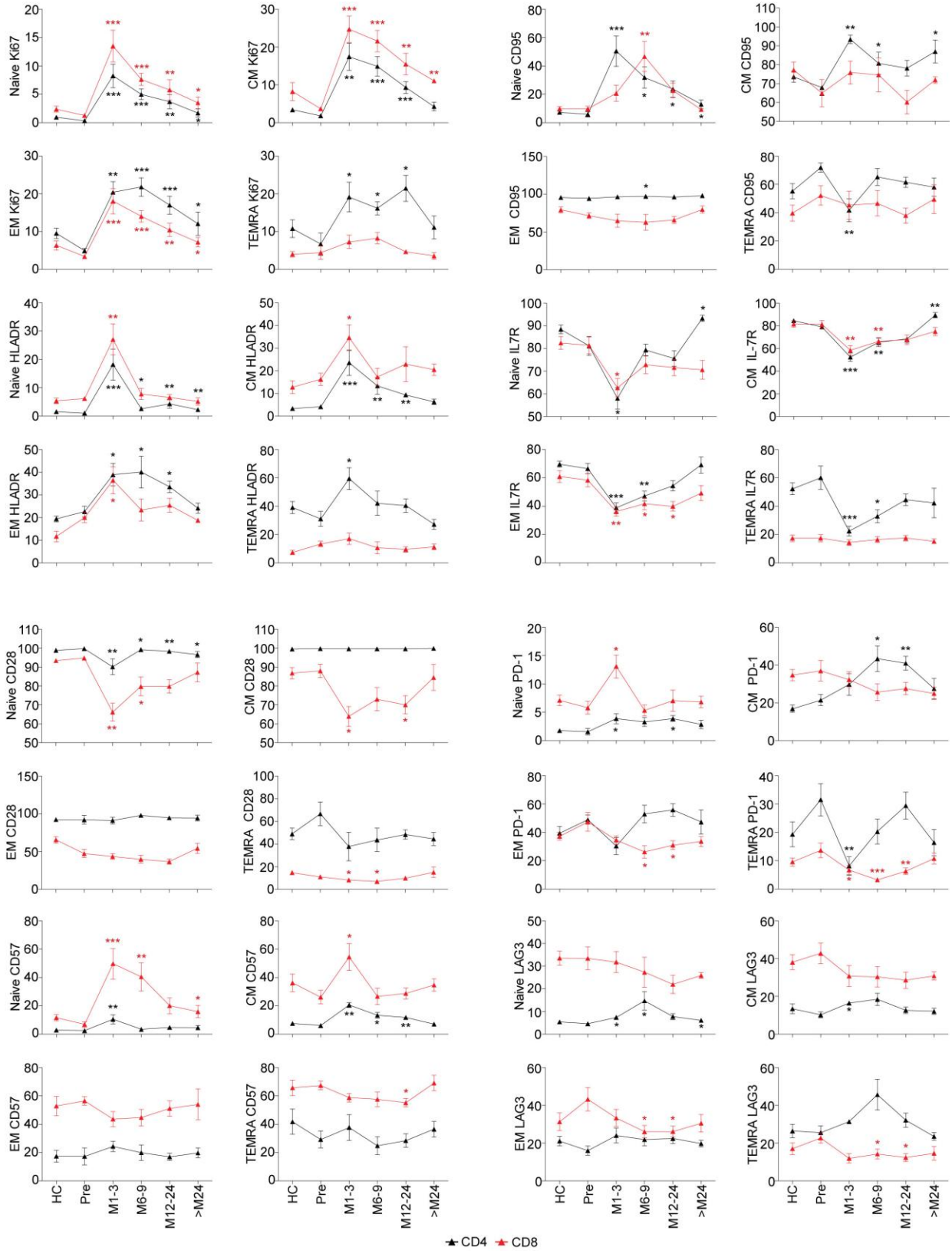
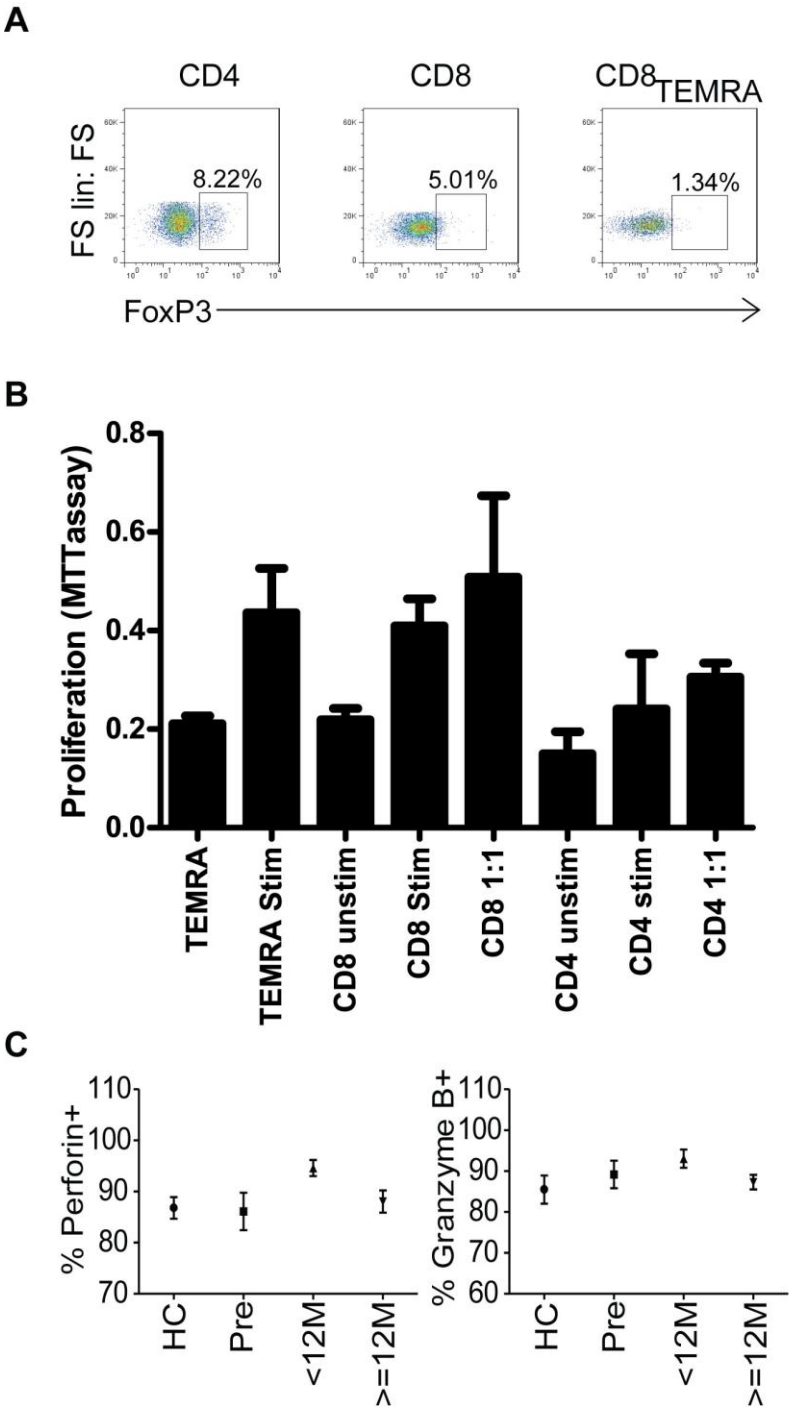
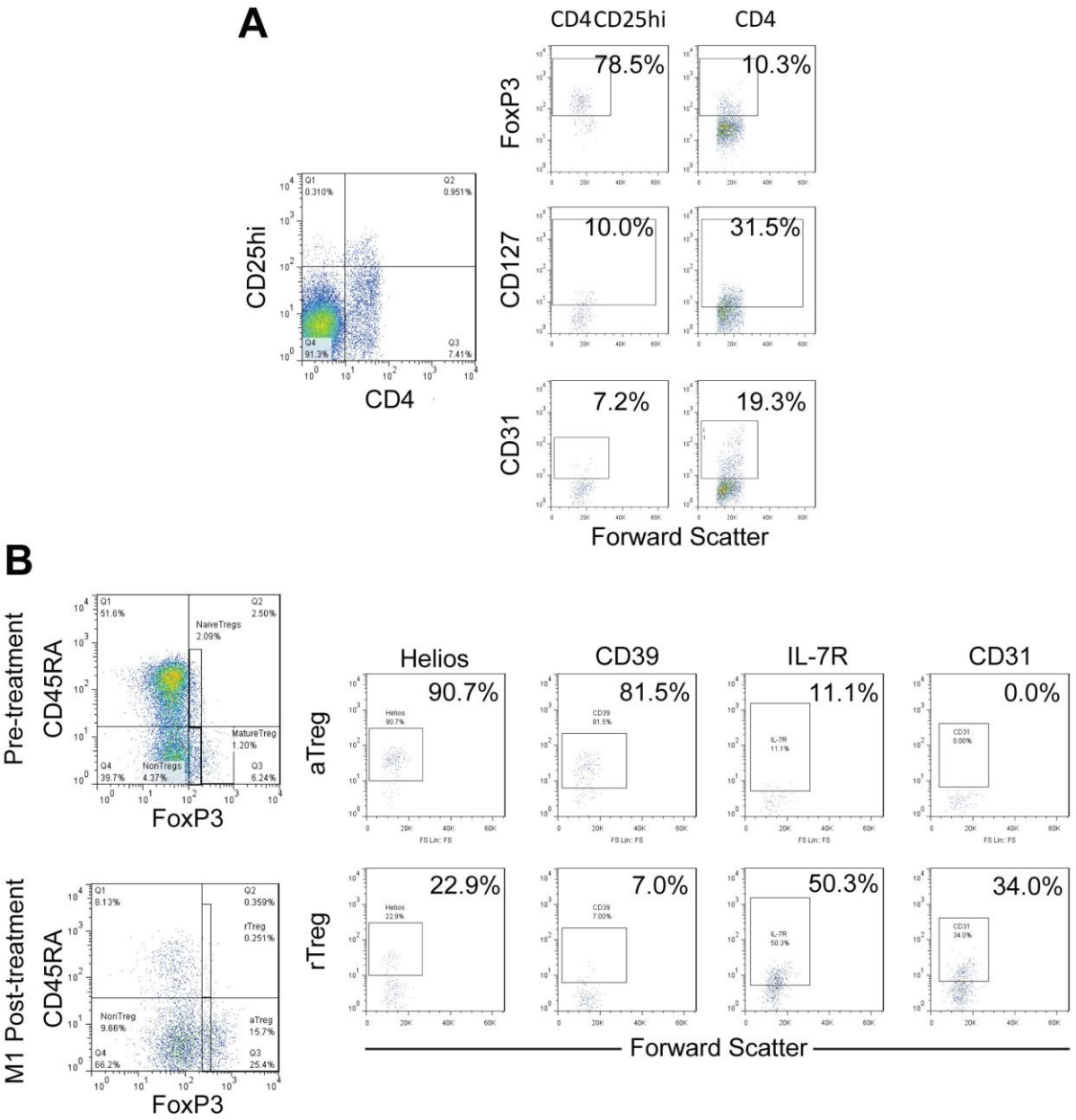


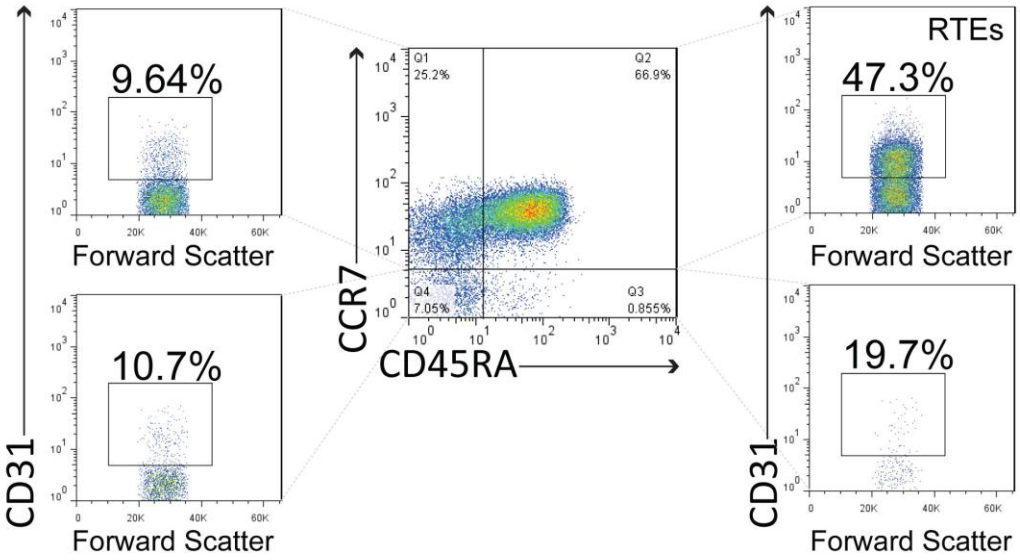
Supplementary Figure 1 Cross-sectional analysis of CD4 and CD8 subsets from 86 RRMS patients pre and post-alemtuzumab and from 29 healthy controls (HC). Graphs show mean percent of: Naive [CD45RA+CCR7+], central memory (CM) [CD45RA-CCR7+], effector memory (EM) [CD45RA-CCR7-] and T effector memory RA (TEMRA) [CD45RA+CCR7-] cells expressing a given marker +/- s.e.m. *P<0.05, ** P<0.01, ***P<0.001.



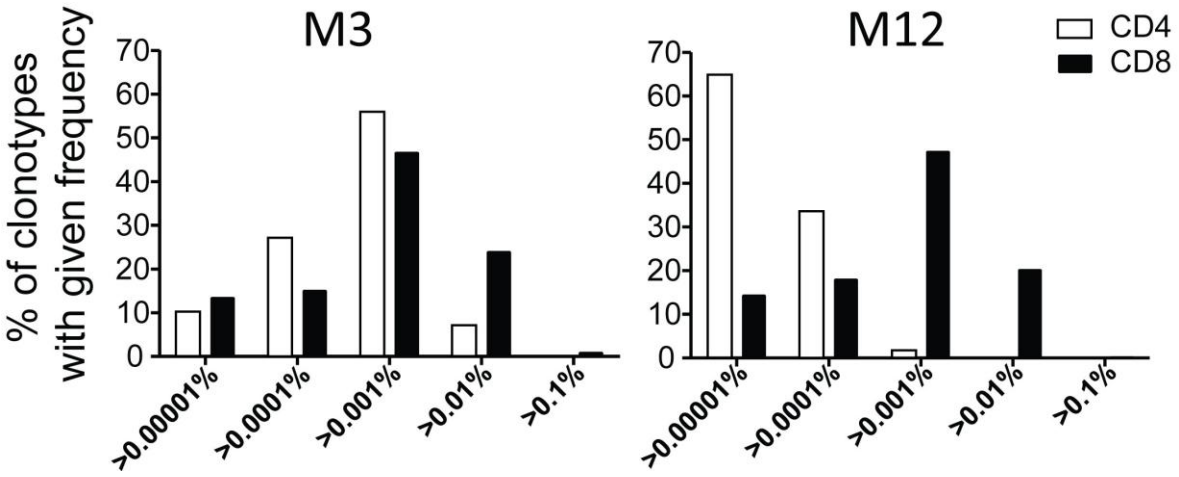
Supplementary Figure 2 $CD8_{TEMRAs}$ post-alemtuzumab do not express high FoxP3, do not suppress T cell proliferation but do contain cytotoxic granules. **(A)** Representative flow plots showing FoxP3 expression in CD4, CD8 and $CD8_{TEMRA}$ subpopulations **(B)** CD4 and CD8 effector populations were cultured with and without CD3/28 bead stimulation, with and without $CD8_{TEMRA}$ (ratio 1:1). Graph shows mean proliferation at 72 hours +/-95% confidence interval. **(C)** Percentage of $CD8_{TEMRA}$ containing perforin and granzyme B, as determined by intracellular FACS from 7 healthy controls (HC) and 20 RRMS patients pre and post-alemtuzumab. Data are mean values +/- s.e.m**.**



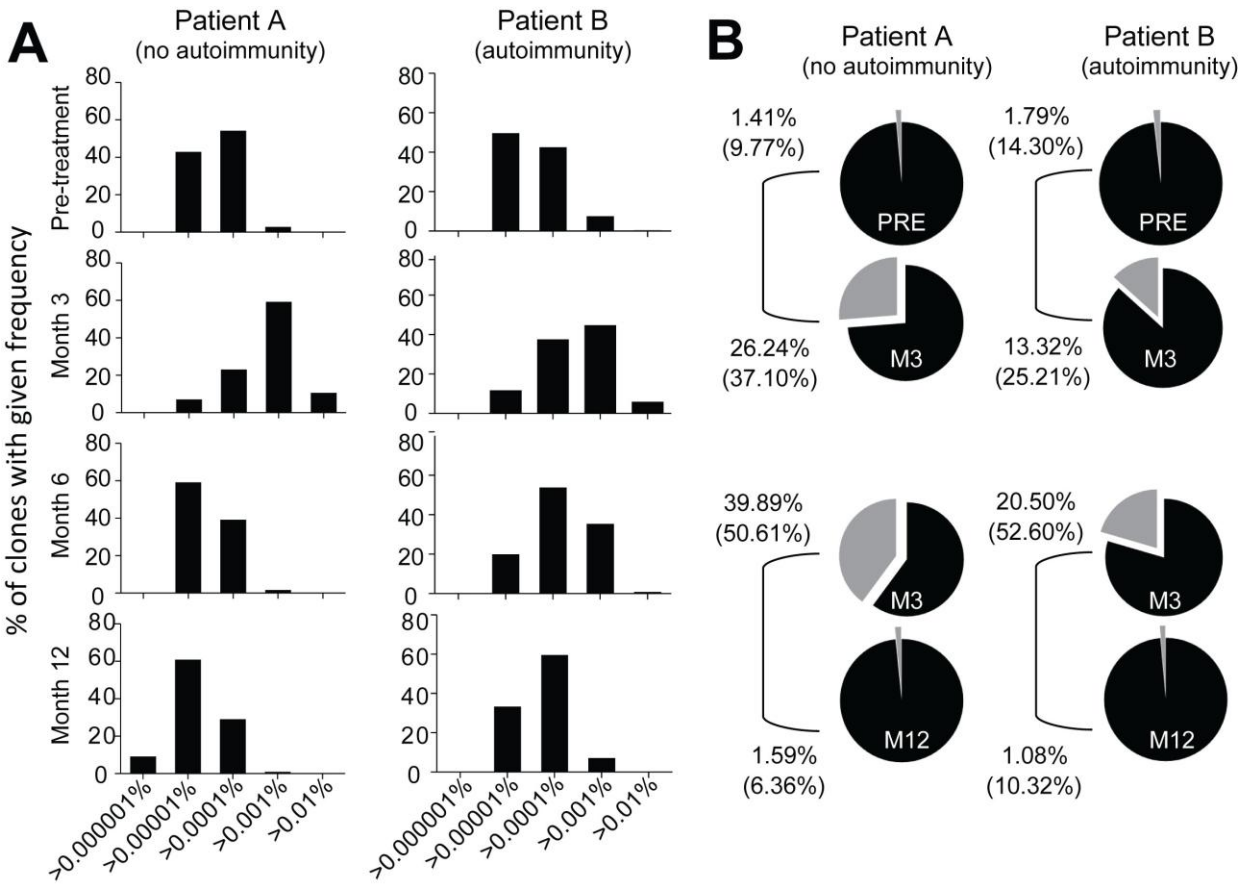
Supplementary Figure 3 Mature aTregs are expanded after alemtuzumab. **(A)** Representative Facs plots showing CD4+CD25hi Tregs, which are increased relative to other CD4 populations after alemtuzumab, express high FoxP3 and low CD127 (IL7R) and CD31 relative to other CD4 populations. **(B)** Representative Facs plots showing Helios, CD39, CD127 (IL7R) and CD31 expression in the aTreg vs. rTreg subpopulations.



Supplementary Figure 4 Recent thymic emigrants are defined as CD4naive (CD4+CD45RA+CCR7+) cells that co-express CD31. CD31 expression is shown for all CD4 subpopulations.



Supplementary Figure 5 T cell clonality is reduced after alemtuzumab. TCR sequencing of CD4 and CD8 separated cells from one patient 3 months after alemtuzumab, and from a second patient 12 months post-treatment. Graphs show percentage of clonotypes expressed at a given frequency. CD4 and CD8 TCRs are restricted at month 3; by month 12, CD4 clonality has improved, whereas CD8 clonality remains restricted.



Supplementary Figure 6 (A) Distribution of clonal frequencies before and at 3, 6 and 12 months post-alemtuzumab for patient A (no autoimmunity) and patient B (autoimmunity). **(B)** Pre-treatment TCR sequences detectable after treatment - expressed as a percentage of total unique sequences (un-bracketed value) and total expressed sequences (bracketed value) for each time-point.

A

Treatment Occasion	1	2	3	4
Number of patients	86	86	39	7
% Patients	99	99	45	8
Number of observations	356	805	382	61
Distribution of Observations per Patient				
- Min	1	1	1	2
- Inter-quartile Range	(3.0, 5.0)	(5.0, 9.0)	(5.5, 14.0)	(5,0, 12.5)
- Max	11	25	21	15
- Mean	4.140	9.384	9.821	8.714

B

Years since first treatment	% of all Patients
0- <4 years	15%
4 - <7 years	39%
>7 years	46%

Supplementary Table 1 a) Distribution of patients and observations by treatment occasion and **b)** length of follow up since first treatment, for patients included in kinetics of T-cell reconstitution studies.

Follow-up period	Treatment Occasion				P-value
	1	2	3	4	
first 18 months					
No. of Patients	85	83	37	7	
mean rate of change	23.05	20.70	14.57	8.47	< 0.001
(95% CI)	(20.08, 25.06)	(17.7, 22.73)	(10.41, 17.76)	(-0.24, 16.22)	
post 18 months					
No. of Patients	3	63	32	5	
mean rate of change	41.07	4.81	1.50	5.94	0.28
(95% CI)	(7.28, 82.79)	(0.55, 17.01)	(-6.11, 17.04)	(-19.38, 39.18)	

Supplementary Table 2. CD4 T cell mean rate of (1x10^6 cells/litre/month) by treatment occasion after adjusting for age at first treatment. Individual patient data were summarised by estimating two rates of change over two observation periods i) up to 18 months, and ii) subsequent to 18 months. This was done using a piecewise linear regression model fitted separately for each treatment occasion. Raw data from 86 patients were used from every treatment occasion and observation period that met the minimum data requirements for this model: (i) two or more cell counts in an observation period, or (ii) at least one cell count in one observation period and two cell counts in the other observation period. Few patients were observed repeatedly for longer than 18 months during their first (only 3 patients) and fourth (only 5 patients) treatment occasions. Therefore, these periods contributed much less information on the rate of change post 18 months than the other treatment occasions. The p-value corresponds to a test of equality among the mean rates of change by treatment occasion.

Follow-up period	Treatment Occasion				P-value
	1	2	3	4	
first 18 months					
No. of Patients	85	83	37	7	
mean rate of change	16.23	11.91	7.07	3.14	< 0.001
(95% CI)	(13.46, 17.99)	(9.12, 13.69)	(3.25, 9.89)	(-4.79, 10.05)	
post 18 months					
No. of Patients	3	63	32	5	
mean rate of change	34.88	-1.49	1.10	1.39	0.12
(95% CI)	(0.4, 58.15)	(-13.39, -0.8)	(-13.36, 4.35)	(-26.6, 18.8)	

Supplementary Table 3. CD8 T cell mean rate of (1x10^6 cells/litre/month) by treatment occasion after adjusting for age at first treatment. Individual patient data were summarised by estimating two rates of change over two observation periods i) up to 18 months, and ii) subsequent to 18 months. This was done using a piecewise linear regression model fitted separately for each treatment occasion. Raw data from 86 patients were used from every treatment occasion and observation period that met the minimum data requirements for this model: (i) two or more cell counts in an observation period, or (ii) at least one cell count in one observation period and two cell counts in the other observation period. Few patients were observed repeatedly for longer than 18 months during their first (only 3 patients) and fourth (only 5 patients) treatment occasions. Therefore, these periods contributed much less information on the rate of change post 18 months than the other treatment occasions. The p-value corresponds to a test of equality among the mean rates of change by treatment occasion.

	Autoimmune disease/ autoantibody	N	Gender (F:M)	Time since last treatment (months)	Severity/Treatment
Clinical (40/87)	Thyroid	36	32:4	20 (IQR 13.5)	2 patients required radioactive iodine 1 patient required thymectomy All other patients responded to standard medical treatment.
	ITP	3	2:1	9 (IQR 1.5)	Patient 1 (M): Immunoglobulin; rituximab and pulsed dexamethasone. Platelets stable >100x10^9/L on mycophenolate. Patient 2 (F): Immunoglobulin and oral steroids. Now off treatment, platelet count >LLN Patient 3 (F): Oral steroids. Now off treatment, platelet count >LLN
	Goodpasture's Syndrome	1	1:0	10 (IQR N/A)	Patient required renal dialysis and renal transplant. Creatinine currently within normal range.
Antibodies (17/87)	Anti-nuclear	8	6:2	9.5 (IQR 7.5)	N/A - no clinical disease
	Anti-mitochondrial	1	0:1	6 (IQR N/A)	
	Anti-smooth muscle	2	2:0	20 (IQR 14)	
	Anti-TPO	6	4:2	27 (IQR 16.5)	

Supplementary Table 4. Clinical details of secondary autoimmunity after alemtuzumab treatment of multiple sclerosis (total patients treated 87). IQR= interquartile range; LLN = lower limit of normal.; anti-TPO = anti-thyroid peroxidase.

A. CD4

	Odds Ratio	95% C.I	P-value of effect
Tertile (Value range)			0.13
First (-12.2 to 18)	1	-	
Second (>18 to 28.8)	2.27	(0.7, 7.98)	
Third (>28.8 to 54.3)	0.72	(0.24, 2.12)	

B. CD8

	Odds Ratio	95% C.I	P-value of effect
Tertile (Value range)			0.52
First (-15.3 to 10.5)	1	-	
Second (>10.5 to 18.3)	1.33	(0.45, 4.01)	
Third (>18.3 to 66)	1.91	(0.63,6)	

Supplementary Table 5. Rate of change in the first 18 months (1x10^6 cells/litre/month) does not predict autoimmunity after alemtuzumab. a) CD4 and b) CD8 rate of change was categorised into tertiles for use as a predictor. Strength of association was measured by the odds ratio, with rate of change in the second and third tertiles expressed in comparison to rate of change in the first tertile (reference category).

	HC	Pre	M1	M3	M6	M9	M12	>M12	>M24
N	29	17	27	27	28	21	27	23	43
Age at FACS (+/-95% CI)	34.55 (+/- 3.56)	35.61 (+/- 4.23)	38.19 (+/-3.45)	35.59 (+/- 3.05)	37.04 (+/- 3.18)	36.05 (+/- 2.91)	36.37 (+/- 2.95)	37.78 (+/- 3.19)	41.07 (+/- 2.62)
Gender (F;M)	19;10	13;4	19;8	18;9	16;12	15;6	17;10	16;7	27;16
Average N. of Cycles	NA	0.00	2.11	2.26	2.25	2.24	2.19	2.26	2.28

Supplementary Table 6. Demographic details of patients included in cross-sectional phenotyping studies.

	Primer Sequence
CD3-out5	5’-ACTGACATGGAACAGGGGAA-3’
CD3-out3	5’-AGCTCTGAAGTAGGGAACATAT-3’
CD3-in5	5’-GGCTATCATTCTTCTTCAAGGTA-3’
Sj-In5	5’-TGATGCCACATCCCTTTCAA-3’
Sj-In3	5’-GTGCTGGCATCAGAGTGTGT-3’
Sj-Out3	5’-ACACTTGCTCCGTGGTCTGT-3’
Sj-Out5	5’-CTCTCCTATCTCTGCTCTGAA-3’
CD3-in3	5’-TTCCTGGCCTATGCCCTTTT-3’
Db1-Out	5’-CTCATCTGGGCCTGTCCTTGT-3’
Db1-In	5’-TGACCCAGGAGGAAAGAAG-3’
1.1-Out	5’-AACCTAGGACCCTGTGGATG-3’
1.1-In	5’-TGTCCTCCATCCTAGCCAGG-3’
1.2-Out	5’-CTCTCTATGCCTTCAATGTG-3’
1.2-In	5’-TCCGTCACAGGGAAAAGTGG-3’
1.3-Out	5’-AAGGGAACACAGAGTACTGGAA-3’
1.3-In	5’-TCCCAACCTCTGCCTGAAT-3’
1.4-Out	5’-TGGACTTGGGGAGGCAGGA-3’
1.4-In	5’-AGGAGTGGAAGGCAGCAGGT-3’
1.5-Out	5’-GAAACTGAGAACACAGCCAAGAA-3’
1.5-In	5’-CTCATAAAATGTGGGTCAGTGGA-3’
1.6-Out	5’-ATCCTCCCTCTTATGTGCATGG-3’
1.6-In	5’-TGAATCCAGGCAGAGAAAGG-3’

Supplementary Table 7 T cell receptor excision circle (TREC) primers

Probes	Sequences
CD3-P1	5’-GGCTGAAGGTTAGGGATACCAATATTCCTGTCTCfluro-3’
CD3-P2	5’-(red 705)CTAGTGATGGGCTCTTCCCTTGAGCCCTTCp-3’
Sj-P1	5’-AATAAGTTCAGCCCTCCATGTCACACTfluro-3’
Sj-P2	5’-(red640)TGTTTTCCATCCTGGGGAGTGTTTCAp-3’
Db1-P1	5’-CTGGGAGTTGGGACCGCCAGAGAGGTfluro-3’
Db1-P2	5’-(red640)TTTGTAAAGGTTTCCCGTAGAGTTGAATCATTGTGp-3’

Supplementary Table 8 LightCycler Probe sequences

BV family	Primer Sequence	Product Size (average bp)
BV2	5’-ATACCTGGCTCGTATGCTGGG-3’	443
BV3	5’-CTCCTCTGCTGTGTGGTCTTCTG-3’	432
BV4	5’-TGCTGTGCGGTTCTCTGTCTC-3’	430
BV5	5’-CTGATCAAAACGAGAGGACAGCA-3’	356
BV6	5’-TC(G/A)G(G/C)CTCCTGTGCTGTG-3’	437
BV7	5’-CAGGTGCTGGAGTCTCCCAG-3’	362
BV7.2	5’-CAGGAGCTGGAGTCTCCCAG-3’	390
BV7.3	5’-CAGATACTGGAGTCTCCCAG-3’	392
BV9	5’-GCAGGCCCAGTGGATTCTG-3’	398
BV10	5’-TGTTCTTCTATGTGGCCCTTTGTC-3’	431
BV11	5’-CGGCC(C/T)TCTGTCTCCTGG-3’	417
BV12	5’-AGGTGACAGAGATGGGACAAGAAGT-3’	359
BV13	5’-CTGCCTGACTCTGCCTGGAAC-3’	459
BV14	5’-TGTCTCCTGGGAGCAAAGCA-3’	414
BV15	5’-TGGCCCTTTGTCTCCTTGG-3’	419
BV18	5’-TCTTCTGGGGGCAGGTCTCT-3’	412
BV19	5’-GGTGCTCTGCTGTGTGGTCC-3’	435
BV20	5’-CTGCTGCTTCTGCTGCTTCTG-3’	435
BV24	5’-CCTGGGAACAGGGTCCATG-3’	407
BV25	5’-GGCTCCTCTGCTACATGGGC-3’	434
BV27	5’-TGGTCCTTTGCCTTCTAGGAGC-3’	419
BV28	5’-CCTGGCTGTAGGCCTCGTAGA-3’	406
BV29	5’-GGACTAGGCTCTGTGTTCAGTGCT-3’	408
BV30	5’-CTCTGCTCTCTCCTTGCCCTTC-3’	432
BC	5’-CTCAAACACAGCGACCTCGG-3’	

Supplementary Table 9 Primer panel of the amplification TCRBV