Differential Response To Rituximab In Anti-Achr And Anti-Musk Positive Myasthenia Gravis Patients

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Differential response to rituximab in anti-AChR and anti-MuSK positive myasthenia gravis patients

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

Tess Deutch Litchman

2020
DIFFERENTIAL RESPONSE TO RITUXIMAB IN ANTI-ACHR AND ANTI-MUSK POSITIVE MYASTHENIA GRAVIS PATIENTS: A SINGLE-CENTER RETROSPECTIVE STUDY
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Response to immunomodulatory therapies in myasthenia gravis (MG) can be variable. A subset of MG patients remains refractory to conventional agents. B-cell targeted therapy with rituximab has demonstrated a durable response in treating refractory myasthenia gravis (MG). This study compares the response to rituximab between patients with acetylcholine receptor autoantibody positive (AChR+) and muscle-specific kinase autoantibody positive (MuSK+) MG.

This retrospective study included 33 patients with either AChR+ or MuSK+ MG who were treated with rituximab from 05/31/2003 to 05/31/2017. Pretreatment and post-treatment immunotherapy regimens, clinical symptoms, and examination findings were evaluated.

Median MGFA Class of II at baseline improved to an asymptomatic median classification at 12-months and last follow-up (p-values <0.001) post-rituximab. Improvement in MGFA class was not significantly different between the groups. Twenty-one patients achieved clinical remission (12/17 AChR+, 9/16 MuSK+) with time to remission of 441.4 ± 336.6 days for AChR+ versus 230 ± 180.8 days for MuSK+ patients (p-value 0.049). The mean prednisone dosage requirement decreased significantly in both groups
post-rituximab (p-value <0.01). AChR+ patients required more hospitalizations for exacerbation post-rituximab (p-value 0.046).

In conclusion, rituximab therapy is observed to have both a clinical benefit and durable response in the majority of AChR+ and MuSK+ MG patients with refractory disease, supporting the role of B cell depletion in the management of MG. While there was no significant difference between these groups in terms of clinical improvement, symptom-free state, and prednisone burden, MuSK+ MG patients may experience greater benefits, including earlier time to remission, fewer exacerbations and hospitalizations post-treatment.
Acknowledgments

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INTRODUCTION

Background

Myasthenia gravis (MG) is an autoimmune disorder of the neuromuscular junction characterized by muscular weakness and fatigue. Autoantibodies bind to the acetylcholine receptors or functionally related proteins in the postsynaptic membrane of the neuromuscular junction, causing decreased uptake of acetylcholine and consequent skeletal muscle weakness\textsuperscript{1-4}. The estimated prevalence of MG is approximately 1-2 per 100,000 with an estimated annual incidence of 7-20 cases per 100,000\textsuperscript{5}.

Muscular weakness is the defining clinical characteristic of MG. Muscles typically involved include the extraocular muscles, leading to ptosis and diplopia, as well as the bulbar muscles, leading to difficulties with speech and swallowing. While oculobulbar involvement is more common, manifestations can generalize to include weakness of the proximal muscles of the extremities and trunk. Generalized muscular involvement is typically symmetric, while ocular involvement is often asymmetric\textsuperscript{1}. Fifteen percent of MG patients have disease restricted only to the ocular muscles, whereas 85% have generalized disease\textsuperscript{6}. Weakness is worsened by repetitive muscle use and varies in severity throughout the day, often worst at the end of the day. Respiratory muscle weakness and consequent respiratory failure can occur with severe disease. However, with adequate treatment, most patients can maintain stable disease characterized only by mild muscle weakness.
**MG Subtypes**

MG is classified into subgroups defined by clinical manifestations, age at onset, thymus pathology, and serology. Up to 80% of MG patients have autoantibodies to the acetylcholine receptor (AChR) and 5-10% have autoantibodies against muscle-specific kinase (MuSK); 20-30% of the patients who do not have autoantibodies against AChR or MuSK have autoantibodies to lipoprotein-receptor-related protein 4 (LRP4)\(^1,7-10\). The remaining 5-10% of MG patients are described as seronegative. Up to 40% of patients who lack autoantibodies to AChR have autoantibodies to muscle-specific kinase (MuSK)\(^10\), making the AChR and MuSK seropositive subtypes the two most common subgroups of MG. The pathogenesis in AChR+ MG is understood to be primarily due to the action of IgG1 autoantibodies which cause a loss of AChR through internalization and localized complement-mediated postsynaptic tissue damage\(^11-13\). MuSK+ MG is characterized by the action of IgG4 antibodies which cause the inhibition of the agrin-LRP4-MuSK pathway by masking binding sites on MuSK, leading to reduced AChR clustering on the postsynaptic membrane\(^14-16\). A complete description of the molecular pathophysiology of myasthenia gravis is beyond the scope of this thesis.

The AChR+ and MuSK+ MG subtypes have been found to have different clinical manifestations. The age of presentation for AChR+ MG follows a bimodal pattern, with peaks at 30 years and 70 years of age. MuSK+ MG patients typically present later with a peak onset after 50 years of age\(^17\). MuSK+ MG has been found to be more common in the Mediterranean and southern Europe than in northern Europe and is more common in the northern regions of Asia than in the southern regions\(^18-20\). These regional differences
are thought to be due to genetic predisposition rather than environmental factors.
Thymoma is typically only seen in AChR+ MG, and AChR+ MG patients respond well
to thymectomy even if no thymoma is found. MuSK+ MG patients usually have no
response to thymectomy, as would be expected due to the less prominent role of the
thymus in the pathophysiology of MuSK+ MG; the lesser role of the thymus in MuSK+
MG is demonstrated histologically by the lack of thymic hyperplasia and other thymic
changes normally seen in AChR+ MG patients\textsuperscript{21-23}. Patients with MuSK+ MG have been
found to have more severe clinical manifestations including more facial and bulbar
muscular involvement, as opposed to the proximal limb weakness seen in AChR+ MG
patients\textsuperscript{21}. Craniobulbar muscle involvement can ultimately be seen in up to 82%-100%
of patients with MuSK+ MG\textsuperscript{24}. Bulbar-onset MuSK+ MG is usually associated with a
more rapidly progressive course\textsuperscript{24}. Patients with MuSK+ MG tend to have more severe
disease manifestations, demonstrated in studies with higher mean QMG scores and
MGFA subclasses\textsuperscript{7,25,26}. Myasthenic crises have been observed to occur 2 to 3 more times
in MuSK+ MG patients than in AChR+ MG patients\textsuperscript{26,27}. Classifying myasthenia into
subgroups can help guide individualized treatment.

\textbf{Current treatment options}

MG was originally thought to be a disorder of hysteria; at that time, the prescribed
treatment was bed rest and avoidance of too much excitement\textsuperscript{28}. Fortunately, much more
is known about MG today. With the first described case most likely dating back to 1664,
as detailed by settlers in Virginia of the “excessive fatigue” and drooping eyelids of
Native American Chief Opechancanough\textsuperscript{29}, MG is one of the oldest identified
neuromuscular disorders, and consequentially its pathophysiology is very well understood. Nonetheless, clinical trials have been challenging to conduct due to the disease’s rarity and fluctuating nature. This has led to significant variation in the management of MG.

While there is general agreement in the neurology community on the use of many treatments for MG, there is no accepted universal standard of care due to the variability in presentation, subtype, and treatment response. In the setting of MG being a rare disease, there have been few randomized controlled trials to date, mainly limited by patient recruitment. The studies that have been conducted\textsuperscript{30-32} often have limited generalizability due to low statistical power, strong patient preferences to take or not take steroids, the long latency of action for many of the agents used in treatment of MG, and the heterogeneous presentation of the disease itself\textsuperscript{33-36}. The ultimate goal of treatment for MG is to render the patient asymptomatic or to lessen the patient’s symptom burden as much as possible while minimizing side effects from medications. Accordingly, treatment response is graded with clinical scoring systems such as the Quantitative Myasthenia Gravis (QMG) and Myasthenia Gravis Foundation of America (MGFA) classification scores. Autoantibody titers following treatment have not been found to correlate with treatment response in AChR+ MG patients\textsuperscript{37}. In this study, AChR autoantibody levels fell in 92% of patients who improved, but also in 63% of patients who did not improve. Thus, following autoantibody levels is not a reliable way to determine treatment response; it is necessary to rely on clinical indicators.
Initial treatment typically includes symptom management with acetylcholinesterase inhibitors, usually pyridostigmine. The mechanism behind these agents is simple: acetylcholinesterase inhibitors delay the degradation of acetylcholine (ACh) in the neuromuscular junction, prolonging the effect of ACh and thereby leading to improvement in strength. Acetylcholinesterase inhibitors only provide symptomatic treatment and are usually not sufficient for disease control for patients with generalized MG. However, some patients can be maintained on pyridostigmine alone. Major side effects include GI upset, but more rarely cholinergic crisis. All groups of MG tend to show some response to acetylcholinesterase inhibitors, but patients with MuSK+ MG have been reported to show a less robust response to pyridostigmine than patients with AChR+ MG. One study noted a nonresponsiveness rate of 71% in MuSK+ MG patients versus 18% of MG patients negative for MuSK+ antibodies, similar to findings in other studies.

For a patient who remains symptomatic on pyridostigmine therapy, the next step in escalation of care is to add immunosuppression. Corticosteroids are often the first-line agent. Treatment effects are dose-dependent, as are side effects. Treatment is typically initiated at a high dose then de-escalated, such as with a transition to an alternate-day regimen. Remission (30%) or marked improvement (45%) occurs in over 75% of patients treated with this regimen. However, many patients with generalized MG require the addition of a nonsteroidal immunosuppressive agent for disease maintenance, including agents such as azathioprine, cyclosporine, mycophenolate mofetil, or tacrolimus. Expert consensus and some randomized controlled trials have
supported azathioprine as the preferred first-line immunosuppressive agent in MG after steroids. Azathioprine can be used in conjunction with prednisone in patients with inadequate response to prednisone or alone in patients with adverse side effects from the steroids. If this regimen fails, patients can be transitioned to mycophenolate mofetil (MMF) or another agent. The delayed onset of action of azathioprine and its adverse effects have spurred interest in alternative agents such as the immunomodulatory drugs. Once patients achieve their treatment goals with their immunosuppressive regimen, the corticosteroid dose can be gradually tapered. Many patients require long-term maintenance with a low-dose corticosteroid. MuSK+ MG patients have been reported to respond less well to immunosuppressive therapy than AChR+ MG patients, experiencing by a lower remission rate and more difficulty in tapering steroids.

Thymectomy, for MG patients with or without thymoma, can also be used as an adjunctive therapy. Thymectomy has been shown to be beneficial in the treatment of generalized AChR+ MG and is recommended for these patients. A recent large randomized controlled trial that compared thymectomy plus prednisone to prednisone alone found improvement in clinical outcomes in patients with non-thymomatous MG, characterized by improvement in clinical status, fewer hospitalizations, and lesser requirements for prednisone and azathioprine therapy. The two-year follow-up of this study similarly found long-lasting benefits of thymectomy in patients with generalized non-thymomatous MG when compared with prednisone alone. Thymectomy is not recommended for patients with MuSK+, LRP4, or ocular forms of MG because no benefit has been established. A recent randomized, retrospective multicenter blinded
review compared thymectomy outcomes for patients with AChR+ vs MuSK+ MG and found that thymectomy was not associated with additional clinical improvement in MuSK+ MG patients\textsuperscript{53}.

Intravenous immunoglobulin (IVIg) and plasma exchange (PLEX) are used as short-term “rescue” therapies in patients with MG experiencing life-threatening acute disease exacerbations characterized by respiratory insufficiency or dysphagia in which rapid response to treatment is necessary, as maintenance therapy for patients not adequately managed by corticosteroids, or as preoperative treatment before thymectomy\textsuperscript{41,54}. A myasthenic crisis is defined as a need for intubation caused by muscle weakness related to the disease. IVIg and PLEX may be given for a severe MG exacerbation and are always indicated for myasthenic crisis. Multiple randomized controlled trials have demonstrated that IVIg and PLEX are likely equally efficacious in the treatment of severe generalized MG\textsuperscript{55,56}. In an emergent situation, expert consensus suggests that PLEX is the preferred agent due to its faster onset of action\textsuperscript{41,57}. Patient factors must be taken into consideration when choosing one agent over the other. For example, PLEX cannot be used in septic patients and catheter placement can be complex due to need for access to large veins. Several contraindications also exist for IVIg, including hypercoagulable states, renal failure, and hypersensitivity to immunoglobulin\textsuperscript{41,58}. IVIg was associated with less severe side effects and found to be cheaper and more convenient in a registry-based observational study\textsuperscript{58}. 
**Treatment-refractory MG**

Despite the wide arsenal of therapies available, a small subset of MG patients remains refractory to conventional therapy. Treatment-refractory MG is characterized by persistent and disabling weakness despite treatment, disease relapses while tapering treatment, or treatment-related adverse effects. International consensus guidelines have established criteria for refractory MG as “PIS [Post-Intervention Status] is unchanged or worse after corticosteroids and at least 2 other immunosuppressive agents, used in adequate doses for an adequate duration, with persistent symptoms or side effects that limit functioning, as defined by patient and physician”\(^4^1\). Clinical studies have typically used the following criteria in their definitions of treatment-refractory MG\(^5^9\)-\(^6^3\), as detailed in a 2018 review written by Mantegazza and Antozzi\(^6^3\):

1) **Insufficient response to conventional therapy, defined by maximal safe doses of steroids and at least one immunosuppressive drug of adequate dose and duration**
2) **Inability to reduce immunosuppressive therapy without clinical relapse or a need for ongoing rescue therapy such as intravenous immunoglobulin G (IVIg) or plasma exchange (PE)**
3) **Severe or intolerable adverse effects from immunosuppressive therapy**
4) **Comorbid conditions that restrict the use of immunosuppressive therapy**
5) **Frequent myasthenic crises even while receiving therapy**

The exact prevalence of treatment-refractory myasthenia gravis is not currently known, but has been observed in approximately 10-15\% of patients with generalized MG in several studies conducted at tertiary referral clinics\(^6^0\)-\(^6^1\),\(^6^4\). Patients with refractory MG
have been observed to more likely be female, anti-MuSK+, thymomatous, and have an early age of onset\textsuperscript{61,64}. Currently, the main treatments in use for refractory generalized MG include rituximab and eculizumab. No consensus exists on the ideal treatment algorithm for treatment-refractory generalized MG.

The humanized monoclonal antibody eculizumab, a complement inhibitor, has recently been approved for use in treatment-refractory generalized AChR+ MG, making it the first immunomodulatory treatment approved for treatment of MG in the United States\textsuperscript{65}. Eculizumab binds to C5 complement protein and inhibits the activation of terminal complement, thus protecting the neuromuscular junction from damage from the complement cascade\textsuperscript{66}. A randomized, double-blind placebo-controlled phase II study reported that refractory patients had improved clinical outcomes as demonstrated by improvement in QMG score post eculizumab treatment\textsuperscript{67}. This was followed by the randomized, placebo-controlled phase III REGAIN trial, which, although did not demonstrate a statistically significant benefit with its prespecified primary endpoint of improvement in activities of daily living (MG-ADL), did support the efficacy of eculizumab with prespecified and posthoc sensitivity analyses and other secondary outcomes for use in treatment-refractory generalized AChR+ MG\textsuperscript{68}. It was later approved by the US Food and Drug Administration (FDA) for adults with generalized AChR+ MG in 2017. Eculizumab would not be expected to work in MuSK+ MG patients because IgG4 autoantibodies are not involved in complement-mediated immunopathology. Notably, given the novelty of the medication, annual costs of eculizumab for one patient are approximately $500,000, with some variation per dose and country, making it one of
the most expensive therapies in the world\textsuperscript{69}. Rituximab, an alternative immunomodulating agent, shows similarly promising results and is less expensive for the patient, as well as provides an option for treatment-refractory patients with generalized MuSK+ MG.

B-cell targeted therapy with rituximab has shown durable response in treating refractory MG. Rituximab is a genetically engineered chimeric mouse-human monoclonal antibody directed against CD20, a transmembrane protein found on the surface of B-lymphocytes\textsuperscript{54,70,71}. CD20 is involved in the initiation of the cell cycle and in the activation, differentiation and growth of B-lymphocytes\textsuperscript{72,73}. The Fab domain of rituximab binds to CD20 cells and recruits immune effector cells for B-cell lysis\textsuperscript{54}. CD20 B-cell levels are decreased for over 6 months following rituximab therapy. Rituximab has been approved for use in non-Hodgkin B-cell lymphoma\textsuperscript{74} and many autoimmune disorders, such as rheumatoid arthritis\textsuperscript{75}, type 1 diabetes\textsuperscript{76}, immune thrombocytopenia\textsuperscript{77}, among others. To note, rituximab has not been approved for use in MG and continues to be administered as an off-label treatment. The increasing use of rituximab in MG still lacks supportive evidence from randomized controlled trials. BeatMG, a recently concluded multicenter double-blind placebo-controlled phase II clinical trial in AChR+ MG, did not provide conclusive evidence in support of rituximab therapy for MG\textsuperscript{78}. The BeatMG study demonstrated a favorable safety profile for rituximab, however, met the primary futility outcome at 52-weeks, suggesting that there would be a low probability of achieving the prespecified steroid-sparing effect difference in a similar mildly
symptomatic cohort on concomitant immunotherapy\textsuperscript{78,79}. To note, this study was not limited to refractory disease or powered to assess efficacy.

The benefits of rituximab in MG have however been reported in multiple case reports and series\textsuperscript{59,62,80-87}. Treatment of MG with rituximab has been shown to result in sustained clinical improvement, as well as allowing for tapering and discontinuing of other immunotherapies\textsuperscript{62,88}. A retrospective meta-analysis conducted in 2015 of MG patients treated with rituximab found an overall response rate of 83.9\% in the patient cohort\textsuperscript{89}. A large retrospective nationwide study recently conducted in Austria demonstrated that over two-thirds of their patient cohort improved to an MGFA-PIS of MM or better after rituximab therapy\textsuperscript{90}. Rituximab is an appealing option because it provides a steroid-sparing therapeutic option with potentially long-lasting effects. The varying responses of the AChR+ and MuSK+ MG subtypes will be discussed in the next section.

\textbf{Differences in response to rituximab between MG subtypes}

MG patients are typically initially treated similarly regardless of their serotype\textsuperscript{84}. As mentioned above, several studies have suggested that MuSK+ patients have a more severe form of the disease and may be more resistant to established treatments than AChR+ MG patients\textsuperscript{26,91-94}.

Several small studies have suggested that MuSK+ MG patients respond well to rituximab and tend to respond better and have more prolonged improved clinical outcomes compared to AChR+ MG patients\textsuperscript{25,50,80,84,94-98}. A multicenter blinded, prospective review
of MuSK+ MG demonstrated better clinical outcomes with rituximab as compared with other treatment regimens, including improved clinical status and ability to taper prednisone. A small single-center study that examined 11 AChR+ MG and 6 MuSK+ MG patients refractory to conventional agents treated with rituximab found demonstrable improvement in clinical status post rituximab treatment in all patients, with longer-lasting effects in the MuSK+ MG patients, i.e. reduced concurrent immunosuppressant requirements and no need for reinfections as compared to the AChR+ MG patients. A 2017 meta-analysis of 169 MG patients treated with rituximab demonstrated clinical status improvement in both AChR+ and MuSK+ groups with fewer relapses in MuSK+ MG post-rituximab compared to AChR+ MG. This same review found that a significantly greater proportion of MuSK+ MG patients as compared with AChR+ MG patients achieved post-rituximab PIS-m of MM or better or CSR or PR, as well as a greater decrease in their QMG scores. This study also found that antibody titers in MuSK+ MG may be a more useful monitoring tool than in AChR+ MG. The aforementioned Austrian study published in 2015 reported that more MuSK+ MG patients achieved remission (71.4% vs 35.9%, p=0.022) compared to AChR+ MG patients after treatment with rituximab.

Current guidelines state that rituximab should be reserved as an escalation therapy for AChR+ MG after failing conventional therapy. Understanding how MuSK+ MG patients respond to rituximab may impact future recommendations and promote the use of rituximab to a first-line agent, or at least for use earlier in the treatment algorithm.
Additional long-term follow-up studies are needed to investigate and further characterize the differential response to rituximab treatments between AChR+ and MuSK+ patients.

In this study, we report our experience with rituximab in 33 patients with generalized MG seen at the Yale Myasthenia Gravis Clinic, 17 with AChR+ MG and 16 with MuSK+ MG, who had an average follow-up of approximately 5 years. We analyzed the difference in treatment response between the AChR+ and MuSK+ MG patients.

**STATEMENT OF PURPOSE**

Targeted therapies, specifically B-cell directed therapeutics, have shown growing promise as effective tools in the management of autoimmune disorders. While small studies and large meta-analyses have been published suggesting a differential response between anti-AChR+ and anti-MuSK+ patients, there is need for more large single-center comparisons between the groups to corroborate this data. We report our experience with the long-term effects of rituximab treatment in thirty-three MG patients followed at the Yale Myasthenia Gravis Clinic. To our knowledge, this retrospective report represents the largest, single-center MG cohort in the U.S. focused on studying possible key differences between these antibody groups. We believe that this work will fill a critical gap in our current knowledge as well as be of significant interest to the neurology community.
Hypothesis:

We hypothesize that there will be a difference in response to rituximab between subgroups and that there will be clinical differences between the MuSK+ and AChR+ subtypes of MG patients that will be reflected in their differential responses to rituximab treatment.

Specific Aims of Study:

**Aim 1:** Time to Clinical Response: We will determine the difference in time from initiation of rituximab treatment to observed clinical benefit between the two groups.

**Aim 2:** Rate of Steroid Reduction: We will determine how quickly each group was able to taper prednisone after initiation of rituximab.

While steroids are considered the mainstay therapy in the treatment of MG, they are associated with many unwanted side effects if used long-term. The current standard of care approach is to transition patients to a steroid-sparing agent. We will look at the ability of patients to reduce their steroid dose after the initiation of rituximab. The differences in rate of this reduction will be determined between the AChR+ and MuSK+ MG groups to shed additional insight on differences in responsiveness to rituximab therapy.

**Aim 3:** Rescue Therapy Rates: We will determine how frequently each group required rescue therapy (IVIG or plasmapheresis).
**Aim 4:** We will quantify how many disease exacerbations and hospitalizations each group experienced.

**METHODS**

**Patients**

This retrospective study included patients with generalized MG treated with rituximab therapy and followed for a minimum of 12 months after completion of the initial set of rituximab treatment cycles (Table 1). We identified 33 patients followed in the Yale Myasthenia Gravis Clinic, New Haven, Connecticut from May 31, 2003 to May 31, 2017. This study was approved by the institutional review board of Yale University as part of an observational study examining the treatment and disease course of MG. All patients provided written informed consent.

Rituximab was administered in cases when the immunotherapy dosage could not be lowered without clinical relapse, adequate clinical control could not be achieved, and/or patients experienced severe adverse effects due to the current immunosuppressive therapy.

Pretreatment and post-treatment immunotherapy regimens, clinical symptoms, and examination findings were evaluated. These included prednisone (which is the standard first-line agent), plasma exchange (PLEX), azathioprine, mycophenolate mofetil, and
intravenous immunoglobulin (IVIg). The number of administered rituximab treatment cycles, time since last treatment cycle, time to relapse, time to remission, and the type of post-relapse treatments were also reviewed. The Myasthenia Gravis Foundation of America (MGFA) clinical classification criteria\textsuperscript{100} and post-intervention status (PIS) were used to assign clinical state 12 months after completion of the initial set of rituximab cycles and at last visit. We defined “improved clinical status” when Complete Stable Remission (CSR), Minimal Manifestations (MM), and/or Improved (I) PIS change was achieved. Remission was defined as when the patient achieved "asymptomatic" status based on the MGFA classification and had not relapsed for 12 months. Exacerbation was defined as when a patient had clinically deteriorated based on a worsened MGFA class.

Rescue therapy was defined as the need for the addition of an immunotherapy (IVIg or PLEX) during a disease exacerbation. This was quantified via chart review by noting for each disease exacerbation which treatments were used. Maintenance therapy was defined as regularly scheduled immunotherapy treatments (IVIg or PLEX). These were quantified via chart review of regularly scheduled treatments for the patient. Concomitant use of other immunosuppressive agents and their daily dosing was also reviewed.

\textit{Rituximab}

Patients were treated with an initial 2- to 4- cycle regimen, except for two patients who only received 1 cycle. Because no established infusion protocol for rituximab use in MG currently exists, the MG clinic used a standard protocol adopted from the non-Hodgkin lymphoma regimen of 4 weekly infusions of 375 mg/m\textsuperscript{2}. One cycle is defined as 1 infusion per week for 4 consecutive weeks. The interval between cycles was 6 months.
The number of rituximab treatment cycles or intervals between cycles was not dictated by B-cell counts but rather based on clinical improvement and patient tolerance of tapering or withdrawal of other immunotherapies (i.e. corticosteroids).

**Statistical Analysis**

Statistical analysis was performed using R version 1.4.3. Descriptive statistics are represented as mean ± standard deviation if not specified otherwise. Wilcoxon-signed rank tests for paired analysis, and Mann-Whitney U-test for unpaired analysis were used for non-parametric data. Paired or unpaired t-test was used for parametric data as applicable. $\chi^2$ test or Fisher’s exact test were used for nominal data as applicable. Kaplan-Meier survival curves were generated with log-rank test to study the duration of response to rituximab. Significance was defined as p-value of $< 0.05$.

**RESULTS**

Thirty-three patients were identified (17 AChR+, 16 MuSK+; mean age 35.9 ± 15.6 years; 24 women and 9 men) with a mean follow-up of 1861 ± 953.4 days. Twenty-seven patients (87.9%) were receiving prednisone prior to initiation of rituximab therapy. The mean number of induction rituximab cycles received was 3.1 ± 1.3.

The AChR+ and MuSK+ groups were well matched overall. There were no significant differences between the AChR+ and MuSK+ groups in terms of age at onset of disease,
gender, length of follow-up, or number of rituximab cycles received. Key differences noted were that the number of patients receiving either IVIg or PLEX maintenance therapy prior to rituximab was higher in the MuSK+ group (4/17 in AChR+ vs. 11/16 in MuSK+, p-value 0.02) and the number of patients who underwent thymectomy was higher in the AChR+ group (15/17 in AChR+ vs. 5/16 in MuSK+, p-value <0.01) (Table 1).

The median baseline MGFA Clinical Class was II for the entire group, which improved to asymptomatic (0) both at 12-months and last follow-up (p-values <0.01) after rituximab treatment (Table 1, Figure 1). The median MFGA Clinical Class for AChR+ patients improved from a baseline of II to asymptomatic (0) at 12-months (p-value <0.01) and at last visit (p-value <0.01) after rituximab treatment. Similarly, MuSK+ patients improved from a median baseline class of II to asymptomatic (0) at 12-months (p-value <0.01) and at last follow-up visit (p-value <0.01) (Figure 1).
Table 1: Demographics and baseline characteristics.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>All patients (n=33)</th>
<th>AChR (n=17)</th>
<th>MuSK (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset, mean (SD)</td>
<td>35.9 (15.6)</td>
<td>33 (25)</td>
<td>34.8 (14.9)</td>
<td>0.70</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>24 (72%)</td>
<td>10 (58%)</td>
<td>14 (87%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Worst MGFA classification, median (range)</td>
<td>III (II-V)</td>
<td>III (II-V)</td>
<td>III (II-V)</td>
<td>0.19</td>
</tr>
<tr>
<td>Thymoma, n (%)</td>
<td>7 (21%)</td>
<td>7 (46%)</td>
<td>0 (0%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Duration of disease in days before rituximab, mean (SD)</td>
<td>1579.4 ± 2423.8</td>
<td>1169.2 ± 762.6</td>
<td>2025.9 ± 3391.1</td>
<td>0.33</td>
</tr>
<tr>
<td>Length of follow-up in days, mean (SD)</td>
<td>1861.5 (953.4)</td>
<td>1196.1 (743.9)</td>
<td>1718.6 (1143.1)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

| Current and previous treatment                    |                      |             |             |         |
| Patients on prednisone when starting rituximab, n (%) | 29 (87%)            | 16 (94%)    | 13 (81%)    | 0.26    |
| Prednisone dose in mg/day, mean (SD)               | 39.1 (23.9)          | 46.8 (20.8) | 30.9 (24.9) | 0.06    |
| Patients on maintenance IVIg and/or PLEX, n (%)    | 15 (46%)             | 4 (24%)     | 11 (69%)    | 0.02    |
| Use of oral steroid sparing agents⁸, n (%)         | 17 (51%)             | 9 (53%)     | 9 (56%)     | 0.86    |
| Patients with previous rescue therapy with IVIg and/or PLEX, n (%) | 29 (87.9%)          | 16 (94%)    | 13 (81.3%)  | >0.9    |
| Previous thymectomy, n (%)                        | 20 (61%)             | 15 (88%)    | 5 (31%)     | <0.01   |
| Time from thymectomy to 1st cycle of rituximab, mean (SD) | 940.6 (1096.9)     | 730.6 (589.3) | 1675.5 (2094.7) | 0.44 |

| Disease exacerbation pre-rituximab                 |                      |             |             |         |
| Number of exacerbations, mean (SD)                 | 1.7 (2.9)            | 2.6 (2.7)   | 0.8 (0.9)   | 0.06    |

| Rituximab therapy                                  |                      |             |             |         |
| Number of rituximab cycles, mean (SD)              | 3.1 (1.3)            | 3.1 (0.9)   | 3.1 (1.7)   | >0.9    |

⁸Azathioprine (6 AChR+, 4 MuSK+); mycophenolate mofetil (3 AChR+, 4 MuSK+); cyclosporine (1 MuSK). IVIg, intravenous immunoglobulin; PLEX, plasma exchange.
The median baseline MGFA Clinical Class was II for the entire group, which improved to asymptomatic (0) both at 12-months and last follow-up (p-values <0.01) after rituximab treatment (Table 1, Figure 1). The median MGFA Clinical Class for AChR+ patients improved from a baseline of II to asymptomatic (0) at 12-months (p-value <0.01) and at last visit (p-value <0.01) after rituximab treatment. Similarly, MuSK+ patients improved from a median baseline class of II to asymptomatic (0) at 12-months (p-value <0.01) and at last follow-up visit (p-value <0.01) (Figure 1).

Figure 1: MGFA classification of AChR+ and MuSK+ patients at baseline, and at 12 months and last visit post-rituximab

Figure 1: MGFA clinical class at baseline, 12-months, and at last visit for AChR+ and MuSK+ patients. (A) The median MGFA clinical class for AChR+ patients improved from a baseline of II to asymptomatic (0) at 12-months (p-value <0.01) and at last follow-up visit (p-value <0.01) post-rituximab. (B) Similarly, MuSK+ patients improved from a median baseline MGFA clinical class of II to asymptomatic (0) at 12-months (p-value <0.01) and at last follow-up visit (p-value <0.01).
MM or better PIS was attained at 12-months in 10 (58.8%) AChR+ patients and 11 (68.8%) MuSK+ patients, respectively (p-value 0.72). At last follow-up MM or better PIS was attained in 11 (64.7%) and 12 (75%) of AChR+ and MuSK+ patient respectively. Twenty-one patients achieved clinical remission (12 AChR+, 9 MuSK+) with time to remission of 441.4 ± 336.6 days for AChR+ versus 230 ± 180.8 days for MuSK+ patients (p-value 0.049) (Figure 2, Table 2). Overall, the MuSK+ group required a shorter period of time to achieve remission, as defined by MGFA class of asymptomatic without any relapse for 12 months.

**Figure 2**: Time to remission between AChR+ and MuSK+ following rituximab treatment.

![Kaplan-Meier survival estimates](image)

**Figure 2**: Time to remission between AChR+ (n=9) and MuSK+ (n=12) patients post-rituximab (days since start of first rituximab infusion). Comparison data shown is only for those that achieved clinical remission.
The mean prednisone dose requirement decreased in both groups following rituximab treatment (AChR+: baseline 46.8 ± 20.8 mg/day, 12-months 11.8 ± 15.3 mg/day, last follow-up 17.9 ± 15.6; MuSK+: baseline 30.9 ± 24.9 mg/day, 12-months 9.5 ± 8.7 mg/day, last follow-up 2.8 ± 3.8 mg/day). While this was significant within groups (p-value < 0.01), there was no significant difference between the two groups (baseline p-value 0.057, 12-months p-value 1, last follow-up p-value 0.75) (Table 1, Table 2, Table 3). There was also no significant difference between the groups in terms of ability to taper off prednisone completely or to reduce the daily dose of prednisone ≤ 10 mg (p-values 0.69 and 0.49 respectively). The length of time required to taper off prednisone completely or ≤ 10 mg/day post-rituximab also did not differ significantly between the groups (p-values 0.4 and 0.08 respectively) (Table 2).

The most commonly used steroid sparing agents in our cohort were azathioprine (AZA) and mycophenolate mofetil (MMF). Only one patient in the MuSK+ group was on cyclosporine only before rituximab infusion. Overall use of steroid sparing agents was significantly reduced following rituximab treatment [18 patients at baseline, 8 patients at 12-months (p-value 0.02), and 5 patients at last follow-up (p-value 0.002)]; however, this did not reach statistical significance when AChR+ and MuSK+ groups were considered separately. There was no significant difference between the groups in terms of successful reduction of concomitant use of steroid sparing agents (Table 2, Table 3).
Table 2: Outcome measures post-rituximab therapy.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>AChR (n=17)</th>
<th>MuSK (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical outcome:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM or better at 12 months, n (%)</td>
<td>10 (58.8%)</td>
<td>11 (68.8%)</td>
<td>0.72</td>
</tr>
<tr>
<td>MM or better at last visit, n (%)</td>
<td>11 (65%)</td>
<td>12 (75%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Patients with clinical remission^, n (%)</td>
<td>12 (70.6%)</td>
<td>9 (56.3%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Days to remission, mean (SD)</td>
<td>441.4 (336.6)</td>
<td>230 (180.8)</td>
<td>0.049</td>
</tr>
<tr>
<td>Patients with relapse, n (%)</td>
<td>10 (58.8%)</td>
<td>6 (37.5%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Days to relapse, mean (SD)</td>
<td>824.1 ± 479.3</td>
<td>1529 ± 1338.4</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Steroid-sparing effect:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone dose at 12-months in mg/day, mean (SD)</td>
<td>11.8 (15.3)</td>
<td>9.5 (8.7)</td>
<td>1</td>
</tr>
<tr>
<td>Prednisone dose at last visit in mg/day, mean (SD)</td>
<td>17.9 (15.63)</td>
<td>2.8 (3.8)</td>
<td>0.75</td>
</tr>
<tr>
<td>Able to taper prednisone to ≤10 mg/day, n (%)</td>
<td>14 (82%)</td>
<td>13 (81%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Time to ≤10 mg/day in days, mean (SD)</td>
<td>361.9 (232.8)</td>
<td>219.1 (152.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Able to taper prednisone completely, n (%)</td>
<td>12 (70%)</td>
<td>9 (56%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Time to taper prednisone completely in days, mean (SD)</td>
<td>811.4 ± 395.6</td>
<td>712.4 ± 395.6</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Disease exacerbation:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of exacerbations, mean (SD)</td>
<td>1.7 (1.2)</td>
<td>1.4 (1.1)</td>
<td>0.42</td>
</tr>
<tr>
<td>Number of patients hospitalized for exacerbations, n (%)</td>
<td>5 (29%)</td>
<td>0 (0%)</td>
<td>0.045</td>
</tr>
<tr>
<td><strong>Steroid sparing agents:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of oral steroid sparing agents, n (%)</td>
<td>3 (18%)</td>
<td>2 (12.5%)</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td><strong>Maintenance therapy:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients requiring maintenance therapy with IVIg and/or PLEX, n (%)</td>
<td>6 (35.3%)</td>
<td>6 (43.8%)</td>
<td>1</td>
</tr>
<tr>
<td>Total number of IVIg and/or PLEX treatment cycles, Mean (SD)</td>
<td>9.8 (31.8)</td>
<td>9.1 (19.8)</td>
<td>0.77</td>
</tr>
<tr>
<td><strong>Rescue therapy:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients requiring rescue therapy with IVIg and/or PLEX, n (%)</td>
<td>7 (41.2%)</td>
<td>3 (18.8%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Total number of IVIg and/or PLEX treatment cycles, Mean (SD)</td>
<td>0.7 (1.1)</td>
<td>0.5 (1.1)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

^ clinical remission was defined as when the patient achieved "asymptomatic" status based on the MGFA classification and had not relapsed for 12 months.
**Table 3:** Comparison of treatment burden between the AChR+ and MuSK+ group

<table>
<thead>
<tr>
<th></th>
<th>AChR</th>
<th></th>
<th></th>
<th>MuSK</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-rituximab</td>
<td>Post-rituximab</td>
<td>p-value</td>
<td>Pre-rituximab</td>
<td>Post-rituximab</td>
<td>p-value</td>
</tr>
<tr>
<td>Prednisone use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone dose (pre-rituximab and 1 year)</td>
<td>46.8 (20.8)</td>
<td>11.6 (14.7)</td>
<td>&lt;0.01</td>
<td>30.9 (24.9)</td>
<td>5.9 (8.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Prednisone dose (last visit)</td>
<td>17.9 ± 15.6</td>
<td></td>
<td>&lt;0.01</td>
<td>2.3 ± 3.8</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Steroid sparing agent use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azathioprine</td>
<td>6</td>
<td>3</td>
<td>0.43</td>
<td>4</td>
<td>2</td>
<td>0.65</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>3</td>
<td>0</td>
<td>0.22</td>
<td>4</td>
<td>0</td>
<td>0.10</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>1</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Maintenance IVIg and PLEX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients on maintenance IVIg and PLEX treatments</td>
<td>4</td>
<td>6</td>
<td>0.71</td>
<td>11</td>
<td>6</td>
<td>0.16</td>
</tr>
<tr>
<td>Total number of maintenance IVIg and PLEX treatments, n (SD)</td>
<td>22.3 (61.3)</td>
<td>9.8 (31.9)</td>
<td>0.29</td>
<td>21.7 (27.2)</td>
<td>9.1 (19.8)</td>
<td>0.21</td>
</tr>
<tr>
<td>Rescue IVIg and PLEX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients on rescue IVIg and PLEX treatments</td>
<td>16</td>
<td>7</td>
<td>&lt;0.01</td>
<td>13</td>
<td>3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total number of rescue IVIg and PLEX treatments, n (SD)</td>
<td>2.1 (1.2)</td>
<td>0.7 (1.1)</td>
<td>&lt;0.01</td>
<td>2.1 (2)</td>
<td>0.5 (1.1)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Figure 3: Time to relapse between AChR+ and MuSK+ following rituximab treatment.

Sixteen (48.5%) patients relapsed after rituximab therapy (Table 2). The relapse rate was 58.8% and 37.5% for AChR+ and MuSK+ patients respectively (p-value 0.22). There was no significant difference in time to relapse between AChR+ and MuSK+ patients (p-value 0.09) (Figure 3B). However, when the cohort was restricted to patients who received ≥ 3 rituximab cycles, MuSK+ patients relapsed later than AChR+ patients (p-value 0.03) (Figure 3C). AChR+ patients had more MG-related hospitalizations as compared to MuSK+ patients during the entire follow-up period (p-value 0.045) (Table 2).

We examined the effect of rituximab treatment on the total burden of IVIg and PLEX required as maintenance therapy. There was no statistically significant reduction compared to pre-rituximab in either the AChR+ or MuSK+ group (p-value 0.29 and 0.21).
respectively). Similarly, the number of patients requiring maintenance IVIg or PLEX was not significantly different in either group post-rituximab therapy compared to pre-rituximab (p-values 0.71 and 0.16 respectively). There was also no difference between the AChR+ and MuSK+ groups in terms of total number of maintenance therapy requirement post-rituximab infusion (p-value 0.77).

Fewer patients required IVIg or PLEX as rescue therapy following rituximab in both groups (p-values <0.01). Similarly, the number of rescue therapies required either with IVIg or PLEX reduced significantly in both AChR+ and MuSK+ patients post-rituximab therapy (p-value <0.01 and 0.02 respectively). However, there was no significant difference between groups in terms of number of patients requiring rescue therapy or the total number of rescue therapy events required post-rituximab (p-values 0.26 and 0.27 respectively) (Table 2, Table 3).

Twenty patients had undergone thymectomy (60.6%), of whom 7 (21.1%) had a thymoma (Table 1). All patients with a thymoma were AChR+. While the thymoma subgroup received more rituximab cycles (3.6 ± 0.9 vs. 2.5 ± 0.8, p-value 0.03), there were no significant differences found between the thymoma vs non-thymoma groups in terms of aforementioned clinical outcomes.

All patients in the study tolerated rituximab treatment without severe adverse side effects identified per chart review.
DISCUSSION

Targeted immunotherapies demonstrate great potential for a subset of MG patients who are refractory to or have adverse effects from conventional immunosuppressive treatments. Depletion of B cells with rituximab, with its established precedence in other autoimmune disorders such as rheumatoid arthritis\textsuperscript{101-103}, pemphigus\textsuperscript{104,105}, autoimmune hemolytic anemia\textsuperscript{106,107}, has availed itself as an appealing next-line option.

In this retrospective analysis of 33 patients with refractory AChR+ or MuSK+ MG and extended follow-up, a long-lasting clinical benefit was again observed following rituximab therapy\textsuperscript{88}. These results support the findings from previous studies demonstrating the benefit of rituximab in managing refractory MG\textsuperscript{62,80,108-110}. Additionally, our findings add valuable insights regarding whether B-cell depletion therapy should be utilized as an induction regimen. The study also provides information on the expected rate of relapse after achieving remission following completion of an induction regimen. The overall relapse rate in our entire cohort was 48.5%, occurring approximately 2.7 years after treatment (992 ± 783.9 days). While the relapse rate was similar to those previously reported\textsuperscript{80,111}, the duration of disease stability was longer in our cohort. Prednisone burden was also successfully reduced in both groups. While concomitant use of steroid-sparing agents was reduced among the whole cohort, statistical significance was not achieved when groups were considered separately, which may be related to small sample size with between-group analysis. There were some trends suggesting that the MuSK+ group may experience greater benefit from rituximab, specifically a faster time to remission and a longer time to relapse, which is further
supported by more frequent MG-related hospitalizations and need for rescue therapy in the AChR+ group.

Despite its interesting observations, this report is limited by the retrospective nature of our study, varied baseline immunotherapy regimens and number of rituximab treatment cycles. Unfortunately, it does not adequately establish the presence of differential response to rituximab between AChR+ and MuSK+ patients or whether antibody status is a predictor of response or durability.

In our healthcare system, rituximab use is mostly restricted to the outpatient setting. While our study included patients with refractory generalized disease, in the attempt to stabilize patients prior to start of infusions, the majority of the patients were categorized as mild-moderate in severity (based on MGFA class) at the time of rituximab initiation. This may have resulted in a scale floor effect masking any possible difference between groups. Due to the MGFA classification only being a gross measure of disease severity, true burden of disease differences could not be assessed as other outcomes were not available for analysis, such as MG Activities of Daily Living (MG-ADL) or Quantitative MG (QMG) scores.

While there is strong evidence that AChR+ and MuSK+ MG are distinct disease entities with unique autoantibody-mediated immunopathologic mechanisms\textsuperscript{10,11}, dramatic clinical differences were not noted with anti-CD20 therapy. B-cell depletion appears to benefit both groups of patients. As a clearer picture emerges regarding pathogenic B-cell
populations and whether these populations return upon B-cell recovery, the application of targeted therapy can be better tailored to each patient.

_Why is there a difference between AChR+ and MuSK+ patients in their response to rituximab?_

Multiple recent studies have corroborated earlier data suggesting that MuSK+ MG patients may have a more robust response to rituximab therapy than AChR+ MG patients. As mentioned above, a multicenter blinded, prospective review of MuSK+ MG conducted in 2017 demonstrated better clinical outcomes with rituximab as compared with other treatment regimens, including improved clinical status and ability to taper prednisone. The same year, a meta-analysis of 169 MG patients treated with rituximab demonstrated clinical status improvement in both AChR+ and MuSK+ groups, with fewer relapses in MuSK+ MG post-rituximab compared to AChR+ MG. These larger studies added to the field of growing evidence that MuSK+ MG patients may respond better to rituximab than AChR+ MG patients. However, this differential treatment effect is still not fully explained.

There are several hypotheses regarding why there may be differences in response to rituximab treatment between MuSK+ and AChR+ MG patients. Various theories regarding disease pathogenesis will be discussed. First, the differential response to rituximab between MuSK+ and AChR+ MG is thought to be related to the role of long-lived plasma cells in the pathogenesis of the two subtypes. A study conducted in 2017 identified the emergence of autoantibody-producing plasmablasts after B-cell depletion in
patients with MuSK+ MG\textsuperscript{113}. Because remission is achieved in a few months despite subsequent repopulation of CD20+ cells, the authors of this study suggest that autoimmunity in MuSK+ MG is driven by short-lived, autoantibody-secreting plasmablasts. This is opposed to AChR+ MG, in which long-lived CD20- plasma cells may contribute to autoantibody production. Rituximab only eliminates CD20+ cells and thus does not affect the long-lived CD20- plasma cells in AChR+ MG. This is corroborated by the results of an earlier study published in 2012 which demonstrated that the MuSK+ MG patients had no decline in their anti-tetanus toxoid (ATT) IgG levels (produced by long-lived plasma cells) after rituximab treatment, despite a concomitant decrease in IgM levels (primarily produced by plasmablasts)\textsuperscript{80,114}. Another hypothesis pertains to the difference in IgG subtype: AChR+ MG is mainly IgG1 and IgG3-mediated, while MuSK+ MG is driven by IgG4 autoantibodies\textsuperscript{13,14}. While it is not completely understood why a difference in IgG subtype would affect rituximab response, this same 2012 study noted that 3 of their MuSK+ MG patients underwent a switch in subtype from IgG4 to IgG1 after rituximab treatment, suggesting that rituximab may have acted more on the IgG4 autoantibodies\textsuperscript{80}. The authors of this study hypothesized that IgG4 antibodies may be produced by short-lived plasma cells. Rituximab has indeed been noted to be highly effective against IgG4 autoantibodies in pemphigus vulgaris as well as in other IgG4-associated systemic diseases\textsuperscript{115}; one study noted drastic reductions in IgG4 levels in 15 out of 18 patients who experienced complete remission following rituximab therapy\textsuperscript{116}. These differences in pathogenesis may explain why the response to rituximab in AChR+ MG is typically more delayed and the autoantibody titer decline less
significant than in MuSK+ MG, as rituximab is primarily acting on the CD20+ cells more prominently involved in the pathogenesis of MuSK+ MG\textsuperscript{80,88}.

Third, differences in cytokine production between AChR+ and MuSK+ MG have been described; investigating these differences in Th1 and Th2 responses may lead to deeper understanding of the differential responses to rituximab therapy. One study investigated the Th2-related pathway in MuSK+ and AChR+ MG by applying ex vivo non-specific T-cell stimulation to peripheral blood mononuclear cells (PBMC) in order to see differential responses of T-cell derived cytokines\textsuperscript{117}. A difference was noted between the two subgroups, with increased secretion of Th1- and Th17-related cytokines in the MuSK+ MG patients, including IFN-\(\gamma\), IL17, and IL21. Another main finding of this study was the down-regulating effect of immunosuppressive therapy on Th1 cytokines (IFN-\(\gamma\), IL12) and up-regulating effect on Th2 cytokines (IL10, IL6) in AChR+ MG patients, but not MuSK+ MG patients\textsuperscript{117}. The authors of this study interpreted these differential cytokine production patterns under treatment as potentially corroborating previously reported observations that MuSK+ MG patients are more resistant to immunosuppressive therapy and have better response to rituximab than AChR+ MG patients. While this research is still in its early stages, identifying differences in T-cell pathophysiology between MG subgroups would be useful because rituximab is postulated to affect both B-cell and T-cell function\textsuperscript{118}. Rituximab is thought to affect T-cell function 1) directly: by depleting the small portion of T-cells which express CD20 on their surface\textsuperscript{119} and 2) indirectly: by impacting B-T cell cooperation via B-cell depletion\textsuperscript{118}. Responsiveness to rituximab has been associated with expansion of the T-regulatory population after
treatment. In one case series, a treatment-responsive MuSK+ MG patient experienced a long-lasting increase in circulating Treg levels, while the nonresponsive AChR+ MG patient was noted to have inconsistent fluctuations in Treg cells\textsuperscript{120}. An important follow-up study will be to perform T-cell repertoire analysis to examine whether rituximab causes modification to the antigen-specific T-cell repertoire and phenotype \textsuperscript{118,121}. Gaining a better understanding of the differences in the pathogenesis of AChR+ and MuSK+ MG and their response to rituximab can pave the way for more personalized treatment plans.

Additional discrete differences are continuing to be identified in the pathogenesis of the two major subtypes of MG. A study published in 2013 identified lower levels of IL-10 producing B-cells in MG patients compared to healthy controls, which correlate with more severe disease\textsuperscript{122}. The subset of B-cells that produces IL-10 has been functionally defined as “regulatory” in mice and humans due to their ability to produce IL-10, which has an important role in controlling immune responses and preventing autoimmunity\textsuperscript{123}. Other autoimmune disorders, including rheumatoid arthritis, Graves’ disease, and systemic lupus erythematosus have been associated with lower levels of IL-10 producing B cells\textsuperscript{124-126}. In this study from 2013, the patients who responded well to rituximab therapy demonstrated a rapid repopulation of B10 cells, while patients who did not respond well to rituximab had a delayed B10 cell repopulation. Although a statistically significant difference was not found, a trend of baseline higher frequencies of B10 cells were seen in patients with AChR+ MG as opposed to MuSK+ MG. Patients with MuSK+ MG also appeared to have a faster repopulation of their B10 cells than AChR+ MG patients, although this also did not reach statistical significance (p=0.072). While the
significance of these findings is still not completely understood, these results suggest fundamental differences in the pathophysiology of AChR+ and MuSK+ MG.

Additionally, a recent investigation identified distinct defects in B-cell tolerance checkpoints in MG, with differing abnormalities unique to the AChR+ and MuSK+ subtypes\textsuperscript{127}. This study performed deep sequencing of the B-cell receptor repertoire and found that AChR+ MG and MuSK+ MG subjects displayed distinct gene segment usage biases in both VH and VL sequences within the naïve and memory compartments, with abnormalities unique to each subtype. However, an earlier study performed by the same group demonstrated that both the AChR+ and MuSK+ subtypes of MG have defects in their central and peripheral B-cell tolerance checkpoints\textsuperscript{128}. While the significance of these findings is not fully understood yet, the results of these studies again suggest fundamental differences in the pathophysiology of AChR+ and MuSK+ MG, which could begin to explain the different disease phenotypes and response to therapy.

Overall these findings suggest various mechanisms behind differences in treatment response to rituximab between MG subtypes. This remains an active field of research with much still to be learned.

\textit{Future Directions}

While rituximab and eculizumab have both shown durable and long-lasting results in patients with treatment-refractory MG, treatment does not stop there. Given the rapidly progressing pace of the field of biologics, there continue to be many new exciting
developments in the area of MG therapy. Further, more research is underway to better understand the pathophysiology of MG and to explain its varied clinical manifestations.

Despite the results from the BeatMG Study, a phase 2 trial of only AChR+ patients, the scientific rationale for B-cell depletion in MG is established based on the totality of published data and experience with CD20-targeted therapy. Further insights from BeatMG Study subgroup analyses and an ongoing placebo-controlled trial of rituximab in new onset generalized MG will aid in better elucidating where B-cell depletion therapy might best fit the overall treatment paradigm. Further, we must further consider whether anti-CD19 as opposed to anti-CD20 may be more effective or durable. The primary objective of B-cell depletion is the elimination of autoantibody producing cells. In the case of anti-CD20 therapy, this goal is partially achieved by preventing the formation of new plasma cells from their precursors. As CD20 is generally not expressed on the majority of autoantibody-producing cells, specifically plasma cells and plasmablasts, this is one potential disadvantage of an anti-CD20 treatment approach, especially if these cells are indeed those driving autoantibody production. CD19 is a transmembrane glycoprotein expressed on early pro-B cells, late pro-B cells, memory B-cells, memory B cells, plasmablasts, and plasma cells (Figure 5).
Therefore, because anti-CD19 therapy depletes both plasmablasts and plasma cells due to the expression of CD19 on these cells, these agents may potentially be more effective at eliminating production of pathogenic autoantibodies than anti-CD20 agents. There are several anti-CD19 therapies currently being investigated in clinical settings for B-cell mediated hematologic malignancies, including blinatumomab, AFM11, MDX-1342, MOR208, SAR3419, and SGN-CD19A, as well as several agents for autoimmune disease including XmAb5871 and MEDI-551 (inebilizumab)\textsuperscript{133}. Inebilizumab has been applied to the experimental autoimmune encephalomyelitis (EAE) animal model of multiple sclerosis with promising results demonstrate a reduced infiltration of leukocytes into the spinal cord and a reduction in the number of autoreactive CD138 plasma cells in the spleen and bone marrow\textsuperscript{134}. Additionally, a recent double-blind randomized placebo-controlled phase 2/3 trial demonstrated the efficacy of inebilizumab in the treatment of neuromyelitis optica spectrum disorder (NMO)\textsuperscript{135}. The randomized controlled phase of this study was actually stopped early before complete enrollment, because of a clear demonstration of efficacy in reducing the risk of an NMOSD attack. To date, anti-CD19...
therapy has not been studied in the treatment of MG. This assumption regarding potential
benefit of anti-CD19 therapy over anti-CD20 needs to be tested in a clinical setting, and
whether this translates into additional or differential clinical benefit between AChR+ and
MuSK+ MG beyond that seen with anti-CD20 therapeutics remains to be seen.

In addition to therapies that directly target cells positive for CD20 and CD19, there has
now been the development of therapies that attempt to prevent the maturation of these
cells. Belimumab, a monoclonal antibody specific against B-cell activating factor
(BAFF), is one such therapy\cite{136}. BAFF is a survival factor expressed by T cells and
dendritic cells that is necessary for cell proliferation and B-cell maturation\cite{137}. Studies
have shown that BAFF levels are elevated in patients with AChR+ and MuSK+ MG\cite{138}.
Belimumab has been approved for use and clinical trials have demonstrated its efficacy in
the treatment of SLE\cite{139-141}. However, a placebo-controlled multicenter double-blind
study studying the use of belimumab versus placebo in patients with generalized MG
who remained symptomatic despite standard of care therapy did not find any clinical
benefit in terms of reduction in QMG scores\cite{136}. While not yet shown to be beneficial in
MG, the use of anti-BAFF therapies in the treatment of MG deserves further research.

The role of combination therapy in treating MG is also an important question that
deserves investigation. While combination biologic therapy has not yet been studied on a
large scale in patients with MG, there have been several studies that suggest its efficacy
in patients with SLE. Two randomized controlled studies are currently underway
examining combination therapy with rituximab and belimumab in patients with SLE: a
phase 3 BLISS-BELIEVE trial comparing combination therapy with belimumab alone\textsuperscript{142} and a phase 2 BEAT-LUPUS\textsuperscript{143} clinical trial assessing the safety of combination therapy. A case series published in 2018 identified 3 patients with active SLE who experienced clinical remission, ability to taper immunosuppressive therapies, and a reduction of BAFF levels after combination therapy with rituximab and belimumab\textsuperscript{144}. The same year, the results of a phase 2A, open-label, single-arm proof-of-concept study were published\textsuperscript{145}. In this study, 16 SLE patients were treated with a combination of rituximab and belimumab. The results were promising: 10 patients achieved a low lupus disease activity state and 14 patients were able to taper concomitant immunosuppressive medications. This study also specifically examined levels of neutrophil extracellular traps (NETs), which play an important role in autoimmunity by delivering autoantigens to the host immune system and for the development of anti-nuclear antibody (ANA), as well as BAFF levels. BAFF levels have been observed to surge after rituximab treatment, which has been theorized to stimulate repopulation of autoreactive B-cells. The findings included that post rituximab and belimumab sequential therapy, patients experienced a reduction of circulating ANAs and excessive NET formation, a restoration of complement C3 and C4 levels to normal, a reduction in BAFF levels, and a complete but transient B-cell depletion with early repopulation of memory B cells and circulating plasma cells. These promising results in the field of SLE identify a new area of research in MG waiting to be pursued.
Non B-cell Therapies

Perhaps with more immediate clinical implications, a study conducted in 2016 demonstrated promising results regarding the use of autologous hematopoietic stem cell transplantation (HSCT) for treatment of severe MG\textsuperscript{146}. This study observed 7 MG patients undergoing autologous HSCT (for MG, 1 for follicular lymphoma with coincident MG) and found that all patients achieved durable MGFA status of complete stable remission. This study indicates that further research looking into HSCT for treatment of MG is necessary.

Most recently, a phase II randomized, double-blind, placebo-controlled multicenter study investigating the safety and efficacy of efgartigimod, an anti-neonatal Fc receptor IgG1 Fc fragment in patients with generalized AChR+ MG, demonstrated that all patients who received the treatment showed a rapid decrease in total IgG and anti-AChR levels. Further, a rapid and long-lasting disease improvement was observed in 75\% of treated patients\textsuperscript{147}. FcRn is a protein within the cell that functions to protect the immunoglobulin molecule from lysosomal degradation by combining with IgG and returning it unharmed to the cell’s surface\textsuperscript{148,149}. An FcRn inhibitor is an antibody that binds to the native FcRN and blocks binding to IgG, which leads to degradation of IgG in the lysosome. This study offers an alternative approach to treating MG via an FcRN-targeted pathway to reduce pathogenic autoantibodies. Above all else, these new developments reaffirm that the field of immunomodulatory therapy for autoimmune disorders is constantly evolving.
The field of monoclonal antibody-based therapies is an exciting and rapidly progressing one. New targets are constantly being identified for the treatment of MG patients. For example, research has now spread into interleukin receptor blockade and proteasome inhibition. Tocilizumab, a monoclonal antibody against IL6, has recently been identified as a promising therapeutic option in a case study of two patients refractory to rituximab\textsuperscript{150}. Bortezomib, a proteasome inhibitor, was observed to be effective in a case study of one patient with MG refractory to rituximab\textsuperscript{151}. Opportunities for more precisely targeted therapies will arise as more immunopathologic targets are identified, likely overtaking rituximab in the future.

CONCLUSIONS

Rituximab therapy is a reasonable option for both AChR+ and MuSK+ generalized MG patients, especially for those with difficult to control disease. While MuSK+ MG patients may experience greater benefits, a significant differential response between groups was not observed in our retrospective study. B-cell targeted therapy continues to be a rational strategy in the MG treatment paradigm. Further investigations are warranted to explore how B-cell depletion therapy is best applied, especially with the advent of other targeted treatment options, to achieve improved outcomes which are both meaningful and sustained in our patients.
CONFLICTS OF INTEREST

Tess D. Litchman reports no conflicts. Richard J. Nowak reports no conflicts directly related to this work. Dr. Nowak has received research support from the National Institutes of Health (NIH), Genentech, Alexion Pharmaceuticals, Ra Pharmaceuticals, Myasthenia Gravis Foundation of America, Momenta, and Grifols. He has served as consultant/advisor for Alexion Pharmaceuticals, CSL Behring, Grifols, Ra Pharmaceuticals, Roivant, and Momenta. Bhaskar Roy reports no conflicts directly related to this work. Dr. Roy has served as consultant/advisor for Alexion Pharmaceuticals. All other authors report no conflicts related to their work.

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