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Title
Intravenous ofatumumab treatment of multiple sclerosis and related disorders: an observational study

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Abstract

Background: Ofatumumab is an anti-CD20 monoclonal antibody approved for subcutaneous administration for the treatment of relapsing multiple sclerosis (MS), but intravenously administered ofatumumab has been investigated in a phase 2 trial and used off-label. The objective of the present study was to assess disease activity and side effects in relation to longer-term intravenous ofatumumab treatment of MS and related disorders.

Methods: We conducted a retrospective study of patients treated off-label with intravenous ofatumumab for MS, neuromyelitis optica spectrum disease (NMOSD) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) at the Danish Multiple Sclerosis Center. Data was retrieved from the Danish Multiple Sclerosis Registry and through medical chart review.

Results: Fifty patients were identified with a median treatment duration of 2.2 years. Annualized relapse rate decreased from 1.03 at baseline to 0.38 during ofatumumab treatment. At 24 months, the probability of having experienced a relapse was 55% and confirmed disability worsening 7%. Frequency of infusion-related reactions was 86% during the first infusion and 42% during the last infusion. Six experienced infections requiring hospitalization.

Conclusion: Our data indicate a reduction of relapse frequency, stabilization of disability worsening and an acceptable safety profile, although we observed a higher frequency of infusion reactions compared to data from other intravenously administered anti-CD20 monoclonal antibodies. The study supports a class effect of anti-CD20 monoclonal antibodies and the hypothesis that complement activation may be associated to a higher frequency of infusion related reactions.

Keywords
Ofatumumab, multiple sclerosis, demyelinating disorders, CD20 depleting therapies
Abbreviations

ADCC: Antibody-dependent cell-mediated cytotoxicity
ARR: Annualized relapse rates
CDC: Complement-dependent cytotoxicity
CDI: Confirmed disability improvement
CDW: Confirmed disability worsening
DMSC: Danish Multiple Sclerosis Center
DMSR: Danish Multiple Sclerosis Registry
DMT: Disease-modifying therapy
MS: Multiple sclerosis
EDSS: Expanded Disability Status Scale
IRR: Infusion-related reactions
IV: Intravenous
MOGAD: Myelin-oligodendrocyte glycoprotein associated disease
NK: Natural killer
NMOSD: Neuromyelitis optica spectrum disease
RRMS: Relapsing-remitting MS
SC: Subcutaneous
1. Introduction

Multiple sclerosis (MS), neuromyelitis optica spectrum disease (NMOSD) and myelin-oligodendrocyte glycoprotein associated disease (MOGAD) are inflammatory and demyelinating disorders of the central nervous system (Wingerchuk et al., 2007; Reich et al., 2018; Höftberger et al., 2020). B cells are implicated in the immunopathogenesis of these disorders, and targeting B cells with anti-CD20 monoclonal antibodies is an increasingly used treatment strategy for MS, NMOSD and MOGAD (Graf et al., 2021a, 2021b).

The surface molecule CD20 is expressed by most B cells and a subset of proinflammatory T cells (Von Essen et al., 2019; Sellebjerg et al., 2020). Ofatumumab is a fully human anti-CD20 monoclonal antibody which depletes CD20+ cells through complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) (Reagan and Castillo, 2011).

Subcutaneous (SC) ofatumumab was approved for relapsing forms of MS with active disease by the U.S. Food and Drug Administration in August 2020 and by the European Medicines Agency in March 2021 based on two phase III trials demonstrating its efficacy on reducing relapse rate, disability worsening and MRI lesion activity (Hauser et al., 2020). Intravenous (IV) ofatumumab, however, has only been studied for MS in a phase II trial including 38 subjects with relapsing-remitting MS (RRMS). The study demonstrated the tolerability of two ofatumumab infusions two weeks apart, and a significant reduction in MRI lesion activity in comparison to placebo during the first 24 weeks after ofatumumab administration (Sorensen et al., 2014). No phase III trial has been conducted, as focus has since shifted to SC ofatumumab administration.

IV ofatumumab (brand name Arzerra®) was approved by the European Medicines Agency in 2010 for treatment of chronic lymphocytic leukemia. Until April 2019, when commercial marketing of the drug was terminated in Europe, selected patients with demyelinating disorders were treated off-label with IV ofatumumab at the Danish Multiple Sclerosis Center (DMSC). The objective of this study was to assess effectiveness and side effects of IV ofatumumab therapy in MS, NMOSD and MOGAD.
2. **Material and methods**

2.1. **Study design**

We conducted a retrospective observational study of all patients who had received IV ofatumumab (Arzerra®, originally developed by Genmab, Copenhagen, Denmark; current marketing authorization holder Novartis Europharm Ltd., Camberley, UK) as treatment for MS, NMOSD or MOGAD at DMSC. Patients were identified through the Danish Multiple Sclerosis Registry (DMSR) (Magyari et al., 2021), and no timeframe restrictions were applied.

2.2. **Treatment and follow up**

The ofatumumab treatment regimen consisted of two induction infusions at a 2-weeks interval followed by a maintenance infusion every 6 months. Standard induction doses were 300 mg followed by 1000 or 300 mg, while maintenance dose was 1000 or 600 mg (before and after April 2016, respectively). Treatment with IV ofatumumab was terminated when marketing of Arzerra® was discontinued in 2019, and many patients treated with ofatumumab were subsequently switched to rituximab (maintenance dose 1000 mg) or ocrelizumab (maintenance dose 600 mg). Patients were pretreated with oral methylprednisolone, fexofenadine and paracetamol before infusions.

At the DMSC, clinical evaluation including Expanded Disability Status Scale (EDSS) scoring is routinely scheduled every 6 months for patients on anti-CD20 monoclonal antibodies. The DMSR is regularly notified by clinicians during regular clinical visits. Immunoglobulin levels (IgA, IgG and IgM) and flowcytometric determination of blood lymphocyte subset counts (CD19+ B cells, CD3+ T cells, CD3+CD4+ T cells, CD3+CD8+ T cells and CD16+CD56+ natural killer (NK) cells) were routinely measured 1-4 weeks before each ofatumumab infusion series. For patients with MS, an MRI re-baseline scan of the brain and spinal cord is generally obtained within 3-6 months after changing or initiating disease-modifying therapy (DMT), and control brain MRI scans are conducted yearly thereafter.

2.3. **Data collection**
Baseline characteristics and blood test results were collected from the latest clinical visit prior to ofatumumab initiation. For relapses, we registered the summed number throughout the year prior to commencement of ofatumumab. Baseline cerebral MRI was defined as the latest scan within 12 months prior to ofatumumab initiation, and re-baseline was defined as the first scan after treatment start.

From the DMSR, we retrieved demographic and clinical data such as sex, diagnosis, date of birth, date of disease onset and diagnosis, relapses and EDSS scores, information on cerebral MRI lesions, initiation and termination of ofatumumab treatment, and prior and subsequent DMT. Additionally, we reviewed electronic medical charts, imaging and laboratory results for participants who continually were affiliated with the DMSC at the time of chart review, cross-validating the data retrieved from the DMSR and collecting information on infections requiring hospitalization, infusion-related reactions (IRRs), immunoglobulin levels, T, B and NK cell counts and assessing for late onset neutropenia.

2.4. Outcomes

Primary outcome was clinical relapses the first year of ofatumumab treatment and annualized relapse rates (ARR) throughout treatment. ARR was calculated for patients who completed at least one year of ofatumumab treatment only and with follow-up from 6 months after the first infusion (re-baseline) until 6 months after the last infusion. Secondary outcomes with follow-up until 6 months after the last infusion were confirmed disability worsening (CDW) or improvement (CDI) for patients with \( \geq 3 \) EDSS assessments, radiological disease activity (gadolinium contrast enhancement and new or enlarging T2 brain MRI lesions) and infections requiring hospitalization. Moreover, we assessed immunoglobulin levels and blood cell counts 4-8 months after each infusion series, late onset neutropenia until up to 2.5 years after termination of ofatumumab treatment, IRRs during the first three infusions and the last infusion and, for patients who were subsequently treated with rituximab or ocrelizumab, IRRs during the first rituximab or ocrelizumab infusion.

CDW was defined as an increase in EDSS score confirmed after \( \geq 6 \) months and meeting the following criteria: \( \geq 1.5 \) points increase for patients with baseline EDSS score = 0, \( \geq 1.0 \) points
increase for baseline EDSS score >0 and ≤5.5, and ≥0.5 points increase for baseline EDSS score >5.5. CDI was defined as a decrease in EDSS score of ≥1 point and was only assessed for patients with baseline EDSS ≥2.0. Assessment of CDW and CDI required a minimum of 3 EDSS scorings within the follow-up period.

B cell counts were truncated at 0.01 × 10^9 cells per liter, as this was the lower limit of detection until 2018. Patients who were treated with another CD20 depleting therapy prior to ofatumumab treatment were excluded from B cell count and IRR results during the first three infusions. IRRs were graded according to a modified version of the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0 (U.S. Department of Health and Human Services, 2017). For immunoglobulin analyses, we excluded measurements during immunoglobulin replacement therapy.

### 2.5. Statistical analyses

Statistical analyses were performed depending on whether data followed a normal distribution or not, which was checked visually by histograms and QQ-plots and statistically by Shapiro-Wilk test. Continuous non-parametric data are reported as medians with their 1st and 3rd quartiles (Q1-Q3) or range, and categorical data as counts and percentages. Relapse rates before and on treatment were calculated using a generalized estimating equation with a negative binomial distribution and log link function. We assumed the working correlation structure to be unstructured. McNemar Test was used for comparing paired categorical data, and Pearson’s Chi-squared test for unpaired categorical data. For survival data, we analyzed differences between survival curves by log-rank test and calculated hazard ratios where applicable. Mixed model analysis was used for repeated measures of continuous outcomes. In statistical analyses p<0.05 was considered significant. Analysis of ARR was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Analyses apart from ARR were conducted in R version 4.1.0 (Gerds, 2019; Pinheiro et al., 2021; R Core Team, 2021), and graphs were made in R and GraphPad Prism version 9.0.0 (GraphPad Software, San Diego, CA, USA).

### 2.6. Protocol approval
The study was approved by the directors of the Neuroscience Center and the Department of Neurology, Copenhagen University Hospital – Rigshospitalet. According to Danish legislation non-interventional register-based studies require neither ethics approval nor informed patient consent. To comply with regulatory definitions for anonymization, results are only reported for groups comprising more than 3 patients. Where relevant, groups ≤3 were pooled.
2. Results

2.1. Patient characteristics

Fifty eligible patients were identified and included, all of whom had been treated between February 2013 and April 2019. Forty-five patients were eligible for chart review, and 37 were subsequently treated with ocrelizumab (n=23) or rituximab (n=14) (Figure 1). Baseline characteristics are summarized in Table 1.

Figure 1
Flow chart outlining patient eligibility and inclusion.

Records (n=52) on patients having received ofatumumab against a demyelinating disease

Excluded (n=2):
- 1 duplicate
- 1 patient treated with subcutaneous ofatumumab

Patients included for data extraction from the Danish Multiple Sclerosis Registry (n=50)

Excluded (n=5):
- No longer affiliated with the Danish Multiple Sclerosis Center

Patients included for medical chart review (n=45)

Excluded (n=8):
- Did not switch to ocrelizumab or rituximab

Patients included for ocrelizumab/rituximab extension (n=37)

Registry data was included for all 50 patients and includes relapses, EDSS changes, MRI data, initiation and termination of ofatumumab treatment, prior and subsequent DMT. Medical chart data was available for 45 patients and includes infections requiring hospitalization, infusion-related reactions, immunoglobulin levels, T, B and NK cell counts and late-onset neutropenia.
Table 1
Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>N</th>
<th>NMOSD/MOGAD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at treatment start, years&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45 (35-51)</td>
<td>42</td>
<td>50 (41-56)</td>
<td>8</td>
</tr>
<tr>
<td>Sex, female (%)</td>
<td>35 (83%)</td>
<td>42</td>
<td>7 (88%)</td>
<td>8</td>
</tr>
<tr>
<td>Disease duration, years&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12 (7-16)</td>
<td>42</td>
<td>6 (4-12)</td>
<td>8</td>
</tr>
<tr>
<td>Phenotype/disorder (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRMS</td>
<td>35 (83%)</td>
<td>42</td>
<td>4 (50%)</td>
<td>8</td>
</tr>
<tr>
<td>Progressive MS</td>
<td>7 (17%)</td>
<td>42</td>
<td>4 (50%)</td>
<td>8</td>
</tr>
<tr>
<td>MOGAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMOSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDSS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 (3.0-5.9)</td>
<td>42</td>
<td>4.0 (3.3-4.4)</td>
<td>8</td>
</tr>
<tr>
<td>Number of DMT changes prior to starting ofatumumab&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 (3.0-5.0)</td>
<td>42</td>
<td>2.0 (1.8-3.3)</td>
<td>8</td>
</tr>
<tr>
<td>Latest DMT prior to ofatumumab</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fingolimod</td>
<td>17 (40%)</td>
<td>42</td>
<td>6 (75%)</td>
<td>8</td>
</tr>
<tr>
<td>Dimethyl fumarate</td>
<td>8 (19%)</td>
<td>42</td>
<td>6 (75%)</td>
<td>8</td>
</tr>
<tr>
<td>Natalizumab</td>
<td>10 (24%)</td>
<td>42</td>
<td>6 (75%)</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>5 (12%)</td>
<td>42</td>
<td>6 (75%)</td>
<td>8</td>
</tr>
<tr>
<td>Reason for switching to ofatumumab</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse effects</td>
<td>9 (23%)</td>
<td>40</td>
<td>7 (88%)</td>
<td>8</td>
</tr>
<tr>
<td>Disease activity</td>
<td>27 (68%)</td>
<td>40</td>
<td>7 (88%)</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>4 (10%)</td>
<td>40</td>
<td>7 (88%)</td>
<td>8</td>
</tr>
<tr>
<td>Relapses previous year, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>14 (33%)</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 1</td>
<td>28 (67%)</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10 (0.84-1.43)</td>
<td>40</td>
<td>0.69 (0.35-1.36)</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Median (Q1-Q3). <sup>b</sup>Estimate (95% confidence interval)

Of the 50 patients included, 4/50 (8%) were diagnosed with NMOSD, 4/50 (8%) with MOGAD and 42/50 (84%) with MS, of which 35/42 (83%) had RRMS and 7/42 (17%) had progressive MS. Median age at treatment start was 46 years (Q1-Q3: 35-52 years), and median EDSS was 4.0 (Q1-Q3: 3.0-5.5). Throughout the year preceding ofatumumab initiation, 17/50 (34%) experienced no relapse.

Baseline brain MRI results were available for 41/42 (98%) of patients with MS. New or enlarging lesions were present in 12/41 (29%), and contrast enhancing lesions in ≤3 of 25 scans with post-contrast sequences.
Before ofatumumab treatment, ≤3 were untreated, ≤3 were treated with rituximab and 45/50 (90%) were treated with another DMT. Overall, the patients had switched DMT a median of 3 times (Q1-Q3: 2-5) prior to initiation of ofatumumab. The most frequent DMTs before switching to ofatumumab were natalizumab (11/50 or 22%), fingolimod (18/50 or 36%) and dimethyl fumarate (8/50 or 16%), and most frequent reasons for discontinuation were disease activity (34/48 or 71%) and adverse events (10/48 or 21%). Median wash-out periods were 1.6 months (Q1-Q3: 1.4-1.8) for natalizumab, 1.6 months (Q1-Q3: 1.2-2.6) for fingolimod, and 2.2 (Q1-Q3: 0.6-5.3) for dimethyl fumarate.

2.2. Disease activity and disability worsening

The first year after initiation of ofatumumab treatment, the relapse-free proportion of patients was 30/50 (60%), which was significantly different from the 34% the year preceding ofatumumab treatment (p=0.009). The second year of ofatumumab treatment, the relapse-free proportion of patients was 27/43 (63%). For patients who completed at least one year of ofatumumab treatment (n=48), the ARR was reduced with 63% (95% CI 47%-74%, p <0.0001) from 1.03 (95% CI 0.79-1.32) at baseline to 0.38 (95% CI 0.26-0.55) during treatment (median follow-up time from re-baseline = 2.1 years). Absolute rate difference was -0.64 (95% CI -0.89 to -0.40, p < 0.0001).

For MS patients separately (n=40), the ARR was reduced with 63% (95% CI 46%-74%, p < 0.0001) from 1.10 (95% CI 0.84-1.43) at baseline to 0.41 (95% CI 0.28-0.60) on treatment. The absolute rate difference was -0.69 (95% CI -0.96 to -0.41, p <0.0001). For MOGAD and NMOSD patients (n=8), the ARR on treatment was 0.27 (95% CI 0.09-0.76). Due to the small sample size, we did not have enough power to reasonably estimate before-and-after differences for this population.

Overall, 30/50 (60%) experienced at least one relapse during follow-up, 8/45 (18%) developed 6 months CDW, and ≤3/45 patients had CDI. For patients who experienced a relapse, median time to first relapse was 6 months (range 23 days-30 months) from treatment start. For MS patients, the probability of having experienced at least one relapse at 24 months was 54% (95% CI 38%-%
69%), and the probability of 6 months CDW was 8% (95% CI 0%-17%) at 24 months. The cumulative risk of CDW and relapse in MS over time is depicted in Figure 2.
Figure 2

Risk of relapse and disability worsening for patients with multiple sclerosis

Kaplan-Meier cumulative incidence curves showing A) the cumulative proportion of multiple sclerosis patients with relapse breakthrough over time, and B) the cumulative proportion of multiple sclerosis patients with six months confirmed EDSS worsening over time.

On re-baseline MRI scans, 5/41 (12%) of MS patients had at least one new or enlarged brain lesion, and ≤3/10 had contrast enhancing lesions. From the re-baseline scan until 6 months after the last infusion, ≤3/29 MS patients developed a new or enlarging lesion. Median MRI follow up
time (i.e. time between re-baseline scan until the last scan within follow up) was 1.9 years (Q1-Q3: 1.3-2.4 years).

Outcomes related to clinical disease activity and worsening are summarized in Table 2.

### Table 2

Intravenous ofatumumab treatment and disease activity

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>MS</th>
<th>N</th>
<th>NMOSD/MOGAD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment duration, years(^a)</td>
<td>2.2 (1.8-2.6)</td>
<td>2.1 (1.8-2.6)</td>
<td>42</td>
<td>3.0 (2.3-3.7)</td>
<td>8</td>
</tr>
<tr>
<td>ARR(^b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence rate ratio</td>
<td>0.38 (0.26-0.55)</td>
<td>0.41 (0.28-0.60)</td>
<td>40</td>
<td>0.27 (0.09-0.76)</td>
<td>8</td>
</tr>
<tr>
<td>Absolute difference</td>
<td>-0.64 (-0.89 to -0.40)</td>
<td>0.37 (0.26-0.54)</td>
<td>39</td>
<td>-0.69 (-0.96 to -0.41)</td>
<td>7</td>
</tr>
<tr>
<td>Relapse-free, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First 6 months</td>
<td>34 (68%)</td>
<td>50</td>
<td></td>
<td>30 (71%)</td>
<td>42</td>
</tr>
<tr>
<td>Months 7-12</td>
<td>38 (79%)</td>
<td>48</td>
<td>48</td>
<td>33 (83%)</td>
<td>40</td>
</tr>
<tr>
<td>Time to first relapse, months(^c)</td>
<td>6 (4-15)</td>
<td>30</td>
<td>50</td>
<td>6 (5-17)</td>
<td>25</td>
</tr>
<tr>
<td>Probability of relapse at 24 months(^a)</td>
<td>55% (69%-41%)</td>
<td>50</td>
<td>54% (38%-69%)</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Time to CDW, months(^a)</td>
<td>28 (18-31)</td>
<td>8</td>
<td>22 (18-29)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Probability of CDW at 24 months(^a)</td>
<td>7% (0%-14%)</td>
<td>45</td>
<td>8% (0%-17%)</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Reason for discontinuing ofatumumab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Marketing terminated</td>
<td>31 (62%)</td>
<td>26 (62%)</td>
<td>42</td>
<td>5 (63%)</td>
<td>3</td>
</tr>
<tr>
<td>Adverse effects</td>
<td>7 (14%)</td>
<td>7 (17%)</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Disease activity</td>
<td>≤3</td>
<td>≤3</td>
<td></td>
<td>≤3</td>
<td>≤3</td>
</tr>
<tr>
<td>Planning pregnancy</td>
<td>≤3</td>
<td>≤3</td>
<td></td>
<td>≤3</td>
<td>≤3</td>
</tr>
<tr>
<td>Other</td>
<td>7 (14%)</td>
<td>6 (14%)</td>
<td>42</td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

\(^a\)Median (Q1-Q3). \(^b\)Estimate (95% confidence interval). \(^c\)p < 0.0001

### 2.3. Adverse and side effects

#### 2.3.1. Immunoglobulin levels

At baseline, 7/44 (16%) had IgG levels below the lower limit of normal (< 6.1 g/L) with a median level of 5.7 g/L (Q1-Q3: 5.0-5.8), 6/43 (14%) had low IgM (< 0.39 g/L) with a median level of 0.27 (Q1-Q3: 0.26-0.32), and ≤3 patients had low IgA (< 0.70 g/L). De novo low IgM was detected in 10/36 (28%), while ≤3 patients developed de novo IgA or IgG hypogammaglobulinemia during ofatumumab treatment. For patients with de novo IgM hypogammaglobulinemia, median treatment time to detection of low IgM was 18 months (Q1-Q3: 10-22), and lowest level was 0.31 g/L (Q1-Q3: 0.28-0.34).
After a median treatment time of 11 months (Q1-Q3: 6-18) for IgG and 6 months (Q1-Q3: 5-6) for IgM, all patients with baseline low IgG or IgM levels either had worsening of the specific hypogammaglobulinemia or new episodes after an initial normalization. Median lowest levels were 5.5 g/L (Q1-Q3: 4.8-5.8 g/L) for IgG and 0.18 g/L (0.14-0.22) for IgM. Serial measurements of immunoglobulin levels are shown in Figure 3.

Figure 3

Serum immunoglobulin levels

Serial immunoglobulin levels as measured within 4-8 months after each ofatumumab infusion series. Immunoglobulin A, G and M level is plotted on the y-axis against infusion series number on the x-axis. Dotted lines mark upper and lower limits of normal range.

2.3.2. Blood cell counts

Seventy-nine percent of patients (27/34) had complete B cell depletion after the first ofatumumab treatment series (i.e. ≤0.01 × 10^9 CD19+ B cells/l), 94% (32/34) after the second, and 94% (32/34) after the third treatment series. Median time of sampling was 5.7 months after the latest infusion series (Q1-Q3: 5.4-5.9) and 0.4 months before the next infusion series (Q1-Q3: 0.3-0.8). Of patients with incomplete B cell depletion after the first infusion series, 3/7 (43%) experienced a relapse during the first 6 months of ofatumumab treatment, and of patients with complete B cell depletion, 10/27 (37%) experienced a relapse (p=0.8).
There were no cases of late onset neutropenia as assessed for up to 2.5 years after the last ofatumumab infusion (median follow up period 2.3 years, Q1-Q3: 2.1-2.4).

Cell counts of CD3+ T cells, CD4+ T cells, CD8+ T cells and NK cells did not change significantly in relation to numbers of infusion series (p=0.38, 0.09, 0.27 and 0.20, respectively). Serial measurements of T, B and NK cell counts are shown in Figure 4.
Figure 4

Blood lymphocyte subset counts

Serial blood cell counts as measured within 4–8 months after each ofatumumab infusion series. Dotted lines indicate upper and lower limits of normal ranges. 4A-D: median cell counts and interquartile ranges for A) CD3+ T cells B) CD8+ T cells, C) CD4+ T cells and D) natural killer cells. 4E: spaghetti plot depicting serial B cell counts for each patient. Line at 0.01 marks the lower limit of detection and complete B cell depletion.

2.3.3. Infusion related reactions

During the first ofatumumab infusion, 6/42 (14%) did not report IRRs, and 34/42 (81%) had moderate to severe IRRs, of which 18/34 (53%) were treated once with one therapeutic, 8/34 (24%) were treated twice or with two therapeutics, and 7/34 (21%) were treated at least three times during the infusion or with at least three therapeutics. There were no cases of life-threatening IRRs, and no patients needed hospitalization for IRRs. Frequency and grading of IRRs are shown in Table 3. The administered doses of the second infusions were available for
39 patients, of whom 28/39 (72%) received 300 mg. For the third infusion, 33/40 (83%) received 600 mg. All the patients who initially received 1000 mg doses were eventually switched to 600 mg maintenance doses.

**Table 3**

Frequency and severity of infusion related reactions.

<table>
<thead>
<tr>
<th>Grade</th>
<th>0: None</th>
<th>1: Mild</th>
<th>2: Moderate</th>
<th>3: Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First infusion</td>
<td>6 (14%)</td>
<td>≤3</td>
<td>30 (71%)</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Second infusion</td>
<td>29 (71%)</td>
<td>5 (12%)</td>
<td>7 (17%)</td>
<td>0</td>
</tr>
<tr>
<td>Third infusion</td>
<td>12 (30%)</td>
<td>≤3</td>
<td>25 (63%)</td>
<td>0</td>
</tr>
<tr>
<td>Last infusion</td>
<td>26 (58%)</td>
<td>4 (9%)</td>
<td>15 (33%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Grade 1: resolved spontaneously without need of intervention or infusion interruption; grade 2: responded to infusion interruption and/or therapy (e.g. antihistamine, methylprednisolone, acetaminophen, inhaled sympathomimetics); grade 3: lasted beyond completion of infusion or recurred after infusion.

Of patients who were subsequently treated with rituximab, 7/14 (50%) experienced no IRRs during their last ofatumumab infusion, and 7/14 (50%) experienced mild to moderate reactions. When switching to rituximab, 9/14 (64%) experienced no IRRs during their first rituximab infusion, and 5/14 (36%) experienced mild to moderate reactions. Median number of ofatumumab infusions before switching to rituximab was 6.5 (Q1-Q3: 5.0-7.0). Of patients who switched from ofatumumab to ocrelizumab, 16/23 (70%) experienced no IRRs during their last ofatumumab infusion, and 7/23 (30%) experienced mild to moderate IRRs. During their first ocrelizumab infusion, 18/23 (78%) experienced no IRRs and 5/23 (22%) experienced mild to moderate IRRs. Median number of ofatumumab infusions before switching to ocrelizumab was 7 (Q1-Q3: 6-8). Overall, the patients who had IRRs after the last administration of ofatumumab were not the same as those who had IRRs after the first administration of rituximab or ocrelizumab, but the number of patients was too low to allow for a detailed analysis.

**2.3.4. Serious infections**

During ofatumumab treatment and until 6 months after the last infusion, 6/45 (13%) were admitted at least once to hospital because of an infection. Median time before acquiring the first infection requiring hospitalization was 16 months (Q1-Q3: 14-17), and ≤3 patients were admitted...
more than once due to an infection. Median duration of hospitalization was 4 days (Q1-Q3: 2.5-6.5 days), and all infections resolved on relevant treatment. Comparison between patients with and without hypogammaglobulinemia at baseline revealed no statistically significant differences. Hazard ratio for an infection requiring hospitalization was estimated to 2.43 (95% CI 0.45-13.30, p=0.3) for patients with low IgG (< 6.1 g/L) at baseline and 1.14 (95% 0.13-9.75, p=0.9) for patients with low IgM (< 0.39 g/L) at baseline.

3. Discussion

Data on IV ofatumumab treatment of demyelinating disorders is limited to a phase II trial of 38 RRMS patients of short duration. We identified 50 patients at the DMSC who had been treated off-label with IV ofatumumab and conducted the first real-world study of its use in patients with MS, MOGAD and NMOSD.

We found a statistically significant change in relapse-free proportion of patients from 34% the year prior to commencement of IV ofatumumab treatment to 60% the first year of treatment. For patients with MS, the ARR decreased from 1.10 at baseline to 0.41 during treatment. This was a smaller reduction than that observed in the ASCLEPIOS I and II phase III trials of SC ofatumumab (Hauser et al., 2020), where ARR was reduced from 1.2 to 0.11 and 1.3 to 0.10, respectively. At 24 months, the cumulated risk of 6 months CDW for the MS group was 8% (95% CI 0%-17%), which is in line with the ASCLEPIOS results (8.2% and 8.0%). However, these risks should be compared with high caution as the MS patients in the present study had a higher age (median 45 years) and EDSS score (median 4.0) at treatment start, and the population consisted of a heterogeneous group of patients with both RRMS and secondary progressive MS, but also primary-progressive MS. Moreover, most had been treated with high-efficacy DMTs prior to switching to ofatumumab, including 24% treated with natalizumab and 40% with fingolimod, and yet; the reason for discontinuation of prior treatment was disease activity in 68% of cases, whereas 41% of the participants in the ASCLEPIOS trials received no DMT prior to enrollment, and for patients who did, interferon-beta was the most frequent DMT. Additionally, ASCLEPIOS required relapses to be accompanied by a clinically relevant increase in EDSS score or functional system scores, whereas relapses in clinical practice are less well-defined.
Seventy-nine percent of patients had complete B cell depletion 4-8 months after the first ofatumumab infusion series. NK and T cell counts did not change overall in relation to number of infusions. As cell counts were assessed 4-8 months after each infusion series, we cannot exclude an earlier impact on these. Interestingly, a study of CD20 depletion with ublituximab found the proportion of NK and T cells to decrease immediately, but transiently, after the first infusion with a subsequent increase to baseline values after two weeks (Lovett-Racke et al., 2019). We found no significant difference between proportion of patients with relapses during the first 6 months between patients with complete versus incomplete B cell depletion, but the longer-term impact could not be determined due to the low number of patients with incomplete depletion after the following infusion series. Moreover, as further cell phenotyping is not part of routine testing, potential changes in cell subsets, including memory B cells, relative frequencies and ratios were not assessed in this study.

During the first ofatumumab infusion, 86% experienced IRRs. This is comparable to the phase II trial of IV ofatumumab for RRMS, where 90.9% of patients who received 300 mg ofatumumab as first infusion experienced drug-related adverse events on first infusion (Sorensen et al., 2014), but somewhat higher than the frequencies reported for IV ocrelizumab (~28%-35%) (Kappos et al., 2011; Hauser et al., 2017; Montalban et al., 2017) and IV rituximab (42%-78.3%) (Bar-Or et al., 2008; Hauser et al., 2008; Hawker et al., 2009), although no head-to-head studies have been conducted. Upon binding of ocrelizumab, CD20+ B cells are primarily depleted through ADCC, whereas ofatumumab and rituximab mainly exert their depletion through CDC. Ofatumumab, however, yields a greater CDC than rituximab and requires a much lower CD20 density for its CDC mediation (Teeling et al., 2006; Klein et al., 2013). This might be of clinical importance, as complement activation is thought to have a role in triggering IRRs (Van Der Kolk et al., 2001; Gelfand et al., 2017).

Serum IgM concentrations showed a decreasing trend related to number of infusions, which is consistent with findings for CD20 depleting therapies in general (Evertsson et al., 2020; Kridin and Ahmed, 2020; Oksbjerg et al., 2021). De novo low IgG and IgA was infrequent. The hazard ratios for infections requiring hospitalization in patients with low IgG and IgM at baseline were non-significant and had very wide confidence intervals. Thus, from this small sample we cannot
make inferences about the association of immunoglobin levels and infections requiring hospitalization. The clinical significance of hypogammaglobulinemia in relation to CD20 depleting therapy in MS is still uncertain. Several studies have indicated that CD20-depletion can induce de novo IgG hypogammaglobulinemia in some patients, and that this may associate with increased infection risk (Salzer et al., 2016; Evertsson et al., 2020; Hauser et al., 2021). On the other hand, other studies did not demonstrate an association between low immunoglobulin serum concentrations and risk of serious infections (Hawker et al., 2009; Oksbjerg et al., 2021). Some studies on rheumatologic cohorts have reported declines of total immunoglobulin levels and hypogammaglobulinemia to be risk factors of serious infections (Besada et al., 2013; Boleto et al., 2018), and one study found immunoglobulin replacement therapy to reduce the risk of serious infections (Barmettler et al., 2018).

The most important limitations of our study are the retrospective nature, the small number of patients and the inherent heterogeneity of the patients included. Some of the patients received 1000 mg for their second infusions and initial maintenance infusions, which may have had an impact on efficacy and adverse effects. However, due to the small number of patients, this could not be validly assessed. Furthermore, there is a risk that the observed reduction in relapse rate is not solely attributable to treatment with ofatumumab but also to a regression-to-the-mean phenomenon. As most patients switched to ofatumumab due to disease breakthrough (71%) and had at least one relapse in the year prior (66%), these sampled patients will by study design have a high ARR, that might not reflect the true population mean ARR. The strengths are the long follow-up periods, continuous assessments and measurements and validation through thorough medical chart review. Additionally, the entire Capital Region of Denmark and Region Zealand use the same electronic medical record system. We thus had access to medical chart notes from hospitalizations and outpatient visits at other hospitals in the regions as well, minimizing the risk of reporting bias. As ofatumumab was an off-label treatment option, the results reflect a patient population for whom the treating neurologists had deemed approved treatment options exhausted, which reduces its generalizability. Accordingly, confounding by indication could compromise estimates of the effect size. Lastly, analyses were limited to overall descriptive trends. Further subgroup analyses examining the effect on disease activity in patients with RRMS, progressive MS, NMOSD and MOGAD separately were not performed due to the risk of type II errors but would be warranted.
**Conclusions**
The results indicate that IV ofatumumab treatment of demyelinating disorders reduces relapse rate and stabilizes disability worsening with an acceptable safety profile. Additionally, the study supports a class effect of anti-CD20 monoclonal antibody treatment of MS, but also suggests that IV treatment with monoclonal antibodies which uses complement activation as an important effector function may be associated to a higher frequency of IRRs.

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**CRediT author statement**
SEM: Methodology, Investigation, Formal analysis, Writing - Original Draft, JRC: Conceptualization, Methodology, Writing - Review & Editing, MM: Data Curation, Methodology, Writing - Review & Editing, MWH: Methodology, Formal analysis, Writing - Review & Editing, FS: Conceptualization, Methodology, Writing - Review & Editing

**Declaration of competing interest**
FS has served on scientific advisory boards for, served as consultant for, received support for congress participation or received speaker honoraria from Alexion, Biogen, Bristol Myers Squibb, Merck, Novartis, Roche and Sanofi Genzyme. His laboratory has received research support from Biogen, Merck, Novartis, Roche and Sanofi Genzyme. JRC has received speaker honoraria from Biogen. MM has served in scientific advisory board for Sanofi, Novartis, Merck, and has received honoraria for lecturing from Biogen, Merck, Novartis, Roche, Genzyme, Bristol Myers Squibb. SEM and MWH have no competing interests to declare.

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CRediT author statement

SEM: Methodology, Investigation, Formal analysis, Writing - Original Draft, JRC: Conceptualization, Methodology, Writing - Review & Editing, MM: Data Curation, Methodology, Writing - Review & Editing, MWH: Methodology, Formal analysis, Writing - Review & Editing, FS: Conceptualization, Methodology, Writing - Review & Editing
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M. Magyari has served in scientific advisory board for Sanofi, Novartis, Merck, and has received honoraria for lecturing from Biogen, Merck, Novartis, Roche, Genzyme, Bristol Myers Squibb.

S. El Mahdaoui and M. Wandall-Holm have no competing interests to declare.

Highlights
- Annualized relapse rate was reduced from 1.03 at baseline to 0.38 during treatment
- Disability worsening was stabilized with a cumulated risk of 7% at 24 months
- Mild-moderate infusion-related reactions were common