Supplementary Information (SI) to:

Subsets of activated monocytes and markers of inflammation in incipient and progressed multiple sclerosis

ID#	Age (y)	Gender	Disease Course	(Newly diagnose	EDSS	Treated	Nr. Of Attacks	Nr. of white	CSF Cells (x
241	25	F	CIS	1	0	Yes	2	3	0
319	19	F	RRMS	1	0	Yes	3	19	3
327	32	F	RRMS	1	1	No	3	22	13
329	45	F	RRMS	1	1	Yes	5	42	5
330	33	F	RRMS	0	1	No	2	5	7
337	47	F	CIS	0	1	No	2	8	2
341	28	F	CIS	0	1	No	4	2	13
342	49	M	CIS	0	3	No	3	15	1
344	50	F	PPMS	1	4,5	No	0	14	2

Supplementary Table SI1. Basic dataset. Excel file above (double click to activate) contains all basic data for the paper. Abbreviations: RRMS (relapsing-remitting MS), PPMS (primary-progressive MS), CIS (clinically isolated syndrome), HC (healthy controls), EDSS (expanded disability status scale), CSF (cerebrospinal fluid), Gender (M=male; F=female), y (years), m (months).

Supplementary Figure SI1. Flowchart of the forty patients and twenty healthy controls in the study cohort. Twenty patients were included at diagnostic work-up, twenty patients were included during follow-ups, as were twenty healthy controls matched for age and gender. Patients were not included if they had other neurologic disease, a medical disease, received methylprednisolone treatment within one month prior to sampling, or had paraclinical isolated syndromes. Patients and healthy controls included in the study cohort were stratified according to disease progression and comprised the following subgroups: RRMS (relapsing-remitting MS), PPMS (primary-progressive MS), CIS (clinically isolated syndrome), and HC (healthy controls).

Supplementary Table SI2. Demographic and clinical data of the patients with MS, CIS, and of healthy controls				
Characteristics (Median)	**RRMS**	**PPMS**	**CIS**	**HC**
No. of subjects (total=60)	n = 25	n = 5	n = 10	n = 20
Gender (M/F)	4/21	2/3	2/8	3/17
Treated (no/yes)[a]	13/12	5/0	9/1	N/A
Age[b]	36	64	34	41
(range)	(19 - 53)	(50 - 66)	(19 - 49)	(19 - 65)
EDSS	1	6	1	N/A
(range)	(0.0 - 3.0)	(3.5 - 6.5)	(0.0 - 3.0)	N/A
Number of attacks[c]	3	N/A	2	N/A
(range)	(2 – 7)	N/A	(1 – 4)	N/A
Disease duration (months)[d]	41	88	13	N/A
(range)	(10 - 176)	(35 - 187)	(4 - 119)	N/A
Time since last attack (months)[e]	10	N/A	11	N/A
(range)	(1 - 92)	N/A	(4 - 119)	N/A
CSF protein (μmol/L)	0.40	0.54	0.37	N/A
(range)	(0.24 - 0.65)	(0.34 - 0.57)	(0.23 - 0.65)	N/A
CSF cells (x 10^6/L)	4	2	2	N/A
(range)	(0 – 18)	(1 – 5)	(0 – 13)	N/A
IgG Index	0.82	0.82	0.56	N/A
(range)	(0.46 - 3.53)	(0.42 - 1.45)	(0.41 - 2.51)	N/A
Number of white matter lesions[f]	10	14	5	N/A
(range)	(2 - 55)	(3 - 45)	(1 - 15)	N/A

Supplementary Table SI2. Demographics and clinical data of the patient cohort. Twenty-five patients with RRMS, five patients with PPMS, and ten patients with CIS as well as twenty HCs were included. [a]Treated patients had received either glatiramer acetate (n=3 RRMS, n=1 CIS), beta interferon 1a (n=8), or beta interferon 1b (n=1). [b]Age (in years) at the time of sampling . [c]Number of attacks: median number of attacks from debut of first symptom(s) to the sampling. [d]Disease duration (in months): the period of time from debut of first symptom(s) to the sampling. [e]Time since last attack: the period of time (in months) from latest attack to the sampling. [f]Number of white matter lesions: Total number of white matter lesions determined by fluid-attenuated inversion recovery (FLAIR) sequences on magnetic resonance imaging (MRI). Abbreviations: RRMS (relapsing-remitting MS), PPMS (primary-progressive MS), CIS (clinically isolated syndrome), HC (healthy controls), N/A (not applicable or available), n (number of persons), EDSS (expanded disability status scale), CSF (cerebrospinal fluid).

Supplementary Figure SI2. Examples of results from flow cytometric analyses of the monocyte subsets from patients with RRMS, PPMS, CIS, and from HC. From left to right: Sample from a representative patient with RRMS, PPMS, CIS and from a healthy control, respectively, were used for this figure. The tree monocyte subsets (classical, intermediate, non-classical) were gated on the "Live Cells" gate (Figure 1).

Supplementary Table SI3. Pearson correlations of cell surface marker expressions to clinical disease measures and soluble markers in serum and CSF												
Characteristics Unless otherwise stated (n=40)	CD40+	CD163+	CD192+	Classical	Intermediate	Non-classical	HERV H3 (classical)	HERV H3 (intermediate)	HERV H3 (non-classical)	HERV W3 (classical)	HERV W3 (intermediate)	HERV W3 (non-classical)
Pearson's correlation coefficients, r, without and with the Bonferroni correction												
Age[a] (n=60) **Level of significance**	0.04 -/-	0.01 -/-	-0.06 -/-	-0.05 -/-	0.19 -/-	0.03 -/-	-0.15 -/-	-0.11 -/-	-0.13 -/-	0.16 -/-	0.15 -/-	0.05 -/-
No. of attacks[b] **Level of significance**	-0.23 -/-	-0.26 -/-	-0.19 -/-	-0.21 -/-	0.03 -/-	0.20 -/-	-0.15 -/-	-0.15 -/-	0.14 -/-	-0.05 -/-	0.01 -/-	0.17 -/-
EDSS **Level of significance**	0.05 -/-	0.06 -/-	0.04 -/-	0.04 -/-	0.05 -/-	-0.01 -/-	0.08 -/-	0.08 -/-	-0.04 -/-	-0.06 -/-	-0.03 -/-	-0.04 -/-
Disease duration[c] **Level of significance**	0.11 -/-	-0.02 -/-	0.10 -/-	0.11 -/-	-0.03 -/-	-0.05 -/-	-0.06 -/-	-0.16 -/-	-0.13 -/-	-0.41 **/-	-0.44 **/-	-0.12 -/-
Time since last attack[d] (n=35) **Level of significance**	-0.21 -/-	-0.20 -/-	-0.19 -/-	-0.19 -/-	0.17 -/-	0.19 -/-	-0.13 -/-	-0.11 -/-	0.01 -/-	-0.14 -/-	0.02 -/-	0.10 -/-
CSF Protein **Level of significance**	0.20 -/-	0.21 -/-	0.19 -/-	0.17 -/-	0.01 -/-	-0.20 -/-	0.12 -/-	0.08 -/-	0.17 -/-	-0.07 -/-	-0.15 -/-	0.05 -/-
CSF Cells **Level of significance**	-0.03 -/-	-0.15 -/-	0.00 -/-	0.02 -/-	-0.23 -/-	0.07 -/-	0.10 -/-	-0.04 -/-	0.05 -/-	-0.11 -/-	-0.26 -/-	-0.01 -/-
IgG Index **Level of significance**	-0.01 -/-	-0.10 -/-	-0.01 -/-	0.03 -/-	-0.20 -/-	0.00 -/-	0.05 -/-	-0.01 -/-	-0.22 -/-	-0.10 -/-	-0.24 -/-	-0.24 -/-
Total number of lesions[e] **Level of significance**	-0.09 -/-	-0.14 -/-	0.01 -/-	0.01 -/-	-0.15 -/-	0.07 -/-	0.12 -/-	0.09 -/-	0.20 -/-	0.03 -/-	-0.08 -/-	0.24 -/-
sCD163 serum **Level of significance**	-0.14 -/-	-0.12 -/-	-0.17 -/-	-0.09 -/-	-0.05 -/-	0.20 -/-	0.13 -/-	0.17 -/-	-0.01 -/-	0.01 -/-	0.25 -/-	0.16 -/-
sCD163 CSF **Level of significance**	-0.06 -/-	-0.13 -/-	-0.18 -/-	-0.16 -/-	0.18 -/-	0.16 -/-	0.24 -/-	0.15 -/-	0.23 -/-	-0.14 -/-	-0.12 -/-	0.16 -/-
sCD163 ratio **Level of significance**	0.22 -/-	0.17 -/-	0.17 -/-	0.14 -/-	0.02 -/-	-0.20 -/-	0.12 -/-	0.01 -/-	0.06 -/-	-0.11 -/-	-0.33 */-	-0.14 -/-
CXCL13 serum **Level of significance**	-0.33 */-	-0.15 -/-	-0.25 -/-	-0.23 -/-	0.03 -/-	0.20 -/-	0.15 -/-	0.21 -/-	0.12 -/-	0.11 -/-	0.06 -/-	0.01 -/-
CXCL13 CSF	-0.06	-0.19	0.02	0.01	-0.29	0.13	-0.16	-0.25	-0.08	-0.23	-0.31	-0.06

Level of significance	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
CXCL13 ratio	0.03	-0.09	0.06	0.07	-0.22	0.04	-0.17	-0.29	-0.10	-0.23	-0.34	-0.08
Level of significance	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	*/-	-/-
Neopterin serum	-0.38	-0.28	-0.66	-0.49	0.74	0.34	-0.05	0.12	0.23	0.03	0.11	0.21
Level of significance	*/-	-/-	***/***	**/-	***/***	*/-	-/-	-/-	-/-	-/-	-/-	-/-
Neopterin CSF	-0.21	-0.20	-0.48	-0.31	0.64	0.17	-0.05	0.02	0.14	-0.15	-0.08	0.14
Level of significance	-/-	-/-	**/-	*/-	***/***	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Neopterin ratio	0.24	0.10	0.26	0.25	-0.13	-0.21	0.11	-0.05	-0.09	-0.23	-0.28	-0.10
Level of significance	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Osteopontin Serum	0.04	0.11	0.08	0.01	-0.05	-0.07	0.17	0.18	-0.11	-0.07	-0.18	-0.21
Level of significance	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Osteopontin CSF	-0.18	-0.35	-0.16	-0.21	-0.13	0.26	-0.13	-0.21	0.39	0.01	0.02	0.44
Level of significance	-/-	*/-	-/-	-/-	-/-	-/-	-/-	-/-	*/-	-/-	-/-	**/-
Osteopontin ratio	-0.14	-0.31	-0.12	-0.16	-0.15	0.21	-0.05	-0.15	0.44	0.06	0.06	0.44
Level of significance	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	**/-	-/-	-/-	**/-
Neurofilament light CSF	0.03	-0.08	0.05	0.07	-0.19	-0.01	0.20	0.07	0.34	0.08	-0.11	0.16
Level of significance	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	*/-	-/-	-/-	-/-

Supplementary Table SI3. Pearson correlations of cell surface marker expressions to clinical disease measures and soluble markers in serum and CSF.
Continuous variables were analysed using Pearson correlations without and with the Bonferroni correction. Each cell surface marker value (Table 1) was correlated to levels of clinical disease measures, and soluble biomarkers in serum and CSF (Table 2) from the included patients. The correlation coefficient, r, is shown with levels of significance: *p<0.05, **p<0.01, and *** p<0.001. [a]Age (in years) at the time of sampling. [b]Number of attacks: median number of attacks from debut of first symptom(s) to sampling. [c]Disease duration (in months): the period of time from debut of first symptom(s) to sampling. [d]Time since last attack: the period of time (in months) from latest attack to sampling. [e]Total number of lesions: total number of lesions determined by fluid-attenuated inversion recovery (FLAIR) sequences on MRI. Abbreviations: CD40+, CD163+, CD192+ positive monocytes, respectively; the three monocyte subsets: classical, intermediate, and non-classical, respectively; and HERV H3 Env and HERV W3 Env expression on each of the three monocyte subsets; RRMS (relapsing-remitting MS), PPMS (primary-progressive MS), CIS (clinically isolated syndrome), HC (healthy controls), n (number of subjects).

Supplementary Figure SI3. Surface marker expression of CD40, CD163, and CD192 on the total monocyte population and on the three monocyte subsets. The differences in expression of CD40, CD163, and CD192 on the total monocyte population (Live cells, Figure 1) **(A)** and the three monocyte subsets: classical **(B)**, intermediate **(C)**, and non-classical **(D)**, were determined as % positive cells of the total monocytes, from the patient group (n=40) and the healthy control group (n=20). Bars represent the mean of the populations and whiskers indicate the standard error of the mean (SEM).

Supplementary Figure SI4. Logistic regression analysis with ROC curve output of newly diagnosed patients with CIS or MS plotted against the HC group. Newly diagnosed patients (n=20) and healthy controls (n=20). AUC, with 95% CI, is given for each parameter. The surface expression of each parameter for patients with CIS or MS are combined as true positives and plotted against HC as true negatives. The diagonal dividing the ROC space represents the random event. A logistic regression analysis with combined parameter results has been performed for "all parameters", parameters with AUC > 0.70, and AUC > 0.75. Abbreviations: ROC (receiver operating characteristic), AUC (area under the curve), RRMS (relapsing-remitting MS), PPMS (primary-progressive MS, CIS (clinically isolated syndrome), HC (healthy controls).

Supplementary Figure SI5. Logistic regression analysis with ROC curve output of patients with CIS and MS plotted against the HC group. Patients (n=20) and healthy controls (n=20). AUC, with 95% CI, is given for each parameter. The surface expression of each parameter for patients with CIS or MS are combined as true positives and plotted against HC as true negatives. The diagonal dividing the ROC space represents the random event. A logistic regression analysis with combined parameter results has been performed for "all parameters", parameters with AUC > 0.70, and AUC > 0.75. Abbreviations: ROC (receiver operating characteristic), AUC (area under the curve), RRMS (relapsing-remitting MS), PPMS (primary-progressive MS, CIS (clinically isolated syndrome), HC (healthy controls).

Supplementary Figure SI6. Logistic regression analysis with ROC curve output of treated patients with CIS and MS plotted against the HC group. Treated patients (n=13) and healthy controls (n=20). AUC, with 95% CI, is given for each parameter. The surface expression of each parameter for patients with CIS or MS are combined as true positives and plotted against HC as true negatives. The diagonal dividing the ROC space represents the random event. A logistic regression analysis with combined parameter results has been performed for "all parameters", parameters with AUC > 0.70, and AUC > 0.75. Abbreviations: ROC (receiver operating characteristic), AUC (area under the curve), RRMS (relapsing-remitting MS), PPMS (primary-progressive MS, CIS (clinically isolated syndrome), HC (healthy controls).

Supplementary Figure SI7. Logistic regression analysis with ROC curve output of untreated patients with CIS and MS plotted against the HC group. Untreated patients (n=27) and healthy controls (n=20). AUC, with 95% CI, is given for each parameter. The surface expression of each parameter for patients with CIS or MS are combined as true positives and plotted against HC as true negatives. The diagonal dividing the ROC space represents the random event. A logistic regression analysis with combined parameter results has been performed for "all parameters", parameters with AUC > 0.70, and AUC > 0.75. Abbreviations: ROC (receiver operating characteristic), AUC (area under the curve), RRMS (relapsing-remitting MS), PPMS (primary-progressive MS, CIS (clinically isolated syndrome), HC (healthy controls).