

# Role of Stem Cell Therapy in Treatment of Muscular Dystrophy

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## INTRODUCTION

Muscular dystrophy connotes a group of disorders due to genetic abnormality of the genes that code for various proteins essential for the integrity of muscle cells. Most of these disorders are hereditary but there can be sporadic mutations as well. Different types of muscular dystrophies are identified based on the core genetic abnormality however they are all characterized by progressive muscle degeneration. Some of the most severe forms of muscular dystrophies are Duchenne's muscular dystrophy (**DMD**) and Congenital muscular dystrophy (**CMD**); the onset of which is early in the childhood, with fast progression and considerably reduced lifespan up to second or third decade of life. The other forms like becker's muscular dystrophy (**BMD**), limb girdle muscular dystrophy (**LGMD**), facioscapulohumeral dystrophy (**FSHD**) have their onset ranging from 2nd to 4th decade of life and the lifespan is largely unaltered but quality of life reduces significantly with severe disability in the later part of life.

# PATHOPHYSIOLOGY OF MUSCULAR DYSTROPHY

Some of the common pathophysiological mechanisms underlying muscular dystrophies are progressive muscle loss, myocellular inflammation and fibrosis (Figure 1). This is a 3 step recovery process of muscles from any injury. Muscle injury triggers resident immune cell response causing phagocytosis. Phagocytes not only clear the debris of the dead cells but also secrete various prostaglandins, cytokines and chemokines triggering an inflammatory response. Usually during muscle repair two types of inflammatory responses are seen the early inflammatory phase and later remodeling phase of myogenesis. In muscular dystrophies the early inflammatory phase is exaggerated due to continuous damage to muscle cells and cell death. Such chronic inflammation exhausts and eventually suppresses satellite cell activation responsible for myogenesis and instead stimulates fibrosis causing fatty replacement of the muscle tissue [1,2]. There is an imbalance of muscle degeneration and regeneration leading progressive muscle weakness.

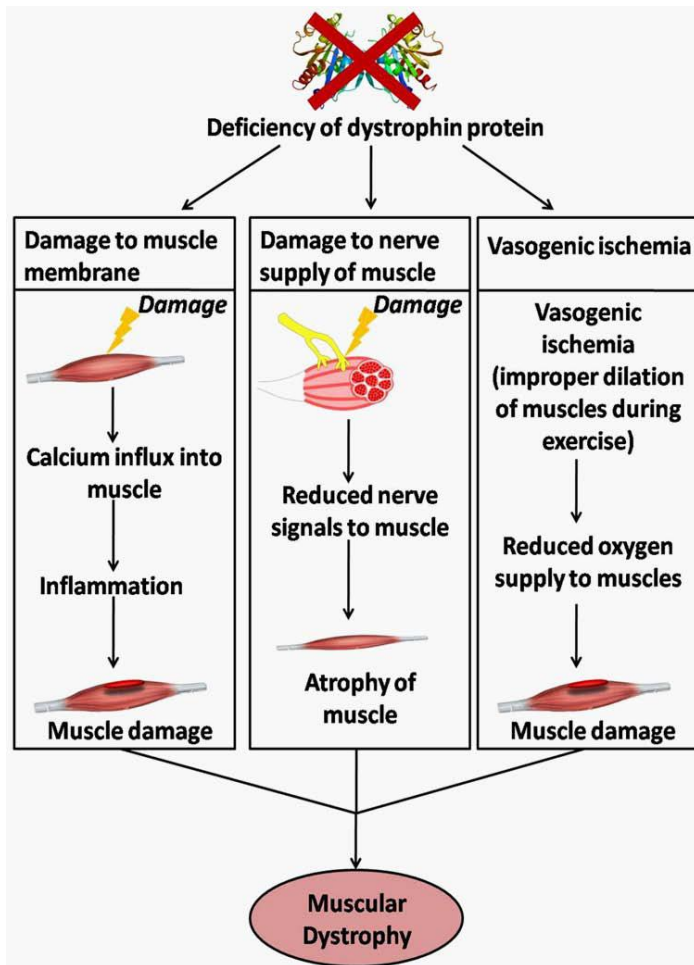


Figure 1: Pathophysiology of muscular dystrophy.

# STRATEGIES FOR ADDRESSING MUSCULAR DYSTROPHY AND NEED OF STEM CELL THERAPY

Over the years various strategies have been devised to combat the pathophysiological processes in muscular dystrophy like use of steroids and anti-fibrotic medications to suppress the inflammation and fibrosis. Different conservative rehabilitative approaches are suggested to minimize muscle damage [3].

## Conservative Management

Multidisciplinary rehabilitation plays a major role in the management of muscular dystrophy. Various nutritional supplements like antioxidants and protein supplements are available to enhance the muscle integrity and muscle building. However, rehabilitative methods and nutritional support alone fall short of altering or halting the disease progression for MD.

## Medical Management

Medications such as steroids and anti-fibrotics have limited role in reducing inflammation and curbing the fibrotic processes.

## Gene Therapy and Replacement of the Deficient Protein

Although the ultimate frontier could theoretically be gene therapy, repairing the gene and getting the functional protein is very complex and currently in the experimental stage. Various factors such as identifying and targeting the exact gene defect, finding optimum vectors for delivering the gene product are few of the challenges being faced by the scientists.

## UNMET MEDICAL NEEDS

None of the above treatment strategies have been able to reverse or prevent the muscle damage in these disorders. Therefore newer treatment strategies are aimed at supporting muscle regeneration through activation of endogenous stem cells or delivery of stem cells exogenously. This highlights the potential use of cellular therapy for the treatment of muscular dystrophies.

Current treatments only alleviate a few symptoms of the disorder but do not alter cellular pathologies. They fail to carry out the repair and regeneration of the damaged muscles to significantly alter clinical outcome of the disease. Also, no treatment corrects the underlying genetic mutation. Although explored extensively, gene therapy has not yet been established in clinical application. No standard therapeutic modality has been successful to halt the progression of the disease or increase the survival. Therefore a treatment strategy that alters the 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 REPLENISHMENT NECESSARY FOR ALL THERAPEUTIC STRATEGIES IN MUSCULAR DYSTROPHY

As explained earlier the core pathology of the muscular dystrophy is uncontrolled muscle damage due to faulty proteins responsible for cytoskeletal integrity. Therefore there is imbalance between the degeneration and regeneration of the muscles. It has been shown in the animal models that muscular dystrophy is a stem cell disease; caused by exhaustion of the stem cells [4]. Leading scientists like Alessandra Sacco from the Stanford-burnham medical center in the field of muscular dystrophy talk about the need of stem cell therapy in the treatment of MD. Through their research they have shown that, the inability of human muscle stem cells to keep up with the ongoing damage caused by the disorder causes progressive muscle weakness. The chronic muscle damage, in a patient with muscular dystrophy initiates a never ending cycle of repair and wasting. The wasting takes over the repair process eventually, since the stem cell compartment in the muscles of an affected individual is insufficient. Hence, they report that muscular dystrophy is definitively a stem cell disorder. Considering this, former postdoctoral fellow Jason Pomerantz, MD, co-corresponding author with Sacco et al. and now an assistant professor at the University of California, San Francisco made a profound statement,

*“If a treatment does not replenish the stem cell compartment, it will likely fail; it would be like pushing the gas pedal to the floor when there is no reserve.”*

The idea that, the symptoms of muscular dystrophy is result of inability of stem cells to repair ongoing damage has some interesting implications. It implies that any successful treatment should begin early, before the stem cell pool is depleted. It suggests that a highly targeted approach to increase telomerase activity in the muscle stem cells could be useful. Finally, it also indicates that researchers and clinicians should investigate stem cell based therapies as well as those aimed at protecting the muscle fibers themselves.

*Hence, strategies for definite treatment of Muscular dystrophy will have to include stem cell replenishment!!*

## IMPLICATIONS OF STEM CELL REPLENISHMENT IN MUSCULAR DYSTROPHY

It is very clear that muscular dystrophy is a multifaceted disease. There are two simultaneous processes that lead to muscle damage. Muscle damage due to lack of dystrophin and exaggerated fibrotic response. Normally these damaged muscles are replaced by dividing muscle stem cells (satellite cells), but repeated rounds of division cause the telomeres to shorten until the stem cells can't fix the damage anymore. Hence, the stem cell pool has to be replenished. Treatments directed solely at repairing the muscle fibers will not suffice and could even exacerbate the disease. The muscle stem cells must be taken into consideration.

Hence, gene therapy alone will not be able to improve muscle strength and muscle longevity. For a successful treatment of muscular dystrophy it must be combined with stem cell therapy.

As we now understand that stem cell therapy forms an integral part of management of muscular dystrophy, let us understand what are stem cells, the types of stem cells and the routes of administration. This is important to elicit, what strategy for stem cell transplantation would be the most appropriate.

## WHAT ARE STEM CELLS?

Stem cells are unique specialized cells with an ability to multiply and develop into many different cell types in the body during early life and growth. They also help in the repair of the body by dividing and replenishing the damaged cells. When a stem cell divides, each new cell has the potential either to remain as a stem cell or to become another type of cell with a more specialized function, such as nerve cell, muscle cell, skin cell, or red blood cell etc.

## MECHANISM OF ACTION OF STEM CELLS (FIGURE 2)

Stem cell therapy shows good promise in reversing the effects of cell death by the ability of cells for differentiation in myocytes and satellite cells, integration into host muscles, and exerting different paracrine effects like immunomodulation, anti-inflammatory effect and angiogenesis [5-7]. Transplanted cells can repair muscle damage and enhance angiogenesis and contribute to neovascularization by producing signaling molecules such as vascular endothelial growth factors (**VEGF**) and fibroblast growth factors (**FGF2**) [8]. Along with increase in angiogenesis, they also promote tissue remodeling, prevent apoptosis, decrease inflammation, release growth factors and activate the satellite cells [9]. In animal studies, these cells have shown to produce the deficient proteins and make new muscle cells which fuse with the host fibers. Satellite cells, the adult skeletal muscle progenitor cells, are commonly considered to be the main cell type involved in skeletal muscle regeneration [10].

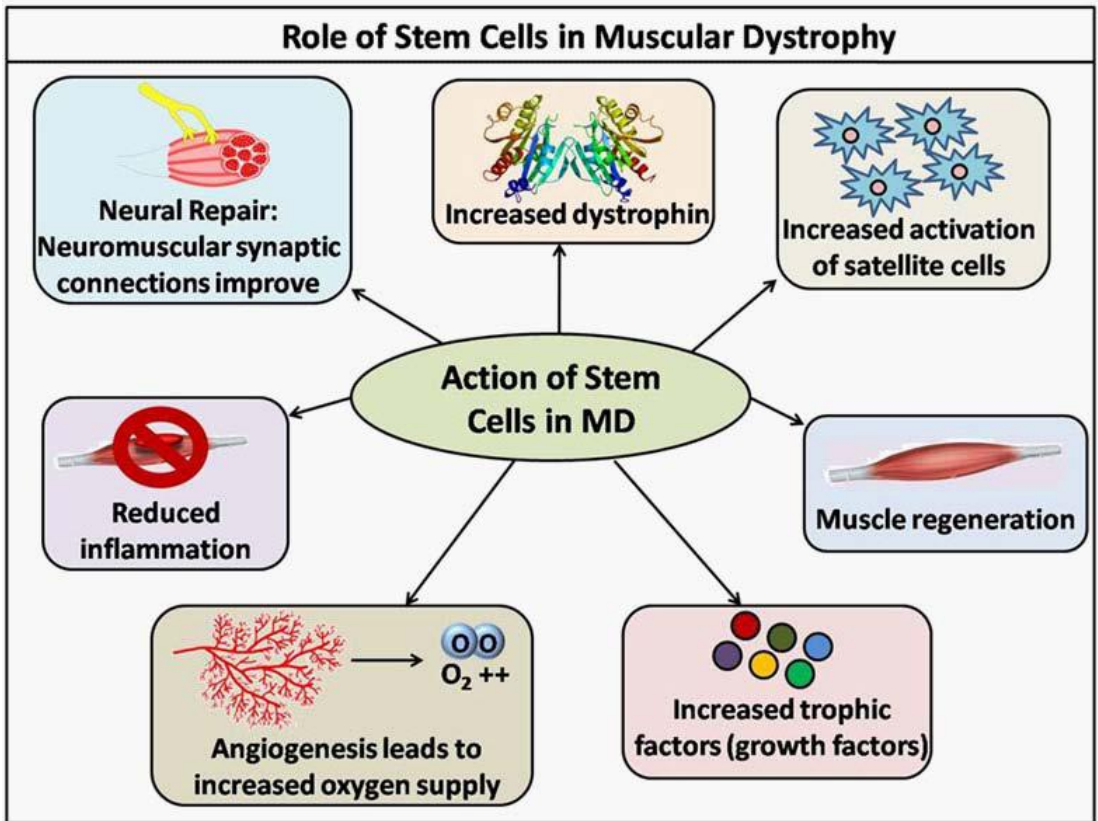


Figure 2: Mechanism of action stem cells in muscular dystrophy.

## WHAT ARE THE TYPES OF STEM CELLS?

Stem cells are of different types, depending on the source from where they are obtained as well as their ability to form different types of cells.

Depending on the source, the stem cells can be broadly classified as Autologous and Allogenic stem cells. Allogenic stem cells are obtained multiple individuals and are transplanted into a new recipient where as autologous stem cells are obtained from the recipients own body.

## TYPES AND SOURCES OF ALLOGENIC STEM CELLS ARE GIVEN BELOW

### Embryonic Stem Cells

These stem cells can be obtained from 3-4 days embryos. This stage in the development of a human is known as blastocyst. Infact, all of us are born from or are a product of stem cells. When we were first conceived (when our mother's egg fertilizes with our father's sperm), we were in one cell stage. Then, we divide and form a clump of cells (16-32 cells), and enter the "blastocyst" stage. The cells obtained from this stage, when grown in a laboratory, has the ability

to form different types of cells of our body (known as having totipotency). Theoretically, these are the most potent stem cells. However, they have certain ethical and medical issues surrounding them. In some religions, derivation of stem cells from blastocyst (which are extra after invitro fertilization), is equivalent to taking a life. Apart from that, these cells have potential to form a type of tumor called ‘teratoma’ (as found in experimental animals).these cells may pose the risk of teratogenicity.

### ***Fetal stem cells***

These cells are isolated from variety of fetal tissue and have a better regenerative capacity than adult stem cells. The ethical concerns that surround embryonic cells may also apply for the fetal stem cells. The investigation of fetal stem cells is still in the experimental phase and has not been used clinically.

### ***Umbilical Cord Stem Cells***

The other source is the umbilical cord blood and placental tissue (Wharton’s jelly).This is a rich source of stem cells. The cord tissue is discarded as a waste during the birth of a child. The option of storing this for “potential” future use for the same child can be considered. It is found to be a good source of stem cells.

However, use of allogenic stem cells increases the risk of immune rejection and lead to poor compatibility with the recipient.

## **TYPES AND SOURCES OF AUTOLOGOUS STEM CELLS**

Autologous sources are bone marrow, adipose tissue and dental pulp. These form a cohort of adult stem cells. Induced pluripotent stem cells are also an attractive possibility of adult, autologous stem cells, wherein cells from the patient’s own body can be reprogrammed to achieve an embryonic cell characteristic. Though theoretically, this remains the “best of both worlds scenario”, practically and clinically, it is still a distant objective.

Hence, in the current realm of translational medicine bone marrow derived mononuclear stem cells, especially, are found to be the safest, most feasible and effective option for use in treatment of various disorders, such as muscular dystrophy. These cells are easily accessible, replenished naturally and containing a varied type of cell population, each with a differing mechanism of action. Together, the multipronged approach towards a multifaceted disease is coming out as the most doable solution.

## **WHAT ARE DIFFERENT ROUTES OF ADMINISTRATION OF STEM CELLS?**

### ***Intramuscular Route***

In this method the stem cells are injected directly into the affected muscles. This creates “local depots” of implanted cells with increased local paracrine activity. Though, this method has been

used for myoblast transplantation, it was done in a very laborious way, with cells injected at very small distances. This is not only impractical, but also very painful for the patient. Hence, a very scientific method of motor point stimulation, followed by injection at those points was devised by physiotherapists and researchers at Neuro Gen BSI.

Motor point is the point at which the main nerve enters the muscle or, in case of deeply placed muscle, the point where the muscle emerges from under covers of the more superficial ones.

### **Facts about motor points**

- Motor points are frequently at the junction of the upper & middle one thirds of the fleshy belly of the muscles, although there are exceptions e.g.: the motor point of vastus medialis, whose nerve enters the lower part of the muscle, is situated a short distance above the knee joint.
- This is the point on the skin region where an innervated muscle is most accessible to percutaneous electrical excitation at the lowest intensity. Deeply placed muscles may be stimulated most satisfactorily where they emerge from beneath the more superficial ones, e.g.: extensor hallucis longus in the lower one third of the lower leg.

Although MD can affect several body tissues and organs, it most prominently affects the integrity of muscle fibers. It causes muscle degeneration, progressive weakness, fiber death, fiber branching and splitting, phagocytosis (in which muscle fiber material is broken down and destroyed by scavenger cells), and in some cases, chronic or permanent shortening of tendons and muscles. Also, overall muscle strength and tendon reflexes are usually lessened or lost due to replacement of muscle by connective tissue and fat. So selection of muscles (motor points) for intramuscular injection depends on manual muscle testing & patient's complaints of weakness & difficulty in activities of daily living.

To inject the stem cells, motor points of each muscle are identified using an electrical stimulator (Figure 3), by an experienced physiotherapist. The cells are then injected at these points (Figure 4).





**Figure 3:** Electric stimulation to mark the motor point.



**Figure 4:** Intramuscular injections in the motor points.

## Intrathecal Route

In the intrathecal route stem cells are transplanted into the cerebrospinal fluid through a lumbar puncture injection. Muscle protein, such as dystrophin have been found in the central nervous system and also in Schwann cells covering the nerves as well. Absence or abnormal dystrophin therefore brings about accelerated damage to the nerves and contributes to muscle weakness.

The co-morbid disorders like that of intellectual disability and cognitive impairment in patients with DMD and BMD suggest neurogenic involvement in muscular dystrophies [11]. 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 atrophied 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 [11].

It has also been noted, that progressive muscle weakness, causes a retrograde degeneration of the nerves, thereby adding a neurogenic component to a pure muscle disease. 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.

A combination of intrathecal and intramuscular injections of stem cells at specific motor points, has been found to be a very good strategy to address the muscle stem cell compartment replenishment as well as for the repair of the nerves.

## Systemic Routes

This is by a simple injection of stem cells intravenous. It is one of the safest, minimally invasive and most widely used routes of administration. However, studies have shown that the cells administered via IV get trapped in organs (e.g. lungs, spleen and liver) other than the target organ. They are also more susceptible to the host immune system.

## RATIONALE FOR THE AUTOLOGOUS BMMNCs INTRATHECAL AND INTRAMUSCULAR INJECTIONS FOR THE TREATMENT OF MUSCULAR DYSTROPHY

Stem cells play a pivotal role in altering the disease pathology. BMMNCs show potential for myogenesis as well as neurogenesis [12-14]; 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 [15]. BMMNCs have been successfully investigated for the treatment of muscular dystrophies with minimal procedure related side effects and no major side effects. Although a muscle disease primarily there is progressive damage neural tissue as well in muscular dystrophy. Various protein complexes that are affected in muscular dystrophy are also part of neural tissue and therefore cause damage to nervous tissue as well [16]. In addition chronic inflammation causes nerve damage at the neuromuscular junction and retrograde degeneration of the nerves. Therefore intramuscular and intrathecal delivery is optimum, and addressed both the neuromuscular components. Autologous cells inherit the genetic abnormality but have shown the potential to alter disease progression [17,18] 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 by host. Therefore autologous cells are a safer option.

# PROTOCOL OF STEM CELL TRANSPLANTATION FOR AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS

Herein, we have described our protocol as we have the maximum experience in treating muscular dystrophy patients using autologous bone marrow mononuclear cells. We have treated more than 886 patients of muscular dystrophy. The cells are injected via intramuscular and intrathecal route of delivery. The procedure for stem cell transplantation at NGBSI is minimally invasive, with simple steps or processes. There is no major surgery or incisions required.

## The Procedure is Carried Out in 3 Steps

### Bone marrow aspiration (Figure 5)

The easiest place to take out the bone marrow is the anterior superior iliac spine. Using a thin bone marrow aspiration needle, 80-120 ml bone marrow is aspirated and collected in heparinized tubes.



**Figure 5:** Bone marrow aspiration.

### Separation of stem cells (Figure 6)

On the same day, within 3-5 hours, stem cells are separated from the bone marrow using density gradient method. The separated cell pellet is analyzed under microscope using Trypan blue to check for viability of the mononuclear cells. Cell viability and cell counting is done manually and confirmed using a cell counter. Sample is then sent for characterization of cell surface marker of CD34+ using FACS analysis.



**Figure 6:** Separation of stem cells.

### **Injection** (Figure 7 and 8)

Stem cells are then immediately injected into L4-5 space. An 18G Touhy needle is inserted into the sub-arachnoid space. After establishing a free flow of CSF, an epidural catheter is inserted into the space and a part of the stem cells are injected into the cerebrospinal fluid (fluid which flows around the brain and spine) and the remaining cells are injected intramuscularly into specific muscles at specific points called motor points.





**Figure 7:** Transplantation of stem cells intrathecally.



**Figure 8:** Transplantation of stem cells intra-muscularly.

# PUBLISHED RESULTS WITH AUTOLOGOUS BONE MARROW MONONUCLEAR CELL INTRATHECAL AND INTRAMUSCULAR TRANSPLANTATION

## Published Data

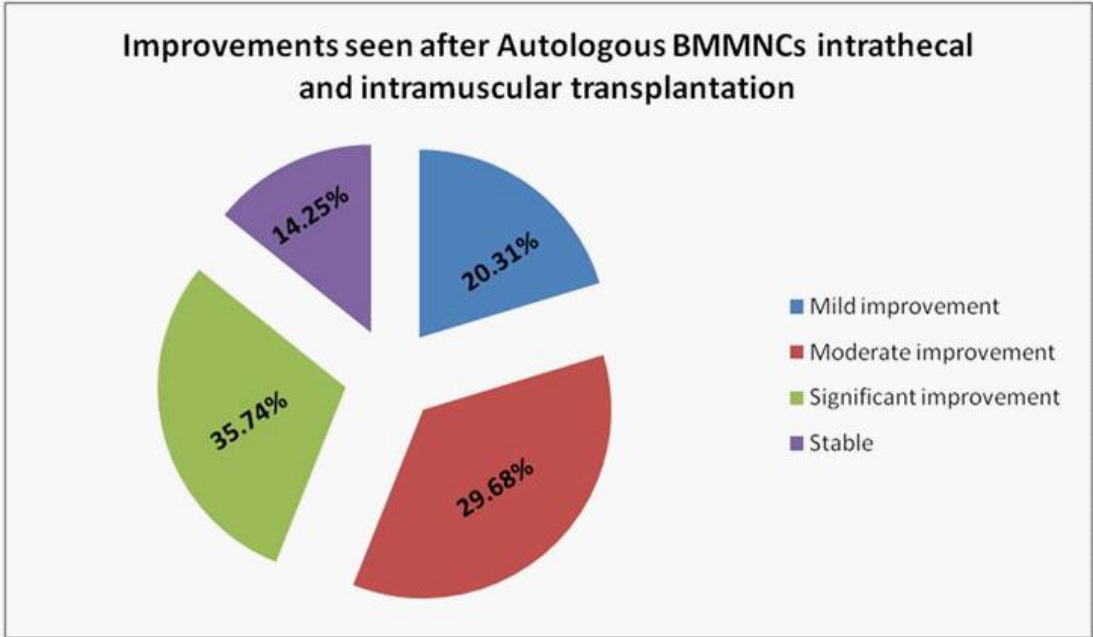
1. A study of 150 patients of DMD, LGMD and BMD were studied for safety and efficacy of autologous bone marrow mononuclear cell intramuscular and intrathecal transplantation. Intramuscular injections were at motor points of the antigravity weak muscles followed by vigorous rehabilitation therapy. There 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  $\pm$  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 [19].

2. Another study analyzed 59 patients of LGMD who underwent cell therapy and rigorous 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 [20].

## 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 9).



**Figure 9:** 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 proximal 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.

Functional 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 10, 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 11). 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

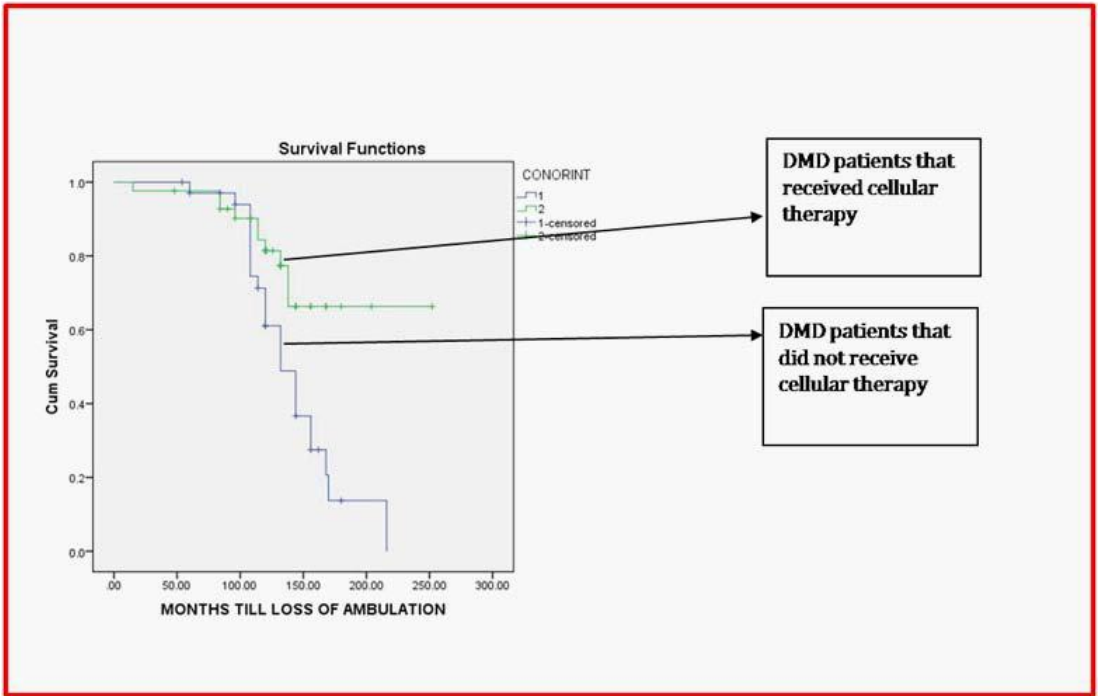
**Table 2:** Matched pair Wilcoxon Sign Rank test analysis of modified manual muscle testing scale.

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

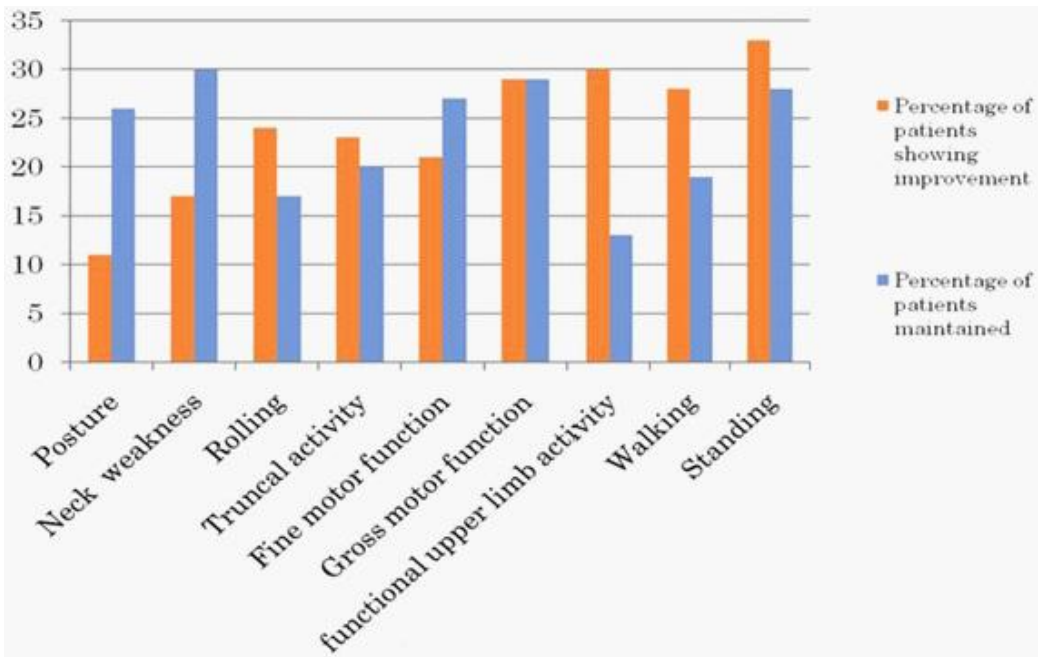
**Table 3:** Kaplan-Meier analysis of time till loss of ambulation for patients with and without stem cell therapy.

	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





**Figure 10:** Kaplan-meier analysis of time till loss of ambulation in patients with and without stem cell therapy.



**Figure 11:** Percentage analysis of symptomatic improvement in the patients with stem cell therapy.

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

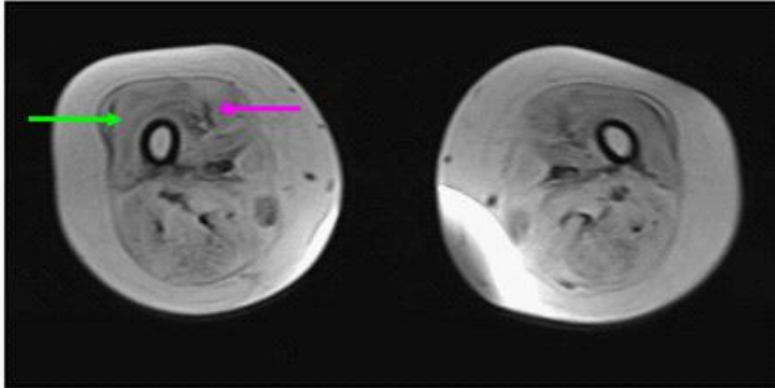
Muscle		Percentage of patients with improved muscle strength	Percentage of patients with deteriorated muscle strength	Percentage of patients with muscle strength maintained
Hip	Flexors	29	15	55
	Extensor	37	13	50
	Abduction	32	8	60
	Adduction	41	6	53
Knee	Flexor	29	5	65
	Extensor	29	10	60
Ankle and Foot	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

## Musculo-skeletal MRI as a monitoring tool

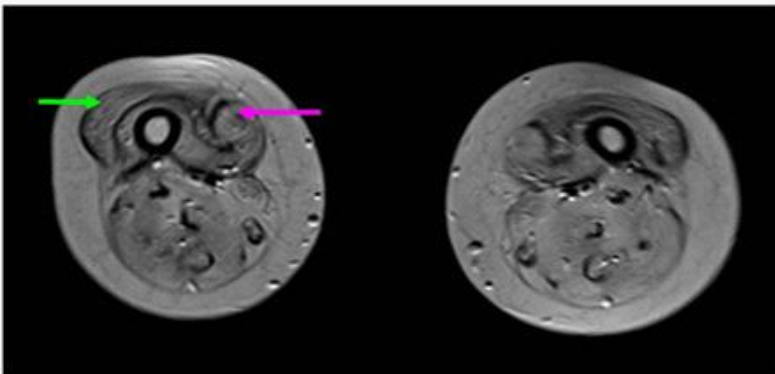
MRI – MSK can be used as an outcome measure, it is an advance technique of radio imaging. MRI has several advantages over other radio imaging techniques like multiplanaraquisition, no need to use ionizing radiation or intravenous contrast. It is non-invasive and is capable of differentiating between 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 [21]. 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 12).

## VASTUS MEDIALIS AND LATERALIS

### PRE STEM CELL THERAPY



### POST STEM CELL THERAPY



**Figure 12:** Improvements on the MRI-MSK after autologous BMMNCs transplantation.

## **REVIEW OF LITERATURE FOR STEM CELL THERAPY IN MUSCULAR DYSTROPHY**

Most extensively studies are the transplantations using satellite cells or myoblast progenies [22]. Huard et al reported presence of dystrophin positive fibers in the host along with transient motor improvement. [23] Gussoni et al studied myoblast transplantation demonstrating that the transplanted myoblasts persisted after injection and their fate was guided by the microenvironment [24-26]. 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 [27-32]. Although, myoblast transfer leads 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. [33] 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 [34].

Hematopoietic stem/progenitor cell populations from adult skeletal muscle also have a therapeutic potential for muscular dystrophy [35]. 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 [36]. 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 muscular 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 altered disease progression [37-41].

## FUTURE DIRECTIONS

Cellular transplantation has also been shown to cause production of fibro-adipogenic precursors which may mature into adipocytes and may lead to fibrotic processes [42]. Thus, more clinical studies for MD should be designed to investigate the fibrotic processes and treatments to alter these. Studies so far have not used imaging outcome measures, imaging modalities can provide a more objective assessments and should be incorporate in future studies. There are different combinations of cell types and routes which can be compared to determine the treatment for the optimum results. The evidence of cellular therapy is scarce and preliminary, therefore more multicenter randomized controlled trials investigating the efficacy of cellular therapy are required.

## References

1. Ceafalan LC, Popescu BO, Hinescu ME. Cellular players in skeletal muscle regeneration. *Biomed Res Int.* 2014; 2014: 957014.
2. Farini A, Sitzia C, Erratico S, Meregalli M, Torrente Y. Influence of immune responses in gene/stem cell therapies for muscular dystrophies. *Biomed Res Int.* 2014; 2014: 818107.
3. Manzur AY, Muntoni F. Diagnosis and new treatments in muscular dystrophies. *J Neurol Neurosurg Psychiatry.* 2009; 80: 706-714.
4. Sacco A, Mourkioti F, Tran R, Choi J, Llewellyn M. Short telomeres and stem cell exhaustion model Duchenne muscular dystrophy in mdx/mTR mice. *Cell.* 2010; 143: 1059-1071.
5. Orlic D, Kajstura J, Chimenti S, Bodine DM, Lerli A. Bone marrow stem cells regenerate infarcted myocardium. *Pediatr Transplant.* 2003; 7: 86-88.
6. Plotnikov EY, Khryapenkova TG, Vasileva AK, Marey MV, Galkina SI. Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture. *J Cell Mol Med.* 2008; 12: 1622-1631.
7. Cselenyák A, Pankotai E, Horváth EM, Kiss L, Lacza Z. Mesenchymal stem cells rescue cardiomyoblasts from cell death in an in vitro ischemia model via direct cell-to-cell connections. *BMC Cell Biol.* 2010; 11: 29.

8. Dellavalle A. Pericytes of human post-natal skeletal muscle are committed myogenic progenitors, distinct from satellite cells, and efficiently repair dystrophic muscle. *Nat. Cell Biol.* 2007; 9: 255-267.
9. Torrente Y, Belicchi M, Sampaolesi M, Pisati F, Meregalli M. Human circulating AC133(+) stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J Clin Invest.* 2004; 114: 182-195.
10. Quattrocelli M, Cassano M, Crippa S, Perini I, Sampaolesi M. Cell therapy strategies and improvements for muscular dystrophy. *Cell Death Differ.* 2010; 17: 1222-1229.
11. Dastur DK, Razzak ZA. Possible neurogenic factor in muscular dystrophy: its similarity to denervation atrophy. *Journal of Neurology, Neurosurgery & Psychiatry.* 1973; 36: 399-410.
12. Torrente Y, Tremblay JP, Pisati F, Belicchi M, Rossi B. Intraarterial injection of muscle-derived CD34(+)/Sca-1(+) stem cells restores dystrophin in mdx mice. *J Cell Biol.* 2001; 152: 335-348.
13. Ferrari G, Mavilio F. Myogenic stem cells from the bone marrow: a therapeutic alternative for muscular dystrophy? *Neuromuscul Disord.* 2002; 12: S7-10.
14. Song S, Song S, Zhang H, Cuevas J, Sanchez-Ramos J. Comparison of neuron-like cells derived from bone marrow stem cells to those differentiated from adult brain neural stem cells. *Stem Cells and Development.* 2007; 16: 747-756.
15. Gnechchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circulation Research.* 2008; 10: 1204–1219.
16. Salam EA, Abdel-Meguid IE, Shatla R, Korraa S. Evaluation of neural damage in Duchenne muscular dystrophy patients. *Acta Myol.* 2014; 33: 13-18.
17. KMD Bushby, D Gardner-Medwin, LVB Nicholson, et al, "The clinical, genetic and dystrophin characteristics of Becker muscular dystrophy: II—correlation of phenotype with genetic and protein abnormalities," *Journal of Neurology.* 1993; 240: 105–111.
18. Sharma A, Sane H, Badhe P, Gokulchandran N, Kulkarni P, et al. A clinical study shows safety and efficacy of autologous bone marrow mononuclear cell therapy to improve quality of life in muscular dystrophy patients. *Cell transplantation.* 2013; 22: S127-S138.
19. Sharma A, Sane H, Paranjape A, Bhagawanani K, Gokulchandran N. Autologous bone marrow mononuclear cell transplantation in Duchenne muscular dystrophy - a case report. *Am J Case Rep.* 2014; 15: 128-134.
20. Sharma Alok, Hemangi Sane, Nandini Gokulchandran, Dipti Khopkar, Paranjape, et al. "The role of cell transplantation in modifying the course of limb girdle muscular dystrophy: a longitudinal 5-year study." *Degenerative Neurological and Neuromuscular Disease.* 2015; 5: 93-102.
21. Kinali M, Arechavala-Gomez V, Cirak S, Glover A, Guglieri M, et al. Muscle histology vs. MRI in Duchenne muscular dystrophy. *Neurol.* 2011; 76: 346-53.
22. Briggs D, Morgan JE. Recent progress in satellite cell/myoblast engraftment -- relevance for therapy. *FEBS J.* 2013; 280: 4281-4293.
23. Huard J, Bouchard JP, Roy R, Malouin F, Dansereau G. Human myoblast transplantation: preliminary results of 4 cases. *Muscle Nerve.* 1992; 15: 550-560.
24. Gussoni E, Pavlath GK, Lanctot AM, Sharma KR, Miller RG. Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. *Nature.* 1992; 356: 435-438.
25. Gussoni E, Blau HM, Kunkel LM. The fate of individual myoblasts after transplantation into muscles of DMD patients. *Nat Med.* 1997; 3: 970-977.
26. Emanuela Gussoni, Richard R. Bennett, Kristina R. Muskiewicz, et al. Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation. *J Clin Invest.* 2002; 110: 807-814.
27. Karpati G, Ajdukovic D, Arnold D, Gledhill RB, Guttmann R. Myoblast transfer in Duchenne muscular dystrophy. *Ann Neurol.* 1993; 34: 8-17.
28. Mendell JR, Kissel JT, Amato AA, King W, Signore L. Myoblast transfer in the treatment of Duchenne's muscular dystrophy. *N Engl J Med.* 1995; 333: 832-838.
29. Tremblay JP, Malouin F, Roy R. Results of a triple blind clinical study of myoblast transplantations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. *Cell Transplant.* 1993; 2: 99-112
30. Neumeyer AM, Cros D, McKenna-Yasek D, Zawadzka A, Hoffman EP. Pilot study of myoblast transfer in the treatment of Becker muscular dystrophy. *Neurology.* 1998; 51: 589-592.
31. Vilquin JT, Marolleau JP, Sacconi S. Normal growth and regenerating ability of myoblasts from unaffected muscles of facioscapulohumeral muscular dystrophy patients. *Gene Ther.* 2005; 12: 1651-1662.

32. JP Tremblay, JP Bouchard, F Malouin. Myoblast transplantation between monozygotic twin girl carriers of Duchenne muscular dystrophy. *Neuromuscular Disorders*. 1993; 3: 583-592.
33. Daniel Skuk, Brigitte Roy, MarlyneGoulet. Dystrophin Expression in Myofibers of Duchenne Muscular Dystrophy Patients Following Intramuscular Injections of Normal Myogenic Cells. *Molecular Therapy*. 2004; 9: 475-482
34. Zhang C, Chen W, Xiao LL, Tan EX, Luo SK. [Allogeneic umbilical cord blood stem cell transplantation in Duchenne muscular dystrophy]. *Zhonghua Yi Xue Za Zhi*. 2005; 85: 522-525.
35. Yang XF, Xu YF, Zhang YB, Wang HM, Lü NW. [Functional improvement of patients with progressive muscular dystrophy by bone marrow and umbilical cord blood mesenchymal stem cell transplantations]. *Zhonghua Yi Xue Za Zhi*. 2009; 89: 2552-2556.
36. Asakura A. Skeletal Muscle-derived Hematopoietic Stem Cells: Muscular Dystrophy Therapy by Bone Marrow Transplantation. *J Stem Cell Res Ther*. 2012; 11.
37. Torrente Y, Belicchi M, Marchesi C, D'Antona G, Cogiமானian F. Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. *Cell Transplant*. 2007; 16: 563-577.
38. A Sharma, P Kulkarni, G Chopra, N Gokulchandran, M Lohia, et al. Autologous Bone Marrow Derived Mononuclear Cell Transplantation In Duchenne Muscular Dystrophy-A Case Report. *Indian journal of Clinical Practice* 2012; 23: 169-72.
39. Alok Sharma, Amruta Paranjape, Hemangi Sane, Khushboo Bhagawanani, Nandini Gokulchandran. Cellular Transplantation Alters the Disease Progression in Becker's Muscular Dystrophy. *Case Reports in Transplantation*. 201: 7.
40. Sharma A, Sane H, Paranjape A, Badhe P, Gokulchandran N. Effect of Cellular Therapy seen on Musculoskeletal Magnetic Resonance Imaging in a Case of Becker's Muscular Dystrophy. *Journal of Case Reports*. 2013; 3: 440-447.
41. Sharma A, Sane H, Paranjape A, Bhagawanani K, Gokulchandran N. Autologous bone marrow mononuclear cell transplantation in Duchenne muscular dystrophy - a case report. *Am J Case Rep*. 2014; 15: 128-134.
42. Penton CM, Thomas-Ahner JM, Johnson EK, McAllister C, Montanaro F. Muscle side population cells from dystrophic or injured muscle adopt a fibro-adipogenic fate. *PLoS One*. 2013; 8: e54553.