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Supplementary Materials for

T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia

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The PDF file includes:

Supplementary Methods

Methods for tumor burden calculation: CLL burden at baseline was estimated as shown in text **Table 1**. The amount of CLL cells were calculated in bone marrow, blood, and secondary lymphoid tissues as described below.

Bone marrow. In healthy adults, the bone marrow represents approximately 5% of total body weight (1, 2). The bone marrow in iliac crest samples has an increasing percentage of inactive (fatty) marrow with age, rising from 20% of the total marrow at age 5 to about 50% by age 35, when it remains stable until age 65, and then rises to about 67% inactive marrow by age 75 (3). The international reference value for the total skeletal weight of active (red) and inactive (fatty) marrow for males at age 35 is currently set at 1.17 kg and 2.48 kg, respectively (4). Adult males between ages 35 to 65 have marrow that represents 5.0% total of body weight, comprised of 1.6% as active (red) marrow and 3.4% as inactive (fatty) marrow (4). Based on the bone marrow biopsy and aspirate specimens, the weight of CLL cells for the 3 patients at baseline was calculated as shown in **Table S9**. These estimates of total CLL marrow mass were then converted to total CLL cell number in the marrow using 1 kg = 10^{12} cells, and the resulting numbers are shown in text **Table 1**. These calculations are based on the assumption that the CLL has a uniform distribution in the bone marrow. For UPN 01, calculations are shown for a marrow biopsy that was obtained before bendamustine chemotherapy, and for an aspirate obtained after bendamustine and pre-CART19 infusion. The numbers are less precise for the day -1 aspirate compared to the day -14 biopsy specimen due to technical limitations of the day -1 aspirate. UPN 02 had a single pre-treatment biopsy specimen showing complete replacement of marrow by CLL. This patient had an unchanged specimen on day 30 post CART19. The marrow burden for UPN 03 was calculated based on a post-chemotherapy and pre-CART19 biopsy.

Blood. Only patient UPN 02 had substantial CLL tumor burden in the blood pre-CART19 infusion. Clinical flow cytometry showed that the cells had a typical phenotype as a clonal population with a dim surface kappa-restricted $CD5^+$ $CD10^-$ $CD19^+$ $CD20(dim)^+$ $CD23(variable)^+$ IgM^- B cell population, and approximately 35% of the CLL cells coexpressed CD38. The CLL burden did not clear with 3 cycles of bendamustine chemotherapy and was present at the time of CART19 infusions. At the time of CART19 infusion, the CLL count in blood was 55,000 cells/μL. Assuming a blood volume of 5.0 L, UPN 02 had $2.75x10^{11}$ CLL cells in blood on day 0. Given the normal overall WBC in patients UPN 01 and 03, we did not calculate the circulating disease burden in these patients, which would lead to a slight underestimate of total body CLL burden.

Secondary lymphoid tissues. The volume of lymphadenopathy and splenomegaly was quantified from axial CT scans using FDA-approved software. The volumes are for chest, abdomen, and pelvis only. Masses from the T1 vertebral body to the level of the bifurcation of the common femoral artery were measured in all patients, and in some, the nodes in the inguinal area were also included. Nodes in the head/neck and extremities were excluded from analysis and excluded from the baseline CLL target cell number, which would also lead to a slight underestimate of total body CLL burden. Patients UPN 01 and 03 have had sustained complete remissions beyond 6 months, and thus the formula (baseline volume - month 3 volume) was used to determine the reduction in tumor burden from baseline; UPN 02 had stable disease in adenopathy, and thus the baseline tumor mass is estimated by subtracting the reference splenic volume from age matched healthy males (5). Baseline tumor mass was converted to CLL cells using a density approach (1 kg/L density, and 1 kg = 10^{12} cells) or a volume approach (CLL cells are 10 μM diameter or 600 fL, assuming spherical shape), and both values are presented in the

text in **Table 1**. The tumor volumes in the 3 patients are shown in **Table S6** as calculated from the available CT scans.

The baseline CT scan for UPN 01 was performed 8 days after 2 cycles of pentostatin/ cyclophosphamide/ rituximab and showed no response to this chemotherapy regimen compared to the previous CT scan. The patient had one cycle of bendamustine before CART19, and thus, the change in tumor volume from Day -37 to Day +31 for UPN 01 cannot exclude the potential contribution of the bendamustine as well as CART19. Similarly, the change in tumor volume for UPN 03 reflects the combined effect of 1 cycle of pentastatin/ cyclophosphamide and CART19.

Method for estimating effective *in vivo* E:T ratio in patients: We used essentially the same approach as in previous pre-clinical studies with humanized mice, where the E:T ratio of infused CAR T cells to the number of tumor cells killed was calculated using the number of tumor cells present at the time of CAR T cell injection and the number of CAR T cells injected (6). For this paper, we have used the number of $CART19^+$ T cells injected (**Table 1**) because, as the $CART19^+$ T cells are dividing, the absolute number of $CART19^+$ T cells present *in vivo* relative to the CLL burden throughout the observed clinical response could not be determined with sufficient accuracy or precision. The available data on CART19 expansion in blood and marrow is robust (**Fig. 2**); however, it was not possible to determine the trafficking of CART19 to other sites such as secondary lymphoid tissues. The calculated values from **Table S10** below were used to derive the effective E:T ratios that are presented in the text.

Sample processing and freezing: Samples (peripheral blood, bone marrow) were collected in lavender top (K_2EDTA,) or red top (no additive) vacutainer tubes (Becton Dickinson) and delivered to the laboratory within 2 hours of draw. Samples were processed within 30 minutes of receipt according to established laboratory SOP. Peripheral blood and marrow mononuclear

cells were purified via Ficoll density gradient centrifugation using Ficoll-Paque (GE Healthcare, 17-1440-03) and frozen in RPMI (Gibco 11875-135) supplemented with 4% human serum albumin (Gemini Bio-Products, 800-120), 2% Hetastarch (Novaplus, NDC0409-7248-49), and 10% DMSO (Sigma, D2650) using 5100 Cryo 1^{o} freezing containers; after 24-72 hours at -80^{o} C, cells were transferred to liquid Nitrogen for long-term storage. Apheresis samples were obtained through the Hospital of the University of Pennsylvania Apheresis/Infusion Unit and processed by Ficoll gradient purification and frozen as above. Viability immediately post-thaw was greater than 85% when assessed. For serum isolation, samples were allowed to coagulate for 1.5-2 hours at room temperature; serum isolated by centrifugation, aliquoted in single use 100 μL aliquots stored at -80^{o} C.

Cell lines: K562 (CML, CD19-negative) was obtained from ATCC (CCL-243). K562/CD19, which is K562 lentivirally transduced at 100% frequency to express the CD19 molecule, was a generous gift of Carmine Carpenito. NALM-6, a CD19-positive non-T, non-B ALL precursor B cell line (7) confirmed to express the CD19 antigen, was a generous gift of Laurence Cooper. The above cell lines were maintained in R10 medium (RPMI 1640 (Gibco, 11875) supplemented with 10% fetal bovine serum (Hyclone), and 1% Pen-Strep (Gibco, 15140-122)). Peripheral mononuclear cells (ND365) from a healthy donor were obtained by apheresis from the Human Immunology Core at the University of Pennsylvania, processed, and frozen as above.

DNA isolation and Q-PCR analysis: Whole-blood or bone marrow samples were collected in lavender top (K_2EDTA) BD vacutainer tubes (Becton Dickinson). Genomic DNA was isolated directly from whole-blood using QIAamp DNA blood midi kits (Qiagen) and established laboratory SOP, quantified by spectrophotometer, and stored at -80^{o}C. Q-PCR analysis on genomic DNA samples was performed in bulk using 123-200 ng genomic DNA/time-point, ABI

Taqman technology and a validated assay to detect the integrated CD19 CAR transgene sequence. Pass/fail parameter ranges, including standard curve slope and r^2 values, ability to accurately quantify a reference sample (1000 copies/plasmid spike), and absence of potential amplification in healthy donor DNA sample were established from the qualification studies and used to define pre-established acceptance ranges for assay performance. Primer/probes for the CD19 CAR transgene were as described (8). To determine copy number/unit DNA, an 8-point standard curve was generated consisting of 10^6 to 5 copies lentivirus plasmid spiked into 100 ng non-transduced control genomic DNA. Each data point (samples, standard curve, reference samples) was evaluated in triplicate with average values reported. For UPN 01, all reported values were derived from a positive Ct value in 3/3 replicates with % CV less than 0.46%. For UPN 02, with the exception of the day +177 sample (2/3 replicates positive, high % CV), all reported values were derived from a positive Ct value in 3/3 replicates with % CV less than 0.72%. For UPN 03, with the exception of the day +1 sample (2/3 replicates positive, 0.8% CV) and the day +3 sample (2/3 replicates positive, 0.67% CV), all reported values were derived from a positive Ct value in 3/3 replicates with % CV less than 1.56%. The lower limit of quantification (LLOQ) for the assay was determined from the standard curve at 2 copies/microgram DNA (10 copies/200 ng input DNA); average values below LLOQ (i.e. reportable not quantifiable) are considered approximate. A parallel amplification reaction to control for the quality of interrogated DNA was performed using 12-20 ng input genomic DNA, a primer/probe combination specific for non-transcribed genomic sequence upstream of the CDKN1A gene (GENEBANK: Z85996) (sense primer: 5'-GAAAGCTGACTGCCCCTATTTG-3', antisense primer: 5'-GAGAGGAAGTGCTGGGAACAAT-3', probe: 5'-VIC- CTCCCCAGTCTCTTT-3'), and an 8

point standard curve created by dilution of control genomic DNA; these amplification reactions produced a correction factor (CF) (ng detected/ng input). Copies transgene /microgram DNA were calculated according to the formula: copies calculated from CD19 standard curve/input DNA (ng) x CF x 1000 ng. Accuracy of this assay was determined by the ability to quantify marking of the infused cell product by Q-PCR according to the formula: Average marking= detected copies/input DNA x 6.3 pg DNA/male somatic cell x CF, versus transgene positivity by flow cytometry using CAR-specific detection reagents. These blinded determinations generated 22.68% marking for the UPN 01 infusion product (22.6% by flow cytometry), 32.33% marking for UPN 02 infusion product (23% by flow cytometry), and 4.3% marking for the UPN 03 infusion product (4.7% marking by flow cytometry).

Cytokine analyses: Quantification of soluble cytokine factors was performed using Luminex bead array technology and kits purchased from Life Technologies (Invitrogen). Assays were performed as per the manufacturer protocol with an 8 point standard curve generated using a 3-fold dilution series. Each standard point and sample was evaluated in duplicate at 1:3 dilution; calculated % CV for the duplicate measures was in most cases less than 5% and always less than 15%. Data were acquired on a Bioplex 200 and analyzed with Bioplex Manager version 5.0 software using 5-parameter logistic regression analysis. Standard curve quantification ranges were determined by the 80-120% (observed/expected value) range. Individual analyte quantification ranges are reported in the Figure legends.

Cellular assay to detect CAR function: Cells were evaluated for functionality after thaw and overnight rest in T cell medium (TCM) (X-Vivo 15 (Lonza, 04-418Q) supplemented with 5% human AB serum (GemCall, 100-512), 1% HEPES (Gibco, 15630-080), 1% Pen-Strep (Gibco, 15140-122), 1% GlutaMax (Gibco, 35050-061), and 0.2% N-Acetyl Cysteine (American Regent,

NDC0517-7610-03) by measuring CD107a degranulation in response to co-culture with CD19 -positive and -negative target cells. Degranulation assays were performed using 1 x 10^6 PBMC and 0.25 x 10^6 target cells in a final volume of 500 μl in 48-well plates for 2 hours at 37^{o}C in the presence of CD49d (Becton Dickinson), anti-CD28, monensin (e-Bioscience) and CD107a-FITC antibody (eBiosciences) as per (9). Following the 2 hour incubation cell mixtures were harvested, washed, and subjected to multi-parametric flow cytometric analysis to identify cell subsets. The gating strategy is presented in **Fig. S3** and involved an initial gate on dump channels (CD14-PE-Cy7, CD16-PE-Cy7, Live/Dead Aqua)-negative and CD3-PE-positive cells, followed by gating on CD8-PE-Texas Red-positive cells.

Antibody reagents: The following antibodies were used for these studies: MDA-CAR, a murine antibody to CD19 CAR conjugated to Alexa647, was a generous gift of Drs. Bipulendu Jena and Laurence Cooper (MD Anderson Cancer Center). For multi-parametric immunophenotyping and functional assays: anti-CD3-A700, anti-CD8-PE-Cy7, anti- PD-1-FITC anti-CD25-AF488, anti-CD28-PercP-Cy5.5, anti-CD57-eF450, anti-CD27-APC-eF780, anti-CD17-APC-eF780, anti-CD45RA-eF605NC, CD107a-FITC (all from e-Bioscience), anti-CD4-PE-Texas Red and Live/Dead Aqua (from Life Technologies) and anti-CD14-V500, anti-CD16-V500 (from Becton Dickinson). For general immunophenotyping: CD3-PE, CD14-APC, CD14-PE-Cy7, CD16-FITC, CD16-PE-Cy7, CD19-PE-Cy7, CD20-PE, all from Becton Dickinson.

Multi-parameter flow cytometry: Cells were evaluated by flow cytometry either fresh after Ficoll-Paque processing or, if frozen, after overnight rest at a density of 2x10^6 cells/ml in TCM. Multi-parametric immunophenotyping was performed using approximately 4x10^6 total cells/condition, using fluorescence minus one (FMO) stains as described in the text. Cells were stained at a density of 1x10^6 cells/100 μL PBS for 30 minutes on ice using antibody and reagent

concentrations recommended by the manufacturer, washed resuspended in 0.5% paraformaldehyde and acquired using a modified LSRII (BD Immunocytometry systems) equipped with Blue (488 nm) Violet (405 nm), Green (532), and Red (633 nm) lasers and appropriate filter sets for the detection and separation of the above antibody combinations. A minimum of 100,000 $CD3^+$ cells were acquired) for each stain. For functional assays, cells were washed, stained for surface markers, re-suspended in 0.5% paraformaldehyde and acquired as above; a minimum of 50,000 $CD3^+$ events were collected for each staining condition. Compensation values were established using single antibody stains and BD compensation beads (Becton Dickinson) and were calculated and applied automatically by the instrument software. Data were analyzed using FlowJo software (Version 8.8.4, Treestar). For general immunophenotyping, cells were acquired using an Accuri C6 cytometer equipped with a Blue (488) and Red (633 nm) laser. Compensation values were established using single antibody stains and BD compensation beads (Becton Dickinson) and were calculated manually. Data were analyzed using C-Flow software analysis package (version 1.0.264.9, Accuri cytometers)

Supplementary Figures

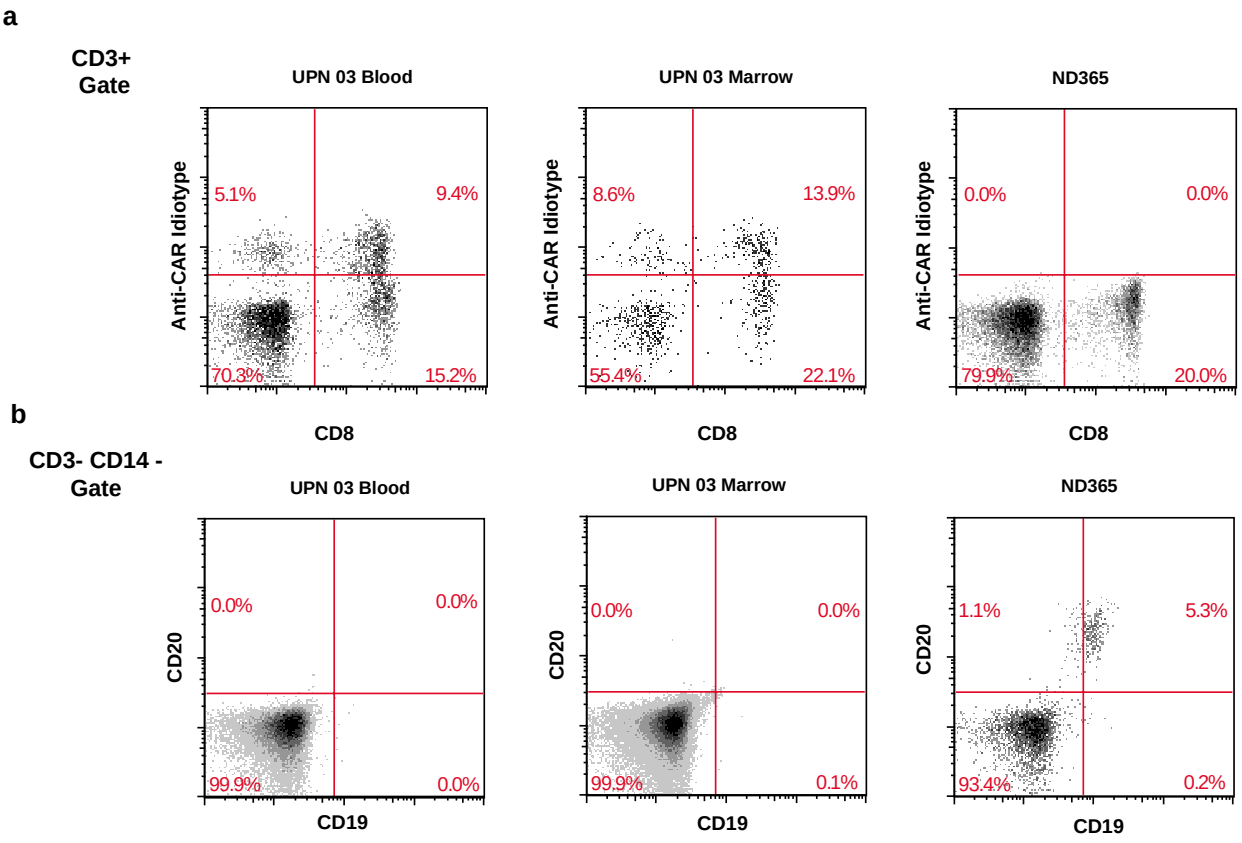

Fig. S1. Prolonged surface CART19 expression and absent B cells in vivo in blood and marrow of UPN 03. Freshly processed peripheral blood or marrow mononuclear cells obtained from UPN 03 at day 169 post-CART19 cell infusion were evaluated by flow-cytometry for surface expression of CAR19 (Panel A) or presence of B cells (Panel B); as a control, PBMC obtained from a healthy donor ND365 were stained. Panel A: To evaluate CAR19 expression in $CD3^+$ lymphocytes, samples were co-stained with antibodies to CD14-PE-Cy7 and CD16-PE-Cy7 (dump channel) and CD3-FITC, positively gated on CD3, and evaluated for CAR19 expression in the $CD8^+$ and $CD8^-$ $CD3^+$ lymphocyte compartments by co-staining with CD8a-PE and the anti-CAR19 idiotype antibody conjugated to Alexa-647. Data in plots are gated on the dump channel-negative/CD3-positive cell population. Panel B: To evaluate the presence of B cells, samples were co-stained with antibodies to CD14-APC and CD3-FITC (dump channels) and evaluated for the presence of B cells in the dump channel-negative fraction by co-staining with antibodies to CD20-PE and CD19-PE-Cy-7. In all cases, negative gate quadrants were established on no-stain controls.

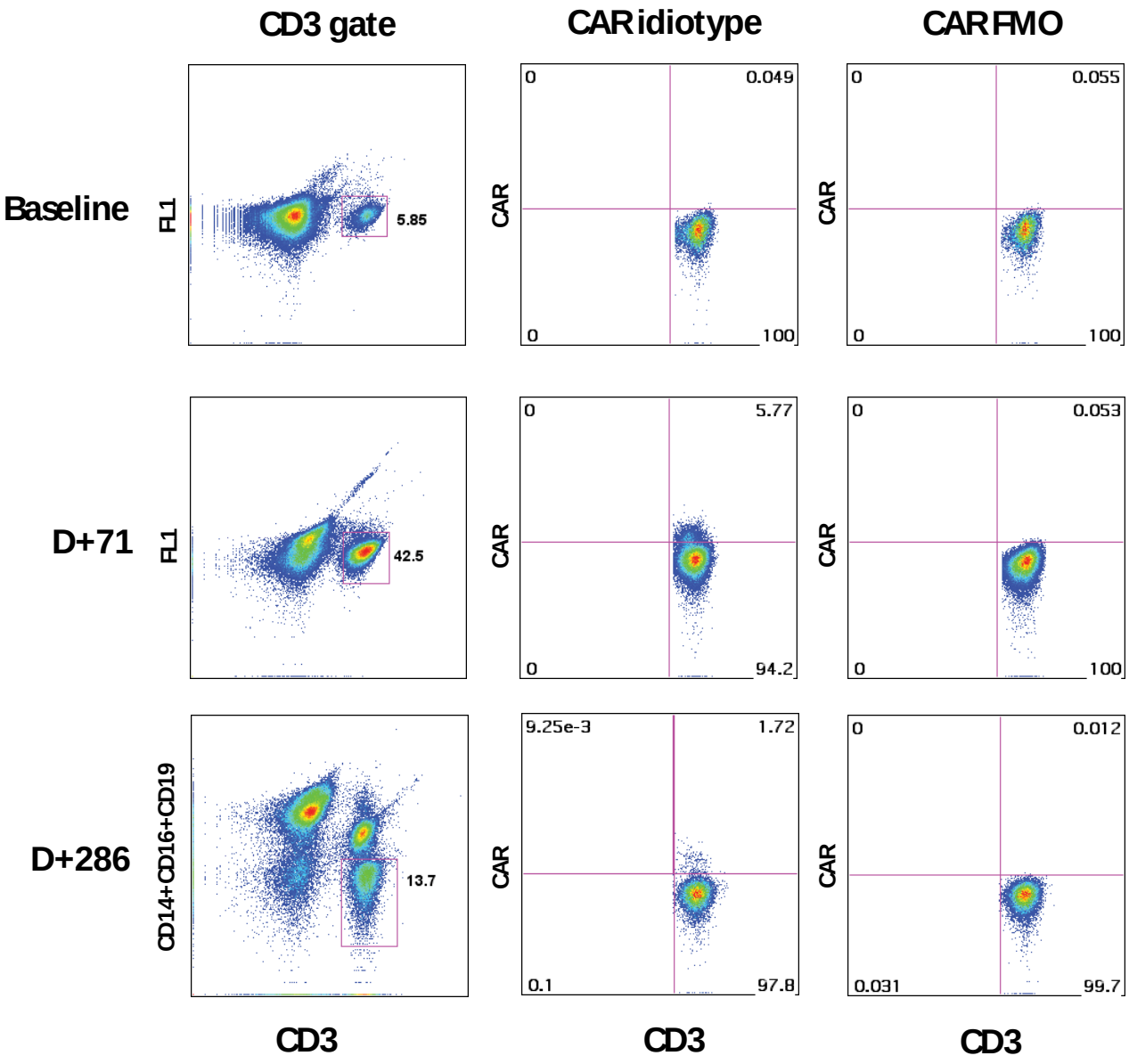

Fig. S2. Direct ex vivo detection of CART19-positive cells in UPN 01 PBMC at 71 and 286 days after T cell infusion. UPN 01 PBMC collected either fresh post-apheresis on day 71 and day 286 post infusion, or frozen at the time of apheresis for manufacture of the T cell product (baseline) and viably thawed prior to the staining, were subjected to flow-cytometric analysis to detect the presence of CART19 cells that express the CAR19 moiety on the surface. To evaluate the expression of CAR19 on lymphocytes, samples were co-stained with CD3-PE and the anti-CAR19 idiotype antibody conjugated to Alexa-647, or co-stained with CD3-PE alone (FMO for CAR19). The primary gate was on all cells, followed by gating on $CD3^+$ cells, showing cells staining for CAR idiotype stain and CAR idiotype FMO. The CAR19-positive idiotype gate was established on the CAR19 FMO samples.

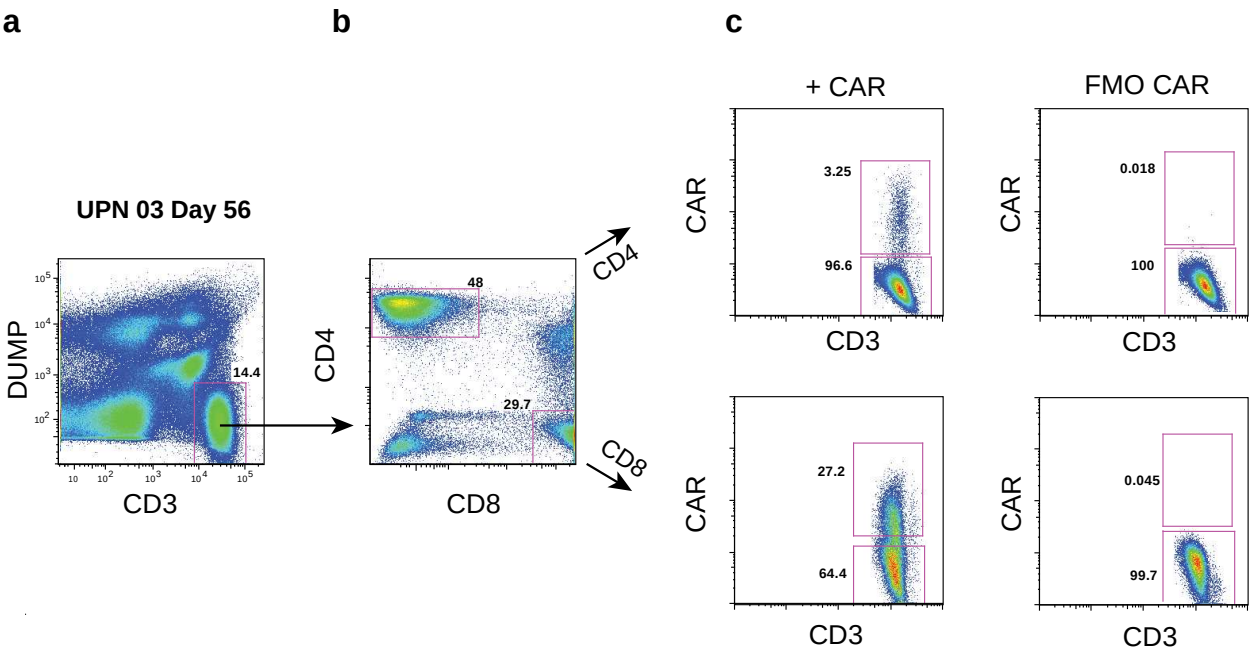

Fig. S3. Gating strategy to identify CART19 expression using polychromatic flow cytometry in UPN 03 blood specimens. The gating strategy for **Fig 4 B, C** is shown for the UPN 03 Day 56 sample and is representative of the strategy used on the UPN 03 Day 169 sample. (**a**) Primary gate: Dump (CD14, CD16, LIVE/dead Aqua) negative, CD3-positive. (**b**) Secondary gates: CD4-positive, CD8-positive. (**c**) Tertiary gates: CAR19-positive and CAR19-negative, established on CAR FMO samples (right-most panels). The gating strategy involved an initial gating on dump channel (CD14, CD16, Live/Dead Aqua)–negative and CD3-positive cells, followed by positive gates on $CD4^+$ and $CD8^+$ cells. Gates and quadrants were established using FMO controls (CAR, CD45RA, PD-1, CD25, CD127, CCR7) or by gating on positive cell populations (CD3, CD4, CD8) and clearly delineated subsets (CD27, CD28, CD57).

Table S1. Apheresis products and CART19 product release criteria.

[1]Dose #2. [2]Assay value at Day 12 below LOQ and had been decreasing from earlier in expansion consistent with carryover of plasmid DNA from vector generation. Submitted to the FDA as an informational amendment. [3]Product release based on surface staining by FACS. [4]Treatment exception granted for release criteria by external DSMC and IRB. [5]data were generated using in-process not final qualified Q-PCR assay.

	Assay	Specification	UPN 01	UPN 02	UPN 03
Apheresis Product					
	Flow Cytometry For $CD3^+$ of $CD45^+$	N/A	4.46%	2.29%	2.67%
CART19 Product					
	Total Cell Number Infused	~2-5 x 10^9	5 x 10^9	1.275 x 10^9 1.275 x 10^9 (1) [2.55 x 10^9 total]	3 x 10^8
	Cell Viability	>= 70%	96.2%	95.3 (90.5)[1]	90.3
	% $CD3^+$ Cells	>= 80%	88.9%	98.8	98.9
	Residual Bead #	<= 100 beads / 3x10^6 Cells	3.95	1	4
	Endotoxin	<= 3.5 EU/mL	<0.5 EU/mL	<0.5 EU/mL	<0.5 EU/mL
	Mycoplasma	Negative	Negative	Negative	Negative
	Sterility (Bactec)	No Growth	No Growth	No Growth	No Growth
	Fungal Culture	No Growth	No Growth	No Growth	No Growth
	BSA ELISA	<= 1 µg/mL	<0.5 ng/mL	<0.5 ng/mL	<0.5 ng/mL
	Replication Competent Lentivirus (RCL)	RCL Not Detectable	Not Detectable	Inconclusive[2]	Inconclusive[2]
	Transduction Efficiency (scFv Expression)	>= 20%	22.6%	23%	4.74%[4]
	Vector DNA Sequence (CART19 PCR)[5]	0.2 - 3 copies/cell	0.15[3]	0.275	0.101

Table S2. Longitudinal measurement of absolute levels for circulating cytokines/chemokines/growth factors in serum from patient UPN 01. Each data point is derived from the average of 2 measurements, with % CV in most cases less than 10%. Reported values are in pg/ml and were calculated by instrument software (Bioplex Manager v5.0). OOR: Out Of Range: Value too high to be quantified (above highest standard curve point and too high to be extrapolated). ND: Not Detected: Value too low to be quantified (below lowest standard curve point and too low to be extrapolated).

	DAY POST-INFUSION															
FACTOR	-1	0	+1	+2	+3	+9	+13	+14	+15	+22	+28	+55	+71	+106	+134	+176
IL-1β	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IL-2	ND	ND	ND	ND	2.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IL-4	ND	ND	ND	ND	ND	3.2	ND	ND	ND	ND	ND	3.2	ND	ND	ND	4.5
IL-5	ND	0.6	0.1	1.1	0.6	0.5	0.1	0.1	ND	0.1	ND	ND	ND	ND	ND	ND
IL-6	6.8	7.0	6.5	5.9	23.5	268.9	46.5	28.7	20.1	15.9	10.9	10.9	2.1	2.7	ND	ND
IL-7	ND	ND	ND	ND	25.2	ND	ND	ND	ND	8.9	ND	25.2	ND	ND	ND	ND
IL-8	12.8	15.3	12.6	15.5	13.3	598.5	282.6	135.5	111.9	125.9	48.5	7.8	11.9	13.7	20.8	15.5
IL-10	14.5	8.5	8.1	11.1	22.3	220.8	41.3	16.7	7.7	6.1	6.9	27.5	3.7	4.9	4.5	4.5
IL-12	783	507	660	638	869	1,119	379	388	266	699	629	484	395	418	397	373
IL-13	ND	ND	ND	ND	ND	13.8	3.1	ND	ND	ND	ND	ND	ND	ND	ND	ND
IL-15	50.8	63.6	64.6	97.3	206.4	533.4	115.1	55.1	14.4	62.0	42.8	44.7	18.5	16.7	5.1	9.7
IL-17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IL-1Rα	909	689	816	765	1,054	2,244	571	487	320	841	833	663	453	537	437	504
IL-2R	6,691	4,319	5,457	5,231	7,680	27,483	22,268	21,887	16,744	8,413	6,264	1,349	674	499	401	313
IFN-α	12.1	21.4	5.3	17.1	25.3	21.4	5.3	5.3	5.3	ND	12.1	21.4	25.3	25.3	28.8	32.2
IFN-γ	2.8	2.8	5.6	5.6	14.8	248.6	2.8	1.4	1.4	1.4	1.4	2.8	2.8	2.8	1.4	2.8
TNF-α	ND	ND	ND	0.3	0.6	3.1	ND	ND	ND	ND	1.0	ND	ND	ND	ND	ND
GM-CSF	ND	ND	ND	ND	ND	64.2	17.8	7.7	14.0	ND	ND	ND	ND	ND	ND	ND

FACTOR	-1	0	+1	+2	+3	+9	+13	+14	+15	+22	+28	+55	+71	+106	+134	+176
G-CSF	60.7	68.5	68.5	33.7	52.4	121.3	132.8	96.5	43.5	75.9	68.5	1,360.0	33.7	52.4	22.4	52.4
MIP-1α	62.6	57.6	56.6	56.6	69.1	233.0	54.6	45.9	22.9	33.5	41.2	52.5	43.6	48.1	36.1	43.6
MIP-1β	112	100	97	101	158	766	43	36	28	43	58	116	64	63	71	73
MCP-1	438	404	458	481	721	2,593	1,093	747	725	753	536	461	564	769	743	657
CXCL9	1,607	1,412	1,682	1,414	1,858	OOR	4,503	2,837	1,375	827	817	1,173	375	359	306	204
CXCL10	530	481	503	596	1,190	OOR	721	446	261	548	361	417	336	388	335	246
VEGF	6.2	6.9	9.0	7.7	9.8	9.4	3.5	5.3	6.4	5.8	10.4	10.9	9.8	6.5	4.2	4.4
RANTES	12,982	9,858	11,569	6,399	OOR	1,927	1,346	1,160	877	2,312	7,197	10,350	10,123	7,576	11,750	11,317
EOTAXIN	75.9	61.2	71.7	58.1	39.7	38.7	46.2	45.6	41.5	66.9	56.5	85.4	117.7	175.8	179.1	219.9
EGF	18.4	18.8	22.5	19.2	53.4	15.8	8.2	1.1	5.9	6.7	19.9	25.1	51.7	43.0	41.2	73.1
HGF	366	337	349	293	372	717	427	397	269	198	186	488	343	285	285	541
FGF-B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table S3. Longitudinal measurement of absolute levels for circulating cytokines/chemokines/growth factors in serum from patient UPN 02. Each data point is derived from the average of 2 measurements, with % CV in most cases less than 10%. Reported values are in pg/ml and were calculated by instrument software (Bioplex Manager v5.0). ND: Not Detected: Value too low to be quantified (below lowest standard curve point and too low to be extrapolated).

	DAY POST-INFUSION											
FACTOR	-1	0	+1	+2	+3	+10	+11	+31	+45	+77	+113	+175
IL-1β	0.9	2.3	0.9	2.1	2.5	1.1	4.5	0.1	0.1	0.9	4.1	ND
IL-2	ND	ND	0.6	1.5	2.3	0.9	1.6	ND	0.1	0.6	0.7	ND
IL-4	6.7	6.7	6.7	1.4	7.6	10.1	8.4	5.9	5.0	8.4	10.1	3.3
IL-5	1.4	1.7	2.2	2.9	2.4	1.6	2.8	1.2	1.1	1.3	1.4	1.2
IL-6	10.9	10.1	14.0	12.5	45.9	16.8	19.5	8.3	8.3	10.7	14.5	9.2
IL-7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IL-8	16.9	14.5	19.7	31.5	39.9	16.4	14.5	5.6	22.4	18.7	77.9	25.1
IL-10	4.6	5.1	5.4	7.5	9.0	8.7	9.6	6.6	6.0	5.4	6.6	5.4
IL-12	189	164	190	167	243	390	368	182	187	282	285	308
IL-13	ND	ND	ND	ND	16.8	10.5	10.5	ND	ND	ND	ND	ND
IL-15	8.6	14.7	21.3	55.0	80.5	22.2	27.1	ND	3.9	ND	1.0	ND
IL-17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IL-1Rα	328	301	371	336	510	683	562	336	301	475	510	527
IL-2R	10,446	9,477	9,883	10,151	12,857	13,598	11,881	9,976	8,008	9,127	10,311	12,178
IFN-α	18.1	12.8	15.6	9.5	18.1	24.6	26.5	22.6	12.8	22.6	18.1	22.6
IFN-γ	ND	ND	ND	ND	1.7	ND	0.4	ND	ND	ND	ND	ND
TNF-α	0.9	0.9	0.6	1.9	3.5	2.6	3.2	ND	ND	1.3	1.3	2.2

FACTOR	-1	0	+1	+2	+3	+10	+11	+31	+45	+77	+113	+175
GM-CSF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G-CSF	16.3	27.3	36.5	ND	66.4	44.7	44.7	36.5	27.3	52.4	59.6	27.3
MIP-1α	66.4	57.3	57.3	57.3	73.0	73.0	74.9	65.4	59.4	71.2	63.5	74.9
MIP-1β	465	370	373	343	351	380	381	440	414	449	200	510
MCP-1	693	561	678	667	724	696	478	455	608	615	646	665
CXCL9	126	126	144	140	317	244	224	79	91	131	196	112
CXCL10	139	115	179	257	410	194	183	123	112	117	145	116
VEGF	9.7	9.0	8.9	6.4	10.0	13.5	13.3	8.4	10.9	13.4	8.2	7.2
RANTES	3,732	3,207	3,162	1,208	4,500	4,491	4,108	3,894	3,860	5,655	4,969	4,230
EOTAXIN	59.8	45.0	40.5	35.3	46.7	63.5	51.3	70.3	73.1	61.7	69.7	66.1
EGF	10.7	10.2	8.3	3.4	27.5	34.0	26.7	16.0	5.4	36.0	19.9	9.7
HGF	296	260	311	542	1,213	452	419	254	290	362	348	371
FGF-B	10.0	10.6	5.3	51.5	5.7	9.7	20.3	ND	9.7	7.1	6.8	4.1

Table S4. Longitudinal measurement of absolute levels for circulating cytokines/chemokines/growth factors in serum from patient UPN 03. Each data point is derived from the average of 2 measurements, with % CV in most cases less than 10%. Reported values are in pg/ml and were calculated by instrument software (Bioplex Manager v5.0) OOR: Out Of Range: Value too high to be quantified (above highest standard curve point and too high to be extrapolated). ND: Not Detected: Value too low to be quantified (below lowest standard curve point and too low to be extrapolated).

	DAY POST-INFUSION														
FACTOR	-1	0	+1	+2	+3	+15	+17	+21	+23	+31	+51	+76	+105	+143	+176
IL-1β	4.0	10.5	ND	8.9	ND	ND	5.5	3.3	ND	ND	ND	ND	ND	ND	ND
IL-2	ND	ND	ND	ND	ND	ND	0.4	ND	ND	ND	ND	ND	ND	ND	ND
IL-4	4.5	ND	ND	ND	ND	4.5	6.9	6.9	8.0	4.5	ND	ND	1.8	ND	1.8
IL-5	0.6	0.6	0.4	0.4	0.4	0.1	0.1	1.0	0.9	1.0	ND	0.4	0.1	ND	ND
IL-6	8.3	8.7	5.3	ND	ND	12.5	52.6	44.2	28.3	10.9	ND	ND	ND	ND	ND
IL-7	37.5	37.5	ND	31.8	33.8	ND	37.5	19.9	56.0	68.5	35.7	ND	33.8	13.3	ND
IL-8	18.6	14.6	8.0	11.7	14.6	69.5	140.7	100.7	47.3	57.3	36.0	45.6	32.2	20.2	19.9
IL-10	1.4	0.7	ND	ND	ND	58.3	178.8	58.1	14.5	ND	ND	ND	ND	ND	ND
IL-12	186	174	170	165	170	246	281	186	157	191	154	165	174	166	158
IL-13	ND	ND	ND	ND	ND	ND	7.5	25.3	27.3	18.8	ND	ND	ND	ND	ND
IL-15	24.2	20.3	13.3	24.5	4.6	114.7	478.3	548.4	294.8	1.4	1.2	ND	1.9	ND	ND
IL-17	ND	ND	ND	10.2	ND	ND	5.1	12.5	ND	ND	ND	ND	ND	ND	ND
IL-1Rα	279	287	254	237	221	546	1,217	1,285	353	279	237	221	237	237	237
IL-2R	644	610	656	633	667	1,115	4,671	16,278	11,008	3,239	261	154	113	118	99
IFN-α	21.4	17.1	17.1	21.4	ND	25.3	33.8	32.2	21.4	25.3	17.1	21.4	12.1	25.3	21.4
IFN-γ	1.4	4.2	2.8	4.2	1.4	28.8	215.1	88.9	21.2	5.6	2.8	1.4	2.8	1.4	1.4
TNF-α	ND	ND	ND	ND	ND	ND	1.3	0.6	ND	ND	ND	ND	ND	ND	ND

FACTOR	-1	0	+1	+2	+3	+15	+17	+21	+23	+31	+51	+76	+105	+143	+176
GM-CSF	ND	ND	ND	ND	ND	ND	2.7	2.7	ND	ND	ND	ND	ND	ND	ND
G-CSF	60.7	68.5	68.5	33.7	43.5	52.4	68.5	89.9	33.7	75.9	52.4	33.7	38.7	33.7	38.7
MIP-1α	52.5	48.1	41.2	41.2	38.7	83.8	159.3	138.8	76.2	54.6	43.6	41.2	41.2	33.5	45.9
MIP-1β	176	174	170	128	168	347	556	225	127	81	85	121	124	96	98
MCP-1	1,212	828	753	622	720	1,352	2,700	1,104	531	521	564	659	748	634	642
CXCL9	177	177	202	163	154	1,631	6,946	OOR	OOR	489	67	48	35	26	44
CXCL10	273	287	253	229	168	918	6,199	OOR	2,484	170	93	111	106	99	106
VEGF	13.3	15.3	9.4	10.2	11.2	9.7	19.4	32.6	29.6	36.4	13.0	13.8	15.3	12.8	8.4
RANTES	10,319	8,983	7,802	8,144	7,051	6,709	6,699	16,683	12,170	9,149	7,080	7,932	8,475	5,316	9,767
EOTAXIN	103.5	82.9	76.5	79.5	88.5	127.5	146.6	106.7	85.4	147.8	114.9	121.6	129.9	128.6	122.6
EGF	119.5	139.6	114.6	73.8	127.6	120.9	119.5	51.7	66.7	262.5	190.5	272.9	186.9	153.1	119.5
HGF	408	381	318	283	360	368	424	482	595	385	324	431	362	341	283
FGF-B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table S5. Longitudinal measurement of absolute levels for marrow cytokines, chemokines, and growth factors in serum obtained from bone marrow samples from patients UPN 01, UPN 02, and UPN 03. Each data point is derived from the average of 2 measurements, with % CV in most cases less than 10%. Reported values are in pg/ml and were calculated by instrument software (Bioplex Manager v5.0). ND: Not Detected: value too low to be quantified (below lowest standard curve point and too low to be extrapolated).

	UPN 01		UPN 02		UPN 03				
FACTOR	+28	+176	-1	+31	-1	+23	+31	+105	+176
IL-1β	ND	ND	2.9	4.7	8.6	ND	ND	ND	ND
IL-2	ND	ND	0.1	3.0	ND	ND	ND	ND	1.0
IL-4	ND	1.8	6.7	5.0	4.5	1.8	4.5	ND	1.8
IL-5	ND	0.1	1.6	1.4	0.9	0.9	1.1	0.1	0.4
IL-6	5.3	1.0	11.2	6.5	7.9	22.7	8.7	ND	ND
IL-7	ND	ND	ND	ND	ND	39.3	60.0	13.3	27.5
IL-8	37.0	8.7	15.9	19.2	15.1	39.3	28.6	16.2	24.1
IL-10	8.8	4.9	4.9	6.0	1.6	15.2	0.4	ND	ND
IL-12	682	350	171	160	170	142	181	172	122
IL-13	ND	ND	ND	ND	7.5	18.8	7.5	ND	ND
IL-15	28.1	15.0	15.0	4.5	34.3	118.6	3.1	1.7	ND
IL-17	ND	ND	ND	ND	7.7	2.3	7.7	ND	ND
IL-1Rα	807	604	319	406	387	270	353	221	403
IL-2R	6,316	359	12,541	10,116	743	10,941	3,472	128	142
IFN-α	25.3	32.2	18.1	18.1	30.5	21.4	32.2	21.4	32.2
IFN-γ	2.8	1.4	ND	ND	4.2	20.0	4.2	2.8	2.8
TNF-α	2.0	6.8	4.0	7.4	5.6	ND	3.5	ND	2.1
GM-CSF	ND	ND	ND	ND	ND	ND	ND	ND	ND
G-CSF	89.9	52.4	16.3	ND	48.0	52.4	43.5	33.7	52.4
MIP-1α	42.4	50.3	61.4	68.3	58.6	79.6	54.6	41.2	51.4
MIP-1β	74	100	640	557	292	103	75	104	97
MCP-1	532	427	612	303	731	363	327	465	339
CXCL9	900	284	79	ND	163	7,234	296	ND	ND
CXCL10	356	354	155	129	431	884	154	82	105
VEGF	1.1	ND	0.7	ND	ND	21.2	24.0	10.4	ND
RANTES	7,223	2,930	2,700	1,280	3,938	8,053	4,767	9,355	2,504
EOTAXIN	58.1	183.6	61.4	56.7	85.5	64.1	112.3	95.7	83.9
EGF	25.1	45.9	25.0	8.3	135.8	58.5	168.9	101.5	123.4
HGF	640	1,886	606	835	1,068	959	1,759	571	1,782
FGF-B	ND	ND	83.1	137.3	ND	ND	ND	ND	ND

Table S6. Calculated CART19 effector/target ratios achieved in vivo. [1]Average of density and volume method. See methods for description. [2]UPN 02 did not respond in bone marrow and had a partial reduction in adenopathy ($3.1x10^{11}$ cells) in the tumor masses measured by CT in spleen and lymph nodes. See **Fig. 5a** for further details.

Patient	Tumor Burden (Baseline and Delta)				$CART19^+$ cells Infused	In Vivo E:T
	Bone marrow Baseline	Blood Baseline	Nodes/Spleen[1] Baseline	Total Change in CLL Burden		
UPN 01	1.70E+12	N/A	8.1E+11	2.51E+12	1.13E+09	1:2200
UPN 02	3.20E+12	2.75E+11	1.6E+12	5.81E+11[2]	5.80E+08	1:1000
UPN 03	8.80E+11	N/A	4.4E+11	1.32E+12	1.42E+07	1:93,000
					Range	1000 - 93,000

Table S7. Percentage and mass of CLL in active bone marrow.

	Wt of Active Marrow (kg)	Wt of Inactive Marrow (kg)	Total marrow (kg)
Normal males (ICRP reference standard)	1.17	2.48	3.65
UPN 01 day -14 (95% cellular)	3.47	0.18	3.65
UPN 02 day -47 (95% cellular)	3.47	0.18	3.65
UPN 03 day -1 (60% cellular)	2.19	1.46	3.65
	Wt of CLL (kg)		
UPN 01 day -14 (70% CLL)	2.43		
UPN 01 day -1 (50% CLL by clot)	1.73		
UPN 02 day -47 (>95% CLL)	3.29		
UPN 03 day -1 (40% CLL)	0.88		

Table S8. Patient tumor volume in secondary lymphoid tissues.

Patient	Study Day	LN volume (mm^3)	Spleen volume (mm^3)	Total volume (mm^3)
UPN 01	-37	239,655	1,619,180	1,858,835
	1 month	105,005	1,258,575	1,363,580
	3 month	65,060	1,176,625	1,241,685
UPN 02	-24	115,990	1,166,800	1,282,790
	1 month	111,755	940,960	1,052,715
UPN 03	-10	239,160	435,825	674,985
	1 month	111,525	371,200	482,725
	3 month	47,245	299,860	347,105

Table S9. Bone marrow plasma cell percentages in patients UPN 01, UPN 02, and UPN 03. Numbers of plasma cells were enumerated in the clinical laboratory by CD138 immunohistochemistry performed on representative bone marrow core biopsies. ND: Not determined, specimen was inadequate. The indicated percentages are estimates determined by an evaluation of an entire bone marrow section. In UPN 01, no $CD138^+$ cells were identified after infusion, whereas in UPN 02 and UPN 03 residual $CD138^+$ cells were present after infusion, at lower levels than in the pre-infusion bone marrows.

Patient	Days after T cell infusion	% plasma cell ($CD138^+$)
UPN 01	-23	1%
	+39	0%
	+116	0%
	+174	0%
UPN 02	-45	2-3%
	-3	2-3%
	+188	1%
UPN 03	-3	3-5%
	+21	1-2%
	+174	1-2%

Table S10. Serum immunoglobulin levels in UPN 01. IgA normal range 50-500 mg/dL, IgM normal range 40-270 mg/dL, IgG normal range 650-2000 mg/dL. Intravenous human immunoglobulin was administered at 500 mg/kg body weight on days +49, +84, +113, +147, +224, +259 post- T cell infusion.

Day From T Cell Infusion	IgA	IgM	IgG
		mg/dL	
-8	<7	12	915
-5	<7	10	765
+5	9	11	623
+43	<7	<4	352
+47	<7	<4	334
+58	<7	<4	733
+85	<7	<4	415
+106	<7	5	623
+114	<7	4	560
+148	<7	5	670
+216	<7	<4	430
+224	<7	<4	372
+230	<7	5	994
+260	<7	<4	525
+286	<7	<4	704

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