Supplementary Materials:

Supplementary Figure 1

Supplementary Figure 2

Supplementary Figure 3

Supplementary Figure 4

Supplementary Table 1

Supplementary Fig. 1: Manufacturing of CD19 CAR-T cells. A) The strategy for manufacturing of CD19 CAR-T cells using the CliniMACs is shown. The leukapheresis product is split into two aliquots. $CD4^+$ cells are selected from one of the aliquots using the CD4 CliniMACs reagent. $CD8^+$ T_{CM} cells are enriched from the second aliquot by a two-step method involving depletion of $CD4^+$, $CD14^+$ and $CD45RA^+$ cells followed by selection of $CD62L^+$ cells from the $CD4^+/CD14^+/CD45RA^+$-depleted fraction. The selected $CD4^+$ cells and $CD4^-/CD14^-/CD45RA^-/CD62L^+$ cells are separately stimulated with anti-CD3/CD28 paramagnetic beads, transduced with a lentiviral vector encoding the CD19 CAR transgene and EGFRt and expanded in vitro for 15 - 20 days before formulation in a $CD4^+/EGFRt^+$: $CD8^+/EGFRt^+$ ratio of 1:1 for infusion. In some patients with severe lymphopenia or circulating blasts, $CD8^+$ cells were selected rather than $CD8^+$ T_{CM} cells (not shown). B) Flow cytometry plots from a representative two-step $CD8^+$ T_{CM} enrichment are shown, illustrating the isolation of the $CD4^-/CD14^-/CD45RA^-/CD62L^+$ fraction that contains T_{CM}-enriched $CD3^+$ cells and $CD3^-$ cells. C) The $CD8^+$ T_{CM} enriched cell product contains $CD3^-$ myeloid cells ($SS^{hi}/CD13^+/CD15^+/CD16^{hi}$; top) and basophils ($SS^{lo}/CD13^+/CD123^+$; bottom). D) Representative flow cytometry plots

show enrichment in EGFRt$^+$ CAR expression in transduced CD4$^+$ (top) and CD8$^+$ (bottom) T cells between LCL stimulation and product release. E) Data from all B-ALL patients (n = 27) showing enrichment of EGFRt$^+$ cells in transduced CD4$^+$ (left) and CD8$^+$ (right) T cells between LCL stimulation (pre-LCL) and product release.

Supplementary Fig. 2: Immunophenotype of infused EGFRt$^+$ CAR-T cells. A) Immunophenotype of CD4$^+$/EGFRt$^+$ CAR-T cells. B) Immunophenotype of CD8$^+$/EGFRt$^+$ CAR-T cells manufactured from bulk CD8$^+$ T cells or CD8$^+$ T_{CM} cells.

Supplementary Fig. 3: Resolution of bulky extramedullary disease after therapy with a low dose of CAR-T cells manufactured from a defined T cell subset composition. PET-CT scans before and after CAR-T cell therapy are shown from 2 patients with extramedullary B-ALL who achieved CR after lymphodepletion chemotherapy and infusion of CAR-T cells at DL 1 (2 x 10^5 CAR-T cells/kg).

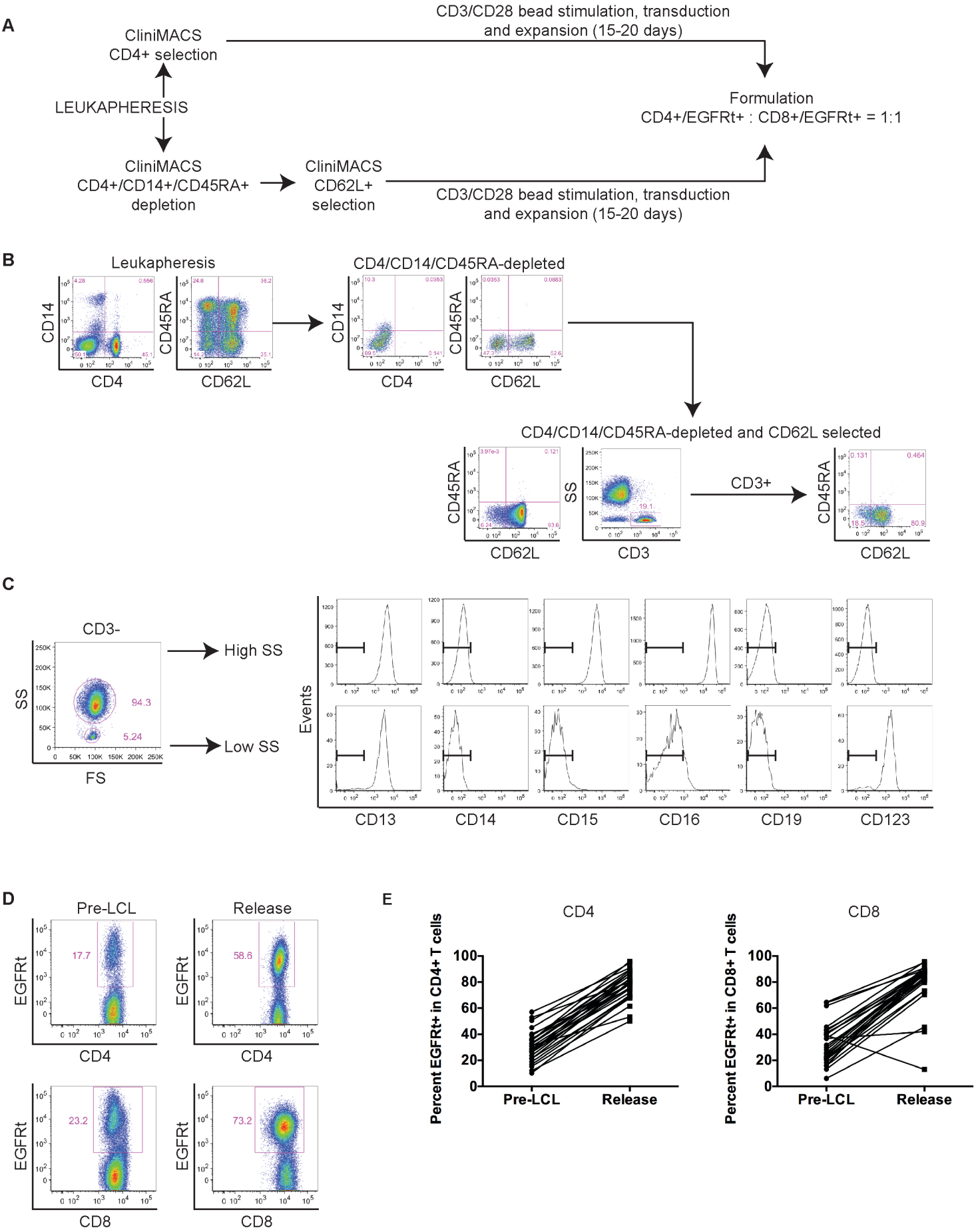
Supplementary Fig. 4: Immune responses directed against the CAR transgene products. A) Pre- and post-infusion PBMC were stimulated twice at weekly intervals with CD19 CAR-transduced autologous T cells. The capacity of the pre-infusion and post-infusion cultured to lyse CD19 CAR-transduced autologous T cells (black) and non-transduced autologous T cells (white) was evaluated by ^{51}chromium release assay. One representative patient of 5 is shown. B-C) The post-infusion T cell line from a patient that exhibited specific lysis of autologous CAR-T cells was stimulated with pools of overlapping peptides from the CAR construct, and peptide pools that induced IFN-γ

secretion higher than that induced by T cells alone in an ELISpot assay were identified. Peptides 51, 55, 59 and 60 are located within the murine scFv. D) Peptides in the murine scFv that are predicted with a percentile rank < 5% (IEDB Analysis Resource, http://tools.immuneepitope.org/mhci) to bind HLA molecules expressed by the patient's CAR-T cells are indicated. Lower percentile scores indicate stronger binding.

Supplementary Table 1:

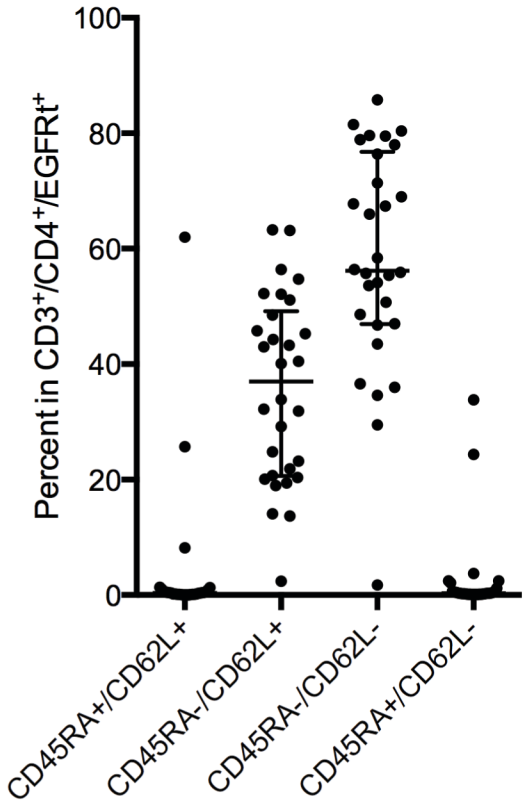
Patients treated with CAR-T cells at DL 1 or 2 who received dexamethasone with or without tocilizumab for CRS and neurotoxicity.

SUPPLEMENTARY FIGURE 1

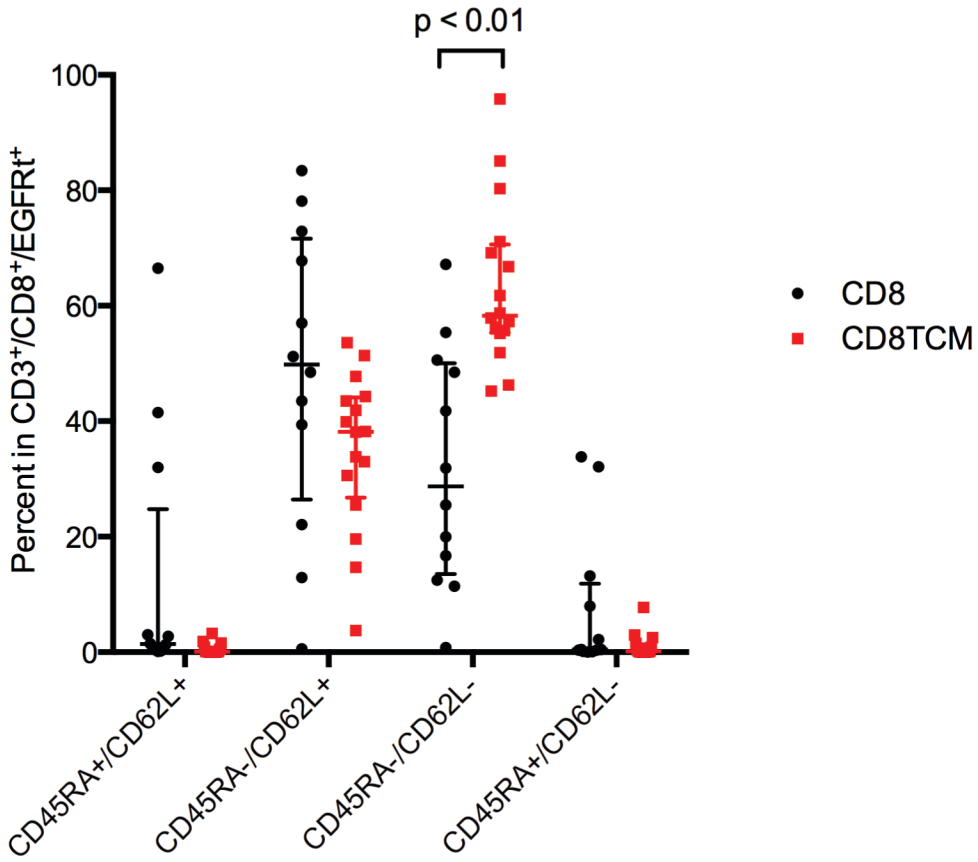


SUPPLEMENTARY FIGURE 2

A



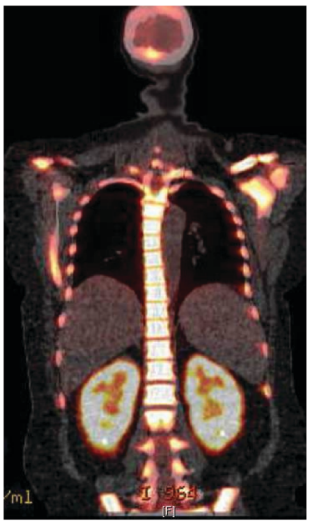
B



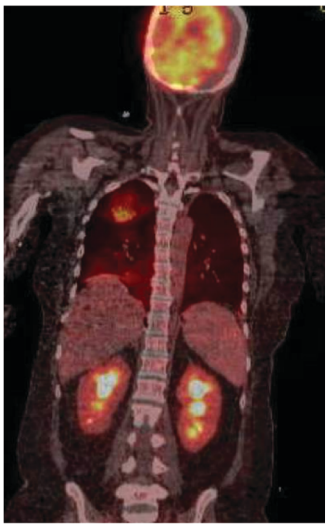
SUPPLEMENTARY FIGURE 3

Patient 1:
Bilateral renal and bone infiltration

Day -6 before 2x10^5 CAR-T cells/kg

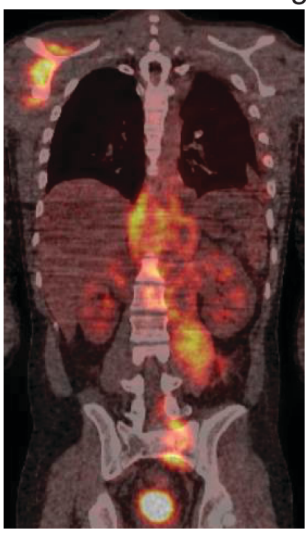


Day 37 after 2x10^5 CAR-T cells/kg

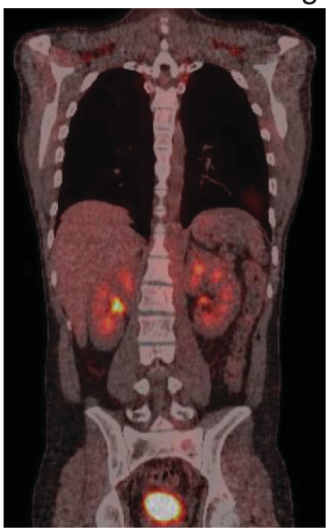


Patient 2:
Retroperitoneal, para-aortic, and right iliac lymphadenopathy and right scapular infiltration

Day -6 before 2x10^5 CAR-T cells/kg

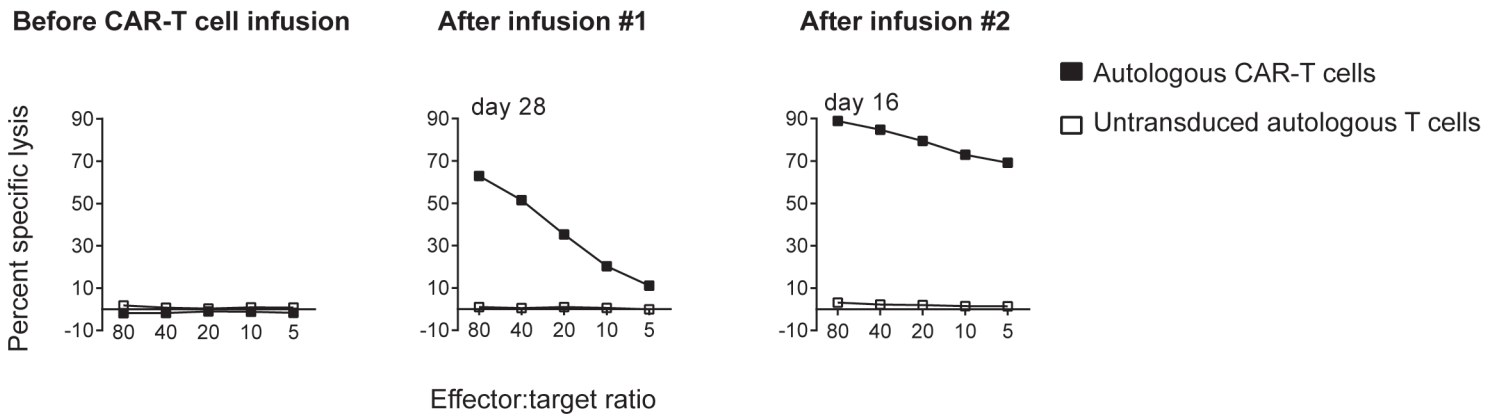


Day 31 after 2x10^5 CAR-T cells/kg



SUPPLEMENTARY FIGURE 4

A



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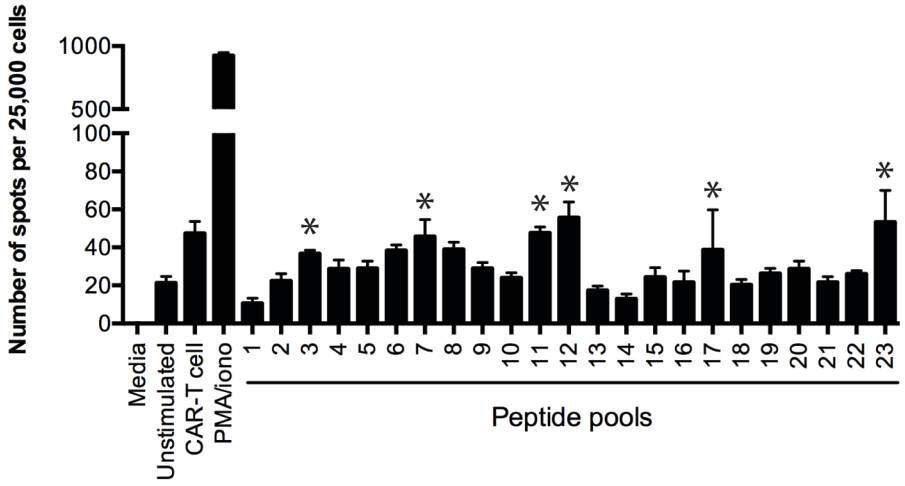

C

Peptide pools

	1	2	3	4	5	6	7	8	9	10	11	12
13	1	2	3	4	5	6	7	8	9	10	11	12
14	13	14	15	16	17	18	19	20	21	22	23	24
15	25	26	27	28	29	30	31	32	33	34	35	36
16	37	38	39	40	41	42	43	44	45	46	47	48
17	49	50	51	52	53	54	55	56	57	58	59	60
18	61	62	63	64	65	66	67	68	69	70	71	72
19	73	74	75	76	77	78	79	80	81	82	83	84
20	85	86	87	88	89	90	91	92	93	94	95	96
21	97	98	99	100	101	102	103	104	105	106	107	108
22	109	110	111	112	113	114	115	116	117	118	119	120
23	121	122	123	124	125							
24												

D

HLA allele	Sequence	Percentile
HLA-A*3002	IYYCAKHYYY	0.15
	AIYYCAKHYY	0.3
	IYYCAKHYY	0.55
	AIYYCAKHY	1
	YYCAKHYYY	1.3
	QTDDTAIYY	2.2
	TAIYYCAKHY	3.3
HLA-A*3010	AIYYCAKHYY	0.3
	AIYYCAKHY	0.6
	IYYCAKHYY	0.8
	IYYCAKHYYY	0.8
	YYCAKHYYY	1.1
	TAIYYCAKHY	1.9
	QTDDTAIYY	2.7
	TTYYNSALK	4.8

SUPPLEMENTARY TABLE 1

Patient number	Fever > 38.3 C (days after CAR-T cell infusion)	Pressor support (days after CAR-T cell infusion)	Intubated (days after CAR-T cell infusion)	Neurotoxicity		Therapy			
				Duration (days after CAR-T cell infusion)	Maximum grade*	ICU care	Tocilizumab (4-8 mg/kg IV; days after CAR-T cell infusion)	Dexamethasone (10 mg IV; days after CAR-T cell infusion)	
								Days	Total doses
7	9-11	11-15	NA	11-16	3	Yes	11	12,13	2
8	1-9,11-16,18,21	NA	NA	7-29	3	No	NA	7,9	2
12	3-7	6-7	NA	6-13	3	Yes	7	7	1
15	0-6	NA	NA	1-10	4	Yes	6	4,6,7,9	4
16	1-7	NA	NA	8-11	4	Yes	NA	8	2
17	0-6	5-8, 10-13	10-15	10-122¶¶	5	Yes	6	6,10-20	21
19	1-5	5	6-17	5-27	4	Yes	5,7	5-16	28
26	1-5	NA	NA	6-12	3	No	NA	6,7	3
27	4-6	NA	NA	5-8	3	No	6	6,7	3
28	5-7	NA	NA	9-12	3	No	7	12,13	4

*NCI CTCAEv4.03

¶¶ Died on day 122 in CR with persistent neurotoxicity