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Advanced therapeutic approaches in sarcoglycanopathies

Martina Scano, Alberto Benetollo, Francesco Dalla Barba and Dorianna Sandonà

Abstract

Sarcoglycanopathies are rare autosomal recessive diseases belonging to the family of limb-girdle muscular dystrophies. They are caused by mutations in the genes coding for α-, β-, γ-, and δ-sarcoglycan. The mutations impair the assembly of a key structural complex, which normally protects the sarcolemma of striated muscle from contraction-derived stress. Although heterogeneous, sarcoglycanopathies are characterized by progressive muscle degeneration, increased serum creatine kinase levels, loss of ambulation often during adolescence, and variable cardio-respiratory impairment. Genetic defects can impair sarcoglycan synthesis or produce a protein that is defective in folding. There is currently no effective treatment available; however, both gene replacement strategy and small molecule-based approaches show great promise and have entered or are starting to enter clinical trials.

Addresses

Department of Biomedical Sciences, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy

Corresponding author: Sandonà, Dorianna (dorianna.sandona@unipd.it)

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Introduction

Sarcoglycanopathies are four autosomal recessive disorders of striated muscle caused by genetic defects of the *SGCA*, *SGCB*, *SGCG*, and *SGCD* genes coding for α-, β-, γ- and δ-sarcoglycan (SG), respectively. Sarcoglycanopathies, are part of the large family of limb-girdle muscular dystrophies (LGMD) and, for this reason, are also known as LGMDR3-6 (numbering according to the gene involved) or LGMD2C–F (old nomenclature) [1]. In the LGMD family, sarcoglycanopathies represent the most severe forms, accounting for approximately 10–25% of all cases [2]. The global prevalence of sarcoglycanopathies is about 1/178,000, but there is a large difference among the four types, with LGMDR3/2D being the most common form in Europe and North America (3.4/100,000); the LGMDR4/2E is ubiquitously distributed (0.8/100,000); the LGMDR5/2C is the most prevalent in the North African and Gypsy population (0.1/100,000); the LGMDR6/2F is the rarest (0.07/100,000) [3,4]. Sarcoglycanopathies are heterogeneous muscular dystrophies affecting mainly the proximal musculature of the upper and lower girdles. The most severe cases are characterized by early onset, usually during the first decade of life, and rapid progression with loss of ambulation during adolescence [2]. Elevated serum creatine kinase (CK) levels are common in the early stages of the disease. Histological evidence of muscle loss and regeneration precedes the gradual replacement of the contractile tissue with fibrotic or adipose tissue. Cardiac and respiratory involvement is commonly late and less frequent in the LGMDR3/2D subtype; intellectual disability has never been reported. Milder forms, characterized by late-onset and slow progression, are known, as well as phenotypic variability among unrelated patients and even between siblings carrying the same SG mutation [5–7]. Importantly, two clinical trials of natural history of sarcoglycanopathies are presently recruiting (NCT04475926; NCT06210672a).

No drug is on the market for sarcoglycanopathy. Nowadays, patients mainly receive supportive treatments aimed at preserving muscle performance for as long as possible, while respiratory and cardiac surveillance/support is mandatory [2]. The aim of this review is to provide an update on the most advanced therapeutic approaches for sarcoglycanopathies. Both gene replacement and conventional pharmacological strategies have been considered in terms of mechanism of action, efficacy, potential target patients, and drug development progression.

The sarcoglycan complex

α-, β-, γ- and δ-SG, are glycoproteins of the sarcolemma of skeletal and cardiac muscle. Two additional

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homologous proteins are known, ε- and ζ-SG, which however participate in the formation of the SG complex in tissues other than striated muscle, and have never been associated with muscular dystrophies. SGs are co-translationally translocated into the endoplasmic reticulum (ER), where folding and glycosylation take place. Once the proper 3D-structure is gained, β- and δ-SG form the core of the SG-complex, at which subsequently binds γ-SG and eventually α-SG. Then, the tetramer moves towards the sarcolemma [8–10], where it is inserted into the large dystrophin-associated protein complex. DAPC links the actin-filaments to the extracellular matrix (ECM) [11,12] assuring connection between adjacent fibers, tendons, and the ECM, and protecting the sarcolemma from contraction-induced damages [8]. In addition to the structural function, DAPC plays a role in cell signaling as it interacts with proteins such as nNOS and integrins; the SG subunits are supposed to be transducers of mechanical information, being post-translationally modified upon muscle contraction [13–15]. Furthermore, it was observed that α-SG possesses ecto-ATPase activity that plays a role in modulating the purinergic signaling of skeletal muscle [16–18].

Types of sarcoglycan mutations and impact on the disease phenotype

The SG complex is acting as a unit. In fact, when a single SG subunit is lacking or mutated, the whole tetramer is destabilized, and there is the secondary loss of the wild type partners.

Figure 1 reports the percentage of unique public variants described in sarcoglycanopathies according to the molecular consequences, as reported in the Leiden Open Variation Database (LOVD[3]) system. When considered globally, missense mutations are the most frequently reported, while defects resulting in protein loss, such as null and frameshift mutations account for approximately a quarter of overall SG mutations. This percentage is highest in the *SGCB* (33%) and *SGCG* (30%) genes.

Several studies have been conducted to identify the impact of the different types of SG mutations on the phenotype. Recently, by using site saturation mutagenesis and high-throughput functional *in vitro* assays, the impact of protein-coding genetic variations in the *SGCB* gene has been checked, allowing the classification of patients' mutations and offering potential utility in the diagnosis and clinical interpretation of novel cases [19]. Even though it is difficult to establish a strong genotype–phenotype correlation [19,20], it is clear that a level of SG expression lower than 30% and an early age of onset are prognostic signs for early loss of ambulation and rapid disease progression [6,7,20–22]. This is less evident in LGMDR3/2D. However, a recent analysis conducted on 120 unique combinations of *SGCA*-mutated alleles, showed that the most severe phenotypes always correlate with the presence of null and frameshift mutations [22]. In the case of homozygous missense mutations, a quote as large as 70% of the reported cases are severe while the remaining are equally divided between mild and intermediate. Even though cardiac involvement is variably described in LGMDR3/2D, this study evidenced that cardiomyopathy was systematically present in patients with at least one mutation in exon 3 [22].

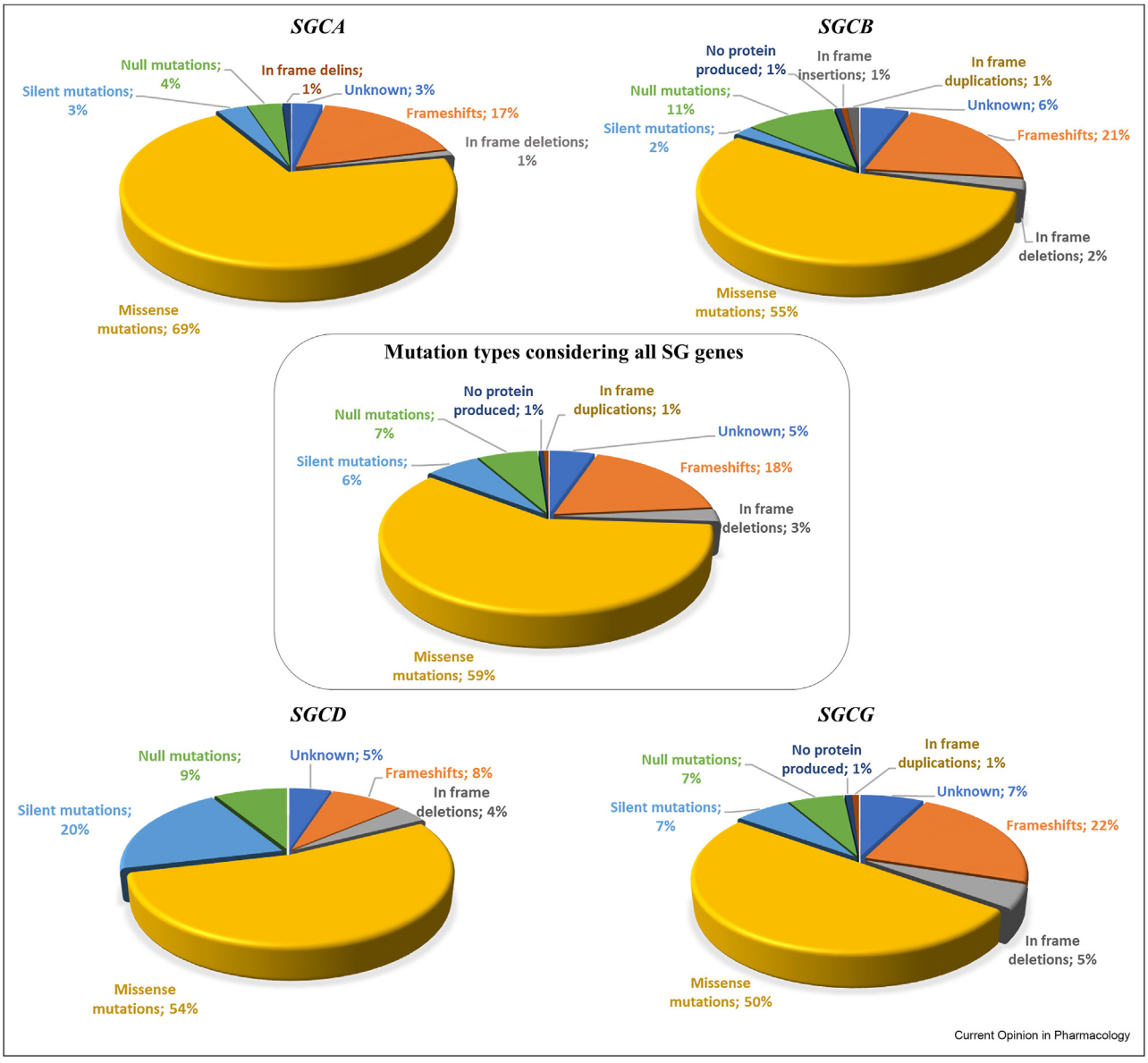
Gene replacement therapies to restore sarcoglycan genes

The gene replacement therapy approach is designed to repair a defect of an existing DNA by delivering an extra copy of the working gene to the cell through a vector system. This is a promising modality, nowadays largely explored, for monogenetic diseases like sarcoglycanopathies [23]. In sarcoglycanopathies, gene therapy is favored by the small dimension of the SG coding regions, well-fitting the loading capacity of the viral vectors presently available. Conversely, a key issue of this approach in muscular dystrophies is the effective gene delivery to the most abundant tissue of the human body, and the duration of the recovery.

The successful use of adeno associated viruses (AAVs) has been reported numerous times in mice, authorizing the first two clinical trials for sarcoglycanopathy that took place between 2006 and 2011. The NCT00494195 study involved six participants, 9–17 years old, with LGMDR3/2D. The aim was to assess the safety and dose of intramuscular administration of a recombinant adeno-associated virus serotype 1 (rAAV1) vector expressing the human alpha-SG gene (hαSG) under the control of the skeletal muscle creatine kinase promoter (tMCK) [24,25]. The second clinical trial, (NCT01344798) evaluated the safety and feasibility of intramuscular injection in nine 16- to 38-years-old, nonambulatory patients with LGMDR5/2C, of the adeno-associated virus serotype 1 (AAV1) carrying the human γ-SG gene (hγSG) under the control of the desmin promoter [26]. The outcome was positive in both clinical trials [25,26], with the expression of the SG proteins persistent up to 6 months post-injection, as evidenced in the NCT00494195 study [24]; no serious adverse event was reported, even though neutralizing antibodies against the virus became evident as early as 15 days postinjection [25], and one patient in NCT01344798 study developed a cytotoxic response towards the AAV1 capsid [26].

Subsequently, a phase I/II dose-ascending open-label clinical trial (NCT01976091) was conducted in 6 patients, 8–13 years old with LGMDR3/2D, by performing isolated limb infusion (ILI) with a rhesus monkey-derived AAV vector carrying the hαSG transgene under the control of the tMCK promoter, (scAAVrh74.tMCK.hSGCA). The

Figure 1



Percentage of unique publicly reported variants for each SG gene, and their average across all genes. Mutations are categorized based on their molecular consequences at the protein level, as reported in the LOVD[3] system. SG, sarcoglycan; LOVD[3], Leiden Open Variation Database.

study evidenced the AAVrh74 serotype to be safe and effective in producing the α-SG protein [27], but also highlighted the difficulties of the ILI method to reach the more proximal muscle targets with the replacement gene, thus limiting the functional recovery [27]. These findings suggested the opportunity for a subsequent systemic delivery and also the potential application to other MD forms. In fact, a phase I/II clinical trial for LGMDR4/2E (NCT03652259), supported by Sarepta Therapeutics started in 2018 and is still ongoing. Six children 4–15 years old, presenting significant symptoms and confirmed *SGCB* mutations on both alleles, were randomly divided into two cohorts. The first cohort received a single intravenous infusion of 1.85×10^{13} vector genome copies kg^{-1}, the second cohort 7.41×10^{13} vector gene copies kg^{-1} of a self-complementary AAVrh74 vector containing a codon-optimized, full-length human *SGCB* transgene, driven by a muscle-specific promoter (MHCK7). The first results are very promising, showing the safety and tolerability of this gene therapy with minimal serious adverse event findings, resolved with standard therapies, and expected increases in AAVrh74 antibodies that, however, did

not correspond to liver enzyme elevation or impact transgene expression. Importantly, transduction was accompanied by sustained expression of the β-SG transgene and muscle functional improvements after 2 years of follow-up [28].

Alongside the clinical trials, preclinical studies were carried out in mice, aimed at identifying the best route of administration and the optimal vectors. One such study evidenced the efficacy and safety of the systemic administration in *sgca* null mice of the recombinant vector scAAVrh74.tMCK.hSGCA, containing a codon-optimized, full-length α-SG, the same used in NCT01976091, using a dose-escalation design [29]. In other preclinical studies, recombinant rAAV, serotype 2/8, was systemically administered to the LGMDR5/2C mouse model to induce the expression of γ-SG controlled by the desmin promoter [30]. Similarly, recombinant AAVrh74 was effective in allowing the γ-SG expression under the control of the MHCK7 promoter in mice [31]. These studies highlighted the safety and effectiveness of the systemic delivery of the transgene in mouse skeletal muscle, with functional restoration of muscle strength and paved the way for future phase I clinical trials, sponsored by ATAmyo therapeutics, which is ready to recruit LGMDR5/2C patients.

Despite the promising results, clinical trials using whole-body administration of AAV particles are quite recent, so long-term evaluation of protein restoration and long-term safety assessment of the vectors are not yet available and will still require years of study [32].

Beyond conventional gene therapy, other approaches combining gene editing and cell replacement deserve attention. Emerging strategies, such as precision gene editing in human muscle stem cells offer a novel approach for autologous cell replacement therapies. In a recent study, human muscle stem cells were isolated from two donors affected by the common *SGCA* c.157G > A mutation, which leads to the skipping of two coregulated exons. Through adenine base editing, the researchers corrected the mutation in cells from both donors with over 90% efficiency. The correction successfully restored splicing, and α-SG expression. Additionally, the corrected patient's cells exhibited the ability to regenerate muscle and contributed to the Pax7+ satellite cell compartment *in vivo* in mouse xenografts [33].

Although the availability of primary human muscular stem cells in early passages and with high regenerative capacity is limited, posing a challenging problem, this approach highlights the potential of autologous gene-repaired human muscle stem cells for cell replacement therapies for muscular dystrophies [33]. This not only represents a significant leap forward in the field, but also underscores the potential of personalized medicine that could offer tailored solutions, mitigating concerns related to immune responses commonly associated with viral vector therapies.

Pharmacological approaches rescuing the mutated sarcoglycan protein

New avenues of therapeutic intervention have become possible thanks to the elucidation of the pathogenic mechanism underlying sarcoglycanopathy, in particular related to missense mutations, and the identification of various components of the endoplasmic reticulum-associated degradation (ERAD) pathway responsible for the degradation of mutated sarcoglycans [34]. The ERAD pathway has been targeted at various stages, including the last, intermediate or initial steps, evidencing the rescue of mutated SGs. In HEK293 cells, expressing disease-causing α-sarcoglycan mutants, the 26S proteasome has been inhibited with MG-132, preserving SG mutants from degradation and recovering the proteins at the plasma membrane [35]. Similarly, by blocking the pathway at an early stage using kifunensine, a specific inhibitor of α-mannosidase I, the recovery of a mutated SG was observed in both cell models and in mice transduced with AAVs expressing the human R77C-α-SG sequence [36,37]. Furthermore, two selective inhibitors of the ER E3-ubiquitin ligase HRD1, specifically involved in the ubiquitination of SG mutants, were effective in the recovery of V247M-α-SG in HEK293 cells and of the entire SG-complex at the sarcolemma of patient-derived myogenic cells [38].

In recent years, cystic fibrosis transmembrane conductance regulator (CFTR) correctors have also gained interest as a pharmacological approach for sarcoglycanopathies. CFTR correctors have the ability to restore the cell surface expression of type II mutants of the chloride channel, which exhibit defects in folding and trafficking [39]. Interestingly, the usefulness of CFTR correctors extends beyond their primary application, as they have been proven effective in enhancing the maturation of various α-SG mutants, leading to their successful recovery at the plasma membrane [40–42]. Notably, in myotubes derived from a LGMDR3/2D patient, the administration of CFTR correctors, either individually or in combination, prompted the re-localization of the whole SG complex [40,41]. Among the CFTR correctors tested, one specific molecule, namely C17, exhibited remarkable efficacy. C17 not only facilitated the re-routing of the mutated R98H-α-SG to the sarcolemma but also showed the ability to recover muscle force in the hind limb muscles of a newly developed LGMDR3/2D murine model [42]. This set the stage for a preliminary structure–function analysis, allowing the derivatization of the molecule suitable for future approaches of fishing for targets, paving the way for mechanistic studies and guiding further refinement of the compound [43]. As C17 has not yet been classified as a

drug, further studies are needed to conclusively rule out potential toxicity, to establish the pharmacological profile, and to determine both the minimal effective dose and optimal routes of administration.

In addition, the knowledge of the pathogenic mechanism of the disease enables the design of strategies that, by targeting various steps of the underlying mechanisms, can eventually rescue the mutant. An illustrative example involves the combination of a low dose of bortezomib a proteasome inhibitor, with the histone deacetylase (HDAC) inhibitor, givinostat [44]. Notably, the functional analysis of the HDAC inhibitor givinostat uncovered its ability to inhibit autophagic degradation of the folding-defective R77C-α-SG, suggesting a synergistic effect with bortezomib that, by inhibiting the proteasome, blocks the other pathway responsible for the disposal of such α-SG mutant [44].

Therapeutic approaches acting on the secondary causes of the disease

Sarcoglycanopathies are exacerbated by immune-mediated damage and sustained oxidative stress, often accompanied by the substitution of the contractile tissue with fibrotic and adipose tissues. Consequently, alternative therapeutic strategies may be directed toward mitigating these features, in combination or as an alternative to the use of classical anti-inflammatory drugs, which, however, have limited efficacy and are often associated with severe side effects.

Mutations that disrupt the SG-complex are thought to impair the ecto-ATPase activity of α-SG [17], thus impacting crucial purinergic signaling pathways. In a normal physiological response, acute muscle injury triggers the release of substantial amounts of ATP that serve as a damage-associated molecular pattern (DAMP). ATP, in this context, initiates inflammation, recruiting inflammatory cells, thus facilitating the clearance of damaged tissues and prompting regenerative processes, aiming to restore normal muscle function. However, in sarcoglycanopathies, the deficiency in ecto-ATPase activity, which would typically regulate extracellular ATP (eATP) levels, may lead to an abnormally high level of eATP, excessively activating P2X7 purinoceptors. This phenomenon not only sustains inflammation but also transforms the potentially compensatory up-regulation of P2X7 in dystrophic muscle cells into a detrimental mechanism that worsens the pathology [45]. *In vivo* blockade of the eATP/P2X purinergic pathway in *sgca*-null mice either by the broad-spectrum P2X receptor-antagonist oATP [46] or by the selective P2X7 antagonist A438079 [47] delayed the progression of the dystrophic phenotype. The blockade of the eATP/P2X7 axis showed a mitigating effect on the muscular inflammatory response, concurrently promoting the recruitment of immunosuppressive regulatory CD4+ T cells expressing forkhead box protein P3 (FOXP3). This resulted in ameliorated inflammatory features enhanced muscle strength, and reduced necrosis and the expression of profibrotic factors [46–48].

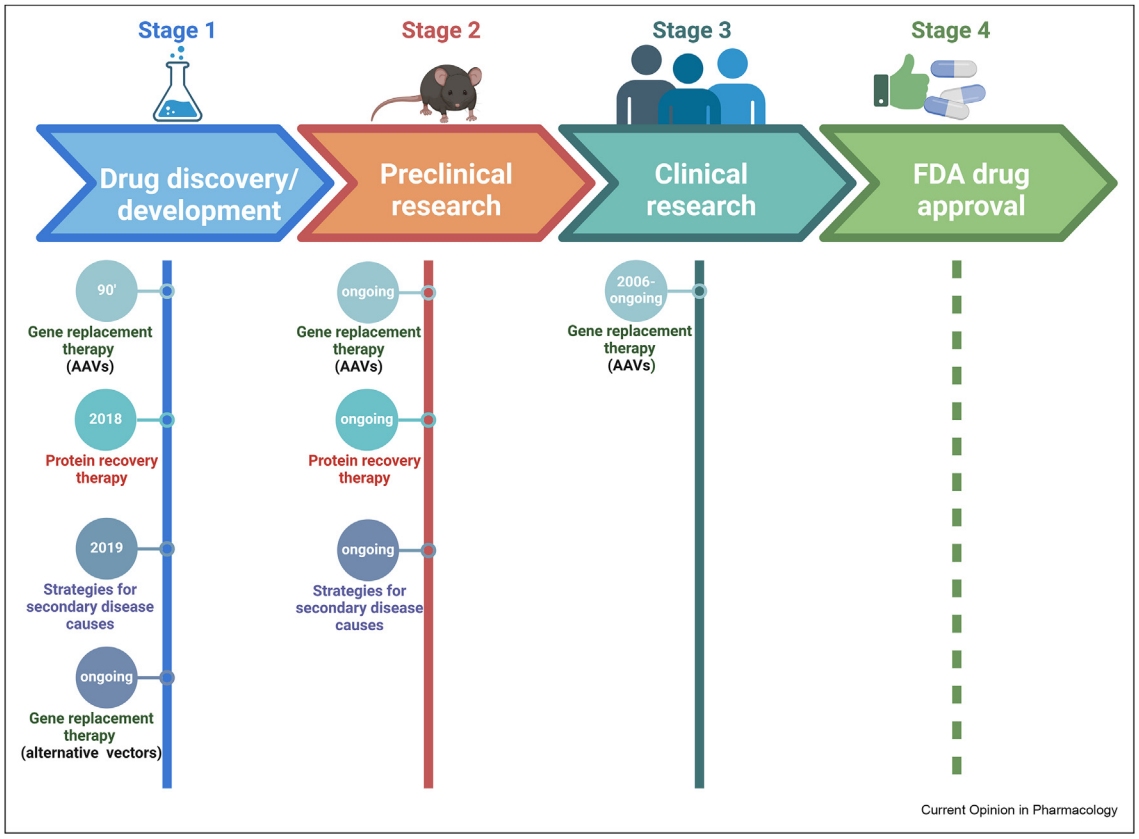
In recent studies, the redox-sensitive high-mobility group box-1 protein (HMGB1) has been identified as overexpressed in muscular dystrophies, including sarcoglycanopathies. Notably, in MD, its redox state tends to be unbalanced toward the proinflammatory oxidized isoform (dsHMGB1), changing its role from the orchestration of tissue regeneration to the exacerbation of inflammation. The HMGB1 knock out, or the intramuscular or intraperitoneal administration of 3S, an engineered nonoxidizable variant of HMGB1, are of potential benefits in *mdx* (DMD murine model) and *sgca*-null mice. In both cases, it was observed an improved functional performance, enhanced muscle regeneration, and a reduction in both inflammation and fibrosis [49].

Fibro-adipogenic precursor cells are muscle-resident stem cells, characterized by the expression of platelet-derived growth factor receptor alpha (PDGFRα). Their activation upon an acute muscle injury is essential during the first phases of muscle regeneration. On the other hand, in MDs, damage and inflammation are persistent with M2 macrophages continuously releasing growth factors; therefore, fibro-adipogenic precursor cells become the major responsible players of the fibrotic and fatty tissue expansion [50]. Nintedanib is a second-generation tyrosine kinase inhibitor (TKi), selectively targeting PDGFRA and PDGFRB, and fibroblast growth factor receptor (FGFR) 2 and 3, and vascular endothelial growth factor receptor (VEGFR). Nitedanib showed efficacy beyond its approved indications for idiopathic pulmonary fibrosis (IPF), diminishing muscle fibrosis and reshaping the prevailing proinflammatory muscle microenvironment in *sgca*-null mice. This led to heightened muscle endurance and strength, contributing to the attenuation of the dystrophic phenotype of these mice [51].

Conclusions

Currently there is no Food and drug administration (FDA) approved pharmacological treatment for sarcoglycanopathies. Nevertheless, as evidenced in Figure 2, many therapeutic approaches show promising expectations. Among them, enormous progress has been made in the field of gene replacement therapy since the first clinical trials started in 2006, with substantial steps taken to enhance gene delivery to skeletal muscle while reducing side effects. This, at the moment, one shot approach is useful for all sarcoglycanopathy patients, but is surely crucial for subjects with genetic defects leading to the loss of the protein (i.e. null and frameshift mutations) that represent 1/4 of the overall

Figure 2



Timeline illustrating the progress in drug development process of therapeutic approaches for sarcoglycanopathies.

SG mutations. However, the long-term safety of the viral vectors and persistance of protein restoration are needed to be defined, especially considering that skeletal muscle accounts for more than 40% of whole-body weight.

Important advancements have been made in the last few years for pharmacological treatments targeting both the primary cause of the disease and secondary effects such as inflammation and fibrosis. In particular, in the last few years, great work has been conducted to repurpose the use of CFTR correctors for recovering mutated SGs. This approach targets the primary cause of the disease and is currently in an advanced preclinical stage. Certainly, it is specifically intended for the sarcoglycanopathy cases due to missense mutations resulting in the production of a folding defective SG that, in most of the cases, is still functional. It is worth noting that these types of genetic defects, according to the data reported in Figure 1, account for 59% of all SG mutations, reaching approximately 69% in the case of *SGCA*.

Finally, it must be underlined that combinatorial strategies can be envisaged, involving the simultaneous or sequential use of multiple therapeutics targeting both the primary and secondary causes of the disease. For example, the combination of gene therapy or CFTR correctors together with compounds addressing inflammation or fibrosis can result in a more robust recovery of the phenotype. The aim of combining multiple strategies recognizes the complexity of managing sarcoglycanopathies, providing a more effective treatment to achieve the therapeutic effect.

The efforts that researchers are carrying out in various fields of therapeutic intervention, together with natural history studies, are a guarantee that in the not too distant future sarcoglycanopathy will be included among the rare diseases that are no longer orphans.

Author contributions

M.S., A.B., F.D.B., D.S. Bibliographic search; M.S., A.B., F.D.B. Writing - Original Draft; M.S. and D.S. Writing -

Review & Editing; D.S. Supervision and funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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