

# Ocrelizumab Treatment Modulates B-Cell Regulating Factors in Multiple Sclerosis

Samantha Ho, MSc, Eva Oswald, BSc, Hoi Kiu Wong, MSc, Atay Vural, MD, PhD, Vuslat Yilmaz, PhD, Erdem Tüzün, MD, Recai Türkoğlu, MD, Tobias Straub, MD, Ingrid Meinl, MD, Franziska Thaler, MD, Tania Kümpfel, MD, Edgar Meinl, MD,\* and Simone Mader, PhD\*

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## Correspondence

Dr. Meinl  
Edgar.Meinl@med.uni-muenchen.de  
or Dr. Mader  
Simone.Mader@med.uni-muenchen.de

## Abstract

### Background and Objectives

Antibodies to CD20 efficiently reduce new relapses in multiple sclerosis (MS), and ocrelizumab has been shown to be effective also in primary progressive MS. Although anti-CD20 treatments efficiently deplete B cells in blood, some B cells and CD20<sup>-</sup> plasma cells persist in lymphatic organs and the inflamed CNS; their survival is regulated by the B cell-activating factor (BAFF)/A proliferation-inducing ligand (APRIL) system. The administration of a soluble receptor for BAFF and APRIL, atacicept, unexpectedly worsened MS. Here, we explored the long-term effects of ocrelizumab on immune cell subsets as well as on cytokines and endogenous soluble receptors comprising the BAFF-APRIL system.

### Methods

We analyzed immune cell subsets and B cell-regulating factors longitudinally for up to 2.5 years in patients with MS treated with ocrelizumab. In a second cohort, we determined B-cell regulatory factors in the CSF before and after ocrelizumab. We quantified the cytokines BAFF and APRIL along with their endogenous soluble receptors soluble B-cell maturation antigen (sBCMA) and soluble transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor (sTACI) using enzyme-linked immunosorbent assays (ELISAs). In addition, we established an in-house ELISA to measure sTACI-BAFF complexes.

### Results

Ocrelizumab treatment of people with MS persistently depleted B cells and CD20<sup>+</sup> T cells. This treatment enhanced BAFF and reduced the free endogenous soluble receptor and decoy sTACI in both serum and CSF. Levels of sTACI negatively correlated with BAFF levels. Reduction of sTACI was associated with formation of sTACI-BAFF complexes.

### Discussion

We describe a novel effect of anti-CD20 therapy on the BAFF-APRIL system, namely reduction of sTACI. Because sTACI is a decoy for APRIL, its reduction may enhance local APRIL activity, thereby promoting regulatory IgA<sup>+</sup> plasma cells and astrocytic interleukin (IL)-10 production. Thus, reducing sTACI might contribute to the beneficial effect of anti-CD20 as exogenous sTACI (atacicept) worsened MS.

### Classification of Evidence

This study provides Class IV evidence that endogenous sTACI in blood and CSF is decreased after ocrelizumab treatment.

\*These authors contributed equally to this work and are co-senior authors and shared corresponding authors.

From the Institute of Clinical Neuroimmunology (S.H., E.O., H.K.W., A.V., I.M., F.T., T.K., E.M., S.M.), Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München; Graduate School of Systemic Neurosciences (S.H.), Ludwig-Maximilians-Universität München, Germany; Department of Neurology (A.V.), Koc University School of Medicine; Department of Neuroscience (V.Y., E.T.), Aziz Sancar Institute of Experimental Medicine, Istanbul University; Department of Neurology (R.T.), Haydarpaşa Numune Education and Research Hospital, Istanbul, Türkiye; Core Facility Bioinformatics (T.S.), Biomedical Center, Ludwig-Maximilians-Universität München, Germany; Munich Cluster for Systems Neurology (SyNergy) (F.T.), Germany.

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### Class of Evidence

Criteria for rating therapeutic and diagnostic studies

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## Glossary

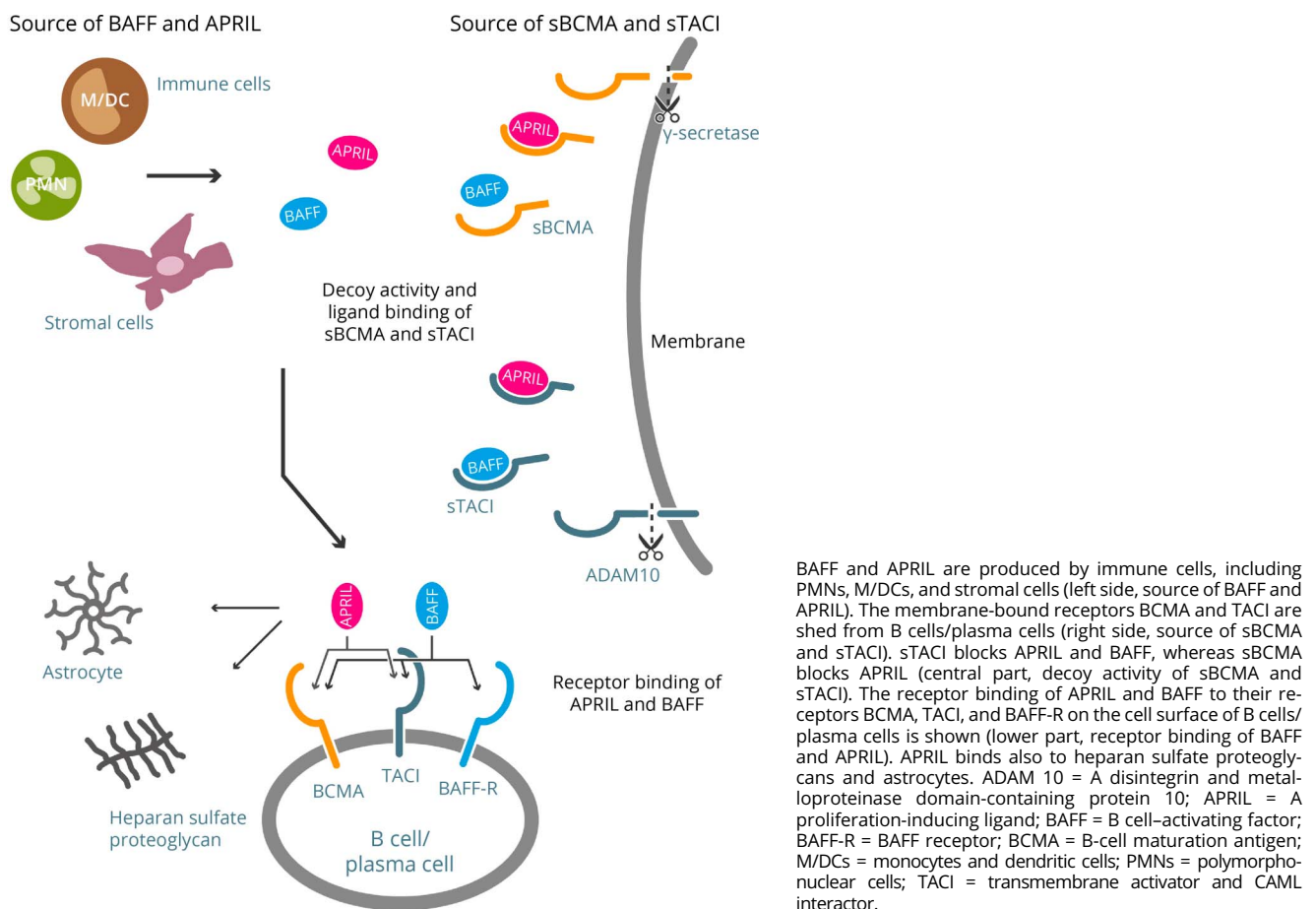
**APRIL** = A proliferation-inducing ligand; **BAFF** = B cell-activating factor; **BAFF-R** = BAFF receptor; **BCMA** = B-cell maturation antigen; **BL** = baseline; **CAML** = calcium-modulator and cyclophilin ligand; **EAE** = experimental autoimmune encephalomyelitis; **ELISA** = enzyme-linked immunosorbent assay; **IL** = interleukin; **MS** = multiple sclerosis; **sTACI** = soluble transmembrane activator and CAML interactor; **sBCMA** = soluble B-cell maturation antigen; **TACI** = transmembrane activator and CAML interactor; **TP1** = time point 1.

Anti-CD20 mAbs are highly efficient in treating relapsing-remitting multiple sclerosis (MS), and the anti-CD20 mAb ocrelizumab is the first approved treatment that slows disability progression in primary progressive MS.<sup>1,2</sup> Anti-CD20 treatment largely depletes circulating B cells and a subset of CD20<sup>+</sup> T cells.<sup>1-7</sup>

In contrast to the almost complete depletion of CD20<sup>+</sup> cells in blood, there is evidence that CD20<sup>+</sup> B cells in the lymphatic organs and the inflamed CNS are not eliminated to the same extent during anti-CD20 treatment.<sup>8,9</sup> Furthermore, CD20<sup>-</sup> plasma cells persist in their survival niches in the bone marrow and the inflamed CNS,<sup>1</sup> and the generation of mucosal IgA<sup>+</sup> plasmablasts continues despite anti-CD20

treatment.<sup>10</sup> The survival of B cells and plasma cells persisting during anti-CD20 is regulated by the B cell-activating factor (BAFF)/A proliferation-inducing ligand (APRIL) system, which comprises the ligands BAFF and APRIL and the receptors B-cell maturation antigen (BCMA), transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor (TACI), and BAFF receptor (BAFF-R).<sup>11</sup> We found previously that the membrane-bound receptors TACI and BCMA are shed from B cells and plasma cells yielding the endogenous soluble receptors, soluble B-cell maturation antigen (sBCMA)<sup>12</sup> and soluble transmembrane activator and CAML interactor (sTACI),<sup>13</sup> reviewed in Ref. 14; these are elevated in the CSF of patients with MS and function as decoys.<sup>12,13</sup> Figure 1 illustrates

**Figure 1** Overview of the BAFF/APRIL System



sources of ligands, soluble receptors, and ligand binding. During anti-CD20 treatment, the interplay between BAFF and APRIL with their soluble receptors regulates the maintenance of remaining B cells and plasma cells. B-cell depletion with anti-CD20 results in elevated BAFF levels in serum,<sup>15</sup> presumably due to reduced consumption,<sup>16</sup> but the influence of anti-CD20 on the soluble receptors is unknown. The strong effect of soluble receptors of the BAFF-APRIL system on MS is evident from unexpected clinical observations: Application of exogenous soluble TACI, atacicept, which decreases B-cell numbers, unexpectedly increased MS activity<sup>17</sup> and enhanced the conversion of optic neuritis to MS.<sup>18</sup> The reasons for the apparently paradoxical effects of atacicept on MS are still not completely understood but may involve regulatory effects of B cells (reviewed in Ref. 19,20) and inhibitory effects mediated via receptors for BAFF and APRIL.<sup>21-23</sup> In the present study, we explored the long-term effects of ocrelizumab on immune cell subsets and on the BAFF-APRIL system focusing on the endogenous soluble receptors sBCMA and sTACI and complexes with their ligands.

## Methods

### Patients

#### First Cohort

From 36 patients with MS (summary in Table 1; individual details in eTable 1, [links.lww.com/NXI/A799](https://links.lww.com/NXI/A799)), blood was obtained before the first administration of ocrelizumab half-dose 300 mg infusion (baseline [BL]), before the second half-dose infusion 2 weeks apart (time point 1 [TP1]), and before every full-dose infusion 600 mg cycle with 6-month intervals (TP2–TP6) for up to 2.5 years. From this cohort, all patients were included in the analysis of immune cell subsets, and we randomly selected 17 of them for our enzyme-linked immunosorbent assay (ELISA) series.

#### Second Cohort

From 19 additional patients with MS (summary in Table 1; individual details in eTable 2, [links.lww.com/NXI/A799](https://links.lww.com/NXI/A799)), CSF and serum were obtained before and 12–19 months after ocrelizumab therapy. The patients received ocrelizumab at 6-month intervals, and CSF was obtained between 2 infusions.

#### Flow Cytometry

Immunophenotyping using anti-CD45 (clone ZD1), anti-CD20 (clone L27), anti-CD3 (clone SK7), and anti-CD19 (clone SJ25C1) was performed in cohort 1 as described.<sup>4</sup>

#### Measurement of Soluble Components of the BAFF-APRIL System

BAFF (DY124), APRIL (DY884), sBCMA (DY193), and sTACI (DY174) were analyzed in serum and CSF by ELISAs (R&D Systems, Minneapolis, MN). To determine whether these ELISAs detect bound or unbound forms of the proteins, we performed each of the ELISAs with a stable physiologic concentration of the protein of interest and added a titration of the interaction partner (including the physiologic concentration) to determine whether the complex formation hinders the detection.

Because we found that the detection of sTACI was blocked by addition of BAFF, we developed an ELISA to quantify the complexes sTACI-BAFF. To this end, we used the capture antibody from the BAFF ELISA kit (841899; DY124) for coating and the detection antibody from the sTACI ELISA kit (841862; DY174) for detection. The quantity of sTACI-BAFF complexes was determined by the quantity of detected sTACI present in the wells. In other words, the quantitative standard of the sTACI-BAFF complexes was conducted as in the sTACI ELISA (DY174). We calculated the total amount of sTACI as sTACI detected in the sTACI ELISA + sTACI detected in sTACI-BAFF complexes. We then calculated the percentage of sTACI in complex with BAFF with this

**Table 1** Summary of Patients' Characteristics

|                 | Patients included in the study <sup>a</sup> | Patients with ELISA analysis <sup>b</sup> | Age at baseline (y) | Sex          | Diagnosis                   | Disease duration at baseline (mo) | Time between the last therapy and the start of ocrelizumab (wk) |
|-----------------|---|---|---------------------|--------------|-----------------------------|-----------------------------------|---|
| <b>Cohort 1</b> | n = 36                                      | n = 17                                    | 41 (23–62)          | 25 F<br>11 M | 26 RRMS<br>8 PPMS<br>2 SPMS | 139 (1–420)                       | 29 (0–574)  |
| <b>Cohort 2</b> | n = 19                                      | n = 19                                    | 47 (27–68)          | 14 F<br>5 M  | 16 RRMS<br>0 PPMS<br>3 SPMS | 149 (64–246)                      | 11 (3–24)   |

Abbreviations: MS = multiple sclerosis; PPMS = primary progressive MS; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive MS.  
<sup>a</sup> First cohort: 36 patients with MS without a previous depleting treatment were recruited from the Ludwig-Maximilians-Universität München Klinikum, Munich, Germany. All patients were included in immune cell phenotype analyses. Patients were analyzed longitudinally from baseline (before initiating ocrelizumab treatment) up to 2.5 years of Ocrelizumab treatment (8 patients were observed for 6–12 months, 5 patients up to 1.5 years, 10 patients up to 2 years, and 13 patients up to 2.5 years after baseline). Second cohort: 19 patients with MS were recruited at baseline and follow-up (12–19 months after the first ocrelizumab infusion from the Haydarpaşa Numune Education and Research Hospital, Department of Neurology, Istanbul, Türkiye). Patients designated as SPMS were in the transition phase from RRMS to SPMS.

<sup>b</sup> Seventeen patients in cohort 1 (as indicated in the table) were included for measurement of B-cell regulatory cytokines with ELISA. All patients from cohort 2 were included for measurement of B-cell regulatory cytokines with ELISA. The patients from cohort 2 were not included for immune cell phenotype analyses.

formula: sTACI-BAFF complex detected (pg/mL)/Total sTACI detected (pg/mL) × 100.

Samples from all available time points of a patient were measured on the same plate. In addition, all 5 ELISAs for each patient were conducted on the same day simultaneously. Two day-to-day controls (sera from 2 healthy controls) were included on every ELISA plate to compare the consistency of ELISA measurements.

## Statistics

All statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). Levels of sBCMA, sTACI, BAFF, APRIL, and sTACI-BAFF complexes are presented as log<sub>2</sub> fold changes compared with BL, which is set as 100%, and were analyzed using a 1-sample *t* test and Bonferroni correction (*n* = 6). Pearson correlation analyses were applied to investigate log<sub>2</sub> fold change of BAFF to log<sub>2</sub> fold change of sTACI as well as log<sub>2</sub> fold change of BAFF to log<sub>2</sub> fold change of sTACI-BAFF complexes in the serum of patients. Differences were considered statistically significant for *p* values of \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, and \*\*\*\**p* < 0.0001 and marked accordingly in the figures.

## Data Availability

After publication, anonymized data will be made available on reasonable request to the corresponding author.

## Standard Protocol Approvals, Registrations, and Patient Consents

All patients provided written informed consent, and the study was approved by the ethical committees of the Medical Faculty of Ludwig-Maximilians-Universität Munich (protocol number 163-16) and Haydarpasa Numune Education and Research Hospital, Istanbul.

## Results

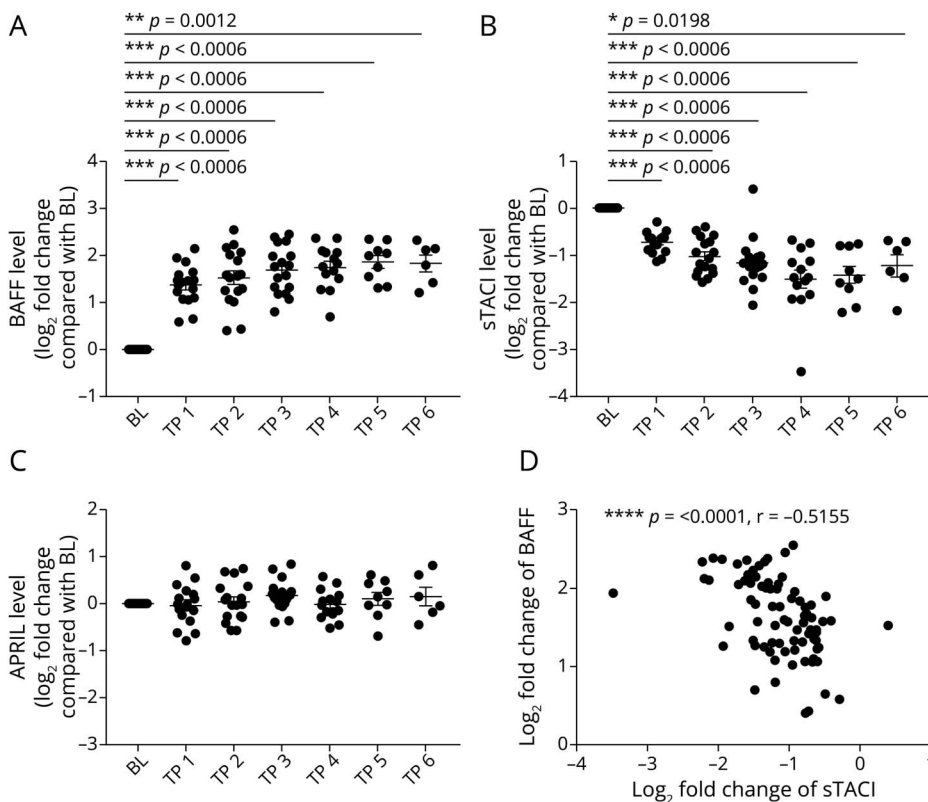
### Effect of Ocrelizumab Treatment on Immune Cell Subsets in Blood

We performed a longitudinal study of the effect of ocrelizumab on CD19<sup>+</sup> B cells, CD3<sup>+</sup> T cells, and CD20<sup>+</sup> T cells in peripheral blood for up to 2.5 years. Our analysis showed that CD19<sup>+</sup> B cells were persistently depleted. The percentage of CD3<sup>+</sup> T cells among all lymphocytes increased, but the absolute number of CD3<sup>+</sup> T cells per microliter blood remained unchanged. The relative increase of CD3<sup>+</sup> T cells was due to the depletion of B cells. CD20<sup>+</sup> T cells were also depleted by ocrelizumab treatment (eFigure 1, links.lww.com/NXI/A798; eTable 3, links.lww.com/NXI/A799).

### Ocrelizumab Treatment Enhances BAFF and Decreases sTACI in Serum and CSF

Ocrelizumab treatment resulted in increased serum levels of BAFF and decreased sTACI, whereas APRIL and sBCMA did

**Figure 2** Effect of Ocrelizumab Treatment on Components of the BAFF/APRIL System in the Serum of Patients



Serum levels of BAFF (A), sTACI (B), and APRIL (C) were evaluated from patient cohort 1 by ELISA from patients receiving ocrelizumab at baseline (*n* = 17), TP1 (mean 18 days; *n* = 17), TP2 (mean 6.7 months; *n* = 17), TP3 (mean 12.9 months; *n* = 17), TP4 (mean 19.1 months; *n* = 14), TP5 (mean 25.2 months; *n* = 10), and TP6 (mean 31.3 months; *n* = 7). Raw values are provided in eTable 3 (links.lww.com/NXI/A799). Data are presented as log<sub>2</sub> fold change compared with baseline. We performed a 1-sample *t* test to compare baseline and follow-up samples. *p* Values are shown if they were <0.05. Data are given as arithmetic mean ± SEM. Negative correlation between BAFF and sTACI levels is shown by the Pearson correlation analyses of log<sub>2</sub> fold change of BAFF to log<sub>2</sub> fold change of sTACI in the serum of patient cohort 1 (D). APRIL = A proliferation-inducing ligand; BAFF = B cell-activating factor; TACI = transmembrane activator and CAML interactor.



not change (Figure 2, A–D; eFigure 2A, [links.lww.com/NXI/A798](https://links.lww.com/NXI/A798); eTable 3, [links.lww.com/NXI/A799](https://links.lww.com/NXI/A799)). The alterations of BAFF and sTACI appeared already at TP1 and were continuously observed up to TP6 (Figure 2, A and B). Because we obtained highly significant results for BAFF, sTACI, and the sTACI-BAFF complexes with cohort 1, we started the evaluation of a second cohort, from whom both serum and CSF were available.

Also in cohort 2, BAFF increased, sTACI decreased, and sBCMA remained unchanged, whereas we noted a slight reduction of APRIL (eFigure 2, B–E, [links.lww.com/NXI/A798](https://links.lww.com/NXI/A798); eTable 3, [links.lww.com/NXI/A799](https://links.lww.com/NXI/A799)). sTACI correlated negatively with BAFF in both cohorts (Figure 2D; eFigure 2F, [links.lww.com/NXI/A798](https://links.lww.com/NXI/A798)). Also in CSF, BAFF increased, and sTACI decreased following ocrelizumab, whereas APRIL and sBCMA remained unaltered (Figure 3, A–D; eTable 3, [links.lww.com/NXI/A799](https://links.lww.com/NXI/A799)). Thus, we consistently observe that ocrelizumab decreases sTACI in serum and CSF.

Having observed that ocrelizumab strongly increased BAFF levels, we asked whether the elevated BAFF could interfere with the detection of sTACI or sBCMA. When we spiked BAFF in recombinant sTACI (Figure 4, A–C) and serum (data not shown), we observed reduced detection of sTACI; This was observed with 2 concentrations of sTACI reflecting sTACI levels in serum (Figure 4A). Although this shows that BAFF reduces the detection of sTACI in our ELISA, this does

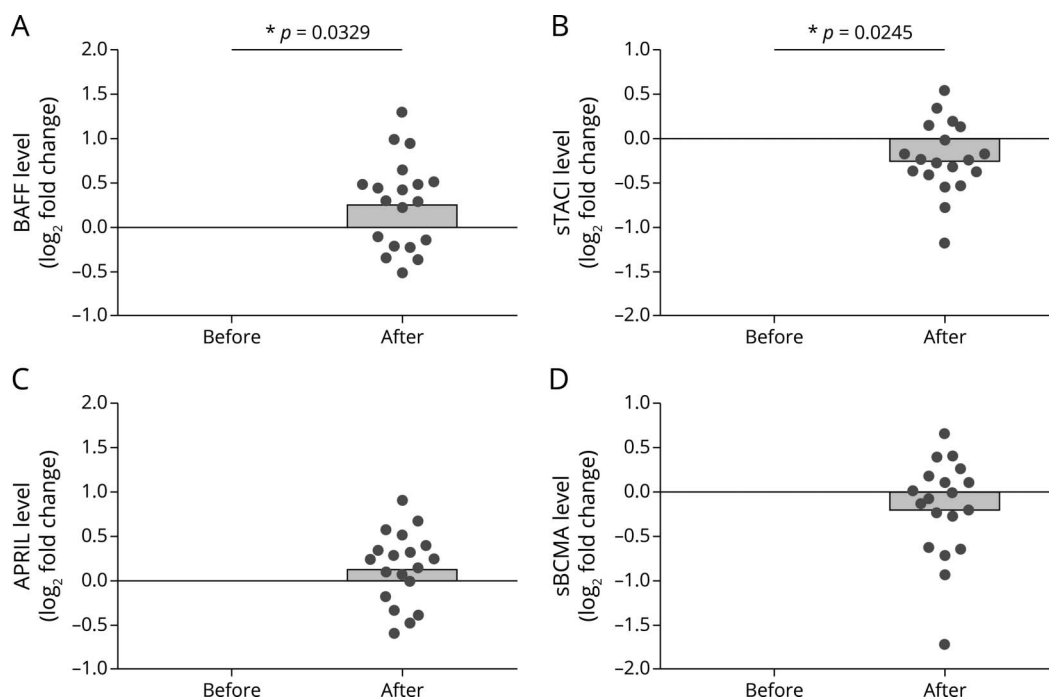
not exclude that the sTACI ELISA partially also detects with low-efficiency sTACI-BAFF complexes. In contrast, the detection of sTACI was not reduced by spiking in APRIL (eFigure 3, [links.lww.com/NXI/A798](https://links.lww.com/NXI/A798)). Consequently, our sTACI ELISA detected free sTACI or sTACI-APRIL complexes, but rather not sTACI-BAFF complexes. The detection of sBCMA, in contrast to sTACI, was neither reduced by BAFF nor by APRIL (eFigure 3, [links.lww.com/NXI/A798](https://links.lww.com/NXI/A798)). Thus, the BCMA detected in our ELISA represents total sBCMA, free sBCMA, or BCMA bound by APRIL or BAFF.

We also noted that the spiking of sTACI reduced the detection of BAFF, but not of APRIL (eFigure 3, [links.lww.com/NXI/A798](https://links.lww.com/NXI/A798)). Thus, our APRIL ELISA detects total APRIL, both free APRIL and the complexes of APRIL and sTACI. The amount of sTACI is decreasing after ocrelizumab therapy in the CSF (Figure 3) and sTACI is a decoy for APRIL; this suggests that the amount of free APRIL measured in our ELISA is increasing.

### Ocrelizumab Induces sTACI-BAFF Complexes

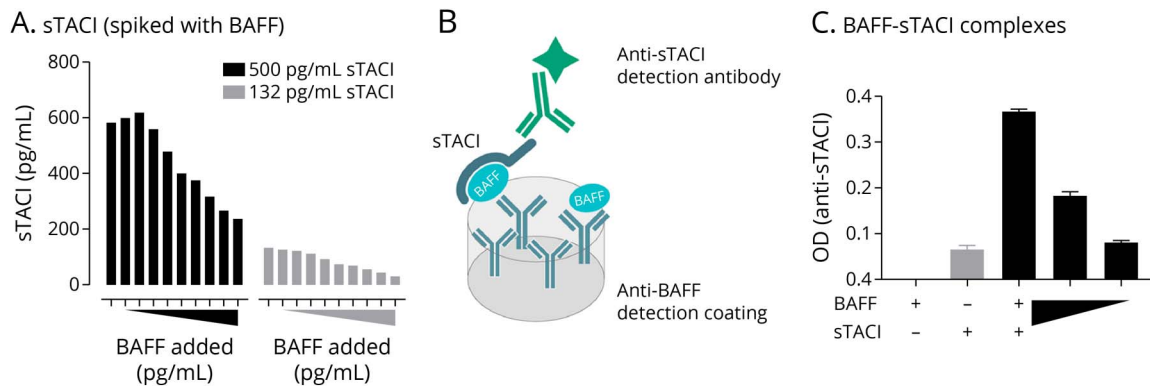
Having found that BAFF reduces the detection of sTACI (Figure 4A), we set up an ELISA to detect sTACI-BAFF complexes by combining well-established antibodies to sTACI and BAFF (Figure 4, B and C). As a result, we found that ocrelizumab induced the formation of sTACI-BAFF complexes in both cohorts (Figure 5, A and B; eTable 3, [links.lww.com/NXI/A799](https://links.lww.com/NXI/A799)). The BAFF levels in serum correlated

**Figure 3** Effect of Ocrelizumab Treatment on Components of the BAFF/APRIL System in CSF



CSF levels of BAFF (A), sTACI (B), APRIL (C) and sBCMA (D) were evaluated from patient cohort 2 by ELISA from patients receiving ocrelizumab at baseline (n = 19) and follow-up (12–19 months after the first ocrelizumab infusion). Data are presented as  $\log_2$  fold change compared with baseline. We performed a 1-sample *t* test to compare baseline and follow-up samples. *p* Values are shown if they were <0.05. Data are given as arithmetic mean  $\pm$  SEM. Raw values are provided in eTable 3 ([links.lww.com/NXI/A799](https://links.lww.com/NXI/A799)). APRIL = A proliferation-inducing ligand; BAFF = B-cell activating factor; sBCMA = soluble B-cell maturation antigen; CSF = cerebrospinal fluid; sTACI = soluble transmembrane activator and CAML interactor.

**Figure 4** Establishment of ELISA Detecting sTACI-BAFF Complexes



(A) Measurement of sTACI (pg/mL) spiked with different amounts of BAFF using an ELISA approach. We added a 2-fold dilution series of BAFF (2,500 to 9.8 pg/mL) to 2 stable concentrations of sTACI (500 pg/ml left part) or 132 pg/mL of (right part) and compared with sTACI alone without BAFF. The left-most column represents no BAFF addition. (B) ELISA design used for the detection of sTACI-BAFF complexes. Anti-BAFF capture antibody and anti-sTACI detection antibody were used to detect sTACI-BAFF complexes. (C) Detection of sTACI-BAFF complexes (OD). Serial dilutions of 1,600, 800, and 400 pg/mL of BAFF were incubated with 500, 250, and 125 pg/mL of sTACI, respectively. BAFF = B cell-activating factor; TACI = transmembrane activator and CAML interactor.

with sTACI-BAFF complexes (Figure 5C). We calculated the percentage of sTACI in complexes with BAFF and observed a steady increase from 43.8% at BL to more than 80% in cohort 1 following ocrelizumab treatment (eTable 4, links.lww.com/NXI/A799). Similarly, also in cohort 2, the percentage of sTACI-BAFF complexes doubled after ocrelizumab therapy (eTable 4, links.lww.com/NXI/A799).

In our cohort 1, we followed the patients longitudinally for up to 2.5 years. Elevation of BAFF, reduction of sTACI, and formation of sTACI-BAFF complexes persist throughout this long observation period, which supports the view that this effect is a consequence of the anti-CD20 treatment and cannot be attributed to treatments before ocrelizumab therapy was started. This study provides Class IV evidence that

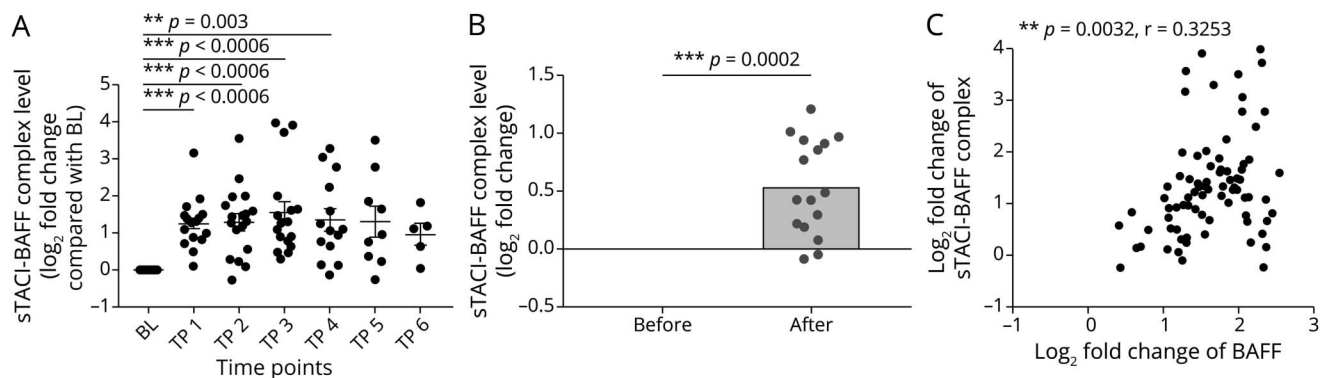
endogenous sTACI in blood and CSF is decreased after ocrelizumab treatment.

## Discussion

Here, we report that endogenous sTACI in blood and CSF is decreased after ocrelizumab treatment. It is tempting to speculate that this contributes to the beneficial effect of ocrelizumab, as the application of exogenous soluble TACI (atacept) worsened MS.<sup>17,18</sup>

By which mechanism could sTACI modulate MS? sTACI functions as a decoy for BAFF and APRIL<sup>13,24</sup> (Figures 1 and 6). Thus, a reduction of sTACI enhances APRIL activity, and

**Figure 5** Identification and Quantification of sTACI-BAFF Complexes



(A) sTACI-BAFF complexes were measured by ELISA in patient cohort 1 in serum samples of patients at baseline (n = 17), TP1 (mean 18 days; n = 17), TP2 (mean 6.7 months; n = 17), TP3 (mean 12.9 months; n = 17), TP4 (mean 19.1 months; n = 14), TP5 (mean 25.2 months; n = 10), and TP6 (mean 31.3 months; n = 7). (B) Serum levels of sTACI-BAFF complex were evaluated in 15 patients from cohort 2 at baseline and follow-up (12–19 months after the first ocrelizumab infusion). Data are presented as log<sub>2</sub> fold change compared with baseline. We performed a 1-sample t test to compare baseline and follow-up samples. Detailed values are given in eTable 3 (links.lww.com/NXI/A799). Data are given as arithmetic mean ± SEM. (C) The BAFF levels in serum correlated with sTACI-BAFF complexes (Pearson correlation analyses of log<sub>2</sub> fold change of BAFF to log<sub>2</sub> fold change of sTACI-BAFF complexes,  $p = 0.0032$ ,  $r = 0.3252$ , cohort 1). BAFF = B cell-activating factor; TACI = transmembrane activator and CAML interactor; TP1 = time point 1.

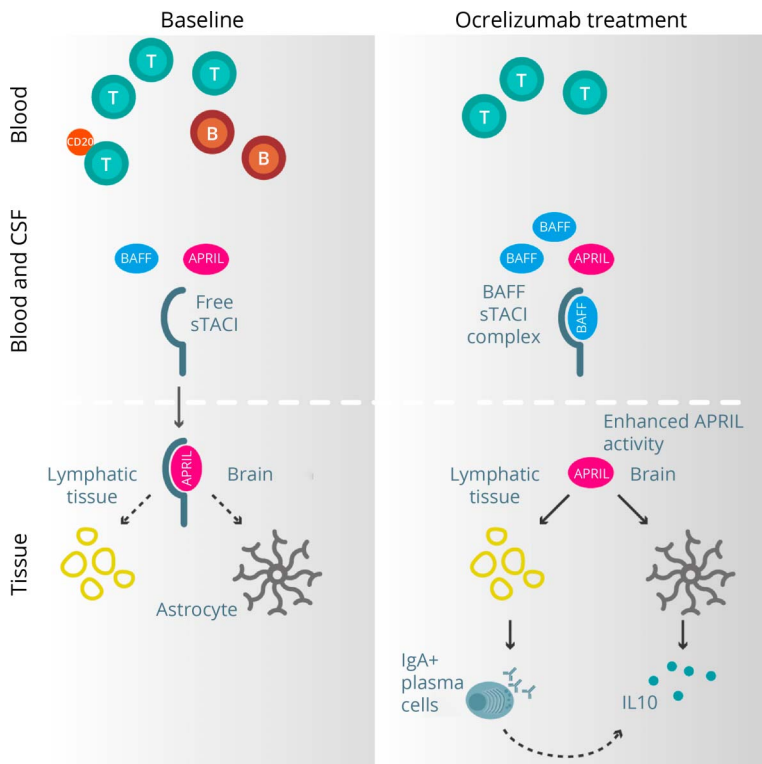
intriguingly, recent work has indicated a beneficial effect of APRIL on CNS inflammation: APRIL, which is produced in MS lesions<sup>23</sup> and by meningeal fibroblasts,<sup>25</sup> stimulates astrocytes to secrete the inhibitory cytokine IL-10 (Ref. 23).

Although BAFF and APRIL share the receptor TACI, there are further aspects to be considered concerning their specific role in neuroinflammation. First, there is *in vitro* evidence that TACI, unlike BAFF-R, is solely activated by oligomeric BAFF and APRIL<sup>26</sup>; BAFF in the serum and CSF of patients with MS is trimeric, not oligomeric.<sup>27</sup> APRIL can be oligomerized via heparan sulfate proteoglycan binding to activate TACI signaling.<sup>11</sup> Second, APRIL exerts functions beyond its binding to TACI, impressively shown by the ability of APRIL to limit atherosclerosis by binding to heparan sulfate proteoglycans.<sup>28</sup> APRIL induces IL-10 production by astrocytes<sup>23</sup> and astrocytes do not express TACI; these APRIL-specific effects are not understood in all details. Consistent with this, APRIL-deficient mice develop a more severe experimental autoimmune encephalomyelitis (EAE).<sup>23</sup>

Furthermore, APRIL promotes IgA class switching<sup>29</sup> and induces regulatory B cells, some of which are IgA positive.<sup>30,31</sup> Importantly, regulatory IgA<sup>+</sup> plasma cells dampen CNS inflammation.<sup>32,33</sup> It was already observed that the production of IgA<sup>+</sup> plasmablasts continues despite anti-CD20 therapy.<sup>10</sup> We propose that the reduced sTACI and enhanced APRIL activity are one explanation for this persistent production of IgA plasmablasts.

We addressed the mechanisms by which ocrelizumab treatment reduces sTACI. We found that ocrelizumab reduces sTACI but leaves sBCMA unaltered. This might be surprising because both sTACI and sBCMA are released from activated B cells and differentiated plasma cells.<sup>12,13</sup> B-cell depletion reduces the amount of newly formed plasmablasts, but the long-lived CD20<sup>-</sup> plasma cells in the bone marrow remain.<sup>1</sup> The unaltered sBCMA levels in blood during anti-CD20 treatment indicate that it is largely derived from long-lived plasma cells, consistent with the observation that IgG in blood derives mainly from long-lived plasma cells and shows little changes during anti-CD20 treatment at least in the first years of treatment.<sup>1</sup> We tested whether the sTACI reduction after ocrelizumab is due to interaction of sTACI with soluble ligands. B-cell depletion with anti-CD20 elevates BAFF, but not APRIL.<sup>15</sup> We assume that this is due to reduced BAFF consumption because the depleted B cells express BAFF-R, which binds BAFF, but not APRIL.<sup>11</sup> We established an ELISA that specifically quantifies sTACI-BAFF complexes and found elevated sTACI-BAFF complexes during ocrelizumab treatment that correlated positively with BAFF and negatively with the sTACI levels. It might be surprising that elevated BAFF levels do not reduce sBCMA, although BCMA binds both BAFF and APRIL.<sup>11</sup> We observed that spiking in BAFF into our sBCMA ELISA had no effect on the measured sBCMA levels. This directly explains why the elevated BAFF does not reduce the detected sBCMA levels. Furthermore, we should consider the lower affinity of BCMA to BAFF

**Figure 6** Illustration Showing Our Findings With the Proposed Consequences



The upper part illustrates our findings: ocrelizumab treatment results in an increase of BAFF and reduced free sTACI in both serum and CSF. In serum, this is associated with increased formation of sTACI-BAFF complexes. sTACI-BAFF complexes were not detected in CSF samples in our study. The lower part links our findings to published work: sTACI is a decoy for APRIL<sup>13</sup> (left). In the absence of the decoy sTACI (right), APRIL might induce IL10 production from astrocytes<sup>23</sup> and promote the development of regulatory IgA plasma cells,<sup>29-31</sup> which can home to the brain and secrete IL-10.<sup>32,33</sup> APRIL = A proliferation-inducing ligand; BAFF = B cell-activating factor; TACI = transmembrane activator and CAML interactor.

compared with APRIL; BAFF binding to BCMA requires a gain of avidity due to oligomerization of BCMA,<sup>34</sup> and therefore, membrane-bound BCMA also binds BAFF. Importantly, sBCMA is a monomer as we have observed before, which binds APRIL, but not BAFF,<sup>12</sup> whereas sTACI binds both BAFF and APRIL.<sup>13</sup> All this explains why the elevation of BAFF has no effect on sBCMA but results in reduction of free sTACI.

Recently, it was observed that anti-CD20 elevated BAFF and this was inversely correlated with CNS inflammation, and it was proposed that anti-CD20 exerts a protective effect by providing a favorable niche for IL-10–producing B cells and suppressive IgA<sup>+</sup> plasma cells.<sup>35,36</sup> We now show that anti-CD20 reduces the decoy sTACI, which may result in enhanced APRIL activity; this could contribute to the proposed mechanism fostering suppressive plasma cells.<sup>18</sup>

We provide information about the concentrations of B-cell regulatory factors and soluble receptors in blood and CSF, but it is a limitation of our study that we cannot measure their concentrations directly in inflammatory lesions. The strong effects of the systemically given atacept, however, argue that the systemic levels of soluble TACI modulate the local microenvironment in the CNS. Although we selected only patients without prior depleting therapy, we cannot exclude the possibility that the therapy had an effect on our results. We show that anti-CD20 therapy reduces sTACI, a decoy for APRIL, but the linkage to local APRIL activity remains to be shown. To study the role of sTACI in neuroinflammation directly, a knock-in mouse expressing a mutated nonshed-dable TACI could be made; such an approach was applied to study the shedding of TNFR1 in EAE.<sup>37</sup>

Altogether, we identify a novel effect of ocrelizumab treatment on the BAFF-APRIL system, a crucial regulatory element for B cells and plasma cells. We describe that the decoy sTACI is reduced over an observation time of up to 2.5 years of ocrelizumab therapy due to formation of sTACI-BAFF complexes. We propose that the reduced decoy sTACI leads to an enhanced local APRIL activity that could induce anti-inflammatory activity in the CNS and support the development and maintenance of regulatory plasma cells (Figure 6).

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## Appendix Authors

| Name                       | Location  | Contribution   |
|----------------------------|---|--|
| <b>Samantha Ho, MSc</b>    | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München; Graduate School of Systemic Neurosciences, Ludwig Maximilians University Munich, Germany | Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data |
| <b>Eva Oswald, BSc</b>     | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Germany  | Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data |
| <b>Hoi Kiu Wong, MSc</b>   | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Germany  | Drafting/revision of the manuscript for content, including medical writing for content, and analysis or interpretation of data   |
| <b>Atay Vural, MD, PhD</b> | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Germany; Department of Neurology, Koc University School of Medicine, Istanbul, Türkiye   | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data  |



## Appendix (continued)

| Name                        | Location  | Contribution   |
|-----------------------------|---|--|
| <b>Vuslat Yılmaz, PhD</b>   | Department of Neuroscience, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Türkiye  | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data  |
| <b>Erdem Tüzün, MD</b>      | Department of Neuroscience, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Türkiye  | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data  |
| <b>Recai Türkoğlu, MD</b>   | Department of Neurology, Haydarpaşa Numune Education and Research Hospital, Istanbul, Türkiye   | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data  |
| <b>Tobias Straub, MD</b>    | Core Facility Bioinformatics, Biomedical Center, Ludwig-Maximilians-Universität München, Germany  | Drafting/revision of the manuscript for content, including medical writing for content, and analysis or interpretation of data   |
| <b>Ingrid Meinel, MD</b>    | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Germany  | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data  |
| <b>Franziska Thaler, MD</b> | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Germany; Munich Cluster for Systems Neurology (SyNergy), Germany | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data  |
| <b>Tania Kümpfel, MD</b>    | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Germany  | Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data  |
| <b>Edgar Meinel, MD</b>     | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Germany  | Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data |
| <b>Simone Mader, PhD</b>    | Institute of Clinical Neuroimmunology, Biomedical Center and University Hospitals, Ludwig-Maximilians-Universität München, Germany  | Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data |

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Samantha Ho, Eva Oswald, Hoi Kiu Wong, et al.

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