Review Article

Restoring the regenerative balance in neuromuscular disorders: satellite cell activation as therapeutic target in Pompe disease

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Abstract: Skeletal muscle is capable of efficiently regenerating after damage in a process mediated by tissue-resident stem cells called satellite cells. This regenerative potential is often compromised under muscle-degenerative conditions. Consequently, the damage produced during degeneration is not efficiently repaired and the balance between repair and damage is lost. Here we review recent progress on the role of satellite cell-mediated repair in neuromuscular disorders with a focus on Pompe disease, an inherited metabolic myopathy caused by deficiency of the lysosomal enzyme acid alpha glucosidase (GAA). Studies performed in patient biopsies as well as in Pompe disease mouse models demonstrate that muscle regeneration activity is compromised despite progressing muscle damage. We describe disease-specific mechanisms of satellite cell dysfunction to highlight the differences between Pompe disease and muscle dystrophies. The mechanisms involved provide possible targets for therapy, such as modulation of autophagy, muscle exercise, and pharmacological modulation of satellite cell activation. Most of these approaches are still experimental, although promising in animal models, still warrant caution with respect to their safety and efficiency profile.

Keywords: Pompe disease; muscle regeneration; satellite cells; regenerative therapies

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Introduction

Pompe disease is an inherited metabolic disorder due to deficiency in the lysosomal enzyme acid alpha glucosidase (GAA). Pompe disease is characterized by progressive degeneration of skeletal muscle in all patients. In classic infantile patients development of hypertrophic cardiomyopathy (1) and involvement of the central nervous system (2,3) is observed. Enzyme replacement therapy (ERT) extends survival for classic infantile patients and improves motor function, although the heterogeneous response between patients remains challenging (4-6). Together, with the cost of therapy and the burden of the regular infusions for patients and caretakers it is clear that novel treatment strategies are warranted. Such novel therapeutic opportunities include improved ERT, substrate-reduction strategies, the use of chaperone, antisense oligonucleotides, and gene therapy approaches (7,8).

Pompe disease is one of the almost 900 different conditions associated with a muscle wasting phenotype (9). Although different muscle disorders are heterogeneous in disease mechanism and in pattern of progression, these conditions often share a defect in muscle regeneration. Muscle-regenerative pathways are common for all patients with neuromuscular disorders and may offer novel

therapeutic opportunities to improve the muscle phenotype across the different diseases. Here, we review muscle regeneration defect(s) in neuromuscular diseases, focusing on Pompe disease. We will also discuss advances in the development of regenerative therapies and the feasibility of using these in the treatment of Pompe disease patients.

Mechanism of the regenerative defect in Pompe disease

Muscle pathology in Pompe disease

Pompe disease is considered a clinical spectrum indicating that the disease can develop at any age and progress at any rate. The onset and progression of disease is roughly correlated with the genotype (10) with classic infantile patients harbouring severe GAA mutations and showing an aggressive course of disease. Deficiency of GAA causes glycogen accumulation that is particularly damaging to skeletal muscle in all patients (11), and to the heart and central nervous system in classic infantile patients (3,12). Skeletal muscles from Pompe disease patients are characterized by vacuolization, irregularly shaped myofibers, and loss of cross-striation (1). During disease progression the lysosomal compartment grows in size and number, replacing healthy cellular content (11). The numerous glycogen-filled lysosomes rupture and release glycogen into the cytoplasm. In the final stages cytoplasmic glycogen has replaced most contractile elements of the muscle cell, resulting in loss of myofiber function. Obviously, glycogen accumulation and lysosomal dysfunction affect other processes that contribute to the pathology, including disturbed macroautophagy (herein referred to as autophagy) and calcium homeostasis, oxidative stress, and mitochondrial abnormalities. Autophagy dysfunction, which is common among lysosomal storage disorders (13), contributes to sarcopenia during aging and will be discussed further below in the context of the muscle regenerative response.

Pathological tissue remodeling, where myogenic tissue is replaced by fat or fibrotic tissue, is a frequent feature of neuromuscular disorders. Adipose deposition of certain muscle groups is observed in most Pompe disease patients at the macroscopic level, and is most prominent in axial muscles, scapular girdle muscles, thigh muscles and specifically tongue (14,15). Pompe disease patients show a typical distribution of affected muscles, with shoulder abductors, abdominal muscles, paraspinal muscles, hip flexors, hip extensors, and hip adductors among the most affected muscles. Hip abductors were affected in more than 80% of all patients (16). The quadriceps femoris was affected in little over half of the patients, while the hand and feet muscle were found to be relatively spared.

The muscle damage in Pompe disease develops predominantly intracellularly, leaving the sarcolemma and basal lamina largely intact. This is in contrast to the increased sarcolemmal fragility, increased sensitivity for contractile damage, and myofiber rupturing that characterizes dystrophic muscle. Ruptured myofibers recruit immune cells that contribute to pathological deposition of adipose and fibrous tissue in dystrophic muscle. A robust intramuscular immune response to progressing disease is absent in Pompe disease, consistent with maintained sarcolemmal integrity. The ruptured and necrotic myofibers in dystrophic muscle also drive the muscle regenerative response and robustly activate MuSCs. Therefore, the lack of robust sarcolemmal damage in Pompe disease may contribute to satellite cell inactivity, as will be delineated further below. The comparison of Pompe disease and dystrophic muscle illustrates that differences in muscle pathology can have profoundly different effects on muscle regeneration.

The role of satellite cells in muscle regeneration

To understand the clinical consequences of distinct defects in muscle regeneration, we will first discuss the role of skeletal muscle stem cells and muscle regeneration in healthy skeletal muscle. Skeletal muscle can regenerate damage by recruiting the activity of tissue-resident stem cells. Skeletal muscle stem cells (MuSCs) or muscle satellite cells are located at the myofiber periphery underneath the basal lamina (17). In homeostasis MuSCs are quiescent, but become rapidly activated after damage and proliferate to generate a large set of progeny that is capable of regenerating damaged myofibers by fusion (*Figure 1*). Quiescent MuSCs express high levels of Pax7, a master regulator of postnatal myogenesis. Upon activation MuSCs downregulate Pax7 and upregulate the determination factor MyoD (18) that drives terminal myogenic differentiation. Part of the activated MuSCs return to quiescence in a process called self-renewal (19) to replenish the stem cell pool and secure long-term regenerative potential. The activated MuSCs proliferate and progress to become a population of myogenic progenitors that eventually start expressing myogenin. Previously, it was demonstrated that, after conditionally depleting MuSCs in a Pax7-dependent manner, skeletal muscle did not or hardly regenerate after

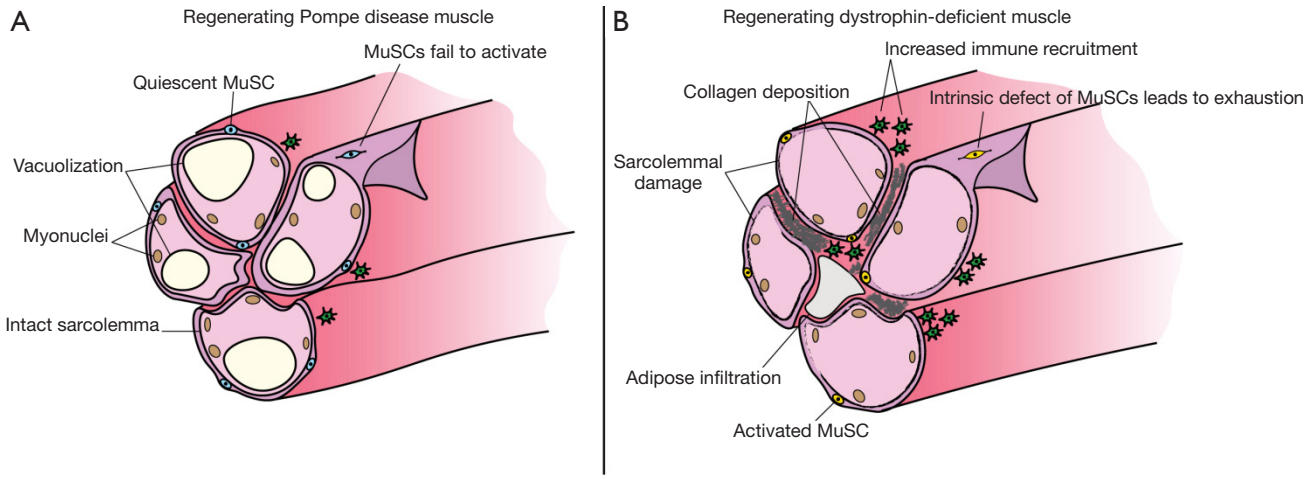

Figure 1 Lack of satellite cell activation in Pompe disease. The figure depicts a cross-sectional view of skeletal muscle in Pompe disease (A) and in dystrophic muscle for comparison (B). While dystrophin-deficient muscle is characterized by MuSC hyperactivation, chronic inflammation, and severe tissue remodeling, Pompe disease is characterized by a failing regenerative response.

damage (20,21). In the absence of MuSCs, injured muscle became infiltrated with inflammatory cells and muscle tissue was replaced with fat and fibrotic tissue, further emphasizing the important role of MuSCs in mediating a healthy regenerative response. Earlier studies already found that skeletal muscle can still regenerate when MuSC numbers are at least 10% of the levels in healthy young animals (22), indicating a large "regenerative reserve" under healthy conditions.

A defect in MuSC activation in Pompe disease

Defects in MuSC activity have been reported for various neuromuscular diseases. These comprise effects on their proliferative potential (23-27), changes in MuSC numbers (25,28-31), and alterations in their potential to properly activate (32) or differentiate (33) (*Table 1*). Animal models for these and other muscle-degenerative diseases were also found to have defects in MuSC activation, proliferation, self-renewal, and differentiation potential (34-39) (*Table 1*). These effects of the failing MuSC population can be irreversible—as in certain dystrophies—or reversible as in Pompe disease.

The current dogma for Duchenne muscular dystrophy (DMD) is that affected muscles undergo continuous rounds of de- and regeneration—as result of contractile myofiber damage—which is thought to eventually exhaust the MuSC pool [see for recent excellent reviews (41,42)]. This proliferative/exhaustive phenotype was observed in myogenic progenitors explanted from dystrophic muscle, with the defect more pronounced in cells isolated from older patients (23,43). Loss of proliferative potential has been attributed to attrition of telomere length (44), which was indeed demonstrated in samples from human DMD subjects (45). The loss of proliferative potential of MuSCs is not restricted to DMD, but also described for other muscle disorders, including myotonic dystrophy (24) or sporadic body inclusion myopathy (25) (*Table 1*). Another mechanism for loss of MuSC function is through premature differentiation of MuSCs, resulting in depletion of the self-renewing stem cell pool, such as has been reported for Emery-Dreifuss dystrophy (46,47). In addition, as it was reported for Myasthenia Gravis, the differentiation potential of MuSC progeny may be compromised, so that muscle regeneration cannot be successfully completed (36) (*Table 1*). In these examples, the MuSC defect is cell-intrinsic and is likely to be irreversible.

Our recent results show that the mechanism of MuSC inactivation in Pompe disease is different and, reversible. We previously described that MuSC numbers are stable in biopsies from Pompe disease patients across a large age-range (2 months–71.7 years) (32) and we and others demonstrated that in mouse models of Pompe disease MuSCs are not exhausted (39,40) (*Table 1*). In fact, MuSC numbers were increased in *Gaa-ko*-mice in two different backgrounds -*fvb/n* and the mixed *c57/bl6* and *129sv* background. Muscle biopsies from Pompe disease patients showed considerable muscle damage which is

Table 1 MuSC defects in various neuromuscular diseases

Type of MuSC defect	Disease	Species	Patients age range*	Number of patients	Animal model	Animals age	Muscles analyzed	Type analysis	Result	Mechanism of MuSC loss of function	Reference
Proliferative defect	DMD [5], PM [5], MD [5], ALS [5]; healthy [7]	Human	30–60	5	–	–	Deltoid, biceps, QF, GAS, Triceps, peroneal	LM and EM	DMD ±6 fold increased and MD/PM ±3-fold increased over adult control	Abnormal proliferation	(28)
	DMD	Human	2–14	9	–	–	Vastus lateralis, vastus medialis	*Ex vivo* isolated cells	80% of DMD clones with long doubling times and aberrant morphology (*vs.* 8% normal clones)	Loss of replicative potential	(23)
	DMD	Human	2–7	7	–	–	vastus lateralis, rectus femoris	IF tissue: Pax7	Increased SC levels	Abnormal proliferation	(29)
	Myotonic Dystrophy	Human	46–54	4	–	–	Vastus lateralis, TA	IF tissue: NCAM	Increased SC numbers in distal muscles, reduced regeneration, proliferative defect	Loss of replicative potential	(24)
	Nemaline myopathy	Human	3 weeks–72 years	12 NM, 21 contrl	–	–	Psoas, diaphragm, quadriceps, abdominal wall, quadriceps, deltoid, TA, intercostal, pectoral, biceps	IF tissue: NCAM1/Pax7	Increased MuSC levels in NM muscle (±10 fold)	Abnormal proliferation	(31)
	Various NMD	Human	16–89	sIBM [39], PM [13], DM [13], MD [10], Ctrl [10]	–	–	Deltoid, biceps brachii, QF, extensor forearm, TA	IF tissue: Pax7	All NMD have increased Pax7-positive cells, MyoD-positive and Myogenin-positive compared to ctrl	Perturbed regeneration/ excessive MuSC activation	(25)
	DMD	Mouse	–	–	*mdx*	6–8 weeks	Hindlimb muscles	IF isolated myofibers: Pax7	Initial increase in mdx, decline after 6 m of age	Loss of replicative potential	(34)
	DMD	Mouse	–	–	*C57Bl/10ScSn-DMDmdx/J*	4 and 8 weeks	GAS	MACS-based isolation of MuSCs	Increased SC levels MDX (early)	Abnormal proliferation	(35)

Table 1 *(continued)*

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Type of MuSC defect	Disease	Species	Patients age range*	Number of patients	Animal model	Animals age	Muscles analyzed	Type analysis	Result	Mechanism of MuSC loss of function	Reference
Functional defect	Limb girdle Muscular dystrophy 2A	Human	2–62	13	–	–	Biceps	IF tissue: Pax7	Increased SC count in fibrotic group	Impaired differentiation	(33)
	Pompe disease	Human	2–71	22	–	–	Vastus Lateralis	IF tissue: Pax7	Equal number, Impaired MuSC activation	Failing MuSC activation	(32)
	Myasthenia gravis	Human+ mouse	–	–	*AchR immunization c57/Bl6*	>10 weeks	TA	IF tissue: Pax7	Increased SC levels; reduced regeneration efficiency	Impaired differentiation	(36)
	Mdx	mouse	–	–	*Mdx*	2–3 months	EDL	IF Isolated myofibers: Pax7	Increased SC/fiber (1.5 fold); reduced asymmetric division	Self-renewal defect	(37)
	Pompe disease	mouse	–	–	*$Gaa^{13Neo/13Neo}$ in FVB/N* (38) *and $Gaa^{6Neo/6Neo}$* (10) *in 129Sv/c57/Bl6*	2–60 weeks	TA, GAS, hindlimb muscles	IF tissue: Pax7; FACS: VCAM	Inceased MuSC, impaired MuSC activation; gaa-deficient MuSC respond to injury and self-renew	Failling MuSC activation	(39)
	Pompe disease	mouse	–	–	*$Gaa^{6Neo/6Neo}$* (10)	6–36 weeks	TA, Triceps Brachii	IF tissue: Pax7	impaired MuSC activation; Gaa-deficient MuSC respond to injury	Failing MuSC activation	(40)

*, age in years unless specified otherwise. ALS, amyotropic lateral sclerosis; DM, Duchenne muscular; DMD, Duchenne muscular dystrophy; EDL, extensor digitorum longus; GAS, Gastrocnemius; MD, myotonic dystrophy; PM, polymyositis; sIBM, sporadic inclusion body myositis; TA, tibialis anterior; QF, quadriceps femoris.

expected to elicit a robust regenerative response, but we found that MuSCs were not activated to repair the damage. Markers of active regeneration, which includes the expression of embryonic myosin heavy chain, were absent (32). Similarly, *Gaa*-deficient mice show muscle wasting, as reflected in decreasing muscle wet weight, and show limited MuSC activation and regeneration (39), reminiscent of the observations in human Pompe disease patients. Interestingly, *Gaa*-ko mice, despite completely lacking functional *Gaa* similarly as classic infantile patients, only develop a muscle phenotype from 15 weeks onwards. At this age, an initial mild regenerative response observed in younger animals, reflected by a gradual increase in myofiber central nucleation and low-level MuSC proliferation, is lost. It can be hypothesized that in young *Gaa*-ko mice the mild regenerative response delayed the onset of muscle wasting.

To determine if the observed regenerative defect could be explained by compromised MuSC function, we as well as Lagalice and colleagues, performed muscle-injury experiments. Both these studies showed that *Gaa*-deficient muscle regenerated efficiently after chemical injury (i.e., using $BaCl_2$ or cardiotoxin), excluding a cell-intrinsic defect of *Gaa*-deficient MuSCs. Moreover, we also showed that *Gaa*-ko muscle regenerated completely after serial injury, indicating that the *Gaa*-deficient MuSCs are also capable of self-renewal (39). Our current work focuses on explaining this apparent paradox between MuSC inactivity during disease progression, while still capable of responding efficiently to exogenous damage. We hypothesize that the failing MuSC activation in Pompe disease may be explained by an inhibitory environment or by insufficient activation signals.

The role of autophagy in MuSC activation and muscle regeneration

In order to maintain the quiescent state, MuSCs require a basal level of autophagic activity (48,49). Disruption of autophagy during aging is shown to affect homeostasis of MuSCs and results in reduction of the pool of quiescent MuSCs in aging individuals (49). In fact, autophagic activity is one of the first processes to be upregulated during MuSC activation. While quiescent cells reside in a low metabolic and energetic state, autophagic activity is rapidly increased after damage to supply the increased demand for ATP, and reducing cofactors and amino acids needed to sustain proliferation (48). Tang and colleagues identified SIRT1 as critical regulator of autophagic flux in activating MuSCs. Loss of SIRT1 resulted in disturbed autophagic activity and delayed MuSC activation. Blocking autophagy disrupted MuSC activation (48,50). Paolini and colleagues found in mice that were genetically modified to express reduced levels of autophagic activity—by ablation of *Atg16l1*, a key component in autophagy—that the muscle regenerative response was delayed (51). The regenerating muscle of these mice contained increased levels of uncommitted MuSCs (Pax7+/MyoD–) and less activated/committed muscle progenitors that expressed MyoD. The authors concluded that failure to upregulate autophagic activity during the early stages of regeneration blocked full MuSC activation. While autophagic activity needs to be increased during the very early stages of MuSC activation, inhibition of autophagy in MuSC progeny is essential for completing regeneration (52). These studies indicate that autophagic activity is tightly controlled during different stages of regeneration, and may even be differentially regulated in different states of MuSC activation. The role of autophagy in MuSC activation and muscle regeneration under diseased conditions is still not entirely clear and warrant further study.

Disruption of autophagic activity contributes to neuromuscular disease

Inhibition as well as excessive activation of autophagy is found to induce muscle atrophy (53,54), suggesting a narrow window of autophagic activity during muscle homeostasis. Genetic inhibition of autophagy was sufficient to induce morphological features of myopathy, including myofiber vacuolization and decreasing fiber diameter (55,56). Disrupted autophagic flux has been observed in biopsies from DMD patients (57), in X-linked myopathy (58), Danon disease (59) and Pompe disease (60,61). Studies in animal models of these diseases support a prominent role of autophagic dysfunction in the disease development. Lysosomal dysfunction in Pompe disease results in accumulation of glycogen as well as autophagic debris, that eventually blocks autophagic flux (62) and disrupts endosomal trafficking. Loss of endosomal trafficking has dramatic effects on cellular health and might also adversely impact uptake of recombinant GAA that is used as ERT in Pompe disease. Defective autophagy also affects mitochondrial functioning, as damaged mitochondria are removed through autophagy—in a process called mitophagy (8). In Pompe disease reduced autophagic activity may contribute to the

mitochondrial abnormalities that are frequently observed in muscle biopsies (11,63). Regulation of autophagy plays a prominent role in Pompe disease progression. More extensive reviews on the topic can be found elsewhere (64,65). It is hypothesized that normalization of autophagy restores the potential to regenerate muscle damage.

The role of the MuSC niche in muscle regeneration

Under homeostatic conditions the MuSC niche is defined by (I) the myofiber that conveys chemical, electrical, and mechanical signals, and (II) the basal lamina, which is largely composed of laminin, collagen, and proteoglycans (*Figure 1*). This polarized protective environment is completely remodeled after myofiber damage exposing MuSCs to several environmental factors that include chemical and electrical signals, auxiliary cells [reviewed in (66)] and matrix components. Interfering with the contact to either the myofiber and/or the basal lamina results in MuSC activation.

The damaged myofiber has an important role in engaging repair after injury. The MuSC-activating potential harnessed within myofibers was already demonstrated by Bischoff in 1986 (67). Bischoff described that exposure to saline extract from crushed myofibers pushed quiescent MuSCs into entering the cell cycle. It is now recognized that disruption of the sarcolemma induces the damaged myofiber to release signals that activate MuSCs, including nitric oxide (NO) (68) and matrix-remodeling proteins MMP-2, MMP-9 and MMP-10 (69,70). The MMP remodeling proteases release growth factors—such as HGF—that are embedded in the matrix. NO that is released by the damaged fiber or by recruited macrophages that also play an important role in liberating HGF (71). HGF binds to c-met—the receptor for HGF—that is expressed on quiescent MuSCs. MuSCs also express fibroblast growth factor receptors 1 and 4 (72), and respond to FGF2 that is expressed by the myofiber and accumulates under basal lamina during aging (73). In fact, purified extracts from aged myofibers compared to those from adult fibers were found to stimulate MuSC proliferation more potently (73), indicating that the changing myofiber niche directly impacts behavior of MuSCs.

The myofiber niche does not solely consist of chemical signals: resident and recruited cells play important roles in mediated muscle homeostasis and regeneration. Among the resident cells are fibro-adipogenic progenitors (FAPs), that are increasingly recognized for their crucial role in regenerative myogenesis (74,75). The role of the immune response after damage has been known for much longer (76). As mentioned above, damaged myofibers recruit immune cells, including neutrophils, eosinophils, and monocytes, that are crucial for proper muscle regeneration (77).

The niche is not only important during MuSC activation but also plays a pivotal role in maintaining quiescence under homeostatic conditions. For instance, heparan sulfate is the glycosaminoglycan component of many proteoglycans in the basal membrane and is dynamically regulated during MuSC activation (78). During aging 6-O sulfation specifically increased, augmenting FGF2-signaling, MuSC activation and age-dependent loss of MuSC quiescence (78). These data show that the composition of the niche can determine and mediate the MuSC response and that the niche composition is responsive to changes in the host.

The role of the niche in Pompe disease

Immunofluorescence analyses of patient biopsies as well as those from *Gaa*-deficient mice show that both the sarcolemma and basal lamina remain intact throughout the course of disease, although we did not analyze changes in niche composition. Also, we did not observe changes in recruitment of major immune populations, including macrophages and lymphocytes (unpublished observations). These findings suggest that myofiber rupturing and necrotic death do not seem to play a major role in Pompe disease muscle pathology. In light of the observed lack of MuSC activation, concomitant with the absence of cell-intrinsic defects (see above), it can be hypothesized that in Pompe disease the MuSC niche either fails to generate signals that activate MuSCs or that the niche actively suppresses MuSC activation. This may suggest that the niche could be a novel therapeutic target in Pompe disease.

The effect of targeting the niche is illustrated in merosin-deficiency or congenital muscular dystrophy 1A (CMD1A). CMD1A is caused by mutations in basal lamina protein LAMA2. It has been demonstrated that expression of integrin A7—that normally binds laminin—restores function and integrity of the basal lamina, and reduces muscle pathology in merosin-deficient mice (79).

Muscle-regenerative therapies for Pompe disease

Various experimental approaches have been developed to target specific regenerative defects in animal models of different muscle disorders. As was discussed above, the

regenerative defect in Pompe disease is distinct from that observed in DMD, with insufficient MuSC activation *vs.* MuSC hyperactivation, respectively. The observed MuSC inactivity in Pompe disease suggests that focusing on safe and efficient methods to activate MuSCs may have the potential to attenuate muscle pathology in Pompe disease. As possible targets for regenerative therapy we will discuss approaches to activate MuSCs directly—providing signals that activate MuSCs—or indirectly, such as by restoring autophagic activity. Some of these approaches bypass the contribution of the endogenous niche, such as injecting growth factors, or involve the niche by inducing myofiber damage—as in the exercise approach. The aim of these strategies is not only to improve muscle morphology and function, but also to reduce the lysosomal damage and improve autophagic/endosomal activity. As a bystander effect, improving muscle morphology and function may positively affect the response to the current ERT therapy.

Activating MuSCs by exercise

Activation of MuSCs and increase in MuSC numbers after resistance exercise are well-documented (80-82), and occur even after a single bout of exercise (83-85). Several factors of the exercise program have been found to affect the MuSC response including mode of exercise—i.e., resistance *vs.* endurance—exercise intensity, duration of the program, age, and sex of the subjects [see also (86)]. Exercise programs are being used and appear well-tolerated for patients with various types of neuromuscular diseases, including DMD (87), Becker muscular dystrophy (BMD) (88), Myasthenia Gravis (89) and myotonic dystrophy (90). Beneficial effects of exercise have also been observed in patients with metabolic myopathies, including glycogen storage diseases (91,92). In our center we performed a 12-week exercise programs with Pompe disease patients consisting of 36 sessions of standardized aerobic, resistance, and core stability exercises (93,94). This study showed that exercise was well-tolerated by patients and improved endurance of patients' muscles—as reflected by increased workload capacity, maximum oxygen uptake capacity and walking distance—and muscle function of hip flexors and shoulder abductors. It will be interesting to determine whether focused and safe exercise programs are capable of sufficiently activating MuSCs to restore the muscle regenerative response in Pompe disease patients.

Concerns exists regarding the safety and potential harmful effects of exercise in patients with increased sensitivity for contractile damage, such as in DMD. Exercise programs for such patients should probably minimize inclusion of lengthening eccentric contractions, which produces greater sarcolemmal disruptions. It should be noted that eccentric—but not concentric contractions—are associated with an efficient MuSC-mediated response (95). Such concerns are not as relevant for Pompe disease patients because of the lack of sarcolemmal fragility. Another concern is immobility of more advanced stage patients that may prevent participation in intensive exercise programs. Interestingly, pathways that were found to be affected by exercise, including AMPKA and PGC1a, can be activated by small molecule reagents—called exercise mimetics—and would offer the possibility of exercising less-mobile patients "pharmacologically". Narkar and colleagues found that treating sedentary *c57/bl6* mice with AICAR and PPARg agonist increased running endurance (96). AMPK signaling as potential target in modulating MuSC activation is supported by observations from Fu *et al.*, who showed that inhibition of AMPK severely impairs satellite cell-mediated muscle regeneration (97).

Exercise programs for neuromuscular patients constitute a minimally invasive therapeutic modality that can be combined with the current state of care or with future therapeutic interventions. As mentioned above, for Pompe disease improving condition of affected muscles may attenuate disease progression and could even positively affect the response to ERT. Obviously, nutrition plays a role in optimizing the response to exercise, although that is beyond the scope of this review.

Activating MuSCs by soluble factors

MuSCs respond to soluble signals that are released into their direct environment following damage, including FGF2, HGF, and NO. Intraperitoneal injection of HGF was found to reverse experimental atrophy in mice and activate MuSCs, as determined by an increase in the fraction of MuSCs that expressed the cell cycle marker Ki67 (98). Interestingly, mTOR activity was reduced in muscle homogenates of treated animals, suggesting that changes in autophagic activity and protein synthesis may also be involved in the observed effect.

Activating MuSCs by targeting autophagy

Autophagic activity is a major determinant of MuSC activation and successful muscle regeneration, and is often

dysregulated in neuromuscular patients, in particular in those with lysosomal storage disorders. The beneficial effects of exercise are also mediated through changes in autophagic activity in the skeletal muscle of mice (99) and humans (100). Exercise activates autophagy through increases in AMPK activity, which in turn inhibits mTORC1—an inhibitor of autophagy (101). The exercise mimetics that were discussed above activate AMPK signaling and function, at least partly, by increasing autophagic activity.

It is thought that glycogen traffics to the lysosome though autophagy (102) and as result of this process cellular debris is taken up as well. Disrupted autophagy as is observed in Pompe disease results in lysosomal accumulation of waste material that eventually terminates the autophagic flux. Based on this, it has been proposed that blocking autophagy would prevent the buildup of autophagic waste material and glycogen in Pompe disease and as such improve the condition of *Gaa*-deficient muscle. Indeed, autophagic buildup and glycogen accumulation were reduced in fast muscle from autophagy-deficient *Gaa*-KO mice, where *Atg*7—a critical member of the autophagy pathway—was depleted. The $Gaa^{-/-}/Atg7^{cKO}$ mice actually appeared more healthy compared to, the "regular" *Gaa*-KO mice (103). Interestingly, the $Gaa^{-/-}/Atg7^{cKO}$ mouse model also showed increased lysosomal glycogen clearing after ERT (103). A recent study showed different strategies targeting autophagy that resulted in removal of autophagic debris, reactivation of autophagic activity and restoration of the response to ERT in *Gaa*-KO mice (104), demonstrating the potential of autophagy as a therapeutic target. To determine the feasibility of autophagy as target for therapy, it will be informative to examine the energetic state and mTOR status of the stem cells in the affected muscles.

Increasing autophagy activity was also found to improve the muscle phenotype in the mouse model of DMD (105). In *mdx* mice autophagic activity is inhibited possibly as result of enhanced levels of oxidative stress, as determined using a Nox2-specific reactive oxygen species (ROS) biosensor. Oxidative stress was found to induce mTOR activity, which is a potent inhibitor of autophagy (105). Genetic inhibition of Nox2, which was found to mediate ROS development in *mdx* mice, reduced oxidative stress levels, reversed autophagic activity, and resulted in improvement of muscle morphological abnormalities and muscle function (105). A similar improvement of the condition of *mdx* mice was observed after a low-protein diet, which was thought to be mediated by attenuation of autophagic dysfunction (57). Together these and other studies suggested that autophagy may be a novel target in the treatment of DMD and Pompe disease and perhaps also for other neuromuscular diseases.

Cell-based therapies and ex vivo gene therapy

The strategies described above harness the regenerative potential of endogenous muscle stem cells. Such strategies are feasible for Pompe disease where the endogenous MuSC population is retained and functional. However, activating MuSCs without gene correction will not constitute a permanent cure.

Advances in gene therapy strategies show promise of achieving gene correction. Current strategies are mediated through adeno-associated virus (AAV) and predominantly target the liver. *Gaa* expressed in the liver is excreted into the circulation and is then taken up by affected muscle in a process called cross-correction (106). Intramuscular injection of AAV into the diaphragm has also been developed. In this regard, clinical trials using AAV-mediated gene therapy are currently ongoing and show a good safety profile, as was recently reported in children (107).

AAV-vectors carrying truncated versions of dystrophin—microdystrophin—were found to be safe and seemed effective in dystrophic mice and dogs (108) and are currently being evaluated in clinical trials. A disadvantage of AAV-vectors is that they do not integrate into the host genome and may be lost on the longer term. Other vector backbones, such as lentiviral vectors (109,110)—that integrate—are investigated for use in gene therapy strategies, including for Pompe disease and DMD. A more detailed discussion of these strategies is beyond the scope of this review and has been reviewed elsewhere (8,111).

Cell-based therapies offer the potential to replenish the MuSC pool with gene-corrected cells via *ex vivo* gene therapy. As during muscle regeneration muscle progenitors fuse to form new myofibers, donor cells share their genetic content with affected fibers. Furthermore, a major advantage of cell-based therapies is the opportunity for long-term improvement of affected muscles. In the 1990s major efforts were undertaken to explore myoblast transfer therapies, based on promising results obtained in the mdx mouse model (112,113). Unfortunately, for a number of reasons the human trials all turned out negative and little if any efficacy was shown (114). In more recent studies, transplantation of MuSCs was found to be much more effective compared to myoblast transfer (115-117),

at least in mouse studies. This reignited interest in the development of a cell-based therapy for muscle-wasting disorders. The major drawback still is that MuSCs lose most of their regenerative potential when expanded *ex vivo* (118). The advent of induced pluripotent stem cells (IPSC) (119) opened novel opportunities of generating tissue-specific progenitor cells without the problems associated with expansion. We have recently described a method to generate skeletal muscle progenitors from Pompe disease patient fibroblasts that retain large expansion potential while maintaining the capacity to differentiate efficiently *in vitro* and engraft *in vivo* (120). The advantage of iPS cells as cell source is that these are amenable for *ex vivo* gene correction using CRISPR-Cas9, for example, to generate gene-corrected myogenic progenitors. Additionally, we used the IPS-derived muscle progenitor model to show the efficacy of an antisense oligonucleotide strategy to increase exon inclusion correcting a frequent splicing mutation found in the Pompe population (121,122). Splicing-targeted therapies for muscle disorders are a very fruitful and promising field and are recently reviewed elsewhere (123). The use of IPS-derived muscle progenitors as sources for cell-based therapies is also considered for other muscle-wasting disorders, including DMD, and has much potential. However, it is currently difficult to fully assess the clinical potential using small animal models, and further studies into the safety of the reprogramming and gene-editing strategies are required.

In vivo gene editing as a therapy

The development of current gene editing tools, in particular CRISPR-Cas9 technology, is rapidly moving forward the field of gene-correction strategies. Recently, this technology has been used to explore the efficacy of gene-editing the genetic defect in *mdx* (124-126). In these studies the CRISPR-Cas9 components were delivered using AAV vectors, and were delivered either directly intramuscularly or systemically. Long and colleagues showed by using this strategy in *mdx* mice that up to 25.5% of TA fibers expressed dystrophin after intramuscular delivery as judged by immunofluorescent analysis. Systemic—intraperitoneal administration in this study—was less efficient (125).

Tabebordbar and colleagues isolated MuSCs from the mdx/Pax7 zgreen^{+} compound reporter mouse, and demonstrated truncated DMD transcripts in MuSC-derived myotubes *in vitro* (126), suggesting that the MuSCs had indeed been targeted *in vivo*. Recently, early results—2 months follow-up—from an *in vivo* gene-editing study in a canine model of DMD using CRISPR/Cas9 technology were reported showing improved histology in four dogs. After systemic delivery dystrophin levels up to 90% of WT levels were found depending on the tissue analyzed (127).

A recent study in a DMD model in nonhuman primates also showed effectivity of *in vivo* gene editing (128). Despite these encouraging early results, caution is warranted. *In vivo* gene-editing is still associated with safety concerns (129) and is still rather inefficient. Further study is required for the development of safe and efficient gene-editing method for future clinical implementation.

Conclusions

In this review we focused on the mechanism of the observed failing regenerative response in Pompe disease and conclude that the failure to efficiently activate MuSCs contributes to ongoing muscle wasting. In the absence of muscle regeneration, muscle damage is not compensated by repair. To restore the regenerative balance, novel therapies may be directed to activate MuSCs directly—through direct administration of MuSC-activating agents—or indirectly, for instance by targeting autophagic activity. In particular, exercise therapy aimed to activate the inactive MuSC population is minimally invasive and has proven to be well-tolerated by patients. Based on the safety profile exercise therapy can be assumed to have clinical feasibility, although studies into their regenerative efficacy are warranted.

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Footnote

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