SUPPLEMENTARY APPENDIX
METHODS

Patient Eligibility

Inclusion Criteria	Exclusion Criteria
1. Relapsed or refractory LBCL, including: ▪ Diffuse large B-cell lymphoma (DLBCL) ▪ Transformed follicular lymphoma (TFL) ▪ Transformed marginal zone lymphoma ▪ Transformed CLL/SLL (Richter's)	1. For patients with prior allogeneic HSCT: ▪ < 100 days post-transplant ▪ Evidence of active GVHD ▪ < 30 days since last immunosuppression
2. ≥ 2 lines of prior therapy, including: ▪ Anthracycline-containing chemotherapy ▪ Anti-CD20 monoclonal antibody	2. For patients with prior CAR T-cell therapy: ▪ < 30 days post-CAR T-cell infusion ▪ > 5% peripheral blood CD3+ CAR+ cells
3. ≥ 1 measurable lesion by Lugano criteria*	3. Unresolved toxicities from prior therapy
4. CD22 expression at any level on lymphoma cells.	4. For patients with prior or current CNS involvement, any symptoms that interfere with the assessment of neurotoxicity.
5. Age ≥ 18 years	5. Active or uncontrolled infection.
6. ECOG performance status ≤ 2	
7. Adequate bone marrow, renal, hepatic, pulmonary, and cardiac function.	
8. Absolute lymphocyte count ≥ 150 cells/μL	

*PMID: 25113753

Surface Antigen Binding Capacity per Cell Quantification

Peripheral blood, cerebrospinal fluid, or fine needle aspirate tissue specimens were processed within 24 hours of collection (mean +/- hours) and stained using the antibody combination listed **(Table M1)** and analyzed on the BD FACSLyric system. Median fluorescence intensity (MFI) for CD19, CD20, and CD22 were determined under saturating conditions and the antigen bound per cell (a.k.a. antigen density) calculated by calibration with BD Quantibrite beads for PE, APC, and BV421 (BD Biosciences, San Jose, CA) were used to determine the median equivalent saturating fluorescence.

Table M1: Quantibrite antibody panel

Antibody	Fluorochrome	Manufacturer	Part Number
Lambda	FITC	BD Biosciences	346586
Kappa	BV605	BD Biosciences	663192
CD22	PE	BD Biosciences	340708
CD34	PERCP	BD Biosciences	347213
CD10	PE-Cy7	BD Biosciences	341102
CD20	APC	BD Biosciences	340940
CD5	APC-R700	BD Biosciences	565121
CD38	APC-H7	BD Biosciences	653314
CD19	BV421	BD Biosciences	659477
CD45	V500-C	BD Biosciences	647450

CD22.BB.z-based Product Manufacturing

CAR22 products were manufactured in the automated closed-system Miltenyi CliniMACs Prodigy (Miltenyi Biotec) in a 7 day manufacturing process. All days provided in this CAR T production section are reflective of the manufacturing schema **(Figure 1A)**. Fresh patient apheresis product was loaded on the Prodigy on manufacturing day 0 (corresponding to day -15

pre-CAR22 infusion). The apheresis product was enriched for CD4 and CD8 T-cells prior to T-cell activation with TransAct (Miltenyi Biotec). On manufacturing day 1, T-cells were transduced with CD22.BB.z lentiviral vector **(Figure S3C)** at an MOI of 18 for Patient 1 and 2, and an MOI of 9 for Patient 3. TransAct was subsequently washed out on manufacturing day 3, followed by a series of media exchanges. On Day 7, when target dose was achieved, the final product was harvested, sampled for QC testing, and cryopreserved. Product release criteria are listed below **(Table M2)**.

Table M2: CAR22 product release criteria

Product Rapid-Release for Infusion Criteria Table	
Release Test	*Acceptance Criteria*
Cell viability	≥ 70%
Cell number	± 20% of planned dose level
% CD3+ Cells	≥ 70%
% CAR+ cells	≥10%
Endotoxin	< 5 EU/kg body weight
Mycoplasma	Negative
Gram Stain	Negative
Preliminary Sterility (3-5 day)	Negative
Preliminary Fungal (3-5 day)	Negative
qPCR-based Replication Competent Lentivirus (RCL)	Negative
VCN	FIO
Product Phenotyping/Composition	FIO
Product Final Release and Lot Disposition Table	
Release Test	*Acceptance Criteria*
Sterility (14 days)	Negative
Fungal (42 days)	Negative
Cell-based RCL	Negative

Phenotyping of Manufacturing Samples at Apheresis, Enrichment, and Final Product Harvest

All samples were washed in FACS Buffer (1× PBS, 2% FBS) and stained for a minimum of 30 minutes at 4ºC, prior to additional washes and running on the MACSQuant flow cytometer (Miltenyi). MACS Comp bead kit (Miltenyi, 130-097-900) was used for compensation controls, with the respective antibody below **(Table M3)**.

Table M3: MACS Comp bead kit antibody-fluorochrome conjugate panel.

Antibody	**Fluorochrome**	**Supplier**	**Part Number**
CD45	VioBlue-REA747	Miltenyi	130-110-637
CD3	FITC-REA613	Miltenyi	130-113-138
CD4	VioGreen-VIT4	Miltenyi	130-113-221
CD8	APC-Vio770-BW135/80	Miltenyi	130-113-155
CD56	PE-REA196	Miltenyi	130-113-312
CD16	PE-REA423	Miltenyi	130-113-393
CD14	APC-REA599	Miltenyi	130-110-520
CD20	PE-Vio770-LT20	Miltenyi	130-113-375

Flow Cytometry for Phenotyping and Exhaustion Profiling of CAR22 Products

All samples were washed in FACS Buffer (1X PBS, 3% FBS), stained for a minimum of 30 minutes at 4ºC, prior to additional washes and running flow cytometry. UltraComp ebeadsTM

(Invitrogen, 01-2222-41) were used for compensation controls, stained with the respective antibody from the antibody index below. Samples were run on the Fortessa X20 (BD Biosciences) and stained using antibodies below **(Table M4)**.

Table M4: CAR22 product immunophenotyping antibody panel

Antigen	Fluorochrome	Supplier	Part Number
CD3	BUV496	BD Biosciences	564809
CD4	APC-Cy7	BioLegend	317418
CD8	BUV805	BioLegend	564912
Recombinant Human Siglec-2/CD22β Fc Chimera	DyLight650	R&D Systems	1968-SL-050
CD45RO	PE-Cy7	eBioscience	25-0457-42
CD45RA	BV711	BD Biosciences	563733
CCR7	FITC	BD Biosciences	561271
CD62L	BV605	BioLegend	562719
CD95	BV421	BioLegend	305624
CD39*	FITC	BioLegend	328206
PD-1*	PE-Cy7	BD Biosciences	561272
CD57*	BV605	BioLegend	393304

* Additional markers used to evaluate for T-cell exhaustion.

Lymphocyte Subset (CAR+ and CAR-) Quantification from Peripheral Blood

A High Dimensional (Hi-D) immuno-phenotyping flow cytometry panel was designed for tracking chimeric antigen receptor (CAR) positive and CAR negative T-cell lineage-specific surface antigens, as well as target B-cell lineage-specific surface antigens in patient samples in real time – referred to as CAR-FACS. Peripheral blood mononuclear cells (PBMCs) were isolated from fresh whole blood by density gradient centrifugation using the Ficoll-Paque Plus system (Sigma-Aldrich; St. Louis, MO). PBMCs (average yield: 2-5×10^6 cells) were then stained with Live/Dead Aqua Fixable Viability Stain (Thermo Fisher; Waltham, MA), then pre-incubated with Fc receptor blocking solution (Human TruStain FcX, BioLegend; San Diego, CA) for 5 minutes. After incubation, the fixed cells were stained at room temperature (RT) in an 11-color, 13-parameter combination with fluorochrome-conjugated monoclonal antibodies (mAb) **(see Table M5)**. CD22.BB.z-transduced cells were used as a positive control and included in daily experiments. Stained and fixed cells were acquired on a BD LSRII analyzer using FACSDiva software (BD Biosciences) and analyzed with Cytobank software (Cytobank, Inc; Santa Clara, CA). The lower limit of quantification for the assay was 1 cell per 10,000 viable PBMCs (0.01%). B-cells were defined as live CD45+, CD3-, CD4-, CD8-, CD14-, and CD19+ and/or CD22+ cells. CD4+ T-cells were defined as live CAR+, CD45+, CD3+, CD4+, CD8-, CD14-, CD19- cells. CD8+ T-cells were defined as live CAR+, CD45+, CD3+, CD4-, CD8+, CD14-, CD19- cells.

Table M5: CAR-FACS antibody panel

Antigen	Fluorochrome	Clone	Supplier	Part Number
CD3	FITC	UCHT1	BioLegend	300406
CD19	PE	SJ25C1	BioLegend	363004
CD14	PE-Cy7	63D3	BioLegend	367112
CD8	PerCP-Cy5.5	SK1	BD Pharmingen	565310
Recombinant Human Siglec-2/CD22β Fc Chimera	DyLight650	CAA42006	R&D Systems	1968-SL-050
CD56	APC-Fire750	5.1H11	BioLegend	362554

Live/Dead-Viability	BV450	--	Thermo Fisher	L-34964
CD22	BV421	HIB22	BioLegend	302524
CD20	BV605	2H7	BioLegend	302334
CD4	BV711	RPA-T4	BioLegend	300558
CD45	BV785	2D1	BioLegend	368528

qPCR Measurement of CAR+ Cells from Peripheral Blood

DNA was extracted from PBMCs (average yield: 2-5×10^6 cells) using QIAmp DNA Mini Kit (Qiagen # 51306) at baseline, and Days 7, 14, 21, 28, 90, and 180 post-CAR22 infusion. CD22.BB.z presence was measured by quantitative polymerase chain reaction (qPCR) using the primer and probe sequences provided **(Table M6)**. For the standard curve, a custom Minigene® plasmid (IDT) was designed containing a partial CD22.BB.z sequence and a partial albumin sequence, which served as a control for normalization. The standard curve contained a ten-fold serial dilution of plasmid between 5×10^5 and 5 copies/µL. Both plasmid and patient DNA from each time point were run in triplicate, with each reaction containing 5 µL of DNA (50 ng total), 100 nM forward and reverse albumin primers (or 200 nM forward and 200 nM reverse CD22.BB.z primers), 150 nM probe suspended in 10 µL of TaqMan Fast Universal PCR Master Mix (2×), No AmpErase® UNG or equivalent (Thermo Fisher Scientific) and 5 µL of TE buffer (Invitrogen # AM9935). The Bio-Rad CFX96 Touch Real-Time PCR Detection System was used for qPCR with 20 µL per reaction. The quality metrics for all qPCR standard curve results were R^2 > 0.99, -3.46 > slope > -3.69 and efficiency > 87%.

Table M6: qPCR reagents for CAR22

Reagent	**Sequence**
Albumin FAM Probe	5' - CCT GTC ATG CCC ACA CAA ATC TCT CC - 3'
Forward Primer Albumin	5' - GCT GTC ATC TCT TGT GGG CTG T - 3'
Reverse Primer Albumin	5' - ACT CAT GGG AGC TGC TGG TTC - 3'
CD22 FAM Probe	5' - /56-FAM/CT GGC GTC G/ZEN/T GGT TGC GGC /3IABkFQ/ - 3'
Forward Primer CD22	5' - GGA CCA AGC TGG AAA TCA AAG C - 3'
Reverse Primer CD22	5' - CGC CGG TGT TGG TGG T - 3'
CD22 Albumin Minigene® Plasmid	AGC TAC AGC ATC CCC CAG ACC TTC GGC CAG GGG ACC AAG CTG GAA ATC AAA GCG GCC GCA ACC ACG ACG CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GCG TCG CAG CCC CTG TCG CTG GCC TTT TGC TCA CAA GCT TGG GGT TGC TGT CAT CTC TTG TGG GCT GTA ATC ATC GTC TAG GCT TAA GAG TAA TAT TGC AAA ACC TGT CAT GCC CAC ACA AAT CTC TCC CTG GCA TTG TTG TCT TTG CAG ATG TCA GTG AAA GAG AAC CAG CAG CTC CCA TGA GTC CCA AGC TAT GTT CTT TCC TGC GTT

Cell-free Tumor DNA Measurement from Patient Plasma

Tumor DNA was extracted from archival formalin-fixed, paraffin-embedded (FFPE) tissue and PCR amplification of IgH-VDJ, IgH-DJ, and Ig Kappa/Lambda regions using universal consensus primers was performed by next generation sequencing (NGS) to determine the tumor clonotype(s) as previously described [PMID: 32605647]. Circulating tumor DNA (ctDNA) was measured from plasma extracted within 6 hours from blood obtained in EDTA tubes using a research application of clonoSEQ (Adaptive Biotechnologies, Seattle, WA) at the following timepoints: pre-LD chemotherapy (day -6), days 0, 7, 14, 21, 28, 60, 90, 180, and 270 post-CAR22 infusion.

Cytokine Measurement from Patient Serum

Serum was isolated from peripheral blood by spinning at 1200×g for 10 minutes at room temperature, and subsequently aliquoted and frozen for future batched analysis. Frozen serum samples were thawed, centrifuged at 14,000 rpm and diluted 3× in 1× PBS, prior to running a 50-plex Luminex bead kit (Affimetrix). A set of 4 AssayChex QC beads were added to each well, each with a unique fluorescence, to provide quality assurance as follows: Chex1 (addition of biotinylated detector antibodies), Chex2 (addition of streptavidin-PE), Chex3 (instrument performance), and Chex4 (non-specific background fluorescence). Additionally, total bead count (>2000) and individual bead counts (>40) are verified and wells below threshold are flagged for possible exclusion. Serum and serial dilutions of cytokine standards were added to respective wells, and plates were incubated for 2 hours with shaking at room temperature, followed by an 18 hour incubation at 4ºC. Plates were then washed and developed according to the manufacturer protocol, and samples were acquired on the Luminex MAP200.

Stanford Post-CAR Toxicity Management, Supportive Care, and Antimicrobial Prophylaxis

Guidelines were initially adopted in December 2017, and subsequently modified in accordance with most up to date evidence base (last June 2020).

CAR T-cell-associated toxicities were managed in accordance with the CARTOX working group recommendations [PMID: 28925994]. Treatment with tocilizumab (8 mg/kg IV per dose) and/or corticosteroids (dexamethasone 10 mg IV per dose or equivalent) was utilized for any patient who developed grade ≥2 CRS and/or neurotoxicity (ICANS), respectively. Dose escalation parameters were specified for worsening CRS and/or ICANS, or if not improving after 24 hours.

Post-hospital discharge monitoring included visits at least weekly in the outpatient infusion center through day 28, followed by outpatient clinic visits and lab monitoring on post-infusion days 28, 60, 90, 180, 270, and 365. Granulocyte colony-stimulating factor (G-CSF) 5 µg/kg per day subcutaneously was administered after lymphodepletion until the absolute neutrophil count (ANC) was >1000 cells per µL, and repeated once daily for three days anytime the ANC was <1000 cells per µL. Initial institutional antimicrobial prophylaxis recommendations were modified from the post-autologous stem cell transplant setting based on best available data. All patients regardless of serologic status received acyclovir 800 mg twice a day or valacyclovir 500 mg twice a day starting with LD chemotherapy until at least 18 months after CAR-T infusion for herpes simplex (HSV) and varicella zoster virus (VZV) infection prophylaxis. Beginning on day 28 post-infusion, all patients without ongoing cytopenias received trimethoprim 80 mg/sulfamethoxazole 400 mg (Bactrim SS) once daily (or atovaquone 1500 mg once daily if ongoing cytopenias were present) until at least 12 months after CAR-T infusion, or until recovering an absolute CD4+ T-cell count >200 cells per µL for *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis. Serum immunoglobulin G (IgG) levels were evaluated every 2-3 months following CAR-T infusion, and IVIG 0.5 g/kg was recommended if recurrent or severe sinopulmonary infections developed with a serum IgG concentration <400 mg/dL.

TABLES

Table S1: Patient and disease characteristics.

Characteristic	Patient 1	Patient 2	Patient 3
Age at enrollment, years	57	53	51
Sex	Female	Female	Male
ECOG PS	1	1	1
Disease Classification			
2016 WHO classification	TFL	HGBCL	HGBCL
IHC and FISH status*	DEL	DHL	DHL
Cell of origin subgroup**	GCB	GCB	GCB
CD19 expression***	>90%	>90%	0%
CD22 expression***	>90%	>90%	>90%
Prior Therapy			
Prior lines of therapy†, n	5	8	6
Prior CAR T-cell therapy	Yes	Yes	Yes
Product(s) received	Axicabtagene ciloclecuel	Lisocabtagene maraleucel	(1) Axicabtagene ciloleucel (2) CD20-19.BB.z
Antigen(s) targeted	CD19	CD19	(1) CD19 (2) CD19 & CD20
Disease Status at Enrollment			
Lugano stage	III	IV	III
IPI score	2	3	2
Response classification‡	Chemorefractory	Primary Refractory	Relapsed
Bulky disease status§	Yes	Yes	No
SPD (cm^2)	68.96	95.26	10.94
Pre-LD LDH (U/L)	402 (1.88 ×ULN)	1531 (7.15 ×ULN)	263 (1.17 ×ULN)
Received bridging therapy‖	No	No	No
CAR22 Efficacy and Toxicity			
Maximum CRS grade¶	2	1	2
Duration of CRS (days)	2	15	7
Maximum ICANS grade¶	0	0	0
Best response achieved#	CR	CR	CR

* Double expressor lymphoma (DEL) was defined as ≥50% staining for MYC *and* BCL2 protein when measured by immunohistochemistry (IHC). Double hit lymphoma (DHL) was defined as the presence of a *MYC* and *BCL2 or BCL6* rearrangement when measured by fluorescent in situ hybridization (FISH) with break-apart DNA probes. -004 and -006 both harbored *MYC* and *BCL2* rearrangements; both patients were also DEL.

** Determined by IHC, using Hans algorithm [PMID: 14504078].

*** Measured by flow cytometry (FCM). CD19 and CD22 expression were assessed by both IHC and flow cytometry; all biopsies had concordant results between modalities.

† See Table S1 for prior treatment history.

‡ Chemorefractory disease was defined as SD or PD to last line of therapy, or relapse ≤12 months following autologous SCT. Primary refractory disease was defined as chemorefractory disease to frontline chemoimmunotherapy.

§ Defined as a single nodal mass ≥10 cm in diameter, or ≥1/3 of the transthoracic diameter.

| Bridging therapy was defined as any lymphoma-directed therapy administered after leukapheresis and before lymphodepletion (LD) chemotherapy.
¶ Graded according to ASTCT concensus criteria [PMID: 30592986].
# Graded according to Lugano criteria [PMID: 25113753].

Abbreviations: ECOG=Eastern Cooperative Oncology Group; PS=performance status; WHO=World Health Organization; TFL=transformed follicular lymphoma; HGBCL=high grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements; IHC=immunohistochemistry; FISH=fluorescent in situ hybridization; DEL=double expressor lymphoma; DHL=double hit lymphoma; GCB=germinal center B-cell-like; CAR=chimeric antigen receptor; CD20-19.BB.z=anti-CD19, anti-CD20 tandem CAR T-cell therapy with 41BB and CD3ζ costimulatory domains; IPI=International Prognostic Index; SPD=sum of product of diameters; LD=lymphodepletion chemotherapy; LDH=serum lactate dehydrogenase; ULN=upper limit of normal; CRS=cytokine release syndrome; ICANS= immune effector cell-associated neurotoxicity syndrome; CR=complete response.

Table S2: Prior treatment history for patients.

Patient 1

Line	Therapy	Best Response*	Outcome
1	DA-EPOCH-R ×2 cycles	NA	Unable to tolerate
1	R-CHOP21 ×4 cycles	CR	Relapsed 5 months after EOT
2	R-DHAP ×3 cycles**	PR	PD 2 months after last cycle
3	Axicabtagene ciloleucel	PR	PD 3 months post-infusion
4	R2 ×2 cycles	PD	No response
5	R-GemOx ×2 cycles	SD	Unable to tolerate
6	CAR22	CR	Ongoing 9 months post-infusion

Patient 2

Line	Therapy	Best Response*	Outcome
1	R-CHOP21 ×6 cycles	PR	Primary refractory disease
2	R-ICE ×2 cycles	PD	No response
3	R-GemOx ×3 cycles	PR	Unable to tolerate
4	Lisocabtagene maraleucel**	PR	PD 3 months post-infusion
5	CC-90002 + rituximab ×1 cycle**	PD	No response
6	R-DHAOx + venetoclax ×2 cycles**	PD	No response
7	Lenalidomide ×1 cycle	NA	Unable to tolerate
8	Pola-BR ×2 cycles	SD	No response
9	CAR22	CR	Ongoing 9 months post-infusion

Patient 3

Line	Therapy	Best Response*	Outcome
1	CHOP ×3 cycles + ISRT	CR	Relapsed 20 years after EOT
2	R-GDP ×4 cycles	PR	PD after 4 cycles
3	Axicabtagene ciloleucel	SD	PD 3 months post-infusion
4	R2 ×1 cycle	SD	No response
5	CAR20.19	CR	Relapse 12 months post-infusion
6	R-GemOx ×1 cycle	PR	Unable to tolerate
7	CAR22	CR	Ongoing 6 months post-infusion

* Graded according to Lugano criteria [PMID: 25113753].

Abbreviations: DA-EPOCH-R=dose-adjusted infusional etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab, cycle length 21 days; R-CHOP21=rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone, cycle length 21 days; R-DHAP=rituximab, dexamethasone, high dose cytarabine, and cisplatin, cycle length 21 days; Axicabtagene ciloleucel= anti-CD19 chimeric antigen receptor T-cell therapy, FMC63.28.z construct (Yescarta); R2=lenalidomide and rituximab, cycle length 28 days; R-GemOx=rituximab, gemcitabine, and oxaliplatin, cycle length 21 days; CAR22=anti-CD22 chimeric antigen receptor T-cell therapy, M971.BB.z construct; NA=not applicable; CR=complete remission; PR=partial response; PD=progressive disease; SD=stable disease; EOT=end of treatment; R-ICE=rituximab, ifosfamide, carboplatin, and etoposide, cycle length 21 days; Lisocabtagene maraleucel=anti-CD19 chimeric antigen receptor T-cell therapy, FMC63.BB.z construct (JCAR017); CC-90002=anti-CD47 monoclonal antibody (Celgene); R-DHAOx=rituximab, dexamethasone, high-dose cytarabine, and oxaliplatin, cycle length 21 days; Pola-BR=rituximab, bendamustine, and polatuzumab vedotin, cycle length 21 days; ISRT=involved site radiotherapy; CAR20.19=anti-CD19, anti-CD20 tandem CAR T-cell therapy.

Table S3: Treatment-emergent adverse events for patients.

	N = 3				
TEAE, n (%)	**Any**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Hematologic disorders					
Neutropenia	3 (100)	0	0	0	3 (100)
Thrombocytopenia	3 (100)	0	0	1 (33.3)	2 (66.7)
Anemia	3 (100)	0	0	3 (100)	0
Cytokine release syndrome					
CRS**	3 (100)	1 (33.3)	2 (66.7)	0	0
Fever	3 (100)	1 (33.3)	1 (33.3)	1 (33.3)	0
Hypotension	2 (66.7)	1 (33.3)	1 (33.3)	0	0
Tachycardia	2 (66.7)	1 (33.3)	1 (33.3)	0	0
Hypoxia	1 (33.3)	0	0	1 (33.3)	0
Chills	1 (33.3)	1 (33.3)	0	0	0
Nervous system disorders					
ICANS**	0	0	0	0	0
Headache	1 (33.3)	0	1 (33.3)	0	0
Infections					
Upper respiratory tract	2 (66.7)	0	2 (66.7)	0	0
Vaginal candidiasis	1 (33.3)	0	1 (33.3)	0	0
Otitis media	1 (33.3)	0	1 (33.3)	0	0
Gastrointestinal disorders					
Nausea	1 (33.3)	1 (33.3)	0	0	0
General disorders					
Fatigue	2 (66.7)	0	2 (66.7)	0	0
Sore throat	1 (33.3)	1 (33.3)	0	0	0
Metabolic disorders					
Hypocalcemia	1 (33.3)	0	1 (33.3)	0	0
Connective tissue disorders					
Myalgia	1 (33.3)	1 (33.3)	0	0	0
Pruritis	1 (33.3)	1 (33.3)	0	0	0
Renal and urinary disorders					
Acute kidney injury	1 (33.3)	1 (33.3)	0	0	0

* Graded per CTCAE v5.0.
** Graded per ASTCT consensus criteria [PMID: 30592986].

Abbreviations: TEAE=treatment-emergent adverse event; CRS=cytokine release syndrome; ICANS=immune effector cell-associated neurotoxicity syndrome; CTCAE=Common Terminology Criteria for Adverse Events; ASTCT=American Society for Transplantation and Cellular Therapy.

Table S3: PET-CT index lesions.

Patient 1

	# 1		**# 2**	
Location	Mesenteric nodal mass		R inguinal LN	
Timepoint	*Diameter(s)**	*SUV*	*Diameter(s)**	*SUV*
Pre-LD	10.3 × 6.6	37.9	1.4 × 0.7	3.4
Day 28	8.1 × 7.3	14.0	1.1 × 0.5	10.4
Month 3	6.8 × 6.2	4.8	1.1 × 0.7	2.9
Month 6	6.6 × 6.4	4.2	1.2 × 0.8	1.4

Patient 2

	# 1		**# 2**	
Location	R pulmonary mass		R subcutaneous mass	
Timepoint	*Diameter(s)**	*SUV*	*Diameter(s)**	*SUV*
Pre-LD	9.1 × 7.6	27.2	9.0 × 2.9	22.7
Day 28	8.6 × 7.7	8.1	9.8 × 3.4	6.1
Month 3	6.3 × 4.7	3.5	6.0 × 2.5	2.8
Month 6	6.3 × 4.8	5.2	5.0 × 1.9	6.0

Patient 3

	# 1		**# 2**		**# 3**		**# 4**	
Location	L axillary LN		R cervical LN		L intraparotid nodule		Mesenteric LN	
Timepoint	Diameter(s)*	SUV	Diameter(s)*	SUV	Diameter(s)*	SUV	Diameter(s)*	SUV
Pre-LD	3.2 × 2.4	20.0	0.8 × 0.8	3.6	2.3 × 1.1	12.0	0.3 × 0.3	7.0
Day 28	1.9 × 0.9	2.6	NI	NI	NI	NI	NI	NI
Month 3	1.5 × 0.7	1.3	NI	NI	NI	NI	NI	NI

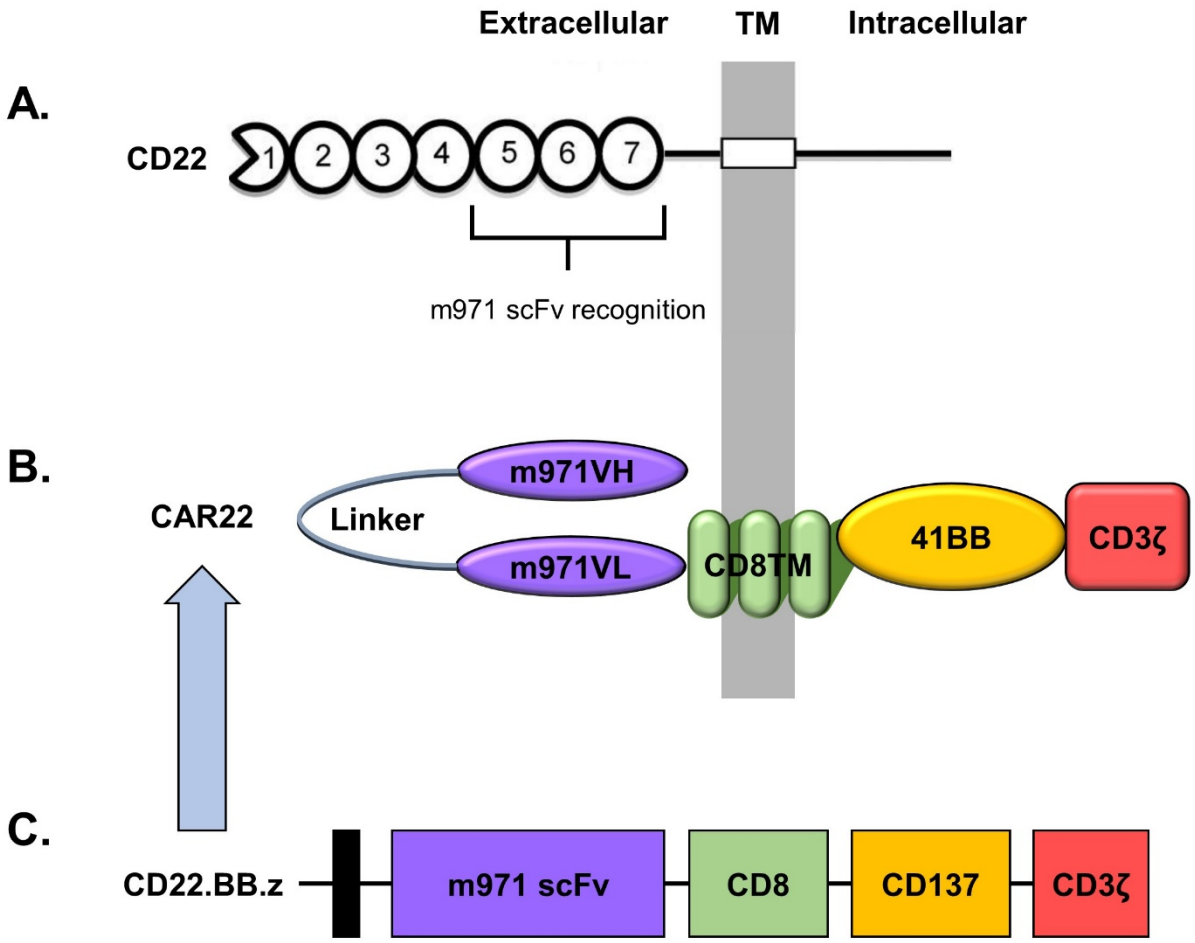
* Measured on CT imaging portion of composite positron emission tomography (PET) scan; dimensions given as long axis × short axis in centimeters.

Abbreviations: PET-CT= composite positron emission tomography-computed tomography imaging; SUV=standardized uptake value; R=right; L=left; LN=lymph node; LD=lymphodepletion chemotherapy; NI=not identified.

FIGURES

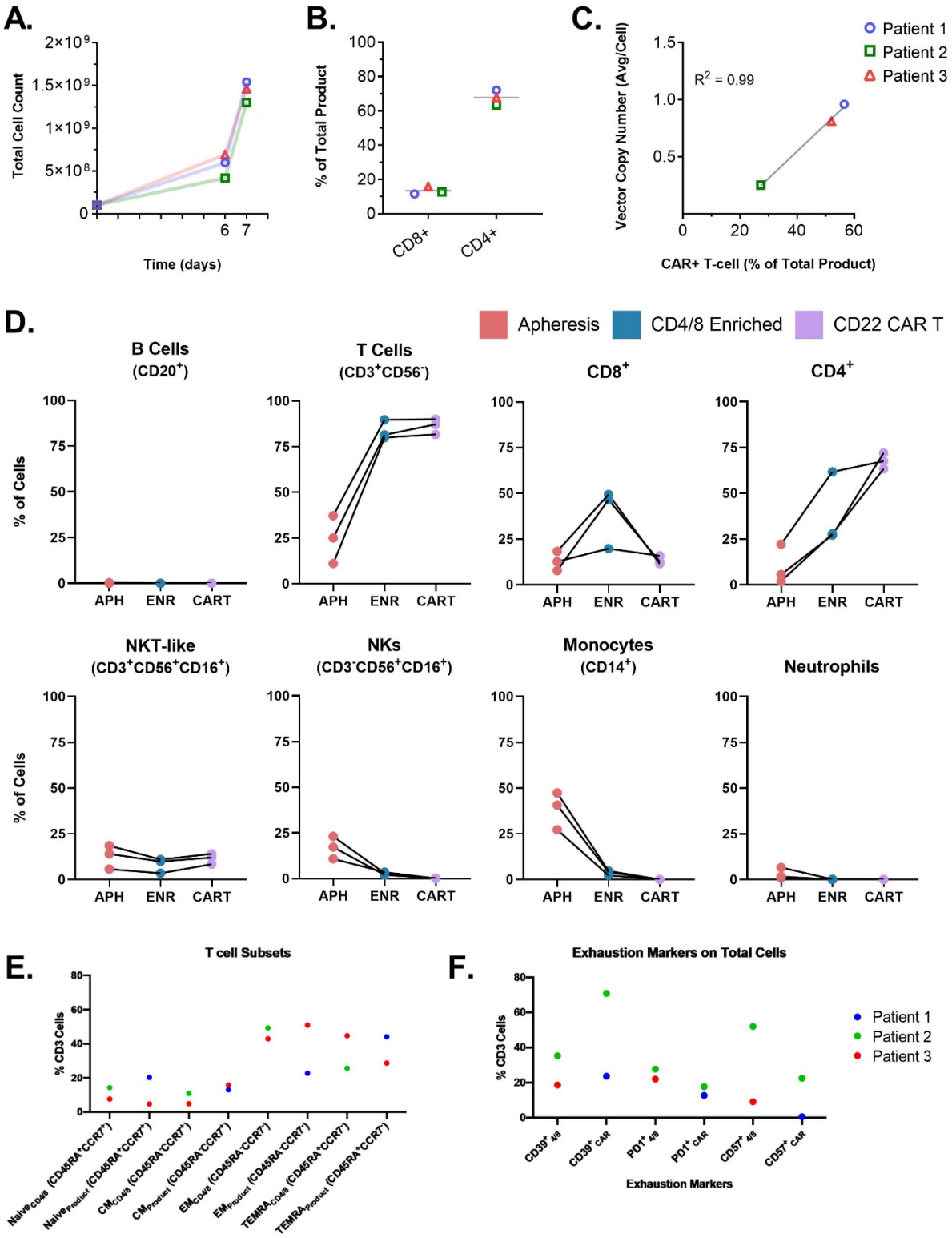
Figure S1: CAR22 vector

The anti-CD22 binding domain (m971) recognizes the proximal extracellular domains of CD22 (A). The CAR22 construct is a second generation CAR modified to incorporate a 41BB costimulatory domain (B) as previously described [PMID: 29155426; 23243285]. In addition to the m971 scFv binding domains connected by a linker sequence, the CD22.BB.z vector also includes a CD8 hinge and transmembrane domain, and a CD3ζ activation domain (C).



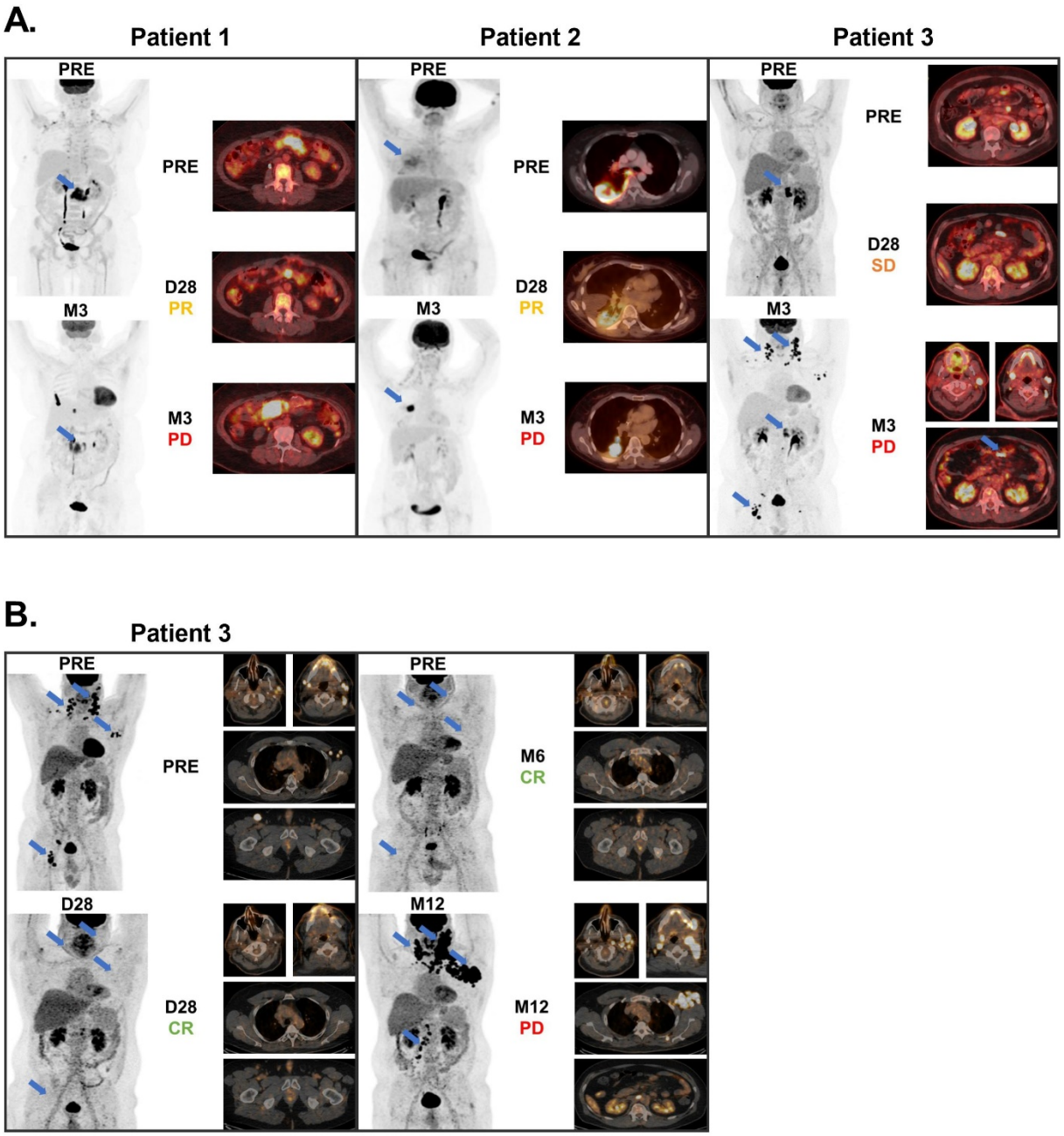
Abbreviations: TM=transmembrane; scFv=single-chain variable fragment; CAR22=anti-CD22 chimeric antigen receptor T-cell therapy; VH=variable region of immunoglobulin heavy chain; VL=variable region of immunoglobulin light chain.

Figure S2: CAR22 product manufacturing characteristics and immunophenotyping
(A) Culture expansion produced effective dosages by 7 days. (B) Phenotyping of CAR22 product demonstrated a skewing towards CD4+ cells. (C) Average transduction efficiency was 45.3%. (D) Composition of apheresis, CD4/8 enriched, and CAR22 products demonstrating the evolution over the course of manufacturing for B-cell (CD20+), T-cell (CD3+, CD56-), CD4+, CD8+, NKT-like (CD3+, CD56+, CD16+), NK (CD3-, CD56+, CD16+), Monocyte (CD14+), and Neutrophil subsets. (E) Phenotyping of T-cell memory subsets reveals a predominance of TEMRA and TEM subsets in CD4/8 enriched samples, with a subset of TN and TCM subsets mildly enriched in the final CAR22 product. (F) Markers of T-cell exhaustion including CD39, PD1, and CD57 were expressed in a minority of cells in the CAR22 product.



Abbreviations: APH=apheresis; ENR=CD4+ and CD8+ T-cell enriched apheresis product; CART=final CAR22 product; CM=central memory T-cell subset; EM=effector memory T-cell subset; TEMRA=CD45RA+ effector memory T-cell subset.

Figure S3: Response to prior CAR T-cell therapy for all patients
(A) Maximum intensity projections (MIP) and PET-CT composite cross-sectional imaging for primary index lesions at specified assessment timepoints (A) following infusion of CAR19 therapy; and in the case of Patient 3 (B) following infusion of CAR20.19 therapy. Two-dimensional MIP images are shown on the left of each panel, with blue arrows indicating index lesions shown in the cross-sectional imaging to the right.



Abbreviations: PRE=pre-infusion/baseline assessment; D28=day 28 post-infusion; M3=day 90 post-infusion; M6=day 180 post-infusion; M12=day 365 post-infusion; CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease; CAR19=anti-CD19 CAR T-cell therapy; CAR20.19=anti-CD19, anti-CD20 CAR T-cell therapy.

Figure S4: Surface antigen expression on neoplastic B-cells
Antigen binding capacity (ABC) or site density on the surface of neoplastic B-cells obtained via FNA of involved sites, as determined by Quantibrite-PE beads and PE-labeled anti-CD19, anti-CD20, and anti-CD22 antibodies using methods described previously. All measurements were taken from biopsies performed at trial enrollment. Dotted blue lines highlight the average ABC of normal control B-cells for comparison.

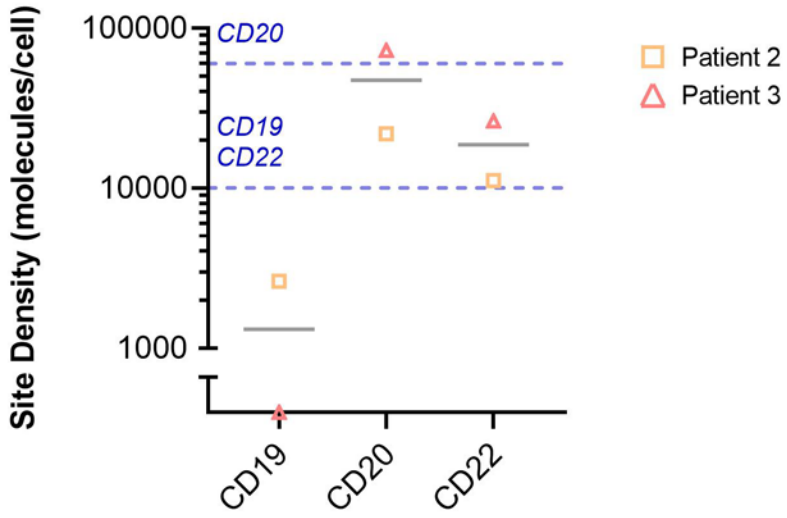
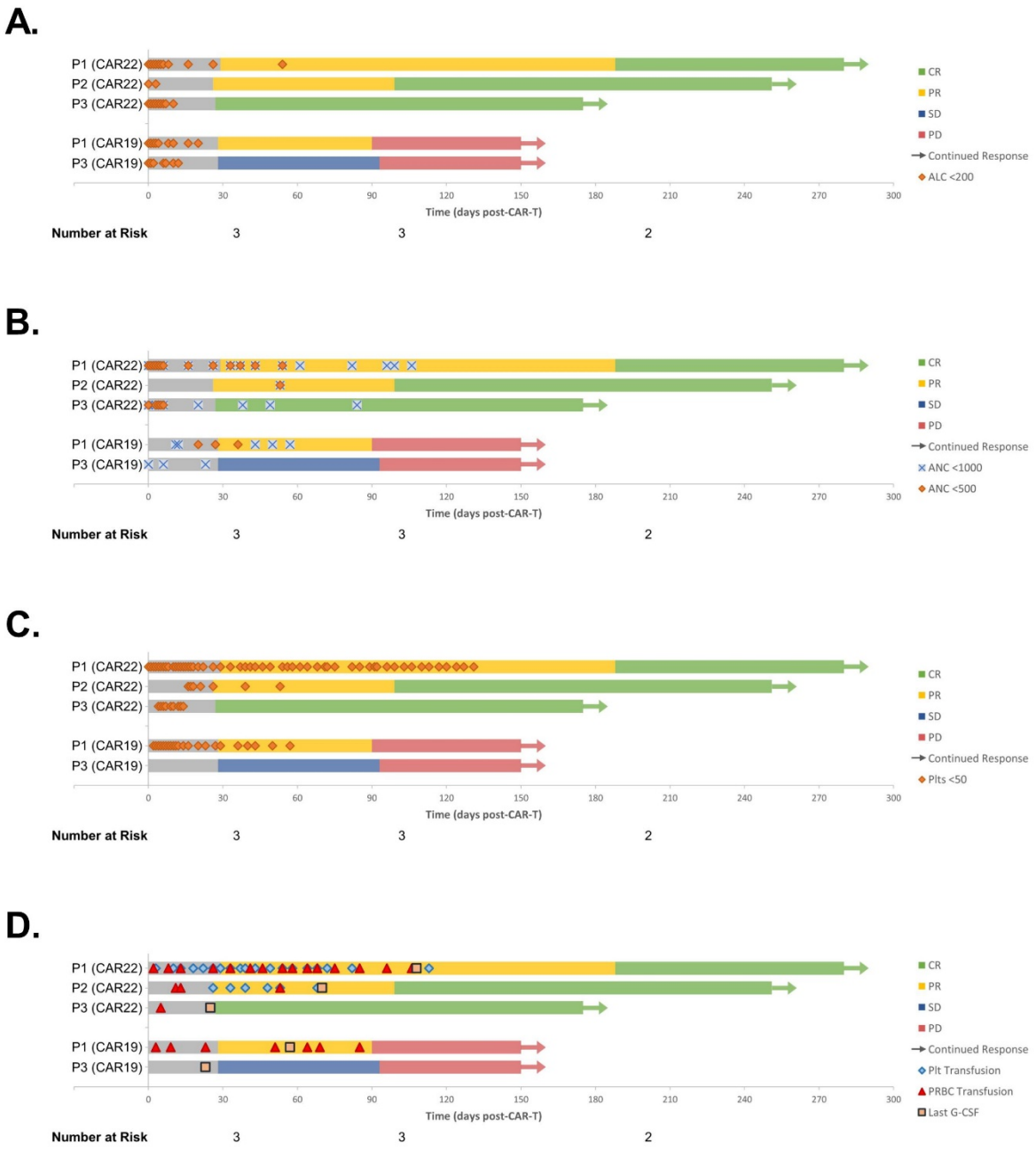


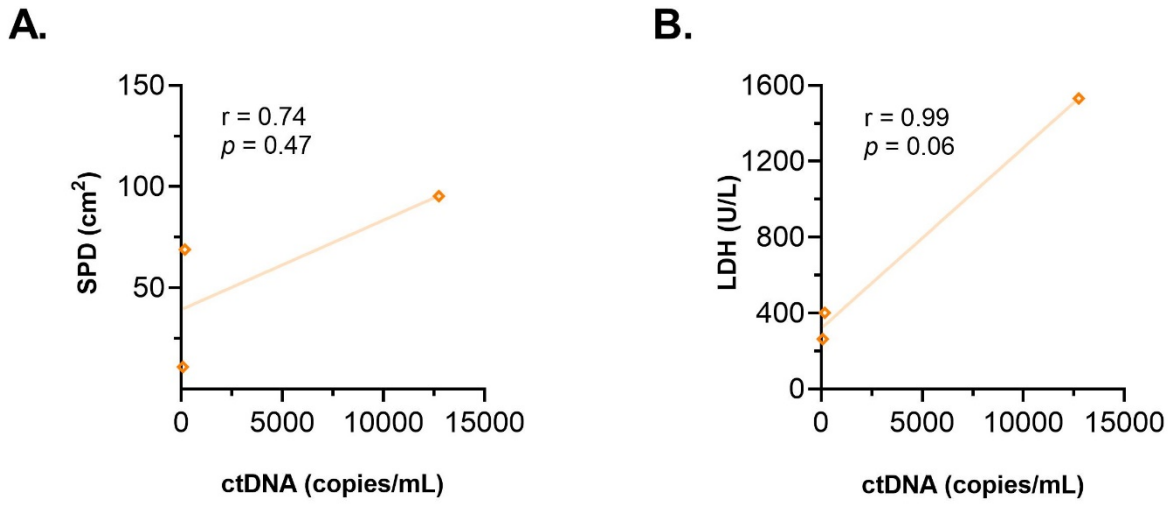
Figure S5: Hematologic toxicity and supportive care utilization for all patients. Swimmer plots showing (A) occurrence of grade 4 lymphopenia; (B) occurrence of grade 3 and 4 neutropenia; (C) occurrence of grade 3 thrombocytopenia; and (D) utilization of blood and platelet transfusions, and time of last growth factor (G-CSF) administration for severe neutropenia. Patients are identified by study number in the left column, and clinical response categorization is indicated by the coloring of the bar. The same data is also displayed for Patient 1 and 3 following their prior CAR19 therapy for comparison.



Abbreviations: CAR19= anti-CD19 chimeric antigen receptor T-cell therapy; CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease; ALC=absolute

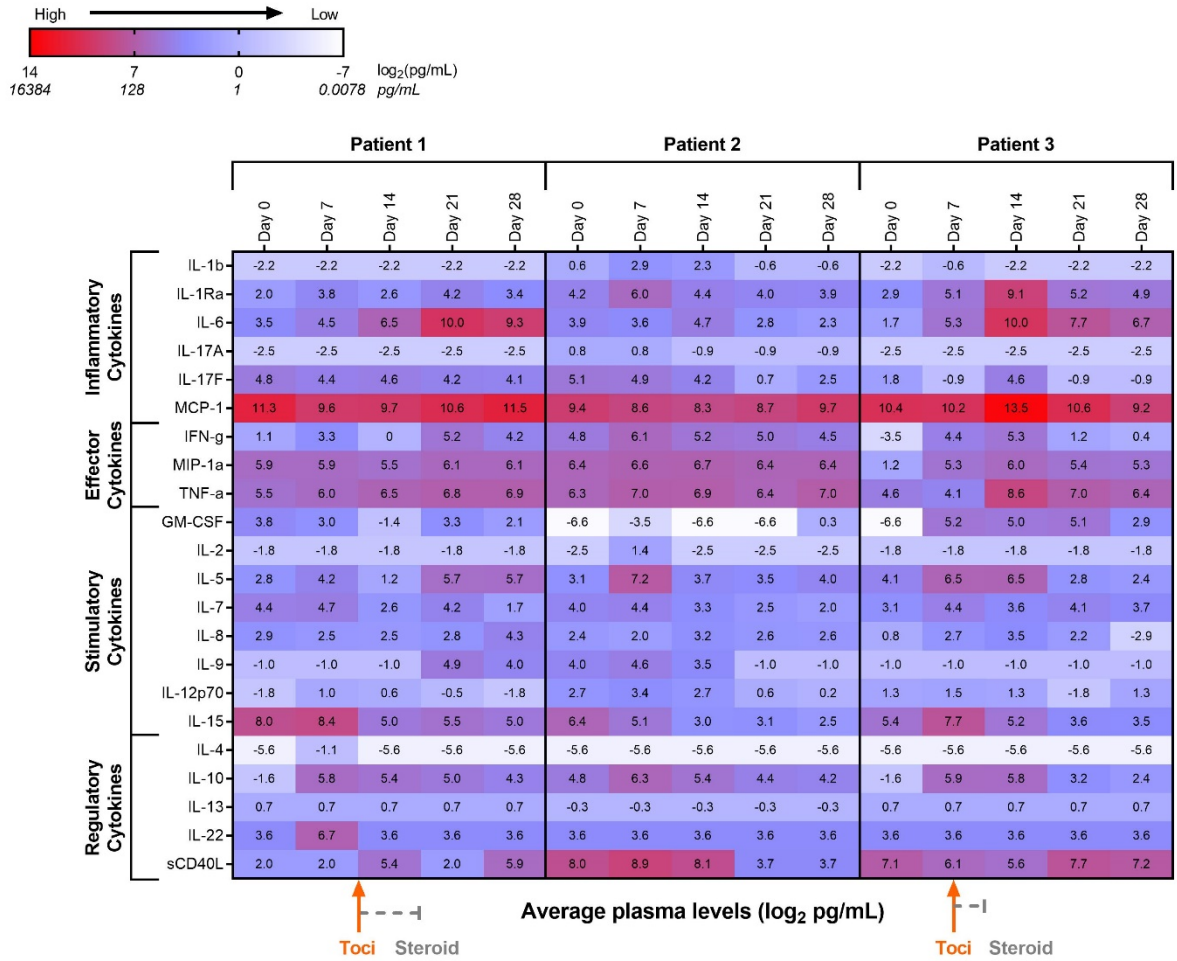
lymphocyte count; ANC=absolute neutrophil count; Plts=platelet count; PRBC=packed red blood cells; G-CSF=granulocyte-colony stimulating factor, filgrastim.

Figure S6: Correlation of ctDNA to other biomarkers of tumor burden.
(A) SPD was determined for index lesions on baseline PET-CT. (B) Serum LDH was obtained prior to the initiation of LD chemotherapy. Lines shown represent linear regression using least squares fitting. Coefficients and P-values shown were obtained by Pearson's correlation analysis.



Abbreviations: SPD=sum of product of diameters; LDH=serum lactate dehydrogenase; ctDNA = circulating tumor DNA.

Figure S7: Post-infusion serum cytokine profiling.
Serial measurements of serum cytokine concentrations were performed prior to infusion, and at days 7, 14, 21, and 28 post-infusion. Orange arrows indicate the timing of tocilizumab administration for grade 2 CRS; gray dotted lines indicate the duration of corticosteroid administration for grade 2 CRS. Average plasma levels at each time point are shown within each cell, and are $\log_2$ transformed for scale.



Abbreviations: IL-1b=interleukin-1 beta; IL-1Ra=interleukin-1 receptor antagonist; IL-6=interleukin-6; IL-17A=interleukin-17A; IL-17F=interleukin-17F; MCP-1=monocyte chemoattractant protein-1; IFN-g=interferon gamma; MIP-1a=macrophage inflammatory protein-1 alpha; TNF-a=tumor necrosis factor alpha; GM-CSF=granulocyte-macrophage colony-stimulating factor; IL-2=interleukin-2; IL-5=interleukin-5; IL-7=interleukin-7; IL-8=interleukin-8; IL-9=interleukin-9; IL-12p70=interleukin-12 p70 heterodimer; IL-15=interleukin-15; IL-4=interleukin-4; IL-10=interleukin-10; IL-13=interleukin-13; IL-22=interleukin-22; sCD40L=soluble CD40 ligand; Toci=tocilizumab, IL-6 receptor antagonist.