Cellular therapies for muscular dystrophies: frustrations and clinical successes.

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ABSTRACT

Cell-based therapy for muscular dystrophies was initiated in humans after promising results obtained in murine models. Early trials failed to show substantial clinical benefit, sending researchers back to the bench, which led to the discovery of many hurdles as well as many new venues to optimize this therapeutic strategy. In this review we summarize recent progresses in pre-clinical cell therapy approaches, with a special emphasis on human cells potentially attractive for human clinical trials. Future perspectives for cell therapy in skeletal muscle are discussed, including the perspective of combined therapeutic approaches.

Keywords

Skeletal muscle, Cell therapy, Clinical trials, Muscular Dystrophy, Regenerative medicine, Stem cells, Transplantation, Myoblast, Satellite cells, Muscular fibrosis

Running head

Cell therapy and muscle diseases

Adult skeletal muscle: an ideal target for cell therapy

The American Society of Gene and Cell Therapy (ASGCT) defines 'cell therapy' as the administration of live whole cells or maturation of a specific cell population in a patient for the treatment of a disease. Historically, the first successful cell therapy trial was obtained as early as 1665 in UK with the first blood transfusion in dogs [1]. Blood transfusions are now considered routine. Bone marrow transplantation has also become a well-established protocol, commonly performed for many kinds of blood disorders [2]. The key to successful bone marrow transplantation is the identification of a good "immunologically matched" donor, who is usually a close relative, such as a sibling. Bone marrow cells of the patient (recipient) are destroyed by chemotherapy or radiation, and cells from the matched donor are infused: the self-renewing stem cells find their way to the bone marrow and begin to replicate and produce cells that mature into the various types of blood cells. The first description of skeletal muscle cell transplantation was performed in mouse and published by T. Partridge in 1978 [3], and the first clinical trials in humans were initiated in the early 90's to restore the missing protein dystrophin in Duchenne muscular dystrophy (DMD) patients.

Skeletal muscle is the most abundant human tissue. It is a highly specialized post-mitotic tissue assuring many physiological functions including locomotion, maintenance of posture, as well as metabolic activity. Skeletal muscle is also a dynamic tissue retaining a remarkable capacity to adapt to physiological demands such as growth and exercise, and to repair and regenerate following injury or disease [4]. This ability is due to the presence of resident mononucleated muscle precursor cells, named satellite cells (SC) [5]. In mature skeletal muscle, SC are located in a specialized niche, between the basal lamina and the sarcolemma of muscle fibers [4]. Normally quiescent in uninjured muscle, SC are able in response to severe damage or disease to activate, express myogenic regulatory factors such as MyoD, Myf5 and MRF4 [6], undergo a proliferative expansion providing a pool of muscle progenitors that fuse to generate

multinucleated myofibers. Discovered by electron microscopy [5] on the basis of their position - beneath the basal lamina - SC are today identified by various protein markers using different techniques (immunofluorescence, muscle tissue digestion, FACS), allowing their identification in different cell state (quiescence, activation and proliferation) in mouse and in humans (for more details see ref [7,8]). Thus skeletal muscle is an adequate target for cell therapy since (1) most of its nuclei are post mitotic, with a (2) limited cell turnover -15,1 year- [9] and (3) muscle stem cells are well characterized. Once restored by therapeutic cells, muscle fibres can produce the therapeutic protein for a long time.

Cell-mediated strategies for muscle diseases

Muscular dystrophies (MD) are a heterogeneous group of disorders, characterized by progressive muscle wasting and weakness, with a wide clinical presentation and severity [10]. More than 30 genetically distinct types of MD have been identified [11, 12] according to the age of onset (paediatric or adults), severity, mode of inheritance, rate of progression, prognosis and the specific muscle groups initially affected. MD are caused by mutations in genes encoding a broad range of proteins located in the extracellular matrix (ECM), at the plasma membrane, in the cytoplasm, at the sarcomere, and in the nucleus of striated muscle cells (http://www.musclegenetable.fr/). Although for many of these MD the genetic defect is now identified and considerable progresses have been made to increase our understanding of muscle genetics, pathophysiology and molecular/cellular partners involved in these pathologies, the pathophysiological mechanisms leading from the genetic mutation to the development of the dystrophic features are still often unknown with no cure available. Several therapeutic strategies have been investigated, including pharmacological, gene-based and cell-based approaches (for pharmacological and gene therapy approaches we suggest to the readers several reviews [13-18]). Cell therapy for MD is based on the delivery of precursor cells

in muscle tissue that will contribute to regeneration and tissue repair. This can be seen as a form of gene therapy, where the functional gene is provided by cells used as vectors. There are basically two ways of getting therapeutic cells: (1) obtained from a healthy donor (allograft), the cells will express the missing protein but will also induce an immune response when grafted in the patient requiring an immune suppression treatment; (2) obtained from the patient itself (autograft), cells will be genetically modified to express the missing protein, avoiding immune suppression (unless the reintroduced protein becomes immunogenic). A third possible option, i.e. the use of non-modified cells in autologous graft, can be envisaged for peculiar MD, such as oculo-pharyngeal muscular dystrophy (OPMD) and this will be detailed in the "Human cell therapy candidates: pre and clinical studies" section of this review. Concerning the route of administration, at least two ways have been explored, intra-muscular or systemic delivery. Intra-muscular injection has been widely used for SC, and seems appropriate if the target is limited. For a broader distribution to the whole body or a range of muscles, systemic or loco-regional delivery via the blood flow is more adapted. This requires that therapeutic cells can extravasate and be distributed within the target tissue. Different criteria for an ideal cell candidate for muscle cell therapy have been identified. An ideal cell has first to harbour a stable myogenic potential: able to fuse with host myofibers after transplantation, the therapeutic cell will synthesize donor-derived proteins; its colonization of the host SC niche will extend the therapeutic effect, since the donor cell will then be able to participate to new cycles of muscle degeneration/regeneration. This stable myogenic profile is essential in order to avoid differentiation into an inappropriate cell type/tissue. A sufficient number of cells, an easy isolation and amplification are three additional properties required for the ideal cell candidate to be used in clinical GMP conditions. If the candidate cell is compatible with a systemic or loco-regional route of administration, homing capacities to the site of muscle regeneration is also highly required. Finally, if autologous cell therapy is

envisaged, a genetically modifiable cell candidate is required to correct the deregulated gene. In the last decades, many stem/progenitor cells other than SC/myoblasts have been studied as potential candidates for cell therapy in MD [8, 19-21]. In this review we will focus on human cell candidates for skeletal muscle (Table 1) (excluding cardiac and smooth muscle studies) that either are already in clinical trials or represent future candidates (IPs and ES). We will highlight hurdles that will need to be solved for efficient clinical trials, including some genetic strategies to eventually modify cells prior to injection.

Human cell therapy candidates: pre and clinical studies

Satellite cells and myoblasts

Discovered more than 50 years ago, physiological muscle precursors i.e satellite cells (SC)/myoblasts, represent the first obvious candidate for cell therapy in MD. Based on pioneer studies showing that intramuscular injection of mouse myoblasts could restore dystrophin in the mdx model (a mouse model for DMD) [22], isolation, characterization and pre-clinical studies of human myoblasts were initiated in the early 90s [23, 24], followed by clinical trials. When heterologous injection of myoblasts was transposed in human clinical trials in DMD patients, this strategy showed safety and evidence that injected myoblasts were able to graft within host tissue but with a transient and limited dystrophin expression and no clinical benefit [8, 19, 25]. Immunosuppression of patients, low migration and poor survival of human injected myoblasts were parameters that began to be taken into account [19, 26, 27]. Since then, several research groups started to develop xenotransplantation strategies in immunodeficient preclinical mouse models in order to analyse human myoblast behaviour and the regulation of key biological events occurring in the host muscle after intramuscular myoblast transplantation in order to design rational strategies to improve grafting [28-30]. For example, we showed that human myoblasts do not disappear massively during the first days after injection

as previously described for mouse myoblast injection [31]. A significant portion of grafted human myoblasts survives and engrafts host muscle, although a cell death peak (occurring 12-24 hours following transplantation) is noted ([32] and unpublished data). Interestingly, we observed an *in situ* proliferation of human myoblasts during the first three days post transplantation that was able to compensate the loss of cells occurring after injection [32]. Preclinical studies have pointed out a poor migration of engrafted myoblasts in both mouse [33,34] and monkey [35] muscles, although some migration has been detected in more recent studies in nonhuman primates [36]. Interestingly, we have shown that the migration of the grafted human myoblasts is limited to the first 3 days after transplantation, then proliferation is down-regulated and grafted myoblasts start to differentiate *in situ*, suggesting that proliferation, migration and differentiation are tightly linked and that modifying one of these three processes has an impact on the others [32]. In preclinical models, muscle damage or irradiation [37] can improve the migration capacities of grafted myoblasts, but such a protocol is not clinically applicable. In clinical settings, Skuk et al, proposed to improve cell dispersion by using a protocol of "high-density" injections based on multiple 1mm-spaced local injections [38, 39]. More recent clinical trials in DMD patients conducted between 2004 and 2007 were based on these settings. Such of protocol remains applicable to small and accessible muscles in DMD patients or in the context of autologous myoblast transplantation for localized muscles in less extended diseases such as OPMD, but seems inappropriate to treat numerous large targets.

Other myogenic stem cells

Myoblasts are often exhausted in dystrophic conditions (such as DMD) [40], which hampers their isolation, modification and amplification for autografts. Furthermore, myoblasts, whatever their origin, cannot be injected systemically, which is the ideal route to target large amounts of tissue [41, 42]. These reasons have encouraged the investigation and the identification

of other types of progenitor/stem cells, distinct from SC but exhibiting myogenic capacities, in a search for candidates with a better performance/efficacy to restore muscle function in MD.

Mesangioblasts/Pericytes Mesoangioblasts are mesodermal progenitors originating from the dorsal aorta of E9.5 mouse embryos contributing to postnatal muscle growth and regeneration [43, 44]. In 2007 its human adult counterpart was identified in a subpopulation of muscle resident cells, previously defined as pericytes [41]. Since their isolation, human mesangioblasts/pericytes have been extensively characterized. They have been isolated from the muscle microvasculature of healthy or young DMD patients on the basis of the expression of pericytes markers such as alkaline phosphatase. *In vivo*, human healthy or DMD mesoangioblasts/pericytes transduced *in vitro* with a lentiviral vector expressing human mini-dystrophin are able after intra-arterial delivery in mice (SCID/mdx) to cross the vessel barrier and migrate into the dystrophic muscle, where they participate to muscle regeneration restoring expression of dystrophin, and colonize the SC niche [41]. Human pericytes are enriched or exhausted in some myopathies [45, 46] and interestingly they seem to be more efficient when injected by the systemic route than when injected directly intra muscularly, making pericytes a promising therapeutic tool for cell therapy [41]. On the basis of all these results a first phase I/II clinical trial (EudraCT No. 2011-000176-33) aiming at assessing safety and efficacy of intra-arterial delivery of HLA-identical allogeneic mesoangioblasts has been completed for DMD patients, although results are not yet published.

CD133 Human blood and skeletal muscle-derived CD133+ cells have been shown to possess myogenic capacities *in vitro* and after implantation *in vivo*. By intramuscular and systemic delivery in immunodeficient mice, they showed ability to contribute to muscle regeneration, including colonisation of SC niche [47-50]. Blood and muscle-derived CD133+ cells isolated from DMD patients have been also genetically corrected and injected in immunodeficient dystrophic mice where they were able to give rise to dystrophin positive fibres [48]. All these

characteristics (systemic delivery, ability to be genetically modified and capacity to colonize the satellite niche) make human CD133+ cells another candidate for cell therapy. In 2007 a double-blinded phase I clinical trial, testing safety of autologous muscle-derived CD133+ in DMD patients, was completed [51]. Results showed an increase in capillary vascularization in four out five injected muscles and no side effects, but no evidence of effective integration in muscle fibres was noted.

ALDH Aldehyde dehydrogenase 1A1 (ALDH) is a detoxifying enzyme involved in the metabolism of aldehydes and of retinoic acid. ALDH activity is one hallmark of human bone marrow, umbilical cord blood, and peripheral blood primitive progenitors presenting high reconstitution capacities *in vivo*. Recently, an ALDH+ cell population has been identified within human skeletal muscles [52]. *In vivo*, after intramuscular injection, ALDH+CD34- cells proliferated and robustly contributed to muscle regeneration, including integrating the pool of SC. If systemic delivery of these cells is possible, their proliferative capacity will represent a considerable advantage in terms of colonization of the host muscle and participation to regeneration.

MuStem A population of muscle-derived stem cells called MuStem cells, was recently isolated from GRMD dogs (the clinically relevant large animal model for DMD) using a pre-plating approach [53]. Intra-arterial injection of canine MuStem in immunosuppressed GRMD dogs resulted in the formation of new regenerated muscle fibers and the restoration of dystrophin expression. MuStem also colonized the SC niche and induced a partial remodelling of the muscle tissue and persistent clinical stabilization of the transplanted GRMD dogs. A study on the global gene expression profile of these MuStem cells 4 months after transplantation, showed an up-regulation of genes enhancing muscle regeneration; this could play an essential role in the stabilization of the dystrophic muscle [54]. Human MuStem have now been identified (Lorant J, Rouger K, personal communication). Although isolated

from different origin, i.e. skeletal muscle for MuStem and muscle blood vessels for mesoangioblasts, and with different methods, common phenotypic properties, such as multilineage potential and homing to damaged muscle when injected systemically (see Table 1), may suggest some similarities between these two types of myogenic cells. Further studies to characterize MuStem cells in terms of heterogeneity and regenerative potential are needed before they can be considered for therapeutic application.

Clinical trial: the example of a phase I/IIa study of autologous myoblast cell transplantation for OPMD

Intramuscular injections of myoblasts - despite their reduced proliferative capacity and migration - always lead to localized tissue repair at the site of injection [25]. OPMD appears as an ideal situation where myoblast transplantation is applicable. Indeed, OPMD is a late-onset autosomal dominant slow progressing inherited dystrophy caused by an abnormal trinucleotide repeat expansion in the PABPN1 gene [55, 56], where a small group of specific muscles (eyelid and pharyngeal) are primarily affected, leading to both ptosis and dysphagia. Affected cricopharyngeal muscle is characterized by exacerbated fibrosis and atrophy [57]. Surgical correction for eyelid and pharyngeal muscle weakness are to date the only therapy to increase the quality of life of these OPMD patients. However surgical myotomy (to fight dysphagia) provides in most cases only a transient benefit with a secondary progressive reoccurrence of dysphagia a few years later [58]. A few years ago, we demonstrated that myoblasts isolated from clinically unaffected muscles of OPMD patients had a normal proliferation and differentiation capacity [59] suggesting that an autologous transplantation of myoblasts isolated from clinically unaffected muscles and implanted into affected pharyngeal muscles may improve their motility and restore some muscle function. A preclinical study in dog confirmed the feasibility of such a protocol [59]. A phase I/IIa clinical study

(ClinicalTrials.gov NCT00773227) was then launched in 2004 with 12 OPMD patients to assess the feasibility and toxicity of autologous myoblast transplantation in pharyngeal muscles following myotomy. The results of the trial demonstrated the safety and good tolerance of the procedure with no adverse side effects. A cell dose dependant functional improvement in swallowing was even observed in this safety study [60]. This study is now being extended to 12 new patients, including patients without a myotomy to decipher the respective contribution of the myoblast injection and the myotomy.

Cell therapy: future perspectives

Improving therapeutic potential of myoblast cell therapy

It is now confirmed that culture conditions modify transplantation efficiency of muscle progenitors: mouse myoblasts are less efficient than the SC they are derived from [61] or even less than the initial SC in its niche [62] and this is probably also true for human cells. Although freshly isolated SC present a high regenerative potential, they will probably not be a therapeutic option because of their limited number. Identifying the modifications arising during the expansion of myoblasts would allow the identification of optimized conditions to improve the regenerative potential of amplified myoblasts. As an example, it has been recently shown that inhibition of p38 signalling pathway during human myoblast expansion in vitro greatly enhances their engraftment *in vivo* [63].

Regarding the niche, the importance of the physiological rigidity of substrates used *in vitro* on the control of stem cell fate has been documented [64]. Gilbert et al, showed that *in vitro* substrate elasticity is a potent regulator of the fate that mouse SC will acquire after transplantation *in vivo*: culturing cells on bioengineered hydrogel substrates that mimic muscle tissue elasticity (12 kilopascals) modulates their stem cell fate, enhances their survival, prevents early differentiation and promotes self-renewal *in vitro*. More importantly, such

conditions of culture result in an increased engraftment ability of these cells when injected *in vivo* in regenerating muscles [65]. Recently, Yennek et al using micropatterns coated with ECM proposed that geometry micropattern plays a predominant effect on the fate of the dividing cells [66]. All these approaches have been tested on myoblasts, but substrate rigidity is known to modify the fate of other stem cells [64]. Although these approaches cannot yet be applied in clinical conditions for various reasons (cost, complexity, scale-up), the involved parameters, from the modulation of signalling to optimization of the substrates, will have to be taken into account for the optimization of large-scale cell cultures for future therapeutic use of stem cells in clinical trials.

Fibrotic environment

If elasticity plays a key role in the SC fate *ex vivo*, it should also be taken into account *in vivo*: transplanting cells in a stiffer environment may modify their behaviour. Studies of muscle fibre rigidity in the mdx mouse showed that dystrophic fibres are more rigid than wild-type, probably du to the absence of dystrophin [67] or/and an increased muscular fibrosis. During fibrosis invasion, functional muscle tissue is progressively replaced by nonfunctional connective tissue. Fibrosis is a complex process present in a large variety of MDs, and characterized by excessive accumulation and modifications of ECM components: we showed recently that advanced fibrosis correlated with modified composition of glycosaminoglycans of muscle ECM in DMD [68]. Moreover, it has been shown that in a fibrotic environment, specialized cells such as SC acquire plasticity toward a fibrogenic fate via a TGFβ-mediated pathway, decreasing their normal regenerative functions [69, 70], and potentially resulting in further increase in tissue fibrosis. Additionally, a sub-population of pericytes have been recently identify as a major source of profibrotic cells during acute muscle tissue injury and in aged skeletal muscle [71, 72]. In mouse it has been shown that muscle-derived fibroadipogenic progenitors (FAPs) rapidly proliferate in response to acute muscle damage, suggesting that

they may play an active role during muscle regeneration [73, 74] and/or in the installation of the fibrosis associated with ECM deposition in the dystrophic mouse model *mdx* [75]. If the presence of FAPs is confirmed in humans, it will be essential to decipher their influence on regeneration, their potential cross-talk with SC and characterize their role in fibrogenesis to ameliorate muscle cell therapy. Indeed muscular fibrosis hampers the success of gene or cell therapy at advanced stages of the disease [78], and pre- anti-fibrotic treatment using tendon modified fibroblasts (expressing angiogenic factors and metalloproteinase) that can restore microcirculation and reduce connective tissue deposition in aged dystrophic mice could ameliorate muscle tissues in patients at advanced stages of the disease [76]. Although it is not yet known how deregulation of ECM components (proteins, glycoproteins, glycosaminoglycans, etc) contribute to worsening of the disease and participate in the formation of fibrosis, improve quality of receiver muscle with anti-fibrotic therapies before receiving the graft will certainly be essential to optimize implanted cell fate in cell therapy.

Human Embryonic and induced pluripotent stem cells: future candidate cells?

Much energy has been put in the last years to increase the myogenic commitment of human pluripotent stem cells embryonic stem cells (hES) [77] and induced pluripotent stem cells (iPS) [78, 79]. *Human ES* cells are isolated from the early embryo (5-6 days) and have the potential to differentiate into all tissues of the three germ layers. Generation and use of hES cells in therapy poses ethical issues, related to the destruction of human embryo, but also safety issues since hES cells can lead to formation of teratomas if they are not fully differentiated [80]. Although myogenic hES capable of muscle engraftment have been reported during the past years (for more details see [81]), most of these strategies used genetic manipulation of hES (lentiviral vector integration,) introducing additional risks, related to the immunogenicity of viral vector proteins and the integration of the transgene in the human genome. Moreover, hES cell-based therapy will have to be an heterologous approach, and the immune privilege of

hES cells is today debated [82] since they seem to increase their immunogenicity as they progress towards differentiation [83].

Generation of *iPS cells* poses less ethical issues than hES since iPS cells are typically generated from adult somatic cells by reprograming them with a defined and limited set of transcription factors (Oct3/4, Sox2, c-Myc, Klf4). Described first in mouse [78, 79], this approach is now applied to human somatic cells [84]. Several studies showed that combination of compounds [85], recombinant proteins fused to cell-penetrating peptide [86], expression plasmids [87] or microRNA [88], or even altering intercellular communication via Notch signalling [89] could partly substitute or entirely eliminate the use of integrative viral vectors, resulting in the production of safer iPS. Like for hES, myogenic differentiation of hiPS can be induced by genetic modifications, further complicating safety issue for possible use of hiPS cells in human trials (see [81] for a comprehensive review). Human hiPS cells have also recently been generated from DMD and BMD patient's fibroblasts [90]. Interestingly, dystrophic iPS can be derived into mesoangioblast-like cells [45], potentially adapted for autologous transplantation. Finally, the engagement of murine and human iPS into myogenesis by treatment with factors has been recently reported, avoiding the use of any transgenesis [91]. Although many problems, including safety, need to be solved before these cells can be used in clinical trials, these results open a new field of investigation for future potential candidates for cell therapy.

Up to now, no universal cell candidate that could be maintained and amplified in vitro and delivered to patients without immune rejection has been described, and the promises raised by the myogenic potential of hiPS still suggest personalized medical treatment involving the generation of hiPS for each patient. Although costs for curing life threatening diseases such as DMD should be analysed in view of their devastating evolution, patient tailored approaches may be variably accessible depending on the country and national health care organization, representing another kind of ethical concern. Independently of the cell candidate chosen, one

solution could be the development of haplobanks matching an as large as possible majority of the population, as suggested for iPS by Wilmut et al in 2015 [92]. These haplobanks however will have to be specific for genetically distinct populations, e.g. an ideal haplobank for Caucasians will be different from that for the Japanese population.

Conclusion

The first demonstration of cell therapy for muscle dystrophy in murine models raised some hope of a clinical application at a time when gene therapy faced many problems (size of the transgene for dystrophin, immune reaction, etc). However, clinical trials for DMD using heterologous myoblasts generated some frustration with the repeated absence of clinical benefit for the patient. Targeting most of the musculature including diaphragm, as required for DMD, turned out to be a real challenge with myoblasts. The nature of the fibrotic receiving tissue added hurdles for the success of cell therapy. However, the recent clinical success of autologous myoblast therapy for OPMD pharyngeal muscles, where a functional improvement of pharyngeal function was observed, provides a proof of concept that cell therapy is still an open clinical approach for defined targets, providing that the therapeutic strategy is adapted to the clinical situation. Several lines of research for the future will concern: 1) the cell type, including systemic delivery of myogenic stem cells to reach a larger amount of tissue; 2) the pre-treatment of the targeted tissue to diminish fibrosis and enhance delivery of the therapeutic agent (this is also true for other approaches, including gene therapy); 3) the combination of strategies, e.g. combined gene and cell therapy to deliver corrected autologous cells, or combined cell types that may together enhance repair and/or revascularization (**Fig. 1**).

AUTHORS CONTRIBUTION

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AUTHOR DISCLOSURE STATEMENT

None

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Cellular therapies for muscular dystrophies: frustrations and clinical successes.

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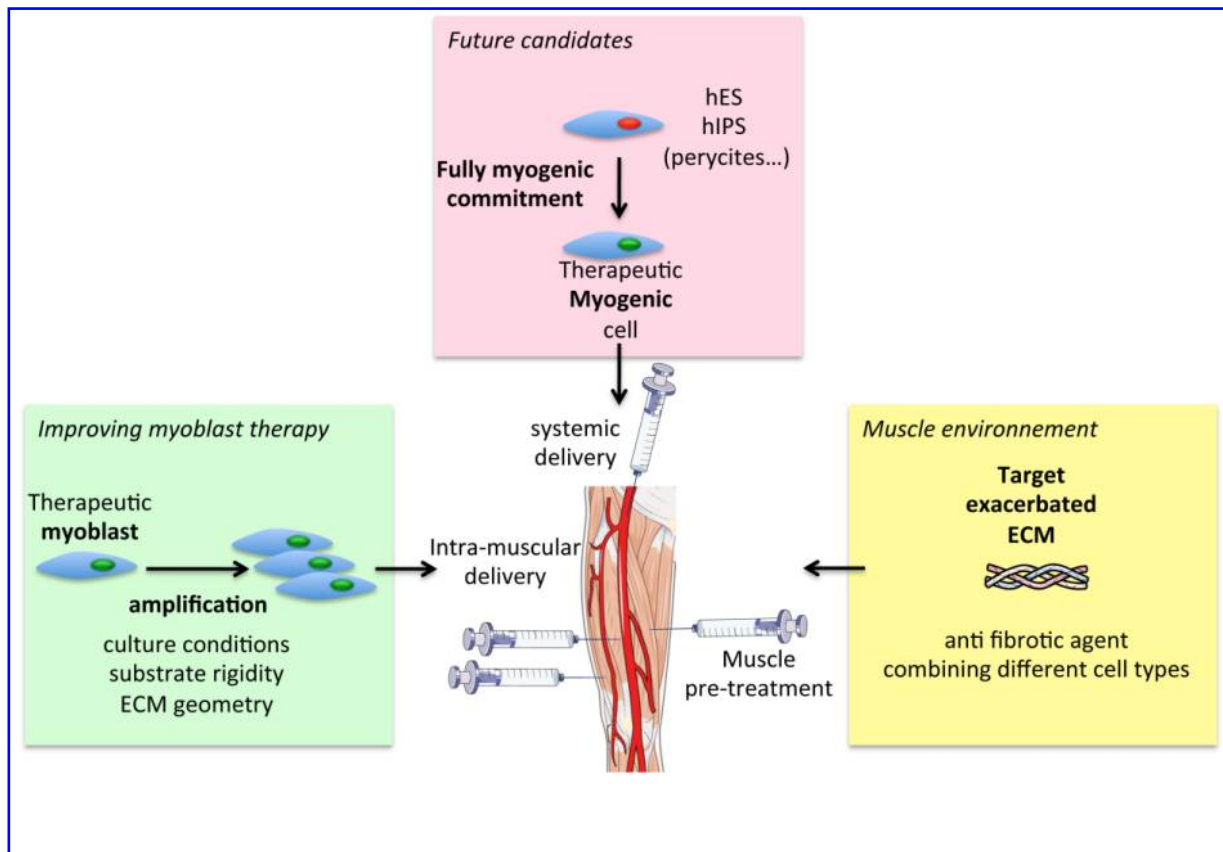
FIGURE LEGEND

Figure 1. Cell therapy for muscular dystrophies. Future directions of cell therapy include improving critical step of amplification of cells (green square), systemic delivery of myogenic candidate cells (pink square) and targeting muscular fibrosis in order to improve muscle quality before the therapy (yellow square). The figure was created using Servier Medical Art (http://www.servier.com).

Table 1. Myogenic cell candidates

Cell type	Origin	Physiological function	Myogenic differentiation	Alternative lineage potential	Systemic delivery in preclinical models	Participation in skeletal muscle regeneration and colonization of satellite cell niche	Clinical trials	Ref.
Myoblast	Skeletal muscle	Myogenesis and skeletal muscle regeneration	Spontaneous	Osteogenic and adipogenic differentiation	No	Yes	Phase II: completed (DMD); Phase I/IIa: on-going (OPMD)	[19, 60]
Mesoangioblast -like/pericyes	Vessel/ Skeletal muscle	Blood flow regulation, microvessel contractility, regulation and control of angiogenesis	Spontaneous for skeletal muscle/vessel-derived pericytes	Chondrogenic, osteogenic and adipogenic differentiation	Yes	Yes	phase I/II: intra-arterial delivery of HLA-identical allogeneic mesoangioblasts. Results not yet published (DMD)	-
CD133+	Blood/ Skeletal muscle	Unknown	Spontaneous but limited (m); Induced by C2C12 co-culture (b)	Myeloid (b) and endothelial differentiation	Yes	Yes	phase I: intramuscular injection, neither local nor systemic adverse effects were reported (DMD)	[51]
ALDH+	Skeletal muscle	Unknown	Spontaneous	Adipogenic differentiation	Not tested	Yes	NA	
MuStem (c)	Skeletal muscle	Unknown	Spontaneous	Osteogenic and adipogenic differentiation	Yes	Yes	NA	-

m: skeletal muscle; b: peripheral blood; c: canine; NA: not available.

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