SUPPLEMENTAL APPENDIX

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Supplemental Table 1. Baseline Characteristics of Patients Treated with anti-CD22 CAR T cells

	Demographics		Prior Therapy					Status				
Pt #	Age (yrs)	Sex	HSCT	CD19 Immunotherapy		CD22 Antibody		Circulating B cells/mcL (non-malignant)	Marrow Blasts (%)*	Extramedullary disease	Blast CD19 Expression	Blast CD22 Site Density (number/cell)
				Agent	Response[£]	Agent	Response[£]					
1	22	M	Y	CD19.BB.z-CAR	CR	Ino	SD	5	99.0**	---	Neg	2,084
2	20	F	Y (2)	CD19.28.z-CAR	CR	---	---	623	5.0^	---	Dim	13,452
3	22	M	Y	CD19.BB.z-CAR	CR	---	---	2	97.0	---	Neg	846
4	22	M	Y	CD19.28.z-CAR	PD	---	---	3	99.0	---	Pos	1,867
5	7	F	Y	CD19.BB.z-CAR	CR	---	---	0	78.0	---	Neg	2,839
6	17	F	Y	CD19.BB.z-CAR	CR	---	---	0	2.6	---	Neg	4,988
7	17	M	Y (2)	---	---	---	---	121	2.6	---	Pos	8,514
8	19	F	Y	---	---	---	---	9	0.3	---	Pos	10,432
9	21	F	Y	CD19.BB.z-CAR	CR	---	---	0	91.0	---	Neg	1,866
10	26	M	Y	Blinatumomab	PD	---	---	48	>99.0	---	Pos	8,430
11	7	M	Y	CD19.28.z-CAR[@^]	PD	Ino	CR	391	19.0	---	Pos	4,816
12	15	M	Y	CD19.28.z-CAR	CR	Ino	CR	17	0.05	Renal mass, Vertebral body masses	Pos	922
13	30	M	Y	CD19.28.z-CAR	CR	---	---	0	27.0	Renal mass, Vertebral body masses, abdominal lymphadenopathy	Neg	3,575
14	18	F	Y	---	---	---	---	488	30.0	---	Pos	7,722
15	8	F	Y	CD19.BB.z-CAR	CR	Ino	CR	0	94.0	Temporal mass	Neg	909
16	26	M	Y	Blinatumomab	CR	---	---	131	77.0	---	Neg	4,568
17	27	M	Y	CD19.28.z-CAR[@]	CR	Ino	CR	1	17.0	Pleural Effusion	Pos	613[#]
18	12	M	N	---	---	---	---	26	75.0	---	Pos	2,267
19	19	M	Y	CD19.BB.z-CAR	CR	---	---	0	87.0	---	Neg	2,196
20	8	F	Y	CD19.BB.z-CAR	CR	---	---	455	70.5	---	Pos	5,779
21	12	M	N	CD19.28.z-CAR	CR	---	---	111	2.6	---	Pos	9,197

*M=male; F=female; HSCT=allogeneic hematopoietic stem cell transplantation; Y=yes; N=no; CR=complete remission; PD=progressive disease; Ino=Inotuzumab; [£] all CRs were MRD negative * % blasts of mononuclear cells by flow cytometry, except when indicated otherwise. ** marrow biopsy, as patient was unaspirable. ^% blasts in peripheral blood that developed following enrollment marrow, prior to therapy. [#]Partial CD22 expression at baseline (post-CD22 targeted antibody therapy) . [@]Also received prior blinatumomab. ^Non-responder to CD19 CAR and blinatumomab.*

Supplemental Table 2. Characteristics of Infused anti-CD22 CAR-T Cell Product

Pt. #	Cell Dose (10^6/kg)	% Transduced (Protein L)	%CD3	% CD4	%CD8	Cryopreserved Prior to Infusion?
1	0.3	50.2%	98.8%	13.4%	84.9%	N
2	0.3	50.1%	99.9%	63.6%	35.4%	Y
3	0.3	41.2%	98.1%	33.1%	63.8%	N
4	0.3	45.0%	99.6%	94.3%	5.0%	Y
5	0.3	31.7%	99.8%	42.8%	56.9%	Y
6	0.3	34.7%	99.3%	36.3%	44.0%	Y
7	1	47.8%	99.8%	70.9%	26.8%	N
8	1	23.4%	99.2%	25.8%	72.7%	Y
9	1	36.9%	99.8%	67.6%	30.8%	Y
10	3	33.4%	96.8%	67%	28.6%	Y
11	3	18.8%	99.9%	78.6%	17.2%	N
12	1	28.8%	99.8%	49%	50.0%	N
13	1	18%	99.6%	75.55	23.6%	N
14	1	40.7%	99.8%	76.8%	22.3%	N
15	1	15.4%	99.5%	60.5%	38%	N
16	1	23.1%	99.6%	51.1%	48.1%	Y
17	1	28.5%	99.7%	56.9%	41.8%	N
18	1	39.1%	99.9%	69.4%	29.3%	Y
19	1	29.2%	99.9%	46.3%	51%	N
20	1	48.7%	99.8%	59.8%	38.1%	N
21	1	33.4%	99.7%	32.5%	66.1%	N

Supplemental Table 3. Grade 3 and 4 Adverse Events at Least Possibly Related to CD22 CAR T cells from Time of Infusion through Day 28

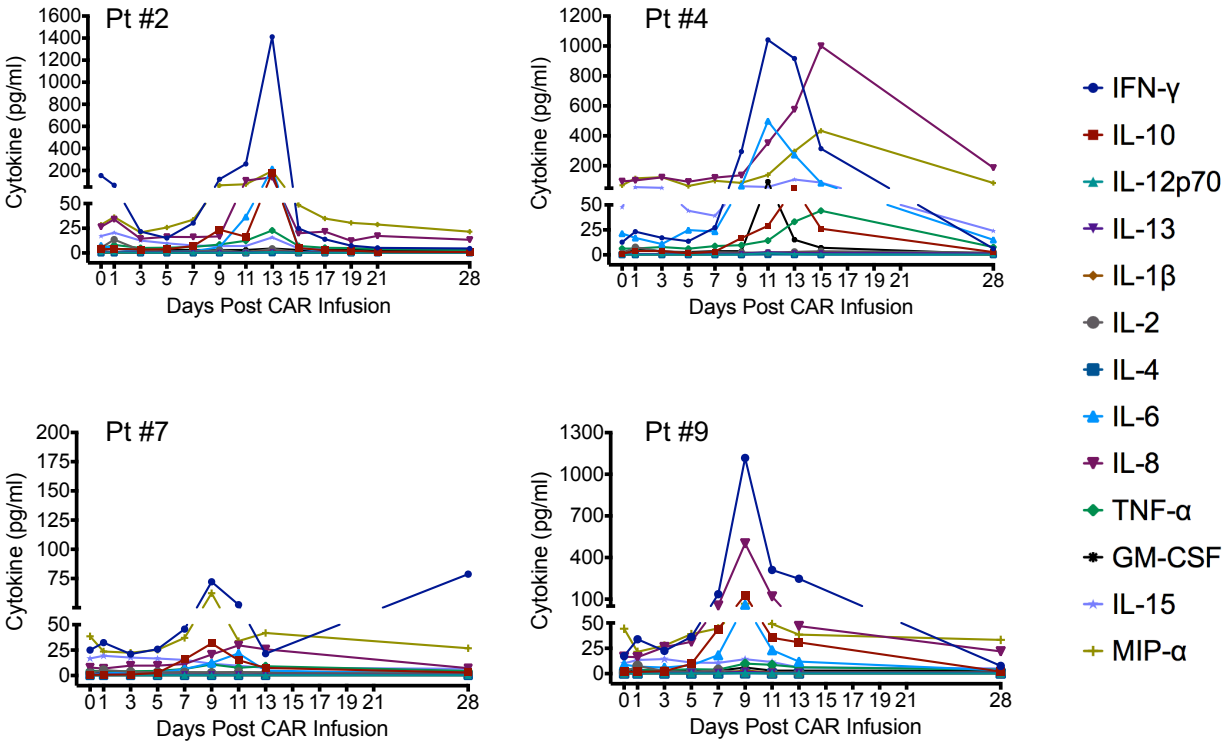
	Dose 1		Dose 2		Dose 3	
	3	4	3	4	3	4
Blood & Lymphatic System Disorders						
Anemia	10		25		1	
Febrile neutropenia	6		14		2	
Cardiac Disorders						
Sinus tachycardia			1			
Gastrointestinal Disorders						
Diarrhea	1		1			
General disorders and administration site conditions						
Fever	2	1	11		2	
Multi-organ failure				1		
Infections and Infestations						
Catheter related infections				1		
Sepsis				1		
Investigations						
Activated partial thromboplastin time prolonged			3			
Alanine aminotransferase increased			1			
Alkaline phosphatase increased			2			
Aspartate aminotransferase increased	1		6	1	1	
Blood bilirubin increased			2			
CPK increased			1			
Fibrinogen decreased			4			
GGT increased				1		
INR increased			1			
Lipase increased			1	1		
Lymphocyte count decreased			13	7	3	1
Neutrophil count decreased	4	6	15	11	4	2
Platelet count decreased	3	2	22	17	8	7
Serum amylase increased			1			
Urine output decreased				1		
Weight gain			1			
White blood cell decreased	6	2	14	11	6	5
Metabolism and nutrition disorders						
Hypertriglyceridemia			6	2		
Hypokalemia	3		4			
Hyponatremia			7		1	
Hypophosphatemia	2	1	26	1	4	
Tumor lysis syndrome			1			
Respiratory, thoracic and mediastinal disorders						
Epistaxis			1			
Hypoxia					2	1
Respiratory failure				1		
Vascular disorders						
Hypotension	1		3		1	

Supplemental Table 4. CAR-T Cell Expansion, Leukemia Response and Toxicity

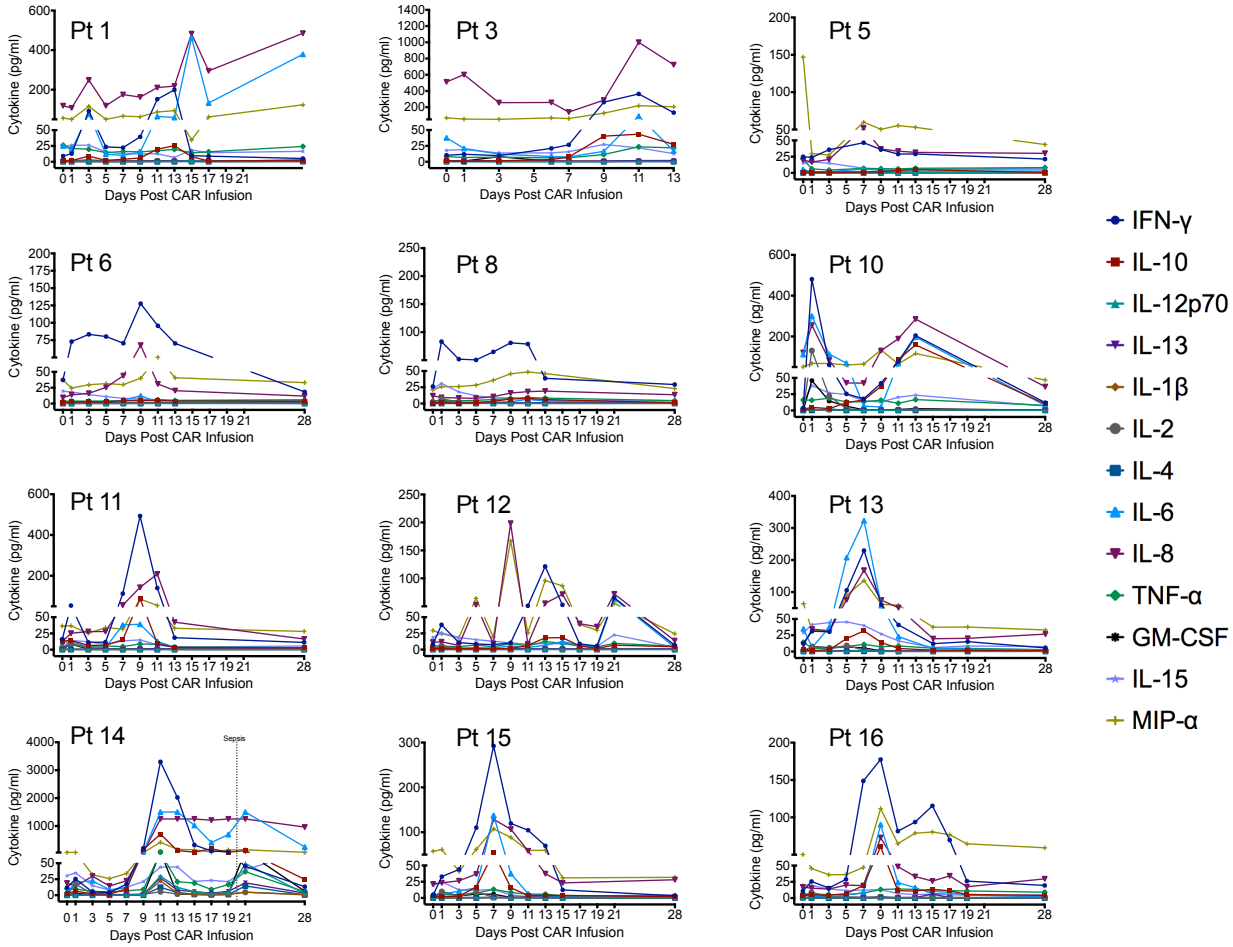
CAR T Cell Dose (10^6 cells/kg)	Pt #	CAR T Cell Expansion					Toxicity		Response	
		Maximum Circulating CAR/μl	Maximum Circulating CAR %[#]	Marrow[$] (%)	CSF[$] (%)	Pleural Fluis[$] (%)	CRS Grade	DLT (Grade)	Maximum Response	Response Duration
0.3	1	2	1.1	0	n/a	---	None	No	PD	n/a
	2	316	52.3	19.5[&]	0.0	---	1	Yes (3)	**CR[£]**	**3 mos**
	3	44	73.0	36.0	32.0	---	1	No	SD	n/a
	4	1	6.0	0	0.0	---	2	No	SD	n/a
	5	0	0.0	1.3	0.0	---	None	No	PD	n/a
	6	9	1.8	2.0	0.0	---	None	No	PD	n/a
1	7	424	62.0	24.0	52.0	---	2	No	**CR[£] +**	**21 mos+**
	8	481	36.2	6.0	26.0	---	1	No	**CR[£]**	**6 mos**
	9	1217	75.0	28.0	6.0*	---	2	No	**CR[£]**	**6 mos**
3	10	15	14.3	0	n/a	---	2	Yes (4)	SD	n/s
	11	732	72	27.6[&]	21	---	1	No	**CR**	**1.5 mos**
1	12	4	2.6	0	0	---	None	No	SD	n/a
	13	105	60	42	69	---	1	No	**CR[£]****	**12 mos**
	14	150	62	n/a	n/a	---	2	Yes (5)[¢]	**CR**	n/a
	15	3593	77	41	61	---	1	No	**CR[£]**	**2 mos**
	16	762	64	40	27	---	1	No	**CR[£] +**	**9 mos+**
	17	2167	89.5	78.2	71.6	73	2	Yes (4)	SD	n/a
	18	0	0	n/a	n/a	---	None	No	PD	n/a
	19	2184	91	71	45	---	2	No	**CR**	**6 mos**
	20	2831	80	25	57	---	1	No	**CR[£]**	**6 mos**
	21	324	76	31	41	---	1	No	**CR[£] +**	**6 mos+**

*[#]% of CD3 cells co-expressing CD22-CAR; [$]% of T cells on Day 28±4; [&]BM obtained at 6 weeks; *CSF obtained at 2 mos*; *CRS=Cytokine Release Syndrome; DLT=dose limiting toxicity; PD=Progressive Disease; CR=complete response; SD=stable disease; PD=progressive disease* [£]MRD negative bone marrow, ***Attained MRD negative remission in the marrow on Day 28 with decreasing PET-avidity seen in the lymphomatous components of the disease that fully resolved by 5 months post-CAR therapy. +Response ongoing,* [¢]death due to sepsis

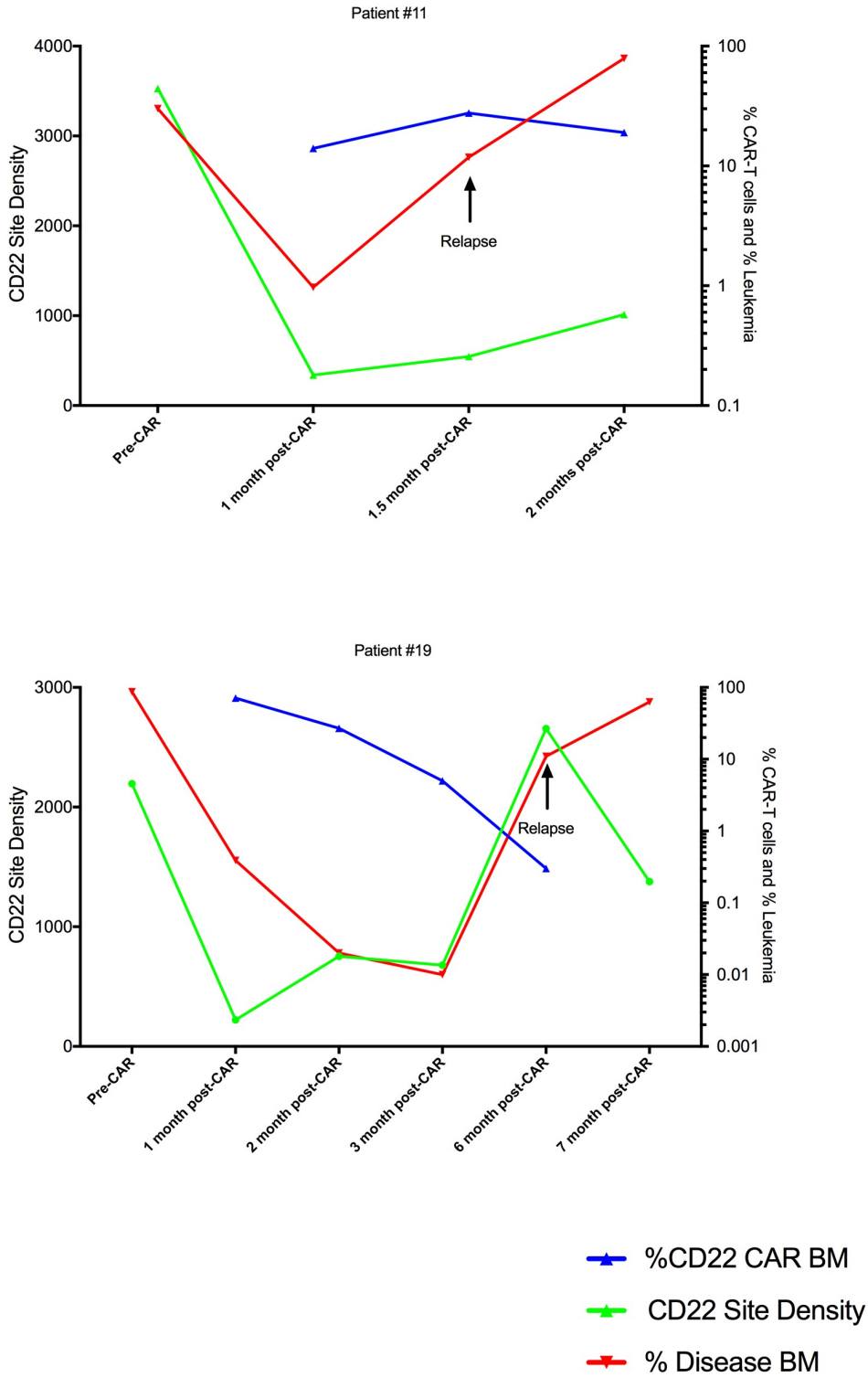
Supplemental Figure 1: ***Serum cytokine profiles following CD22 CAR T cells demonstrate multiple distinct patterns.*** Serial cytokines were prospectively measured on all patients and representative profiles are shown here. Patients #2, 7, and 9 were all responders and experienced cytokine release syndrome. Of note, patient 7 had low burden leukemia at time of treatment, patient #2 had intermediate leukemic burden and patient #9 had high leukemic burden. Patient #4 had high leukemic burden prior to CD22 CAR treatment, demonstrated cytokine release syndrome with elevations of cytokines as illustrated, and experienced a transient reduction in leukemic blasts following CD22-CAR therapy but did not attain remission.



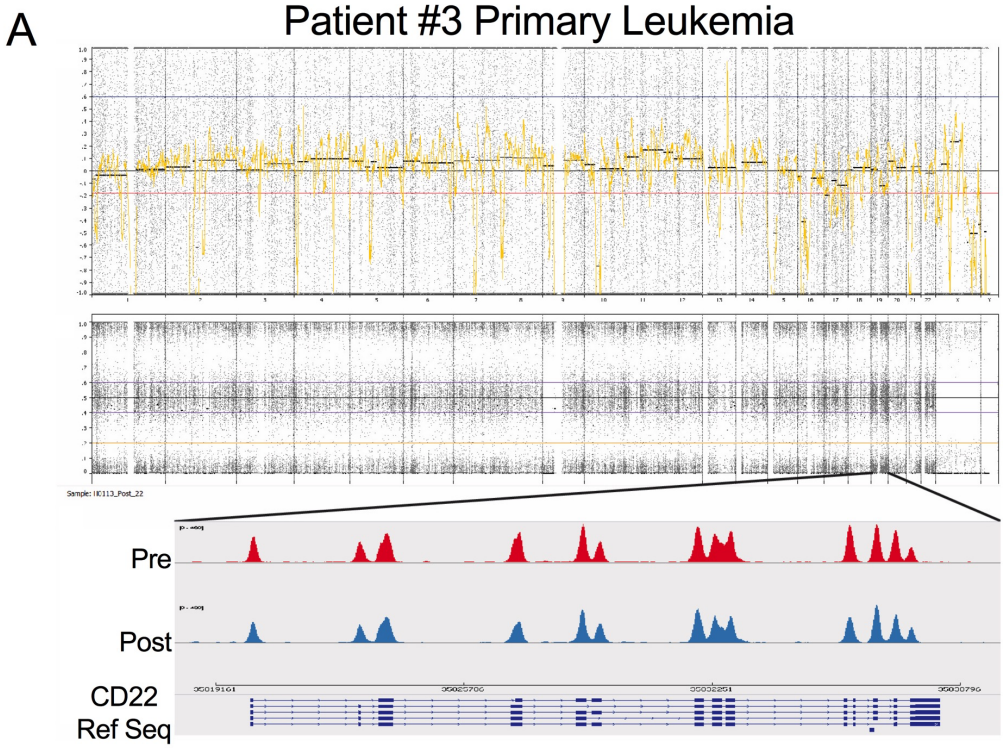
Supplemental Figure 2: ***Serum cytokine profiles following CD22 CAR T cells demonstrate multiple distinct patterns.*** Patients #8, 11, 13, 14, 15 and 16 all attained complete remission.

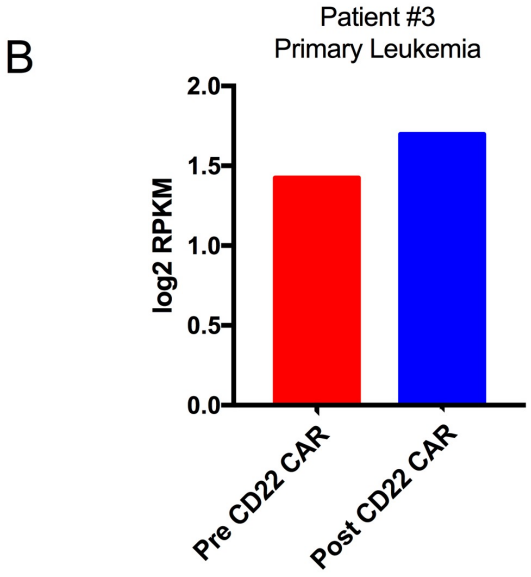


Supplemental Figure 3: ***Serial measurements of CD22 site density on leukemic blasts in the presence of CD22 immune pressure demonstrate variability over time.***

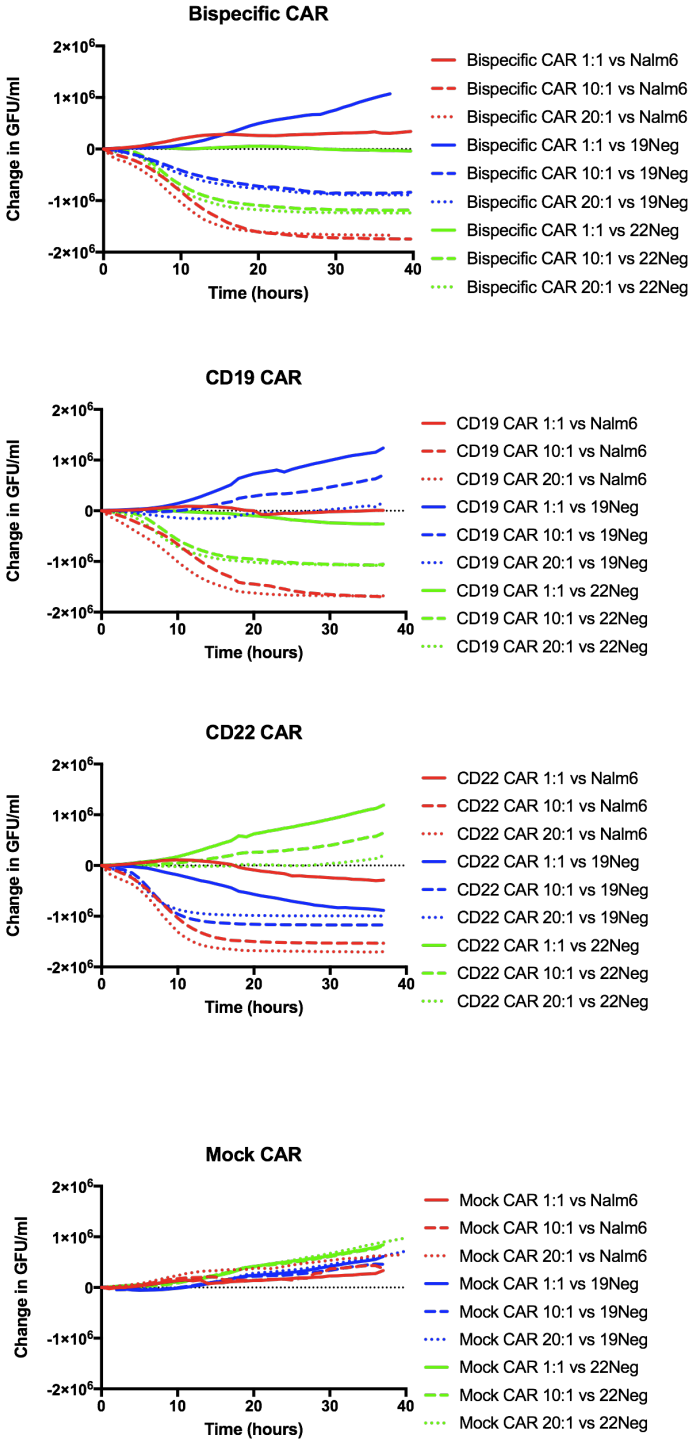


Supplemental Figure 4. ***Whole Exome and RNAseq profiling of CD22 in Primary Patient Sample recurring in the presence of CD22 CAR Immune Pressure.*** (a) Genome-wide copy number profiling of Patient #3 demonstrates no gains or losses with maintenance of the ploidy status before and after therapy. Neither mutations or focal copy number changes were observed in the CD22 gene locus for patient #3. (b) CD22 transcript levels were noted to slightly increase after CD22 CAR treatment.





Supplemental Figure 5. ***In vitro killing of CD19+/CD22+ ALL and single antigen expressing targets by CD19 CAR, CD22 CAR and a CD19xCD22 bispecific CAR.*** Killing of parental Nalm6 (CD19+/CD22+) and Nalm6 with crispr/Cas9 mediated deletion of CD19 or CD22 (CD19Neg and CD22Neg) by CD19 CAR, CD22CAR and the bispecific CD19xCD22 CAR at various effector to target ratios. Killing is measured as loss of GFP positive tumor cells over time.



Supplemental Figure 6. ***Sequence of CD19xCD22 bispecific CAR.***

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