Complement inhibition in Myasthenia – from basics to RCT data

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ABSTRACT

Myasthenia gravis (MG) is the prototypic autoimmune neurological disorder causing fatiguable muscle weakness either limited to the ocular muscles or becoming generalised involving the limb and bulbar muscles. Nine out of ten generalised MG patients have IgG1 or IgG3 antibodies against the acetylcholine receptor (AChR). AChR antibodies cause neuromuscular weakness by internalisation of AChR, receptor blockade and activation of the complement pathway. Complement activation causes formation of the membrane attack complex (MAC), leading to degradation of the neuromuscular junction (NMJ). Several animal models have confirmed the role of complement in the pathogenesis of MG, with the experimental autoimmune MG models (EAMG) often needing complement inhibitory therapies to prevent or reverse the disease. Various molecules that target the complement system have now been developed to treat myasthenia gravis. The vast majority of the currently studied molecules target the C5 protein, thereby preventing the formation of MAC and subsequent NMJ destruction. The currently studied anti-complement therapies for MG include Eculizumab, Zilucoplan, Ravulizumab, Pozelimab, Cemdisiran, Gefurilimab, Danicopan and a few others in the pipeline. Eculizumab has been shown in clinical trials to be effective in the treatment of refractory MG, but further sub-group analysis and real-life experience have shown that this drug can be beneficial in various patients including those receiving regular intravenous immunoglobulin (IVIG), plasma exchange or Rituximab. It was approved for use by the FDA in October of 2017. Ravulizumab is a long-acting monoclonal antibody which has similar mechanism of action to Eculizumab and was approved for use in MG by the FDA in April 2022. Zilucoplan is a macrocyclic peptide which can be given subcutaneously and binds to C5 and C5b, thus preventing terminal complement activation (FDA new drug application accepted in Nov 2022). Many of these have also been shown to have long-term benefit in different sub-groups of patients with MG. Patients would need to be vaccinated against Neisseria meningitidis because of the risk of Gram-negative septicaemia, although no major safety signatures have been noted in the studies so far. Future studies may be able to identify specific biomarkers which might aid in selecting the most appropriate patients who might respond to these therapies.

Keywords: Myasthenia Gravis, Complement, Eculizumab, Ravulizumab, Zilucoplan

Introduction

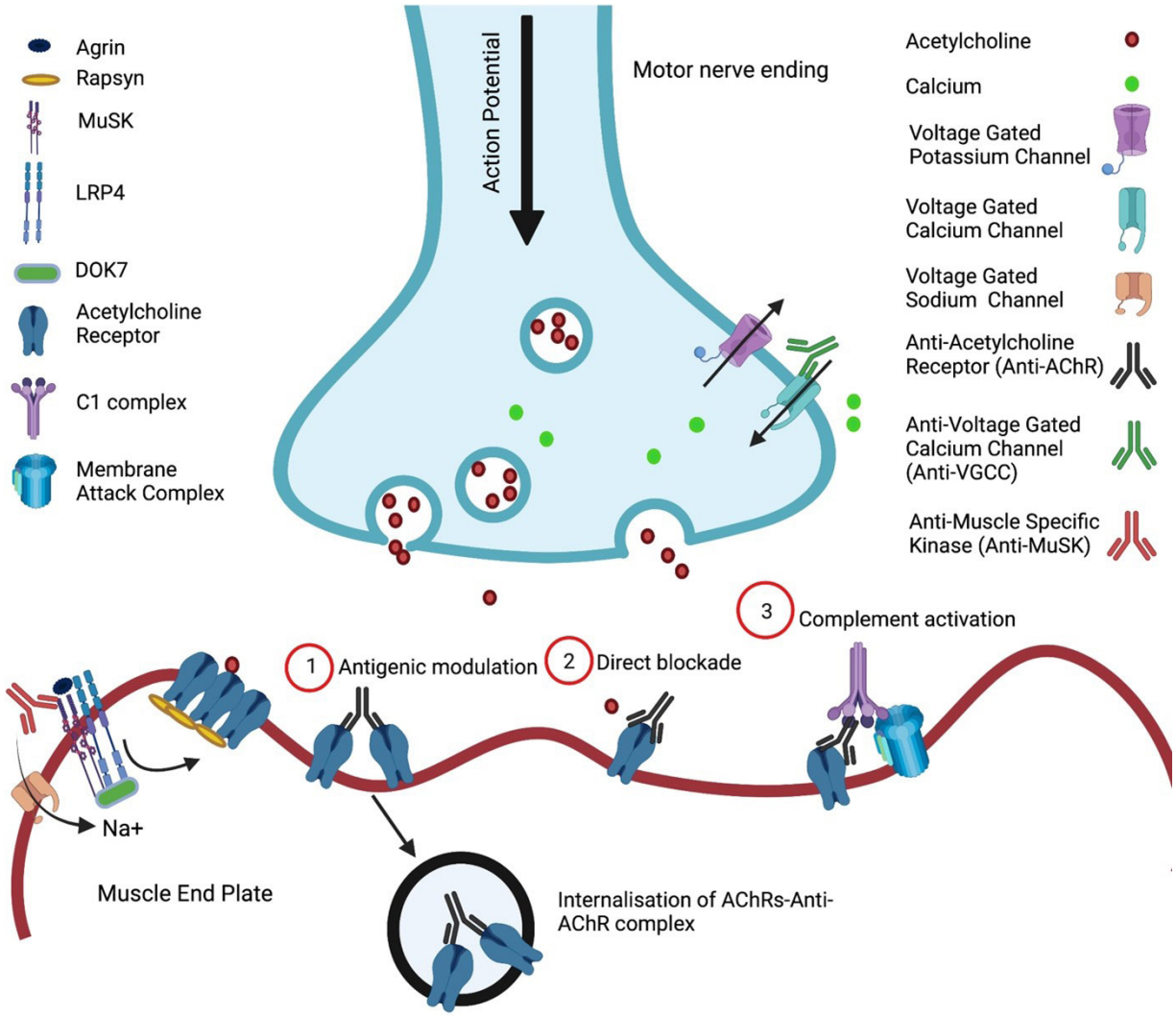
Myasthenia gravis (MG) is the most well recognised autoimmune nervous system disease characterised by fatiguable muscle weakness.[1] Patients can have symptoms localised to the eye muscles causing ptosis and double vision (ocular MG) or progress to develop weakness in the limbs or bulbar muscles causing dysphagia, dysarthria and breathing difficulties (generalised MG, gMG). Generalised MG is caused by antibodies against the nicotinic acetylcholine receptor (AChR) in over 85% of patients.[2] Other main antibodies involved in myasthenic syndromes include those against the muscle-specific tyrosine kinase (MuSK), which is seen in 5-8% of generalised MG, and the pre-synaptic voltage gated calcium channels (VGCC), causing the related Lambert Eaton Myasthenic syndrome (LEMS). Low density lipoprotein receptor related protein 4 (LRP4) antibodies are seen in up to 2% of generalised MG patients. [1] Antibodies against several other molecules have been described including acetylcholinesterase, agrin, ColQ, titin, ryanodine, Kv1.4 and cortactin, but their exact pathophysiological role is unknown. [3, 4] The main molecules involved in neuromuscular transmission and the pathogenetic mechanisms in MG are schematically represented in **Figure 1.**

The action potential arriving at the pre-synaptic terminal opens voltage gated calcium channels (VGCC) triggering release of Agrin and Acetylcholine receptor (AChR) to the synaptic cleft. The binding of ACh to its receptor (AChR) opens voltage gated sodium channels leading to muscle contraction. The clustering of AChRs at the neuromuscular junction (NMJ) is promoted by Agrin binding to the MuSK-LRP4 complex. There are three main mechanisms by which AChR antibody causes neuromuscular damage: antigenic modulation where Anti-AChR cross links AChRs, increasing the internalisation of AChRs (1), direct blockade when Anti-AChR blocks the ligand binding site of Acetylcholine to AChR (2) and complement activation (3). Anti-AChR-AChR complex activates the complement system, leading to the destruction of muscle end plate by Membrane Attack Complex.

Other NMJ syndromes include Anti-MuSK MG (Anti-MuSK binds MuSK-Lipoprotein Receptor Related Protein 4 (MuSK-LRP4), interferes with interaction of MuSK with other NMJ molecules and reduces AChR clustering) and LEMS (anti-VGCC binds VGCC at motor nerve terminal, blocking the calcium influx and calcium driven AChR vesicle release into NMJ).

There are three possible ways by which the AChR antibodies are likely to impair neuromuscular transmission.[5] These include:

Figure 1
Neuromuscular transmission and immunopathogenesis of neuromuscular junction disorders



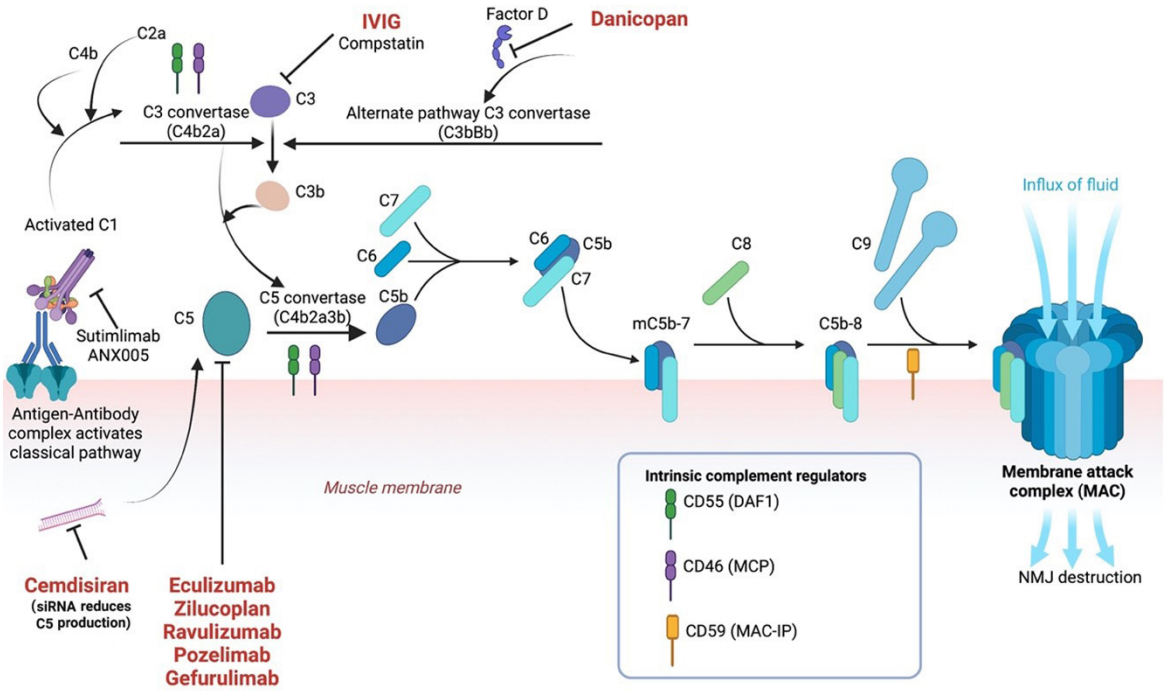
1. Antigenic modulation –antibodies cross-link the receptors, accelerating internalisation and degradation of AChR
2. Direct blockade – antibodies prevent the acetylcholine from binding to the AChR
3. Reduction of AChR density –activation of the complement cascade causes lysis of the post-synaptic membrane and simplification of the neuromuscular junctional folds

It is currently not easy to demonstrate in vivo which of the three mechanisms might be the predominant component in an individual MG patient, although complement activation is thought to play the major role in the pathogenesis, at least in AChR-MG patients.[5-7] Antibodies against AChR, LRP4 and VGCC are predominantly of the IgG1 sub-class and are more likely to fix complement as opposed to MuSK antibodies, which are usually IgG4. The vast majority of complement inhibition studies in MG have been done on AChR antibody positive patients and hence this review primarily focuses on this sub-group of MG.

Complement pathway

The complement system is an integral part of innate immunity and is composed of over fifty proteins primarily responsible for defending the host from infections by eliminating pathogenic organisms. It also serves as a link between innate and adaptive immunity by interacting with the T and B-cell receptors or by dendritic cell modulation.[8] The complement system is also involved in the clearance of immune complexes and dead cells.[9] This delicate balance can occasionally be disrupted, leading to autoimmune neurological disorders and may contribute to some neurodegenerative conditions (e.g.: Alzheimer's disease, amyotrophic lateral sclerosis and Huntington's disease).

Figure 2
The classical complement pathway and molecules used to inhibit this pathway in Myasthenia Gravis



This has now led to a growing interest in complement modulatory therapies in various neurological diseases, involving the peripheral (e.g.: Guillain-Barre syndrome, chronic demyelinating neuropathies, dermatomyositis) and central nervous system (e.g.: neuromyelitis optica, autoimmune encephalitis, multiple sclerosis). [6]

A detailed review of the complement pathway is beyond the scope of this article. The main aim of the complement pathway is the formation of the membrane attack complex (MAC), which leads to destruction of microorganisms or tissue damage when triggered by autoimmunity. The activation of the pathway involves three different initiation loops [6], although we will concentrate on the one responsible for myasthenia pathogenesis in more detail:

1. **Classical pathway** – C1 activation after binding of antigen-antibody complexes, which leads to a cascade of reactions explained below.
2. **Mannose Binding Lectin (MBL) pathway** – Lectin binds mannose or other carbohydrates (e.g.: ficolins or collectins) on the bacterial surface, activating the Mannan-binding lectin serine proteases (MASP1 and MASP2) and leading to the formation of C3 convertase, with subsequent steps common with the classical pathway.
3. **Alternate pathway** – Spontaneous activation of C3 leads to a low rate, "tick-over" pathway which is an integral part of innate immunity. Unlike the other two pathways, C1, C2 and C4 are not needed with Factor B and properdin Factor D helping to produce the alternate C3 convertase (C3bBb). This, when combined with high concentrations of C3b, leads to the production of alternate C5 convertase (C3bBbC3b).

Classical pathway and terminal complement complex

The activation of C1 complex by the multi-valent C1q binding to the Fc portion of the AChR-bound antibody (usually IgG1 or 3, less commonly IgG2), generates enzymatically active C1r and C1s. C1s cleaves C4 to C4a and the larger C4b, and the combination of C1r, C1s and C4b converts C2 to C2a and C2b. The C4b2b complex is called C3 convertase because it cleaves C3 to C3a and C3b, the latter combining with the C3 convertase to form C5 convertase. C5 convertase (C4b2a3b) initiates the terminal complement pathway by cleaving C5 into C5a and C5b. C5a is a chemoattractant protein and is involved in anaphylactic reactions along with the C3a released earlier. The C5b component sequentially accepts C6 and C7, and then translocates to the outer lipid bilayer of cell membrane, exposing its lipophilic structure due to the transmembrane location. C8 and several (up to 17) molecules of C9 are added, widening the pore size and subsequent formation of the osmolytic membrane attack complex (MAC, C5b-9)[6] **(Figure 2)**. MAC formation at the post-synaptic membrane leads to lysis and disruption of the NMJ folds.

Complement regulators

To avoid spontaneous activation of the complement pathway that leads to cell injury, there are several inhibitory molecules in the plasma (C4 binding protein and factor H) and cell surface (CD55 - decay-accelerating factor (DAF1), including CD46 – membrane co-factor protein (MCP) – and CD59 – membrane attack complex inhibitory protein (MAC-IP)). CD55 and CD46 are concentrated at the NMJ and inactivate C3 and C5 convertases, whereas CD59 inhibits C9 polymerisation and hence the formation of the MAC complex. From a clinical point of view, the complement regulators are expressed less abundantly at the extraocular muscle NMJs, possibly suggesting the predilection of these muscles in myasthenia.[10]

The binding of AChR to ACh activates C1 leading to the formation of C3 convertase (C1C4b2Ca) which cleaves C3 to form C3b. The C3b binds to the C4b2a complex forming the C5 convertase (C1C4bC2aC3b), which cleaves C5 to C5a and C5b. C5b initiates the lytic pathway leading to the formation of membrane attack complex (MAC). The intrinsic complement regulators which prevent spontaneous activation of the pathway and the targets of some of the main anti-complement therapies in MG are shown (IVIG – Intravenous immunoglobulin). Factor D is a serine protease which cleaves Factor B to Bb and helps in the formation of the alternate pathway C3 convertase, which is a potential signal amplification pathway of the complement pathway.

Evidence for the role of complement in Myasthenia

Experimental autoimmune myasthenia gravis (EAMG) models have been established in rodents to investigate the pathogenesis of MG. The animal models mirror human MG in that the rodents develop fatigable muscle weakness and show decremental response on repetitive nerve stimulation. EAMG models can be made either by immunising with purified AChR or its sub-unit (active) or by transferring antibodies from patients with MG (passive). In addition to a clinical response similar to human MG, the EAMG models show deposition of immunoglobulins and complement components (C3 and MAC) at the neuromuscular junction of affected animals, with destruction of the end-plate and also reduction in the miniature endplate potential (MEPP) amplitude.[11, 12]

EAMG induction can be inhibited either by depleting the complement by giving cobra venom factor or by knocking out complement components C3, C5 or C6.[13-16] Animals show serum AChR antibodies and also deposition of IgG but not the corresponding complement components at the end-plates. Similarly, animals lacking the complement regulators (e.g.: DAF1 and CD59) are known to be susceptible for EAMG, with severe end-plate damage, loss of AChR and significant complement deposition seen in the double knock-out models.[17-19]

The role of complement in human myasthenia has been established since the 1970s by the demonstration of C3 and MAC deposition at the NMJ, causing degenerated junctional folds.[11, 20, 21] Patients are shown to have depleted serum complement components and the neurophysiology often correlates with the serum complement-fixing capacity demonstrated in-vitro.[19, 22] More recently, by measuring the serum levels of complement proteins and regulators, it has been shown that the inflammatory pathogenesis in MG is associated with activation of the complement pathway, especially in AChR antibody positive MG patients [23, 24]. Techniques are being developed to identify complement activity in individual patients using modified cell lines [25] or CH50 hemolysis assays [26], so that appropriate patients can be selected for complement therapies.

Complement inhibition as therapy for MG

Experimental models

Initial experiments confirmed that the administration of anti-complement therapies reduces clinical weakness in EAMG models and minimises complement deposition at the neuromuscular endplates. This can be achieved either by using inhibitors of the classical pathway (anti-C1q) or the terminal lytic pathway (anti-C6, anti-C5) or by using siRNA which causes prolonged suppression of the liver C5 expression.[27-30]

Clinical trials

Eculizumab

The first anti-complement therapy studied in MG is an IgG2/4 monoclonal antibody directed against the C5 protein. Binding of Eculizumab to C5 prevents its breakdown to C5a and C5b, thereby reducing chemotaxis by inflammatory cells and formation of MAC, respectively. Eculizumab has already been in clinical use for other complement-mediated conditions like paroxysmal nocturnal haemoglobinuria (PNH) and atypical haemolytic uremic syndrome (aHUS). The initial phase II study in MG (NCT00727194) was done using 14 patients for 16 weeks followed by a cross-over, with significant improvement in the Quantitative Myasthenia Gravis (QMG) scores in the Eculizumab patients, which was rapid and clinically meaningful. [31]

The encouraging Phase II results led to the phase III study in a multi-centre, randomised double-blind, placebo-controlled fashion (REGAIN, Safety and Efficacy of Eculizumab in AChR positive Refractory Generalised Myasthenia Gravis; NCT01997229) followed by an open label extension (OLE).[32, 33] REGAIN enrolled 125 AChR antibody positive refractory MG patients to either Eculizumab or placebo for 26 weeks. The induction dose of Eculizumab was 900 mg on day 1, weeks 1, 2 and 3 and 1200 mg in week 4, and thereafter maintenance dose of 1200 mg every 2 weeks. The primary endpoint assigned was the change in Myasthenia Gravis Activity of Daily Living (MG-ADL) score from baseline to week 26 using worst-rank ANCOVA and the secondary endpoints assessed were the change from baseline in the total scores of QMG,

Myasthenia Gravis Composite (MGC) and Myasthenia Gravis Quality of Life 15 (MG-QOL-15), and the proportion of responders.[32]

REGAIN failed to attain significance for the primary endpoint (mean rank of 56.6 vs 68.3, p=0.0698). However, the intervention group showed significantly better secondary outcomes including changes in QMG (p=0.0129) and MGQoL-15 (p=0.0281) scores but without significant change in MGC. In the pre-specified sensitivity analysis, significant difference in all scores was noted between the two groups in favour of Eculizumab starting as early as week 1 and sustained through week 26. A major drawback detected in the trial design and possibly the reason for the negative result in primary endpoint was the use of the worst rank analysis. This relegated all patients who discontinued therapy to the lowest rank irrespective of the reason for such discontinuation. This was notable in the eculizumab group where 3 patients who had a good therapeutic response discontinued due to side effects other than myasthenic worsening, namely prostatic carcinoma, Moraxella bacteremia and bowel perforation. The side effects were mild to moderate, with headache, upper respiratory infection and nasopharyngitis being the most common and reported equally in both the groups. No patients developed Meningococcal infection. Fewer patients in the eculizumab group needed rescue therapy for MG exacerbations.[32]

117 patients from the double-blind phase of REGAIN (56 in Eculizumab/ Eculizumab group and 61 in the placebo/ Eculizumab group) entered the OLE phase for up to 4 years. After a blinded induction phase (active drug provided as 1200 mg every 2 weeks for Eculizumab group and 900 mg on day 1 and weekly for 3 weeks for the previous placebo group), all patients were continued on 1200 mg once in 2 weeks. The primary endpoint was the change in mean MG-ADL score over time. Interim analysis showed a reduction of 75% in the episodes of myasthenic worsening compared to the baseline. Infections of specific interest occurred in less than one-fifth of the study group and none had meningococcal meningitis. Improvements in myasthenia scores and quality of life scores were sustained with rapid improvements in the patients who switched over from placebo to Eculizumab after the double-blind phase (called the placebo/ Eculizumab group above).[33]

Various post hoc analysis of the REGAIN trial and OLE have underlined the efficacy and broad-spectrum responses with Eculizumab. In the REGAIN trial, Eculizumab-treated patients were two times more likely to have achieved minimal manifestation post intervention status compared to placebo at week 26In the OLE at 130 weeks, a substantial majority (88%) patients had attained improved status and 57.3% had reached minimal manifestation status. [34] Minimal symptom expression defined as MG-ADL score of 0-1 or MG-QOL-15 score of 0-3 was attained by a significantly higher proportion of Eculizumab-treated patients at week 26 of REGAIN.[35]

By week 12 of the randomised control trial (RCT), 67.3% and 56.1% Eculizumab-treated patients were classified as responders based on clinically meaningful improvements in MG-ADL (≥3 points) or QMG scores (≥5 points), respectively. While the majority were early responders (i.e. response within 12 weeks), new responders continued to emerge with longer term therapy. At the end of the OLE, the corresponding numbers were 84.7% and 71.4%, showing sustained response to treatment.[36]

Eculizumab was shown to be beneficial in subgroups of subjects in REGAIN and OLE who presumably had the worst spectrum of refractory MG as defined by failed use of chronic IVIg therapy and Rituximab. Eculizumab was administered in both these subsets after a sufficient washout period. The 17 patients on chronic IVIg who completed OLE (8 in Eculizumab/Eculizumab and 9 in placebo/Eculizumab groups respectively) had a higher exacerbation rate in the year preceding randomization compared to the total REGAIN cohort. Eculizumab in the REGAIN and OLE produced rapid and sustained improvement in the majority and reduced the exacerbation rate by more than two-thirds between pre-treatment years and during treatment (i.e. reduced from 150 to 47 exacerbation per 100 patient-years).[37] In addition, 14 patients who were previously exposed to Rituximab did not show any difference from the unexposed group in terms of efficacy or safety of Eculizumab.[38] There are also reports of successful transitioning from thrice-weekly plasmapheresis (PLEX) to Eculizumab.[39]In one study, three ventilator-dependent AChR-MG patients who were previously resistant to other immunotherapies, IVIg and PLEX were given Eculizumab. While two achieved minimal manifestations status in 4 to 6 weeks of therapy, the third had partial amelioration of symptoms allowing transition to non-invasive ventilation.[40] Eculizumab has also been found to be useful in refractory myasthenic crisis.[41]

More recent real-world evidence has shown improvement in MG-ADL scores (4.4 vs 6.33) and reduction in exacerbations (7 vs 42) at 12 months (vs baseline) in 15 treatment-refractory AChR-MG patients. The average exacerbations per patient/year reduced from 2.8 to 0.46, with a mean reduction of Prednisolone dose of 23.33 mg/ day. In addition, the mean single breath count improved from 28.13 to 50.26 seconds with IVIG being discontinued in all 6 patients receiving them and 9/15 patients could also come off the Pyridostigmine.[42]

In a retrospective 24-month observational study, 57 MG patients treated with Rituximab and 20 with Eculizumab were compared. The primary end point of change in QMG scores as well as more frequent minimal manifestation state were achieved by the Eculizumab cohort, although the risk of myasthenic crisis remained the same in both groups.[43]

The role of Eculizumab as rescue therapy in refractory MG has been firmly established via the RCT and OLE, various subgroup analysis and case reports, but its role as

a first-line agent and duration of therapy are still unclear. It is currently licensed to be used in generalised AChR-MG (USA, FDA approval – Oct 2017), refractory AChR-MG (EU) and AChR-MG unresponsive to IVIG/PLEX (Japan). Even though all the current approvals are for AChR antibody positive patients, Eculizumab has also been successfully used in some seronegative patients.[44] Paediatric and thymoma-associated MG patients may need to be studied further although early anecdotal reports are promising.[45, 46] The annual cost of therapy, which exceeds half a million US dollars, has been a major deterrent to the wider use of this drug around the world.[47, 48]

Ravulizumab

Ravulizumab, a recombinant human monoclonal antibody, is a long-acting C5 complement inhibitor with a similar mechanism of action to Eculizumab. The long half-life of this molecule necessitates fewer intravenous infusions for maintenance (once every 8 weeks, as opposed to every 2 weeks for Eculizumab). This drug was previously approved for treatment of PNH and is under investigation for atypical HUS and IgA nephropathy.[49]

175 adults with symptomatic AChR antibody positive gMG were recruited to receive Ravulizumab infusion versus placebo (1:1) in the phase 3 randomized placebo-controlled CHAMPION-MG study (NCT03920293). The dosage of Ravulizumab was weight-based given as 2400 – 3000 mg single loading dose on day one followed by maintenance doses of 3000 – 3600 mg every 8 weeks starting from day 15. The primary efficacy endpoint of significant improvement in MG-ADL and the secondary outcomes were achieved in the treatment group at 26 weeks. No marked difference in adverse effects was noted between the two groups.[50] The open label extension phase of the study is ongoing. Ravulizumab is currently approved for use in MG by the FDA (Apr 2022) and potentially can be used for a wider range of patients.

Zilucoplan

Zilucoplan prevents the terminal activation of the complement cascade by two mechanisms. It binds to the C5 complement component to prevents its cleavage and binds to the existing C5b to prevent its attachment to C6. It is a small macrocyclic peptide given as a subcutaneous (SC) injection. The advantages of this molecule are its ability to be self-administered, good NMJ penetration because of its small size, and the ability to concomitantly administer IVIg therapy or neonatal Fc receptor (FcRN) inhibitors as this is not an antibody, unlike Eculizumab and Ravulizumab.[51]

In the phase 2 clinical study over 12 weeks in symptomatic adult AChR-MG patients, 44 patients were randomized and received one of the three interventions: once daily SC injection of Zilucoplan at 0.3 mg/kg, once daily Zilucoplan at 0.1 mg/kg or placebo. The main efficacy endpoints were changes in MG-ADL and QMG scores and the high dose Zilucoplan group showed a rapid and statistically significant improvement in the scores compared to placebo (MG-ADL 3.4 vs 1.1; QMG 6.0 vs 3.2). They also had reduced need for rescue therapies. No serious treatment emergent adverse reactions were reported with Zilucoplan.[52] The phase 3 study to study the efficacy and tolerability of 0.3 mg/kg Zilucoplan (n=86) versus placebo (n=88) (RAISE; NCT04115293) has been completed, with significant benefits shown in the primary outcome (MG-ADL, $p<0.001$) and also the secondary outcomes (QMG, $p<0.001$; MGC, $p=0.0023$; MG-QoL15r, $p=0.0128$). Clinically meaningful improvement in the MG-ADL score (≥3 points) was achieved in 73.1% of Zilucoplan patients versus 46.1% of those receiving placebo. The corresponding QMG improvement (≥5 points) was seen in 58% patients receiving the active drug (vs 33%). [53]

Pozelimab

Pozelimab is a fully humanized IgG4 monoclonal antibody which blocks C5 and can be used alone or in combination with Cemdisiran, a small siRNA which interfere with the hepatic production of C5. Cemdisiran reduces the circulating C5 protein levels and Pozelimab blocks any remaining C5, thus preventing the MAC deposition at NMJ. Loading dose of Pozelimab at 15 mg/kg IV followed by four repeat doses of Pozelimab at 400 mg SC administered once weekly was found to inhibit complement activation in healthy volunteers.[54] In animal studies, combination of Pozelimab with Cemdisiran allowed lower doses and decreased dosing frequency compared to use of the individual agents separately.[55] The phase 3 randomized controlled trial of the combination (intravenous Pozelimab loading followed by 4 weekly SC injections along with Cemdisiran subcutaneous 4 weekly) versus placebo in gMG is ongoing (NCT05070858).

Other anti-complement therapies

The main complement therapies in MG are summarised in **Table 1.** Of the existing immunomodulatory therapies for MG, IVIG has multiple actions along the complement cascade. These include binding of C1q, neutralisation of C3a and C5a leading to uptake, inhibition of C3b and C4b and prevention of MAC deposition.[56]

The newer therapies which are under various stages of clinical trials (although not necessarily in MG) include Tesidolumab, Crovalimab, Zimura, Gefurulimab and Nomacopan (all anti-C5), SKY59 (anti-C5 and also inhibits FcRn), Compstatin (family of cyclic peptides which inhibits C3), ANX005 (anti-C1q), Cinryze, Berinert and Ruconest (all anti-C1r/s), and Sutimlimab (anti-C1s). Danicopan (anti-Factor D) and Avacopan (anti-C5aR1), are orally administered complement blockers.[57, 58]

Table 1
Complement therapies currently used or being studied in Myasthenia Gravis

Molecule	Mechanism of action	Target group	Route and dose of administration	Current evidence
Eculizumab	Recombinant humanised IgG2/4 monoclonal antibody against C5 complement	AChR+ MG	IV induction of 900 mg weekly for 4 weeks followed by 1200 mg maintenance every 2 weeks	QMG: Eculizumab Vs Placebo = 54.7 Vs 70.7 (P=0.0129); MG-QoL15: Eculizumab Vs Placebo = 55.5 Vs 69.7 (P=0.0281) **(REGAIN)** Approved for treatment of adults with AChR+ gMG
Ravulizumab	Long-acting recombinant humanised monoclonal antibody against C5 complement	AChR+ MG	IV weight-based dose. Single loading dose of 2400 – 3000 mg and maintenance doses of 3000 – 3600 mg every 8 weeks	QMG total scores improved by 5 points or more – 30% in treated group Vs 11.3% in placebo group **(CHAMPION MG)** Approved for treatment of adults with AChR+ gMG
Zilucoplan	Macrocyclic peptide binding C5 and C5b complement components	AChR+ MG	SC, once daily dose of 0.3 mg/kg	Phase 3 study showed positive results **(NCT04115293, RAISE)** Primary outcome (MG-ADL, p<0.001) Secondary outcomes (QMG, p<0.001; MGC, p=0.0023; MG-QoL15r, p=0.0128)
Pozelimab	Fully humanised IgG4 monoclonal antibody inhibiting C5 complement	AChR+ or LRP4+ MG	SC alone or in combination with Cemdisiran	Phase 3 study is ongoing **(NCT05070858)**
Cemdisiran	siRNA suppressing hepatic C5 synthesis	AChR+ or LRP4+ MG	SC alone or in combination with Pozelimab	Phase 3 study is ongoing **(NCT05070858)**
Gefurulimab (ALXN1720)	Anti-C5 humanised bi-specific VHH antibody (nanobody)	AChR+ MG	SC weight-based dose once weekly	Phase 3 study is underway (**NCT05556096**)
Danicopan (ALXN2050)	Small molecule complement pathway factor D inhibitor	AChR + MG	Oral, multiple dosages in trial	Phase 2 study ongoing (**NCT05218096**)

(Abbreviations: AChR – Acetylcholine receptor, MG – Myasthenia Gravis, MG-ADL – Myasthenia Gravis Activities of Daily Living score, MGC – Myasthenia Gravis Composite score, MG-QoL15 – Myasthenia Gravis Quality of Life Score, gMG – generalised Myasthenia Gravis, LRP-4 – Low-density Lipoprotein receptor related protein 4)

Safety

Anti-C5 complement therapies have been in use for over a decade for PNH and more than five years in MG. No major safety markers have been identified, even in patients receiving other immunosuppressive therapies like Rituximab. The main risk is the development of Gram-negative infections, especially meningococcal sepsis since MAC formation is the primary defence against these organisms. Subsequently, meningococcal vaccinations are mandatory prior to initiation of complement therapies, and many countries stipulate the use of prophylactic antibiotics to prevent any serotypes which may not be covered by the vaccine. No safety concerns have been raised in pregnancy and lactation.[59] When using antibiotics, fluoroquinolones and macrolides are best avoided to minimise MG exacerbations. If complement therapy is used in children in the future, additional vaccinations (e.g.: against Streptococcus pneumoniae and Haemophilus Infleunzae type B) may be required. So far, clinically significant neutralising antibodies have not been identified.

Biomarkers for complement therapy

Currently, there is a dearth of biomarkers which will predict the sub-group of patients who may respond better to complement inhibitory therapies. Serological studies assessing circulating C3 levels, C5 functional activity and total complement activity estimated by CH50, or a combination of these assays (e.g.: C3:CH50 ratio) are currently being studied. A CRISPR/Cas9 genome modified HEK293T cell line with reduced complement regulator expression has been used to develop a novel assay that may be helpful to assess complement activity in AChR-antibody positive patients, thereby helping to identify patients who may benefit from anti-complement therapies. [25] Rare missense C5 heterozygous variants (c.2654 G →A; c.2653 C →T) have been shown to replace Arginine with Histidine or Cysteine on C5, preventing its ability to bind Eculizumab making the drug ineffective. Similarly, complement related gene panels may help identify the ideal "complotype" which will help develop personalised medicine.[60] A new bioassay is currently being developed enabling functional characterisation and complement-mediated neuromuscular synaptic damage.[61] It has to be noted that the complement activity as measured using the available assays do not correlate well with disease severity or AChR antibody levels [26], even though older papers suggested a link between C3 levels and disease severity. [62]

Summary

The existing model for treatment in myasthenia revolves around three main actions – inhibiting ACh breakdown by cholinesterase inhibitors, suppressing the immune system by steroids and immunomodulatory therapies and thymectomy to modify specific autoimmune activity, especially in AChR antibody-positive patients. Current steroid-sparing immunotherapies in MG are limited by their slow onset of action (often taking several months to be effective) and rescue therapies like plasma exchange/ IVIG are unlikely to be useful for long term management. The newer complement-mediated therapies are useful for selective blocking of one of the main mechanisms of antibody-mediated myasthenic syndromes. These have had extensive experimental and pre-clinical evidence and more recently have had consistently positive results in Phase II and Phase III studies. Even though there is a theoretical risk of infections with Gram-negative organisms like Neisseria, this has not been shown to be a major concern in studies so far. Future studies may be able to identify biomarkers predicting which patients might be better suited for these targeted therapies.

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Disclosures

SJ has served as an international advisory board member for Alexion, Alnylam, Argenx, Regeneron, Immunovant and UCB pharmaceuticals, is currently an expert panel member of Myasthenia Gravis consortium for Argenx pharmaceuticals and has received speaker fees from Argenx, Eisai, Terumo BCT and UCB pharmaceuticals.

References

1. Gilhus, N.E., et al., *Myasthenia gravis.* Nat Rev Dis Primers, 2019. **5**(1): p. 30.
2. Lazaridis, K. and S.J. Tzartos, *Autoantibody Specificities in Myasthenia Gravis; Implications for Improved Diagnostics and Therapeutics.* Front Immunol, 2020. **11**: p. 212.
3. Takamori, M., *Myasthenia Gravis: From the Viewpoint of Pathogenicity Focusing on Acetylcholine Receptor Clustering, Trans-Synaptic Homeostasis and Synaptic Stability.* Front Mol Neurosci, 2020. **13**: p. 86.
4. Koneczny, I. and R. Herbst, *Myasthenia Gravis: Pathogenic Effects of Autoantibodies on Neuromuscular Architecture.* Cells, 2019. **8**(7).
5. Ruff, R.L. and R.P. Lisak, *Nature and Action of Antibodies in Myasthenia Gravis.* Neurol Clin, 2018. **36**(2): p. 275-291.
6. Dalakas, M.C., H. Alexopoulos, and P.J. Spaeth, *Complement in neurological disorders and emerging complement-targeted therapeutics.* Nat Rev Neurol, 2020. **16**(11): p. 601-617.
7. Howard, J.F., Jr., *Myasthenia gravis: the role of complement at the neuromuscular junction.* Ann N Y Acad Sci, 2018. **1412**(1): p. 113-128.
8. Kemper, C. and J.P. Atkinson, *T-cell regulation: with complements from innate immunity.* Nat Rev Immunol, 2007. **7**(1): p. 9-18.

9. Botto, M. and M.J. Walport, *C1q, autoimmunity and apoptosis.* Immunobiology, 2002. **205**(4-5): p. 395-406.

10. Kaminski, H.J., et al., *Complement regulators in extraocular muscle and experimental autoimmune myasthenia gravis.* Exp Neurol, 2004. **189**(2): p. 333-42.

11. Sahashi, K., et al., *Ultrastructural localization of immune complexes (IgG and C3) at the end-plate in experimental autoimmune myasthenia gravis.* J Neuropathol Exp Neurol, 1978. **37**(2): p. 212-23.

12. Engel, A.G., et al., *Passively transferred experimental autoimmune myasthenia gravis. Sequential and quantitative study of the motor end-plate fine structure and ultrastructural localization of immune complexes (IgG and C3), and of the acetylcholine receptor.* Neurology, 1979. **29**(2): p. 179-88.

13. Lennon, V.A., et al., *Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis.* J Exp Med, 1978. **147**(4): p. 973-83.

14. Tuzun, E., et al., *Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis.* J Immunol, 2003. **171**(7): p. 3847-54.

15. Christadoss, P., *C5 gene influences the development of murine myasthenia gravis.* J Immunol, 1988. **140**(8): p. 2589-92.

16. Chamberlain-Banoub, J., et al., *Complement membrane attack is required for endplate damage and clinical disease in passive experimental myasthenia gravis in Lewis rats.* Clin Exp Immunol, 2006. **146**(2): p. 278-86.

17. Morgan, B.P., et al., *The membrane attack pathway of complement drives pathology in passively induced experimental autoimmune myasthenia gravis in mice.* Clin Exp Immunol, 2006. **146**(2): p. 294-302.

18. Kaminski, H.J., et al., *Deficiency of decay accelerating factor and CD59 leads to crisis in experimental myasthenia.* Exp Neurol, 2006. **202**(2): p. 287-93.

19. Jacob, S., et al., *Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis.* Arch Neurol, 2012. **69**(8): p. 994-1001.

20. Engel, A.G., E.H. Lambert, and F.M. Howard, *Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis: ultrastructural and light microscopic localization and electrophysiologic correlations.* Mayo Clin Proc, 1977. **52**(5): p. 267-80.

21. Nakano, S. and A.G. Engel, *Myasthenia gravis: quantitative immunocytochemical analysis of inflammatory cells and detection of complement membrane attack complex at the end-plate in 30 patients.* Neurology, 1993. **43**(6): p. 1167-72.

22. Romi, F., et al., *The role of complement in myasthenia gravis: serological evidence of complement consumption in vivo.* J Neuroimmunol, 2005. **158**(1-2): p. 191-4.

23. Ozawa, Y., et al., *Changes in serum complements and their regulators in generalized myasthenia gravis.* Eur J Neurol, 2021. **28**(1): p. 314-322.

24. Stascheit, F., et al., *Complement activation profiles in anti-acetylcholine receptor positive myasthenia gravis.* Eur J Neurol, 2023. **30**(5): p. 1409-1416.

25. Obaid, A.H., et al., *Heterogeneity of Acetylcholine Receptor Autoantibody-Mediated Complement Activity in Patients With Myasthenia Gravis.* Neurol Neuroimmunol Neuroinflamm, 2022. **9**(4).

26. Fichtner, M.L., et al., *Myasthenia gravis complement activity is independent of autoantibody titer and disease severity.* PLoS One, 2022. **17**(3): p. e0264489.

27. Tuzun, E., et al., *Pros and cons of treating murine myasthenia gravis with anti-C1q antibody.* J Neuroimmunol, 2007. **182**(1-2): p. 167-76.

28. Biesecker, G. and C.M. Gomez, *Inhibition of acute passive transfer experimental autoimmune myasthenia gravis with Fab antibody to complement C6.* J Immunol, 1989. **142**(8): p. 2654-9.

29. Zhou, Y., et al., *Anti-C5 antibody treatment ameliorates weakness in experimentally acquired myasthenia gravis.* J Immunol, 2007. **179**(12): p. 8562-7.

30. Kusner, L.L., et al., *Investigational RNAi Therapeutic Targeting C5 Is Efficacious in Pre-clinical Models of Myasthenia Gravis.* Mol Ther Methods Clin Dev, 2019. **13**: p. 484-492.

31. Howard, J.F., Jr., et al., *A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis.* Muscle Nerve, 2013. **48**(1): p. 76-84.

32. Howard, J.F., Jr., et al., *Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study.* Lancet Neurol, 2017. **16**(12): p. 976-986.

33. Muppidi, S., et al., *Long-term safety and efficacy of eculizumab in generalized myasthenia gravis.* Muscle Nerve, 2019. **60**(1): p. 14-24.

34. Mantegazza, R., et al., *Post-intervention Status in Patients With Refractory Myasthenia Gravis Treated With Eculizumab During REGAIN and Its Open-Label Extension.* Neurology, 2021. **96**(4): p. e610-e618.

35. Vissing, J., et al., *'Minimal symptom expression' in patients with acetylcholine receptor antibody-positive refractory generalized myasthenia gravis treated with eculizumab.* J Neurol, 2020. **267**(7): p. 1991-2001.

36. Howard, J.F., Jr., et al., *Long-term efficacy of eculizumab in refractory generalized myasthenia gravis: responder analyses.* Ann Clin Transl Neurol, 2021. **8**(7): p. 1398-1407.

37. Jacob, S., et al., *Response to eculizumab in patients with myasthenia gravis recently treated with chronic IVIg: a subgroup analysis of REGAIN and its open-label extension study.* Ther Adv Neurol Disord, 2020. **13**: p. 1756286420911784.

38. Siddiqi, Z.A., et al., *Eculizumab in refractory generalized myasthenia gravis previously treated with rituximab: subgroup analysis of REGAIN and its extension study.* Muscle Nerve, 2021. **64**(6): p. 662-669.

39. Greenwood, G.T. and Z. Lynch, *Successful Transition from Plasma Exchange to Eculizumab in Acetylcholine Receptor Antibody- and Muscle-Specific Kinase (MuSK) Antibody-Negative Myasthenia Gravis: A Case Report.* Am J Case Rep, 2020. **21**: p. e921431.

40. Usman, U., et al., *The use of eculizumab in ventilator-dependent myasthenia gravis patients.* Muscle Nerve, 2021. **64**(2): p. 212-215.

41. Hofstadt-van Oy, U., et al., *Complement inhibition initiated recovery of a severe myasthenic crisis with COVID-19.* J Neurol, 2021. **268**(9): p. 3125-3128.

42. Katyal, N., N. Narula, and R. Govindarajan, *Clinical Experience with Eculizumab in Treatment-Refractory Acetylcholine Receptor Antibody-Positive Generalized Myasthenia Gravis.* J Neuromuscul Dis, 2021. **8**(2): p. 287-294.

43. Nelke, C., et al., *Eculizumab versus rituximab in generalised myasthenia gravis.* J Neurol Neurosurg Psychiatry, 2022. **93**(5): p. 548-554.

44. Datta, S., S. Singh, and R. Govindarajan, *Retrospective Analysis of Eculizumab in Patients with Acetylcholine Receptor Antibody-Negative Myasthenia Gravis: A Case Series.* J Neuromuscul Dis, 2020. **7**(3): p. 269-277.

45. Velez-Santamaria, V., et al., *Eculizumab as a promising treatment in thymoma-associated myasthenia gravis.* Ther Adv Neurol Disord, 2020. **13**: p. 1756286420932035.

46. Amano, E., et al., *Eculizumab improved weakness and taste disorder in thymoma-associated generalized myasthenia gravis with anti-striational antibodies: A case report.* eNeurologicalSci, 2019. **14**: p. 72-73.

47. Mantegazza, R. and P. Cavalcante, *Eculizumab for the treatment of myasthenia gravis.* Expert Opin Biol Ther, 2020. **20**(9): p. 991-998.

48. Tice, J.A., et al., *The effectiveness and value of eculizumab and efgartigimod for generalized myasthenia gravis.* J Manag Care Spec Pharm, 2022. **28**(1): p. 119-124.

49. McKeage, K., *Ravulizumab: First Global Approval.* Drugs, 2019. **79**(3): p. 347-352.

50. Tuan Vu, A.M., Renato Mantegazza, Djillali Annane, Masahisa Katsuno, Rasha Aguzzi, Ahmed Enayetallah, Kathleen N. Beasley, Nishi Rampal and James F. Howard, Jr., for the CHAMPION MG Study Group, *Terminal Complement Inhibitor Ravulizumab in Generalized Myasthenia Gravis.* NEJM Evidence 2022. **1**(5): p. EVIDoa2100066.

51. Howard, J.F., Jr., et al., *Zilucoplan: An Investigational Complement C5 Inhibitor for the Treatment of Acetylcholine Receptor Autoantibody-Positive Generalized Myasthenia Gravis.* Expert Opin Investig Drugs, 2021. **30**(5): p. 483-493.

52. Howard, J.F., Jr., et al., *Clinical Effects of the Self-administered Subcutaneous Complement Inhibitor Zilucoplan in Patients With Moderate to Severe Generalized Myasthenia Gravis: Results of a Phase 2 Randomized, Double-Blind, Placebo-Controlled, Multicenter Clinical Trial.* JAMA Neurol, 2020. **77**(5): p. 582-592.

53. https://clinicaltrials.gov/ct2/show/NCT04115293. 2023.

54. Weyne, J., et al., *A Randomized, Double-Blind, Placebo-Controlled Phase 1 Study of the Pharmacokinetics and Pharmacodynamics of REGN3918, a Human Antibody Against Complement Factor C5, in Healthy Volunteers.* Blood, 2018. **132**(Supplement 1): p. 1039-1039.

55. Devalaraja-Narashimha, K., et al., *Pharmacokinetics and pharmacodynamics of pozelimab alone or in combination with cemdisiran in non-human primates.* PLoS One, 2022. **17**(6): p. e0269749.

56. Lunemann, J.D., F. Nimmerjahn, and M.C. Dalakas, *Intravenous immunoglobulin in neurology--mode of action and clinical efficacy.* Nat Rev Neurol, 2015. **11**(2): p. 80-9.

57. Dalakas, M.C., *Role of complement, anti-complement therapeutics, and other targeted immunotherapies in myasthenia gravis.* Expert Rev Clin Immunol, 2022. **18**(7): p. 691-701.

58. Zelek, W.M., et al., *Compendium of current complement therapeutics.* Mol Immunol, 2019. **114**: p. 341-352.

59. Langereis, J.D., et al., *Eculizumab impairs Neisseria meningitidis serogroup B killing in whole blood despite 4CMenB vaccination of PNH patients.* Blood Adv, 2020. **4**(15): p. 3615-3620.

60. Mantegazza, R., et al., *Complement Inhibition for the Treatment of Myasthenia Gravis.* Immunotargets Ther, 2020. **9**: p. 317-331.

61. Plomp, J.J., et al., *A bioassay for neuromuscular junction-restricted complement activation by myasthenia gravis acetylcholine receptor antibodies.* J Neurosci Methods, 2022. **373**: p. 109551.

62. Liu, A., et al., *Correlation of C3 level with severity of generalized myasthenia gravis.* Muscle Nerve, 2009. **40**(5): p. 801-8.