A clinical perspective on muscle specific kinase antibody positive myasthenia gravis

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The discovery of autoantibodies directed against muscle-specific kinase (MuSK) in "seronegative" myasthenia gravis (MG) patients marked a milestone in MG research. In healthy muscle, MuSK regulates a phosphorylation pathway, which is essential for the development and maintenance of acetylcholine receptor (AChR) clusters at the neuromuscular junction. Autoantibodies directed against MuSK are predominantly of the IgG4 subclass, but there is increasing evidence that IgG1-3 could also contribute to the pathology underlying MuSK-MG. MuSK-IgG4 are monovalent and block the binding site for LRP4 on MuSK, thereby inhibiting the downstream phosphorylation pathway and compromising the formation of AChR clusters. Clinically, MuSK-MG is commonly associated with the predominant involvement of bulbar, facial, shoulder and neck muscles. Cholinesterase inhibitors should be avoided in MuSK-MG due to the risk of clinical impairment and cholinergic crisis. Corticosteroids and other non-steroidal immunosuppressants are less effective with the need for higher doses and prolonged treatment. Rituximab, by contrast, has been shown to be particularly effective and is now often used early in the disease course. Its use is associated with a significant improvement in the clinical outcome of MuSK-MG patients over time. This review aims to describe the pathophysiology underlying MuSK-MG and provide a comprehensive overview of the clinical features and therapeutic options.

1 Introduction

The first known documentation of myasthenia gravis (MG) by Thomas Willis dates back to the second half of the seventeenth century, and around 200 years later W.H. Erb (1879) and S. Goldflam (1893) published the first detailed descriptions of the disease ([1–3]). In 1895, Jolly first theorized that an impairment of signal transmission at the

neuromuscular junction (NMJ) was the underlying pathomechanism (4), but it was not until the 1970s that the acetylcholine receptor (AChR) antibody (Ab) was identified (5). In the year 2001, Hoch et al. followed with the publication of their discovery of Abs against muscle specific kinase (MuSK) (6). Both discoveries were significant milestones: the identification of AChR-Abs proved the proposition of John Simpson in 1960 that MG was an autoimmune disease (7), and the discovery of MuSK-Abs confirmed hints of clinical distinctions between "AChR positive" and previously "seronegative" MG.

MG with underlying MuSK-Abs (MuSK-MG) is characterized by specific clinical features and a distinct treatment response when compared to patients with AChR-Abs (8). Muscle weakness and fatigability primarily affect bulbar, respiratory and neck muscles (9–14). Atrophy of affected muscle groups was observed in later stages of the disease, although imaging studies have also revealed early subclinical muscular wasting (12, 14–19). Before the identification of MuSK-Abs, the treatment of "seronegative" MG patients followed the standard regimen for AChR-Ab positive MG (AChR-MG). However, part of that regimen, namely thymectomy and cholinesterase inhibitors, was ineffective in MuSK-MG (20–22). Cholinesterase inhibitors were even associated with the potential to worsen symptoms of MuSK-MG patients (10, 12, 22, 23). Advancements in our understanding of the molecular mechanisms underlying MuSK-MG have been successfully translated into clinical practice and led to improved treatment strategies. Clinical guidelines on MG now take the antibody status into consideration (24, 25). Different recommendations for AChR-MG and MuSK-MG have been implemented, respectively, which has led to a significant improvement of the clinical outcome of MuSK-MG patients over time (25, 26). In light of these developments, this review aims to address our understanding of the pathomechanisms involved in MuSK-MG, the clinical features, as well as current and future therapeutic approaches.

2 MuSK is a crucial component of the AChR clustering pathway

Muscle specific tyrosine kinase is a 120 kDa transmembrane protein located in the postsynaptic muscle cell membrane and plays a crucial role in the development, function, and maintenance of the NMJ (27–29). Structurally, MuSK is composed of three Ig-like domains followed by a frizzled-like cysteine-rich domain in the N-terminal extracellular regions, a transmembrane region, and a cytoplasmic domain with kinase activity (30–33).

2.1 The agrin/LRP4/MuSK/Dok7 pathway as a positive regulator of AChR clustering

Neural agrin, a large heparan-sulfate proteoglycan secreted from the motor nerve terminal, and the low-density lipoprotein receptor-related protein 4 (LRP4), another transmembrane protein of the muscle cell membrane, interact with MuSK molecules in a

2:2:2 configuration (34–39). In a recently published study analyzing the structure of the agrin/LRP4/MuSK complex using cryogenic electron microscopy, LRP4 was shown to act as a "clamp" resulting in a direct interaction between agrin and MuSK, thereby promoting MuSK dimerization and (auto)phosphorylation of its cytoplasmic domain (37). Downstream of tyrosine kinase 7 (Dok7) is recruited and binds to the juxtamembrane cytoplasmic region of MuSK. Upon phosphorylation, Dok7 fosters full MuSK activation by enhancing MuSK dimerization (40). Subsequently, MuSK activation induces tyrosine-phosphorylation of 43 kDa receptor-associated protein of the synapse (rapsyn), which then undergoes liquid-liquid phase separation (LLPS) to form membraneless condensates (41). These co-condense with AChR subunits and cytoskeletal proteins, facilitating the formation of rapsyn-AChR-aggregates together with components of the cytoskeleton (41). Different intermediate proteins including Crk, Abl, Src and Rho are recruited by MuSK activation and further amplify the process of LLPS and AChR clustering (42–49). Ultimately, rapsyn interacts with the cytoplasmic loops of all AChR subunits and connects receptors by up to three bridges, which is the minimum number required to form a 2D network (50–52). Many of these pathways are involved in actin remodeling and cytoskeletal rearrangements to anchor and immobilize the AChR clusters within the postsynaptic plasma membrane (52, 53) (Figure 1).

2.2 Negative regulators of AChR clustering

It is important to acknowledge that AChR clustering underlies a homeostatic regulation of positive and negative regulators. As highlighted before, the reciprocal activation of MuSK and Dok7 is the strongest promoter of AChR clustering. Acetylcholine (ACh), by contrast, is a strong negative regulator. Sustained cholinergic stimulation associated with prolonged calcium influx through the AChRs (e.g., in the absence of acetylcholine esterase in the synaptic cleft) with accumulation of intracellular calcium activates a calpain-Cdk5 pathway, which mediates AChR dispersal (54–58). This pathway is counterbalanced by rapsyn, which was reported to interact with calpain and inhibit its activity, thereby stabilizing AChR clusters in an agrin-dependent manner (54). Another negative regulator of AChR clustering is the SRC homology two domain-containing phosphotyrosine phosphatase 2 (SHP2). Agrin-stimulation of MuSK was shown to phosphorylate signal regulatory protein a 1 (SIRPa 1), an activator of SHP2, which, in a negative feedback loop, reduces MuSK phosphorylation and activity (59). Endocytosis of MuSK was suggested to be another important regulator of AChR clustering and is directly linked to its activation, specifically in response to stimulation with agrin (60, 61). Other factors promoting MuSK endocytosis include Wnt, a family of glycoproteins secreted from the nerve terminal that regulate AChR clustering and play a critical role in neuromuscular development (62). MuSK also acts as a receptor for the bone morphogenic protein (BMP). The MuSK-BMP pathway has been suggested to inhibit Ca^{2+} signaling via regulator of G protein signaling 4 (RGS4) (63). Together, all these mechanisms constitute a complex network of pathways that contribute to the fine-tuned regulation of AChR clustering and cholinergic signaling at the NMJ (Figure 1).
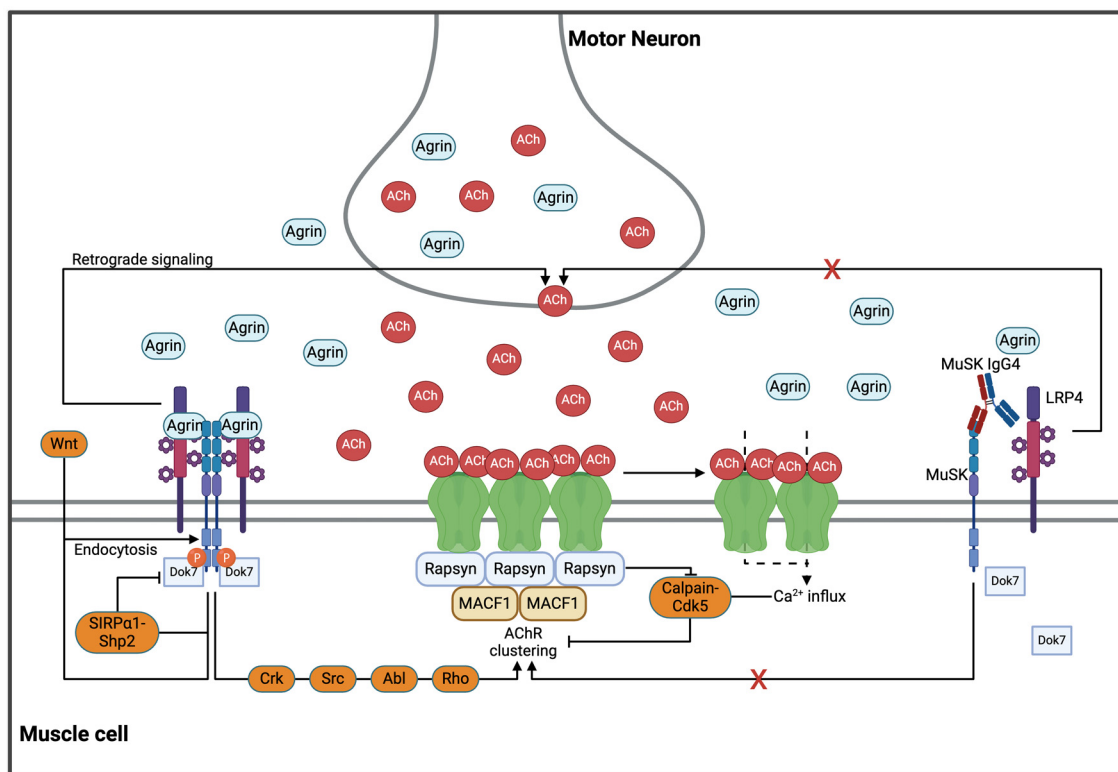
FIGURE 1

The AChR clustering pathways in health and MuSK-MG. MuSK, agrin and LRP4 bind in a 2:2:2 configuration, facilitating transmembrane autophosphorylation of MuSK. Recruitment of Dok7 initiates a signaling cascade through various pathways (including Src, Crk, Abl and Rho) which in turn enhances rapsyn-condensation and subsequent AChR clustering. The two-dimensional AChR network is connected through rapsyn, which forms up to three bridges with all AChR subunits. This network is further stabilized in the third dimension by the cytoskeleton via MACF1. Formation and stabilization of AChR clusters is essential for physiological neuromuscular activity. However, excessive cholinergic activation leads to a prolonged Ca^{2+} influx through the AChRs, thereby activating the Calpain/Cdk5 pathway and dispersing AChR clusters. Further upstream, MuSK-dimerization is inhibited by the SIRPa1/SHP2 pathway in a negative feedback loop. Endocytosis of MuSK, either mediated by agrin or Wnts, is suggested as another important negative regulator. LRP4-MuSK is hypothesized to increase the quantal content, which balances AChR clustering. However, the exact mechanisms underlying this effect are yet to be unraveled. Monovalent IgG4 antibodies inhibit MuSK dimerization and the downstream (and upstream) pathways important for AChR clustering. Abl, Abelson murine leukemia viral oncogene homolog 1; ACh, acetylcholine; AChR, acetylcholine receptor; Cdk5, Cyclin-dependent kinase 5; Crk, CT10 regulator of kinase; Dok7, Downstream of tyrosine kinase 7; LRP4, low-density lipoprotein receptor-related protein 4; MACF1, microtubule actin crosslinking factor 1; MuSK, muscle specific kinase; Rho, Ras homology; SHP2, Src homology region 2 domain-containing phosphatase-2; SIRPa 1, signal regulatory protein α1; Src, from "sarcoma", Wnt, wingless-related integration site. Created in BioRender.com.

3 Unravelling the pathogenicity of MuSK antibodies

The pathogenicity of MuSK-Abs is related to the nature of the antibodies, which are predominantly of the IgG4 subclass and therefore unable to bind complement or activate immune cells via interaction with Fcγ receptors (64, 65). Instead, IgG4 is considered to mediate anti-inflammatory tolerance effects; in general, by competitive blocking of epitopes on autoantigens, IgG4 can prevent the binding of other antibody classes and neutralize their effector mechanisms (64). In the case of MuSK-MG, the pathogenicity of MuSK-IgG4-Abs relies on their ability to block the MuSK-LRP4 interaction and prevent MuSK activation, which disturbs the balance between MuSK-induced clustering and ACh-induced dispersal of AChR clusters (66, 67).

3.1 MuSK antibodies are monovalent and undergo Fab-arm exchange

MuSK-IgG4-Abs are, unlike IgG1-3 antibodies, functionally monovalent. Due to variations in the amino acid sequence (Ser228), IgG4 are characterized by a greater stereometric flexibility in the hinge region, which causes an instability of the interchain disulfide bridges, and the alternative formation of more stable intrachain bridges under reducing conditions (68–70). As a result, IgG4-Abs dissociate into two half-molecules, each consisting of a heavy and a light chain, which then recombine randomly into chimeric, bi-specific antibodies (64, 68, 71). This mechanism is further facilitated by another single amino acid change in the CH3 region (Arg409) that reduces the non-covalent interaction between both heavy chains. Taken together, these amino acid changes allow for ~99% of MuSK-IgG4-Abs to undergo Fab-arm exchange (71, 72), which are therefore monovalent for their antigen.

3.2 MuSK-IgG4 antibody inhibition of LRP4-MuSK signaling and complex interactions with both pre- and post-synaptic proteins

The reactivity of MuSK-Abs can be directed against several extracellular MuSK domains (64, 73), with epitope spreading reported in 19% of MuSK-MG patients (73). Disease severity, however, is determined by reactivity of the autoantibodies against the MuSK-Ig-like domain 1 (MuSK-Ig1) (66, 67, 73, 74), which is required for MuSK to bind LRP4 (38). MuSK-IgG4-Abs block the interaction between MuSK and LRP4 and prevent MuSK phosphorylation and activation (Figure 1). As a result, MuSK becomes unable to respond to agrin-LRP4 stimulation, and the agrin-LRP4-MuSK-Dok7 cascade does not form, resulting in reduced AChR clustering at the NMJ (66, 67). The disrupted LRP4-MuSK interaction was also shown to correlate with a lack of the compensatory presynaptic increase of released acetylcholine vesicles (i.e., quantal content, QC) in *in vivo* models of MuSK-MG, which, by contrast, is commonly observed in AChR-MG disease models and in patient muscles (75–77). A potential explanation is based on the function of LRP4 as a retrograde signal molecule (78, 79). LRP4-MuSK is suggested to increase the QC, which would balance the LRP4-MuSK induced AChR clustering by increasing AChR-dispersal. MuSK-Abs could then disrupt this mechanism and prevent the compensatory increase of the quantal content, although this has not been shown directly so far (76, 77, 80).

The loss of acetylcholinesterase (AChE) has also been proposed as a pathomechanism in MuSK-MG. MuSK interacts with collagen Q (ColQ), which is arranged as trimers to form a triple helix in the synaptic cleft, where each ColQ molecule binds and anchors a tetramer of AChE to the basal lamina (81, 82). The MuSK-Ig1 and -Fz domains have been determined as the binding sites for ColQ (74) with MuSK-Abs shown to block these interaction sites and inhibit the binding of MuSK to ColQ (83). This could lead to the loss of AChE from the extracellular matrix of the NMJ and increase the availability of acetylcholine in the synaptic cleft, which, as a negative regulator of AChR clustering, could facilitate dispersal of the receptors. This was hypothesized to be responsible for the hypersensitivity of many MuSK-MG patients towards AChE inhibitors, although there was no correlation between drug hypersensitivity and autoantibodies against specific epitopes in a longitudinal epitope mapping study in MuSK-MG (73).

3.3 MuSK-IgG1-3 antibodies could contribute to the pathogenic process in some MuSK-MG patients

MuSK-IgG1-3 can be detected at low concentrations in most MuSK-MG patients and have been proposed to contribute to the pathogenic process in MuSK-MG (67, 84). They were shown to inhibit agrin-induced AChR clustering in C2C12 cells, surprisingly without affecting LRP4-MuSK interaction. Both IgG1-3 and IgG4 prevented AChR cluster formation in C2C12 cells or in C2C12 cells overexpressing Dok7, which induces dimerization and activation of

MuSK and thereby facilitates AChR clustering independent of the agrin-LRP4 pathway (67). In a more recent study, MuSK IgG1-3 induced a similar phosphorylation time course for MuSK, Dok7 and the AChR β-subunit as agrin but still prevented the formation of AChR clusters induced by agrin in C2C12 cells (84). Further evidence for the potential pathogenic role of MuSK IgG1-3 antibodies derives from experiments utilizing monoclonal antibodies isolated from MuSK-MG patient B cell clones (85–87). Here, even in the absence of agrin, divalent antibodies recognized the MuSK-Ig-like domain 2 and enhanced MuSK phosphorylation but inhibited AChR clustering, irrespective of the subclass backbone (86). In another study, divalent MuSK-Abs were found to target the MuSK Ig-like 1 domain and again resulted in increased MuSK phosphorylation. However, instead of inhibiting the clustering of AChRs, these antibodies induced low levels of AChR clustering, which was explained by the ability of divalent antibodies to crosslink MuSK molecules (85). These findings could indicate different downstream MuSK phosphorylation and AChR clustering pathway aspects depending on the IgG subclass or on the different MuSK epitopes targeted by the monoclonal or polyclonal (patient serum) antibodies. This hypothesis is supported by experiments analyzing MuSK mutations identified in patients with congenital myasthenic syndromes. Here, a mutation in the MuSK Fz-like domain ablated MuSK phosphorylation and AChR clustering, whereas another mutation in the kinase domain of *MUSK* was found to increase MuSK phosphorylation while impairing AChR clustering (88). Therefore, the modification of different MuSK domains or epitopes by mutations or antibodies could differentially affect the conformation and activation of MuSK and, thereby, also the downstream signaling pathway required for AChR clustering (87, 89, 90).

The activation of complement could represent another mechanism underlying MuSK IgG1-3 pathogenicity, with evidence deriving from *in vivo* disease models. MuSK-immunized mice show elevated levels of complement-fixing anti-MuSK IgG isotypes in sera and NMJs, and complement deposition can be observed widely in muscle samples (91, 92). Moreover, MuSK-immunized IgG1 knock-out mice (where mouse IgG1 is the murine analogue of human IgG4 with poor complement activation potency) and WT both developed comparable disease severities, serum MuSK-Ab levels, and NMJ immunoglobulin and complement deposition ratios (91). However, *in vivo* experiments performed by another group revealed the induction of experimental autoimmune MG in mice by the passive transfer of MuSK-IgG4 but not MuSK-IgG1-3 together with complement (93), although the MuSK-specific IgG1-3 antibodies could not be detected in the mouse sera. It was therefore suggested that this model was not representative of the situation in MuSK-MG patients, in which MuSK-IgG1-3 antibodies are commonly detectable (67, 93).

4 Epidemiology of MuSK-MG

MuSK-Abs can be detected in up to 70% of generalized MG patients without AChR-Abs (6, 10), and LRP4-Abs can be found in 7-33% of double-seronegative patients without AChR- and MuSK-Abs (94). Double positive patients with MuSK- and LRP4-Abs were found more frequently than double positive patients with AChR-

and LRP4-Abs (14.9% versus 7.5%) (94). The overall female-to-male ratio of MuSK-MG ranges between 3:1 and 9:1 (10, 12, 13), with higher rates of male MG patients in late-onset disease (9, 95). In contrast to AChR-MG, a bimodal pattern of incidence is not common in MuSK-MG (10), with only one study from China reporting a bimodal distribution of disease onset (9). Prevalence ratios vary significantly across countries (Table 1), with differences between ethnic groups. In a Norwegian and Dutch study, MuSK-MG was more frequent in Asian immigrants than in patients of European descent (8% vs. 0-4%) (96). In an US-American study, 50% of patients with MuSK-MG were African-American, as compared to only 10% within the AChR-MG cohort (97). Interestingly, different rates of MuSK-MG were reported within individual countries. In a Chinese study, only 4% of AChR-Ab negative patients in the HuBei province had MuSK-Abs (98), while another study reported a rate of 26.7% among AChR-Ab negative patients in Northeast China (99). However, these differences are likely due to the use of different assays in the two studies, with cell-based assays resulting in a lower proportion of MuSK-Ab positives compared to enzyme-linked immunosorbent assays. A particularly high prevalence (43.0 per 1,000,000 population) of MuSK-MG was reported in Sardinia (100). The same study reported a much higher overall incidence and prevalence of MG in Sardinia compared to other regions/countries (96, 100–104). Notably, Sardinia is burdened by particularly high frequencies of other autoimmune disorders, such as multiple sclerosis (MS) and systemic lupus erythematosus (SLE) (105, 106). The *TNFSF13B* variant, resulting in an overexpression of the B cell activating factor (BAFF), was found to be associated with MS and SLE in a genome-wide association study in Sardinia (107) and can help to explain the higher frequencies of myasthenia gravis. Differences in the frequencies of specific HLA alleles across populations could also explain the geographical variations. A meta-analysis published in 2018 concluded that DQ5-DR14 and DQ5-DR16 haplotypes are associated with an increased risk of developing MuSK-MG, whereas DQ3-DR4 and DQ3-DR11 were found to be protective (108).

TABLE 1 Prevalence of MuSK-MG *.

Region	Publication year	Prevalence (per million)
China, HuBei Province (98)	2007	0.02**
Netherlands (101)	2007	1.9
Greece (102)	2009	2.9
Italy, Trento (173)	2011	1.9
Northern Europe (Norway & Netherlands) (96)	2016	3.6
Northern Portugal (103)	2016	5.8
Latvia (174)	2017	8.9
Italy, Sardinia (100)	2024	43.0

* Only epidemiological studies with available prevalence estimations or with figures that permitted prevalence calculations were included.
** Another study from Northeast China reported higher rates of MuSK-MG (99); this study, however, did not permit prevalence calculations and was thus not included in the table.

5 MuSK-MG patients present with distinct clinical features

MuSK-MG patients are characterized by specific clinical symptoms that differ from those of MG patients with AChR-Abs. An acute or subacute onset of extraocular or bulbar muscle weakness is common, with bulbar muscles often remaining severely affected throughout the disease (9–13, 95). In contrast to AChR-MG, extraocular muscle paresis is mostly conjugated in MuSK-MG, manifesting as symmetrical upward or horizontal gaze limitation and, in some cases, progressing to complete ophthalmoplegia (109). In patients with a purely ocular onset, symptoms usually generalize within 1-3 months (109). Neck muscles are also often affected resulting in head drop (11, 13), whereas neck flexors can only be mildly involved (8, 110). Limb muscle affection is less severe and inconsistent (10, 12), and fluctuations of myasthenic symptoms may be less evident than in AChR-Ab positive MG patients (8). Myasthenic crises, by contrast, are frequent and often occur early in the disease course (9, 12, 13). Compared to LRP4-MG, MuSK-MG has been associated with a more severe clinical phenotype at disease onset, with MuSK/LRP4-Abs double positive patients being more severely affected than single positive MuSK-MG patients (94). Muscle atrophy and fatty replacement have been reported to occur frequently in chronic MuSK-MG patients, often affecting facial and bulbar muscles (16). Subclinical changes may even be detected at early disease stages by magnetic resonance imaging (111).

6 Diagnostic considerations in MuSK-MG

Diagnosis of MuSK-MG is based on the clinical presentation together with electrophysiological studies and the detection of MuSK-IgG-Abs. Repetitive nerve stimulation (RNS) and single-fiber jitter recording are helpful in supporting the diagnosis of MG but must be applied in affected muscles in order to prevent false-negative results in MuSK-Ab positive MG, with the sensitivity of RNS reported to increase up to 70-80% when tested in facial muscles and ranging around 20% in (unaffected) limb muscles (97, 112–114). Edrophonium/neostigmine testing is also commonly performed in MuSK-MG patients to enhance neuromuscular transmission by increasing the synaptic concentration of acetylcholine, and was shown to be positive in up to 60-70% of tested MuSK-MG patients (10, 12), which is generally lower than in AChR-Ab positive MG patients (115). However, edrophonium/neostigmine testing should be performed with caution as nicotinic side effects are common (23, 116).

Three different assays are available for the detection of MuSK-IgG-Abs, which represents the gold standard for confirmation of MuSK-MG diagnosis in suspected cases. The radioimmunoprecipitation assay (RIPA) utilizes immunoprecipitation of the extracellular domain of ^{125}I-radiolabelled MuSK upon incubation with patients' sera (117). This method is characterized by a specificity of almost 100% (118) and allows the quantification of autoantibody titers by counting

the ^{125}I precipitation at different serum concentrations. However, the strict regulations of radiation disposal limit its application. As a non-radioactive method, enzyme-linked immunosorbent assays (ELISA) are commercially available, and their application do not require specific licenses for diagnostic laboratories. MuSK-ELISA plates are coated with the extracellular domain of MuSK and allow quantification of MuSK-IgG titers, but the sensitivity and specificity of the assay were shown to be suboptimal compared to RIPA and cell-based assays (CBAs) (71, 119), indicating potential limitations in their diagnostic accuracy for MuSK-MG. Cell-based assays, by contrast, are characterized by higher diagnostic accuracy compared to RIPA and ELISA (120). Here, HEK293 cells are commonly used to express full-length MuSK with appropriate posttranslational modifications and conformation in the more physiological environment on the cell surface. In contrast to fixed cells, the application of live cells in CBAs was shown to be associated with higher sensitivity (121), and the use of IgG-specific secondary antibodies is recommended to avoid detection of non-specific binding of IgM to MuSK, which can be a problem with anti-human IgG antibodies that include anti-IgM activity (122). Ideally, CBAs should be used as a first-line assay in the diagnosis of MuSK-MG, as well as in the evaluation of patients tested negative by RIPA or ELISA (120).

7 Current therapeutic options in MuSK-MG

Specific considerations have to be taken into account for the successful treatment of MuSK-MG, owing to its distinct pathophysiological mechanisms that differ from those of MG with AChR-Abs (Figure 2). Gaining insight into these pathomechanisms has helped to improve the clinical management of MuSK-MG, which has evolved from an AChR-antibody negative, severe, and difficult-to-treat disease to a MuSK-antibody positive condition with a beneficial outcome in most cases. The excellent treatment response of MuSK-MG to rituximab is one major factor in this development (26).

7.1 Acetylcholinesterase inhibitors

Acetylcholinesterase inhibitors (AChEI) are often ineffective and associated with an increased risk of adverse events in MuSK-MG (10, 12, 23). In a retrospective cohort analysis of 165 MuSK-MG patients receiving AChEI, only 4% reported an initial clinical improvement, whereas 34% reported an impairment of muscle weakness. Side effects were experienced by 77% of patients, with cholinergic crises occurring in 7% of the cohort (23). Impaired tolerance against AChEI could be explained by an increased sensitivity to cholinergic agents or an inherent cholinergic hyperactivity in MuSK-MG patients. Electrophysiological studies revealed a higher frequency of repetitive compound action potentials in MuSK-MG patients using AChEI (116, 123), which correlated with poor tolerance to pyridostigmine (123). These clinical data are in line with data from MuSK-immunized mice

that display neuromuscular hyperactivity upon administration of AChEI (124). In another study, pyridostigmine treatment potentiated the MuSK-IgG-induced decline in AChR density and endplate potential (EPP) amplitude, whereas treatment with 3,4-diaminopyridine elevated EPP amplitude without exacerbating the MuSK-IgG-induced loss of AChRs (125). Some patients have also been reported to carry a polymorphism in the promoter region of the *ACHE* gene that encodes the catalytic subunit of AChE associated with increased susceptibility to adverse events upon (even low dose) AChEI treatment (126).

7.2 3,4-diaminopyridine

3,4-diaminopyrdine improves neuromuscular transmission by increasing the presynaptic release of acetylcholine. Evidence for effectiveness in MuSK-MG derives from animal models (125), case reports (127, 128), and a randomized-controlled study on 7 patients (129). Here, treatment with 3,4-diaminopyrdine in a daily dose of 30-60mg was associated with improvement of disease severity according to different clinical scores including the Quantitative MG score and the MG-specific Activities of Daily Living score. However, as the number of patients was very low in this study, there is a need for confirmation of the results in a large multi-center trial.

7.3 β-adrenergic agonists

Sympathomimetics have been shown to maintain NMJ structure by increasing the shape and size of the postsynaptic folds (130, 131), and to improve NMJ function (131). The β-adrenergic agonists salbutamol and ephedrine have been well established for the therapy of congenital myasthenic syndromes with *AGRN*, *COLQ*, *DOK7*, *LRP4* and *MUSK* mutations (132, 133), but with only anecdotal clinical experience of salbutamol in MuSK-MG (134, 135). When tested in 5 patients who had not tolerated pyridostigmine, there was a notable clinical response in 3 patients, whereas treatment had to be discontinued in 2 patients due to side effects or lack of efficacy after six months (134).

7.4 Corticosteroids and non-steroidal immunosuppressants

Immunosuppression is the mainstay treatment in both AChR-MG and MuSK-MG. Despite the lack of randomized-controlled trials, there is general consensus that MuSK-MG patients respond to corticosteroids and/or non-steroidal immunosuppressants (13, 136, 137), which are recommended and often used as the first-line therapy in MuSK-MG (24, 25). However, the frequency of exacerbations during corticosteroid tapering is notably higher in patients with MuSK-MG compared to those with AChR-MG, often requiring higher dosages and prolonged treatment durations (136, 138, 139). Non-steroidal immunosuppressants such as azathioprine, mycophenolate mofetil, cyclosporine, methotrexate and tacrolimus
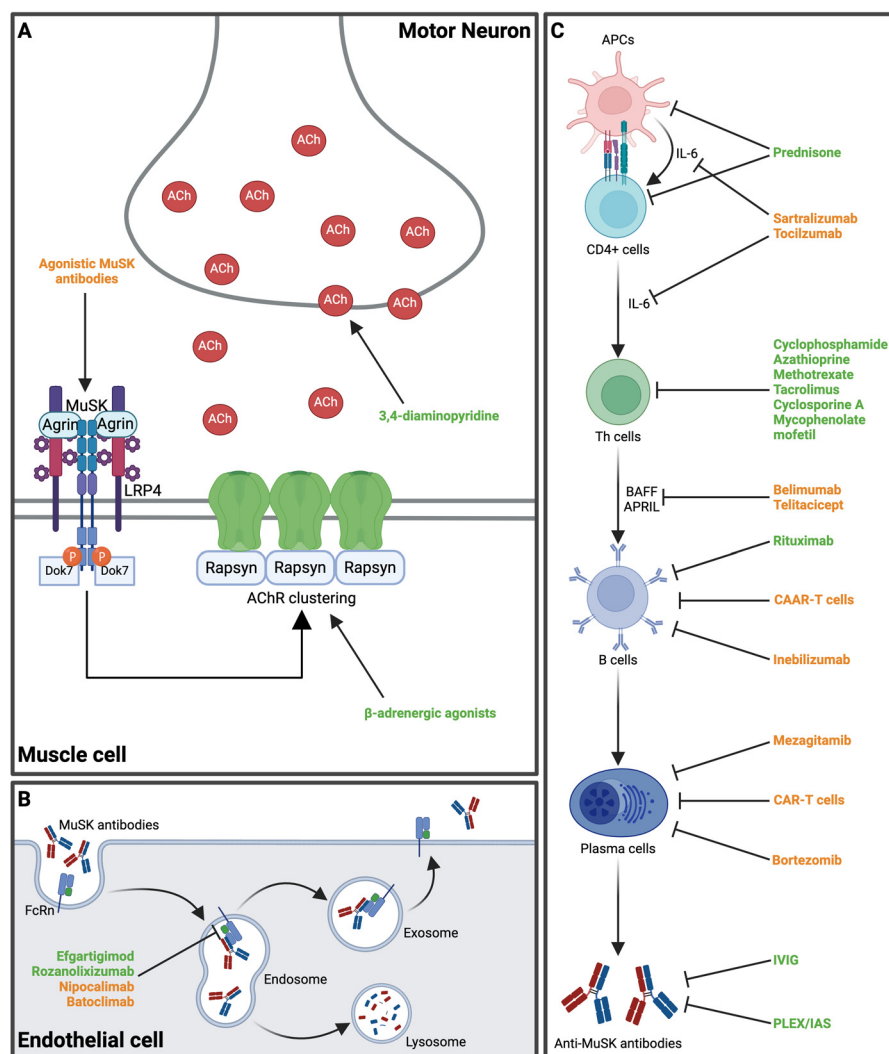
FIGURE 2

Main mechanisms of action of several established and novel therapeutic approaches in MuSK-MG. **(A)** At the neuromuscular endplate, 3,4-diaminopyridine increases pre-synaptic acetylcholine release. β-adrenergic agonists (e.g., salbutamol) stabilize AChR-clustering and increase shape and size of post-synaptic folds. Agonistic MuSK-antibodies may induce MuSK-dimerization and have been shown to reduce disease activity in a preclinical mouse model. **(B)** The FcRn are expressed on endothelial cells and mediate recycling of MuSK-Abs, thereby extending their half-life. FcRn inhibitors block the IgG-FcRn interaction and expedite the clearance of MuSK-Abs. **(C)** The pathway from antigen presentation to naïve T cells and subsequent antibody production presents different target points for therapeutic interventions. Various novel therapeutics target B cell activation and B cell lineages (see also Table 2). Available treatment options are highlighted in green, while novel therapeutic agents proposed for MuSK-MG are highlighted in orange. ACh, acetylcholine; AChR, acetylcholine receptor; APCs, antigen-presenting cells; APRIL, A proliferation-inducing ligand; BAFF, B cell activating factor; CAAR, chimeric autoantibody receptor; CAR, chimeric antigen receptor; CD, cluster of differentiation; Dok7, Downstream of tyrosine kinase 7; FcRn, neonatal fragment crystallizable receptor; IAS, immunoadsorption; IL-6, interleukin-6; IVIG, intravenous immunoglobulin; LRP4, low-density lipoprotein receptor-related protein 4; MuSK, muscle specific kinase; PLEX, plasma exchange; Th cells, T helper cells. Created in BioRender.com.

are therefore often introduced early in the disease course as steroid-sparing agents to avoid side effects associated with long-term administration of high-dose corticosteroids (13, 136, 139). However, a significant proportion of MuSK-MG patients on high-dose immunosuppression with corticosteroids alone or in combination with azathioprine have a refractory disease (136, 139). Periodic relapses and myasthenic crises can be effectively treated with acute plasma exchange or the administration of intravenous immunoglobulins (IVIG) (25), with evidence for higher rates of clinical improvement associated with plasma exchange as compared to IVIG treatment (10, 13, 140).

7.5 Anti-CD20 monoclonal antibodies

Rituximab is a mouse/human monoclonal chimeric IgG1 targeting CD20, a transmembrane phosphoprotein expressed on the surface of developing B lymphocytes but not on progenitor cells and mature plasma cells (141). Upon binding to CD20, immune effector cells are recruited and cause B cell lysis and depletion. Rituximab has been shown to be particularly effective in IgG4 related diseases (142–144). In MuSK-MG, there is currently class IV evidence for a beneficial effect upon treatment with rituximab deriving from a prospective blinded multicenter study (145).

TABLE 2   Clinical studies on novel therapeutic agents for the treatment of MuSK-MG.

Target	Name	Study identifier and phase
CD19 (B cells)	Inebilizumab (MEDI-551)	NCT04524273, phase III
CD38 (Plasma cells, B & T cells)	Mezagitamib (TAK-079)	NCT04159805, phase II
IL-6	Sartralizumab Tocilizumab	NCT04963270, phase III NCT05067348, phase II
BAFF	Belimumab Telitacicept	NCT01480596, phase II NCT04302103, phase II
B cell maturation antigen (BCMA)	CAR-T cells	NCT05451212, phase IIb
B cells expressing MuSK	MuSK-CAAR-T	NCT05451212, phase I
FcRn	Batoclimab Nipocalimab	NCT05403541, phase III NCT04951622, phase III

Especially in patients with high disease activity or refractory disease, rituximab treatment should therefore be considered early in the disease course (24, 25), which together with corticosteroids and immunosuppression results in an overall favorable outcome in most MuSK-MG patients (26). The intravenous application of rituximab is generally well tolerated and safe (146), with a rapid and sustained effect (147, 148) that correlates with a reduction of MuSK-IgG4 titers, but not with total IgG4 titers (148, 149). This may be related to MuSK-Ab production by short-lived Ab-secreting plasmablasts derived from CD20^{+} memory B cells (86, 150), in contrast to AChR-MG, where autoantibody production is maintained by long-lived plasma cells (151, 152). Relapse after treatment with rituximab was shown to correlate with the occurrence of antibody-secreting CD27^{+} CD38^{+} plasmablasts (150), and CD27^{+} memory B cell and MuSK-Ab titer monitoring were proposed as a sensitive biomarker for prediction of clinical relapses and for adjustment of rituximab infusion frequencies (150, 153). Moreover, MuSK-Ab secreting B cell clones were shown to survive B cell depletion therapy and recirculate up to several months prior to clinical relapse (154). These clones could differentiate from CD20^{low} plasmablasts (154), and their survival might be explained by the ineffectiveness of rituximab to reach tissue-based B cells (155–157).

7.6 FcRn blockade

The neonatal Fc receptor (FcRn) binds to the Fc region in a pH-dependent manner and protects IgG from lysosomal degradation, allowing it to return to the circulation. FcRn inhibitors such as efgartigimod and rozanolixizumab prevent FcRn from interacting with IgG, thereby disrupting the recycling process and leading to increased degradation and clearance of IgG from the circulation (158). In MuSK-MG, there is both preclinical and clinical evidence for the therapeutic potential of FcRn inhibition. Efgartigimod-treated MuSK-myasthenic mice functionally outperformed control mice, and electromyography demonstrated improved decrement

and compound muscle action potentials upon treatment, which correlated with reduced MuSK-IgG4 levels (159). Efgartigimod has been tested in the phase III ADAPT trial including 129 AChR-MG and 38 AChR-Ab negative MG patients, among which 6 patients were MuSK-Ab positive and 3 patients received efgartigimod (160). However, all MuSK-MG patients responded to the therapy as defined by a ≥2 point improvement in the MG Activities of Daily Living (MG-ADL) score, including those in the placebo-group. Rozanolixizumab has also been tested in a phase III trial (MycarinG) including 21 MuSK-MG patients, with 13 patients receiving rozanolixizumab and 8 patients in the placebo group (161). All 12 MuSK-MG patients in the treatment group with available data were MG-ADL responders, in contrast to only one of seven responding patients in the placebo group. Despite the low number of MuSK-Ab positive patients included in these clinical trials, the available data suggest that FcRn inhibitors pose a promising therapeutic option for MuSK-MG and can be considered in refractory patients with unsatisfactory response to rituximab (25).

8 Future therapies

Despite the availability of various treatment options, a significant proportion of MuSK-MG patients remain symptomatic or experience periodic clinical exacerbations. Therefore, there is an urgent need for novel therapeutic strategies targeting the underlying immune machinery that produces the autoantibodies (162).

Given the pivotal role of B cell pathophysiology in MuSK-MG, as highlighted by the efficacy of rituximab, expanding therapeutic options to include agents that directly or indirectly target B cell lineages may provide valuable treatment alternatives. Inebilizumab (MEDI-551), an anti-CD19 monoclonal antibody, is currently under investigation in a phase III trial (MINT trial, NCT04524273). Mezagitamib (TAK-079), targeting CD38 expressed on plasma cells, completed a phase II trial in 2023 (results pending, NCT04159805). Targeting IL-6 to inhibit B cell proliferation and activation, the monoclonal antibody satralizumab (phase III LUMINESCE trial completed in 2024, preliminary results published (163), NCT04963270) and tocilizumab (ongoing phase II trial, NCT05067348) may also have therapeutic potential in MuSK-MG. Preliminary results from the LUMINESCE trial indicate significant improvement in clinical scores in generalized MG (163). However, only 9 MuSK-MG patients were included, and they were not considered in the analyses. Telitacicept (RC18), an inhibitor of the B cell activation factor (BAFF), demonstrated efficacy in reducing clinical severity in generalized MG in a phase II study (164), but this study was not able to recruit MuSK-MG patients. Similarly, in a phase II trial on the BAFF inhibitor belimumab, the primary endpoint was unmet, and MuSK-MG patients were limited to the placebo arm (165). Bortezomib, a proteasome complex inhibitor, was successfully used in a single case with MuSK-MG (166).

A novel approach utilizes chimeric antigen receptor (CAR) T cells that were genetically engineered to target the B cell maturation antigen and cause mature plasma cells to undergo apoptosis. In a

prospective, open-label phase 1b/2a proof-of-concept study (MG-001), the safety and efficacy of this approach was investigated in 14 patients with generalized MG, of whom 2 patients were MuSK-Ab positive (167). Both patients improved in all clinical scales, without any dose-limiting toxicity, neurotoxicity, or cytokine release syndrome across the whole cohort. A similar approach was tested in an experimental autoimmune MuSK-MG mouse model. T cells were engineered to express the MuSK chimeric autoantibody receptor (CAAR) with CD137-CD3ζ signaling domains for precision targeting of B cells expressing anti-MuSK-Abs (168). The results were promising and indicated MuSK-specific B cell depletion without decreasing total B cell numbers or total IgG levels. In another recently published preclinical study, agonistic MuSK antibodies were successfully utilized to treat mice with severe disease activity after passive transfer of pathogenic MuSK-Abs derived from patients with MuSK-MG (169). Overall, these innovative approaches have the potential to specifically target the underlying immune mechanisms responsible for autoantibody production or directly target the disease mechanism.

C5 complement inhibitors have demonstrated efficacy in treating generalized AChR-Ab positive MG (161, 170–172). MuSK-Abs, by contrast, are predominantly of the IgG4 subclass and do not activate complement (64); therefore, the use of complement inhibitors does not appear to be indicated in MuSK-MG. However, since MuSK-IgG1-3 can also be detected at low concentrations in most MuSK-MG patients (67, 84), and given the evidence for complement activation in animal models (91, 92), complement inhibition may also represent a potential therapeutic approach for MuSK-MG with IgG1-3, which has not yet been validated in clinical trials.

9 Conclusion

Due to a better understanding of the underlying pathophysiological mechanisms and due to advances in the clinical management of the disease, MuSK-MG has become a condition with a favorable outcome in most cases. However, MuSK-MG patients are underrepresented in clinical trials and comparative investigations between existing drugs are yet to be performed, rendering attempts to develop treatment algorithms challenging. The implementation of consensus guidelines for clinical trials on MuSK-MG would help to standardize research

efforts, ensuring more robust and comparable data across studies. Biomarker research on refractory disease may further bolster decision making processes.

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