Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
eFigure 1. Flow Diagram for Recruitment, Inclusion, and Exclusion of Patients

Abbreviation: OCT: optical coherence tomography; CSF: cerebrospinal fluid.
In total, frequencies of CD4\(^+\) T cells (CD4\(^+\) TC), CD8\(^+\) T cells (CD8\(^+\) TC), CD19\(^+\)CD138\(^-\) B cells (CD19\(^+\) BC), CD19\(^+\)CD138\(^+\) plasma cells (CD138\(^+\) PC), CD56\(^{bright}\) natural killer cells (CD56\(^{bright}\) NK), CD56\(^{dim}\) natural killer cells (CD56\(^{dim}\) NK) and CD56\(^-\)CD14\(^+\) monocytes (MΦ) were studied. Gates for CD56\(^{bright}\) and CD56\(^{dim}\) NK cells were set according to populations in the peripheral blood.
eFigure 3. Correlation of Additional Retinal Layer Volumes With Cerebrospinal Fluid Parameters
Cohort 1, n=65 patients, 103 eyes, 7 patients missing due to technical issues. Plot of various intrathecal immune cell subsets and the corresponding common ganglion cell and inner plexiform layer (GCIPL) volume (A) or inner nuclear layer volume (INL) (B); Kendall-rank correlation analysis corrected for age, sex, EDSS at baseline, disease duration, and treatment group (A, B).
## eTable 1. Retinal Layer Correlations at Baseline in Cohorts 1 and 2

### Cohort 1

<table>
<thead>
<tr>
<th>Layer</th>
<th>pRNFL (µm)</th>
<th>TMV (mm³)</th>
<th>RNFL (mm³)</th>
<th>GCIPL (mm³)</th>
<th>INL (mm³)</th>
<th>OPL (mm³)</th>
<th>ONL (mm³)</th>
<th>PR (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRNFL (µm)</td>
<td>$r = 0.56$ (0.37-0.70)**</td>
<td>$r = 0.47$ (0.27-0.63)**</td>
<td>$r = 0.66$ (0.50-0.77)**</td>
<td>$r = 0.04$ (-0.19-0.27)</td>
<td>$r = 0.05$ (-0.28-0.18)</td>
<td>$r = 0.13$ (-0.10-0.36)</td>
<td>$r = 0.12$ (-0.11-0.35)</td>
<td></td>
</tr>
<tr>
<td>TMV (mm³)</td>
<td>$r = 0.56$ (0.37-0.70)**</td>
<td>$r = 0.50$ (0.30-0.66)**</td>
<td>$r = 0.84$ (0.76-0.90)**</td>
<td>$r = 0.46$ (0.25-0.62)**</td>
<td>$r = 0.19$ (-0.04-0.41)</td>
<td>$r = 0.48$ (0.28-0.64)**</td>
<td>$r = 0.34$ (0.11-0.53)**</td>
<td></td>
</tr>
<tr>
<td>RNFL (mm³)</td>
<td>$r = 0.47$ (0.27-0.63)**</td>
<td>$r = 0.50$ (0.30-0.66)**</td>
<td>$r = 0.59$ (0.42-0.73)**</td>
<td>$r = -0.09$ (-0.32-0.15)</td>
<td>$r = 0.01$ (-0.23-0.24)</td>
<td>$r = -0.18$ (-0.40-0.05)</td>
<td>$r = 0.14$ (-0.10-0.36)</td>
<td></td>
</tr>
<tr>
<td>GCIPL (mm³)</td>
<td>$r = 0.66$ (0.50-0.77)**</td>
<td>$r = 0.84$ (0.76-0.90)**</td>
<td>$r = 0.59$ (0.42-0.73)**</td>
<td>$r = 0.30$ (0.07-0.50)*</td>
<td>$r = 0.14$ (-0.08-0.38)</td>
<td>$r = 0.15$ (-0.05-0.40)</td>
<td>$r = 0.18$ (-0.05-0.40)</td>
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<tr>
<td>INL (mm³)</td>
<td>$r = 0.04$ (-0.19-0.27)</td>
<td>$r = 0.46$ (0.25-0.62)**</td>
<td>$r = -0.09$ (-0.32-0.15)</td>
<td>$r = 0.30$ (0.07-0.50)*</td>
<td>$r = 0.21$ (-0.01-0.43)</td>
<td>$r = 0.22$ (-0.01-0.43)</td>
<td>$r = 0.20$ (-0.03-0.41)</td>
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<tr>
<td>OPL (mm³)</td>
<td>$r = 0.05$ (-0.28-0.18)</td>
<td>$r = 0.19$ (-0.04-0.41)</td>
<td>$r = 0.01$ (-0.23-0.24)</td>
<td>$r = 0.14$ (-0.10-0.36)</td>
<td>$r = 0.21$ (-0.01-0.43)</td>
<td>$r = -0.36$ (-0.55-0.14)**</td>
<td>$r = 0.05$ (-0.18-0.28)</td>
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</tr>
<tr>
<td>ONL (mm³)</td>
<td>$r = 0.13$ (-0.10-0.36)</td>
<td>$r = 0.48$ (0.28-0.64)**</td>
<td>$r = -0.18$ (-0.40-0.05)</td>
<td>$r = 0.15$ (-0.08-0.38)</td>
<td>$r = 0.22$ (-0.01-0.43)</td>
<td>$r = -0.36$ (-0.55-0.14)**</td>
<td>$r = -0.02$ (-0.26-0.21)</td>
<td></td>
</tr>
<tr>
<td>PR (mm³)</td>
<td>$r = 0.12$ (-0.11-0.35)</td>
<td>$r = 0.34$ (0.11-0.53)**</td>
<td>$r = 0.14$ (-0.10-0.36)</td>
<td>$r = 0.18$ (-0.05-0.40)</td>
<td>$r = 0.20$ (-0.03-0.41)</td>
<td>$r = 0.05$ (-0.18-0.28)</td>
<td>$r = -0.02$ (-0.26-0.21)</td>
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</tbody>
</table>

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Cohort 2

<table>
<thead>
<tr>
<th>pRNFL (µm)</th>
<th>TMV (mm³)</th>
<th>RNFL (mm³)</th>
<th>GCIPL (mm³)</th>
<th>INL (mm³)</th>
<th>OPL (mm³)</th>
<th>ONL (mm³)</th>
<th>PR (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRNFL (µm)</td>
<td>( r = 0.56 ) ((0.46-0.63)^{***})</td>
<td>( r = 0.54 ) ((0.45-0.63)^{***})</td>
<td>( r = 0.72 ) ((0.65-0.77)^{***})</td>
<td>( r = 0.11 ) ((-0.01-0.24))</td>
<td>( r = 0.02 ) ((-0.10-0.15))</td>
<td>( r = 0.03 ) ((-0.10-0.15))</td>
<td>( r = -0.02 ) ((-0.15-0.11))</td>
</tr>
<tr>
<td>TMV (mm³)</td>
<td>( r = 0.56 ) ((0.46-0.63)^{***})</td>
<td>( r = 0.47 ) ((0.37-0.56)^{***})</td>
<td>( r = 0.81 ) ((0.76-0.85)^{***})</td>
<td>( r = 0.55 ) ((0.46-0.63)^{***})</td>
<td>( r = 0.19 ) ((0.06-0.31)^{**})</td>
<td>( r = 0.55 ) ((0.45-0.63)^{***})</td>
<td>( r = 0.17 ) ((0.04-0.29)^{**})</td>
</tr>
<tr>
<td>RNFL (mm³)</td>
<td>( r = 0.54 ) ((0.45-0.63)^{***})</td>
<td>( r = 0.47 ) ((0.37-0.56)^{***})</td>
<td>( r = 0.62 ) ((0.53-0.69)^{***})</td>
<td>( r = -0.01 ) ((-0.14-0.11))</td>
<td>( r = 0.02 ) ((-0.10-0.15))</td>
<td>( r = 0.02 ) ((-0.32-0.08)^{**})</td>
<td>( r = -0.21 ) ((-0.33-0.08)^{**})</td>
</tr>
<tr>
<td>GCIPL (mm³)</td>
<td>( r = 0.72 ) ((0.65-0.77)^{***})</td>
<td>( r = 0.81 ) ((0.76-0.85)^{***})</td>
<td>( r = 0.62 ) ((0.53-0.69)^{***})</td>
<td>( r = 0.37 ) ((0.26-0.48)^{***})</td>
<td>( r = 0.12 ) ((0.02-0.25))</td>
<td>( r = 0.10 ) ((-0.02-0.22))</td>
<td>( r = -0.01 ) ((-0.14-0.11))</td>
</tr>
<tr>
<td>INL (mm³)</td>
<td>( r = 0.11 ) ((-0.01-0.24))</td>
<td>( r = 0.55 ) ((0.46-0.63)^{***})</td>
<td>( r = -0.01 ) ((-0.14-0.11))</td>
<td>( r = 0.37 ) ((0.26-0.48)^{***})</td>
<td>( r = 0.12 ) ((0.02-0.25))</td>
<td>( r = 0.32 ) ((0.20-0.42)^{***})</td>
<td>( r = 0.12 ) ((-0.01-0.24))</td>
</tr>
<tr>
<td>OPL (mm³)</td>
<td>( r = 0.02 ) ((-0.10-0.15))</td>
<td>( r = 0.19 ) ((0.06-0.31)^{**})</td>
<td>( r = 0.02 ) ((-0.10-0.15))</td>
<td>( r = 0.12 ) ((0.02-0.25))</td>
<td></td>
<td>( r = -0.12 ) ((-0.25-0.01))</td>
<td>( r = 0.02 ) ((-0.1-1.15))</td>
</tr>
<tr>
<td>ONL (mm³)</td>
<td>( r = 0.03 ) ((-0.10-0.15))</td>
<td>( r = 0.55 ) ((0.45-0.63)^{***})</td>
<td>( r = -0.2 ) ((-0.32-0.08)^{**})</td>
<td>( r = 0.10 ) ((-0.02-0.22))</td>
<td>( r = 0.32 ) ((0.20-0.42)^{***})</td>
<td>( r = -0.12 ) ((-0.25-0.01))</td>
<td>( r = 0.15 ) ((0.02-0.27)^{*})</td>
</tr>
<tr>
<td>PR (mm³)</td>
<td>( r = -0.02 ) ((-0.15-0.11))</td>
<td>( r = 0.17 ) ((0.04-0.29)^{**})</td>
<td>( r = -0.21 ) ((-0.33-0.08)^{**})</td>
<td>( r = -0.01 ) ((-0.14-0.11))</td>
<td>( r = 0.12 ) ((-0.01-0.24))</td>
<td>( r = 0.02 ) ((-0.1-1.15))</td>
<td>( r = 0.15 ) ((0.02-0.27)^{*})</td>
</tr>
</tbody>
</table>

Baseline retinal layer values correlations were evaluated in cohort 1 (n=72) and cohort 2 (n=240). Abbreviations: pRNFL: peripapillary retinal nerve fiber layer; TMV: total macular volume; RNFL: macular retinal nerve fiber layer; GCIPL: common
ganglion cell and inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; PR: photoreceptor layer. R-values (95% confidence interval); *p<.05, **p<.01, ***p<.001.
**Table 2. Cerebrospinal Fluid Parameters Cohort 1**

<table>
<thead>
<tr>
<th>CSF Analysis at Baseline</th>
<th>Cohort 1</th>
<th>Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count per µL</td>
<td>6.0 (3.0-12.0)</td>
<td>0-5</td>
</tr>
<tr>
<td>IgG index</td>
<td>0.78 (0.53-1.07)</td>
<td>&lt;0.7&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgA index</td>
<td>0.27 (0.24-0.33)</td>
<td>&lt;0.041&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgM index</td>
<td>0.08 (0.05-0.14)</td>
<td>&lt;0.061&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>% CD4&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>67.0 (62.3-71.8)</td>
<td>50.1-83.3&lt;sup&gt;a,4&lt;/sup&gt;</td>
</tr>
<tr>
<td>% CD8&lt;sup&gt;+&lt;/sup&gt; T cells</td>
<td>17.6 (13.6-21.9)</td>
<td>6.2-30.2&lt;sup&gt;a,4&lt;/sup&gt;</td>
</tr>
<tr>
<td>% CD19&lt;sup&gt;+&lt;/sup&gt; B cells</td>
<td>1.96 (1.45-4.20)</td>
<td>0.0-1.4&lt;sup&gt;a,4&lt;/sup&gt;</td>
</tr>
<tr>
<td>% CD19&lt;sup&gt;+&lt;/sup&gt;CD138&lt;sup&gt;+&lt;/sup&gt; plasma cells</td>
<td>0.52 (0.14-0.94)</td>
<td>0.0-0.3&lt;sup&gt;a,4&lt;/sup&gt;</td>
</tr>
<tr>
<td>% CD14&lt;sup&gt;+&lt;/sup&gt; monocytes</td>
<td>0.31 (0.12-0.69)</td>
<td>0.0-21.6&lt;sup&gt;a,4&lt;/sup&gt;</td>
</tr>
<tr>
<td>% CD56&lt;sup&gt;bright&lt;/sup&gt; NK cells</td>
<td>1.29 (0.61-2.33)</td>
<td>0.85-2.95&lt;sup&gt;b,5&lt;/sup&gt;</td>
</tr>
<tr>
<td>% CD56&lt;sup&gt;dim&lt;/sup&gt; NK cells</td>
<td>0.75 (0.36-1.11)</td>
<td>1.7-4.5&lt;sup&gt;b,5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Cell count, IgG, IgA, IgM indices were analyzed in 72 patients, cytometric analysis of cell subsets in 65 patients (7 patients missing due to technical issues); median (25%-75% interquartile range). Abbreviations: CSF: cerebrospinal fluid; CD: cluster of differentiation; Ig: immunoglobulin; NK: natural killer; a: reference values as mean ± 2 standard deviations; b: reference values as 25-75% interquartile range.
Table 3. Parameters Associated With Confirmed Disability Worsening as Measured by EDSS in Cohort 2

<table>
<thead>
<tr>
<th></th>
<th>Cohort 2 (n=240)</th>
<th></th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stable EDSS (n=203)</td>
<td>Worse EDSS (n=37)</td>
<td></td>
</tr>
<tr>
<td>Sex, female (%)</td>
<td>132 (65)</td>
<td>30 (81)</td>
<td>.07</td>
</tr>
<tr>
<td>Age, years</td>
<td>35.0 (29.0-42.0)</td>
<td>32.0 (28.0-38.5)</td>
<td>.10</td>
</tr>
<tr>
<td>Disease duration, months</td>
<td>37 (22.5-60.5)</td>
<td>26.0 (15-48.0)</td>
<td>.29</td>
</tr>
<tr>
<td>EDSS</td>
<td>1.0 (0-2.0)</td>
<td>1.0 (0-1.5)</td>
<td>.08</td>
</tr>
<tr>
<td><strong>Disease activity during follow-up (per year)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapses</td>
<td>0 (0-0)</td>
<td>0.4 (0-0.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Increase in T2 lesions</td>
<td>0 (0-1.0)</td>
<td>1.0 (0-3.7)</td>
<td>.002</td>
</tr>
<tr>
<td>Gd+ lesions</td>
<td>0 (0-0)</td>
<td>0 (0-0.5)</td>
<td>.02</td>
</tr>
<tr>
<td><strong>Disease modifying therapies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None, n (%)</td>
<td>29 (14)</td>
<td>5 (14)</td>
<td>.99</td>
</tr>
<tr>
<td>1st line therapy, n (%)</td>
<td>129 (64)</td>
<td>22 (59)</td>
<td>.71</td>
</tr>
<tr>
<td>2nd line therapy, n (%)</td>
<td>45 (22)</td>
<td>10 (27)</td>
<td>.53</td>
</tr>
<tr>
<td><strong>OCT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRNFL, µm</td>
<td>101.0 (92.5-108.0)</td>
<td>98.0 (92.0-103.5)</td>
<td>.13</td>
</tr>
<tr>
<td>TMV, mm³</td>
<td>8.7 (8.5-9.0)</td>
<td>8.7 (8.4-8.9)</td>
<td>.40</td>
</tr>
<tr>
<td>RNFL, mm³</td>
<td>0.86 (0.80-0.94)</td>
<td>0.87 (0.75-0.94)</td>
<td>.53</td>
</tr>
<tr>
<td>GCIPL, mm³</td>
<td>2.01 (1.89-2.11)</td>
<td>1.94 (1.78-2.05)</td>
<td>.03</td>
</tr>
<tr>
<td>INL, mm³</td>
<td>0.98 (0.94-1.03)</td>
<td>0.99 (0.95-1.03)</td>
<td>.79</td>
</tr>
<tr>
<td>OPL, mm³</td>
<td>0.81 (0.77-0.86)</td>
<td>0.82 (0.77-0.84)</td>
<td>.85</td>
</tr>
<tr>
<td>ONL, mm³</td>
<td>1.82 (1.70-1.94)</td>
<td>1.83 (1.72-2.03)</td>
<td>.26</td>
</tr>
<tr>
<td>PR, mm³</td>
<td>2.24 (2.20-2.27)</td>
<td>2.24 (2.22-2.27)</td>
<td>.61</td>
</tr>
</tbody>
</table>

Median (25%-75% interquartile range IQR) if not otherwise stated; Fisher’s exact test, Mann-Whitney U test; Abbreviations: EDSS: expanded disability status scale; Gd+: Gadolinium enhancing; OCT: optical coherence tomography; pRNFL: peripapillary retinal nerve fiber layer; TMV: total macular volume; RNFL: macular retinal nerve fiber layer; GCIPL: common ganglion cell and inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; PR: photoreceptor layer.
References


