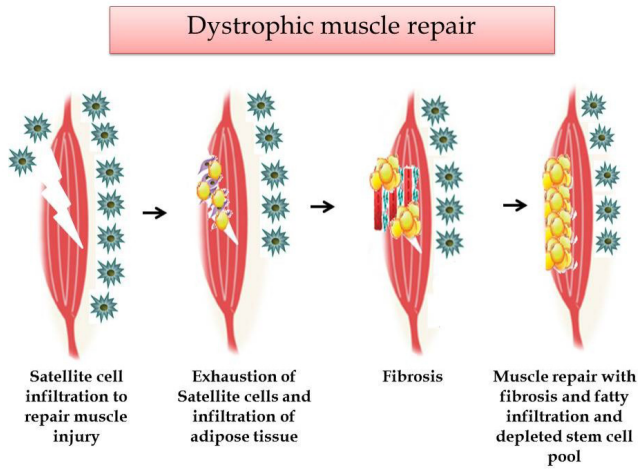


Figure (1b)

Figure 1: Muscle repair in non-dystrophic muscles with adequate stem cells and dystrophic muscles showing exhaustion of stem cells.

Pathophysiology of Muscular Dystrophy

The core pathology of muscular dystrophy is genetic abnormality and resultant abnormal protein transcription. The abnormal protein alters the structure of DGC compromising the cell integrity. Differences in the protein involved and its role in the DGC results in differences in the symptoms of muscular dystrophies [3,4]. Cells are then prone to damage even with minimal contractile stress. Damaged muscles cells are repaired by resident stem cells, satellite cells. Continuous damage leads to fast depletion of the satellite cells. Increasing deficit between the damage and repair causes progressive loss of muscle fibers which are replaced by fibrosis and adipose tissue. This is exhibited as muscle weakness [3,4]. Constant damage and disintegration of cells leads to immune cell infiltration and inflammatory cytokines secretion. The cycle continues over the years and leads to chronic inflammation where the cell repair process is altered and there is increased inflammation [5-7]. In response to the chronic ischemia and inflammation, apoptotic processes are upregulated in muscular dystrophy triggering accelerated cell death [8]. Muscular dystrophies also affect smooth muscle functioning and therefore impair the movement of the blood vessel walls causing altered blood supply to the muscles and can lead chronic ischemia [9]. The muscle repair in muscular dystrophy is different in comparison to regular muscles.

After the inflammatory necrosis it follows a fibrotic pathway of remodeling with fat cell replacement. Persistence of myofibroblasts and fibroblasts in the injured microenvironment due to chronic muscle injury leads to muscle fibrosis and contractures observes in MD. These are predominant in more severe forms of MD like DMD [10,11]. The degenerative processes are not only dystrophic but also atrophic. Proteins of DGC are present in neural tissue and play an important role in synaptic structure [12,13]. Many pathways of neurotransmission involve DGC and impaired DGC can lead to abnormal neurotransmission, abnormal synaptic activity and problems in the formation of neuromuscular junction causing muscle wasting.

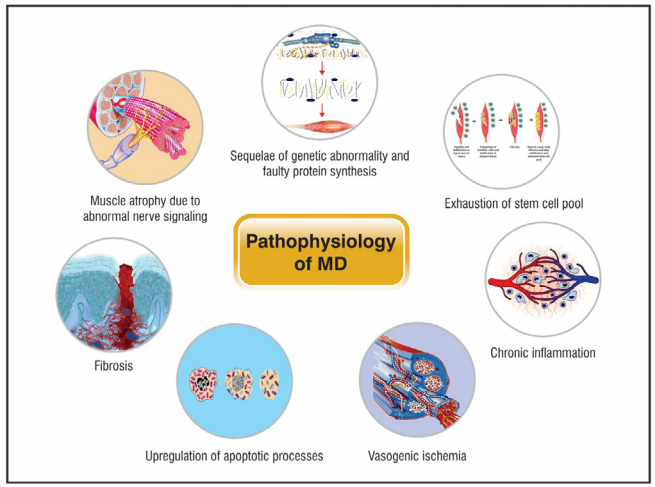


Figure 2: Pathophysiology of Muscular Dystrophy.

Current Treatments For Muscular Dystrophy

Muscular dystrophy remains largely untreated due to the core genetic abnormality and complex pathophysiology. The ultimate cure for the disease will be through gene therapy and stem cell therapy. Currently, viral mediated gene delivery, exon skipping via antisense oligonucleotides and viral mediated exon skipping are the strategies being explored in for the treatment of muscular dystrophies to bypass or correct the core genetic pathology [14]. However currently these are under investigation and their clinical implementation is quiet farfetched.

The only drug approved by the Food and drug administration of United States of America is Eteplirsen which acts through exon skipping of exon 51 and can be used specifically for patients with these mutations and deletions [15]. The results from early randomized double blind controlled trial of safety and efficacy were published in 2011 by Cirak et. al. 2011 [16]. The study showed that the intravenous application was well tolerated, induced skipping of exon 51 and led to dystrophin expression in the new muscle fibers [16] Authors also demonstrated the functionality of expressed dystrophin [17]. The study showed dose dependent response. Clinical studies of Eteplirsen show a modest benefit in 6 minute walk distance and slower disease progression as compared to the control group. There was no reversal of symptoms reported with the therapy. Out of 186 only 13 patients were eligible for exon skipping using eteplirsen, so the drug can only be used for a very small population of DMD children. It cannot be used for any other forms of muscular dystrophies. Some concerns have been raised about the methodology revealing discrepancies in reporting of the efficacy study of Eteplirsen [18]. Application of this drug is limited to a small subset of MD. The efficacy is debated and it does not reverse the symptoms due to inability to replenish the stem cell pool. Therefore treatment with gene therapy alone is inadequate and needs to be combined with approaches that conserve and replenish stem cell pool.

Pharmacological strategies are symptomatic or address the secondary pathophysiology of inflammation and fibrosis. Several clinical trials have demonstrated the clinical outcomes and adverse events of steroid treatment [19-22]. A Cochrane review of the randomised controlled trials, non-randomised control trials and good quality cohort studies demonstrated moderate benefit in strength and function of children with DMD at the end of 6 and 12 months. Only one of these trials assessed a long term effect of steroids and effect on disease progression measured with time taken for loss of ambulation. Time to loss of ambulation was prolonged by 13 months in the group treated

with deflazacort administration every alternate day. There is insufficient evidence to demonstrate effect of steroids on disease progression. All the studies have reported adverse events of steroids. The reported adverse events include weight gain, osteoporosity, vertebral fractures, cataract, cushingoid features, excess hair growth and behavioral symptoms [23]. Medicines that upregulate the utrophin are also used however the efficacy is limited [24]

Surgical intervention is used in case of contractures. Apart from medical and surgical intervention, rehabilitative intervention is heavily relied upon. Exercise promotes muscle growth, reduces fibrosis, has an anti-inflammatory effect and facilitates functional independence.

Available treatments have very limited benefit and several adverse events. There is insufficient evidence to suggest any effect of disease progression and modification of disease pathology. None of these treatments are successful in reversing the symptoms of MD or alter the core pathology.

Unmet Medical Needs

Despite available medical, surgical and rehabilitative interventions MDs are still incurable. Muscle death and progressive loss cannot be prevented with these therapies. Although gene therapies are promising solution for the cure only one gene therapy is approved which can be used

for a very small population DMD has moderate benefit on disease progression and does not reverse the symptoms of the disease. Genetic therapies alone are insufficient and treatment strategies that address replacement of stem cell pool are essential for a positive outcome and potential reversal of the disease.

There is an immediate need for treatment strategies that can alter disease pathology, slow down or halt disease progression by addressing the core pathology of muscular dystrophy i.e. imbalance between degeneration and regeneration of stem cells and exhaustion of stem cell pool is required.

Stem Cell Replacement as a Therapeutic Strategy for Muscular Dystrophies

As suggested previously the imbalance between degeneration and regeneration of muscles leads to progressive muscle damage in muscular dystrophy. Repeated division of satellite cells can shorten telomerase causing faulty protein transcription. Treatments that solely repair the existing muscle fiber may not be sufficient. Stem cell replacement is necessary to provide a viable solution for progressive muscle loss, however, such replacement should begin at an early phase of the disease to prevent stem cell pool

exhaustion. Direct approach may benefit more than a systemic or general administration of stem cells.

Understanding Stem Cells

Stem cells are unique undifferentiated cells that possess the capacity to proliferate and self renew to produce large numbers of functional cell progeny [25]. The entire human body develops from these stem cells. Throughout life stem cells bring about repair of tissue and replaces damaged tissue. Stem cells can differentiate either to produce new stem cells capable of further differentiation or to produce specialized differentiated tissue.

Types of Stem Cells

Stem Cells can be Classified Based on Several Criteria

Based on their ability to differentiate they are categorized as totipotent, pluripotent, multipotent and unipotent. **Totipotent** cells are capable for differentiating to form any tissue including embryonic tissue, e.g. cells of the morula. **Pluripotent** stem cells can also differentiate to form any cell except the embryonic tissue and placenta. Recently some of the multipotent cells have been genetically reprogrammed to achieve pluripotency. These cells are known as **induced pluripotentstem cells** (i-PSCs). **Multipotent** stem cells can produce cells from more than one lineage but have limited differentiation capability. **Unipotent** stem cells are highly specialized stem cells that are committed to a single lineage and can only differentiate into one cell type.

If cells procured from recipients own body then they categorized as **autologous** cells, whereas, if stem cells are procured from a different host they are categorized as **allogenic** cells.

Based on the source they are classified into **embryonic stem cells, fetal stem cells, umbilical cord stem cells** and adult stem cells. Adult stem cells are the stem cells that are present throughout the human body and can be procured from various sources such as bone marrow, adipose tissue, dental pulp and peripheral blood.

There are various ethical concerns surrounding using embryonic and fetal cells for research and therapy. There are also some concerns regarding the safety of the pluripotent embryonic and fetal cells due to their tumorigenic properties.

Routes of Administration

Stem cell transplantation has been explored through various routes of administration, however, for the purpose of this chapter we will discuss the routes that are commonly used in pre-clinical and clinical research of muscular dystrophy.

Intramuscular

Intramuscular route of administration can be considered most appropriate as muscular dystrophy is primarily a muscle disease. The cells can be injected in several points in the muscle alternatively they can be injected in the motor point of the muscle.

Motor point

A motor point is the point at which the motor branch of the innervating nerve enters the muscle (Figure 3). It is the point with the highest concentration of motor endplates and myoneural synapses. Due to high numbers of neuromuscular junctions at this point, a muscle contraction can be easily elicited using minimal electric stimulus. Motor points can therefore be identified as superficial points directly over the points on the muscles with help of external electrical stimulation. Limitation of this method is that only superficial muscles can be stimulated using this method [26,27].

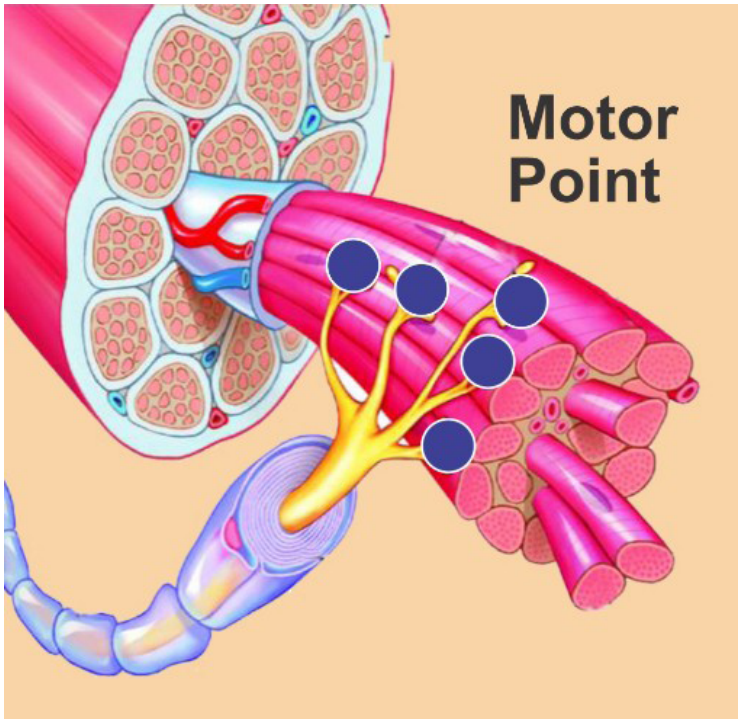


Figure 3: Motor point.

Intrathecal

Cells are administered in the cerebrospinal fluid through lumbar puncture procedure. There is a strong evidence to support presence of DGC in nervous tissue. Intrathecal approach targets the neural component of muscular dystrophies.

Systemic

Cells are administered through systemic routes i.e in-

travenous. However there are several disadvantages of using these routes in case of muscular dystrophy. The cells administered are diluted and therefore large numbers of cells need to be administered for a positive outcome. Also the cells are entrapped in liver, lungs and spleen and therefore a very small fraction of injected cells has a potential to reach the target organ. Since muscular dystrophy involves all muscles to different extents the delivery should be targeted at weaker muscles.

Benefits of Stem Cells in Muscular Dystrophy

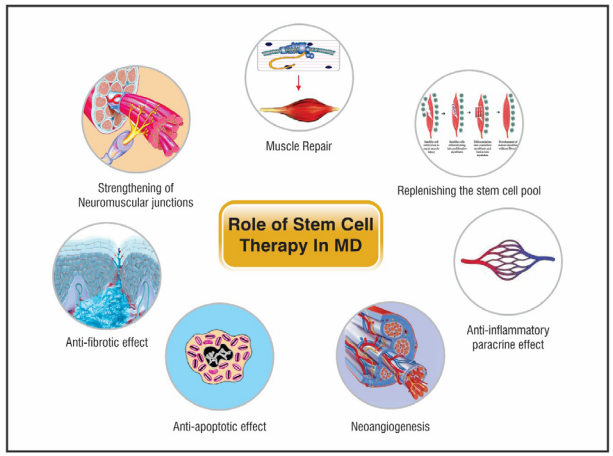


Figure 4: Role of stem cell therapy in Muscular Dystrophy.

Pathologic mechanism of muscular dystrophy	Physiological benefits of stem cell transplantation
Rapid muscle degeneration as a sequale of genetic abnormality	Transplanted cells have myogenic potential i.e. the cells can differentiate into a mature myocyte and therefore can repair and regenerate muscle fibers [28,29].
Lack of dystrophin expression in the muscle or faulty dystrophin production	Preclinical evidence suggests that stem cell transplantation can restore dystrophin expression in mouse model of muscular dystrophy. Such dystrophin expression can lead to formation of muscle fibers that are resistant to easy damage and degeneration [30].
Exhaustion of stem cell pool	Exogenous stem cell transplantation replenishes the stem cell pool. Transplanted cells can also stimulate the resident satellite cells [31].
Chronic inflammation	In addition to the actual regeneration of muscle cells and replacement of resident stem cells, transplanted cells also exhibit numerous beneficial paracrine mechanisms. Stem cells secrete various anti-inflammatory cytokines and various growth factors that are myoprotective. Vasculoendothelial growth factor is a growth factor that promotes neoangiogenesis. In addition anti-inflammatory and neo-angiogenetic effect, cells have immunomodulatory and anti-apoptotic effects on neighboring cells. Transplanted cells also stimulate muscle plasticity and remodeling therefore prevents fibrosis [32-37]. These paracrine effects are also catalyzed by exosomes secreted by MSCs [32-34].
Fibrosis	
Vasogenic ischemia	
Neurogenic atrophy	

What Does the Current Literature Evidence Say About Stem Cell Therapy in Muscular Dystrophy?

Most extensively studied are the transplantations using satellite cells or myoblast progenies [38]. Huard et al reported presence of dystrophin positive fibers in the host along with transient motor improvement. [39] Gussoni et al studied myoblast transplantation demonstrating that

the transplanted myoblasts persisted after injection and their fate was guided by the microenvironment [40-42]. They also documented the ability of exogenous human bone marrow cells to fuse into skeletal muscle and persist up to 13 years after transplantation. Similar results were recorded for various other studies on myoblast transfer [43-48]. Although, myoblast transferleads to some degree of improvement in muscle strength and enables transient dystrophin delivery, there are various limitations for such a transfer. Survival rates are very poor, there is a risk of immune rejection and targeted delivery may limit the spread of the cells. Hence, other sources of stem cells such as bone marrow and umbilical cord are being explored by the researchers.

Feasibility data of umbilical cord stem cell transplantation in DMD was published by Zhang et al. 2009 and Yang et al 2009 investigated the feasibility of employing double transplantations of autologous bone marrow mesenchymal stem cells (BMSC) and umbilical cord mesenchymal stem cells (UMSC) in the treatment of progressive muscular dystrophy (PMD). Total effective rate was 82.9% concluding it as a safe and effective treatment [49,50].

Hematopoietic stem/progenitor cell populations from adult skeletal muscle also have a therapeutic potential for muscular dystrophy [51]. Torrente et al (2007) studied the safety of autologous transplantation of muscle-derived CD133+ cells. They recorded increased ratio of capillary per muscle fibers with a switch from slow to fast myosin-

positive myofibers [52]. Sharma et al published the results of autologous bone marrow derived mononuclear cells intrathecally and intramuscularly in 2 patients with DMD and 2 with BMD as individual case reports showing functional improvements along with improvement in MRI and electrophysiological tests. They also published case series on different types of MD and LGMD. 150 patients with different types of musclular dystrophies like DMD, BMD and 56 patients with LGMD were studied. The results showed mild improvements in muscle strength and improvement in symptoms like static balance, walking, bed mobilities and stamina. The most important finding was that there was slower disease progression [53-57].

Our Experience in Treating Muscular Dystrophy with Stem Cells

Protocol

After carefully reviewing the available evidence and our experience we have designed a protocol for stem cell transplantation at NeuroGen brain and spine institute. The protocol consists of 4 steps. We have the experience of treating maximum number of patients with muscular dystrophy. We use intrathecal and intramuscular transplantation of autologous bone marrow mononuclear cells combined with rehabilitation for the treatment of muscular dystrophy. The procedure for harvesting and trans-

planting the cells is minimally invasive and there are no major adverse effects of this procedure.

Bone Marrow Aspiration

Under local anesthesia maintaining aseptic conditions, 80 – 120 ml of bone marrow is aspirated from anterior superior iliac spine.

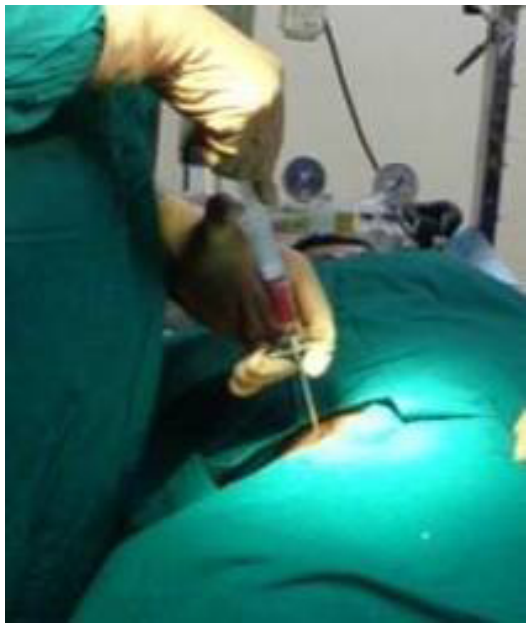


Figure 5: Bone marrow aspiration.

Separation of Stem Cells

Stem cells are separated using density gradient method. The separated cell pellet is analyzed under microscope using Trypan blue to check for viability of the mononuclear cells. CD34+ cells are identified using FACS analysis. Cell viability, cell count and percentage of CD34+ cells is calculated. The separation of cells is performed within 3 to 5 hours of aspiration on the same day.

Figure 6: Separation of stem cells.

Injection

Separated cells are then transplanted intrathecally and intramuscularly. The cells are divided into two portions and are diluted in the cerebrospinal fluid. One portion is

used for intrathecal transplantation via lumbar puncture at the level between fourth and fifth lumbar vertebra. The other portion is further divided into multiple smaller portions for intramuscular injections at the motor points of the muscles.

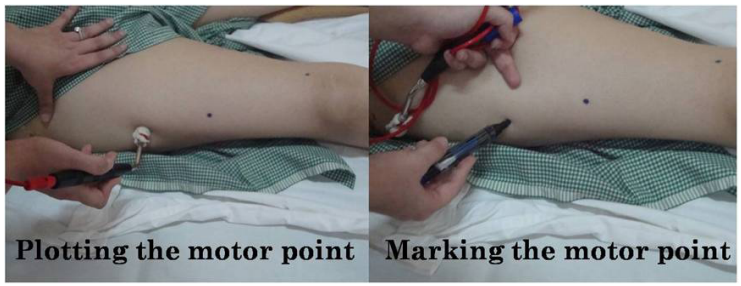


Figure 7: Identification of the motor points.

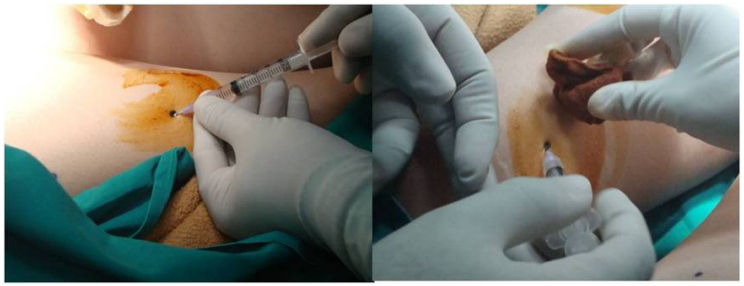


Figure 8: Transplantation of stem cells intra-muscularly.

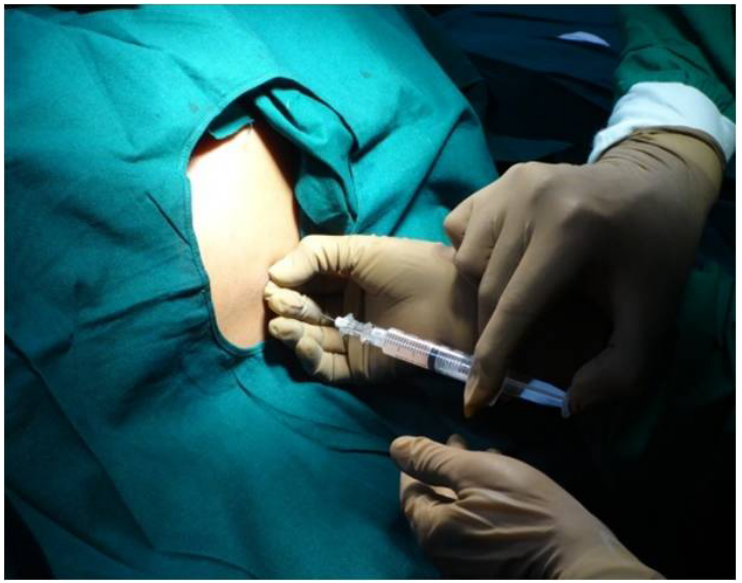


Figure 9: Transplantation of stem cells intrathecally.

Motor points to be injected are pre-determined a day prior to the procedure by an experienced physiotherapist. Weakest of the muscles that are most relevant to regaining function are chosen and motor points are marked using electrical stimulation.

Rigorous Rehabilitation

The day after the transplantation rehabilitative therapies like physiotherapy, occupational therapy, speech therapy, aquatic therapy, psychological counseling and

diet and nutrition advice are provided. Patient is asked to continue the therapies as a home program preferably under professional supervision at regular intervals.

Rationale for the protocol

Autologous Cells

Autologous cells inherit the genetic abnormality but have shown the potential to alter disease progression [54,55] and have no major irreversible side effects or risk of immune rejection upon transplantation. Allogenic cells show the promise of regenerating muscle cells without faulty proteins but it is only at an experimental level and with current routes of delivery it is impossible to deliver these cells in every muscle of the body. Allogenic cells also pose a great risk of immune rejection. Therefore autologous cells are a safer option.

Bone Marrow Mononuclear Cells (BMMNCs)

BMMNCs show potential for myogenesis as well as neurogenesis [58,59]; they have various paracrine effects like promoting angiogenesis, release of anti-inflammatory cytokines, various neurotrophic and myotrophic factors as well as growth factors, immune-modulation and stimulation of resident satellite cells [60]. MNCs are combination of multiple cell fractions; some of these are mesenchymal cells, vascular endothelial progenitors, very small embryonic like cells, fibroblasts and hematopoeitic cell precursors [61].

One of these cells is Mesenchymal cells (MSCs). MSCs secret exosomes that influence angiogenesis, neurogenesis and reduce inflammation [62]. MSCs derived exosome secretion is stimulated by inflammation. In a chronic inflammatory condition like that of MD, MSCs exert an exosome mediated immune modulation and inhibit fatty infiltration along with fibrotic injury [63]. Exosomes also promote formation of new muscle fibers by enhancing myogenesis and angiogenesis in the skeletal muscles [64].

BMMNCs have been successfully investigated for the treatment of muscular dystrophies with minimal procedure related side effects and no major side effects.

Intrathecal Delivery

The co-morbid disorders like that of intellectual disability and cognitive impairment in patients with DMD and BMD suggest neurogenic involvement in muscular dystrophies [65]. Dastur and Razzak 1973, highlight the myopathological similarities between atrophies and dystrophies. They analyzed 1348 cases of muscular dystrophies and anterior horn cell lesions and observed that 23% of the patients with dystrophy showed group atrophy in histological examination; 30% of the patients with denervation atrophy showed myopathic changes in the histological examination. There were similar numbers of atro-

phied and hypertrophied muscles in both the dystrophic and atrophic muscles. Depletion of Type II muscle fibers in dystrophic muscles was observed. These findings suggest the overlap between the denervation and myopathic pathology in these conditions. This study highlights the myopathic as well as neuropathic pathology of muscular dystrophies [65]. In addition to this several studies have explored presence of DGC in schwann cells, purkinje fibers and non-neural tissue like kidney, retina as well as glial cells [1,3,4,12,13]. Progressive muscle weakness may cause retrograde degeneration of the nerves.

Hence, from our experience, the intrathecal delivery of cells ensures nerve repair and tightening of neuromuscular junctions. It improves axial muscle and core muscle strength along with overall balance in the body. This method is minimally invasive and is the safest targeted mode of transplantation.

Rehabilitation Therapies

It is important that stem cell therapy be complimented with rehabilitative therapies like physiotherapy, occupational therapy, aquatic therapy, speech therapy, psychological intervention and nutritional advice. Preclinical studies have shown upregulation of utrophin, satellite cells mobilization and improved muscle strength following physical exercise like wheel running [66,67]. Regular exercise improves muscle strength, functional independence, respiratory function; promotes satellite

cell mobilization and enhances the regenerative capacity of the muscles in patients with muscular dystrophy [68-71]. Rehabilitative exercises have a resonating effect with the paracrine effects of cellular therapy. Exercise brings about myoprotective, neuroprotective, anti-inflammatory, antioxidant, antiatherogenic and neoangiogenetic effect systemically [66-75]. Exercise promotes mobilization of heamatopoeitic stem cells and endothelial progenitor cells; increases their percentage in peripheral circulation and promotes their migration to the damaged muscles for repair and regeneration [76-78]. Exercise also promotes neural stem cells migration and proliferation [79]. Clinical study evaluating effects of physical activity compared to the effects of structured exercise program showed that there is greater improvement in muscle strength, endurance, lung function and quality of life in patients with structured exercise program post hematopoietic stem cell transplantation. Several other studies have highlighted the benefits of exercise post cell transplantation [80,81].

Repeat Transplantation

Since muscular dystrophy is a progressive disorder and there is continual stem cell pool depletion. In our experience repeat dose of transplantation is required to alter the progression of the disease. The second transplantation can be repeated after 6 months of the first transplantation. There is insufficient evidence to conclude how many transplantations are required and the most optimum time frame to repeat the dose.

Adverse Effects

The protocol is safe and there are no major adverse effects of cellular transplantation. Some minor procedure related adverse effects like pain and swelling at the site of injection and aspiration can be experienced by a small percentage of patients. Other procedure related minor adverse effects include nausea, headache and vomiting. All the adverse events are completely treatable and usually subside within 48 hours of the procedure.

Published Results

Published results with Autologous Bone Marrow Mononuclear Cell Intrathecal and Intramuscular Transplantation

Published data

1. A study of 150 patients of DMD, LGMD and BMD were studied for safety and efficacy of autologous bone marrow mononuclear cell intramuscular and intrathecal transplantation. Intramuscular injections were at motor points of the antigravity weak muscles followed by vigorous rehabilitation therapy. There were no significant adverse events. Assessment after transplantation showed neurological improvements in trunk muscle strength, limb strength on Manual Muscle Testing (MMT), with Gait improvements and a shift on assessment scales such as Functional Independence Measure (FIM) ; Brooke and Vignos scale. Further, Imaging and Electrophysiological studies also showed significant changes in selective cases. On a mean follow up of 12 months ± 1 month, overall 86.67% cases showed symptomatic and functional improvements, with 6 patients showing changes with respect to muscle regeneration and decrease in fatty infiltration on musculoskeletal Magnetic Resonance Imaging (MRI) and 9 showing improved muscle electrical activity on Electromyography (EMG). 53% cases showed increase in trunk muscle strength, 48% showed increase [82].
2. Another study analyzed 59 patients of LGMD who underwent cell therapy and rigourous rehabilitation. Detailed subjective and objective analysis was done using neurological assessment and outcome measures like Functional Independence Measure (FIM) and Manual Muscle Testing (MMT). The study was undertaken over the period of 5 years, with a follow up range from 9 months to 4.5 years. Mean age of the group was 32 with minimum of 16 and maximum of 57 years. Mean age of onset was 18 with minimum age of onset of 3, to maximum of 36 years. The comparison of FIM scores of the patients post procedure yielded

no significant difference suggestive of maintained function over the time. There was a statistically significant improvement in the muscle strength of major body muscles like, hip and knee muscles, upper abdominals and shoulder muscles. The key finding of this study was the demonstration of a plateau phase in their progression. There were no significant adverse events noted. The results show that autologous BMMNCs may be a novel, safe and effective treatment approach to control the rate of progression of LGMD, thus improving the functional outcomes and enhancing their quality of life [83].

Unpublished Results

512 patients diagnosed with muscular dystrophy were analyzed. Symptomatic analysis was done for the core symptoms of the disease. These included changes in ambulatory status, hand functions, balance, stamina/fatigue, trunk activation and standing. They were graded as no change, mild, moderate and significant change. On follow up, out of 332 patients, 85.74% of patients showed improvements while 14.25% of patients remained stable without any deterioration in any of the symptoms. Mild improvements were observed in 20.31% of patients, moderate in 35.74% of patients, whereas, 29.68% of patients showed significant improvements (Figure 10).

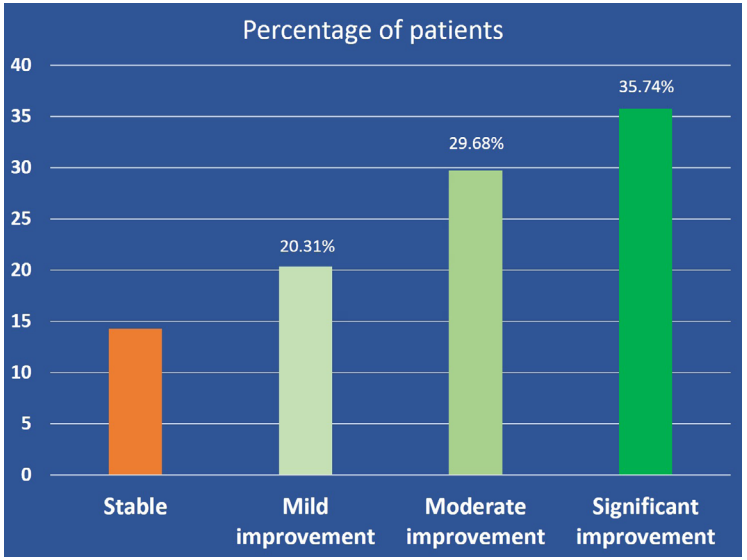


Figure 10: Distribution of improvements seen after autologous BMMNCs transplantation

Duchenne Muscular Dystrophy

Total of 139 boys detected with DMD underwent Autologous bone marrow mononuclear cell intrathecal and intramuscular transplantation. Mean age of the group was 11 years, ranging from 3 to 23 years. 39 boys were below the age of 10 years at admission, 77 were between 10 to 15 years and 23 boys were over the age of 15 years. 57 boys were ambulatory at assessment and 81 were non-ambulatory. Genetic testing was available for 64 boys, 38 of which showed distal rod ,45-55 exon deletions, 7 showed proxi-

mal rod, 3-21 exon deletion, 2 showed both proximal and distal rod, 4 showed deletion of exons in other regions and 13 patients showed no deletions but mutations.

Funtional status and muscle strength were assessed using, functional independence measure (FIM) scale, Brooke and Vignos scale and Manual muscle testing. In addition to these outcome measures the time till ambulation was compared with 35 age matched patients that chose not to undergo Stem cell therapy after initial consultation.

The changes in the scales were analysed statistically using Matched pair Wilcoxon Sign Rank test (Table 1 and 2). There was no statistically significant deterioration in these scales suggesting the delayed progression of the disease. Kaplan-Meier Survival Analysis was used to compare the age at loss of ambulation (Figure 11, Table 3). There was a statistically significant difference in the time till loss of ambulation for children that underwent stem cell therapy from those that did not. The average predicted age at the time till loss of ambulation was 142 months for children that did not undergo stem cell therapy; whereas it was significantly higher, 204 months, in children that underwent stem cell therapy. Percentage analysis was performed for the symptomatic improvement in these children (Table 4, Figure 12). This analysis suggested that majority of the patients had shown improvement or halting of the progression in postural deviations, neck weakness, bed mobility, trunk activity, gross and fine motor function, functional

upper limb activity, walking and standing. The pre and post therapy measurements were performed at a median follow up of 6 months.

Table 1: Matched pair Wilcoxon Sign Rank test analysis of outcome measures pre and post therapy.

Outcome measure	Pre Therapy Mean Score	Post Therapy Mean Score	Statistical Significance
Functional Independence Measure	71	76	0.001
Brooke Scale	3.07	3.27	0.076
Vignos Scale	6.5	6.8	0.245

Table 2: Matched pair Wilcoxon Sign Rank test analysis of modified manual muscle testing scale.

Muscle Group	Pre Therapy Mean Score	Post Therapy Mean Score	Statistical Significance
Hip flexors	6	6.69	0.001
Hip Abductors	5.42	6.08	0.001
Hip Adductors	4.21	5	0.001
Knee Flexion	9.1	9.48	0.004
Knee Extension	5.26	5.69	0.003
Shoulder Adduction	5.26	6.02	0.04
Shoulder internal rotation	7.23	7.79	0.001
Biceps	7.96	8.32	0.01
Upper Abdominals	3.8	4.21	0.005

Table 3: Kaplan-Meier analysis of time till loss of ambulation for patients with and without stem cell therapy.

	Comparison Group	Intervention Group	Test statistics
Total no. of patients	35	42	-
Percentage of patients currently non- ambulatory	65%	23%	-
Predicted time till loss of Ambulation	142 months	204 months	0.004

Table 4: Percentage analysis of modified manual muscle testing scale .

Muscle		Percentage of patients with improved muscle strength	Percentage of patients with deteriorated muscle strength	Percentage of patients with muscle strength maintained
Hip	Flexors	29	15	55
	Extensor	37	13	50
	Abduction	32	8	60
	Adduction	41	6	53
Knee	Flexor	29	5	65
	Extensor	29	10	60
Ankle and Foot	Peronei	27	10	63
	Tibialis Anterior	27	10	63
	Tibialis Posterior	26	12	63
	Plantar Flexors	9	3	88
	EHL	14	9	77
	EDL	14	8	78
Shoulder	Deltoids	28	17	55
	Adduction	24	10	65
	Internal Rotation	24	4	72
	External rotation	26	5	69
Elbow	Biceps	19	8	73
	Triceps	26	14	60
Wrist and Fingers	Wrist Flexors	10	4	86
	Wrist Extensors	12	3	86
	Supinators	12	4	85
	Pronators	5	4	91
	Palmar Interossei	12	12	77
	Dorsal Interossei	10	12	78
	Lumbricals	10	5	85
Trunk	Upper abdominals	36	8	56
	Lower abdominals	26	18	56

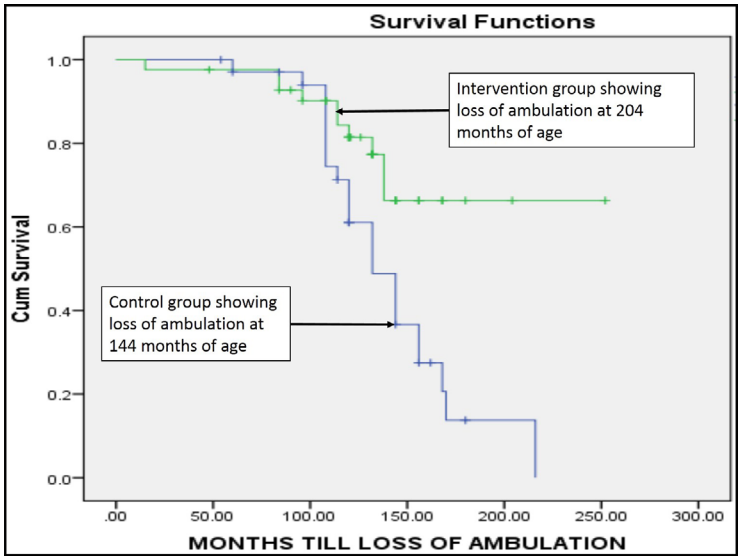


Figure 11: Kaplan-meier analysis of time till loss of ambulation in patients with and without stem cell therapy.

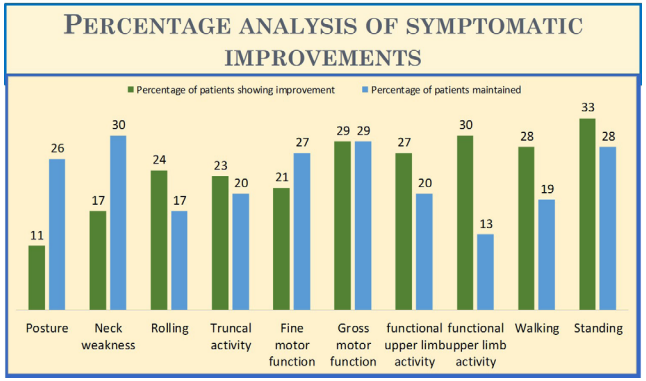


Figure 12: Percentage analysis of symptomatic improvement in the patients with stem cell therapy.

Musculo-Skeletal MRI as a Monitoring Tool

MRI – MSK can be used as an outcome measure, it is an advance technique of radio imaging. MRI has several advantages over other radio imaging techniques like multiplanaraquisition, no need to use ionizing radiation or intravenous contrast. It is non-invasive and is capable of differentiating between the soft tissue and muscle fiber with high resolution. Different studies have assessed the progression of the disease on MRI in diseases like BMD and DMD [84].

We studied therapeutic benefits of autologous BMMNCs transplantation in a patient with BMD. MRI-MSK was used as an outcome measure. 8 months later increased muscle fiber was noted to peronei, gastro-soleus and triceps which also correlated with the clinical improvement. (Figure 13).

Another case of a 28 year old male with BMD who underwent adult autologous bone marrow mononuclear cell intrathecal transplantation showed regeneration in Peroneous longus, brevis, gastrocnemius, soleus and triceps as demonstrated on MRI-MSK 6 months post transplantation [85]. Clinically he showed improvement in standing endurance, ambulation, exercise tolerance, muscle strength and fatiguability (Figure 13&18).

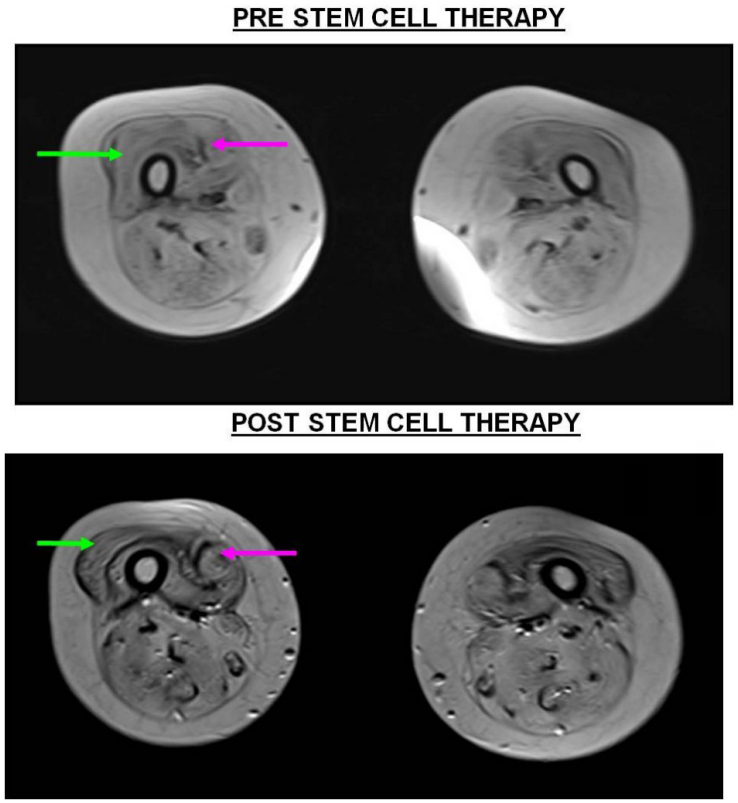


Figure 13: Improvements on the MRI-MSK after autologous BMMNCs transplantation.

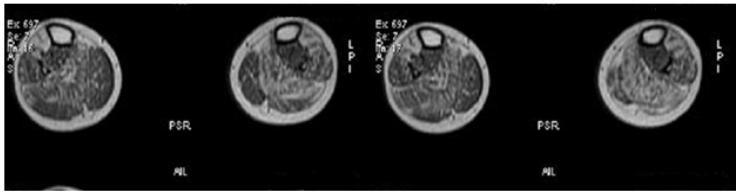


Figure 14: T1 weighted axial musculoskeletal MRI images of Peroneous Longus and Brevis before Autologous BMMNCs transplantation.

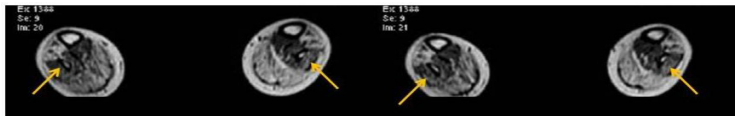


Figure 15: T1 weighted axial musculoskeletal MRI images of Peroneous Longus and Brevis after Autologous BMMNCs transplantation, arrown showing muscle regeneration and reduced fatty infiltration.

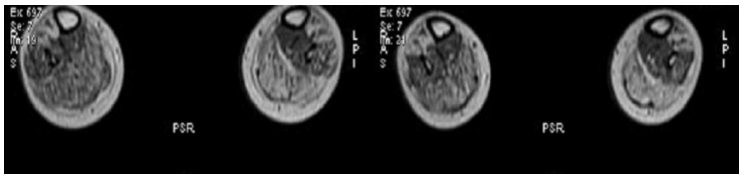


Figure 16: T1 weighted axial musculoskeletal MRI images of Gastrocnemius and Soleus before Autologous BMMNCs transplantation.

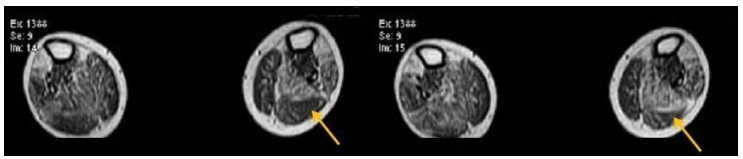


Figure 17: T1 weighted axial musculoskeletal MRI images of Gastrocnemius and Soleus after Autologous BMMNCs transplantation; arrown showing muscle regeneration and reduced fatty infiltration.

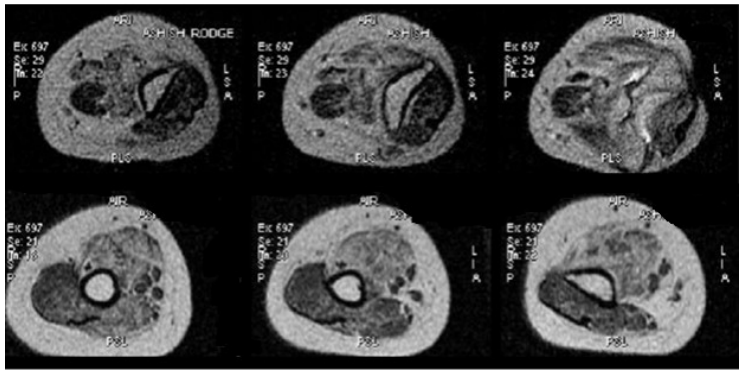


Figure 18: T1 weighted axial musculoskeletal MRI images of Left and Right Long, Medial and Lateral head of Triceps before Autologous BMMNCs transplantation.

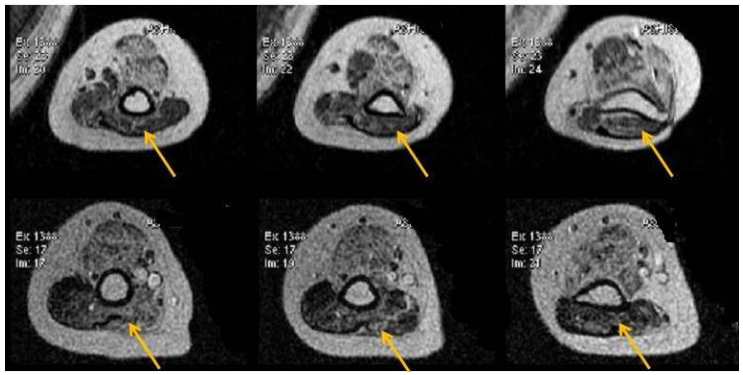


Figure 19: T1 weighted axial musculoskeletal MRI images of Left and Right Long, Medial and Lateral head of Triceps after Autologous BMMNCs transplantation; arrows showing muscle regeneration and reduced fatty infiltration.

Future Directions

Clinical studies have demonstrated efficacy of different types of cells and routes of administration individually, however there is no comparative studies. Future studies should be focused at determining the optimum cell type, route of delivery and dosage of delivery. Muscular dystrophy is a progressive disorder therefore repeat dosing may be required, frequency for repeat dose of stem cell transplantation needs to be determined in future. Trials should be initiated to assess the effect of stem cell therapy in combination with gene therapy drugs like Eteplirsen. Apart from usual outcomes that focus on muscular endurance and strength, outcomes that assess the effectiveness of therapy on cardio-respiratory systems should also be assessed.

Conclusion

Current treatment options are inadequate to cure or alter the pathology of muscular dystrophy. Muscular dystrophy can be considered as a stem cell disease as symptoms of the disease are evident once the stem cell pool is depleted. Therefore it is essential to include stem cell therapy for any treatment strategy to be effective. Stem cell replacement should be combined with structured and supervised rehabilitation to attain optimum results. As muscular dystrophy is a progressive disorder treatment earlier in the phase of the disease will have a better effect.

Repeated transplantation of cells may be required and additional benefit of repeat transplantation should be studied further. MRI-MSK can be an effective monitoring tool to assess the effects of stem cell therapy.

Adult autologous bone marrow mononuclear cells intrathecal and intramuscular transplantation is safe and can slow down the progression of the disease in muscular dystrophy. This treatment can delay the loss of ambulation in children with DMD. Stem cell transplantation in combination with current treatments may improve quality of life, time till loss of ambulation and survival of the patients.

The available evidence for benefits of stem cell therapy is limited and therefore more clinical trials and studies are required that use rigorous methodologies to find out the effect of stem cell therapy in different forms of muscular dystrophies using different cell types, dosages, routed of administration and transplantation frequency.

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