

Supplemental Methods

Additional clinical details on the HALT-MS study. Twenty-five subjects were enrolled on the HALT-MS clinical trial (1). Patients had an average age of 37 yrs, (range 27-53); there were 17 females and 8 males. The average disease duration was 6.4 yrs. (range 1.0-13.0). The baseline EDSS (expanded disability status scale) ranged from 3.0 to 5.5 with a mean value of 4.5.

All 25 subjects were mobilized and an autologous graft collected. The graft, Auto-CD34+HPC, was manufactured under a Type II Drug Master File (FDA BBIND-11821, V1.0 July 14, 2004) (2). CD34+ cells were selected using the Baxter Isolex device. The Certificate of Analysis specified viable CD34+ selected cells of > 70% with other cells including T cells (CD3+), B cells (CD19+), NK cells (CD56+) and monocytes (CD14+) < 30% combined.

Twenty-four subjects proceeded to transplantation. Subjects received a standard BEAM preparative regimen of BCNU (300 mg/m², day -6), etoposide (100 mg/m²/day, BID days -5 to -2), ara-C (100 mg/m²/day, BID days -5 to -2) and melphalan (140 mg/m², day -1) with rabbit antithymocyte globulin (ATG, 2.5 mg/kg/day on days -2 and -1). On day 0, the autologous graft was thawed and infused. Patients received a minimum of 2.0 x 10⁶ CD34+ cells/kg of patient body weight per the protocol; the median viable CD34+ dose was 5.1 (range 3.9 – 12.8) x 10⁶ cells/kg, and the median viable CD3+ dose was 2.34 x 10⁴ cells/kg (2). HALT-MS subjects engrafted neutrophils with an ANC of greater than 500 cells/uL at a median of day 11, and platelets at greater than 20,000/uL with no platelet transfusions in the preceding seven days, at a median of day 18. Treatment failure was defined as one or more of: death from any cause, MS clinical relapse, EDSS

progression as evidenced by an increase of greater than >0.5 in EDSS confirmed at 3 months or later, or presence of 2 or more independent MRI lesions indicative of disease activity.

References for Supplemental Methods

1. Nash RA, Hutton GJ, Racke MK, Popat U, Devine SM, Steinmiller KC, Griffith LM, Muraro PA, Openshaw H, Sayre PH, et al. High-dose immunosuppressive therapy and autologous HCT for relapsing-remitting MS. *Neurology*. 2017;88(9):842-52.
2. Keever-Taylor CA, Heimfeld S, Steinmiller KC, Nash RA, Sullivan KM, Czarniecki CW, Granderson TC, Goldstein JS, and Griffith LM. Manufacture of Autologous CD34(+) Selected Grafts in the NIAID-Sponsored HALT-MS and SCOT Multicenter Clinical Trials for Autoimmune Diseases. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2017;23(9):1463-72.

Supplemental Table 1. TCR β productive reads and re-arrangements for CSF

msid	timept	smtp	total_productive_reads	productive_rearrangemen
P1	M00	CSF	1,081,367	15,817
P1	M24	CSF	49,286	70
P1	M48	CSF	4,658	19
P2	M00	CSF	19,655	82
P2	M24	CSF	6,716	42
P2	M48	CSF	5,753	93
P3	M00	CSF	452,232	3,934
P3	M24	CSF	13,481	54
P4	M00	CSF	13,964	73
P4	M24	CSF	32,875	168
P5	M00	CSF	413,563	21,185
P5	M24	CSF	338,724	1,313
P5	M48	CSF	993,356	3,741
P6	M00	CSF	2,583,215	12,989
P6	M24	CSF	125,241	640
P6	M48	CSF	1,651,976	5,543
P8	M00	CSF	2,576,621	7,587
P8	M24	CSF	1,172,362	3,460
P9	M00	CSF	1,828,100	13,995
P9	M24	CSF	477,499	3,063
P10	M00	CSF	1,595,274	25,978
P10	M24	CSF	41,289	260
P10	M48	CSF	11,862	259
P11	M00	CSF	878,455	3,586
P11	M24	CSF	145,494	433
P12	M00	CSF	363,864	3,570
P12	M24	CSF	94,878	293
P14	M00	CSF	898,866	26,972
P14	M24	CSF	909,409	4,597
P15	M00	CSF	142,480	395
P15	M24	CSF	26,177	269
P16	M00	CSF	337,644	19,923
P16	M24	CSF	64,789	267
P19	M00	CSF	20,735	78
P19	M24	CSF	966,922	13,379
P19	M48	CSF	59,495	1,651
P23	M00	CSF	496,492	6,476
P23	M24	CSF	59,405	1,150
P24	M00	CSF	269,105	1,417
P24	M24	CSF	61,859	258
P25	M00	CSF	225,839	1,019
P25	M24	CSF	30,134	301
P25	M48	CSF	22,607	396

Supplemental Table 2: TCR β productive reads and re-arrangements for blood

msid	timept	smptp	total_productive_reads	productive_rearrangement
P1	M00	CD4	7,440,098	78,584
P1	M12	CD4	62,372	9,170
P1	M24	CD4	5,840,310	118,544
P1	M48	CD4	4,744,848	115,479
P2	M00	CD4	9,201,556	203,260
P2	M12	CD4	3,956,786	153,144
P2	M24	CD4	8,075,810	251,285
P2	M48	CD4	6,358,942	133,234
P3	M00	CD4	11,286,649	104,325
P3	M12	CD4	1,785,620	29,882
P3	M24	CD4	5,332,708	152,614
P4	M00	CD4	7,040,392	183,705
P4	M12	CD4	3,442,148	49,860
P4	M24	CD4	7,863,741	106,562
P5	M00	CD4	19,482,412	162,773
P5	M12	CD4	3,890,698	183,241
P5	M24	CD4	4,843,212	212,603
P5	M48	CD4	2,688,938	77,376
P6	M00	CD4	17,740,397	171,206
P6	M12	CD4	5,747,151	239,327
P6	M24	CD4	5,161,325	203,171
P6	M48	CD4	2,263,158	54,198
P7	M00	CD4	8,297,014	105,539
P7	M12	CD4	5,790,777	80,052
P8	M00	CD4	5,306,056	152,598
P8	M12	CD4	6,896,122	116,977
P8	M24	CD4	5,129,463	128,354
P9	M00	CD4	11,236,157	184,862
P9	M12	CD4	6,423,648	237,181
P9	M24	CD4	5,995,291	210,455
P9	M48	CD4	3,465,904	80,444
P10	M00	CD4	14,373,964	222,892
P10	M12	CD4	11,045,085	143,616
P10	M24	CD4	7,025,708	356,620
P10	M48	CD4	3,495,295	125,822
P11	M00	CD4	6,412,481	193,796
P11	M12	CD4	8,260,734	121,392
P11	M24	CD4	3,182,954	66,044
P12	M00	CD4	6,882,408	146,744
P12	M12	CD4	4,975,161	184,249
P12	M24	CD4	2,112,274	43,431
P14	M00	CD4	8,553,619	214,340
P14	M12	CD4	5,325,016	359,581
P14	M24	CD4	6,168,942	247,004
P15	M00	CD4	13,246,503	228,078
P15	M12	CD4	3,923,814	99,167
P15	M24	CD4	2,903,975	79,229
P16	M00	CD4	10,445,819	228,443
P16	M12	CD4	2,861,604	118,188
P16	M24	CD4	2,448,021	135,563
P17	M00	CD4	4,173,133	151,954
P17	M12	CD4	2,482,317	121,278
P17	M24	CD4	5,806,240	168,667
P18	M00	CD4	5,387,807	161,343
P18	M12	CD4	3,237,543	135,015
P18	M24	CD4	3,756,000	100,730
P19	M00	CD4	9,942,215	309,951
P19	M12	CD4	7,594,902	218,079
P19	M24	CD4	3,375,948	211,095
P19	M48	CD4	6,487,993	142,818
P20	M00	CD4	7,772,064	121,787
P20	M12	CD4	4,637,596	229,842
P20	M24	CD4	6,968,663	133,848
P21	M00	CD4	9,659,071	235,817
P21	M12	CD4	4,122,281	80,581
P21	M24	CD4	8,478,402	251,417
P23	M00	CD4	10,272,413	224,346
P23	M12	CD4	7,167,462	140,660
P23	M24	CD4	2,379,412	87,994
P24	M00	CD4	3,000,325	141,903
P24	M12	CD4	4,038,748	137,317
P24	M24	CD4	3,247,939	207,891
P25	M00	CD4	5,206,654	76,267
P25	M12	CD4	4,265,905	27,454
P25	M24	CD4	4,323,900	84,928
P25	M48	CD4	4,726,740	77,024

msid	timept	smptp	total_productive_reads	productive_rearrangement
P1	M00	CD8	5,111,962	23,528
P1	M12	CD8	5,733,421	23,624
P1	M24	CD8	4,838,382	43,908
P1	M48	CD8	5,935,003	44,317
P2	M00	CD8	14,081,689	98,022
P2	M12	CD8	3,724,604	33,054
P2	M24	CD8	6,651,072	60,440
P2	M48	CD8	2,500,915	50,936
P3	M00	CD8	6,308,060	98,791
P3	M12	CD8	2,501,501	47,875
P3	M24	CD8	4,407,850	157,283
P4	M00	CD8	6,626,331	112,994
P4	M12	CD8	5,333,549	16,011
P4	M24	CD8	9,203,837	23,475
P5	M00	CD8	6,036,054	55,854
P5	M12	CD8	4,149,057	61,094
P5	M24	CD8	4,255,371	62,401
P5	M48	CD8	3,140,368	59,305
P6	M00	CD8	3,452,809	64,294
P6	M12	CD8	3,652,863	79,866
P6	M24	CD8	3,722,007	90,656
P6	M48	CD8	1,997,070	28,351
P7	M00	CD8	4,439,954	87,043
P7	M12	CD8	3,226,827	37,526
P8	M00	CD8	7,182,462	158,484
P8	M12	CD8	2,834,305	25,788
P8	M24	CD8	3,313,489	20,311
P9	M00	CD8	6,901,342	131,738
P9	M12	CD8	9,972,157	54,938
P9	M24	CD8	2,899,539	21,885
P9	M48	CD8	2,995,177	22,058
P10	M00	CD8	4,140,957	141,901
P10	M12	CD8	5,137,537	85,939
P10	M24	CD8	3,600,176	45,086
P10	M48	CD8	3,723,451	118,174
P11	M00	CD8	2,572,502	71,784
P11	M12	CD8	4,008,777	14,613
P11	M24	CD8	3,194,039	11,487
P12	M00	CD8	3,205,380	58,236
P12	M12	CD8	4,095,498	90,389
P12	M24	CD8	1,462,192	43,931
P14	M00	CD8	5,494,697	222,417
P14	M12	CD8	5,480,782	192,666
P14	M24	CD8	7,083,576	356,858
P15	M00	CD8	5,911,245	161,589
P15	M12	CD8	2,708,215	26,645
P15	M24	CD8	1,251,204	38,692
P16	M00	CD8	5,696,461	94,365
P16	M12	CD8	2,567,505	33,989
P16	M24	CD8	5,059,874	74,624
P17	M00	CD8	3,522,839	42,564
P17	M12	CD8	5,271,760	49,137
P17	M24	CD8	2,456,967	59,981
P18	M00	CD8	4,321,210	81,627
P18	M12	CD8	4,699,821	73,960
P18	M24	CD8	4,879,598	112,709
P19	M00	CD8	7,992,668	141,844
P19	M12	CD8	4,022,396	99,044
P19	M24	CD8	1,080,804	42,113
P19	M48	CD8	6,161,514	128,245
P20	M00	CD8	4,246,153	165,841
P20	M12	CD8	5,613,785	117,257
P20	M24	CD8	3,693,189	95,589
P21	M00	CD8	9,370,023	170,975
P21	M12	CD8	4,297,320	16,997
P21	M24	CD8	9,283,926	168,060
P23	M00	CD8	5,849,063	138,788
P23	M12	CD8	7,479,006	31,804
P23	M24	CD8	2,832,517	37,224
P24	M00	CD8	9,690,114	174,384
P24	M12	CD8	5,069,316	35,277
P24	M24	CD8	2,815,269	67,472
P25	M00	CD8	1,453,413	25,002
P25	M12	CD8	14,816,580	7,883
P25	M24	CD8	5,268,396	20,267
P25	M48	CD8	5,961,180	25,587

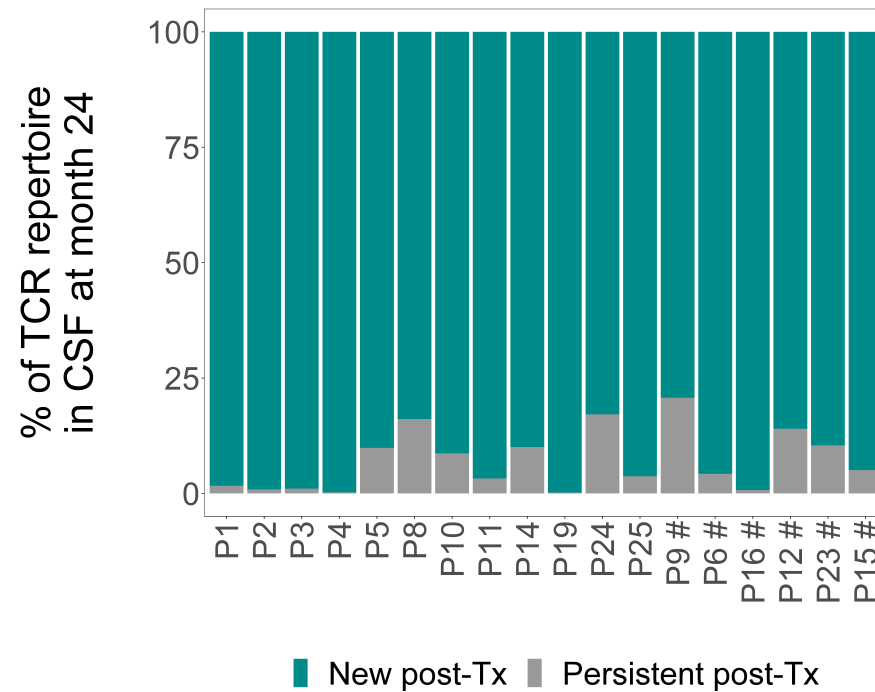
Supplemental Table 3: Median and range of TCR β productive reads and re-arrangements for blood and CSF samples

productive reads

smptp	timept	min	median	max
CD4	M00	3,000,325	8,553,619	19,482,412
CD4	M12	62,372	4,265,905	11,045,085
CD4	M24	2,112,274	5,145,394	8,478,402
CD4	M48	2,263,158	4,111,018	6,487,993
CD8	M00	1,453,413	5,696,461	14,081,689
CD8	M12	2,501,501	4,297,320	14,816,580
CD8	M24	1,080,804	3,707,598	9,283,926
CD8	M48	1,997,070	3,431,910	6,161,514
CSF	M00	13,964	432,898	2,583,215
CSF	M24	6,716	63,324	1,172,362
CSF	M48	4,658	22,607	1,651,976

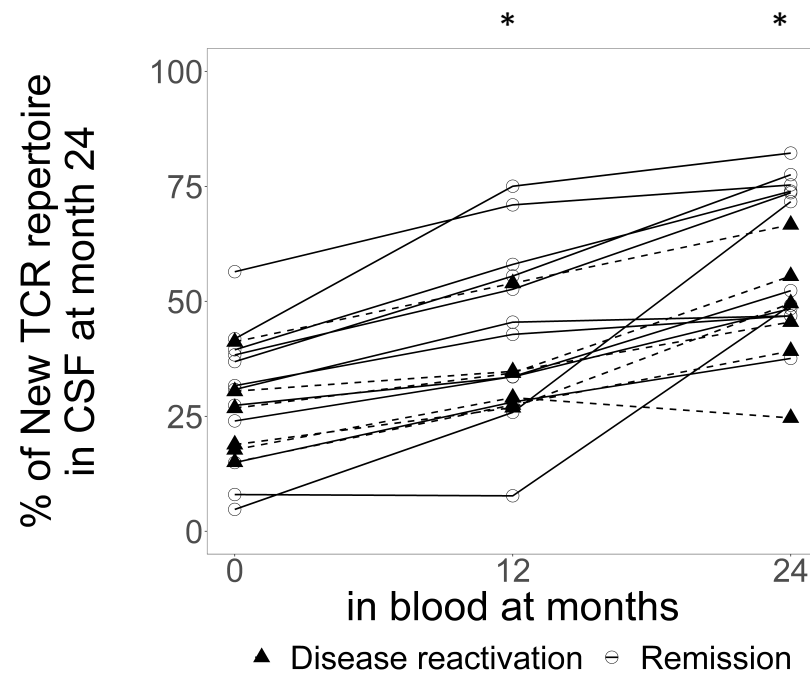
re-arrangements

smptp	timept	min	median	max
CD4	M00	76,267	171,206	309,951
CD4	M12	9,170	135,015	359,581
CD4	M24	43,431	144,089	356,620
CD4	M48	54,198	97,962	142,818
CD8	M00	23,528	98,791	222,417
CD8	M12	7,883	37,526	192,666
CD8	M24	11,487	52,534	356,858
CD8	M48	22,058	47,627	128,245
CSF	M00	73	5,205	26,972
CSF	M24	42	297	13,379
CSF	M48	19	396	5,543



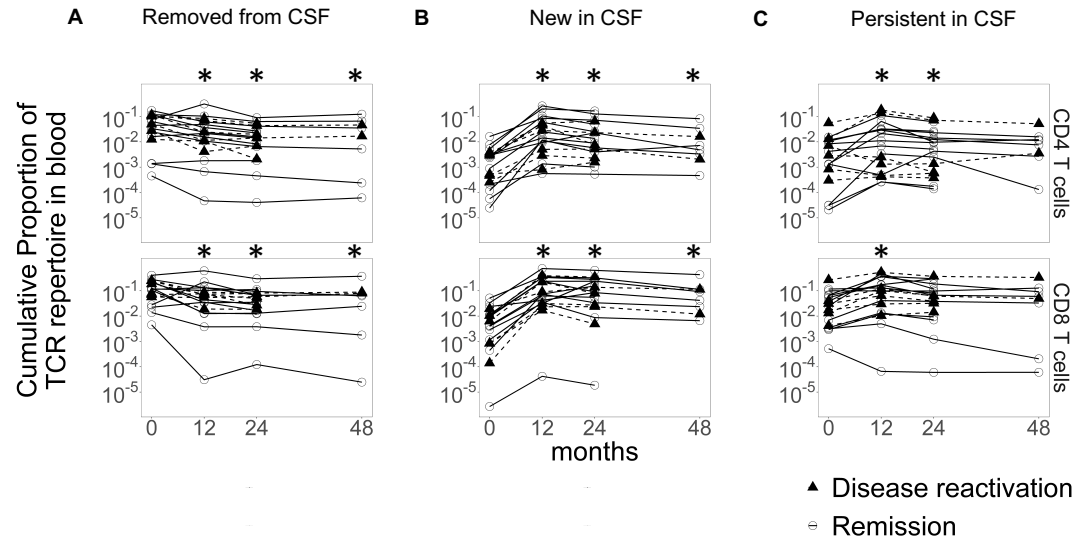
Supplemental Figure 1.

TCR overlap percentage was used to determine the percent of clones that were undetectable, termed new (teal) versus detected, termed persistent (grey) in the CSF repertoire at month 24 compared to pre-therapy for each participant as in Figure 3A. To ensure the robust changes observed were not due to differences in sampling depth between time points, randomized, permutation testing was performed to match the sampling depths for CSF samples collected pre-therapy and at month 24 post-transplant. Data represent the mean from 1000 permutations of down-sampled pre-therapy repertoire to match the sampling depth at month 24 post-transplant.



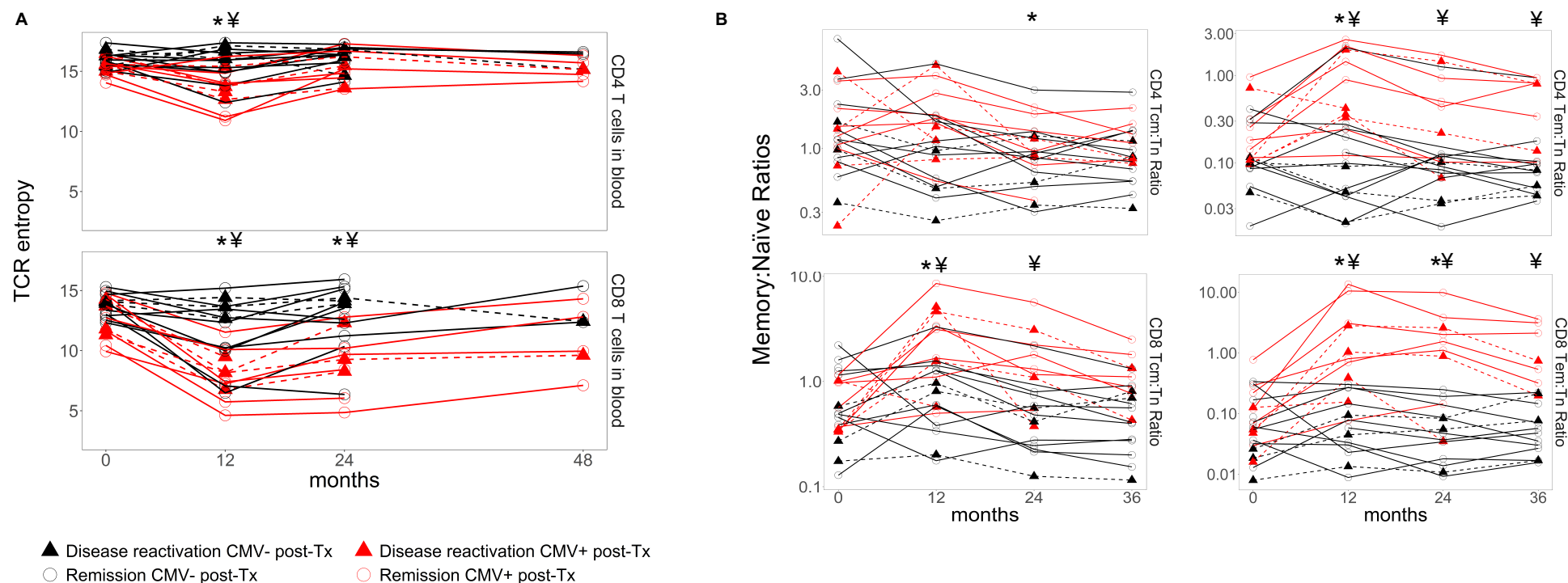
Supplemental Figure 2 (individual lines for Figure 3C).

Longitudinal evaluation of new clones in CSF at month 24 as the percentage detected by ultra-deep sequencing in CD4⁺ or CD8⁺ T cells in blood. Filled triangles are participants that met the primary endpoint for the HALT-MS study before month 60 post-transplant, and open circles are participants that stayed in remission from active MS until the last follow-up. *p<0.05 between month 0 pre-therapy versus months 12 and 24 post-transplant using mixed model for repeated measures.

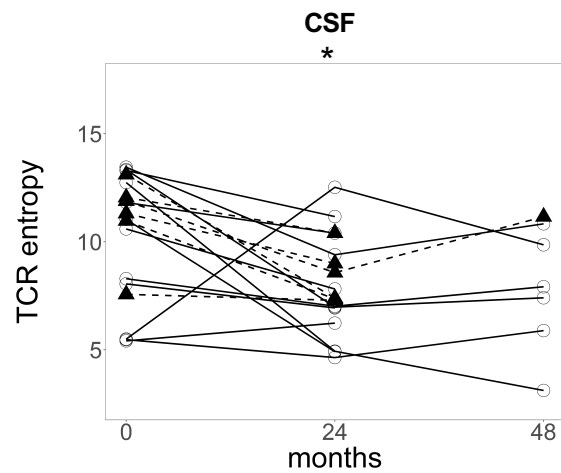


Supplemental Figure 3 (individual lines for Figure 4).

Proportions of TCR clones pre-existing in blood that are (A) pre-existing in CSF pre-therapy and undetectable in CSF at month 24, termed removed, (B) new in CSF at month 24 post-transplant, and (C) persisting in CSF at month 24 post-transplant. Proportions were aggregated per subject within circulating CD4⁺ or CD8⁺ T cell repertoires pre-to-post transplant and then log-transformed. Filled triangles are participants that met the primary endpoint for the HALT-MS study before month 60 post-transplant, and open circles are participants that stayed in remission from active MS until the last follow-up. The line represents the mean of participants evaluated. * $p < 0.05$ between month 0 pre-therapy versus months 12, 24, and 48 post-transplant using mixed model for repeated measures.



Supplemental Figure 4 (individual lines for Figure 5). CMV infection post-AHSCT therapy is associated with impaired TCR diversification and an increased ratio of effector memory to naïve cell subsets in circulating CD8 T cells. (A) TCR repertoire diversity was analyzed using the Shannon entropy index in blood CD4 and CD8 T cells before therapy and at months 12, 24 and 48 post-transplant. (B) Ratios of circulating CD4 (top) and CD8 (bottom) central memory (Tcm) and effector memory (Tem) to naïve (Tn) cells were analyzed by flow cytometry as previously reported (Harris et al 2018). Filled triangles are participants that met the primary endpoint for the HALT-MS study before month 60 post-transplant, and open circles are participants that stayed in remission from active MS until the last follow-up. Participants were further stratified based on CMV infection status post-transplant. Black shapes are participants that were CMV negative, and red shapes are participants that were CMV positive, by PCR post-transplant. The line represents the mean of all participants evaluated at each time point. * $p < 0.05$ between month 0 pre-therapy versus months indicated post-transplant using mixed model for repeated measures in panels A and B. # $p < 0.05$ between CMV+ vs. CMV- groups post-transplant at each visit using mixed model for repeated measures in panels A and B.



Supplemental Figure 5 (individual lines for Figure 6A). **TCR diversification in CSF is reduced at month 24 post-AHSCT therapy.** TCR repertoire diversity was analyzed using the Shannon entropy index in CSF before therapy and at months 24 and 48 post-transplant. Filled triangles are participants that met the primary endpoint for the HALT-MS study before month 60 post-transplant, and open circles are participants that stayed in remission from active MS until the last follow-up. * $p < 0.05$ between month 0 pre-therapy and at month 24 post-transplant.