The Role of Rituximab in the Treatment of Myasthenia Gravis

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Abstract
Rituximab, a chimaeric monoclonal antibody against CD20, depletes B cells. It was initially approved for the treatment of B-cell lymphomas, but more recently has been approved for use in rheumatoid arthritis. It has been used extensively ‘off-label’ for the treatment of other autoimmune diseases with some evidence of efficacy, but there remain some as yet unanswered concerns about safety. Myasthenia gravis is the paradigm of an antibody-mediated disorder, and B cells are believed to play a crucial role. This article reviews experience of the efficacy and safety of rituximab in myasthenia gravis and considers predictive factors for the success and failure of rituximab in this disease.

Keywords
Rituximab, myasthenia gravis, antiacetylcholine receptor (anti-AChR), antimuscle-specific tyrosine kinase (anti-MuSK), predictive factors, clinical response

Disclosure: The authors have no conflicts of interest to declare.

Received: 20 September 2010 Accepted: 8 November 2010 Citation: European Neurological Review, 2010;5(2):95–100 DOI:10.17925/ENR.2010.05.02.95

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Myasthenia gravis (MG) is an autoimmune disease associated with circulating antibodies, either against the nicotinic acetylcholine receptor (anti-AChR; 80% of patients with generalised MG) or muscle-specific tyrosine kinase (anti-MuSK, 10% of patients), that induce a dysfunction of neuromuscular transmission owing to loss of functional receptors. Less commonly, MG remains confined to the ocular muscles. Only about 50% of such patients have antibodies detectable by standard assay (almost invariably anti-AChR) and most respond well to moderate doses of steroids without the need for more aggressive immunosuppression. In addition to anticholinesterase drugs, most patients with generalised MG require long-term treatment with steroids and immunosuppressive drugs, of which the most commonly used include azathioprine, mycophenolate mofetil and ciclosporin.24 Between 5 and 10% of patients remain refractory to such treatment.24 Other immunosuppressive drugs may then be considered, including cyclophosphamide, tacrolimus and etanercept, whose efficiency has not been assessed on the basis of double-blind clinical trials. Intravenous immunoglobulins (IVIg)25 and plasma exchange26 are used for acute exacerbations while waiting for other treatments to become effective, but have no sustained beneficial effect. Newer effective molecules with a good safety profile are undoubtedly needed.

Rituximab (RTX), a chimaeric monoclonal antibody specific for human CD20 that targets B lymphocytes, was first developed (and licensed) for the treatment of B-cell lymphomas27–29 and is used at a dose of 375mg/m2/body surface area once weekly for four weeks. It was noted that in patients with lymphoma treated with RTX and concomitantly suffering from autoimmune diseases (rheumatoid arthritis [RA] or MG)) the autoimmune diseases were ameliorated. Subsequent to these early reports, RTX has been used in many autoimmune diseases where B cells seem to play a role, not only in RA. These pivotal studies30–32 led to the molecule being licensed in cases of RA resistant to antitumour necrosis factor (anti-TNF) first-line therapy (RTX 1g on days one and 15), and also being used (off-label) in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis,33 multiple sclerosis,34 systemic lupus erythematosus (SLE),35 immune thrombocytopenic purpura (ITP)36 and pemphigus.37

To date, the experience of the use of RTX in MG has been mainly in the form of single case reports,38–44 short series of patients35,45 and a report of all 10 MG patients in the UK who were identified through personal contact as having received RTX,46 representing overall to date 53 RTX-treated MG patients (see Table 1). Among them, 47 were reported as being improved after a follow-up of six to 48 months (see Table 1), with some in complete stable remission. Of the other six patients, three were unchanged, two worsened and one died after RTX treatment (see Table 1). It is highly probable that there is reporting bias and that failures of RTX in MG are not published (either not submitted by authors or rejected by journals), a notable exception being the compilation of all UK-treated RTX MG patients (see Table 1). It is noteworthy that four of the six failures recorded to date were described in this study.46 To circumvent this likely positive reporting bias and to better evaluate the efficacy and safety of RTX in refractory MG, we are currently conducting a prospective phase II open trial (identifier: NCT00774462). Given that RTX is clearly not 100% effective, it is appropriate to ask whether there are predictive factors for response and, more generally, where if at all RTX should stand in the order of treatment options.
Neuromuscular Disease

Mechanisms of Action of Rituximab

CD20 (the target of RTX) is expressed from the early pre-B-cell stage and remains present in mature B cells. It is not present on stem cells and is lost before the differentiation of B cells into plasma cells. RTX is known to deplete B cells by three mechanisms:

- antibody-dependent cellular cytotoxicity (ADCC): natural killer cells, macrophages and monocytes are recruited through their Fcγ receptors bound to surface CD20, inducing B-cell lysis;
- complement-dependent cytotoxicity as a result of complement activation by the B cell–RTX complex and the generation of a membrane attack complex, again leading to B-cell lysis; and
- direct apoptosis of B cells induced by RTX binding.

B-cell depletion following RTX lasts for eight months on average in the peripheral blood, after which a new ontogeny repopulates the B-cell pool. This is characterised by the appearance of immature B cells (CD38+, CD10+, CD24+), followed by naïve B cells (CD27−), while memory B cells (CD27+) may remain reduced for up to two years.

B Cells in Myasthenia Gravis – Role of Rituximab

No correlation has been found between the serum antibody titre and disease severity in MG. Nevertheless, MG remains the paradigm of an antibody-mediated disorder. Several lines of evidence demonstrate the humoral immune-mediated nature of MG:

- the anti-AChR antibodies lead to loss of acetylcholine receptors and reduced efficiency of neuromuscular transmission;
- human IgG is found at the neuromuscular junctions of MG muscles;
- placental transfer of these antibodies in women with myasthenia can cause foetal or neonatal weakness and, occasionally, severe deformities;
- plasma exchange produces a striking clinical benefit in MG;
- the plasma, or IgG purified from it, is able to transfer features of MG to mice; and
- immunisation against AChRs consistently induces evidence of MG in experimental animals.

B cells are not directly responsible for the production of these antibodies (anti-AChR and anti-MuSK). However, on activation and cross-linking of surface immunoglobulins by a specific antigen, B cells undergo proliferation and differentiation to produce plasma cells. These are non-dividing, specialised cells whose only function is to secrete immunoglobulins. Plasma cells are divided into two groups: short-lived cells (a few days) producing IgM and long-lived cells (years) producing IgA and IgG.

Although the percentage of B cells in the peripheral blood of patients with MG is similar to that of healthy subjects, the percentage of B cells that expressed CD71, a transferrin receptor marker of B-cell activation, was greater in MG patients compared with controls. It is well established that for some anti-AChR+ patients, their thymus is implicated in the physiopathogenesis of MG. In those thymuses, the presence of germinal centres with strong overexpression of CD23 and Bcl-2 indicates that B-cell activation and proliferation are occurring. Within germinal centres, B cells are in close contact with dendritic cells (DCs) and are influenced by soluble signals produced by DC such as B-cell-activating factor (BAFF), the level of which was increased in MG patients compared with controls. Moreover, B cells may function as antigen-presenting cells and provide important co-stimulatory signals (such as cytokines) required for CD4+ T-cell clonal expansion and effector functions. T cells also play an important role in the physiopathogenesis of MG (see below).

By depleting B cells, RTX may benefit MG through one of several RTX mechanisms. First, RTX depletes B cells, the precursors of plasma cells. With time, one may hypothesise that, as plasma cells are not replaced, antibody titres will decrease. In 29 MG cases the antibody titres before and after RTX were reported (see Table 1). It is noteworthy that in the vast majority of cases (except for three patients) antibody titres decreased after RTX, in six patients to undetectable levels (see Table 1).

This effect of RTX has also been described in other autoimmune diseases, with disappearance of autoantibodies in some but not all patients. For instance, in a recent controlled trial of RTX (versus cyclophosphamide, n=197) for ANCA-associated vasculitis, 47% of those in the RTX group became ANCA-negative by six months. In RA, RTX treatment was associated with a large and rapid decrease in rheumatoid factor levels. Concerning the global level of immunoglobulins, in the pivotal trial of RTX for RA (n=161), 121 treated with RTX, by six months levels of immunoglobulins (IgG, IgM and IgA) did not change substantially and there was no effect on antitetanus antibody titres. However, with increasing cycles of RTX (up to seven years), hypogammaglobulinaemia can occur, a side effect that can be controlled by IVIg injections. It also has to be noted that IgM levels fall more than IgG levels (10% fall after a first cycle of RTX, 19% after a second and 24% after a third), as reported in an open-label extension of three controlled trials (n=1,039). This may be due to the fact that plasma cells producing IgM have a relatively short half-life. Second, B-cell activation, especially in germinal centres of the thymus, should disappear with B-cell depletion, at least in patients where the depletion of B cells is achievable in primary (such as thymus) or secondary (lymph nodes) lymphoid organs. This B-cell depletion, usually effective in peripheral blood, can vary widely in different tissues. Third, the antigen-presenting role of B cells and cytokine production, implicated in T-cell functions, can be abrogated by B-cell depletion.

T Cells in Myasthenia Gravis – Role of Rituximab

MG is driven by ACHR-specific T cells. Specific (mostly CD4+) T cells to different ACHR epitopes are found in the thymus and peripheral blood of MG patients. However, self-specific T cells (even for ACHR epitopes) are also frequently found in the peripheral blood of healthy individuals. These T cells do not induce injury because of mechanisms of peripheral tolerance, achieved in large part through the action of regulatory T (Treg) cells. Many studies in different human autoimmune diseases have reported abnormalities in Treg, in their frequency, in their function or both. Proof of principle of Treg cell therapy has been achieved in different animal models and now human trials are starting or are planned (despite difficulties, such as in Treg production). Concerning MG, a severe defect of Treg functions (with normal Treg numbers) was found in peripheral blood and in the thymus.

RTX has the potential to influence T-cell response by several different mechanisms, but to date the significance of those mechanisms has not been explored in RTX-treated MG patients. First, RTX can increase
# Table 1: Characteristics and Response to Rituximab Treatment in 53 Patients with Myasthenia Gravis

<table>
<thead>
<tr>
<th>Ref</th>
<th>Age, Sex</th>
<th>MG Type</th>
<th>Previous Treatments</th>
<th>MGFA CC Immediately Before RTX</th>
<th>Ab Titre Before RTX</th>
<th>Initial Dose of RTX</th>
<th>Other Cycles of RTX (n)</th>
<th>Follow-up Duration Post-first RTX (months)</th>
<th>MGFA PS After RTX</th>
<th>Ab Titre After RTX</th>
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<tbody>
<tr>
<td>25</td>
<td>27, M</td>
<td>AChR+</td>
<td>S, ASTC, CA, I</td>
<td>IVb</td>
<td>55nM</td>
<td>BL</td>
<td>N</td>
<td>6</td>
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<td>15nM</td>
</tr>
<tr>
<td>26</td>
<td>9, F</td>
<td>AChR+</td>
<td>S, P, I, A</td>
<td>IVb</td>
<td>7.3nM</td>
<td>BL</td>
<td>N</td>
<td>22</td>
<td>MM</td>
<td>1.5nM</td>
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<tr>
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<td>C, S</td>
<td>Estimated III</td>
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<td>BL</td>
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<td>Im</td>
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<td></td>
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</tr>
<tr>
<td>27</td>
<td>56, F</td>
<td>MuSK+</td>
<td>S, I, P, A, M</td>
<td>IVb</td>
<td>NR</td>
<td>BL</td>
<td>Y (1)</td>
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<tr>
<td>28</td>
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<td>MuSK+</td>
<td>S, A, P, I, CA, M, C</td>
<td>IVb</td>
<td>NR</td>
<td>BL</td>
<td>Y (1)</td>
<td>18</td>
<td>CSR</td>
<td>NR</td>
</tr>
<tr>
<td>29</td>
<td>21, F</td>
<td>MuSK+</td>
<td>S, I, P, A, M</td>
<td>Estimated IVb</td>
<td>NR</td>
<td>BL</td>
<td>Y (1)</td>
<td>13</td>
<td>CSR</td>
<td>NR</td>
</tr>
<tr>
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<td>46, F</td>
<td>AChR+</td>
<td>S, I, CA</td>
<td>V</td>
<td>2471M</td>
<td>BL</td>
<td>N</td>
<td>9</td>
<td>MM</td>
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<td>S, A, CA, M, P</td>
<td>IVb</td>
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<td>NR</td>
<td>N</td>
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<td>Im</td>
<td>NR</td>
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<td>32</td>
<td>75, M</td>
<td>AChR+</td>
<td>S, I, Mx</td>
<td>Ilia</td>
<td>&gt; 8 U</td>
<td>BL</td>
<td>Y (7)</td>
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<td>MM</td>
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<tr>
<td>33</td>
<td>50, F</td>
<td>AChR+</td>
<td>S, Mx, A, I, P</td>
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<td>MM</td>
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<tr>
<td>34</td>
<td>11, M</td>
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<td>S, I, A, M</td>
<td>Estimated IVb</td>
<td>NR</td>
<td>BL</td>
<td>N</td>
<td>NR</td>
<td>W</td>
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<td>After RTX Failure, Successfully Treated by ASCT</td>
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<tr>
<td>35</td>
<td>14, F</td>
<td>AChR+</td>
<td>S, A, M, C, ASC</td>
<td>Estimated IVb</td>
<td>NR</td>
<td>BL</td>
<td>Y (2)</td>
<td>25</td>
<td>CSR</td>
<td>NR</td>
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<tr>
<td>36</td>
<td>76, M</td>
<td>AChR+</td>
<td>S, I, P</td>
<td>V</td>
<td>192IU/ml</td>
<td>1g x 2</td>
<td>N</td>
<td>18</td>
<td>MM</td>
<td>NR + undetected</td>
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<tr>
<td>37</td>
<td>71, M</td>
<td>AChR+</td>
<td>P, Mx</td>
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<td>310nM</td>
<td>BL</td>
<td>Y (1)</td>
<td>14</td>
<td>CSR</td>
<td>13nM</td>
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<tr>
<td>RTX Given for Lymphoma</td>
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<tr>
<td>38</td>
<td>52, F</td>
<td>MuSK+</td>
<td>S, A, CA, M, C, I</td>
<td>IVb</td>
<td>7.5nM</td>
<td>BL</td>
<td>Y (2)</td>
<td>9</td>
<td>MM</td>
<td>2nM</td>
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<td>CSR</td>
<td>NR</td>
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<td>19.8nM</td>
<td>N</td>
<td>24</td>
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<td>41</td>
<td>59, NR</td>
<td>MuSK+</td>
<td>S, I, P</td>
<td>Estimated IVb</td>
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<td>BL</td>
<td>Y (9)</td>
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<tr>
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<td>S, I</td>
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<td>BL</td>
<td>Y (9)</td>
<td>24</td>
<td>CSR</td>
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</tr>
<tr>
<td>43</td>
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<td>S, CA, M</td>
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<td>&gt;100nM</td>
<td>BL</td>
<td>Y (1)</td>
<td>48</td>
<td>CSR</td>
<td>60nM</td>
</tr>
<tr>
<td>44</td>
<td>54, F</td>
<td>MuSK+</td>
<td>S, I, M</td>
<td>Estimated IVb</td>
<td>8.2nM</td>
<td>BL</td>
<td>N</td>
<td>60</td>
<td>I</td>
<td>3.6nM</td>
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<tr>
<td>45</td>
<td>12, F</td>
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<td>S, I, M</td>
<td>Estimated IVb</td>
<td>46nM</td>
<td>BL</td>
<td>Y (2)</td>
<td>29</td>
<td>D</td>
<td>9.3nM</td>
</tr>
<tr>
<td>46</td>
<td>19, M</td>
<td>AChR+</td>
<td>S, A, CA, I</td>
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<td>212nM</td>
<td>BL</td>
<td>Y (1)</td>
<td>12</td>
<td>I</td>
<td>350nM</td>
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<tr>
<td>47</td>
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<td>S, A, CA, M, P</td>
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<td>75nM</td>
<td>BL x1/2</td>
<td>Y (3)</td>
<td>33</td>
<td>MM</td>
<td>110nM</td>
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<td>48</td>
<td>45, F</td>
<td>MuSK+</td>
<td>S, I, P</td>
<td>Estimated IIIb</td>
<td>NR</td>
<td>Y (3)</td>
<td>27</td>
<td>MM</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>21, F</td>
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<td>S, A</td>
<td>Estimated IIIb</td>
<td>220IU/ml</td>
<td>Y (3)</td>
<td>24</td>
<td>MM</td>
<td>150nM</td>
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<td>AChR+</td>
<td>S, I, M</td>
<td>Estimated IVb</td>
<td>NR</td>
<td>1g x 2</td>
<td>N</td>
<td>24</td>
<td>MM</td>
<td>0.9nM</td>
</tr>
<tr>
<td>51</td>
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<td>S, CA, M</td>
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<td>NR</td>
<td>MM</td>
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<td>&gt;1000nM</td>
<td>BL</td>
<td>Y (1)</td>
<td>48</td>
<td>CSR</td>
<td>60nM</td>
</tr>
<tr>
<td>53</td>
<td>31, F</td>
<td>AChR+</td>
<td>S, I, M</td>
<td>Estimated IVb</td>
<td>8.2nM</td>
<td>BL</td>
<td>N</td>
<td>30</td>
<td>D</td>
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<tr>
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<td>Estimated IVb</td>
<td>46nM</td>
<td>BL</td>
<td>Y (2)</td>
<td>12</td>
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<td>350nM</td>
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<tr>
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<td>BL</td>
<td>Y (2)</td>
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<td>I</td>
<td>NR</td>
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<td>Patient 3 Also Treated for a Malignant Thymoma</td>
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<td>BL</td>
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<td>NR</td>
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<td>NR</td>
<td>BL</td>
<td>Y (1)</td>
<td>6</td>
<td>IM</td>
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<td>NR</td>
<td>BL</td>
<td>Y (4)</td>
<td>24</td>
<td>MM</td>
<td>NR</td>
</tr>
<tr>
<td>59</td>
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<td>BL</td>
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<td>MM</td>
<td>NR</td>
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<td>BL</td>
<td>Y (3)</td>
<td>15</td>
<td>MM</td>
<td>0.19nM</td>
</tr>
<tr>
<td>61</td>
<td>55, F</td>
<td>AChR+</td>
<td>S, A, P</td>
<td>Estimated IVb</td>
<td>NR</td>
<td>BL</td>
<td>Y (2)</td>
<td>6</td>
<td>IM</td>
<td>NR</td>
</tr>
</tbody>
</table>

The Role of Rituximab in the Treatment of Myasthenia Gravis
Factors Predictive of Response to Rituximab

A potential cause of failure of RTX is a polymorphism of the Fcγ receptor III gene (substitution of a phenylalanine for a valine at position 158). This is predictive of failure in the treatment of B-cell lymphoma and SLE, but not in chronic lymphocytic leukaemia or in Sjögren’s syndrome. This polymorphism has not been studied in MG.

The quality of B-cell depletion seems to influence the response to RTX. In RA, a recent study (n=60) showed that rapid (at day 15 before the second 1g RTX injection) and complete B-cell depletion measured using a highly sensitive flow cytometry assay (below 0.0001x10^9/l versus 0.05x10^9/l in conventional assays) was predictive of a better clinical outcome. Interestingly, patients in whom B cells were depleted only after the second infusion did no better than those in whom depletion was never complete. It could be that patients who have difficulty achieving peripheral clearance may be more likely to have difficulty clearing other lymphoid organs and/or inflamed tissues, with a subsequent poorer response. B-cell depletion is more depleted only after the second infusion did no better than those in the macaque monkey, greater B-cell depletion is achieved by increasing the first injection dose (mimicking the RA regimen) than repeating the doses (such as during the standard B lymphoma regimen; personal observation). So, whether to prescribe 375mg/m^2 four times or 1g twice may depend on the disease being treated and individual variations in response. The past history of the disease and its treatment may also influence response. Long-term refractory disease in patients who have received prior immunosuppressant drugs may be associated with the selection of particular memory B-cell clones (CD27+IgD- class-switched memory B cells) that could be more resistant to RTX. These memory B cells, which first reappear during B-cell reconstitution, seem to correlate with a poorer outcome in RA. Similarly, a higher number of anti-TNF agent failures in RA seems to be associated with poorer clinical outcome after RTX.

Moreover, the subtype of autoantibodies accompanying an autoimmune disease may influence RTX response. For example, in RA the presence of rheumatoid factors rather than anti-CCP antibodies was associated with better clinical response after RTX. Considering MG, in the 53 RTX-treated patients so far reported (see Table 1), 20 (38%) were anti-MuSK+, a much higher proportion than in the general MG population (10%). This may reflect observations that MuSK+ MG tends to be more severe and resistant to conventional immunosuppressants than AChR+ MG, but in addition, because of positive reporting bias, that RTX may be more effective in MuSK+ MG than AChR+ MG. Furthermore, five of the six MG patients whose antibodies reached undetectable levels after RTX were MuSK+ (see Table 1).

Finally, RTX is a chimaeric monoclonal antibody (i.e. murine antibody origin for a portion of the variable region and human origin for the remaining variable and constant regions), and human anti-chimaeric antibody (HACA) can be induced by RTX injection. The proportion of patients with HACA positivity was 9.2% after more than one cycle of RTX in RA. However, there was no clear evidence that the presence of HACA interfered with the safety or efficacy of additional courses of RTX, and HACA positivity does not appear to be a significant concern in determining whether a patient should receive additional RTX courses, at least in RA. In SLE, the proportion of patients developing HACA seems higher; in one trial of 24 patients

### Table 1: (continued)

<table>
<thead>
<tr>
<th>Ref</th>
<th>Age, Sex</th>
<th>MG Type</th>
<th>Previous Treatments</th>
<th>MGFA CC Immediately Before RTX</th>
<th>AB Titre Before RTX</th>
<th>Initial Dose of RTX</th>
<th>Other Cycles of RTX (n)</th>
<th>Follow-up Duration Post (first) RTX (months)</th>
<th>MGFA PS</th>
<th>Ab Titre After RTX</th>
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<tr>
<td>45</td>
<td>11, F</td>
<td>AChR+</td>
<td>S, P, I</td>
<td>IVa</td>
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<td>NR</td>
<td>NR</td>
<td>CSR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>AChR+</td>
<td>S, A, M, P, C</td>
<td>V</td>
<td>BL, NR</td>
<td>NR</td>
<td>NR</td>
<td>CSR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>AChR+</td>
<td>S, A, I, P</td>
<td>Ibid</td>
<td>BL, NR</td>
<td>NR</td>
<td>NR</td>
<td>U</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>29</td>
<td>F</td>
<td>MuSK+</td>
<td>S, A, CA, I, P</td>
<td>Ibid</td>
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<td>NR</td>
<td>NR</td>
<td>PR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>34</td>
<td>F</td>
<td>AChR+</td>
<td>S, A, Mx, M, I</td>
<td>Ibid</td>
<td>BL, NR</td>
<td>NR</td>
<td>NR</td>
<td>U</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>35</td>
<td>F</td>
<td>AChR+</td>
<td>S, A, Mx, CA, I, P</td>
<td>Ibid</td>
<td>BL, NR</td>
<td>NR</td>
<td>NR</td>
<td>W</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>36</td>
<td>F</td>
<td>AChR+</td>
<td>S, A, I</td>
<td>V</td>
<td>BL, NR</td>
<td>NR</td>
<td>NR</td>
<td>Im</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>40</td>
<td>F</td>
<td>MuSK+</td>
<td>S, M, I</td>
<td>V</td>
<td>BL, NR</td>
<td>NR</td>
<td>NR</td>
<td>Im</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>46</td>
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<td>AChR+</td>
<td>S, A, I</td>
<td>V</td>
<td>BL, NR</td>
<td>NR</td>
<td>NR</td>
<td>U</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>52</td>
<td>F</td>
<td>MuSK+</td>
<td>S, A, M, I, P</td>
<td>Ibid</td>
<td>BL, NR</td>
<td>NR</td>
<td>NR</td>
<td>Im</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

Patient 9 Had a Malignant thymoma

A = azathioprine; Ab = antibodies; AChR+ = anticholinesterase-receptor-antibody-positive; ASTC = allogenic stem cell transplantation; BL = standard dose of RTX for B lymphoma (375mg/m^2, every week for four consecutive weeks); CA = cyclophosphamide; CA = cyclosporine A; CSR = complete stable remission; D = died; I = intravenous immunoglobulins; Im = improved; MGFA CC = Myasthenia Gravis Foundation of America clinical classification; AA = MM = minimal manifestation; MuSK+ = antimuscle-specific tyrosine kinase-antibody-positive; M = methotrexate; N = no; NR = not reported; P = plasma exchange; PR = pharmacological remission; RA = rheumatoid arthritis; RTX = rituximab; S = steroids (prednisone or prednisolone); T = unchanged; VGKC+ = antivoltage-gated potassium channel antibody-positive; W = worse; Y = yes.

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The Role of Rituximab in the Treatment of Myasthenia Gravis

10 (42%) developed the condition, with two of them having a serum-sickness-like syndrome. This syndrome, comprising a triad of fever, rash and polyarthritis, develops within a couple of weeks of the RTX injection and responds to corticosteroids. Fewer than 20 cases have been reported so far, mostly associated with the treatment of autoimmune diseases as opposed to lymphoma.

Unanswered Questions
The use of RTX for MG is in its infancy. Clinical trials are lacking, arguably in part because of a lack of support from the pharmaceutical industry, so our experience is based on 53 reported cases (see Table 1) and personal observations. The evidence relates to refractory, severe, generalised MG. All patients had failed to respond adequately to at least two conventional immunosuppressant drugs (on average RTX was used as the fifth-line immunosuppressant treatment; see Table 1). The MG was severe despite prior therapies (mostly Myasthenia Gravis Foundation of America (MGFA) grade IV; see Table 1). In all cases RTX was never used alone but rather in combination with different conventional immunosuppressants. Thus, the frequent improvement observed (see Table 1) cannot be attributed solely to the RTX. Many questions remain unanswered.

Would Rituximab Be Effective If Used as a Single Therapy?
To answer this question, a controlled trial of RTX versus steroids at an earlier stage of the disease (MGFA grade II/III) would be required.

What Is the Best Combination of Immunosuppressants to Use with Rituximab in Severe Myasthenia Gravis?
As a result of the rarity of the disease and inter-individual variation of response, there may not be a single answer to this question and the choice will depend on the past experience of each patient. We also have to keep in mind that the risk of severe side effects, notably of progressive multifocal leucoencephalopathy (PML), increases with combination therapy (see below). In MG, RTX has been mostly used at B lymphoma doses (375mg/m²x4) but has sometimes been used at RA doses (1g x 2), without a clear difference in efficacy (see Table 1).

What Is the Best Regimen for Myasthenia Gravis?
The first approved dosing schedule for RTX was based on the original trial in B-cell lymphoma. However, it is likely that a course of two infusions (of 1g) may become the standard dosing regimen for patients with autoimmune diseases, as it has been approved for RA. This is the schedule being used in the MG trial we are conducting.

The clinical response in patients with severe MG is not rapid (personal observation) and is typically between one and four months, but sometimes slower or quicker until further RTX cycles (see Table 1). This delay may depend on the past history of the MG, associated immunosuppressants and other as yet undefined factors. It seems highly likely that some patients will be resistant to RTX.

Are There Any Identifiable Factors that Usefully Predict the Clinical Response?
We have noted some of the factors known from treating autoimmune diseases and lymphoma (see above). None can be performed routinely, except the B-cell count in peripheral blood (measured by flow cytometry). The lack of complete depletion seems to be associated with a poorer outcome. The effect of RTX is transient and, after a variable period, many patients (with different autoimmune diseases) will have a relapse.

What Is the Proportion of Myasthenia Gravis Patients in Complete Stable Remission After One Cycle of Rituximab?
This depends on the past history of the patient, but maybe also on the type of antibodies (are MuSK+ MG patients more sensitive to RTX?). Future clinical trials will address this question.

Is the B-cell Reconstitution in Peripheral Blood a Predictive Factor for Relapse?
In RA, 50% of the patients relapsed coincident with the reappearance of B cells in the peripheral blood, but relapse was delayed (up to 33 months after the reappearance of B cells) for the others. Absence of B cells thus correlates well with lack of relapse, but the reappearance of B cells does not accurately predict relapse, or at least the timing of it. However, it is probably of help to monitor this parameter over time.

In severely affected MG patients we and others (see Table 1) have repeated RTX before evidence of relapse to try to maintain stable remission. Experience in RA shows that patients treated with repeated courses of RTX (up to seven years) have sustained clinical responses with no new adverse events.

How Can We Reduce the Risk of Relapse?
One may distinguish two situations. The first is the case of patients in whom a flare-up will not be life-threatening, in which case RTX can be repeated ‘à la carte’, guided by the clinical signs of relapse, as rheumatologists have done for RA. By contrast, in cases of life-threatening disease where a relapse should as far as possible be avoided, it is appropriate to consider repeating RTX in a preventative manner (e.g. 1g every six months). Apart from consideration of cost, the latter approach can be justified only if such long-term treatment does not carry with it unacceptable risks and side effects.

Side Effects of Rituximab and General Recommendations for Its Use in Myasthenia Gravis
Presumably as RTX does not affect stem or plasma cells, cytopenia (notably neutropenia) or hypogammaglobulinaemia giving rise to an increased risk of infections are rare (although occasionally described). The risk of infection is higher when RTX is combined with chemotherapy or other immunosuppressants. The overall tolerability profile of RTX is good. Nevertheless, a major concern is the risk of PML. To date, 61 PML cases in association with RTX have been reported. Of those cases, 56 patients were treated for a lymphoproliferative disorder with RTX in combination with chemotherapy, and the remaining five for autoimmune diseases (two SLE, one RA, one ITP and one autoimmune pancytopenia). Among the latter, one received RTX with corticosteroids without other immunosuppressants. The fatality rate was 90% with a median time to death of two months after PML diagnosis. Another risk is of hepatitis B reactivation. For occult carriers (HBSAg-negative, HBCab-positive), RTX can be prescribed under lamivudine prophylaxis.

In practice, the general recommendations before and during RTX treatment are to check for hepatitis B infection, pregnancy (RTX does not appear to be teratogenic but crosses the placental barrier and induces foetal B-cell depletion), immunoglobulin levels, routine blood
cell counts and B-cell count by flow cytometry. With respect to PML, brain magnetic resonance imaging and cerebrospinal fluid analysis should be performed if any clinical signs develop.

Conclusions

RTX is generally well tolerated and has already proved its efficacy as salvage therapy for severe, resistant MG (see Table 1). Many questions still remain (listed above). In our opinion there are sufficient data to justify the use of RTX in patients with severe MG that has proved resistant to steroids, and appropriate trials in terms of dose and duration of two of the standard immunosuppressant drugs. Although cost has been raised as an issue in some centres, it should be remembered that a course of RTX currently costs about one-third of a five-day course of either IVg or plasma exchange. Furthermore, the long-term health-associated costs of a patient with severe MG, notably relating to repeated hospital and intensive care admissions, are potentially enormous (never mind social care issues, employment, etc.). More difficult are the issues relating to long-term side effects from RTX therapy, most notably PML but also hypogammaglobulinaemia. We believe that current evidence favours the use of RTX in the situations stated and of course following appropriate discussions with the patient.

What is currently unanswerable is the place of RTX in the management of milder MG, and in particular whether its use should be considered before standard immunosuppressant drugs, including steroids. Although steroids are the established mainstay of treatment, they have significant limitations in terms of both efficacy and safety. Both cost and long-term side effects become more of an issue in this patient group. We believe that it is appropriate for multicentre trials to be undertaken to answer these issues. Ad hoc use and single-case reporting should be discouraged; that approach, as reviewed above, was arguably a necessity for the relatively rare situation of severe refractory MG, but to answer these important questions in this larger group of patients demands a more formal approach.

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### Neuromuscular Disease