

# Practical Management for Use of Eculizumab in the Treatment of Severe, Refractory, Non-Thymomatous, AChR + Generalized Myasthenia Gravis: A Systematic Review

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**Abstract:** Myasthenia gravis (MG) is a rare autoimmune disorder caused by specific autoantibodies at the neuromuscular junction. MG is classified by the antigen specificity of these antibodies. Acetylcholine receptor (AChR) antibodies are the most common type (74–88%), followed by anti-muscle specific kinase (MuSK) and other antibodies. While all these antibodies lead to neuromuscular transmission failure, the immuno-pathogenic mechanisms are distinct. Complement activation is a primary driver of AChR antibody-positive MG (AChR+ MG) pathogenesis. This leads to the formation of the membrane attack complex and destruction of AChR receptors and the postsynaptic membrane resulting in impaired neurotransmission and muscle weakness characteristic of MG. Broad-based immune-suppressants like corticosteroids are effective in controlling MG; however, their long-term use can be associated with significant adverse effects. Advances in translational research have led to the development of more directed therapeutic agents that are likely to alter the future of MG treatment. Eculizumab is a humanized monoclonal antibody that inhibits the cleavage of complement protein C5 and is approved for use in generalized MG. In this review, we discuss the pathophysiology of MG; the therapeutic efficacy and tolerability of eculizumab, as well as the practical guidelines for its use in MG; future studies exploring the role of eculizumab in different stages and subtypes of MG subtypes; the optimal duration of therapy and its discontinuation; the characterization of non-responder patients; and the use of biomarkers for monitoring therapy are highlighted. Based on the pathophysiologic mechanisms, emerging therapies and new therapeutic targets are also reviewed.

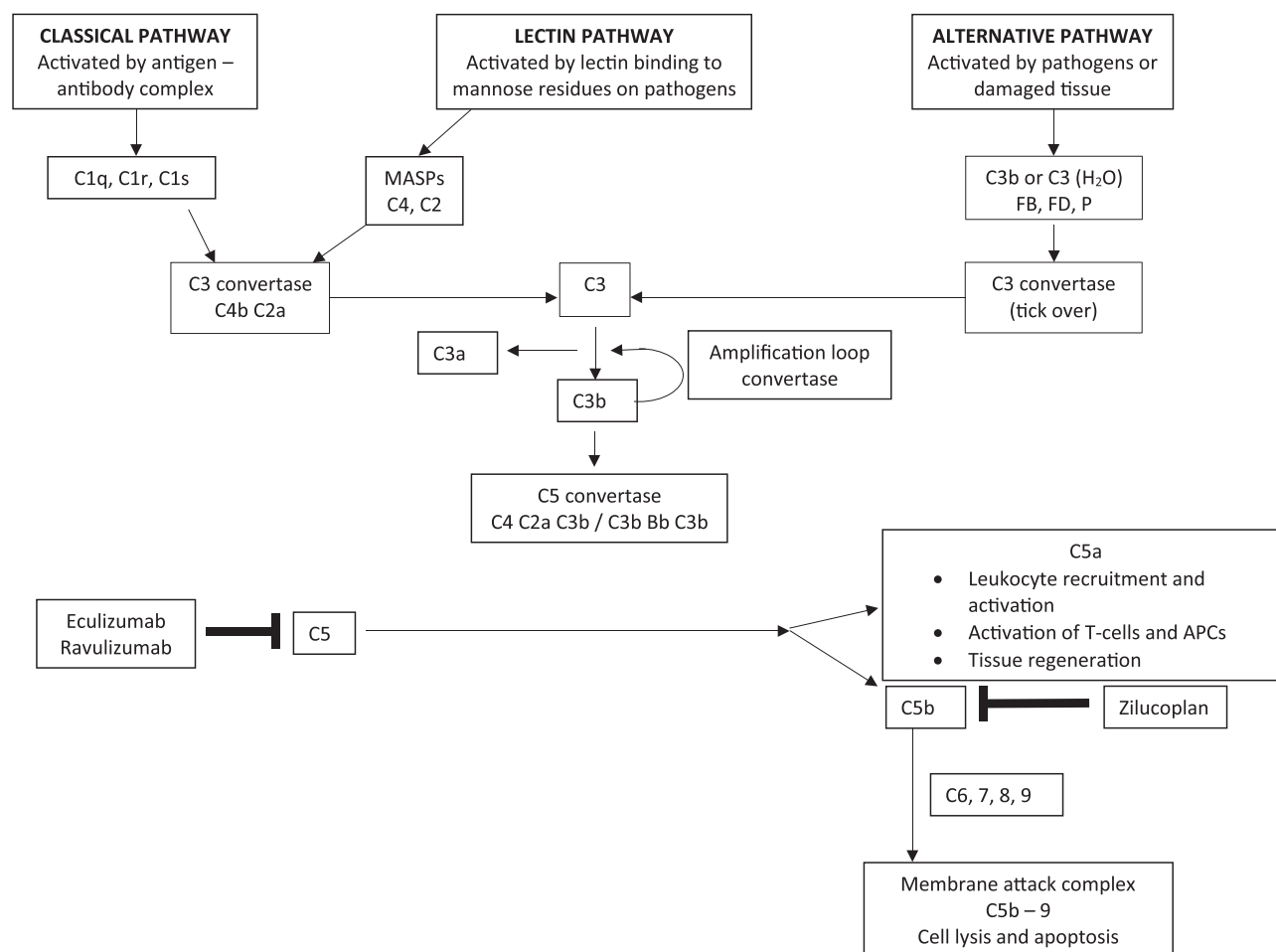
**Keywords:** myasthenia gravis, pathophysiology, autoantibodies, complement, eculizumab

## Introduction

Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular junction (NMJ), with an estimated prevalence of 70–163 per million for acetylcholine receptor (AChR) MG (AChR+ MG), and 1.9–2.9 per million for muscle-specific kinase (MuSK) MG (MuSK+ MG). Women are affected more frequently than men, with a female-to-male ratio of 3:1 for AChR+ MG and 9:1 for MuSK+ MG.<sup>1</sup>

The characteristic feature of MG is fatigable skeletal muscle weakness, predominantly affecting the ocular muscles, with a risk of progression to generalized weakness within 2 years after disease onset. Respiratory muscle involvement leading to a myasthenic crisis happens in up to 20% of the cases of AChR+ MG. The diagnosis is based on the presence of known autoantibodies, electrophysiological tests (single-fiber electromyography and repetitive nerve stimulation [RNS]), and improvement of symptoms with the use of acetylcholinesterase (AChE) medications or after the ice pack test.<sup>2,3</sup>

The role of complement in MG (Figure 1) has been demonstrated in animal models and is further highlighted by the recent approval of eculizumab, a humanized monoclonal antibody, which by targeting the C5 complement protein



**Figure 1** Complement and its role in MG. This figure shows different pathways and associated regulatory proteins involved in the successful formation of MAC, including factors B and D (FB, FD), properdin (P), and mannan-binding lectin serine protease 1 (MASP-1).

intercepts the formation of C5b-9 or the membrane attack complex (MAC). This systematic review focuses on the role of complement in MG, describes the relevant clinical data, and provides practical recommendations for the use of eculizumab in the management of MG. Moreover, we also describe the relevant pathophysiology of MG, which provides a rationale for future therapies in MG.

## Pathophysiology of MG (Figure 2)

MG is an antibody-mediated disease with T-cell-driven immune pathogenesis and complex interactions between CD4+ T-cells and B-cells. The pathogenic mechanisms can be subdivided based upon the type of underlying antibody present.

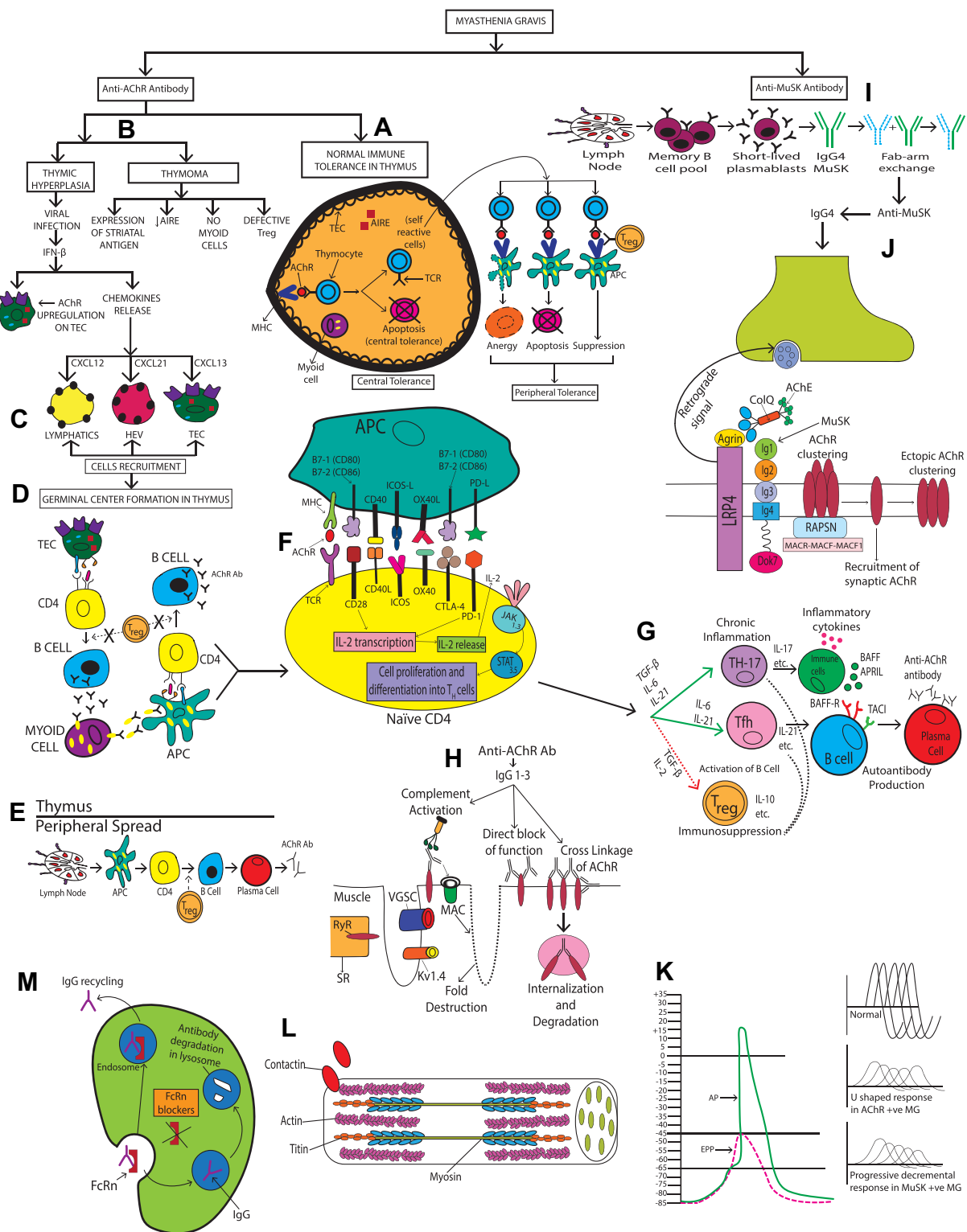
### Anti-AChR Antibody-Associated MG

#### Role of Thymus

The thymus is affected in most patients with AChR+ MG; histologically or radiologically, approximately 70% of the patients have thymic follicular hyperplasia, 10% have thymoma and the remainder have either a normal or an atrophic thymus.<sup>4</sup>

#### Normal Thymus Gland Anatomy and Immune Tolerance (Figure 2A)

T-cell maturation occurs in the thymus and involves collaborations between developing T-cells called thymocytes and thymic epithelial cells (TECs), and other stromal cells such as dendritic cells and myoid cells. Myoid cells are distinguished by striations, and are the only known cells, besides skeletal muscles, to express folded AChR subunits, and may play a role in inducing central immune tolerance to muscle proteins.



**Figure 2** Pathogenesis of MG – The potential cascade of events in AChR+ and MuSK+ MG: **(A)** Normal Immune tolerance in thymus. **(B and C)** Thymic pathology, including thymic hyperplasia and thymoma in AChR+ MG. In thymic hyperplasia, a potential sequence of events leading to tertiary lymphoid genesis has been discovered which is initiated by an inciting event, such as a viral infection in a susceptible environment, leading to the creation of a suitable environment for autoimmune pathology. Altered properties of neoplastic epithelial cells in thymoma favor autoimmunity. **(D)** Sensitization to the AChR and germinal center formation. **(E)** Peripheral spread of the thymus-initiated autoimmune process. **(F and G)** B-cell- and T-cell-mediated factors contributing to the pathogenesis of MG. **(H)** Mode of action of AChR-specific autoantibodies. **(I and J)** Activation and physiologic functions of MuSK. **(K)** Safety factor and neuromuscular transmission failure. **(L)** Additional MG-associated antibodies. **(M)** FcRn blockers. Red square – AIRE; Yellow oval– folded AChR; Blue oval – unfolded AChR.

Multigene transcription factors, such as the autoimmune regulator (AIRE), which is expressed in the thymic medulla, aid in the detection of self-antigens in the thymus. AIRE leads to the expression of unfolded AChR subunits on TECs. T-cells that identify these proteins (or self-antigens) are targeted for negative selection and apoptosis, providing the basis for central tolerance to the AChR. Self-reactive T-cells that escape central tolerance are suppressed in the periphery (peripheral tolerance) by either apoptosis, anergy (functional unresponsiveness due to lack of co-stimulatory signal B7 on antigen-presenting cells), or by a subset of CD4<sup>+</sup> cells outsourced from the thymus gland called regulatory T-cells (Tregs).<sup>4,5</sup>

### Thymic Follicular Hyperplasia (Figure 2B)

Under physiological conditions, B-cells are nearly non-existent in the thymus. However, in the majority of AChR<sup>+</sup> MG, the thymus displays germinal centers (GCs) with a high number of B-cells (thymic hyperplasia). GCs are typically found in B-cell-producing secondary lymphoid organs, like lymph nodes, and are responsible for the generation of the humoral immune response resulting in the production of antibodies and durable memory B-cells. These findings not only support the presence of thymic inflammation but also modification of its role from T-cell maturation to the development of an adaptive immune response, ie, the thymus becomes a tertiary lymphoid structure.<sup>5,6</sup>

The sequence of events given below has been proposed as leading to the genesis of tertiary lymphoid structures in the thymus of MG patients.

### Cellular Recruitment (Figure 2C)

The mechanisms leading to the selective production of muscle autoantibodies in MG are unclear. This could begin with an initiating event, such as a viral infection, which in the presence of a certain predisposing background (human leukocyte antigen D-related genotype, estrogen, etc.) triggers the release of cytokines, particularly interferon-beta (IFN- $\beta$ ), causing upregulation of thymic expression of alpha-AChR (the main immunogenic region [MIR] in MG) and overproduction of ligands of the chemokine (CXCL) family. The latter contribute to neogenesis (CXCL 12 and CXCL 21 for lymphatics and specialized blood vessels called high endothelial venules respectively) and chemoattractant (CXCL13) upregulation, especially for B lymphocytes on TEC. The combination of neogenesis and chemoattractant upregulation provides an extensive vascular network and optimal environment for peripheral antigen-presenting cells (APCs), B-cells and T-cells to find their niche in the thymus.<sup>7</sup>

### Sensitization to AChR and Germinal Center Formation (Figure 2D)

Sensitization to the AChR involves a two-step model: 1) hyperplastic major histocompatibility complex (MHC) class II expressing TECs exhibit unfolded AChR subunits and activate autoreactive CD4 T-cells, leading to the production of early AChR antibodies; and 2) thymic myoid cells expressing intact AChR affected by these antibodies release AChR-immune complexes, which activate APCs, leading to further activation of autoreactive CD4 T-cells and B-cells. These organize into GCs and contribute to the production of subsequent epitope diversification. Autoreactive B-cells and T-cells, and anti-AChR antibodies, exit the thymus and attack the AChR on peripheral muscles, leading to damage to NMJ and MG symptoms.<sup>4-6</sup>

### Peripheral Spread of Thymus-Initiated Autoimmune Process (Figure 2E)

The thymus-initiated autoimmune process later spreads to peripheral secondary lymphoid organs. This accounts for the continuous disease activity even after thymectomy, which is mediated by autoantibody-producing B-cells that have emigrated from the thymus as well as functionally defective Treg cells.<sup>4</sup>

### Role of Cytokines (Figure 2F and G)

Activated T-cells, B-cells, plasma cells, and related cytokines play central roles in the production of pathogenic autoantibodies in MG. Collectively, the imbalance of cytokines from TH1 (IFN  $\gamma$ ), TH17 (interleukin [IL] 17), T follicular helper (Tfh) cells (IL-21 and IL-4), B-cell survival and differentiation promoters (B-cell-activating factor [BAFF] and a proliferation-inducing ligand (APRIL) produced by activated immune cells and downregulation of Treg cells may all contribute to the sustenance of the pathogenesis of MG.<sup>7</sup> This is further discussed in the section on future pathophysiologic target-based interventions.

## Thymoma (Figure 2B)

The altered properties of neoplastic epithelial cells in thymomas, including the defective expression of AIRE, reduced or absent thymic myoid cells, variable expression of striational antigen epitopes (including titin and various AChR subunits), and the defective generation of Tregs, all profoundly affect the selection of thymocytes and result in the export of autoreactive T-cells that replace the more tolerant T-cells.<sup>4</sup>

## AChR-Specific Autoantibodies (Figure 2H)

AChR-specific autoantibodies predominantly belong to the IgG1 and IgG3 subclasses and originate from long-lived plasma cells. They mediate tissue damage at the NMJ by binding to AChR, which leads to focal endplate lysis through complement activation and MAC formation, and cross-linking of adjacent AChRs, which leads to their internalization and degradation, thereby directly blocking the acetylcholine (ACh) binding site.<sup>5</sup>

## Anti-MuSK MG

### Physiologic Function and Activation of MuSK (Figure 2I and J)

Anti-MuSK antibodies are detected in 1–10% of the patients with generalized MG. The MuSK activation process starts with the binding of lipoprotein-receptor-related protein 4 (LRP4) to neural-derived Agrin. Pre-synaptically, through a retrograde signal to the motor neuron, LRP4 contributes to an increase in the clustering of acetylcholine vesicles. Post-synaptically, LRP4 interacts with MuSK leading to its dimerization and auto-phosphorylation. Activated MuSK, stabilized by Docking Protein 7 (DOK7), triggers a series of additional intracellular signaling steps leading to phosphorylation of AChR and the synaptic protein, rapsyn. Self-aggregation of rapsyn provides a scaffold required for anchoring the AChR with the actin cytoskeleton via microtubule-actin cross-linking factor 1 (MACF1), thus leading to the formation of mature AChR clusters. In addition, MuSK also interacts with acetylcholinesterase-associated collagen (ColQ), which is the collagen-tail subunit of AChE found in the neuromuscular junction.<sup>8</sup>

The prevention of extra-synaptic AChR clustering, as well as overstimulation of muscles, is provided by negative regulators of clustering pathways including AChE itself (which downregulates AChR expression), as well as MuSK-activated Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2). SHP2 is hypothesized to have a role during embryonic development by limiting the establishment of AChR clusters in non-motor nerve terminal extra-synaptic locations.<sup>8</sup>

### Features of Anti-MuSK Antibodies (Figure 2I and J)

The anti-MuSK antibodies have the following unique features<sup>8</sup>: 1) belong to the IgG4 subclass; 2) half antibody (Fab arm) whereby they recombine after disassociating, resulting in antibodies with two distinct antigen-binding variable regions (bispecific). As a result, IgG4 MuSK antibodies are monovalent for their antigen, rather than divalent, preventing divalent-dependent cross-linking and antigen internalization, 3) Do not involve the thymus (hence, there is no role of thymectomy in MuSK+ MG) and instead, the production of pathogenic antibodies occurs in the secondary lymphoid tissues, predominantly by short-lived plasmablasts. They lead to NMJ failure through disruption of the Agrin-MuSK–DOK7-rapsyn pathway required for AChR clustering and postsynaptic muscle membrane maintenance. Several additional features are characteristic of IgG4 anti-MuSK antibodies. These include a) loss of retrograde signaling to motor neurons leading to the absence of pre-synaptic increase of ACh vesicles. This is manifested by a progressive decremental response pattern in RNS studies (compared to partial recovery giving a U-shaped appearance on RNS studies in AChR+ MG)<sup>9</sup>, and could create an opportunity for presynaptic potentiation with the use of the potassium channel blocker 3,4-diaminopyridine, as a symptomatic therapy for patients with MuSK + MG.<sup>10</sup> (Figure 2K); and b) blockage of ColQ-MuSK binding, leading to excessive ACh at the neuromuscular synapse from loss of AChE, which contributes to the dispersal of AChR and hypersensitivity in MuSK+ MG to use of AChE inhibitors, manifested by fasciculations, an unsatisfactory clinical response, and repetitive compound motor action potentials (after discharges) during nerve conduction studies.<sup>11</sup> Moreover, additional proposed mechanisms of disease production involving the less common IgG1 and IgG3 anti-MuSK antibodies include complement-mediated damage, AChR cross-linkage, endocytosis of MuSK and direct blockage of function. Additionally, divalent binding of commercial or cloned (monospecific) MuSK



antibodies, irrespective of their original subclass, has been proposed as a mechanism of disease causation through agrin-independent MuSK dimerization and auto-phosphorylation, which leads to the formation of ectopic, extra-synaptic AChR clusters that fail to participate in neuromuscular transmission due to lack of interface with motor neuron terminals.<sup>8</sup>

## Safety Factor and NMJ Transmission Failure (Figure 2K)

The activation of AChRs by the release of the quantal contents of presynaptic vesicles containing ACh leads to localized depolarization of the postsynaptic membrane, causing the ultimate generation of the end plate potential (EPP). The amplitude of the EPP usually exceeds the threshold required for the activation of voltage-gated sodium channels present at the depths of the postsynaptic folds, resulting in the generation of the muscle fiber action potential. This excess of EPP at the NMJ, called the safety factor, ensures consistent translation of the nerve action potential into a muscle action potential. The reduction of the safety factor, due to the mechanisms interfering with nerve-to-muscle crosstalk mentioned above, in AChR and MuSK-associated MG, results in neuromuscular transmission failure manifested clinically as fatigable weakness affecting different body segments.

## Other MG-Associated Antibodies (Figure 2L)

Additional antibodies identified through increased use of cell-based assays in MG include the following: anti-LRP4, anti-agrin, ColQ, anti-titin, anti-ryanodine receptor (RyR), anti-contactin, anti-heat shock protein-70, anti-matrix metalloproteinases and anti-voltage-gated potassium channel (Kv1.4); their pathogenic significance remains unknown.<sup>5</sup>

## Complement and Its Role in Myasthenia Gravis (Figure 1)

### The Complement Cascade

The complement system consists of hepatically synthesized plasma proteins, which play a major role in the innate immune system as well as in inflammation, more generally. Physiologically, the complement cascade is made up of three distinct pathways – classical, lectin and alternative, all of which have unique triggers. The classical pathway is activated by antigen–antibody complexes, the lectin pathway is activated by the interaction between sugar molecules in microbial surfaces and mannose-binding lectin, and the alternative pathway is activated by microbe surface molecules. All three pathways result in the cleavage of C3 into C3a and C3b. C3b acts as the body's primary opsonin, enhancing phagocytosis of bacteria and immune complexes. C3b leads to the formation of C5 convertase, which cleaves C5 into C5a and C5b, which ultimately leads to the formation of MAC that is responsible for cell lysis and cytotoxicity. Physiologically, several molecules, including decay-accelerating factor (CD55) and C1 esterase inhibitor, act to prevent inappropriate complement activation directed towards self cells.<sup>12,13</sup>

## The Role of Complement in MG

### Preclinical Data

The role of complement in the pathophysiology of MG was first identified in the 1970s when Engel and others visualized anti-AChR antibody, C3, and MAC bound to the debris of the post-junctional membrane in MG patients.<sup>14</sup> Much of the evidence for the role of complement in MG comes from animal models of experimental autoimmune myasthenia gravis (EAMG). Mice genetically deficient in the complement are resistant or less susceptible to EAMG.<sup>15</sup> Inhibiting MAC formation, using anti-complement antibodies, protects rats from developing muscle weakness, electrophysiological abnormalities, and AChR loss typical of EAMG.<sup>16</sup> Later, in-vivo studies found that MG patients have higher serum levels of C5b-9 and that C5a levels are positively correlated with MG disease severity.<sup>17,18</sup>

### Development of Eculizumab as a Complement Blocker

After complement was identified as an important player in the pathophysiology of MG, researchers endeavored to find a therapeutic target within the complement pathway. C5 was identified as an optimal target, because its blockade effectively terminates the complement cascade regardless of which upstream pathway is activated, and its blockade does not inhibit the important upstream function of C3b-mediated opsonization. Eculizumab is a recombinant humanized monoclonal antibody that binds to C5, preventing its cleavage and ultimately to the formation of the MAC.<sup>19</sup> It has been studied in the treatment of

other complement-mediated diseases, including paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and neuromyelitis optica spectrum disorder, and found to be generally safe and effective.<sup>20–23</sup> Eculizumab obtained FDA approval for the treatment of these conditions in 2007, 2011, and 2019, respectively.

## Role of Eculizumab in MG

### Study Populations

Eculizumab has been best studied in patients with severe, treatment-refractory, non-thymomatous, AChR+ generalized MG (gMG). In these studies, treatment-refractory is operationally defined as patients who have received at least two immunosuppressant therapies (ISTs), or at least one IST in addition to intravenous immunoglobulin (IVIg) or plasma exchange (PLEX), given at least four times per year for 12 months, without symptom control. Studies of eculizumab in this patient population have shown it to be safe and effective, with particularly rapid and sustained control of disease manifestations.

### Phase II Trial<sup>24</sup>

The study involved 14 subjects with AChR+ gMG who were randomized to either eculizumab or placebo for 16 weeks (Period 1), with a 5-week washout period before switching to the other group (Period 2). Primary efficacy endpoint of a  $\geq 3$ -point reduction in quantitative MG (QMG) score was achieved in 86% of the eculizumab-treated patients, compared to 57% of the placebo group, suggesting a therapeutic effect of the drug in gMG. The efficacy of eculizumab was rapid and sustained; 57% of the patients in the eculizumab group achieved the primary endpoint by 1 week, 100% achieved it by 3 weeks, and the clinical improvement was sustained throughout the remainder of the study period. Additionally, patients in the eculizumab group from treatment period 1 did not return to a baseline QMG score after the 5-week washout period, suggesting a “carryover” effect of eculizumab.

### Phase III REGAIN Trial<sup>25</sup>

The phase III REGAIN trial was a 26-week multicenter, randomized, double-blind, placebo-controlled study in gMG patients. Patients were randomized to either the eculizumab or placebo group. The primary efficacy endpoint was a change in MG–Activities of Daily Living (MG-ADL) score from baseline to 26-weeks. Secondary efficacy endpoints included changes from baseline in QMG, Myasthenia Gravis Composite (MGC) and Myasthenia Gravis Quality of Life (MG-QOL15) scores. Safety endpoints included incidence of adverse events, serious adverse events, hospital admissions and clinical deterioration (defined as MG crisis, substantial symptomatic worsening or health in jeopardy without rescue therapy). The study narrowly missed its primary efficacy endpoint ( $p=0.0698$ ), but the eculizumab group did achieve statistically significant improvement in the QMG score compared to the placebo group ( $\geq 5$ -point improvement in the QMG score, 45% versus 19%) and in the MG-QOL15 score, with the most treatment effect achieved by week 12 and sustained through week 26.

The safety profile of eculizumab was also reaffirmed in the REGAIN trial. The most common adverse events were headache, upper respiratory tract infection, nasopharyngitis, and gastrointestinal upset (nausea and diarrhea). The incidence was similar across treatment groups. Serious adverse events occurred in 15% of the eculizumab group compared to 29% of the placebo group. Serious adverse events included MG exacerbations and crises; infections (upper respiratory tract infection, bacteremia, acute cholecystitis, diverticulitis, endocarditis, gastritis, gastroenteritis, tonsillitis, bacterial urinary tract infection, and varicella); hematologic/oncologic effects (deep vein thrombosis, pulmonary embolism, lymphopenia, bone metastases, and prostate cancer), surgical adverse events (intestinal perforation); and constitutional/metabolic events (pyrexia, apnea, hyperglycemia, and general physical health deterioration). Of the serious adverse events, infections were most common, occurring in 3% of the eculizumab group and 10% of the placebo group. Additionally, the incidence of hospital admissions, MG exacerbations, and rescue therapy was less in the eculizumab group. There were no deaths or meningococcal infections during the study period.

### Open-Label-Extension (OLE) of REGAIN Trial<sup>26</sup>

After REGAIN, study participants were invited to enter into its 3-year OLE study, provided they entered within 2 weeks of completing REGAIN. Patients in the eculizumab/eculizumab group did not experience significant changes in their MG disease assessment scores, compared to the end of the REGAIN trial; their improvements compared to the pre-REGAIN

baseline were sustained for the duration of the OLE. Patients in the placebo/eculizumab group achieved statistically significant changes in MG disease assessment scores similar in magnitude to those of the REGAIN treatment group. The efficacy of ecolizumab was again shown to be rapid and sustained. Patients in the placebo/eculizumab group met the efficacy endpoints as early as 1 week after initiating ecolizumab; over half of the treatment effect was observed by 3 months, and treatment effects were sustained for the duration of the 3-year study period.

Furthermore, of all patients treated with ecolizumab in the OLE, 55% experienced a clinically meaningful response in ADLs, 39% experienced a clinically meaningful response in muscle strength, and perhaps most importantly, 56% achieved either “minimal manifestations” or “pharmacologic remission” status. Evaluation of final data from REGAIN and its OLE revealed that the proportion of patients who achieved “improved” or “minimal manifestations” Myasthenia Gravis Foundation of America status increased with increased duration of ecolizumab therapy. Of those patients who received 130 weeks of treatment, 88% achieved “improved” status, 57% achieved “minimal manifestations” status, and 2 patients achieved “pharmacologic remission” status.<sup>27</sup> Rates of MG exacerbation, rescue therapy administration, and MG-related hospitalization were markedly reduced compared to the pre-REGAIN baseline (75%, 65%, and 83% reductions, respectively).

The OLE also re-demonstrated the favorable safety profile of ecolizumab, consistent with REGAIN data, and found that the most common adverse events were headache and nasopharyngitis. There were higher rates of common adverse events (30% compared to 15%) and serious adverse events (44% compared to 15%) in the OLE compared to REGAIN, but no new safety signals emerged.<sup>28</sup> Eighteen percent of all patients experienced an infectious adverse event of special interest. Importantly, there were no cases of meningococcal infection during the data acquisition period. There was one case of meningococcal meningitis that occurred after the interim data cut-off date despite appropriate vaccination, but it resolved with antibiotic treatment. In total, three patients died during REGAIN and its OLE, but there was no identifiable relationship between these deaths and ecolizumab treatment.

## Post Hoc Analysis of Eculizumab Studies in MG

### Minimal Symptom Expression

A post-hoc analysis of the REGAIN and OLE data found that beyond statistically significant clinical improvement, a significant proportion (17–25%) of patients treated with ecolizumab across the two trials achieved “minimal symptom expression” as defined by MG-ADL of 0–1 or MG-QOL15 of 0–3.<sup>29</sup> These scores had previously been used to represent disease remission.<sup>30</sup> Moreover, the proportion of patients who achieved “minimal symptom expression” was sustained throughout the 2.5 years of the OLE.

### Efficacy of Eculizumab in Study Participants Previously Treated with Rituximab

A post-hoc subgroup analysis of REGAIN and its OLE examined the efficacy of ecolizumab in study participants who had previously been treated with rituximab, which has historically been used off-label with uncertain efficacy for the treatment of severe, treatment-refractory, AChR+ gMG.<sup>31,32</sup> This analysis found that ecolizumab was no less effective in patients previously treated with rituximab, which again speaks to its profound potential for those patients who previously had severely limited treatment options.

Another retrospective observational study involving generalized, therapy-refractory anti-AChR-ab-mediated MG compared rituximab (57 patients) vs ecolizumab (20 patients), with otherwise similar clinical and demographic characteristics. Eculizumab was associated with a better outcome compared to rituximab as measured by change in the QMG score, and minimal disease manifestations after 12 and 24 months of treatment. However, the risk of myasthenic crisis was not ameliorated in either group.<sup>33</sup> A systematic review and meta-analysis by Feng et al explored the optimal therapies for refractory MG. They found no significant difference between the efficacy, as well as the incident density of MG exacerbation/crisis, between rituximab and ecolizumab. Rituximab was found to have a better safety profile than ecolizumab, with a reduced rate of side effects.<sup>34</sup>

It is important to mention that rituximab, a monoclonal antibody against B-cell membrane marker CD20, has historically been used off-label to treat severe, treatment-refractory MG. It appears to be particularly effective in patients with MuSK+ MG, rather than in AChR + MG. MuSK+ MG patients often respond relatively poorly to first-line



immunosuppressive therapies. This preferential response could be explained by different pathogenesis of MG in these antibody subtypes. Complement fixing IgG1 and IgG3 are the main antibody subclasses in AChR+MG, whereas in MuSK+MG the prevalent subtype is IgG4, which acts by disrupting the function of the target or the interaction between the target and partner protein, without the ability to fix complement or cross-link antibodies. The effectiveness of rituximab has also been established in other IgG4-mediated disorders, such as chronic inflammatory demyelinating polyneuropathy associated with nodal and paranodal antibodies. Other explanations for the difference in efficacy of rituximab between AChR+ and MuSK+ MG include the involvement of different subsets of T-helper cells (T-helper 1 cells responsible for the generation of IgG1 and IgG3 vs T-helper 2 cells for the production of IgG4); and the preferential production of IgG4 by short-lived plasma cells, which are depleted by rituximab, while having little or no effect on long-lived plasma cells.<sup>35</sup>

### Efficacy of Eculizumab in Treating Fatigue

Fatigue is known to be a problematic symptom for patients with gMG, as it contributes significantly to decreased quality of life.<sup>36</sup> A post-hoc analysis of REGAIN and its OLE examined the effect of eculizumab versus placebo on fatigue in these patients with severe, treatment-refractory AChR+ gMG as measured by change from baseline in the Neuro-QOL Fatigue subscale score. In the REGAIN trial, patients in the eculizumab group achieved a mean change of -16.3 compared to -7.7 in the placebo group. By week 4 of the OLE, patients in the placebo/eculizumab group achieved a mean change of -17.4 compared to -17.8 in the eculizumab/eculizumab group. These improvements in fatigue were maintained through week 52 of the OLE.<sup>37</sup>

### Efficacy of Eculizumab in an Asian Cohort

Asian patients with MG have clinical (higher incidence of juvenile-onset and ocular MG) and genetic (HLA antigens) differences compared to Caucasian patients. These differences may influence immune-pathogenesis, and therefore a response to immune-mediated treatments, in this patient population. A post-hoc subgroup analysis of REGAIN and its OLE evaluated the safety and efficacy of eculizumab in Japanese study participants compared to Caucasian study participants and found that eculizumab was similarly well-tolerated and effective among these patient populations.<sup>38–41</sup>

### Effect of Eculizumab on Concomitant Immunosuppressive Therapy

Study participants in the OLE were on a stable regimen of ISTs (including corticosteroids, azathioprine, mycophenolate mofetil, methotrexate, cyclosporine, tacrolimus, and cyclophosphamide) at the time of enrollment. At the OLE baseline visit, 98.3% of the participants were using at least one IST, and adjustment of ISTs was permitted throughout the course of the trial. A post-hoc analysis of the OLE data found that a significant proportion of patients (48.7%) treated with eculizumab were able to decrease or even discontinue concomitant ISTs. Notably, of the study participants using corticosteroids at the OLE baseline visit, 47.9% were able to decrease the dose of corticosteroids, and 11.1% were able to discontinue corticosteroids. Additionally, clinical improvement occurred independently of the type of IST or change in IST, suggesting that this improvement was due to the treatment effect of eculizumab.<sup>42</sup>

### Response to Eculizumab in Patients with gMG Recently Treated with Chronic IVIg

Similar to rituximab, IVIg has historically been used in the treatment of severe, treatment-refractory, AChR+ gMG. A post hoc subgroup analysis of REGAIN and its OLE examined the efficacy of eculizumab in study participants who had previously received IVIg at least four times per year. This analysis found that eculizumab was no less effective in patients who previously required chronic IVIg treatment.<sup>43</sup>

### Real-World Evidence for the Use of Eculizumab in MG

Real-world studies of eculizumab in MG have demonstrated efficacy and safety similar to that reported in the Phase II, phase III, and OLE trials. A retrospective review of 15 patients with treatment-refractory AChR+ gMG treated with eculizumab at the University of Missouri between 2016 and 2019, showed reductions in MG-ADL scores in all patients, decreased number of MG exacerbations, an increase in single-breath count test score in all patients, achievement of QMG classification of “none” or “mild” in all patients within 12 months of eculizumab initiation, and reductions in concomitant

MG medications, including corticosteroids, in all patients. This retrospective review also demonstrated similar safety data, with 10 mild adverse events reported.<sup>44</sup>

A post-marketing interim analysis of 40 patients with treatment-refractory AChR+ gMG treated with eculizumab in Japan between 2017 and 2019 similarly showed reductions in mean MG-ADL and QMG scores, as well as reductions in IVIg treatments. This study also demonstrated safety data at par with the phase II, phase III, and OLE trials, with 16 adverse events reported.<sup>45</sup>

## The Future of Eculizumab in gMG

Eculizumab obtained FDA approval for the treatment of AChR+ gMG on October 23, 2017. In 2020, the American Academy of Neurology published an updated version of the International Consensus Guidance on Management of Myasthenia Gravis, which formally recommends the use of eculizumab for patients with severe, treatment-refractory, AChR+ gMG.<sup>46</sup> While the approval of eculizumab undoubtedly represents a huge step forward in the treatment of severe, refractory MG, many unanswered questions about the use of this medication still remain.

### Characterization of Eculizumab Non-Responders

Despite the marked efficacy of eculizumab demonstrated in REGAIN and its OLE, 25% of the patients with the treatment-refractory disease did not respond to the medication. Details about these non-responders' baseline characteristics, MG history, and co-morbidities could be useful in determining positive predictors of treatment benefit or lack of response. Studies have already evaluated the feasibility of using serologic measures of complement levels and activity to monitor treatment response to anti-complement therapies.<sup>47</sup> Further research into these serologic measures may provide a reliable biomarker to monitor MG disease activity and accurately predict treatment response to eculizumab. Genetic and epigenetic factors represent other avenues to predict treatment response to anti-complement therapies. Genetic variants that lead to eculizumab resistance have already been identified in PNH patients,<sup>48</sup> so it is reasonable to hypothesize that similar genetic variants will impede eculizumab efficacy in MG patients. MicroRNAs (miRNAs) have a lot of potential as epigenetic biomarkers to predict treatment response to anti-complement therapies. Several miRNAs have already been found to play a role in the regulation of complement-dependent cytotoxicity.<sup>49,50</sup>

### Optimal Dose, Timing and Treatment Duration with Eculizumab

The studies of eculizumab in severe, treatment-refractory AChR+ gMG all used the same dosing regimen; this is further discussed in the section on dosing of eculizumab. Eculizumab is used at a lower dose in the treatment of PNH, so further studies regarding optimal dose, timing and treatment duration in MG are required.<sup>20,21</sup> According to some experts, after 6 months of successful treatment and confirmation of maximal responsiveness, eculizumab infusion intervals could be lengthened.

### Tapering and Discontinuation of Eculizumab

Similarly, there is a paucity of data regarding optimal tapering and discontinuation of eculizumab in MG. In REGAIN and its OLE, most clinical benefits were observed within 3 months, so clinicians should consider discontinuation of eculizumab if significant improvement is not observed in that period. Since eculizumab is unlikely to alter the antibody production, tapering is suggested as sudden discontinuation may lead to the rapid return of weakness.<sup>51</sup>

### Use of Eculizumab in Subtypes of MG Including Seronegative and Thymoma-Associated Disease

Eculizumab is currently only approved and recommended for treatment-refractory AChR+ gMG in the United States, but there is a lot of potential, and in fact some early data, to suggest its efficacy in other MG subtypes. A retrospective analysis of eculizumab in AChR-gMG resulted in clinically meaningful improvements in MG-ADL score, QMG score, and respiratory function in all patients. Additionally, all patients experienced a statistically significant reduction in MG exacerbations, and all were able to reduce steroid dose and discontinue at least one other MG medication.<sup>52</sup> Greenwood et al reported a case of a treatment-refractory seronegative gMG patient who after transitioning from thrice-weekly plasma exchange to eculizumab showed significant and sustained decrease in MG-ADL scores.<sup>53</sup> The phase II and phase III trials of eculizumab excluded patients with a history of thymoma or thymic neoplasm, but a case report of a patient

with severe, treatment-refractory, thymomatous AChR+ gMG found eculizumab to be a well-tolerated and effective treatment. The patient experienced significant improvement in motor symptoms within 8 weeks of initiating eculizumab therapy, as well as improvement in QMG and MG-ADL scores, and those improvements were maintained for 1 year. Additionally, the patient experienced no further MG crises requiring admission, and was able to decrease the corticosteroid requirement, and increase the interval between IVIg treatments.<sup>54</sup> Amano et al reported a case of treatment-refractory thymomatous AChR+ and anti-striational antibody+ gMG who achieved minimal manifestations of disease with the addition of eculizumab.<sup>55</sup>

Rigorous studies of eculizumab in the treatment of other MG subtypes are needed, and there is good reason to expect that it will play a major role in the field moving forward.

### Eculizumab Use in Different Stages of MG

Rapid improvement was seen during the induction phase in the REGAIN placebo group patients who were switched to eculizumab in the OLE study; statistically significant benefits were observed as early as after the first infusion. Given this rapid effect, the role of eculizumab during MG exacerbations or as a bridge to longer-acting treatments needs to be determined. Several case reports and case series have also suggested eculizumab to be an effective rescue treatment for myasthenic crisis.<sup>56,57</sup> Similarly, its use as a single agent rather than add-on therapy needs to be studied.

## Practical Considerations for the Use of Eculizumab in the Treatment of Refractory, Non-Thymomatous, AChR + Generalized Myasthenia Gravis Required Vaccinations (Figure 3)

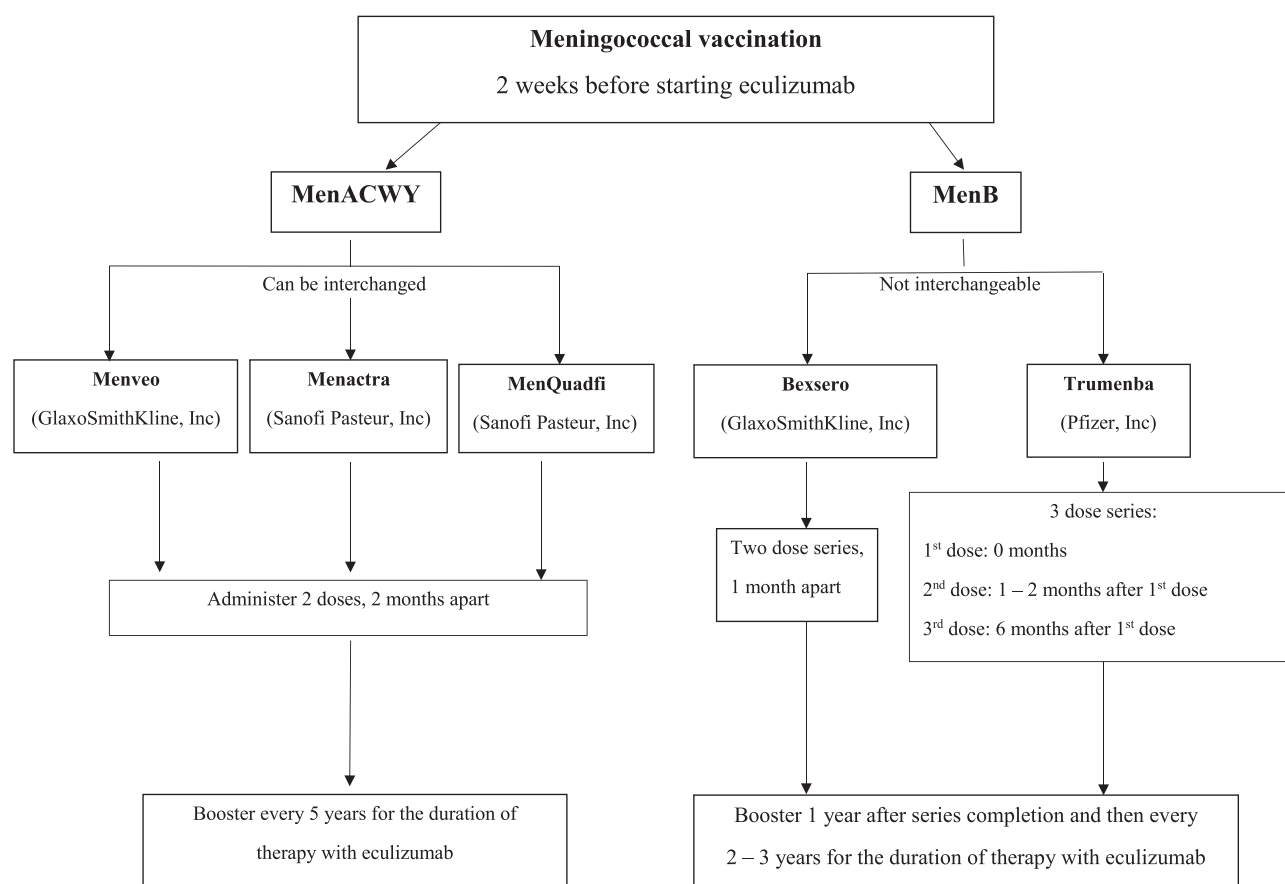
Therapy with eculizumab raises the risk of life-threatening Neisserial infections, such as *N. meningitidis*, and has been linked to a 1000- to 2000-fold increase in the incidence of meningococcal illness. Therefore, before treatment initiation with eculizumab, patients must receive two types of meningococcal vaccinations, at least 2 weeks before their first dose.

The first type is the quadrivalent meningococcal conjugate vaccine against several serotypes of *N. meningitidis* (Men ACWY). The brand names of this vaccine in the United States are Menactra® (Sanofi Pasteur, Bridgewater, NJ, USA), and Menveo® (GlaxoSmithKline LLC, Philadelphia, PA, USA).<sup>46,58</sup> It is recommended to administer all doses using the same vaccine, but quadrivalent vaccination can be interchangeable.<sup>59</sup> Two doses need to be administered, 2 months apart. A booster dose of Men ACWY is recommended every 5 years for the duration of therapy with Eculizumab.<sup>59,60</sup>

The second type of vaccine is for *N. meningitidis* serotype B (MenB). The brand names for type B vaccines in the United States are Bexsero® (GlaxoSmithKline LLC, Philadelphia, PA, USA) and Trumenba® (Pfizer, New York, NY, USA). The two brands are not interchangeable. Bexsero is a two-dose series, with vaccines given at least 1 month apart. Trumenba is a three-dose series, where the second dose is 1–2 months after the first dose and the third dose is due in the sixth month.<sup>46,55</sup> It is recommended that patients get a booster dose of MenB vaccine a year after the completion of the series, and then every 2–3 years while on treatment with eculizumab.<sup>59,60</sup>

Neurologists should be vigilant that vaccination decreases, but does not eliminate, the risk of meningitis. Enrollment and certification with the eculizumab REMS (Risk Evaluation and Mitigation Strategy) program is also required before starting treatment. Patients must be counseled of the risk and symptoms of meningococcal meningitis, and the use of a safety bracelet/information card is strongly encouraged.<sup>55</sup>

In situations when urgent start of treatment is warranted, and the patient has received vaccinations within 2 weeks prior to starting eculizumab, then 2 weeks of antibacterial prophylaxis is recommended. The recommended antibiotic and dosing is Penicillin VK 250–500 mg every 12 hours.<sup>46</sup> If the patient has a penicillin allergy, a macrolide antibiotic is recommended instead, such as azithromycin 500 mg daily or erythromycin 500 mg twice a day.<sup>46,61</sup> Fluoroquinolone antibiotics, such as ciprofloxacin 500 mg daily, can also be used as a penicillin alternative.<sup>46</sup> Both macrolide and fluoroquinolone antibiotics in patients with penicillin allergy should be prescribed with extreme caution under the supervision of a neuromuscular physician, as these antibiotics have the potential of causing MG exacerbation.<sup>46</sup>



**Figure 3** Vaccination for *Neisseria meningitidis*.

## COVID 19 Vaccination in Mg Patients

### Safety of COVID-19 Vaccination in Patients Receiving Eculizumab Treatment for MG

In general, vaccination for MG patients including for COVID-19 is recommended, especially for those receiving immunosuppressive agents.<sup>62</sup> While the details of the individual immune therapy were not provided, a retrospective Italian cohort study in MG patients compared the MG-ADL scores before and after the administration of COVID-19 vaccines; 90% received the BNT162b2 vaccine (Pfizer-BioNTech), 7.5% were given the mRNA-1273 vaccine (Moderna), and one patient received the ChAdOx1 nCoV-19 vaccine (AZD1222, AstraZeneca). The study showed no significant change in the MG-ADL scores, suggesting a favorable short-term safety profile, as well as the efficacy of these vaccines in avoiding life-threatening complications, such as MG exacerbation and COVID-19 pneumonia. Worsening of MG-ADL scores in only a few patients following vaccination was found to be either coincidental or due to other contributing factors such as exacerbation of chronic obstructive pulmonary disease, radiculopathy, or refusal of part of the treatment. In conclusion, the use of the COVID-19 vaccine was supported by data presented in the study, even in the presence of active immunomodulating therapies.<sup>63</sup> While COVID-19 vaccination appears to be safe in MG, due to the possibility of potential flare-up of existing MG, it is reasonable to postpone vaccination in patients with myasthenic crisis and severe bulbar symptoms.<sup>64</sup> Future prospective trials are required to confirm these observations.

### Efficacy of COVID Vaccination in Eculizumab-Treated MG Patients

There is a dearth of high-quality data regarding the efficacy of COVID-19 vaccination in Eculizumab-treated MG patients. Currently, there are no protocols in place for COVID-19 vaccinations before beginning Eculizumab treatment. A case report measuring the immunogenic response to a course of an mRNA COVID-19 vaccine in a patient treated with mycophenolate, prednisone, and eculizumab found that an immune response was initially not achieved after BNT162b2 (Pfizer-BioNTech)

vaccination. It was only after the discontinuation of eculizumab, and the administration of two doses of an alternative mRNA vaccine (M1273, Moderna) that a specific IgG response to the SARS CoV-2 spike protein was seen. The authors concluded that while eculizumab might have contributed to the lack of an immune response to the first vaccine, it was more likely related to concomitant treatment with mycophenolate and prednisone.<sup>65</sup> The risk of delaying vaccination, and thus failing to mitigate COVID-19 risk, should be balanced against a possible blunted response to the vaccine. As part of a collaborative decision-making process, this choice should be personalized. While there is a lack of scientific evidence in this instance, in a patient with stable MG treatment with eculizumab could be held for 1–2 weeks before COVID-19 vaccination.

## Use of Eculizumab in Patients with Active COVID-19 Infection

Increased susceptibility to COVID-19 infection in eculizumab-treated MG patients has been reported,<sup>66</sup> however, owing to the blockade of C5 complement, which is thought to be elevated in COVID-19 infection, eculizumab could conceivably have a beneficial therapeutic effect in active COVID-19 infection.<sup>67,68</sup> The use of Eculizumab in patients actively infected with COVID-19 is also currently being studied in the SOLID-C19 trial.<sup>69</sup> According to available published research, eculizumab could be continued in MG patients with active COVID infection.

## Pharmacokinetics of Eculizumab

The elimination half-life of eculizumab is approximately 270–375 hours and plays a role in the need for every 2-week dosing.<sup>70</sup> The clearance of eculizumab is increased almost to 250-fold, and elimination half-life is reduced to 1.26 hours, in patients receiving fresh frozen plasma (FFP) or PLEX, thus necessitating supplemental doses in these circumstances.<sup>70</sup> Eculizumab does not have known active metabolites.<sup>71</sup>

## Dosing of Eculizumab

The medication is given intravenously, and dose during the *Induction phase* is 900 mg every week for 4 doses. For the *interim phase*, week 5, patients receive a 1200 mg infusion. The *maintenance phase* starts from week 6 onwards, where the dose is 1200 mg every 2 weeks.<sup>72</sup> A hospital-designed medication order set that specifies the dose at each interval, helps to reduce the chance for errors regarding the dose and corresponding intervals (Table 1).

## Dose Adjustment of Eculizumab During Concomitant Treatment with PLEX

For patients who are receiving PLEX, additional doses are needed depending on the most recent dose. Extra doses of eculizumab 600 or 300 mg are given within 60 minutes after PLEX, if the last doses given were equal to or greater than 600 or 300 mg, respectively.<sup>70</sup>

## Dose Adjustment of Eculizumab During Treatment with FFP Infusion

For patients receiving an FFP infusion, an additional eculizumab dose of 300 mg is given within 60 minutes before the infusion, if the last dose given was equal to or greater than 300 mg.<sup>70</sup>

## Contraindications to Use of Eculizumab

Eculizumab use is contraindicated in patients who lack *N. meningitidis* vaccination unless the benefit of treatment outweighs the risk of infection in patients who are unable to undergo vaccinations.<sup>70</sup>

## Eculizumab in Pregnancy

A case report discussed the successful treatment of refractory gMG before, during, and after pregnancy.<sup>72</sup> No adverse events were reported in the mother or newborn, suggesting a favorable benefit versus risk profile for eculizumab treatment during pregnancy.<sup>72</sup> Treatment of MG during pregnancy with eculizumab may be beneficial, with a reduction in maternal complications and high fetal survival.<sup>73</sup> However, further studies are warranted to confirm this finding, and benefit versus risk should be assessed in pregnant patients on a case-by-case basis.



**Table I** Eculizumab Therapy Plan

Eculizumab (Soliris) for Myasthenia Gravis											
Provider information											
<ul style="list-style-type: none"> <li>Soliris Risk Evaluation and Mitigation Strategy (REMS) Program: <i>providers must enroll in the program.</i> <ul style="list-style-type: none"> <li>1-888-SOLIRIS (1-888-765-4747) or solirisrems.com</li> </ul> </li> <li>Indications: <ul style="list-style-type: none"> <li>Generalized myasthenia gravis (gMG, anti-acetylcholine receptor antibody-positive)</li> </ul> </li> <li>Confirm vaccination status</li> </ul>											
Pre-medications											
<ul style="list-style-type: none"> <li>Patients are pre-medicated at least 30 minutes before infusion but not longer than 1 hour before infusion: <ul style="list-style-type: none"> <li>Acetaminophen (Tylenol) 650mg PO once</li> <li>Diphenhydramine (Benadryl) 50mg PO once or alternative medication if needed</li> <li>Methylprednisolone 40mg IV once</li> </ul> </li> </ul>											
IV Line Care											
<ul style="list-style-type: none"> <li>0.9% sodium chloride infusion 250mL IV at 10 mL/hr run continuously to keep vein open</li> <li>Heparin 500 units intracatheter prn for IV line care per nursing policy</li> </ul>											
Dosage/Administration instructions											
<ul style="list-style-type: none"> <li><b>Myasthenia Gravis Dosing</b> <ul style="list-style-type: none"> <li>Soliris must be given at the recommended regimen time points or within 2 days</li> </ul> </li> </ul>											
Refractory gMG Adult (≥18 years of age) Dosing Schedule											
Pretreatment		Induction Phase				Interim Phase	Maintenance Phase				
≥2 weeks before induction	Week	1	2	3	4	5	6	7	8	9	q14d
<i>Neisseria meningitidis</i> vaccination	Soliris Dose	900 mg	900 mg	900 mg	1200 mg	1200 mg	–	1200 mg	–	1200 mg	
Supplemental dose required in case of plasmapheresis, plasma exchange or fresh frozen plasma infusion.											
<ul style="list-style-type: none"> <li><b>Administration</b> <ul style="list-style-type: none"> <li>Do not administer as IV Push or Bolus</li> <li>Diluted to a final concentration of 5mg/mL <ul style="list-style-type: none"> <li>Eculizumab (Soliris) 900mg in 0.9% sodium chloride 180mL IV infusion over 35 minutes</li> <li>Eculizumab (Soliris) 1200mg in 0.9% sodium chloride 240mL IV infusion over 35 minutes</li> </ul> </li> <li>Pediatric Patients: Administration over 1–4 hours</li> <li>Infusion reactions: Infusion rate can be slowed but <b>cannot exceed 2 hours total infusion time.</b></li> </ul> </li> </ul>											
Monitoring Needs											
<ul style="list-style-type: none"> <li>Complete vital signs (BP, pulse, temperature) prior to infusion and 1 hour after infusion. <ul style="list-style-type: none"> <li>Repeat vital signs with infusion rate change if applicable.</li> </ul> </li> <li>Monitor signs/symptoms of hypersensitivity during infusion and for 1 hour post-infusion</li> <li>If infusion reaction occurs, monitor patient for respiratory and cardiovascular reactions for 2 hours post infusion.</li> </ul>											
Common Side effects with corrective actions and emergency medications											
<ul style="list-style-type: none"> <li>Nausea → Ondansetron (Zofran) 8mg IV once prn</li> <li>Pain/Headache/Fever → Acetaminophen 325–650mg oral prn</li> <li>Peripheral Edema</li> <li>Infusion Reactions</li> <li>Muscle/Back pain</li> <li>Sinusitis</li> <li>Cough</li> <li>Constipation</li> </ul>											
Emergency medications											
<ul style="list-style-type: none"> <li>Diphenhydramine (Benadryl) 25–50mg PO or 50mg IV prn per nursing judgment for infusion reaction</li> <li>Epinephrine (Adrenalin) 0.3mg IM once prn for severe infusion reactions and anaphylaxis</li> <li>Acetaminophen (Tylenol) 325–650mg oral prn for infusion reaction, pain, headache, or fever</li> <li>Ondansetron (Zofran) 8mg IV once prn</li> </ul>											

(Continued)

**Table I** (Continued).

Acute infusion reactions and corrective measures		
Mild Hives, pruritus, throat itching, headache, nausea, dizziness (NO hypotension), hyperemia, urticarial, GI upset	Moderate Wheezing, rigors, dysphagia, mild dyspnea (No hypotension), chest discomfort, palpitation, elevated temp	Severe Significant hypotension and chest discomfort, significant shortness of breath, bronchospasm, angioedema, stridor, abdominal cramping with nausea and vomiting accompanied by hypotension
<ol style="list-style-type: none"> <li>1. Pause infusion</li> <li>2. Send covering provider FYI page</li> <li>3. Administer diphenhydramine, acetaminophen, or ondansetron if need</li> <li>4. Check vital signs, and then monitor q 15 min till within normal limits (WNL)</li> <li>5. If the patient does not return to baseline then page on-call provider</li> <li>6. If symptoms resolve and the patient returns to baseline then re-challenge at half the rate</li> </ol>	<ol style="list-style-type: none"> <li>1. Stop infusion</li> <li>2. Call on-call provider</li> <li>3. Administer diphenhydramine, acetaminophen, or ondansetron if need</li> <li>4. Monitor VS q 5 min till WNL</li> <li>5. Discuss with provider how to proceed: if symptoms resolve can consider restarting at a slower rate not to exceed a total infusion time of 2 hours</li> </ol>	<ol style="list-style-type: none"> <li>1. Stop infusion</li> <li>2. Have someone call code (111) then the on-call provider</li> <li>3. Administer epinephrine, diphenhydramine, acetaminophen, or ondansetron if need</li> <li>4. Stabilize patient till code team arrives, continue monitoring VS</li> <li>5. Continue with code procedure</li> </ol>

## Eculizumab and Breastfeeding

Eculizumab was not detected in the breast milk of nursing women who were on treatment for PNH.<sup>73</sup>

## Eculizumab and Renal/Hepatic Impairment

The pharmacokinetics of eculizumab is not affected by renal impairment; no adult studies to date have assessed the effect of hepatic impairment on the pharmacokinetics of eculizumab.<sup>70</sup>

## Cost of Treatment with Eculizumab

The current average cost of eculizumab (brand name Soliris<sup>®</sup>, Alexion Pharmaceuticals Inc. Boston, MA, USA) per mL is \$260.92. It has been reported that it is the most expensive drug to treat MG, with the cost being more than \$60,000/month in the United States. Putting this into perspective, the monthly cost of treatment with high-dose prednisone is \$15, and that of ISTs such as mycophenolate mofetil and azathioprine is in the hundreds of dollars. The annual cost of treatment with rituximab ranges between \$20,000 and \$40,000. A course of treatment with 2g/kg of IVIg in a 70kg patient, or treatment with 5 PLEX treatments, ranges between \$20,000 and \$35,000. It is still to be determined if the cost of treatment with eculizumab can be offset by reducing other related healthcare costs in MG patients.<sup>28</sup>

## Future Complement Based Therapies (Figure 1)

### Ravulizumab (Brand Name Ultomiris<sup>®</sup>, Alexion Pharmaceuticals Inc. Boston, MA, USA)

Ravulizumab is another recombinant humanized monoclonal antibody that binds to and inhibits the C5 complement protein. It differs from eculizumab by amino acid substitutions in the Fc region of eculizumab that provide high affinity for C5, and an immediate and sustained reduction in C5.<sup>74,75</sup> This amino acid sequence alteration also leads to a longer half-life of the antibody, due to recycling through the neonatal Fc receptor (FcRn) pathway, thus reducing the frequency of administration to every 8 weeks, compared to every 2 weeks for eculizumab. Treatment with ravulizumab requires a weight-based loading dose, followed by weight-based maintenance doses 2 weeks later and then every 8 weeks thereafter. It is currently approved for the treatment of aHUS and PNH. On the basis of a beneficial outcome in the phase III clinical trial to investigate its efficacy and safety in MG, ravulizumab has now been FDA approved as of April 28,

2022.<sup>76</sup> The cost of ravulizumab is estimated to be much higher than that of eculizumab per mL. The projected average cost per mL of ravulizumab (Intravenous) is \$2,561.60.

## Zilucoplan

Zilucoplan is a synthetic macrocyclic peptide that inhibits MAC formation by a dual mechanism: 1) it binds to the C5 complement protein, thereby blocking the cleavage to C5a and C5b; and 2) it directly inhibits the first step of MAC assembly (ie, C5b-C6 binding).<sup>74</sup> The binding site for zilucoplan on C5 is different from that for eculizumab, as demonstrated by its ability to bind C5 in blood samples of patients who are genetically resistant to eculizumab. The phase III RAISE trial was recently concluded, showing favorable efficacy, safety and tolerability in gMG patients. The dose of zilucoplan used in the 12-week placebo-controlled RAISE trial was 0.3mg/kg daily by subcutaneous administration.<sup>77</sup>

## Future Pathophysiologic Target-Based Interventions in MG

1. Traditional therapeutic interventions in MG include the use of pharmacological agents such as AChEs, corticosteroids and other immunosuppressants; thymectomy; and use of interventions for disease exacerbations, which include PLEX and IVIg. These treatment approaches will not be discussed here but can be reviewed in previously published articles.<sup>4,5</sup>
2. Therapies in MG employing disease-specific mechanistic approaches include the following:

### Anti-IFN Therapies (Figure 2C)

Since thymic overexpression of Type-I interferon (IFN-I), especially IFN- $\beta$ , seems to be the main orchestrator of thymic changes, treatment with monoclonal antibodies against IFN-I (rontalizumab or sifalimumab) could be considered. Further, there is an increasing recognition of the role of miRNAs in EAMG. miRNAs regulate post-transcriptional gene expression by binding and degrading messenger RNA, leading to inhibition of translation and protein expression. MiR-29 subtypes play a role in modulating the IFN-I signal in the thymus of mice with EAMG by favoring increased expression of IFN- $\beta$  and emergence of pro-inflammatory Th17 cells. This strategy could be considered not only for monitoring but also for possible future therapy of MG.<sup>78</sup>

### Therapy with Chemokine Antagonists (Figure 2C)

Since chemoattractant (CXCL13) upregulation on TEC is critical for cellular recruitment, the use of CXCL13 and other chemokine targets could be another potential therapeutic strategy in MG.<sup>79</sup>

### T-Cell Co-Stimulatory Pathway-Based Therapies (Figure 2F)

Following the initial T-cell receptor (TCR) and peptide-MHC interaction, co-stimulation enhances or inhibits T-cell activation. Positive co-stimulatory pathways include B7-CD28, CD40 ligand (L)-CD40, inducible T-cell co-stimulator protein (ICOS) ICOS-ICOS-L, and OX40-OX40L. Negative co-stimulatory pathways include B7-cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death (PD)-1-PD-L1. These interactions activate downstream events, leading to increased release and binding of IL-2 to the IL-2 receptor (CD25), which through activation of the Janus kinases (JAK) – Signal Transducer and Activator of Transcription system promote lymphocyte proliferation and differentiation into T-helper cells. Potential therapeutic targets include monoclonal antibodies against CD80/86 (abatacept [Orencia<sup>®</sup>, Bristol-Myers Squibb Company, Princeton, NJ, USA]), CD40 (iscalimab), ICOS, CD25 (daclizumab) and JAK (tofacitinib [Xeljanz<sup>®</sup>, Pfizer Inc. New York, NY, USA]). Another treatment approach is the use of the chimeric antigen receptor (CAR) T-cell therapy in which T-cells from patients are harvested and genetically modified to express a CAR designed to recognize and bind to a target antigen, which allows CAR T-cells to identify and attack target cells.<sup>80,81</sup>

## T-Cell-Associated Cytokines (Figure 2G)

Upregulation of Th17 and Tfh, along with their cytokines in MG can provide a basis for treatment with an IL-6 receptor antagonist (tocilizumab [ACTEMRA<sup>®</sup>, Genentech, Inc. San Francisco, CA, USA]) or monoclonal antibodies against IL-17 (brodalumab, inekizumab). The downregulation of Tregs can potentially be treated with vitamin D, rapamycin or granulocyte-macrophage colony-stimulating factor.<sup>81</sup>

## Agents Targeting B-Cells (Figure 2G)

This involves direct targeting with biologics, including inhibition of CD20 using a first-generation agent (rituximab) or next-generation agents (ocrelizumab, ofatumumab). CD20 is present on all B-cells except for stem cells, pro-B-cells, and plasma cells. Also targeted could be CD19 using inebilizumab (Uplizna<sup>®</sup>, Horizon Therapeutics Ireland DAC, Dublin, Ireland), which depletes almost all B-cells, including plasmablasts, but not plasma cells and stem cells. Of potential therapeutic benefit might also be the use of indirect B-cell inhibitors that target BAFF (belimumab [Benlysta<sup>®</sup>, GlaxoSmithKline LLC, Philadelphia, PA, USA]) and the BAFF/APRIL receptor (atacept) which block transmembrane activator, calcium modulator and cyclophilin ligand interactor (TACI); and use of a proteasome inhibitor such as bortezomib, which causes apoptosis of highly active plasma cells.<sup>82</sup>

## Fc and Neonatal FcRn Receptor Modulation (Figure 2M)

Fc receptors are involved in antibody-mediated effector functions and complement activation. Engineered IVIg preparations with increased sialic acid levels (glycosylation) of Fc core IgG molecules lead to suppression of inflammation through upregulation of inhibitory FcγRIIB receptors on different immune cells.<sup>83</sup> Since the IgG recycling pathway (Figure 2M) is mediated by the FcRn, treatment with FcRn blockers (eg, efgartigimod [Vyvgart<sup>®</sup>, Argenx BV, Zwijnaarde, Belgium], rozanolixizumab, nipocalimab and batoclimab) prompts the degradation of IgG in lysosomes, resulting in a decrease in IgG levels (chemical plasmapheresis).<sup>81</sup>

## Modification of Motor Endplate-Related Specific Factors (Figure 2H–J)

The clustering of AChRs on the postjunctional membrane of the NMJ can potentially be strengthened by the use of SHP2 inhibitors (which are negative regulators of the AChR clustering pathway) and β2-adrenergic agonists (via stabilization of AChRs at the postsynaptic membrane, mediated through protein kinase A, a downstream effector of β2 receptors).<sup>84</sup> Another approach is the potential use of antisense oligonucleotides (ASOs) against the AChE-R isoform.<sup>85</sup> Under physiological conditions, splicing of the AChE gene produces the predominantly AChE-S isoform. However, acute AChE treatment shifts the splicing of the AChE pre-mRNA to the rare, AChE-R variant, which through increased ACh hydrolysis restores the balance between the ACh and AChE levels. Targeting exon 2 of the AChE mRNA, using the ASO “EN101”, results in excessive destruction of AChE-R mRNA and restores ACh levels in the synaptic cleft.

## Conclusions

The success of the eculizumab clinical trials is a paradigm shift in the management of MG and might alter the future of MG treatment away from the current use of the generalized non-specific immunosuppressive therapies to pathophysiological mechanism-based directed therapies. Infection, particularly meningococcal meningitis, is the most serious adverse effect. While eculizumab has demonstrated its efficacy in the management of refractory gMG as the first complement inhibitor, many unanswered questions need to be addressed about the use of this medication. Future use of pharmacogenetics, and the potential use of biomarkers, would be useful to tailor and monitor mechanistic-based targeted therapies in the different subtypes of MG.

## Abbreviations

ACh, acetylcholine; AChE, acetylcholinesterase; AChR, acetylcholine receptor; aHUS, atypical hemolytic uremic syndrome; AIRE, autoimmune regulator; APC, antigen-presenting cell; APRIL, a proliferation-inducing ligand; ASO,

antisense oligonucleotides; BAFF, B-cell-activating factor; CAR, chimeric antigen receptor; CD55, complement decay-accelerating factor 55; ColQ, collagen Q; CXCL, chemokine ligands; DOK7, docking protein 7; EPP, end plate potential; EAMG, experimental autoimmune myasthenia gravis; FcRn, neonatal Fc receptor; FFP, fresh frozen plasma exchange; GC, germinal center; gMG, generalized myasthenia gravis; ICOS, inducible T-cell co-stimulator protein; IFN- $\beta$ , interferon beta 1A; IgG, immunoglobulin G; IL, interleukin; IST, immunosuppressant therapies; IVIg, intravenous immunoglobulin; JAK, janus kinase inhibitors; Kv1.4, potassium voltage-gated channel subunit 4; L, ligand; LRP4, low-density lipoprotein receptor-related protein 4; MAC, membrane attack complex; MACF1, microtubule-actin crosslinking factor 1; MG, myasthenia gravis; MG-ADL, Myasthenia Gravis Activities of Daily Living; MGC, myasthenia gravis composite; MG-QOL, Myasthenia Gravis Quality of Life; MHC, major histocompatibility complex; MIR, main immunogenic region; miRNAs, Micro RNAs; MuSK, muscle-specific kinase; NMJ, neuromuscular junction; OLE, open-label extension; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PLEX, plasma exchange; PNH, paroxysmal nocturnal hemoglobinuria; QMG, quantitative myasthenia gravis; REMS, risk evaluation and mitigation strategy; RNS, repetitive nerve stimulation; RyR, ryanodine receptor; SHP2, Src homology-2-domain-containing protein tyrosine phosphatase 2; TCR-T, cell receptor; TEC, thymic epithelial cell; Tfh, T follicular helper cell; TH-17, T-helper 17 cell; Treg, regulatory T-cell.

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## Author Contributions

All authors made significant contributions to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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