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Supplementary Materials for

Massive clonal expansion of medulloblastoma-specific T cells during adoptive cellular therapy

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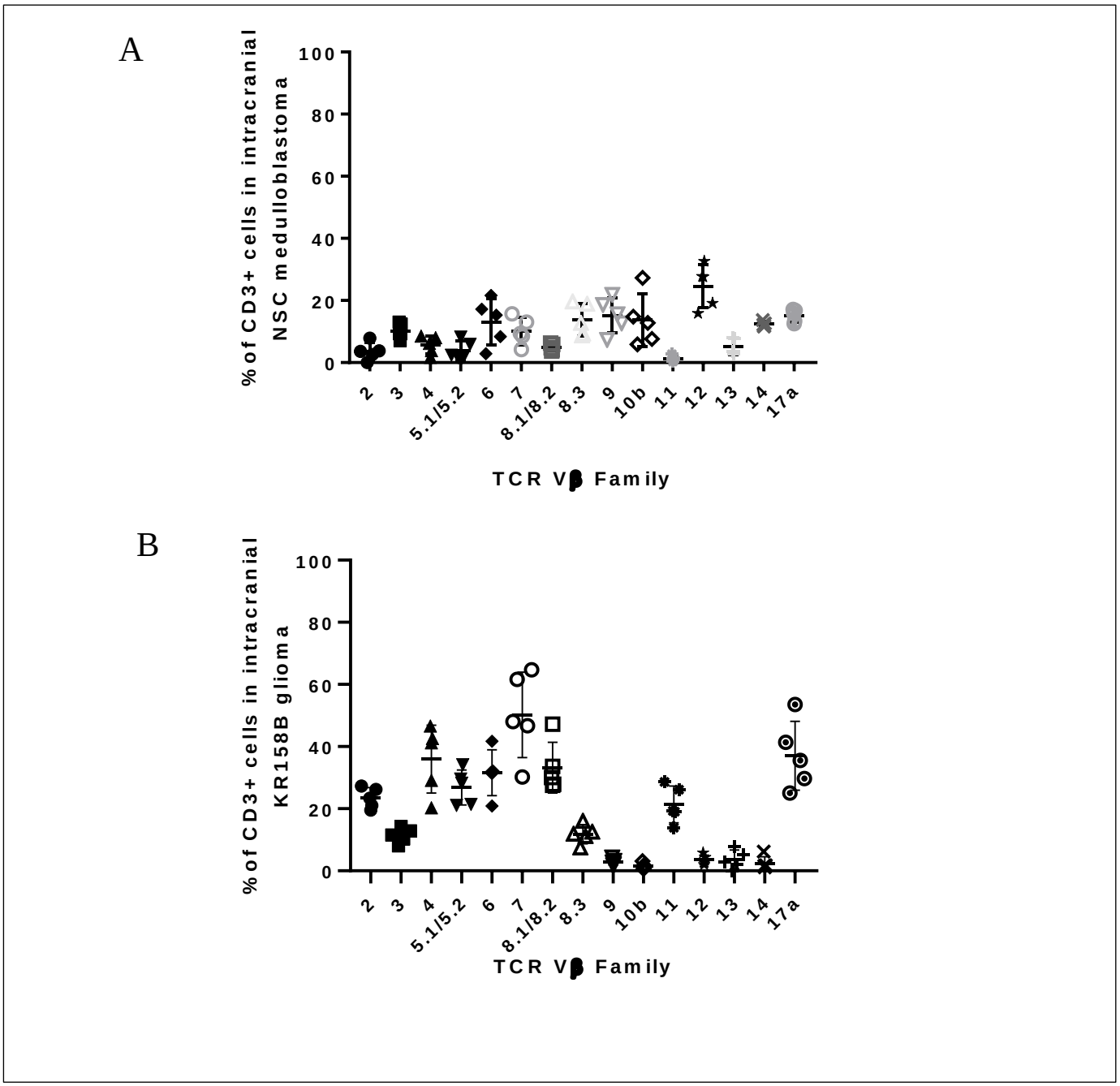

Fig. S1. TILs in untreated mice. A) C57BL/6 mice received orthotopic NSC medulloblastoma and left untreated until symptomatic of disease. Tumors were harvested and processed into single cell suspension for flow cytometry. The relative frequency of fifteen TCR Vβ families was determined from the tumor tissue (n=5 mice. This was repeated three additional times). **B**) C57BL/6 mice received orthotopic KR158B high-grade glioma and left untreated until symptoms of disease were observed. The relative frequency of fifteen TCR Vβ families was determined by flow cytometry from the tumor tissue (n=5 mice).

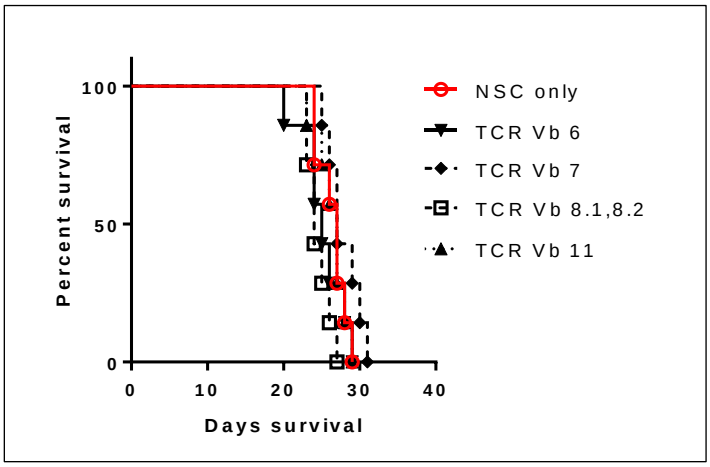

Fig. S2. Adoptive transfer of TCR Vβ6, 7, 8.1/8.2, or 11 does not provide survival benefit against NSC. NSC tumor-bearing mice received either no treatment or adoptive cellular therapy with TCR Vβ 6+, Vβ 7+, Vβ 8.1/8.2, or Vβ 11+ T cells. No survival benefit was observed in any groups over untreated controls (n=7 mice/group).

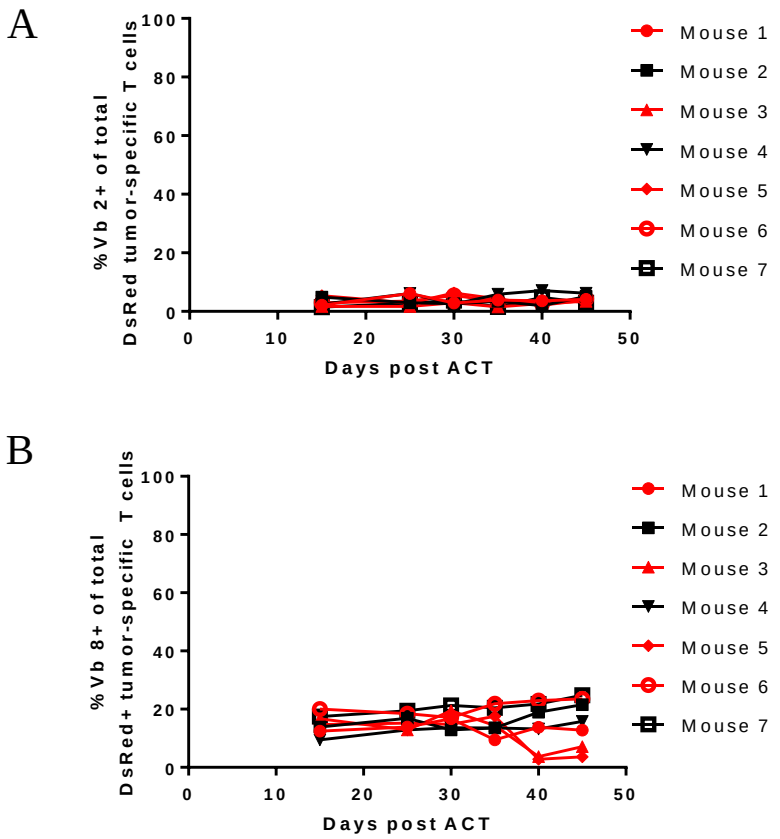

Fig. S3. Relative expansion of TCR Vβ families in peripheral blood after ACT. KR158B tumor-bearing mice received ACT using DsRed+ tumor-reactive T cells. DsRed^{+} TCR Vβ families were measured over time until humane endpoint. Neither **A**) TCR Vβ 2^{+} DsRed^{+} T cells, nor **B**) TCR Vβ 8.1/8.2^{+} DsRed^{+} T cells expanded in peripheral blood over time in treated mice.

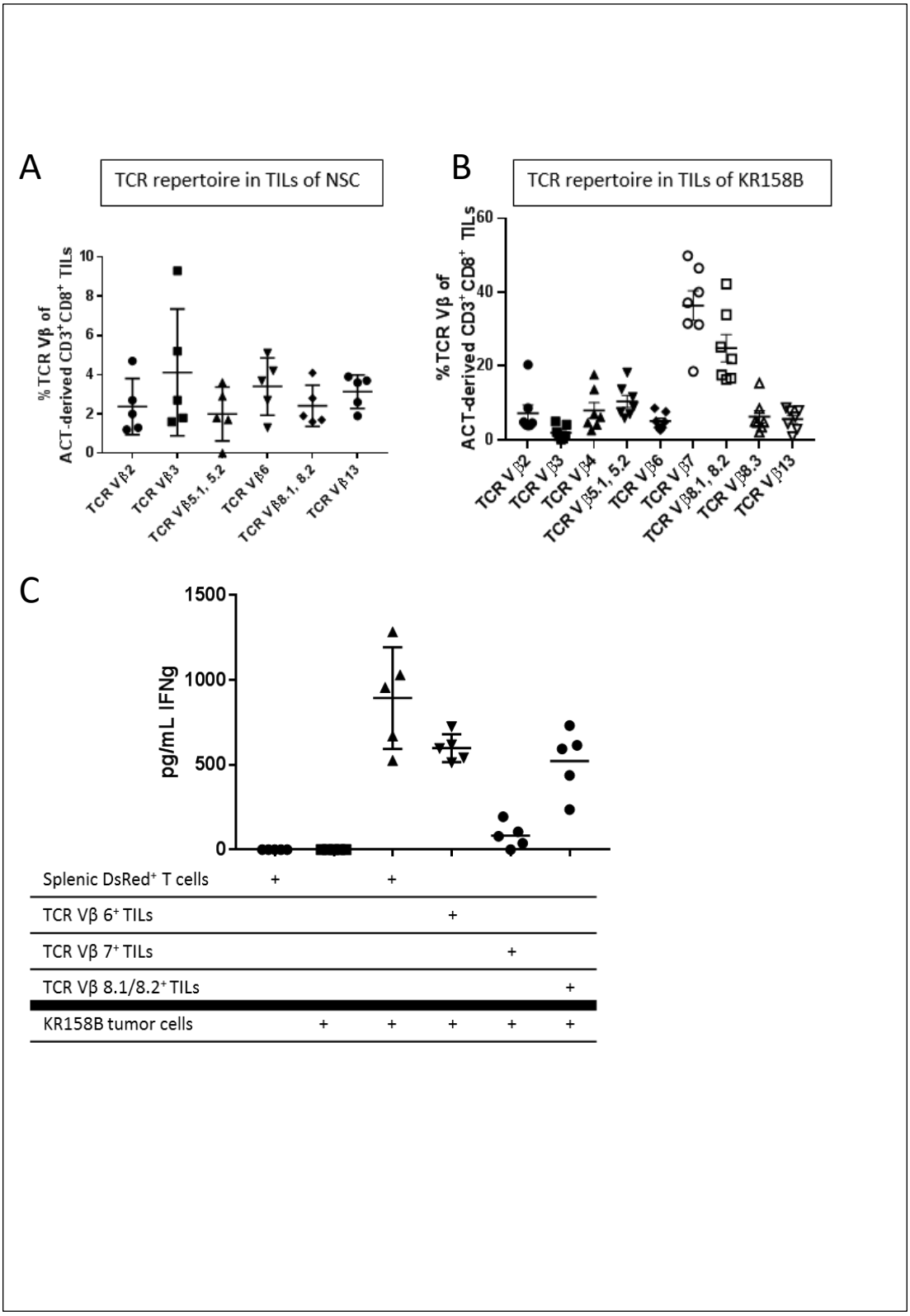

Fig. S4. Tumor-reactive T cells retain function within tumor. C57BL/6 mice received orthotopic NSC medulloblastoma or KR158B high-grade glioma then treated with adoptive cellular therapy using tumor-reactive T cells generated using DsRed+ mice. Responders to therapy had no detectable tumor by bioluminescent imaging. In non-responders to ACT, tumors were harvested at endpoint and the tumor infiltrating lymphocytes from DsRed+ T cells was spectratyped for TCR Vβ family expansion. **A**) In NSC tumor-bearing mice that did not respond to ACT, we found no hyper-expansion of any TCR V β families derived from the adoptively transferred cells. **B**) In KR158B tumor-bearing mice, we found relative expansion of TCR Vβ 7^+ and $8.1/8.2^+$ DsRed+ T lymphocytes. **C**) TCR Vβ 7^+ and $8.1/8.2^+$ $DsRed^+$ T lymphocytes were then FACS isolated from the tumor. Each cell type was then used as effectors in an *in vitro* functionality assay to determine if they maintained anti-reactivity. IFNγ secretion was measured as an indication of function. Splenic T cells from a completely naïve C57BL/6 mouse were used as a negative control. These experiments were repeated twice with n=5 tumors.

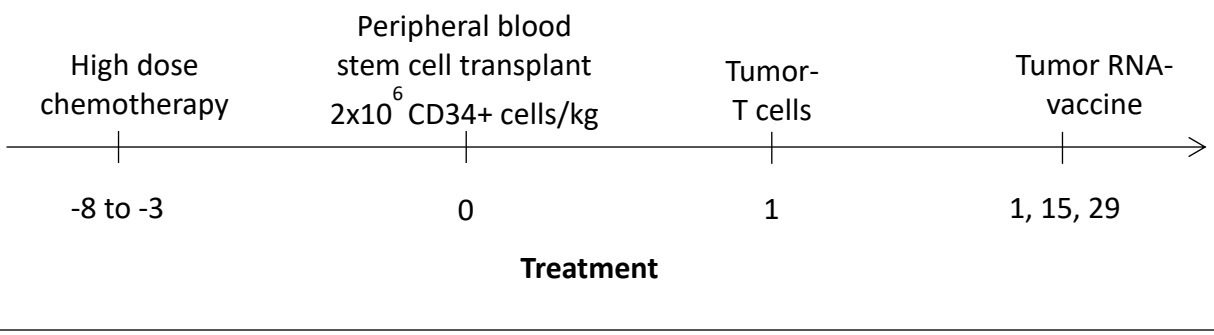

Fig. S5. ACT in recurrent medulloblastoma and PNET. Study schema of adoptive cell therapy using autologous *ex vivo* activated and expanded T cells in children with recurrent medulloblastoma and PNETs. An 11 year old child with recurrent medulloblastoma received surgery followed by high dose chemotherapy and peripheral blood stem cell transplant ($2x10^6$ CD34+ cells/kg). This was followed by adoptive transfer of autologous tumor-reactive T cells and three autologous tumor RNA-pulsed dendritic cell vaccines. Peripheral blood was drawn at 2, 4, 6, and 16 weeks post-adoptive cellular therapy.

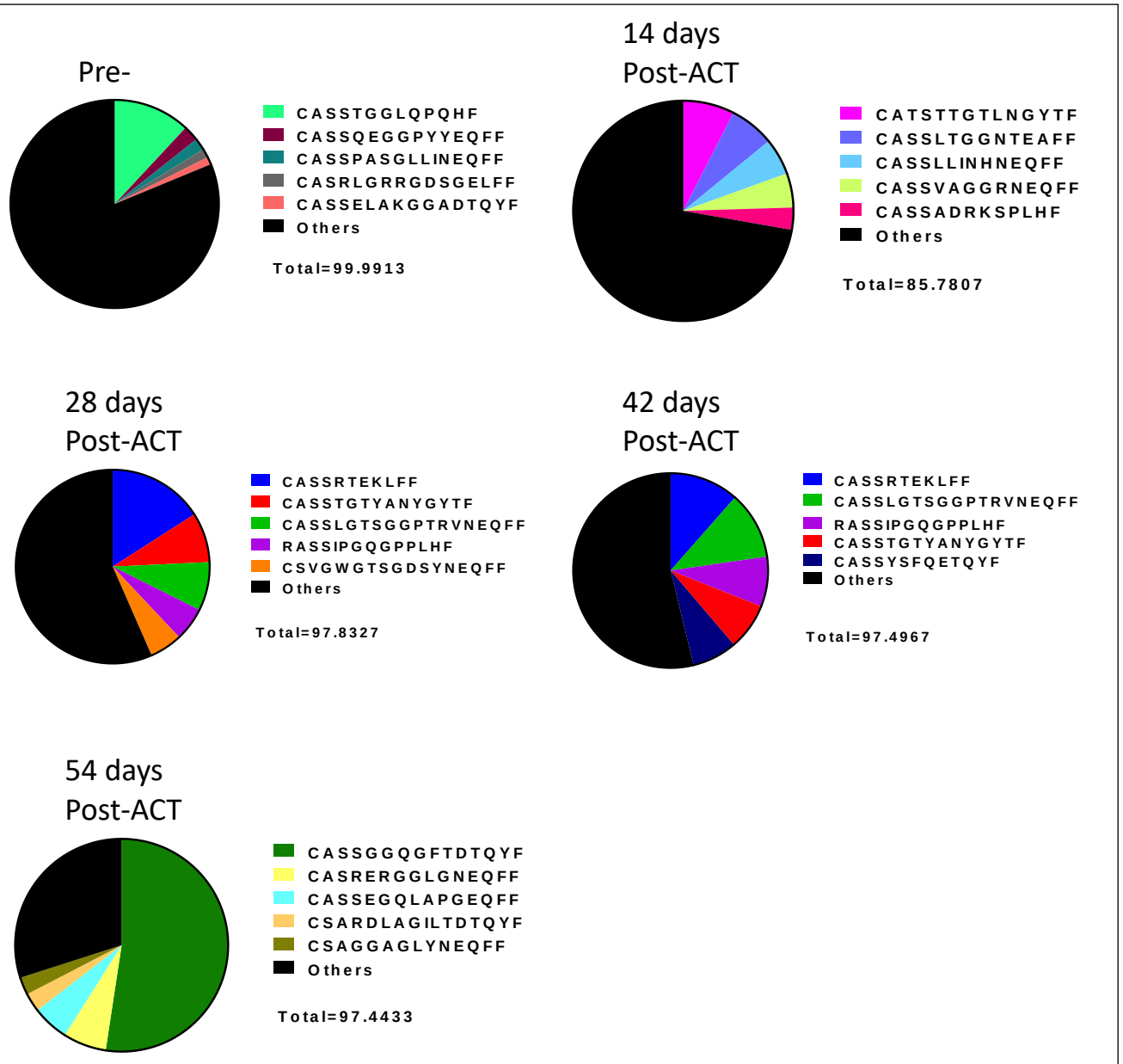

Fig. S6. Lack of single clonal expansion over time in nonresponder to ACT. A patient with recurrent medulloblastoma was treated with adoptive cell therapy (ACT) using autologous *ex vivo* activated T cells and experienced no response to therapy with <70 days to progression. TCR sequencing was conducted on the patient's tumor-reactive T cells, and on patient peripheral blood mononuclear cells (PBMC) taken at pre-ACT, then at 14, 28, 42, and 54 days post-adoptive cellular therapy. Clonal analysis revealed hyper-expansion of different TCR clones at each time point and that no hyper-expansion of a single clone over time was observed in this non-responder to ACT.

Table S1. Clonal expansion in patient PBMCs after ACT.

Clonotype Identifier	Reference code	Pre-ACT	Tumor-specific T cells	2 weeks Post-ACT	4 weeks Post-ACT	6 weeks Post-ACT	16 weeks Post-ACT	V Family	J Family
		Productive frequency of total PBMCs							
qAGGTRg.14426B03B02S07L14	LJ23	0.000077	0.042056	0.146576	5.9237	9.171714	7.787833	BV03	BJ02-07
lFNRGRn.12644B27S01B02S01L13	SC30	0.002525	0.00032	<0.00001	0.129883	7.357996	7.698551	BV27-01	BJ02-01
sASGGPh.23441B09S01B01S05L13	KD35	0.001377	<0.00001	<0.00001	0.010187	0.0317564	7.708697	BV09-01	BJ01-05
sRISGGVq.526441B05S01B02S05L13	KI11	0.002448	0.00016	0.008795	0.002547	8.921264	3.619983	BV05-01	BJ02-05

Table S2. Productive frequency of TCR Vβ families in patient MNCs before ACT, ex vivo expanded ttRNA T cells, and at follow-up after T cell infusion.

	Pre-expansion	TTRNA T cells Infusion	2 Weeks Post-T Cell Infusion	4 Weeks Post-T Cell Infusion	6 Weeks Post-T Cell Infusion	16 Weeks Post-T Cell Infusion
TCR Vb 09-01	4.4	5.15	5.41	8.15	6.57	13.4
TCR Vb 03	4.36	5.59	11.9	5.22	12.9	10.4
TCR Vb 27-01	4.35	4.62	2.78	2.11	9.3	10.2
TCR Vb 20	7.48	7.39	6.43	8.06	7.51	8.46
TCR Vb 05-01	5.05	5.4	6.66	5.88	11.5	6.42
TCR Vb 02-01	5.26	7.42	4.78	6.13	3.83	4.63
TCR Vb 06	4.13	3.19	4.02	5.26	4.78	3.93
TCR Vb 12	3.3	4.04	3.34	4.31	3.73	3.85
TCR Vb 19-01	4.48	4.04	3.7	3.89	4.44	3.03
TCR Vb 07-02	2.99	2.33	5.43	5.41	3.37	2.86
TCR Vb 11-02	2.19	1.78	1.28	2.31	2.41	2.29
TCR Vb 29-01	1.91	1.41	1.55	1.06	0.74	2.19
TCR Vb 21-01	0.535	0.653	0.827	1.82	2.31	1.96
TCR Vb 28-01	4.07	3.78	3.49	2.67	2.02	1.84
TCR Vb 18-01	3.02	5.74	3.41	2.01	1.42	1.68
TCR Vb 04-01	2.65	1.99	3.04	3.45	1.87	1.58
TCR Vb 06-05	4.7	3.59	2.13	1.86	1.58	1.55
TCR Vb 07-08	1.31	1.21	1.94	2.26	1.91	1.53
TCR Vb 07-09	4.55	5.38	7.29	2.15	1.32	1.53
TCR Vb 05-05	0.696	0.378	1.53	1.61	1.67	1.46
TCR Vb 05-04	2.16	1.24	2.04	2.01	1.84	1.33
TCR Vb 04-02	1.69	2.27	2.88	2.28	1.4	1.21
TCR Vb 07-03	2.36	1.36	1.44	1.18	1.02	1.21
TCR Vb 14-01	0.898	0.64	0.704	0.736	0.627	1.2
TCR Vb 06-01	2.73	1.53	1.57	1.65	1.12	1.1
TCR Vb 10-03	1.76	1.95	1.34	1.19	1.04	1.08
TCR Vb 04-03	1.63	0.888	3.42	1.28	1.17	1.04
TCR Vb 06-06	1.67	1.28	1.08	0.846	0.704	0.848
TCR Vb 12-05	0.363	0.31	0.777	0.907	0.593	0.747
TCR Vb 05-08	0.571	0.404	0.196	0.308	1.04	0.741
TCR Vb 05-06	1.89	2.18	1.28	0.973	0.557	0.595
TCR Vb 24	2	1.63	1.25	0.95	0.493	0.584
TCR Vb 15-01	1.41	0.963	1.42	1.14	0.674	0.507
TCR Vb 07-06	0.984	0.665	0.522	0.354	0.304	0.361
TCR Vb 11-03	0.716	0.584	0.369	0.494	0.277	0.333
TCR Vb 11-01	0.449	0.376	0.167	0.191	0.126	0.28
TCR Vb 25-01	0.86	0.605	0.765	0.298	0.221	0.262
TCR Vb 06-04	0.603	1.06	0.276	0.367	0.275	0.258
TCR Vb 20-01	0.558	0.33	0.49	0.252	0.16	0.256
TCR Vb 10-02	0.423	0.426	0.226	0.132	0.09	0.207
TCR Vb 10-01	0.55	0.462	0.22	0.15	0.108	0.181
TCR Vb 07-07	0.378	0.504	0.425	0.285	0.177	0.17
TCR Vb 13-01	0.808	2.22	0.308	0.288	0.157	0.168
TCR Vb 23-01	0.226	0.224	0.237	0.115	0.0622	0.0933
TCR Vb 05-03	0.102	0.0803	0.173	0.11	0.134	0.0609
TCRVb 25	0.0204	0.0064	0.135	0.132	0.113	0.0568
TCR Vb 30-01	0.101	0.292	0.085	0.0891	0.0655	0.0528
TCR Vb 01-01	0.0692	0.0457	0.114	0.0586	0.0671	0.0467
TCR Vb 16-01	0.102	0.0587	0.0762	0.0713	0.0213	0.0386
TCR Vb 12-02	0.0754	0.04	0.0293	0.0255	0.018	0.0345
TCR Vb 06-09	0.0218	0.0134	0.0469	0.0331	0.0278	0.0325
TCR Vb 05	0.0568	0.0225	0.044	0.028	0.0295	0.0284
TCR Vb 05-07	0.0158	0.00608	0.0147	0.0229	0.0295	0.0223
TCR Vb 07-05	0.0223	0.0382	0.0117	0.0102	0.00491	0.0203
TCR Vb 12-01	0.0337	0.0262	0.0469	0.0102	0.0131	0.0142
TCR Vb 06-07	0.0418	0.0353	0.0528	0.028	0.00818	0.0101
TCR Vb 06-08	0.0162	0.0114	0.0117	0.00509	0.00327	0.0101
TCR Vb 07-01	0.0235	0.000612	0.00016	0.0127	0.00982	0.00812
TCR Vb 07-04	0.0207	0.0283	0.0235	0.0153	0.00818	0.00812