

## Supplementary Materials for

### **Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia**

David L. Porter,\* Wei-Ting Hwang, Noelle V. Frey, Simon F. Lacey, Pamela A. Shaw, Alison W. Loren, Adam Bagg, Katherine T. Marcucci, Angela Shen, Vanessa Gonzalez, David Ambrose, Stephan A. Grupp, Anne Chew, Zhaohui Zheng, Michael C. Milone, Bruce L. Levine, Jan J. Melenhorst, Carl H. June\*

\*Corresponding author. E-mail: david.porter@uphs.upenn.edu (D.L.P.); cjune@exchange.upenn.edu (C.H.J.)

Published 2 September 2015, *Sci. Transl. Med.* **7**, 303ra139 (2015)  
DOI: 10.1126/scitranslmed.aac5415

#### **This PDF file includes:**

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#### **Other Supplementary Material for this manuscript includes the following:**

(available at

[www.sciencetranslationalmedicine.org/cgi/content/full/7/303/303ra139/DC1](http://www.sciencetranslationalmedicine.org/cgi/content/full/7/303/303ra139/DC1))

- Table S6. Source data for Fig. 1 (Excel).
- Table S7. Source data for Fig. 2 (Excel).
- Table S8. Source data for Fig. 3 (Excel).
- Table S9. Source data for Fig. 4A (Excel).
- Table S10. Source data for Fig. 4C (Excel).

Table S11. Source data for Fig. 5 (Excel).

Supplemental Figures

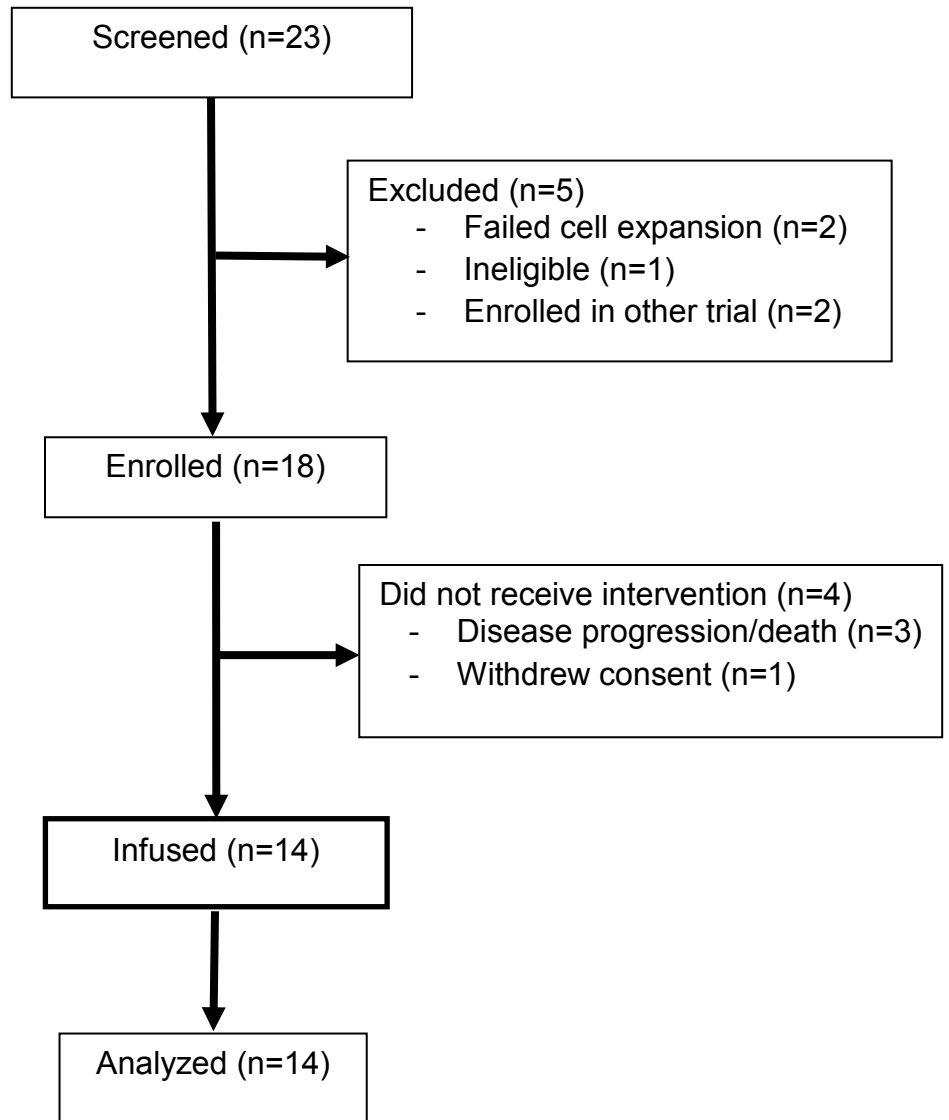
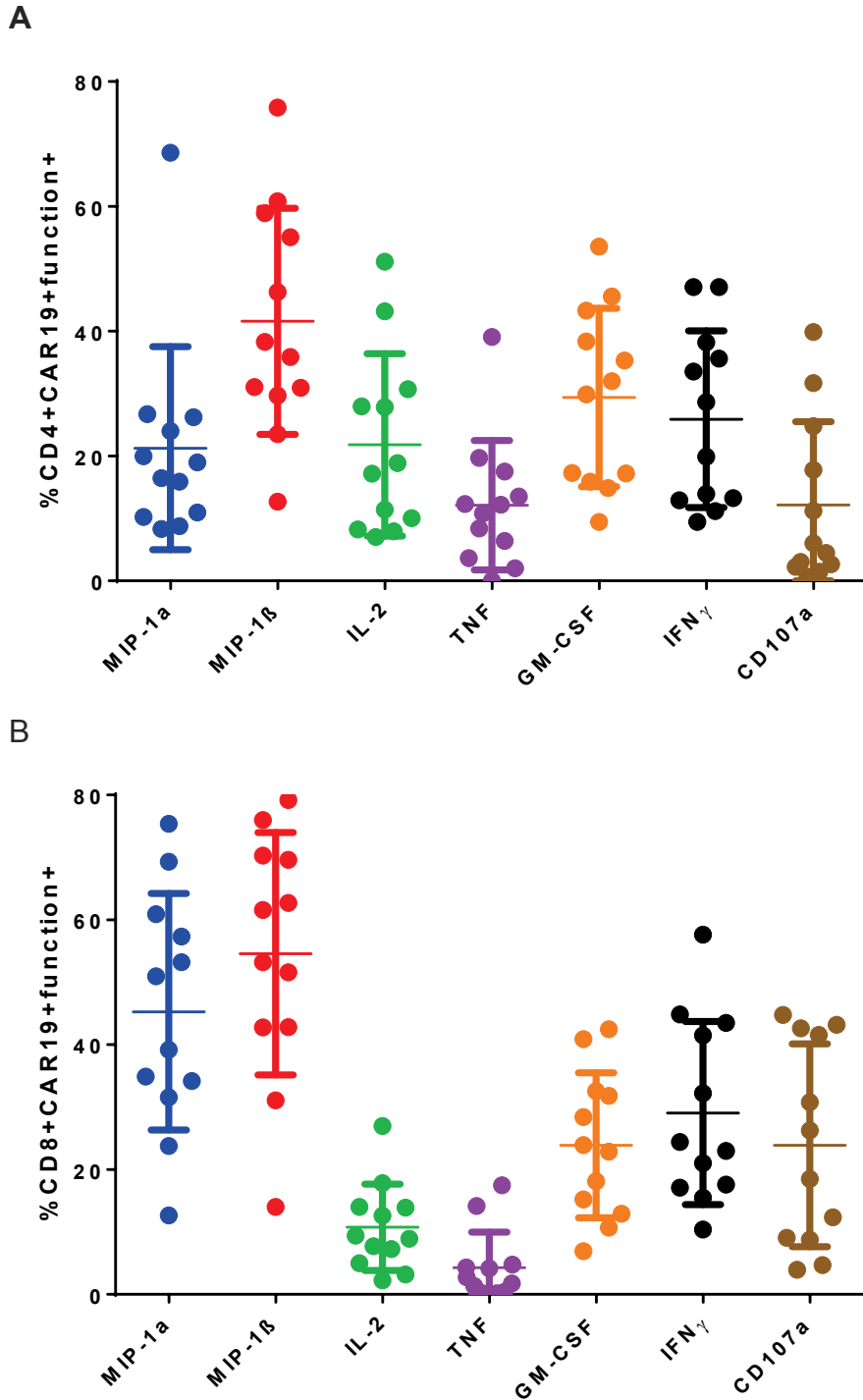
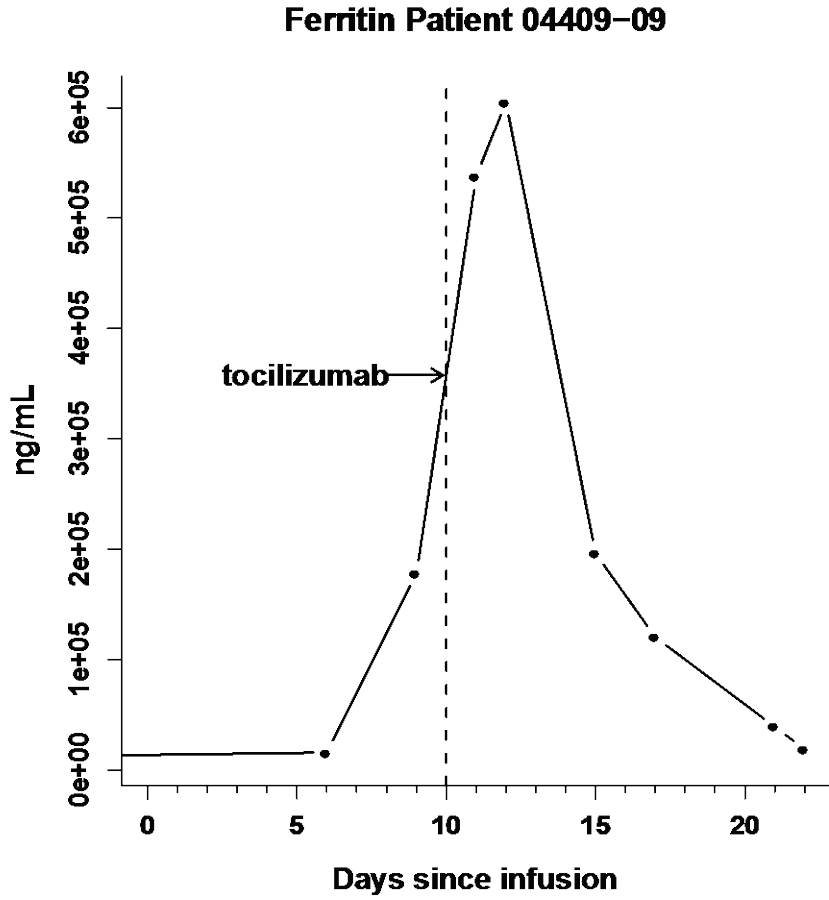


Figure S1: Consort diagram.

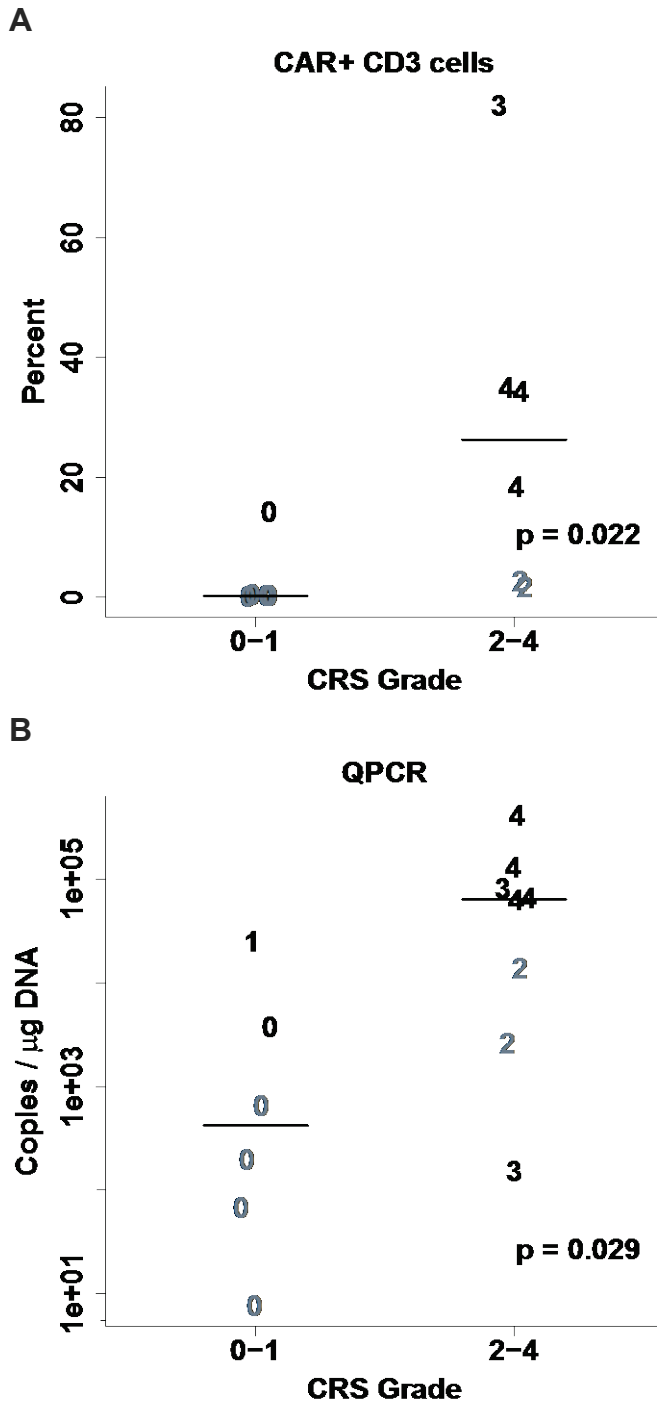


**Figure S2: CTL019 cells are polyfunctional.**

Manufactured CTL019 cells from 12 subjects (cells were not available for subjects 02 and 03) were stimulated for 6 hours with CD19-expressing target cells in the presence of cytokine secretion blockers plus fluorescently conjugated anti-CD107a antibody after which the cells were harvested, stained, and analyzed for intracellular cytokine expression. Shown here are the frequencies of CD4+ (A) or CD8+ (B) CAR19+ T cells expressing the indicated cytokines or degranulating upon stimulation.



**Figure S3: Example of serum ferritin response to tocilizumab in a patient with CRS. Tocilizumab was administered on day 10 after CTL019 infusion.**



**Figure S4: Association of peak CTL019 expansion with CRS and the response observed within the first 3 months after infusion.** Peak CTL019 expansion measured by flow cytometry based on 11 subjects (A) and by qPCR based on 14 subjects (B). The grade of CRS is indicated numerically by symbol; gray symbols represent non-responders, black symbols are those who achieved a complete or partial remission. Flow cytometry detection was not available for subjects 01, 02, and 03 because an antibody to detect CTL019 cells was not available at the time of their treatment. Wilcoxon rank-sum p-values are provided.

**Table S1: Cell dose and product characteristics (N = 14).**

Total cells infused	
<b>Mean (SD)</b>	1.7 x 10 <sup>9</sup> (1.7 x 10 <sup>9</sup> )
<b>Median (range)</b>	7.5 x 10 <sup>8</sup> (1.7 x 10 <sup>8</sup> 5.0 x 10 <sup>9</sup> )
Total CTL019 infused	
<b>Mean (SD)</b>	2.8 x 10 <sup>8</sup> (3.1 x 10 <sup>8</sup> )
<b>Median (range)</b>	1.6 x 10 <sup>8</sup> (1.4 x 10 <sup>7</sup> -1.1 x 10 <sup>9</sup> )
Transduction efficiency, %	
<b>Mean (SD)</b>	19.1 (10.1)
<b>Median (range)</b>	20.1 (4.7-39.2)
Post harvest viability,%	
<b>Mean (SD)</b>	95.2 (3.6)
<b>Median (range)</b>	96.1 (85.7-98.9)
CD3+CD45+,%	
<b>Mean (SD)</b>	98.1 (2.9)
<b>Median (range)</b>	99.1 (88.9-99.9)
CD3+CD4+CD45+,%	
<b>Mean (SD)</b>	82.0 (8.6)
<b>Median (range)</b>	81.3 (67.7-97.0)
CD3+CD8+CD45+,%	
<b>Mean (SD)</b>	19.2 (8.6)
<b>Median (range)</b>	20.0 (4.6-33.9)
Vector DNA sequence, copy/cell	
<b>Mean (SD)</b>	0.31 (0.41)
<b>Median (range)</b>	0.17 (0.03-1.46)
CD19 expression*	
<b>Mean (SD)</b>	47405 (30415)
<b>Median (range)</b>	35180 (11604-105339)

**Table S2: Tumor burden assessed by IgH deep sequencing analysis of blood and bone marrow of all study subjects.**

ID	Sample type	Timepoint	Total productive IgH reads	Unique productive IgH reads	Tumor clone reads	CLL clone % of total	Cell equivalents sequenced
1	PB	baseline	408,579	48	407,592	99.76%	
		mo 6	285,305	7362	0	0.00%	
		mo 12	41	12	0	0.00%	
		yr 3	174	6	0	0.00%	298,667
		yr 3.5	123	8	0	0.00%	350,171
	BM	d 28	179	3	0	0.00%	408,838
		mo 6	202,535	4451	0	0.00%	
		yr 1	18,506	231	0	0.00%	
		yr 2	88	2	0	0.00%	
2	PB	baseline	1,385,340	4544	1,285,862	92.82%	
		mo 32	88	8	0	0.00%	317,714
		yr 3	160	8	0	0.00%	346,057
		yr 4	212	10	0	0.00%	308,419
	BM	yr 1	5	2	0	0.00%	
		yr 2	601	25	0	0.00%	
3	BM	d -1	73,094	15	72,773	99.56%	359,924
		d 28	38,012	17	37,793	99.42%	372,114
5	PB	d -28	644,936	363	640,284	99.28%	219,276
		mo 2	83,946	27	83,083	98.97%	264,838
		mo 4	803,040	1411	775,298	96.55%	254,629
		mo 9	5,427,049	1328	5,409,125	99.67%	652,343
	BM	baseline	144,603	275	142,345	98.44%	
		d 28	3,248	35	2,098	64.59%	225,219
		mo 3	230,318	349	222,934	96.79%	
		mo 6	359,965	844	348,739	96.88%	
6	PB	d 14	1,822,321	347	1,817,320	99.73%	285,410
	BM	d 28	1,701,147	67	1,699,900	99.93%	126,933
7	PB	d -28	236,746	73	235,738	99.57%	333,105
		d 14	318,115	74	317,746	99.88%	565,333
		d 28	330,137	423	326,674	98.95%	325,333
9	PB	d 1	1,090,418	21	1,089,770	99.94%	566,552



ID	Sample type	Timepoint	Total productive IgH reads	Unique productive IgH reads	Tumor clone reads	CLL clone % of total	Cell equivalents sequenced
		d 6	843,460	26	842,887	99.93%	314,362
		d 10	58,061	9	57,989	99.88%	381,714
	BM	d -1	315,752	26	315,418	99.89%	
		d 28	162	7	0	0.00%	
		mo 3	114	8	0	0.00%	
		mo 6	175	10	0	0.00%	537,295
		yr 1	458	13	0	0.00%	368,914
10	PB	d -28	1,955,943	289	1,949,846	99.69%	292,724
		d 14	1,866,354	239	1,861,305	99.73%	462,324
		d 28	412,286	34	405,605	98.38%	403,657
		mo 2	1,368,305	57	1,347,305	98.47%	527,238
		d -1	1,037,111	38	1,020,951	98.44%	367,390
		d 14	1,751,246	51	1,724,159	98.45%	651,733
		d 51	145,019	24	143,816	99.17%	360,381
		mo 3	59	4	0	0.00%	393,905
		mo 6	119	9	0	0.00%	383,238
		mo 9	299	10	0	0.00%	350,324
		d 450	154	8	0	0.00%	590,629
		d 540	143	7	0	0.00%	518,248
	BM	d 28	2,067,856	88	2,066,136	99.92%	459,124
		d 360	93	7	0	0.00%	354,438
12	PB	d 1	3,571,895	802	2,942,544	82.38%	415,543
		mo 4	1,370	10	945	68.98%	605,410
		mo 6	2,852	18	1,534	53.79%	
	BM	d -1	521,981	49	422,361	80.92%	
		mo 1	280,316	32	187,560	66.91%	
		mo 2	10,395	8	7,522	72.36%	253,867
		mo 3	17,076	22	12,342	72.28%	271,086
mo 6	66,236	23	49,689	75.02%	661,486		
14	PB	d -1	9,696,164	248	9,688,506	99.92%	1,269,790
		d 14	10,213,223	265	10,204,841	99.92%	1,269,790
	BM	d -7	7,585,592	215	7,579,747	99.92%	1,269,790
		d 28	30,410,311	83	3,040,153	10.00%	547,657
17	PB	d -1	954,450	63	954,332	99.99%	794,971
		mo 2	55,404	34	54,835	98.97%	460,495

ID	Sample type	Timepoint	Total productive IgH reads	Unique productive IgH reads	Tumor clone reads	CLL clone % of total	Cell equivalents sequenced
	BM	mo 6	456,879	58	456,291	99.87%	634,819
		d 14	84,346	20	84,215	99.84%	249,752
		d 28	427,129	151	425,910	99.71%	977,981
		mo 3	422,140	291	418,999	99.26%	488,686
18	PB	d -1	1,398,315	287	1,395,144	99.77%	1,201,067
		mo 2	4,712,625	660	4,698,243	99.69%	970,210
	BM	d 28	4,593,644	685	4,582,005	99.75%	1,269,790
22	PB	d -1	1,883,771	60	1,850,747	98.25%	561,524
		mo 2	112	7	0	0.00%	609,676
		mo 4	46	4	0	0.00%	402,590
		mo 5	92	7	3	3.26%	400,000
	BM	d -1	316,980	49	312,133	98.47%	
		d 28	513	19	41	7.99%	673,219
		mo 3	1,984	58	220	11.09%	917,943
		mo 6	6,745	71	3,343	49.56%	756,114
		mo 9	628,128	25	620,398	98.77%	490,210
25	PB	d -1	3,065,971	60	3,064,760	99.96%	624,914
		d 7	3,318,243	61	3,316,886	99.96%	912,610
		d 21	37,751	19	37,517	99.38%	735,848
		mo 2	732,672	89	730,496	99.70%	862,476
	BM	d 28	554,950	52	553,611	99.76%	408,533

Abbreviations: BM, bone marrow; d, day; mo, month; PB, peripheral blood; wk, week; yr, year.

**Table S3: Association of pretreatment characteristics with response.**

Characteristic	Responders N=8	Non-Responders N=6	P-value†
Age at infusion, year			0.96
Mean (SD)	67 (9.7)	67 (7.3)	
Median (range)	66 (59–78)	67 (51–78)	
Number prior therapies			0.55
Mean (SD)	4.8 (2.3)	5.5 (2.7)	
Median (range)	5.5 (1–9)	5.5 (1–8)	
del 17p	8 (50%)	2 (33%)	0.63
IgHV unmutated**	4 (57%)	5 (83%)	1.00
Rai stage			1.00
Rai 1	3 (38%)	2 (33%)	
Rai 4	5 (62%)	4 (67%)	
Binet stage			0.75
Binet A	0	1 (16%)	
Binet B	3 (38%)	1 (16%)	
Binet C	5 (62%)	4 (67%)	
Lymphocytes in BM, %*			0.17
Mean (SD)	82 (23.6)	77 (12.0)	
Median (range)	90 (40–95)	80 (60–90)	
Total CTL019 infused (x10 <sup>8</sup> )			0.25
Mean (SD)	3.8(3.7)	1.5(1.1)	
Median (range)	2.8 (0.1–11.3)	1.3 (0.2–2.8)	
CD19 expression***			0.87
Mean (SD)	47904(31870)	46405(31938)	
Median (range)	41779 (11604–105339)	33152 (25657–93659)	

†: Based on Fisher's exact test for categorical variables, Wilcoxon rank-sum test for continuous variables except for age at infusion which a t-test was used. \* Missing value for three responders and one non-responder. \*\*: Missing value for one responder. \*\*\*: Antibody binding capacity, approximately equal to the number of CD19 molecules on the surface of the cells. Missing value for two no CRS subjects.

**Table S4A: Penn Grading System for CTL019-associated CRS.**

Grade 1	Mild reaction: Treated with supportive care such as anti-pyretics, anti-emetics
Grade 2	Moderate: Requiring IV therapies or parenteral nutrition; some signs of organ dysfunction (i.e. gr 2 Cr or gr 3 LFTs) related to CRS and not attributable to any other condition. Hospitalization for management of CRS related symptoms including fevers with associated neutropenia.
Grade 3	More severe reaction: <u>Hospitalization required for management of symptoms related to organ dysfunction</u> including gr 4 LFTs or gr 3 Cr related to CRS and not attributable to any other conditions; excludes management of fever or myalgias. Includes <u>hypotension treated with intravenous fluids*</u> or <u>low-dose pressors</u> , <u>coagulopathy requiring FFP or cryoprecipitate</u> , and <u>hypoxia requiring supplemental O2 (nasal cannula oxygen, high flow O2, CPAP or BiPAP)</u> . Patients admitted for management of suspected infection due to fevers and/or neutropenia may have grade 2 CRS.
Grade 4	Life-threatening complications such as hypotension requiring “high dose pressors” **, hypoxia requiring mechanical ventilation.
Grade 5	Death

\*Defined as: multiple fluid boluses for blood pressure support

\*\* See Supplemental Table 1b for definition of “High dose” vasopressors

**Supplemental Table 4B: Definition of high-dose vasopressors.**

Vasopressor	Dose for ≥ 3 hours
Norepinephrine monotherapy	≥ 0.2 mcg/kg/min
Dopamine monotherapy	≥ 10 mcg/kg/min
Phenylephrine monotherapy	≥ 200mcg/min
Epinephrine monotherapy	≥ 0.1 mcg/kg/min
If on vasopressin	High-dose if vaso + NE equivalent of ≥ 0.1 mcg/kg/min (using VASST formula)
If on combination vasopressors (not vasopressin)	Norepinephrine equivalent of ≥ 20 mcg/min (using VASST formula*)

**\*VASST Trial Vasopressor Equivalent Equation:**

Norepinephrine equivalent dose = [norepinephrine (mcg/min)] + [dopamine (mcg/kg/min) ÷ 2] + [epinephrine (mcg/min)] + [phenylephrine (mcg/min) ÷ 10] (adapted from <sup>28</sup>)

**Table S5: Association of pretreatment characteristics with CRS (N = 14).**

Characteristic	CRS N=9	No CRS N=5	P-value †
Age at infusion, year			0.18
Mean (SD)	69 (9.8)	63 (7.3)	
Median (range)	66 (58–78)	64 (51–70)	
Number prior therapies			0.89
Mean (SD)	5 (2.6)	5.8 (3.3)	
Median (range)	6 (1–9)	5 (1–8)	
del 17p	5 (56%)	1 (20%)	0.30
IgHV unmutated	3 (33% <sup>**</sup> )	1 (20%)	1.00
Rai stage			1.00
Rai 1	3 (33%)	2 (40%)	
Rai 4	6 (67%)	3 (60%)	
Binet stage			1.00
Binet A	1	0	
Binet B	2 (23%)	2 (40%)	
Binet C	6 (67%)	3 (60%)	
Lymphocytes in BM, % <sup>*</sup>			0.56
Mean (SD)	79 (21.1)	80 (10)	
Median (range)	90 (40–95)	80 (70–90)	
Total CTL019 infused (x10 <sup>8</sup> )			0.55
Mean (SD)	3.4(3.1)	1.8(1.5)	
Median (range)	1.6 (0.1–11.3)	1.6 (0.17–3.9)	
CD19 expression <sup>***</sup>			0.41
Mean (SD)	44982(30964)	54673(33874)	
Median (range)	28696 (11604–105339)	37919 (32441–93659)	

†: Based on Fisher's exact test for categorical variables, Wilcoxon rank-sum test for continuous variables except for age at infusion which a t-test was used. \*: Missing value for two CRS subjects and 2 no CRS subjects. \*\*: Missing value for one CRS subject. \*\*\*: Antibody binding capacity, roughly equal to the number of CD19 molecules on the surface of the cells. Missing value for two no CRS subjects.