**Treatment Outcomes With Rituximab in 100 Patients With Neuromyelitis Optica**

**Influence of FCGR3A Polymorphisms on the Therapeutic Response to Rituximab**

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**IMPORTANCE** Despite the increased use of rituximab therapy in neuromyelitis optica spectrum disorder (NMOSD), the overall efficacy and safety of long-term rituximab treatment in a large group of patients is uncertain. Furthermore, the identification of a predictor of rituximab response is an important issue for assessing the individual risk-benefit of therapy and making treatment decisions.

**OBJECTIVE** To assess the long-term clinical efficacy and safety of rituximab treatment in patients with NMOSD and the influence of fragment c gamma receptor 3A (FCGR3A) polymorphisms on rituximab response.

**DESIGN, SETTING, AND PARTICIPANTS** A retrospective review of 100 patients with relapsing NMOSD treated with rituximab for at least 6 months, from February 1, 2006, to January 31, 2015, at the institutional referral center. After induction therapy, a single infusion of rituximab (375 mg/m²) as maintenance therapy was administered whenever a reemergence of CD27 memory B cells among peripheral blood mononuclear cells occurred. Using an allele-specific polymerase chain reaction–based method, the gene polymorphisms FCGR3A-V158F were assessed.

**MAIN OUTCOMES AND MEASURES** The primary end point was annualized relapse rate; disability (Expanded Disability Status Scale score), safety of rituximab treatment, event of insufficient memory B-cell depletion following rituximab, and time to retreatment of rituximab were secondary end points.

**RESULTS** By January 31, 2015, a total of 100 patients received repeated rituximab treatment during a median of 67 months. Of these patients, 41 had more than 5 years’ follow-up and 24 had more than 7 years’ follow-up. The annualized relapse rate was reduced significantly by 96% (mean [SD] annualized relapse rate of prerituximab vs postrituximab, 2.4 [2.0] vs 0.1 [0.6]) and disability improved or stabilized in 96% of patients. Rates of adverse events were generally stable. The FCGR3A-F allele was associated with a risk of relapse while receiving rituximab treatment (additive model, \( P < .05 \); recessive model, \( P = .04 \); maximum, \( P = .03 \)) and insufficient memory B-cell depletion (additive model, \( P = .03 \); recessive model, \( P = .03 \); maximum, \( P = .03 \)).

**CONCLUSIONS AND RELEVANCE** Repeated rituximab treatment for NMOSD was observed in an increasing number of patients and increasing duration of exposure and maintained good efficacy and a safety profile consistent with previous reports. The finding of a relationship between FCGR3A genetic polymorphisms and rituximab response suggests the importance of individualized rituximab treatment strategies in NMOSD.


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Rituximab is a chimeric monoclonal anti-CD20 antibody that has been proposed as an effective therapy for neuromyelitis optica spectrum disorder (NMOSD). Previous data have been based mostly on small case series with short-term follow-up. There has been concern about the variability of rituximab responses. Previously, we reported the therapeutic efficacy of rituximab in 30 patients with NMOSD across a 5-year period and the depletion of memory B cells in peripheral blood was associated with a clinical response to rituximab. However, not all patients respond to rituximab treatment and the durability of the memory B-cell depletion is variable. The reason for this variability in rituximab response and the question of who will benefit from a lower and more cost-effective dose of rituximab is unresolved.

Rituximab-coated B cells are eliminated by the following mechanisms: antibody-dependent cell cytotoxicity (ADCC) by natural killer cells, complement-dependent cytotoxicity, and apoptosis. In particular, the prevailing mechanism of B-cell depletion is believed to be ADCC, mediated by effector cells that engage the fragment c portion of rituximab via the fragment c gamma receptor, which is present on immune cells. Previous studies have suggested that the therapeutic activities of rituximab may be affected by patients’ biological characteristics, such as fragment c gamma receptor 3A (FCGR3A) gene polymorphisms. A valine (V)/phenylalanine (P) substitution at position 158 of FCGR3A is the polymorphism that affects the affinity of receptors in human IgG binding and the 158F allele has a lower affinity for human IgG. In hematologic disease, carrying 1 or 2 FCGR3A-158F alleles was associated with a poorer response to rituximab therapy. Nevertheless, to our knowledge, no reported genetic study has examined the association between FCGR3A polymorphisms and rituximab responses in NMOSD.

In the current study, we report our experience with repeated rituximab treatment in 100 patients with NMOSD across a median period of 5 years. The objectives of this study were to determine the clinical efficacy and safety in a larger number of patients compared with what has been previously reported and explore the influence of FCGR3A genotypes on rituximab response.

Methods

Study Population

Patients with NMOSD (definite NMO, using the 2006 revised diagnostic criteria or the limited form of NMO with anti-aquaporin 4 [AQP4] antibodies) received rituximab therapy for at least 6 months at the National Cancer Center, Korea from February 1, 2006, to January 31, 2015. The original study cohort reported in 2011 included 30 patients with NMOSD who began rituximab treatment before January 2009. Since then, 56 patients who newly began rituximab treatment and 14 patients who initiated rituximab treatment before 2009 but were excluded from the original report owing to prior mitoxantrone treatment were also included in the current study. Consequently, 100 total patients with NMOSD were included. Clinical data from the patients were evaluated retrospectively. At the last review, only 4 patients discontinued rituximab treatment. Two patients moved to a long-distance location after 24 and 35 months of rituximab, 1 patient died of pneumonia, and 1 patient discontinued rituximab treatment after 9 months owing to ongoing relapses. These patients’ data on rituximab treatment were not censored in our analysis. This study was approved by the institutional review board of the National Cancer Center. Written informed consent was obtained from all patients.

Rituximab was administered according to our published protocol. The following 2 regimens were used as induction treatment: 375 mg/m² infused once weekly for 4 weeks and 1000 mg infused twice during a 2-week interval. After induction therapy, a single infusion of rituximab (375 mg/m²) as maintenance therapy was administered whenever the frequency of reemerging CD27+ memory B cells in peripheral blood mononuclear cells measured with flow cytometry exceeded 0.05% in the first 2 years and 0.1% thereafter.

Assessment

The primary end point was the annualized relapse rate (ARR). Secondary endpoints were the neurological status indicated by the Expanded Disability Status Scale (EDSS) score, proportion of patients who were relapse free, and safety of rituximab. Relapse was defined as a new neurological disturbance that increased the EDSS score by at least half a point or when the worsening of 1 point in 2 functional systems or 2 points in 1 functional system occurred and lasted for at least 24 hours in the absence of fever or infection. If a new neurological change accompanied a corresponding new magnetic resonance imaging lesion, it was also considered to be relapse, regardless of disability change. Immediate relapses were treated with high-dose intravenous methylprednisolone. If a severe disability persisted after corticosteroid therapy, plasma exchange was performed. Clinical adverse events were recorded throughout the study. Serum levels of immunoglobulins were measured every year.

In terms of biological responses to rituximab treatment, a patient who had more than 2 events of incomplete memory B-cell depletion at 6 to 10 weeks after administration of rituximab was considered to have insufficient memory B-cell depletion. The time to retreatment, which was determined by the degree of memory B-cell depletion and repopulation, was considered to be another end point of the biological rituximab response. In the analysis of the clinical response according to genotype, relapses associated with delayed retreatment (against the retreatment protocol) were excluded.

Flow Cytometric Analysis

Peripheral blood samples were obtained every 6 weeks throughout the first year, every 8 weeks throughout the second year, and every 10 weeks thereafter to evaluate lymphocyte subsets, including CD27+ memory B cells. Details are provided in eAppendix 1 in the Supplement.

FCGR3A Genotype Determination

Of the 100 patients, the following 9 did not provide consent for genetic analyses: 2 moved to long-distance locations, 1 died,
and 6 continued taking rituximab. Determination of the FCGR3A-V158F polymorphism was done blindly on a coded specimen by polymerase chain reaction followed by direct sequencing. Details are provided in eAppendix 2 in the Supplement. The distributions of the VV, VF, and FF genotypes of FCGR3A were 8%, 35%, and 57%, respectively.

Statistical Analyses
The ARR and EDSS scores before and after receiving rituximab were compared using the Wilcoxon signed rank test. Univariable logistic regression analysis was used to test for association among values. Baseline demographic and clinical differences between the genotypes were compared using the parametric Mann-Whitney or Kruskal-Wallis test for continuous variables and the Fisher exact test for categorical variables. Genetic associations were analyzed using a Cochran-Armitage trend test with an additive and recessive model. Because it is known that a single test statistic optimal for 1 model may experience a substantial loss of power, we attempted to apply an efficiency robust test by taking the maximum test statistics of additive and recessive models.20 A P value less than .05 was considered to indicate statistical significance and the SAS, version 9.3 (SAS Institute, Inc) and R, version 3.0.2 software packages were used for all analyses.

Results
Patient Characteristics
Clinical and demographic profiles of the patients are outlined in Table 1. Of the 100 patients with NMOSD, 56 patients were immunosuppressant naive, 44 patients received 1 or more immunosuppressants before beginning rituximab therapy, 22 patients received mitoxantrone at a median cumulative dose of 104 mg/m2, and 28 patients received azathioprine or mycophenolate mofetil treatment.

Treatment Outcome
As of January 31, 2015, 100 patients had received rituximab across a median period of 67 months (range, 9-108 months), 41 patients continued rituximab treatment across 5 years, and 24 patients continued rituximab treatment across 7 years. Of the 100 total patients, 70 (70%) were relapse free. The mean (SD) prerituximab ARR was 2.4 (2.0), and the mean (SD) postrituximab ARR was 0.1 (0.6) (P < .001). Of 100 patients, 94 (94%) showed a marked reduction in the ARR (<25% of the preimmunotherapy ARR; Figure). The median EDSS score was 4 (range, 0-8.5) before rituximab treatment and 3 (range, 0-8.0) after treatment (P < .001). The EDSS score improved in 58 patients and stabilized in 38 patients. Worsening of the EDSS score after rituximab was observed in only 4 patients.

The median number of retreatments after induction was 7 treatments (range, 1-17 treatments). The mean interval between treatments was 29 weeks, 23 weeks (range, 8-56 weeks) during the initial 2-year study, and 37 weeks (range, 15-81 weeks) thereafter. Among the 96 patients who continued treatment, 3 patients switched from rituximab to mitoxantrone therapy after 6, 14, and 20 months of rituximab, respectively, owing to frequent events of insufficient memory B-cell depletion. After monthly administration of as much as 72 mg/m2 of mitoxantrone for 6 months, these 3 patients switched back to receiving rituximab therapy. Two of the 3 patients revealed prolonged retreatment intervals of rituximab therapy after mitoxantrone therapy. One patient combined cyclosporine and steroid treatment for psoriasis while receiving rituximab treatment in the dermatology clinic. None of the patients were given concomitant immunosuppressants while receiving rituximab other than these 4 patients.

Relapses During Treatment and Clinical Factors Associated With Rituximab Responses
In total, 49 relapses occurred in 30 of 100 patients receiving rituximab. Eleven patients had more than 2 relapses and 5 had more than 3 relapses while taking rituximab. First, 6 relapses in 5 patients occurred during the early treatment stages (within 6 weeks) when the depletion of memory B-cells was not yet sufficient. Second, 7 relapses in 5 patients were associated with delayed retreatment. Finally, the remaining 36 relapses in 25 patients occurred despite patients following the treatment protocol, 25 relapses in 18 patients occurred in conjunction with insufficient depletion or unexpected rapid repopulation of memory B cells, and 11 relapses in 9 patients occurred during periods where memory B cells were fewer than the therapeutic target. Most relapses were of mild to moderate severity (EDSS score of <6.0 or a visual acuity better than 20/200 Snellen units at the nadir attack) and only 7 relapses in 4 patients required plasma exchange following steroid treatment. According to the univariable regression analysis, the patients’ baseline characteristics, including onset age, anti-AQP4 antibody status, disease duration, prior treatment, pre-ARR, and rituximab duration were not associated with relapse while receiving rituximab treatment or post-ARR (data not shown). Re-

Table 1. Baseline Clinical Characteristics of 100 Patients Treated With Rituximab

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>43 (11)</td>
</tr>
<tr>
<td>Onset age, mean (SD), y</td>
<td>38 (11)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>92 (92)</td>
</tr>
<tr>
<td>Seropositivity for anti-aquaporin 4 antibody, No. (%)</td>
<td>94 (94)</td>
</tr>
<tr>
<td>Disease duration, mean (SD), y</td>
<td>11 (5)</td>
</tr>
<tr>
<td>Interval from disease onset to rituximab treatment, median (range), mo</td>
<td>50 (2-228)</td>
</tr>
<tr>
<td>Total attacks prior to rituximab treatment, No. (%)</td>
<td>9 (7)</td>
</tr>
<tr>
<td>Total attacks prior to any immunosuppressive treatment, No. (%)</td>
<td>8 (7)</td>
</tr>
<tr>
<td>Previous immunosuppressive treatment history, No. (%)</td>
<td>44 (44)</td>
</tr>
<tr>
<td>Mitoxantrone treatment</td>
<td>22 (22)</td>
</tr>
<tr>
<td>Azathioprine or mycophenolate mofetil</td>
<td>28 (28)</td>
</tr>
<tr>
<td>Previous interferon beta treatment history, No. (%)</td>
<td>56 (56)</td>
</tr>
<tr>
<td>ARR before rituximab, mean (SD)</td>
<td>2.4 (2.0)</td>
</tr>
<tr>
<td>ARR before immunotherapy, mean (SD)</td>
<td>2.7 (2.3)</td>
</tr>
<tr>
<td>EDSS score before rituximab, median (range)</td>
<td>4 (0-8.5)</td>
</tr>
</tbody>
</table>

Abbreviations: ARR, annualized relapse rate; EDSS, Expanded Disability Status Scale.
Regarding biological responses, previous mitoxantrone treatment was associated with a prolonged interval to retreatment not only in the initial 2 years (34 vs 22 weeks; \( P < .001 \)) but also thereafter (46 vs 37 weeks; \( P = .02 \)). However, other clinical variables were not associated with time to retreatment or insufficient memory B-cell depletion.

Safety

Of 100 patients, no case of progressive multifocal leukoencephalopathy was observed. The most frequent adverse events were infusion-related reactions during the first infusion (26%) but the incidence declined with subsequent infusions. No patient had a severe infusion reaction leading to drug withdrawal. Five cases of herpes zoster infection, 2 cases of pneumonia, and 1 case of thyroid cancer were observed during rituximab treatment; however, these events did not result in discontinuation of rituximab. Three patients had psoriasis before the onset of NMOSD. They were relapse free while receiving rituximab treatment but showed no improvement in the preexisting psoriasis. One patient, a woman in her mid-sixties, died of pneumonia after taking rituximab for 6 years. She had been taking more than 1 year of corticosteroids and cyclosporine for severe psoriasis during rituximab treatment. Five months after the last retreatment of rituximab, she was admitted to a local clinic for dyspnea and aggravation of psoriasis. Despite empirical antibiotic treatment, pneumonia rapidly progressed to septic shock and the patient died. There were 2 planned pregnancies that occurred during rituximab treatment. After more than 4 years of rituximab treatment, 2 patients became pregnant 3 and 4 months after the last retreatment with rituximab, respectively. Rituximab treatment was reinitiated within 1 month after delivery and the repopulation of memory B cells was observed when treatment was re-initiated (0.21% and 0.18%, respectively). Each patient delivered a healthy baby and no relapse occurred during or after pregnancy. In 41 patients treated with rituximab for more than 5 years, 53%, 29%, and 10% had low IgM, IgG, and IgA levels, respectively. Additionally, of the 24 patients with more than 7 years of treatment, 46%, 42%, and 25% had low IgM, IgG, and IgA levels, respectively. However, no increase in infection rates was observed in patients with low IgM or IgG levels.

Rituximab Response According to FCGR3A Genotypes

Not all patients responded to rituximab treatment in our cohort and the interval to retreatment varied among patients. To explore the predictive factors for rituximab response, we investigated the influence of the FCGR3A genotypes on rituximab efficacy. The demographic and disease characteristics did not differ across genotypes (Table 2). The FCGR3A-158F allele was associated with a risk of insufficient memory B-cell depletion and a short retreatment interval during the initial 2 years (Table 3). To exclude potential prolonged effects of previous mitoxantrone treatment on rituximab response, we analyzed the association between biological response and genotype in a sub-
The results of subgroup analysis revealed a stronger association of the FCGR3A-F allele with risk of insufficient memory B cells and short time to retreatment (Table 3). In terms of clinical response, the FCGR3A-158F allele was associated with a risk of at least 1 relapse while receiving rituximab treatment but no association was observed between the FCGR3A genotype and more than 2 relapses post-ARR or EDSS worsening (Table 4).

Discussion

In the current study, we described treatment outcomes of 100 patients with NMOSD who were treated with rituximab across 5 years. To our knowledge, this is the largest cohort study with the longest follow-up of rituximab treatment in patients with NMOSD. The inclusion of 41 patients with more than 5 years of...
treatment and 24 patients with more than 7 years of treatment provided a high level of confidence in the efficacy and safety of long-term rituximab treatment. The relapse rate was reduced significantly by 96% and 94% of patients had a marked reduction in the ARR (<25% of the prerituximab ARR). In addition, 70% of patients were relapse free and disability improved or stabilized in 96% of patients. The high response rate in the current study suggests that more patients with NMOSD than previously thought6,7 may benefit from rituximab therapy if its use is tailored. The high-response rate might be partly attributed to the particularly high seropositive rate (94%) for anti-AQP4 antibodies in our cohort. Serious adverse events did not increase across time or with multiple retreatments. Although 1 death due to pneumonia was found in our cohort, the effect of rituximab treatment in relation to the death is unclear owing to the comorbidity of severe psoriasis21 and other immunosuppressive treatments comitant with rituximab. A decrease in immunoglobulin levels was observed in some patients following rituximab treatment but the clinical consequences of this are unclear.

Regarding the retreatment strategy of rituximab, we previously suggested a treatment-to-target approach using memory B cells in peripheral blood.3,5 Likewise, in the current study involving an increased number of patients, most clinical relapses occurred following memory B-cell repopulation. Despite rituximab treatment, an insufficient depletion of memory B cells was associated with poor clinical response. Furthermore, the degree of memory B-cell depletion and rituximab retreatment intervals varied among individuals. These findings suggest that regular retreatment regardless of disease activity might pose a risk of insufficient efficacy in some patients and overtreatment in others. Accordingly, there is an unmet need to identify patients likely to require frequent administration of rituximab.

In the present study, we found that the FCGR3A genotype was an independent predictor of peripheral B-cell depletion and the clinical response to rituximab. The FCGR3A-F allele was associated with greater probabilities of insufficient depletion of memory B cells, a short retreatment interval, and relapse during rituximab treatment. These results are consistent with previous findings in patients with lymphomas or rheumatoid arthritis in which the response to rituximab appeared to be poorer in patients with FCGR3A-FF genotype, likely a result of the low-antibody affinity of natural killer cells and decreased ADCC efficacy.16,22-25 Our results suggest that a similar mechanism of B-cell depletion with ADCC activity is likely to be important in rituximab therapy for NMOSD. Nonetheless, the finding that the FCGR3A-FF genotype does not inevitably lead to insufficient memory B-cell depletion may be explained by lower yet still sufficient ADCC activity or, more likely, by other B-cell depletion mechanisms.17

Despite the risk of insufficient depletion of memory B cells following rituximab in patients with the FCGR3A-FF genotype, clinical outcomes, including more than 2 relapses post-ARR and EDSS worsening, were not significantly different in patients with this genotype. The notable clinical response to rituximab therapy among our patients with the FF genotype might be explained by the individualized tailoring of rituximab retreatment to maintain therapeutic B-cell depletion through more frequent retreatment than was used in FCGR3A-V allele carriers. Consequently, the patients with the FCGR3A-FF genotype underwent significantly shorter retreatment intervals than did FCGR3A-V allele carriers. Previously, Dall’Ozzo and colleagues11 also suggested that adjusting the rituximab dose or administration schedule in patients with the FCGR3A-FF genotype would achieve a better clinical response. Thus, patients with the FCGR3A-FF genotype compared with FCGR3A-V allele carriers may require more intensive rituximab therapy to achieve efficacious B-cell depletion in NMOSD.

Conclusions

These observations from 100 patients with NMOSD treated with rituximab across 5 years, including a substantial num-

### Table 4. Clinical Outcome According to FCGR3A Genotype in 91 Total Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relapse ≥1 OR (95% CI)</th>
<th>P Value</th>
<th>Relapse ≥2 OR (95% CI)</th>
<th>P Value</th>
<th>Postrituximab ARR Coefficient Estimate (95% CI)</th>
<th>P Value</th>
<th>EDSS Worsening P Value</th>
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</thead>
<tbody>
<tr>
<td><strong>FCGR3A</strong></td>
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<tr>
<td>Additive VF vs VV genotypes</td>
<td>2.35 (1.07 to 5.85)</td>
<td>.05b</td>
<td>1.17 (0.49 to 4.90)</td>
<td>.58</td>
<td>0.12 (−0.07 to 0.31)</td>
<td>.20</td>
<td>.99</td>
</tr>
<tr>
<td>FF vs VV genotypes</td>
<td>5.50 (1.14 to 24.24)</td>
<td>.04b</td>
<td>1.88 (0.24 to 24.01)</td>
<td>.65</td>
<td>0.24 (−0.13 to 0.62)</td>
<td>.19</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Recessive V carriers vs FF genotypes</td>
<td>0.35 (0.12 to 0.91)</td>
<td>.03b</td>
<td>0.42 (0.21 to 3.30)</td>
<td>.56</td>
<td>−0.16 (−0.4 to 0.08)</td>
<td>.18</td>
<td>.88</td>
</tr>
<tr>
<td>MAX*</td>
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<tr>
<td>Additive VF vs VV genotypes</td>
<td>4.82 (1.61 to 21.29)</td>
<td>.01b</td>
<td>2.17 (0.61 to 13.82)</td>
<td>.30</td>
<td>0.14 (−0.09 to 0.38)</td>
<td>.23</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>FF vs VV genotypes</td>
<td>23.22 (2.58 to 453.24)</td>
<td>.01b</td>
<td>4.72 (0.38 to 191.03)</td>
<td>.38</td>
<td>−0.19 (−0.5 to 0.11)</td>
<td>.22</td>
<td>.99</td>
</tr>
<tr>
<td>Recessive V carriers vs FF genotypes</td>
<td>0.18 (0.04 to 0.63)</td>
<td>.12b</td>
<td>0.47 (0.07 to 2.25)</td>
<td>.99</td>
<td>−0.19 (−0.5 to 0.11)</td>
<td>.22</td>
<td>.99</td>
</tr>
<tr>
<td>MAX*</td>
<td></td>
<td>.01b</td>
<td>.32</td>
<td>.25</td>
<td>.93</td>
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</table>

Abbreviations: ARR, annualized relapse rate; EDSS, Expanded Disability Status Scale; MAX*, maximum test statistic of additive and dominant models.

*Wide confidence interval owing to low-cell frequency.

bP < .05.

Subgroup analysis in 71 patients without prior mitoxantrone treatment history.
Treatment Outcomes With Rituximab in Patients With Neuromyelitis Optica

Original Investigation Research


