**Supplementary Tables**

**eTable 1: Detailed clinical information of patients of cohort 1**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patients included in study a** | **Patients with ELISA analysis b** | **Age at baseline (years)** | **Sex** | **Diagnosis** | **Disease duration at baseline (month)** | **Therapy** | | **Time between last therapy and start of OCR (weeks)** |
| **n=36** | **n=17** | **41 (23-62)** | **25 F 11 M** | **26 RRMS**  **8 PPMS**  **2 SPMC** | **139 (1-420)** | **Therapy before OCR c** | **Last therapy before OCR** | **29 (0-574)** |
| Patient 1 | Yes | 49 | F | RRMS | 1 | none | None |  |
| Patient 2 |  | 36 | M | PPMS | 60 | LAQ | LAQ | 22 |
| Patient 3 | Yes | 23 | F | PPMS | 33 | NAT | NAT | 7 |
| Patient 4 |  | 49 | F | RRMS | 381 | IFN, GLAT | GLAT | 1 |
| Patient 5 | Yes | 54 | F | PPMS | 58 | none | None |  |
| Patient 6 | Yes | 36 | F | RRMS | 214 | IFN, IFN, (Rebif, Avonex), NAT, DMF | DMF |  |
| Patient 7 |  | 30 | M | RRMS | 74 | DMF, NAT | NAT | 6 |
| Patient 8 | Yes | 52 | F | SPMS\* | 250 | IFN, GLAT, IVIg | NAT | 7 |
| Patient 9 | Yes | 42 | F | RRMS | 263 | IFN, FINGO, NAT | NAT | 6 |
| Patient 10 | Yes | 36 | M | RRMS | 80 | IFN, NAT | NAT | 7 |
| Patient 11 | Yes | 52 | M | PPMS | 95 | ICST, LAQ, ICST | ICST | 19 |
| Patient 12 | Yes | 41 | F | PPMS | 47 | IFN | IFN | 33 |
| Patient 13 | Yes | 30 | F | RRMS | 35 | IFN, NAT | NAT | 8 |
| Patient 14 |  | 32 | F | RRMS | 167 | IFN, GLAT, FINGO, IVIg | IVIg | 48 |
| Patient 15 | Yes | 52 | M | RRMS | 227 | IFN, FGL, NAT | NAT | 6 |
| Patient 16 |  | 44 | F | RRMS | 251 | IFN, GLAT, DMF, NAT | NAT | 9 |
| Patient 17 | Yes | 62 | M | SPMS\* | 408 | GLAT, MOX | MOX | 574 |
| Patient 18 | Yes | 60 | F | RRMS | 420 | IFN, DMF | DMF | 53 |
| Patient 19 | Yes | 39 | F | RRMS | 168 | IFN, GLAT, FINGO | FINGO | 5 |
| Patient 20 | Yes | 25 | M | RRMS | 48 | DMF, NAT | DMF | 2 |
| Patient 21 |  | 23 | F | RRMS | 68 | GLT, DMF | DMF | 2 |
| Patient 22 |  | 38 | M | RRMS | 132 | IFN, DMF | DMF | 2 |
| Patient 23 | Yes | 53 | F | RRMS | 228 | IFN, AZA, GLAT, IVIg, ICST, DMF, NAT | NAT | 7 |
| Patient 24 |  | 30 | F | RRMS | 17 | GLAT | GLAT | 0 |
| Patient 25 |  | 50 | F | PPMS | 17 | none | None |  |
| Patient 26 |  | 50 | F | RRMS | 264 | IFN, NAT | NAT | 6 |
| Patient 27 |  | 49 | F | RRMS | 36 | TERI | TERI | 4 |
| Patient 28 |  | 26 | F | RRMs | 49 | IFN | IFN | 9 |
| Patient 29 | Yes | 25 | F | RRMS | 85 | GLAT, IFN, DMF,TERI, FINGO, NAT | NAT | 6 |
| Patient 30 |  | 27 | M | RRMS | 27 | TERI, NAT | NAT | 6 |
| Patient 31 |  | 39 | F | RRMS | 133 | IFN, Fingo, GLAT, NAT | NAT | 6 |
| Patient 32 |  | 43 | M | PPMS | 73 | none | none |  |
| Patient 33 |  | 40 | F | RRMS | 145 | IFN, NAT | NAT | 5 |
| Patient 34 |  | 30 | M | RRMS | 111 | IFN, NAT | NAT | 6 |
| Patient 35 |  | 41 | F | PPMS | 110 | IFN, GLAT, NAT | NAT | 7 |
| Patient 36 |  | 57 | F | RRMS | 230 | GLAT, IFN, FINGO, NAT | NAT | 6 |

**a** cohort 1: 36 MS patients (26 RRMS, 8 PPMS and 2 SPMS) without a previous B cell depleting treatment were recruited from the LMU 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). We only included patients without prior depleting therapy, including rituximab, daclizumab and alemtuzumab.

**b** 17 patients (as indicated in the table) were included for measurement of B-cell regulatory cytokines with ELISA.

**c** Abbreviations: AZA = azathioprine, DMF= dimethyl fumarate, FINGO= fingolimod, GLAT= glatiramer acetate, IFN= interferon-beta, IVIg= intravenous immunoglobulin, ICST = pulsatile high dose steroid treatment, LAQ = laquinimod, MOX = mitoxantrone, NAT= natalizumab, OCR= ocrelizumab, TERI= teriflunomide

\* patients were in the transition phase from RRMS to SPMS

**eTable 2: Detailed clinical information of patients of cohort 2**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patients included in study a** | **Patients with ELISA analysis b** | **Age at baseline (years)** | **Sex** | **Diagnosis** | **Disease duration at baseline (months)** | **Therapy** | | **Time between last therapy and start OCR (weeks)** |
| **n=19** | **n=19** | **47 (27-68)** | **14 F**  **5 M** | **16 RRMS**  **3 SPMS** | **149 (64-246)** | **Therapy before OCR c** | **Last therapy before OCR** | **11 (3-24)** |
| 1 | Yes | 53 | F | RRMS | 246 | IFN, AZA, TERI, DMF | DMF | 5 |
| 2 | Yes | 27 | F | RRMS | 68 | GLAT, FINGO | FINGO | 12 |
| 3 | Yes | 33 | M | RRMS | 64 | IFN, TERI, FINGO | FINGO | 8 |
| 4 | Yes | 48 | F | SPMS\* | 130 | IFN, FINGO | FINGO | 4 |
| 5 | Yes | 50 | F | RRMS | 126 | GLAT, NAT, FINGO, DMF | DMF | 3 |
| 6 | Yes | 40 | M | RRMS | 97 | IFN, FINGO | FINGO | 10 |
| 7 | Yes | 42 | M | RRMS | 81 | GLAT, FINGO | FINGO | 8 |
| 8 | Yes | 46 | M | RRMS | 162 | IFN, FINGO | FINGO | 5 |
| 9 | Yes | 36 | M | RRMS | 188 | GLAT, NAT, FINGO | FINGO | 7 |
| 10 | Yes | 67 | F | RRMS | 194 | GLAT, IFN | IFN | 8 |
| 11 | Yes | 61 | F | SPMS\* | 158 | IFN, TERI | TERI | 5 |
| 12 | Yes | 57 | F | SPMS\* | 210 | AZA | AZA | 5 |
| 13 | Yes | 46 | F | RRMS | 156 | IFN, NAT, FINGO | FINGO | 10 |
| 14 | Yes | 46 | F | RRMS | 116 | IFN, FINGO | FINGO | 24 |
| 15 | Yes | 68 | F | RRMS | 109 | IFN, GLAT, FINGO | FINGO | 24 |
| 16 | Yes | 43 | F | RRMS | 183 | IFN, FINGO | FINGO | 24 |
| 17 | Yes | 42 | F | RRMS | 227 | GLAT, FINGO | FINGO | 12 |
| 18 | Yes | 54 | F | RRMS | 135 | IFN, GLAT, FINGO | FINGO | 16 |
| 19 | Yes | 36 | F | RRMS | 188 | IFN, FINGO, NAT | NAT | 20 |

**a** cohort 2: 19 MS patients (16 RRMS, 3 SPMS, mean age 47.2 years old, 14 female and 5 males) were recruited at baseline and follow up (12-19 months after first ocrelizumab infusion from the Haydarpasa Numune Education and Research Hospital, Department of Neurology, Istanbul, Turkey.

**b** From all 19 patients CSF was included for measurement of B-cell regulatory cytokines with ELISA. From 16 out of 19 patients the serum was available for ELISA measurement. The patients from cohort 2 were not included for immune cell phenotype analyses.

**c** Abbreviations: AZA = azathioprine, DMF= dimethyl fumarate, FINGO= fingolimod, GLAT= glatiramer acetate, IFN= interferon-beta, IVIg= intravenous immunoglobulin, ICST = pulsatile high dose steroid treatment, LAQ = laquinimod, MOX = mitoxantrone, NAT= natalizumab, OCR= ocrelizumab, TERI= teriflunomide,

\* patients were in the transition phase from RRMS to SPMS

**eTable 3: Effects of ocrelizumab on lymphocyte subsets (raw data) and on levels of BAFF, sTACI, APRIL, sBCMA and sTACI-BAFF-complexes.**

**Cohort 1:**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **lymphocytes (%) a** | **BL b** | **TP1** | **TP2** | **TP3** | **TP4** | **TP5** | **TP6** |
| **CD3+ T cells** | 69.33 (48 - 86) | 83.73 (59 - 97) | 84.02 (60 - 97) | 85.11 (58 - 97) | 85 (62 - 97) | 85.66 (59 - 97) | 79.92 (52 - 97) |
| **CD3+ CD20+ T cells** | 3.62 (0.5 - 11) | 0.23 (0.1 - 0.5) | 0.82 (0.5 - 4) | 0.75 (0.5 - 3) | 0.67 (0.5 - 2) | 0.73 (0.5 - 2) | 0.53 (0.5 - 1) |
| **CD19+ B cells** | 16.61 (7 - 31) | 0.13 (0.1 - 0.5) | 0.69 (0.1 - 4) | 0.36 (0.1 - 3) | 0.36 (0.1 - 3) | 0.2 (0.1 - 1) | 0.46 (0.1 - 4) |
| **CD19+ CD20+ B cells** | 15.96 (7 - 31) | 0.1 (0.1 - 0.1) | 0.64 (0.1 - 4) | 0.29 (0.1 - 2) | 0.34 (0.1 - 3) | 0.19 (0.1 - 1) | 0.46 (0.1 - 4) |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Absolute cell count (cells/µl) a** | **BL** | **TP1** | **TP2** | **TP3** | **TP4** | **TP5** | **TP6** |
| **CD3+ T cells** | 1310 (386 - 3130) | 1176 (292 - 2463) | 1085 (373 - 1834) | 1137 (296 - 2041) | 1084 (448 - 1957) | 1106 (432 - 1611) | 940 (311 - 1890) |
| **CD3+ CD20+ T cells** | 70 (8 - 207) | 3 (0 - 9) | 10 (3 - 73) | 10 (2 - 50) | 8 (4 - 29) | 9 (4 - 28) | 6 (3 - 13) |
| **CD19+ B cells** | 357 (69 - 1125) | 1 (0 - 8) | 8 (1 - 43) | 4 (0 - 23) | 4 (1 - 38) | 2 (1 - 9) | 3 (1 - 24) |
| **CD19+ CD20+ B cells** | 334 (69 - 1125) | 1 (0 - 3) | 7 (1 - 38) | 3 (0 - 15) | 3 (1 - 38) | 2 (1 - 9) | 3 (1 - 24) |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Absolute levels c** | **BL** | **TP1** | **TP2** | **TP3** | **TP4** | **TP5** | **TP6** |
| **sTACI (pg/ml)** | 120.11 (62.16 - 303.69) | 75.52 (38.59 - 197.36) | 61.33 (24.41 - 132.22) | 56.76 (20.19 - 149.25) | 47.75 (7.13 - 168.86) | 34.30 (18.13 - 61.02) | 39.73 (18.57 - 56.63) |
| **BAFF (pg/ml)** | 300.92 (183.06 - 509.86) | 780.24 (517.44 - 1,293.77) | 870.00 (465.04 - 1,389.21) | 961.06 (607.76 - 1,526.25) | 979.39 (589.34 - 1,512.08) | 1.022.25 (612.95 - 1,479.08) | 968.84 (570.25 - 1,370.97) |
| **sBCMA (ng/ml)** | 19.01 (10.80 - 26.46) | 18.77 (11.07 - 38.68) | 19.44 (11.22 - 35.28) | 17.63 (10.89 - 25.84) | 18.08 (11.74 - 24.69) | 17.27 (11.88 - 20.46) | 16.11 (10.89 - 22.72) |
| **APRIL (ng/ml)** | 1.54 (0.69 - 3.86) | 1.44 (0.78 - 2.24) | 1.56 (0.87 - 2.90) | 1.69 (1.09 - 2.99) | 1.49 (0.94 - 2.69) | 1.48 (0.76 - 2.57) | 1.50 (1.04 - 2.27) |
| **sTACI-BAFF (pg/ml)** | 93.70 (9.37 - 256.49) | 203.51 (82.02 - 363.32) | 202.94 (96.41 - 518.51) | 266.03 (109.25 - 1,115.12) | 220.25 (77.57 - 722.88) | 175.29 (83.47 - 301.53) | 228.06 (162.00 - 264.06) |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Time from Baseline** | **BL (days)** | **TP1 (days)** | **TP2 (months)** | **TP3 (months)** | **TP4(months)** | **TP5 (months)** | **TP6 (months)** |
| **Lymphocyte data** | 0 | 17 (13 - 42) | 6.6 (5 - 7.4) | 12.9 (10.7 - 14.2) | 19.8 (17.9 - 32.4) | 25.6 (23.8 - 27.7) | 31.2 (30 - 32) |
| **ELISA data** | 0 | 18 (13 - 42) | 6.7 (6.2 - 7.4) | 12.9 (12.2 - 13.5) | 19.1 (18 - 19.9) | 25.2 (24.1 - 25.9) | 31.3 (30.2 - 32) |

**Cohort 2:**

|  |  |  |
| --- | --- | --- |
| **Sera d** | **Before ocrelizumab** | **After ocrelizumab** |
| **sTACI (pg/ml)** | 353.87 (176.54 - 559.15) | 184.83 (47.62 - 330.96) |
| **BAFF (pg/ml)** | 393.32 (211.86 - 548.07) | 913.18 (424.98 - 1668.23) |
| **sBCMA (ng/ml)** | 34.14 (14.32 - 48.82) | 31.08 (14.32 - 50.74) |
| **APRIL (ng/ml)** | 1.01 (0.13 - 5.82) | 0.55 (0.01 - 4.57) |
| **sTACI-BAFF (pg/ml)** | 86.38 (40.94 - 123.22) | 121.74 (7.06 - 235.74) |

|  |  |  |
| --- | --- | --- |
| **CSF d** | **Before ocrelizumab** | **After ocrelizumab** |
| **sTACI (pg/ml)** | 47.09 (23.49 - 96.42) | 40.59 (18.81 - 113.19) |
| **BAFF (pg/ml)** | 65.83 (21.06 - 174.49) | 76.35 (32.91 - 157.89) |
| **sBCMA (pg/ml)** | 441.3 (130.97 - 798.4) | 383.28 (129.56 - 815.4) |
| **APRIL (pg/ml)** | 41.1 (11.22 - 72.63) | 41.01 (0 - 72.39) |

**a** T cells (CD3+ and CD3+CD20+) and B cells (CD19+ and CD19+CD20+) are shown as percent of all lymphocytes and as absolute cell count per µl. Data are shown as average and minimum to maximum.

**b** Time points during treatment includedBL**,** TP 1 (n=34, 17 average days, from 13 to 42 days), TP 2 (n=36, 6 average months, from 5 to 7.4 months), TP 3 (n=35, 12.9 average months, from 10.7 to 14.2 months), TP 4 (n=28, 19.8 average months from 17.9 to 32.4 months), TP 5 (n=23, 25.6 average months from 23.8 to 27.7 months), TP 6 (n=13, 31.2 average months from 30 to 32 months) for immune cell subsets.

**c** The absolute levels of BAFF, sTACI, APRIL, sBCMA and sTACI-BAFF complexes measured at each time point are shown as average and minimum to maximum. For cohort 1, time points of ELISA measurements included TP 1 (n=17, 18 average days, from 13 to 42 days), TP 2 (n=17, 6.7 average months, from 6.2 to 7.4 months), TP 3 (n= X 17, 12.9 average months, from 12.2 to 13.5 months), TP 4 (n=14, 19.1 average months from 18 to 19.9 months), TP 5 (n=10, 25.2 average months from 24.1 to 25.9 months), TP 6 (n=7, 31.3 average months from 30.2 to 32 months). Patients 5, 12 and 17 were only measured up to TP4. Patient 1, 7, 8, 30, 37 were only measured up to TP5. Patients 19, 23, 25, 26 were only measured up to TP6. sTACI in serum in cohort 2 is higher than in cohort 1. We noted that the baseline of sTACI in serum in cohort 2 was higher than in cohort 1. Importantly, the samples of each cohort were measured together, and we consistently observed the increase of BAFF and the decrease of sTACI after ocrelizumab therapy.

**d** ELISA data are shown for cohort 2 in serum and in CSF at baseline (serum n=16, CSF n=19) and at follow up (serum n=16, CSF n=19, 15 average months, from 12.5 to 19.8 months after infusion). BL= baseline, TP= time point.

**eTable 4: Estimation of the amount of sTACI complexed with BAFF**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Cohort 1** | **BL** | **TP1** | **TP2** | **TP3** | **TP4** | **TP5** | **TP6** |
|  |  |  |  |  |  |  |  |
| sTACI(pg/ml) | 120.11 | 75.52 | 61.33 | 56.76 | 47.75 | 34.3 | 39.73 |
| sTACI in sTACI-BAFF complexes (pg/ml)**a** | 93.7 | 203.51 | 202.94 | 266.03 | 220.25 | 175.29 | 228.06 |
| Sum of sTACIand sTACI-BAFF complexes(pg/ml)**b** | 213.81 | 279.03 | 264.27 | 322.79 | 268.00 | 209.59 | 267.79 |
| % of sTACI in complexes with BAFF **c** | 43.82 | 72.93 | 76.79 | 82.42 | 82.18 | 83.63 | 85.16 |

|  |  |  |
| --- | --- | --- |
| **Cohort 2 (serum)** | **Before ocrelizumab** | **After ocrelizumab** |
| sTACI(pg/ml) | 353.87 | 184.83 |
| sTACI in sTACI-BAFF complexes (pg/ml) **a** | 86.38 | 121.74 |
| Sum of sTACIand sTACI-BAFF complexes(pg/ml)**b** | 440.25 | 306.57 |
| % of sTACI in complexes with BAFF **c** | 19.62 | 39.71 |

Values shown in the upper two rows represent values of eTable3

a sTACI in sTACI-BAFF complexes = sTACI-BAFF (pg/ml)

b Total sTACI (pg/ml) = sTACI (pg/ml) + sTACI-BAFF (pg/ml)

c % of sTACI in complexes with BAFF (%) = sTACI-BAFF (pg/ml) / Total sTACI (pg/ml) \* 100