SUPPLEMENTARY MATERIALS

Supplementary Methods

Reagents and protocols for flow cytometry: Antibodies for CAR T cell detection panels were anti-CD45 V450 (clone HI30), anti-CD14 V500 (clone M5E2), anti-CD56 Ax488 (clone B159), anti-CD4 PerCP-Cy5.5 (clone RPA-T4), anti-CD8 APC-H7 (clone SK1) (all from BD Bioscience). Also, anti-CD3 BV605 (clone OKT3), anti-HLA-DR BV711 (clone L243), anti-CD19 PE-Cy7 (clone H1B19) were used from Biolegend. CART-BCMA expression was assessed by using a bis-biotinylated BCMA-Fc recombinant protein and the secondary staining reagent Streptavidin-PE from BD Bioscience (cat#554061). Cells were resuspended in 100 µL PBS containing 1% fetal bovine serum, 0.02% sodium azide and bis-biotinylated BCMA-Fc and incubated for 30 min on ice, washed, resuspended in 100 µL PBS containing 1% fetal bovine serum, 0.02% sodium azide, surface antibodies and SA-PE, and incubated for 30 minutes on ice, washed, resuspended in 250ul PBS containing 1% fetal bovine serum and 0.02% sodium azide and acquired using a Fortessa flow cytometer equipped with a violet (405 nm), blue (488 nm), a green (532 nm), and a red (628 nm) laser. Data were analyzed using FlowJo software (Version 10, Treestar). Compensation values were established using eBioscience UltraComp eBeads (eBioscience cat#01-222-42) and DIVA software.

Quantitative PCR: Genomic DNA was isolated directly from whole blood or marrow aspirate, and qPCR analysis was performed using ABI TaqMan technology and a validated assay to detect the integrated CAR transgene sequence as described(1) using triplicates of 200 ng of genomic DNA per time point for peripheral blood and marrow samples. To determine copy number per unit DNA, an eight-point standard curve was generated consisting of 5 to 106 copies of lentivirus plasmid spiked into 100 ng of non-transduced control genomic DNA. The number of copies of plasmid present in the standard curve was verified using digital qPCR with the same primer/probe set and performed on a QuantStudio 3D digital PCR instrument (Life Technologies). Each datapoint (sample and standard curve) was evaluated in triplicate with a positive Ct value in three of three replicates with percent coefficient of variation of less than 0.95% for all quantifiable values. To control for the quality of interrogated DNA, we performed a parallel amplification reaction using 20 ng of genomic DNA and a primer/probe combination specific for a non-transcribed genomic sequence upstream of the CDKN1A (p21) gene as described (1). These amplification reactions generated a correction factor to adjust for calculated versus actual DNA input. Copies of transgene per microgram of DNA were calculated according to the formula: copies per microgram of genomic DNA = (copies calculated from CART-BCMA standard curve) × correction factor/(amount DNA evaluated in nanograms) × 1000 ng.

Measurement of serum cytokines: Human cytokine magnetic 30-plex panel (LHC6003M) was from Life Technologies. Serum samples collected at baseline and at scheduled time-points out until 28 days post-infusion were cryopreserved at -80°C. Batched samples were thawed and analyzed according to the manufacturers' protocols. Assay plates were measured using a FlexMAP 3D instrument, and data acquisition and analysis were done using xPONENT software. Data quality was examined based on the following criteria. The standard curve for each analyte has a 5P R^2 value > 0.95 with or without minor fitting using xPONENT software. To pass quality control, the results for an in-house control serum needed to be within the 95% of CI (confidence interval) derived from historical in-house control data for >25 of the tested analytes. No further tests were done on samples with results out of range low (<OOR). Samples with results that were out of range high (>OOR) or greater than two times the standard curve

maximum value (SC max) were re-tested at higher dilutions. Results that passed the above quality controls or retests were reported.

Measurement of soluble BCMA, BAFF, and APRIL: Antibody sets for human BCMA (DY193), APRIL (DY884B) and BAFF (DT124-05) were from R&D Systems. ELISA bead-strip and 4-column reservoir (SOW-A16735) were from Assay Depot. ELISA substrate ADHP (10010469) was from Cayman Chemical. Assay plates (OX1263) were from E&K Scientific. All ELISA reagents were prepared according to the protocols for DuoSet ELISA except for Color Reagent B, which was supplemented with ADHP at 100 uM. Due to limited volumes of sera and lack of availability of a Luminex assay for BCMA, APRIL and BAFF, the three analytes were measured using ELISA bead-strips. Instead of coating the capture antibody (cAB) to the wells of an ELISA plate, it was coated on the surfaces of macrospheres, which enabled the measurement of all three analytes using 100ul of serum. Assays were set up using bead-strips in assay plates based on an assay map following the protocol for antibody sets. At the end of the assay, one substrate plate per 12 bead-strips was prepared by adding 100 ul/well of substrate solution (1:1 of color reagent A and ADHP). Each bead-strip was placed in one column of the substrate plate according to the assay map. Color development was for 10 to 30 minutes. Plates were read on a FLUO STAR OMEGA instrument. Data quality control was performed as described for Luminex data.

Assessment of bone marrow myeloma cells, including BCMA expression: Flow cytometry assessment of bone marrow aspirate material was performed directly on aspirate following a brief ammonium chloride red blood cell lysis step. The procedure was adapted from the EuroFlow protocol (2). Briefly, up to 2mls of bone marrow aspirate was diluted with 48mls of Pharm Lyse solution (BD Biosciences Cat# 555899) and incubated for 15 minutes at room temperature on a shaking device. The cells were then collected by centrifugation for 10 minutes at 800g, washed twice with flow cytometry buffer (PBS with 1% fetal bovine serum), stained with L/D Aqua viability dye (Thermo Fisher Cat# L34957). Surface staining was done with a mixture of antibodies to CD45, CD19, CD138, CD38, CD14, CD56, CD20, CD3, CD269 (BCMA), CD274 (PD-L1). FMO (fluorescence minus one) secondary only controls were used for BCMA evaluation. Aliquots of normal donor PBMC cells were stained in parallel as controls. The cells were then washed before permeabilization/fixation using Cytofix/Cytoperm reagent (BD Biosciences) for 20 minutes at room temperature, washed, and stained with a mixture of antibodies to kappa and lambda immunoglobulin light chains. The samples were then washed before resuspension in PBS and acquisition on a 17-color LSR Fortessa Special Order Research Product flow cytometer (BD) equipped with a violet, blue, green, and red laser. A minimum of 5 x 10^6 cells were acquired per sample. List mode files were analyzed using either FlowJo (Treestar) or FCS Express.

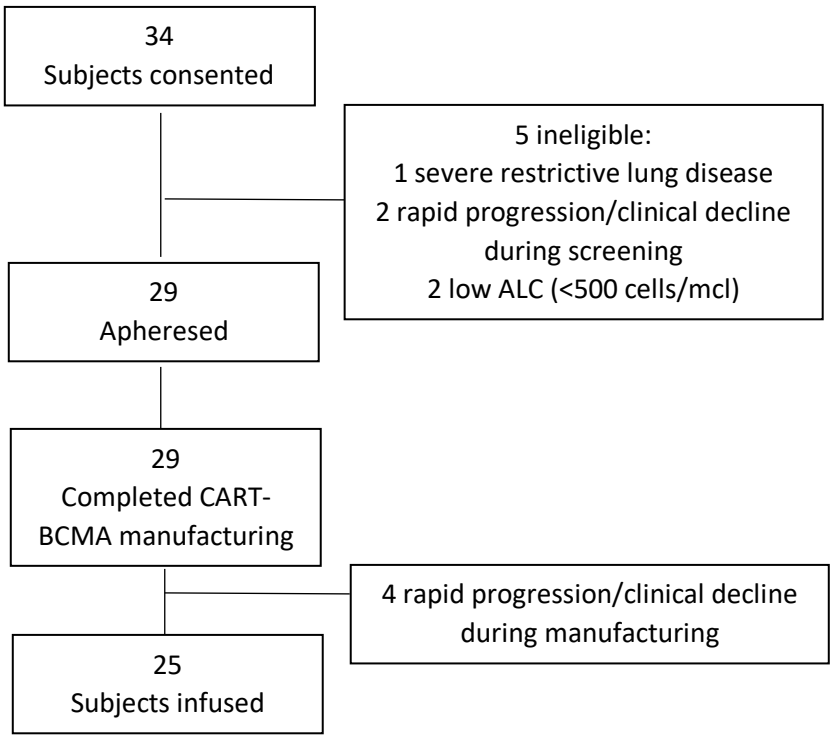

Figure S1: CONSORT diagram showing subject enrollment

ALC = absolute lymphocyte count

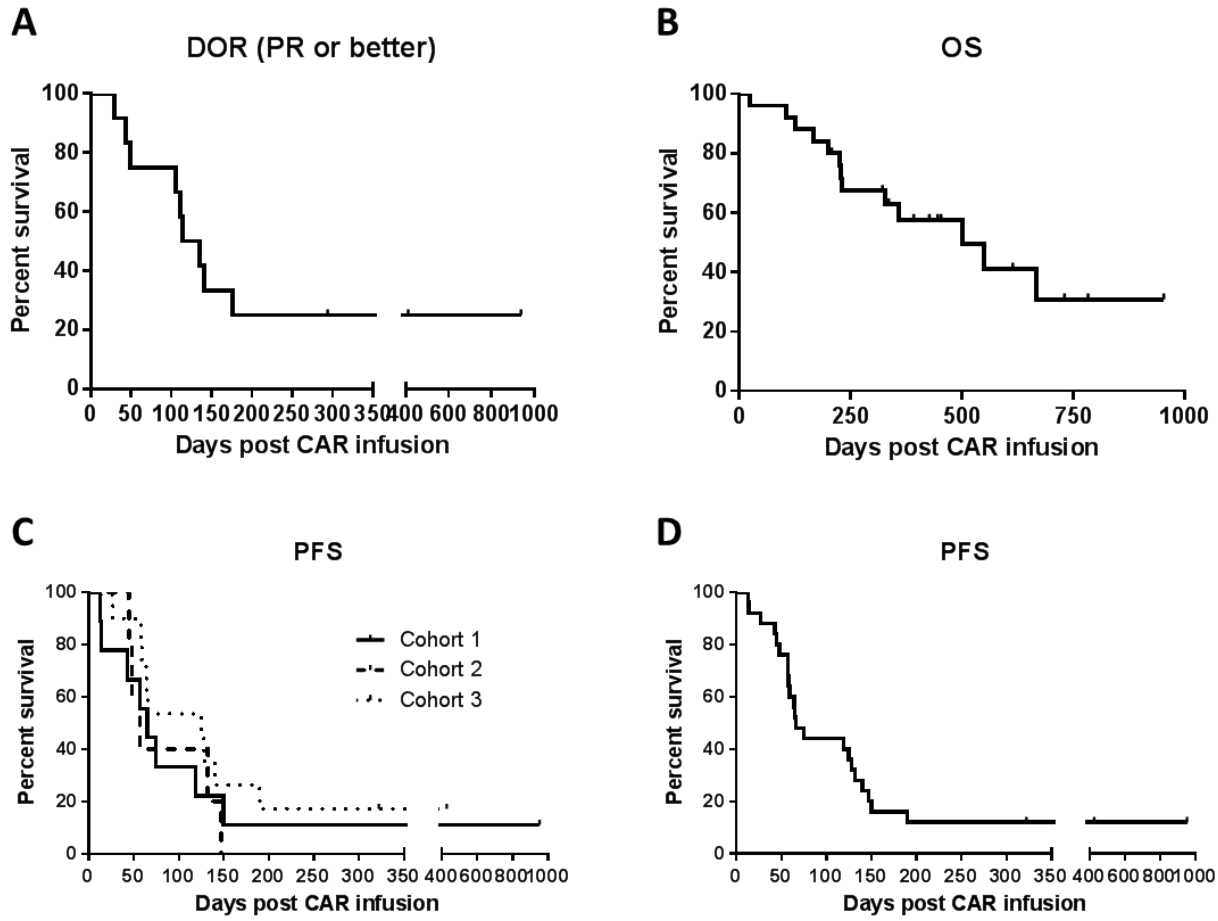

Figure S2: Additional clinical outcomes for treated subjects. A: Duration of response (DOR) for all subjects with partial response (PR) or better. B. Overall survival (OS) for all subjects. C. Progression-free survival (PFS) by cohort. D. PFS for all subjects. Curves derived by Kaplan-Meier method.

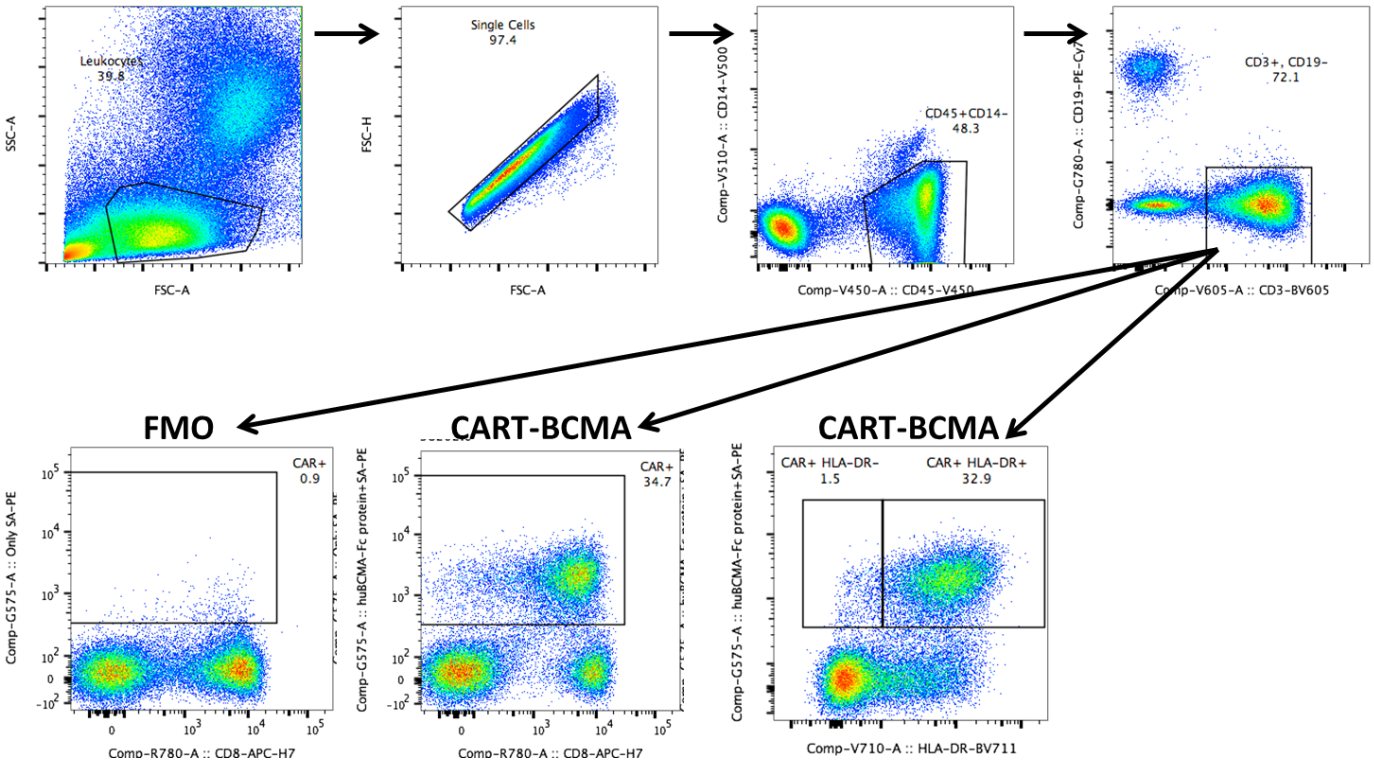

Figure S3: Representative gating and staining for CART-BCMA cells. Staining is shown for peripheral blood from subject 01, day +7 after first CART-BCMA infusion. Cells are gated by forward and side scatter, then singlets, then CD45+CD14- leukocytes, then T cells (CD3+CD19-). CART-BCMA+ cells were identified using biotinylated recombinant human BCMA-Fc and streptavidin-PE. Negative control was an FMO (fluorescence minus one) tube (lacking biotinylated BCMA-Fc) with streptavidin-PE. The % of CD3+ T cells expressing CART-BCMA was calculated by subtracting CAR+ cells in FMO tube from CAR+ cells in tube with biotinylated BCMA-FC (i.e. in this example 34.7 – 0.9 = 33.8). Activation status of CART-BCMA+ cells was identified by staining for HLA-DR (bottom right panel). The % of CAR+ cells that were activated at each timepoint was calculated by dividing %HLA-DR+ by (%HLA-DR+ plus %HLA-DR-) (i.e. in this example 32.9/(32.9 + 1.5) = 95.6%).

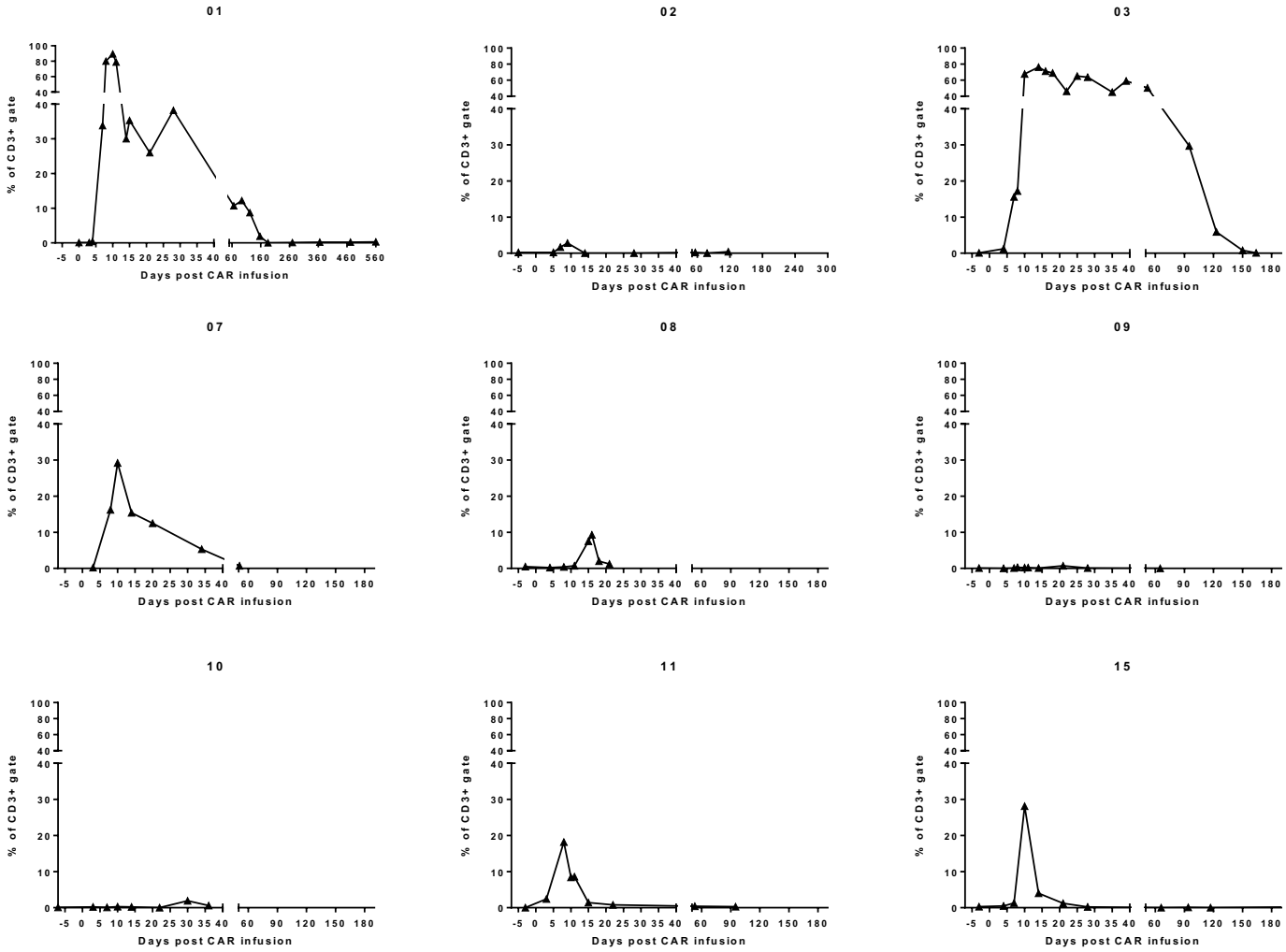

Figure S4: Expansion of CART-BCMA cells: Cohort 1. The frequency of CAR+ T cells within all peripheral blood CD3+ T cells, as measured by flow cytometry, is depicted for each subject.

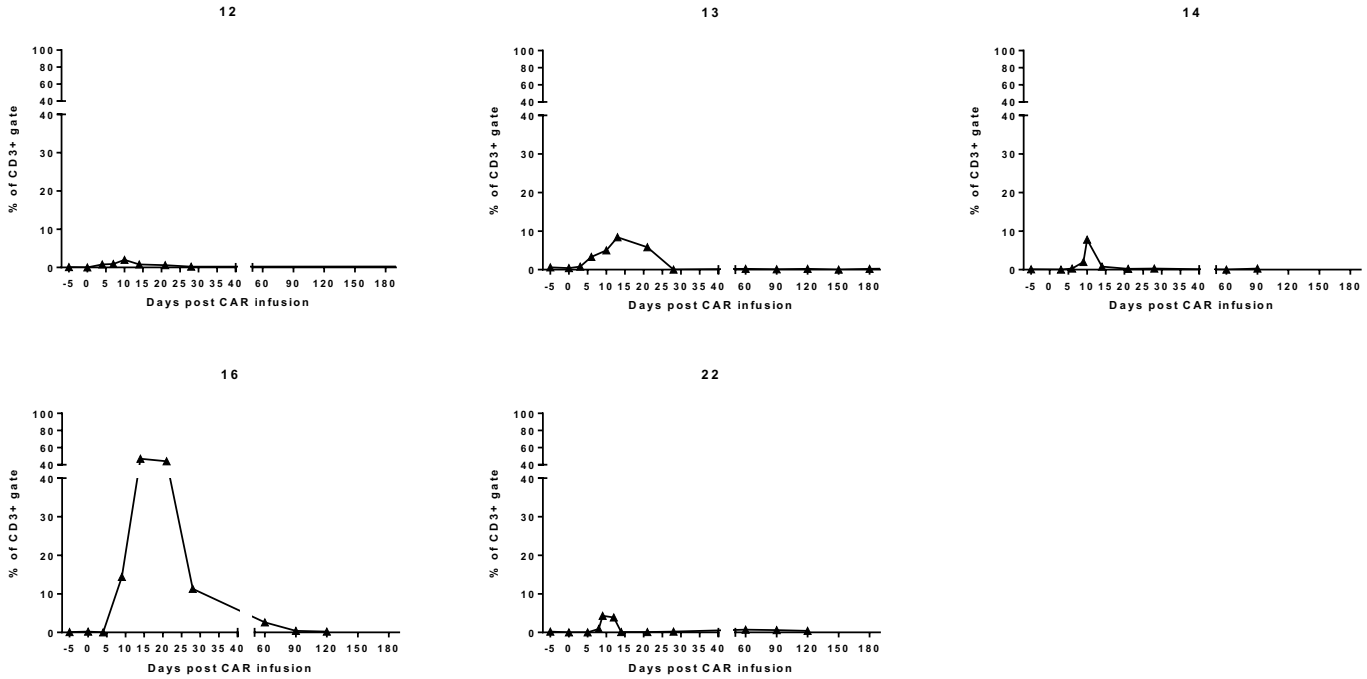

Figure S5: Expansion of CART-BCMA cells: Cohort 2. The frequency of CAR+ T cells within all peripheral blood CD3+ T cells, as measured by flow cytometry, is depicted for each subject.

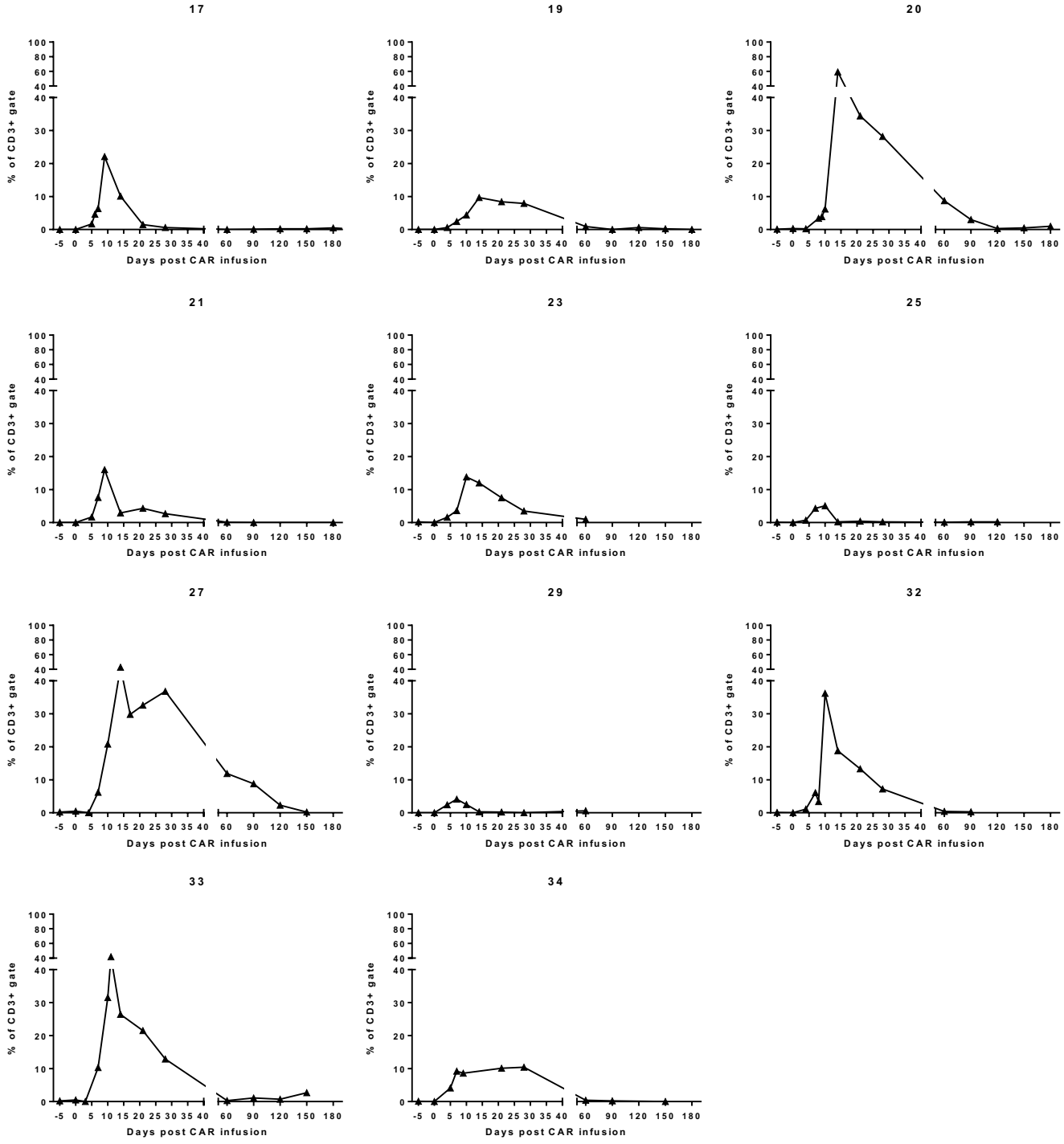

Figure S6: Expansion of CART-BCMA cells: Cohort 3. The frequency of CAR+ T cells within all peripheral blood CD3+ T cells, as measured by flow cytometry, is depicted for each subject.

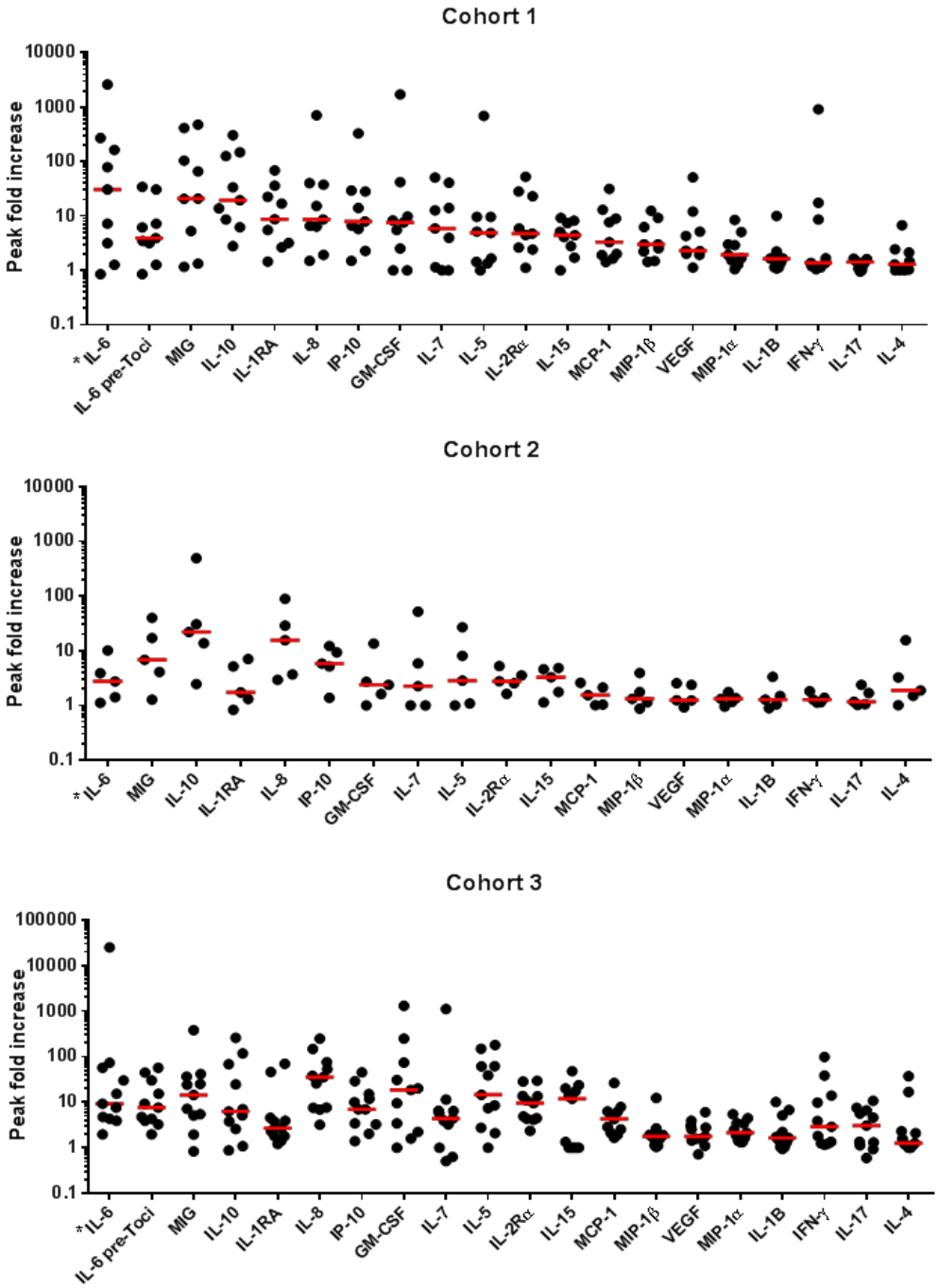

Figure S7: Serum cytokine changes after CART-BCMA treatment. Concentrations (pg/ml) of peripheral blood cytokines were assessed at multiple time-points by Luminex assay. The peak fold increase over baseline for the most frequently elevated cytokines over first 28 days post-infusion are shown, based on cohort. Red line depicts median. *Peak fold-increase of IL-6 at any time post-infusion. IL-6 pre-Toci = for subjects who got tocilizumab (n=6) or siltuximab (n=1), only pre-tocilizumab or pre–siltuximab IL-6 values are included. No subject in Cohort 2 received tocilizumab or siltuximab.

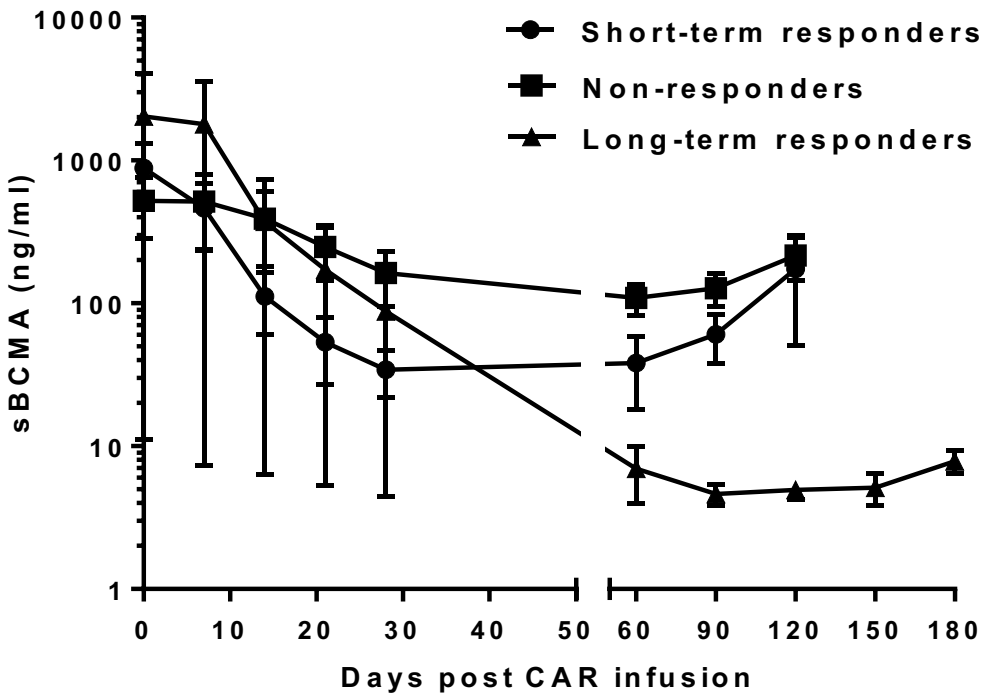

Figure S8: Serial soluble BCMA concentrations reflect disease response and progression. Serum concentration of soluble BCMA (sBCMA) was assessed by ELISA at multiple time-points post-CART-BCMA infusion. Groups are divided into Short-term responders (PR or better lasting ≤ 6 months, n=9), Long-term responders (PR or better lasting >6 months, n=3), or Non-responders (MR, SD, or PD, n=13). Mean concentration (ng/ml) + SEM are depicted. Only 3 Short-term responders and 1 Non-responder had data at day 150 and 180, so these are not depicted. Before day 28, serial sBCMA concentrations decline at a similar trajectory for both short-term and long-term responders, and both decline more rapidly than non-responders ($p=0.03$ for long-term responders vs. non-responders; $p<0.001$ for short-term responders vs. non-responders). After day 28, the slopes of the curves are not significantly different between short-term responders and non-responders ($p=0.848$), but the slope remains different for long-term responders ($p=0.05$ for long-term responders vs. non-responders; $p=0.016$ for long-term responders vs. short-term responders). The estimation was based on a linear random intercept mixed effects model on log10-transform sBCMA that included two piecewise linear splines connected at day 28; p-values were determined based on z test for the regression coefficient of interest or a linear combination of the coefficients.

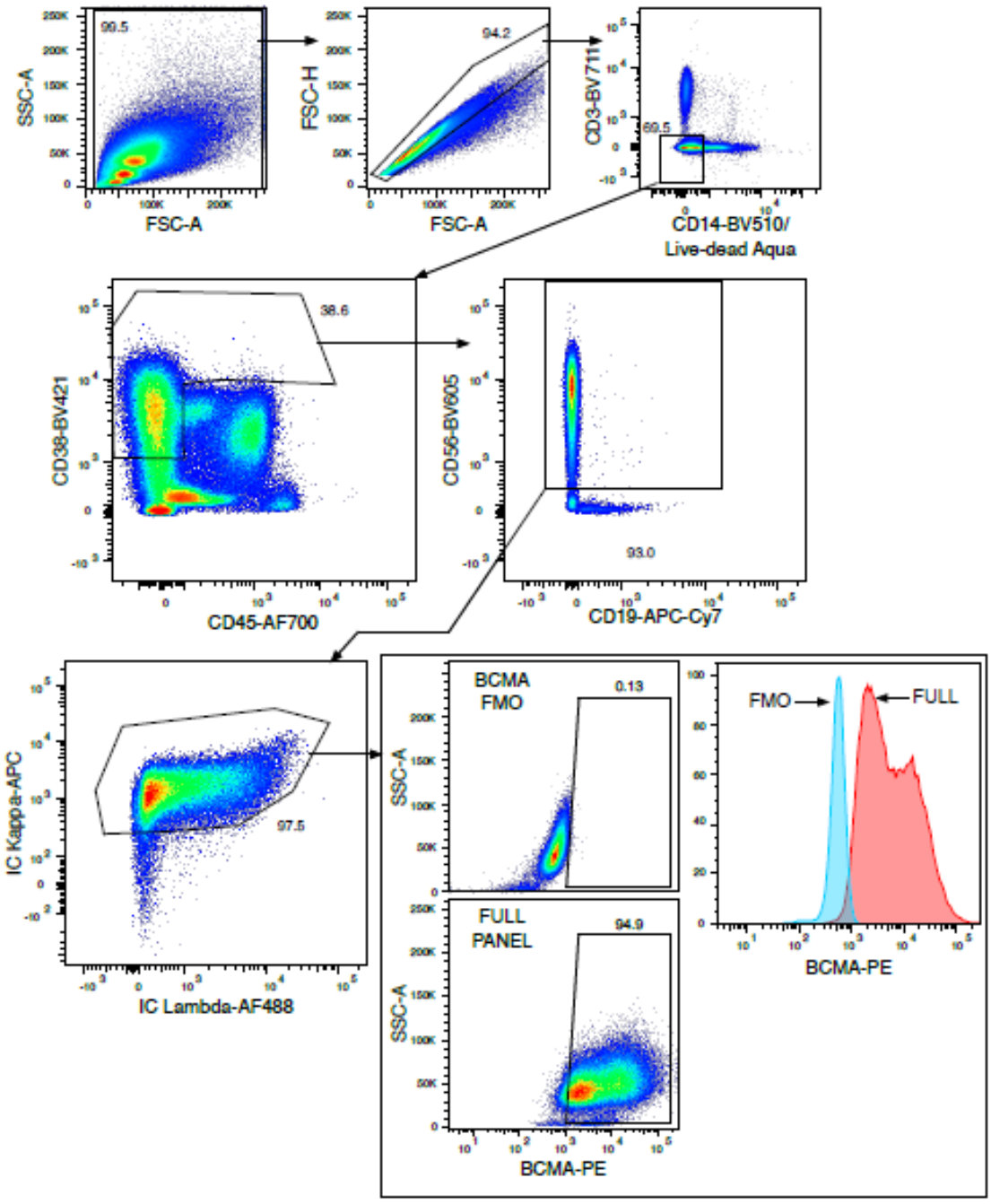

Figure S9: Representative gating for myeloma cells and BCMA staining. Bone marrow aspirate cells were gated by forward and side scatter, then by singlets, then on CD3-CD14- cells. Myeloma cells were identified by gating first on CD38^{hi} cells, then by gating on clonal plasma cells using CD19, CD56, and kappa/lambda staining. In this example, myeloma cells are CD19-CD56+kappa+. The % BCMA + was determined using an FMO tube lacking anti-BCMA antibody.

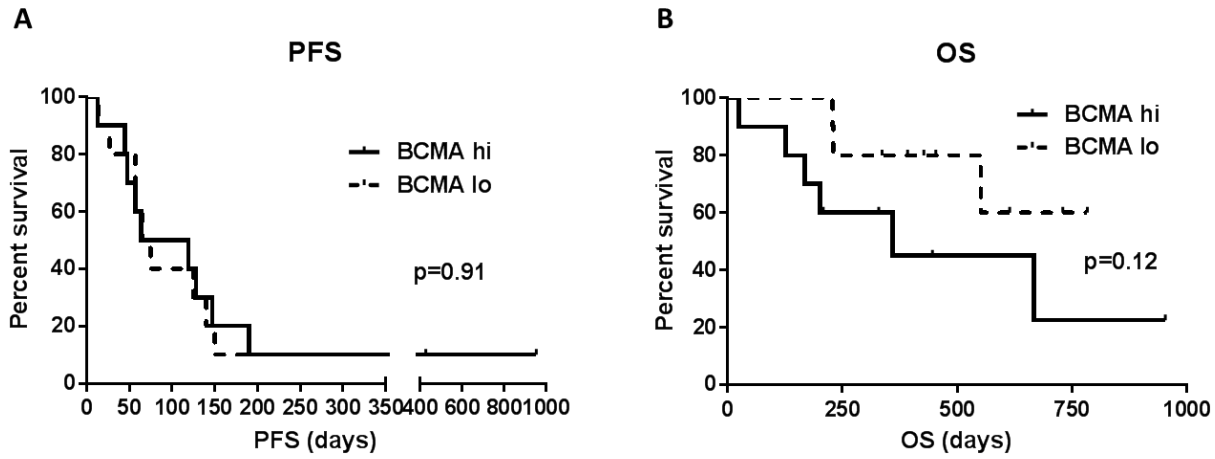

Figure S10: PFS and OS based on baseline BCMA intensity: Subjects evaluable for baseline BCMA expression by flow cytometry (n=20) were divided into BCMA hi (MFI> median value of 3741) and BCMA lo (MFI < median) and assessed for progression-free (A) and overall (B) survival. Curves derived by Kaplan-Meier method. P-values determined by log-rank test (p-value <0.05 considered significant).

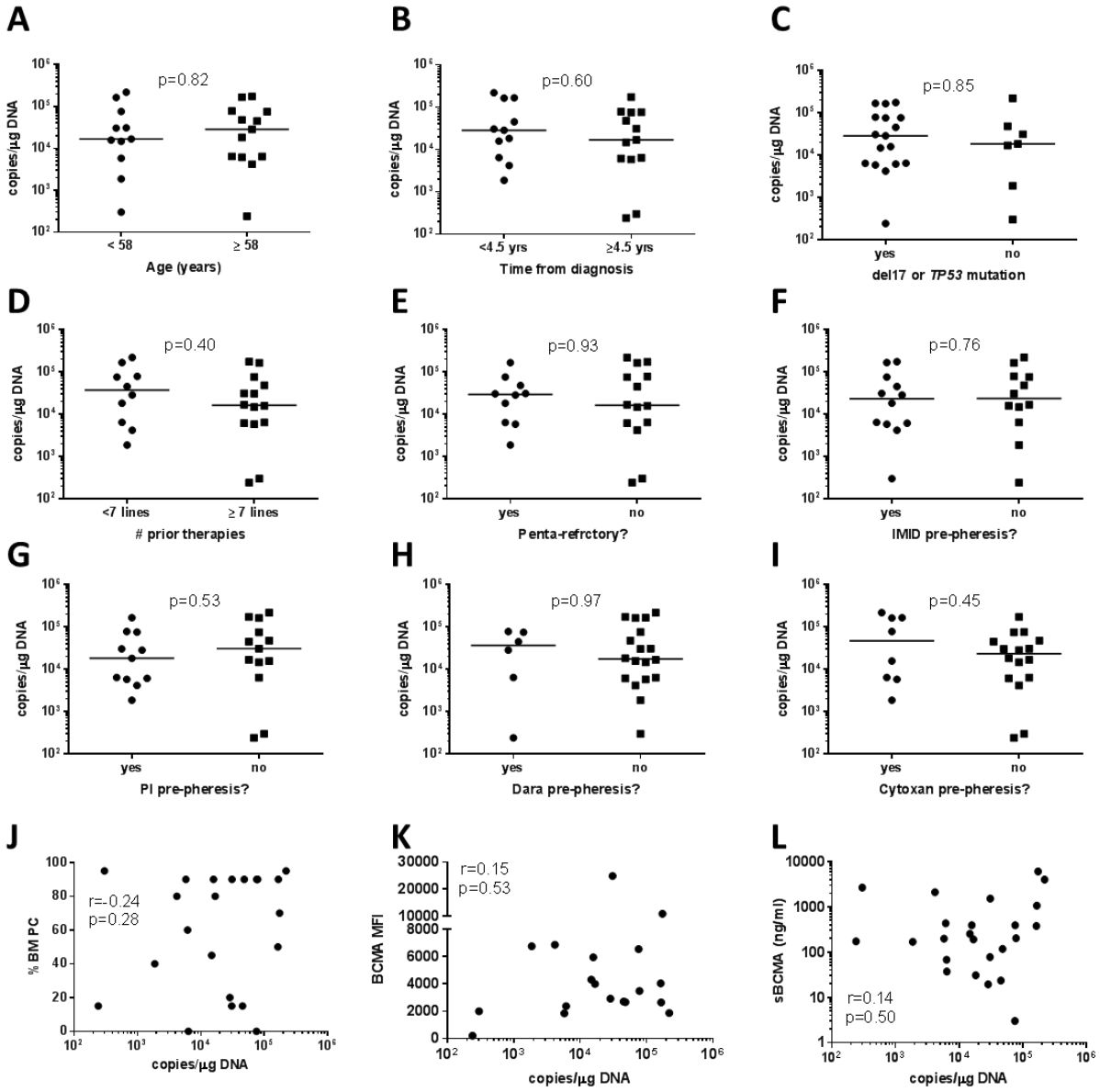

Figure S11: Peak CART-BCMA expansion is not associated with baseline clinical characteristics, baseline BCMA expression or sBCMA concentration. Peak CART-BCMA level (copies/µg genomic DNA) by qPCR was not significantly associated with (A) age at enrollment (above or below median); (B) years from diagnosis (above or below median); (C) presence of del17p by FISH or *TP53* mutation by sequencing; (D) number (#) of therapeutic lines (above or below median); (E) being penta-refractory to 2 proteasome inhibitors (PIs), 2 immunomodulatory drugs (IMiDs) and daratumumab (dara); receiving therapy just prior to leukapheresis that contained (F) an IMiD, (G) a PI, (H) dara, or (I) cyclophosphamide (Cytoxan); (J) percentage of pre-treatment bone marrow plasma cells (%BM PC); (K) baseline BCMA mean fluorescence intensity (MFI) on BM PC; or (L) baseline serum soluble BCMA (sBCMA) concentration. For A-I, analysis by Mann-Whitney test; line represents median value. For J-L, analysis by Spearman correlation.

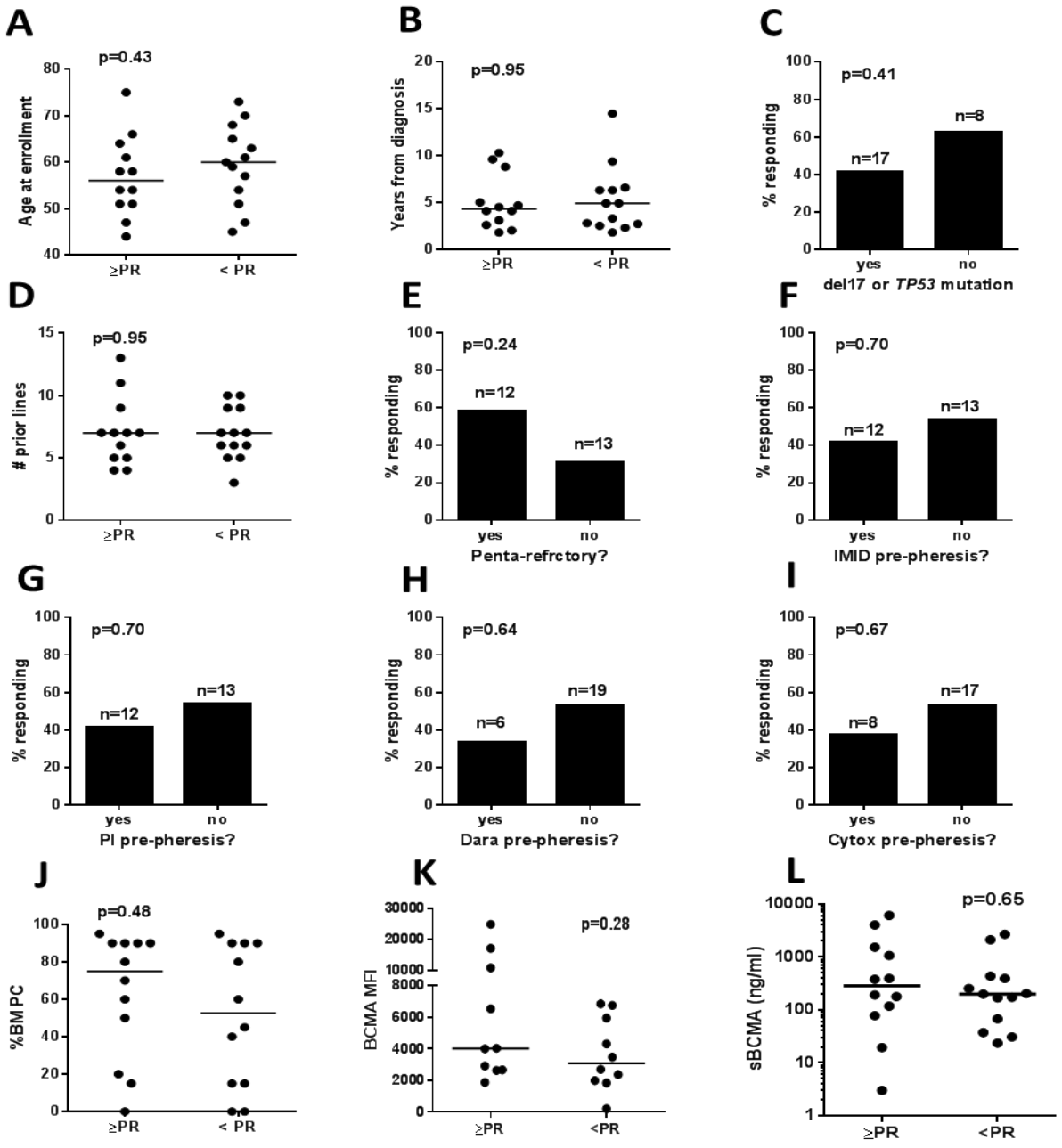

Figure S12: Response is not associated with baseline clinical characteristics, baseline BCMA expression or sBCMA concentration. Clinical response (≥ partial response (PR)) was not significantly associated with (A) age at enrollment (B) years from diagnosis (C) presence of del17p by FISH or *TP53* mutation by sequencing; (D) number (#) of therapeutic lines (E) being penta-refractory to 2 proteasome inhibitors (PIs), 2 immunomodulatory drugs (IMiDs) and daratumumab (dara); (F-I) receiving a regimen just prior to leukapheresis that contained an IMiD, a PI, dara, or cyclophosphamide (Cytoxan); (J) percentage of pre-treatment bone marrow plasma cells (%BM PC); (K) baseline BCMA mean fluorescence intensity (MFI) on BM PC; or (L) baseline serum soluble BCMA (sBCMA) concentration. For C, E-I, analysis by Fisher Exact test. For A, B, D, J-L, analysis by Mann-Whitney test; line represents median value.

Table S1

University of Pennsylvania CRS Toxicity Grade				
1	**2**	**3**	**4**	**5**
Mild reaction: Treated with supportive care such as anti-pyretics, anti-emetics	Moderate reaction requiring IV fluids or parenteral nutrition; some signs of organ dysfunction (i.e., grade 2 creatinine or grade 3 liver function tests [LFTs] related to CRS and not attributable to any other condition). Hospitalization for management of CRS related symptoms including fevers with associated neutropenia.	More severe reaction: Hospitalization required for management of symptoms related to organ dysfunction including grade 4 LFTs or grade 3 creatinine related to CRS and not attributable to any other conditions. This excludes management of fever or myalgias. Includes hypotension treated with IVFs* or low-dose pressors, coagulopathy requiring fresh frozen plasma (FFP) or cryoprecipitate, and hypoxia requiring supplemental oxygen (nasal cannula oxygen, high flow oxygen, Continuous Positive Airway Pressure [CPAP] or Bilateral Positive Airway Pressure [BiPAP]). Patients admitted for management of suspected infection due to fevers and/or neutropenia may have grade 2 CRS.	Life-threatening complications such as hypotension requiring high dose pressors or hypoxia requiring mechanical ventilation.	Death

*Defined as hypotension requiring multiple fluid boluses for blood pressure support.

Table S1. Penn grading system for Cytokine Release Syndrome (CRS).

Table S2

Sub.	Age	Sex	Race	Yrs from Dx	Iso-type	Karyo-type	FISH	Mutation	# lines*	Last tx before pheresis, infusion**
Cohort 1										
01	66	M	white	9.6	IgGK	46XY	+11, -16q, -17p	*NRAS, TP53, TP53*	11	Pom/Dex Pom/Dex
02	68	M	white	6.3	IgAK	46XY	+1q, +4p, -17p	n/a	9	Carfilz/Len/Dex Carfilz/Cyclo/Dex
03	54	F	white	3.1	IgAL	n/a	+1q, t(4;14), -16q	n/a	4	D-PACE D-AC
07	44	M	white	1.8	K	n/a	+1q, -4, +11, -14, -16	*NRAS, TP53*	9	Carfilz/Pano Pom/Dex-ACE
08	70	M	white	3.3	IgAL	complex	+1q, -1p, -4, -17p	n/a	6	Carfilz/Pom/Dex Carfilz/Pom/Dex
09	61	M	white	6.6	K	46XY	t(11;14), -16q, -17p	none	9	Daratumumab Pulse Dex
10	47	M	white	4.9	IgGL	n/a	+1q, t(11;14)	*KRAS, SF3B1*	7	Pom/Dex Pom/Cyclo/Dex + plasma exchange
11	57	F	AA	6.3	IgGL	46XX	+1q, t(4;14), -17p	n/a	10	VDT-PACE VDT-PACE
15	51	F	Asian	4.7	IgAK	46XX	+1q, t(11;14)	*NRAS, KRAS, BIRC3, KIT*	7	Pembro/Pom/Dex Pom/Dex
Cohort 2										
12	51	F	white	2.8	IgAL	46XX	+1q, -1p, t(4;14), +17p	*TET2*	5	CyBorD CyBorD
13	59	M	white	9.4	IgGL	46XY	+11, -16q, -17p	n/a	10	Salvage ASCT VD-CE
14	54	F	white	4.9	IgGK	46XX	t(11;14), -16q, -17p	n/a	7	Venetoclax none
16	54	M	white	4.1	IgGK	complex	+1q,	*BRAF*	7	D-CE

							-16q, -17p			D-CE + radiation
22	45	M	white	2.7	IgGL	46XY	t(11;14), +1q, -17p	*n/a*	7	D-ACE none
Cohort 3										
17	64	F	white	5.0	IgAK	46XX	t(14;16), +1q, -1p, +11	n/a	7	CPI-610 D-AC
19	58	M	white	4.1	L	46XY	r(11;14), +1q, +17, -17p	n/a	5	Dara/Ixa/Pom/Dex Dara/Ixa/Pom/Dex
20	61	M	white	2.6	IgGL	n/a	t(4;14), +1q, -17p	n/a	6	Carfilz/cyclo/pom/dex VD-AC
21	73	M	white	2.5	IgAK	46XY	+1q, +14, +17, -17p	*TET2*	5	Dara/Pom/Dex Bort/Pom/Dex
23	63	M	white	2.3	IgGK	46XY	none	none	6	Carfilz/Pom/Dex/Nelfin VD-PCE
25	51	M	AA	4.5	IgGK	46XY	t(11;14), +1q	*FLT3*	7	Selinexor/Dex Bort/Venetoclax/Dex + XRT
27	58	F	AA	10.3	IgGK	complex	t(11;14), +1q, -1p, -16, -17p	*BRAF, TET2*	13	Carfilz/Venetoclax/Dex Carfilz/Venetoclax/Dex
29	60	F	white	1.8	IgGK	45X,-X	none	*TET2, RB1, TP53*	3	VDT-PACE none
32	65	F	white	14.5	IgGL	46XX	t(4;14), +1q, -17	*TET2, TET2*	6	Carfilz/Cyclo/Dex Carfilz/Cyclo/Dex
33	75	M	white	8.8	IgGK	hyperdip	+5, +9, +11, +15, -17p	*RAD21*	5	Pembro/Len/Dex none
34	47	M	white	2.0	IgGL	46XY	+1q, -1p, +14q	*MYCN, TPMT*	4	Carfilz/Pom/Dex VD-PACE

Table S2: Individual subject characteristics. *A line of therapy was defined as per IMWG criteria. Radiation was not counted as a line. **Most recent therapy received before T cell collection ("pheresis" – top line) and CART-BCMA infusion ("infusion" – bottom line). All therapy was held for at least 2 weeks prior to pheresis and again for at least 2 weeks prior to infusion (4 weeks for monoclonal antibodies).
AA = African-American; ASCT = autologous stem cell transplant; Bort = bortezomib; Carfilz = carfilzomib; cyclo = cyclophosphamide; CPI-610 = investigational BET inhibitor; CyBorD = cyclophosphamide, bortezomib, dexamethasone; D-AC = dexamethasone + infusional doxorubicin and cyclophosphamide; D-ACE = dexamethasone + infusional doxorubicin, cyclophosphamide, and etoposide; D-CE: dexamethasone + infusional cyclophosphamide and etoposide; D-PACE = dexamethasone + infusional cisplatinum, doxorubicin, cyclophosphamide, and etoposide; Dara = daratumumab; Dex = dexamethasone; Dx = diagnosis; hyperdip = hyperdiploid; ixa = ixazomib; K = kappa light chain; L = lambda light chain; Len = lenalidomide; Nelfin = nelfinavir; Pano = panobinostat; Pembro = pembrolizumab; Pom = pomalidomide; Pom/Dex-ACE = pomalidomide, dexamethasone + infusional doxorubicin, cyclophosphamide, and etoposide; Tx = treatment; VD-AC = bortezomib, dexamethasone + infusional doxorubicin and cyclophosphamide; VD-CE = bortezomib, dexamethasone + infusional cyclophosphamide and etoposide; VD-PCE = bortezomib, dexamethasone + infusional cisplatinum, cyclophosphamide, etoposide; VDT-PACE = bortezomib, dexamethasone, thalidomide + infusional cisplatinum, doxorubicin, cyclophosphamide, and etoposide; Yrs = years.

Table S3

Sub.	%CD3+ in seed culture	CD4: CD8 in seed culture	%CD3+ at harvest	CD4: CD8 at harvest	Fold exp	Pop dblgs	Trans Eff (%)	Total #CAR+ given (x 10^8)	Best re-sponse
Cohort 1									
01	52.6	2.04	98.4	2.40	43.5	4.54	9.6	2.0*	sCR
02	63	0.40	69.1	1.31	12.7	3.67	18.2	5.0	MR
03	58.4	2.47	97.6	1.72	17.9	4.16	25.9	2.0*	VGPR
07	20.3	1.47	94.9	1.60	38.9	5.28	24.2	5.0	PR
08	20.8	0.31	92.5	2.99	7.9	2.98	22.7	3.5	PD
09	86.8	0.49	87.5	1.69	10.0	3.33	22.2	5.0	SD
10	47.3	1.07	96.7	1.32	11.5	3.52	16.5	1.77	PD
11	21.4	0.81	96.9	1.20	8.7	3.11	19.4	5.0	MR
15	90.8	0.71	96	1.24	22.6	4.50	33.3	2.0*	VGPR
Cohort 2									
12	80	0.38	96.2	3.23	31.7	4.98	21.7	0.5	SD
13	72	0.50	86.9	1.72	14.0	3.84	24.2	0.5	MR
14	94.6	0.81	98.9	2.21	60.4	5.92	31.8	0.5	SD
16	50.9	1.06	98.1	0.84	30.6	4.94	21.9	0.5	PR
22	65.1	0.46	98.4	1.39	49.3	5.62	11	0.5	SD
Cohort 3									
17	84.4	0.59	97.3	1.87	56.8	5.83	27.4	5.0	PR
19	88.1	0.91	94.9	2.09	16.8	4.07	13.7	5.0	CR
20	70	0.70	97.4	2.17	32.3	5.02	8.4	5.0	VGPR
21	83.5	0.46	98.9	3.26	45.9	5.52	7.5	5.0	SD
23	74.7	0.42	97.6	1.46	45.1	5.77	11	5.0	MR
25	69.2	0.54	99	0.93	23.7	4.57	10.7	2.0*	PR
27	69	0.91	97.9	0.90	25.1	4.65	15	5.0	VGPR
29	29.8	2.49	91.7	4.55	23.0	4.52	8.3	5.0	SD
32	71	1.23	98.3	1.87	40.0	5.32	8.9	5.0	MR
33	83.6	0.33	96.5	1.78	31.1	4.96	8.6	5.0	PR
34	62.7	1.73	96.4	4.02	29.3	4.87	14.7	5.0	VGPR

Table S3: CART-BCMA manufacturing and product details. Frequency of total CD3+, CD3+CD4+, and CD3+CD8+ cells was assessed by flow cytometry at the start of manufacturing ("seed culture," after elutriation), and at the end of manufacturing ("at harvest"). Aph = apheresis product; fold exp = fold expansion; pop dblgs = population doublings; trans eff = transduction efficiency. MR = minimal response; PD = progressive disease; PR = partial response; sCR = stringent complete response; SD = stable disease; VGPR = very good partial response

*Subjects 01, 03, 15, and 25 received only 40% of planned dose due to fevers/early CRS.

Table S4

Sub.	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Cohort 1					
01	Dry eye Dry mouth Vomiting Flu-like symptoms Metabolism disorder – other (iron deficiency) Myalgia Headache Ischemia cerebrovascular Nervous system disorder – other (confusion) Vascular disorder – other (edema)	DIC Vertigo Ileus Nausea Fever Lung infection Creatinine increased Thrombocytopenia Musculoskeletal disorder – other (generalized muscle aches) Urinary retention Respiratory disorder – other (pulmonary edema and atelectasis) Skin/SQ disorder – other (skin tear) Hypertension	Anemia Fatigue CRS Alk phos increased AST increased Hypocalcemia Hypokalemia Hypophosphatemia	Lymphopenia	
02	Sinus tachycardia Gastrointestinal disorders – other (loose stool) Nausea Fatigue CRS Dizziness Headache Respiratory disorder – other (nasal and sinus congestion, cough)		Thrombocytopenia		
03	Sinus bradycardia Ear disorder – other (ears feel full, hard to hear) Eye disorder – other (difficulty reading) Constipation	SVT Fatigue Infection – other (S. mitis/S. oralis bacteremia) Upper respiratory infection Lymphopenia	CRS UTI Fibrinogen decreased Hypophosphatemia Seizure Pleural effusion Hypertension	Neutropenia Leukopenia Hypocalcemia RPLS Delirium	

	Edema Fever Hypokalemia Hypomagnesemia Bone pain Concentration impairment Headache Hoarseness Skin/SQ disorder – other (ecchymosis)	Hypernatremia Musculoskeletal disorder – other (deconditioning, pain base of skull) Intracranial hemorrhage Respiratory disorder – other (post-nasal drip) Hypotension			
07	Infection – other (upper respiratory infection) Skin/SQ disorder – other (rash on face)	CRS Dyspepsia Hypoalbuminemia Hypocalcemia Respiratory, thoracic disorders – other (hemothorax)	Anemia Hypokalemia Hyponatremia		
08	Sinus tachycardia General disorder – other (rigors)		Lung infection Delirium	CRS Thrombocytopenia Encephalopathy Infection – other (fungemia)	Death NOS
09	Constipation Chills Fatigue Thrombocytopenia Anorexia Hypokalemia	Anemia Pericardial effusion CRS Neutropenia Hypocalcemia Headache	Lung infection		
10	Chills Fever	Thromobocytopenia			
11	Nausea Fatigue Thrombocytopenia Headache Respiratory disorder – other (cough, congestion)	CRS Infection – other (Clostridium difficile) Hypocalcemia	Hypophosphatemia		
15	Hypocalcemia Hypokalemia Headache Cough Dyspnea	Fatigue CRS Infection – other (positive test for Shiga toxin) Alk phos increased	Thrombocytopenia Hypoglycemia	Neutropenia	

	Respiratory disorders – other (nasal congestion) Alopecia	Hypotension			
Cohort 2					
12	Anemia Vomiting Fatigue Fever Hypocalcemia	Infection – other (adenovirus)	Febrile neutropenia	Sepsis Neutropenia Leukopenia	
13	Diarrhea Fatigue ALT increased AST increased Anorexia Dizziness Pruritis Skin/subcutaneous tissue disorder – other (lesions on bilateral ears)	Upper respiratory infection	Neutropenia		
14	Back pain Pain in extremity	CRS	Anemia Febrile neutropenia	Neutropenia Leukopenia	
16	Colitis Hypocalcemia Hypokalemia Hypomagnesemia Paresthesia Confusion Hoarseness Sore throat Hypotension	Flu-like symptoms CRS Hypoalbuminemia Epistaxis	Febrile neutropenia	Lymphopenia Neutropenia Thrombocytopenia Leukopenia	
22	Fatigue Myalgia Insomnia	Nausea CRS	Febrile neutropenia	Lymphopenia Leukopenia	
Cohort 3					
17	Diarrhea Hypocalcemia Hypokalemia Back pain Headache Peripheral sensory neuropathy Insomnia	Chills CRS Arthralgia		Neutropenia Leukopenia	

19	Nausea Chills	Ear and labyrinth disorders – other (seasonal allergies) Gastrointestinal disorders – other (food poisoning) Fever Sinusitis Upper respiratory infection	CRS Lymphopenia Hypophosphatemia Hypertension		
20	Constipation Diarrhea Vomiting Chills CPK increased Hypokalemia Back pain Allergic rhinitis	Fatigue ALT increased Hypophosphatemia Dysphasia Lethargy Confusion Hypotension	CRS Hyponatremia	AST increased	
21	Alk phos increased Hypocalcemia Hyponatremia Epistaxis	CRS Skin disorders – other (cellulitis)	Lymphopenia Neutropenia Thrombocytopenia Leukopenia		
23	Anal hemorrhage Nausea Edema limbs Hypoalbuminemia Hypocalcemia Tremor	CRS Abdominal pain Colitis Diarrhea Vomiting Pain Thrombocytopenia Hypokalemia	Febrile neutropenia		
25	Nausea Facial pain Fatigue Hypomagnesemia Headache Peripheral sensory neuropathy	Hypoalbuminemia Hypokalemia Hypercalcemia	CRS Hypocalcemia Papilledema		
27	Diarrhea Nausea Oral pain Fatigue Pain Infections – other (upper respiratory infection)	Sinus bradycardia Edema limbs Urinary tract infection Creatinine increased Neutropenia Hypernatremia	CRS SVT AST increased Ejection fraction decreased Lymphopenia Leukopenia Hypoalbuminemia	Anemia Thrombocytopenia Hyperglycemia Hypocalcemia Hypokalemia Pleural hemorrhage	

	Back pain Myalgia Pain in extremity Dry skin Hematoma	Wheezing	Hyponatremia Encephalopathy Hypertension		
29	Nausea Fatigue Pain Respiratory disorders – Other (scratchy throat, slight cough)	Chills CRS Hypoalbuminemia	Abdominal pain Hyponatremia Hypophosphatemia	Lymphopenia Leukopenia	
32	Nausea Fatigue Alopecia	Diarrhea Encephalopathy Allergic rhinitis	Anemia CRS Neutropenia Hypoalbuminemia Hypocalcemia Hyponatremia Hypophosphatemia	Lymphopenia Thrombocytopenia Leukopenia	
33	Oral pain Diarrhea Fatigue Flu-like symptoms Arthralgia Flank pain Allergic rhinitis	Chills CRS Hypoalbuminemia	Febrile neutropenia	Neutropenia	
34	Diarrhea Nausea Sinus tachycardia Confusion ALT increased	CRS Thrombocytopenia Hypocalcemia Flu-like symptoms AST increased	Leukopenia Hypophosphatemia Hyopnatremia	Lymphopenia Neutropenia	

Table S4. Individual subject adverse events. All events regardless of attribution are listed. If an event occurred more than once in same patient, highest grade is reported. Alk phos = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatine phosphokinase; CRS=cytokine release syndrome; DIC = disseminated intravascular coagulation; NOS = not otherwise specified; RPLS = reversible posterior leukoencephalopathy syndrome (also known as posterior reversible encephalopathy syndrome (PRES)); SQ = subcutaneous; SVT = supraventricular tachycardia; UTI = urinary tract infection

	Grade 1	Grade 2	Grade 3	Grade 4	All (n, %)
CRS (total, n=25)	2	12	7	1	22 (88%)
Cohort 1 (n=9)	1	4	2	1	8 (89%)
Cohort 2 (n=5)	0	3	0	0	3 (60%)
Cohort 3 (n=11)	1	5	5	0	11 (100%)
Neurotoxicity (total, n=25)	3	2	1	2	8 (32%)
Cohort 1 (n=9)	1	0	0	2	3 (33%)
Cohort 2 (n=5)	1	0	0	0	1 (20%)
Cohort 3 (n=11)	1	2	1	0	4 (36%)

Table S5. Cytokine release syndrome (CRS) and neurotoxicity by cohort. All events regardless of attribution are listed. CRS graded as per Penn grading scale (Suppl. Table S1).

Sub.	Day of peak	%CAR+ of CD3+	%CAR+ of CD4+	%CAR+ of CD8+	%HLADR+ of CAR+	Best response
Cohort 1						
01	10	89.5	8.6	92.3	97	sCR
02	9	2.8	1.4	5.0	82	MR
03	14	76.2	13.7	85.9	97	VGPR
07	10	29.2	7.3	39.3	89	PR
08	16	9.3	0.4	16.9	95	PD
09	11*	*	*	*	*	SD
10	30	1.9	0.1	2.0	21	PD
11	8	18.2	14.6	25.2	94	MR
15	10	28.1	6.3	51.0	96	VGPR
Cohort 2						
12	10	2.0	0.8	2.3	76	SD
13	13	8.4	2.9	13.2	92	MR
14	10	7.8	3.5	10.6	95	SD
16	14	47.1	12.1	50.3	98	PR
22	9	4.3	0.6	8.6	94	SD
Cohort 3						
17	9	22.1	10.4	27.4	96	PR
19	15	9.7	8.9	10.2	87	CR
20	15	59.2	22.4	65.1	99	VGPR
21	9	16.0	9.2	19.9	97	SD
23	10	13.8	6.6	17.3	93	MR
25	10	5.1	3.8	5.9	85	PR
27	14	42.9	4.9	47.3	88	VGPR
29	7	4.1	2.8	15.0	77	SD
32	10	36.2	6.1	46.4	97	MR
33	11	41.8	29.8	46.9	91	PR
34	**	**	**	**	**	VGPR

Table S6. Characteristics of peripheral blood CART-BCMA+ cells at peak expansion. CART-BCMA cells were assessed by flow cytometry as in Fig. S3. At day of peak expansion, the frequency of CAR+ cells within CD3+, CD4+, and CD8+ populations are listed. Activation status at peak expansion (as measured by % of CAR+ cells expressing HLA-DR) is also shown. MR = minimal response; PD = progressive disease; PR = partial response; sCR = stringent complete response; SD = stable disease; VGPR = very good partial response

*Peak determined by qPCR; CAR+ cells not detectable by flow. **No sample available between days 10-21 so peak could not be determined.

Sub.	Day 28 blood	Day 28 BM	3 month blood	3 month BM	Other (site, day)
Cohort 1					
01	23447.33	10458.66	4373.95	n/a	n/a
02	454.57	383.62	334.90	n/a	n/a
03	220081.08*	109964.97*	9517.67	9934.97	CSF, D4: 3285.88 CSF, D16: 158038.74 Pleural fluid, D8: 23941.97 Pleural fluid, D18: 144516.62
07	1757.60	2140.31	n/a	n/a	n/a
08	n/a	n/a	n/a	n/a	n/a
09	32.15	16.75	n/a	n/a	n/a
10	62.64	0.00	n/a	n/a	n/a
11	731.44	n/a	218.26	n/a	n/a
15	724.49	1084.16	32.58	99.48	n/a
Cohort 2					
12	140.60	25.59	n/a	n/a	n/a
13	193.80	43.64	94.70	27.04	n/a
14	66.69	255.76	8.80	0.00	n/a
16	25919.10	22077.89	n/a	n/a	n/a
22	34.56	22.09	77.71	63.76	n/a
Cohort 3					
17	1639.98	1398.82	1392.25	649.69	n/a
19	12987.83	6578.03	153.88	117.53	n/a
20	43463.65	28086.46	4107.95	5223.76	n/a
21	8372.96	1161.92	96.11	n/a	n/a
23	5946.81	n/a	102.68	n/a	n/a
25	256.33	259.97	149.20	160.33	n/a
27	51014.95	41242.37	14271.48	9634.66	Pleural fluid, D15: 58511.99
29	738.84	210.05	n/a	n/a	n/a
32	6983.98	7055.94	58.15	15.72	n/a
33	7539.99	5142.13	230.66	39.09	n/a
34	5199.07	1512.03	n/a	n/a	n/a

Table S7. CART-BCMA engraftment by qPCR in blood, bone marrow, and other sites. CART-BCMA levels (copies/μg genomic DNA) were generally comparable in blood and marrow at tested timepoints. CART-BCMA was found at high levels in CSF and pleural fluid of subject 03, and pleural fluid of subject 27. BM = bone marrow; CSF = cerebrospinal fluid. n/a = not available. *Assays performed on day 45.

Cytokine	Median peak fold increase: CRS Gr 0-2	Median peak fold increase: CRS Gr 3-4 or Gr 2 + toci	p-value
IL-6*	3.88	76.26	<0.0001
IFN-γ	1.26	11.95	<0.0001
IL-2Rα	4.13	18.40	<0.0001
MIP-1α	1.35	3.47	<0.0001
IL-15	1.71	12.81	0.0004
IL-1RA	2.33	19.68	0.0036
MCP-1	2.11	6.99	0.0044
GM-CSF	2.69	36.27	0.0052
IL-1β	1.28	2.23	0.0052
MIG	6.81	39.14	0.008
MIP-1β	1.49	2.39	0.013
IL6 pre-Toci**	3.88	11.48	0.014
IL-5	2.84	35.32	0.019
VEGF	1.93	3.68	0.022
IL-17	1.16	3.13	0.026
IL-7	2.24	6.18	0.10
IP-10	6.63	10.97	0.10
IL-8	8.65	30.72	0.20
IL-4	1.29	1.95	0.24
IL-10	13.84	15.82	0.53

Table S8: Peak fold increase in serum cytokines and severity of cytokine release syndrome (CRS). Serum cytokine concentrations in pg/ml through day 28 were measured by Luminex assay. The median peak fold increase over baseline for each cytokine listed for subjects with no CRS, grade 1 CRS, or grade 2 CRS not receiving tocilizumab (CRS gr 0-2) was compared to median peak fold increase for subjects with grade 3-4 CRS or grade 2 CRS receiving tocilizumab (CRS Gr 3-4 or Gr 2 + toci). Exact p-value by Mann-Whitney test is listed when applicable. *Peak fold-increase of IL-6 at any time post-infusion. **IL-6 pre-Toci = for subjects who got tocilizumab (n=6) or siltuximab (n=1), only pre-tocilizumab or pre–siltuximab IL-6 values were included in analysis. Because the statistical analyses performed here are exploratory and hypothesis-generating in nature, no adjustment of the p-values was made for multiple comparisons.

Cytokine	Median peak fold increase: No neurotox	Median peak fold increase: Any neurotox	p-value
IL-6*	3.92	76.26	0.0002
IFN-γ	1.28	15.77	0.0002
IL-1RA	2.33	19.68	0.0015
MIP-1α	1.53	3.47	0.006
GM-CSF	3.46	36.27	0.013
IL-15	1.76	10.54	0.016
IL-1β	1.32	3.70	0.018
IP-10	5.80	20.44	0.019
IL-7	3.29	10.28	0.019
IL-2Rα	4.46	20.86	0.019
IL-6 pre-Toci**	3.92	20.08	0.032
VEGF	1.93	3.68	0.050
MIP-1β	1.56	2.24	0.056
MIG	7.17	39.14	0.057
MCP-1	2.13	6.41	0.066
IL-8	8.65	36.77	0.12
IL-17	1.32	1.57	0.19
IL-5	5.15	8.54	0.26
IL-4	1.47	2.20	0.40
IL-10	13.70	27.51	0.41

Table S9: Peak fold increase in serum cytokines and neurotoxicity. Serum cytokine concentrations in pg/ml through day 28 were measured by Luminex assay. The median peak fold increase over baseline for each cytokine listed for subjects with no neurotoxicity (neurotox) was compared to median peak fold increase for subjects with any grade neurotoxicity. Exact p-value by Mann-Whitney test is listed. *Peak fold-increase of IL-6 at any time post-infusion. **IL-6 pre-Toci = for subjects who got tocilizumab (n=6) or siltuximab (n=1), only pre-tocilizumab or pre–siltuximab IL-6 values were included in analysis. Because the statistical analyses performed here are exploratory and hypothesis-generating in nature, no adjustment of the p-values was made for multiple comparisons.

Sub	Pre % +	Pre FMO MFI	Pre BCMA MFI	D28 % +	D28 FMO MFI	D28 BCMA MFI	D90 % +	D90 BCMA MFI	D90 BCMA MFI	Best response
Cohort 1										
01	95	568	10864	n/a	n/a	n/a	n/a	n/a	n/a	sCR
02	93	355	2375	97	483	3254	n/a	n/a	n/a	MR
03	84	265	1873	n/a	n/a	n/a	19*	78*	268*	VGPR
07	n/a	n/a	n/a	84	273	1426	n/a	n/a	n/a	PR
08	99	124	6859	n/a	n/a	n/a	n/a	n/a	n/a	PD
09	28	53	206	94	204	1116	n/a	n/a	n/a	SD
10	83	192	1998	99	54	2140	n/a	n/a	n/a	PD
11	81	332	1844	62	49	832	n/a	n/a	n/an	MR
15	99	249	24842	n/a	n/a	n/a	100	362	30685	VGPR
Cohort 2										
12	100	-142	6755	100	187	7268	n/a	n/a	n/a	SD
13	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	MR
14	99	192	4317	98	172	2388	n/a	n/a	n/a	SD
16	97	366	4039	48	394	850	93	488	3819	PR
22	100	321	5947	99	271	4947	100	126	5599	SD
Cohort 3										
17	87	646	2667	27	295	784	76	350	1543	PR
19	98	429	2923	65	316	992	n/a	n/a	n/a	CR
20	89	394	2643	29	369	944	36	185	377	VGPR
21	99	314	2704	86	252	1108	n/a	n/a	n/a	SD
23	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	MR
25	97	283	4000	54	259	833	92	223	2501	PR
27	100	618	6548	74	242	964	70	159	616	VGPR
29	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	SD
32	92	176	3482	85	169	1472	n/a	n/a	n/a	MR
33	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	PR
34	100	303	17109	92	1607	5001	100	309	11232	VGPR

Table S10. Details of BCMA expression on myeloma cells. Bone marrow myeloma cells were gated and BCMA expression analyzed as per Suppl. Figure S8. The percentage of myeloma cells expressing BCMA (% +), as well as the mean fluorescence intensity (MFI) for BCMA and FMO (fluorescence minus one) negative control are depicted. n/a = not available. Pre=pre-treatment. D28 = day 28 post-treatment. D90 = day 90 post-treatment. Sub = subject. *actually D164.

Supplementary References

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2. Flores-Montero J, et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia.* 2017;31(10):2094-103.