

ABSTRACT

“USE OF CART19 TO DEplete NORMAL B CELLS TO INDUCE TOLERANCE”

The present invention provides compositions and methods for inducing tolerance in a human. The invention includes administering a genetically modified T cell expressing a CAR wherein the CAR comprises an antigen binding domain, a transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain.

Figure 1A

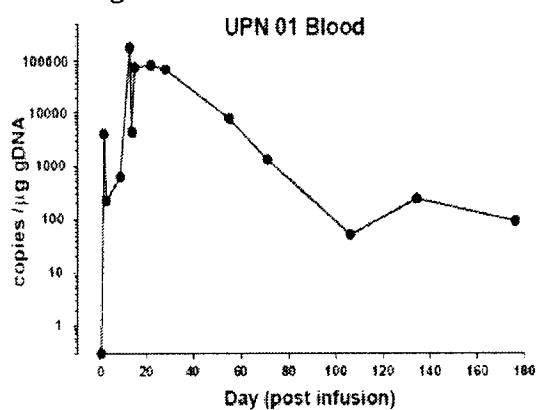


Figure 1B

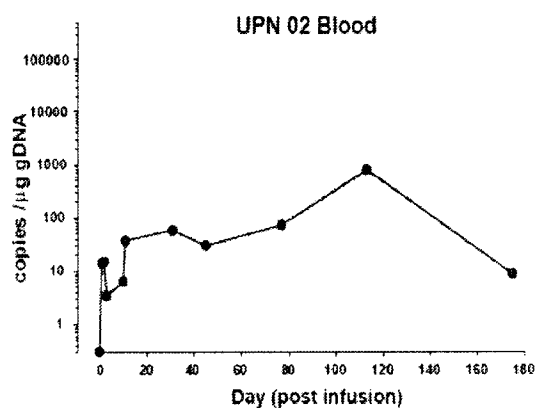


Figure 1B

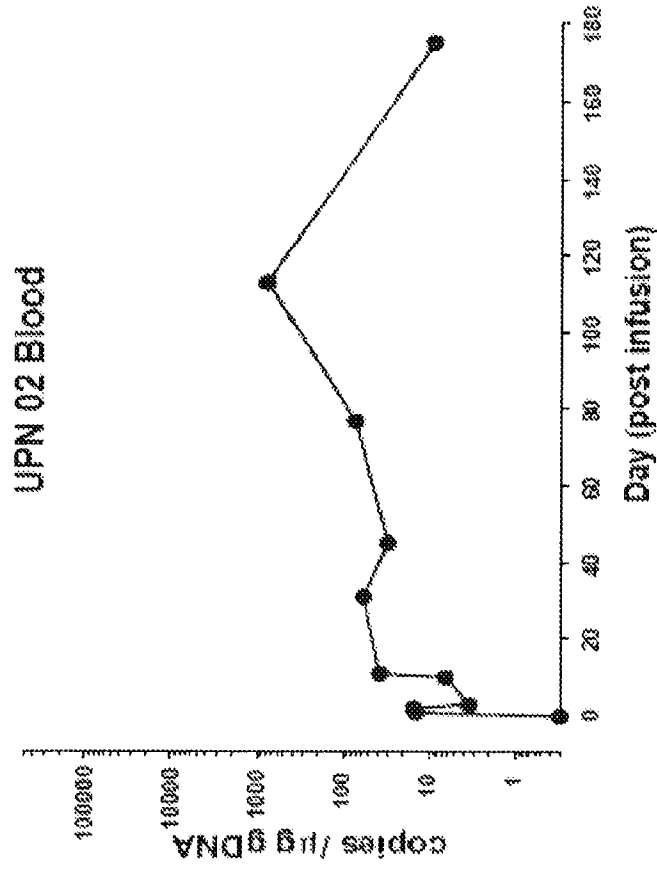


Figure 1A

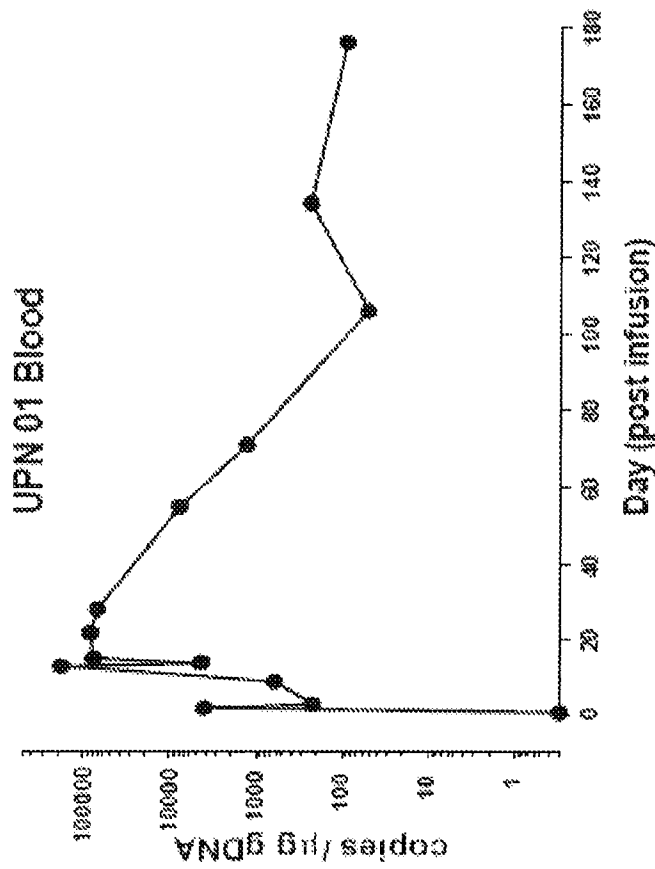


Figure 1D

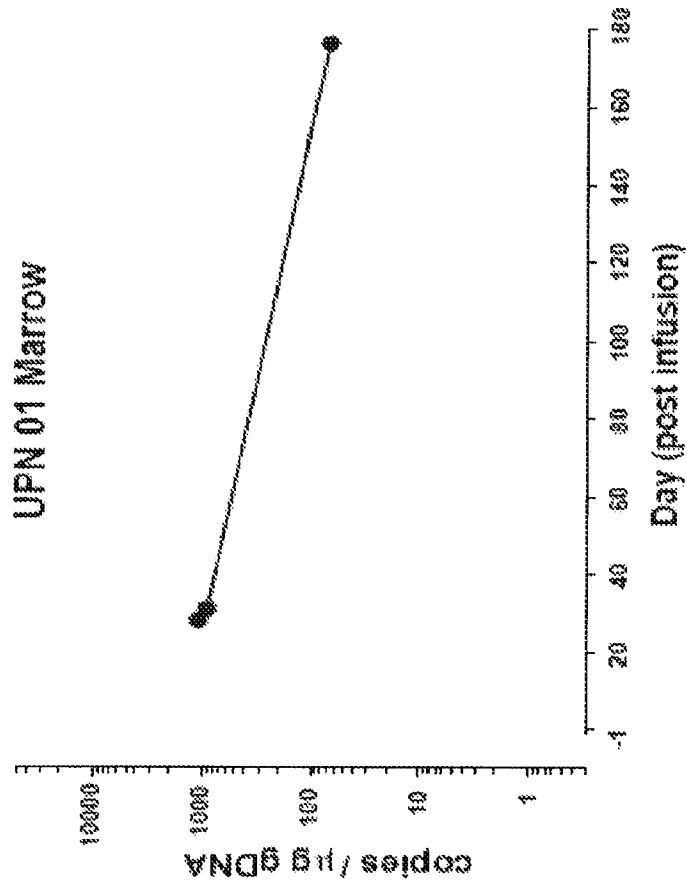


Figure 1C

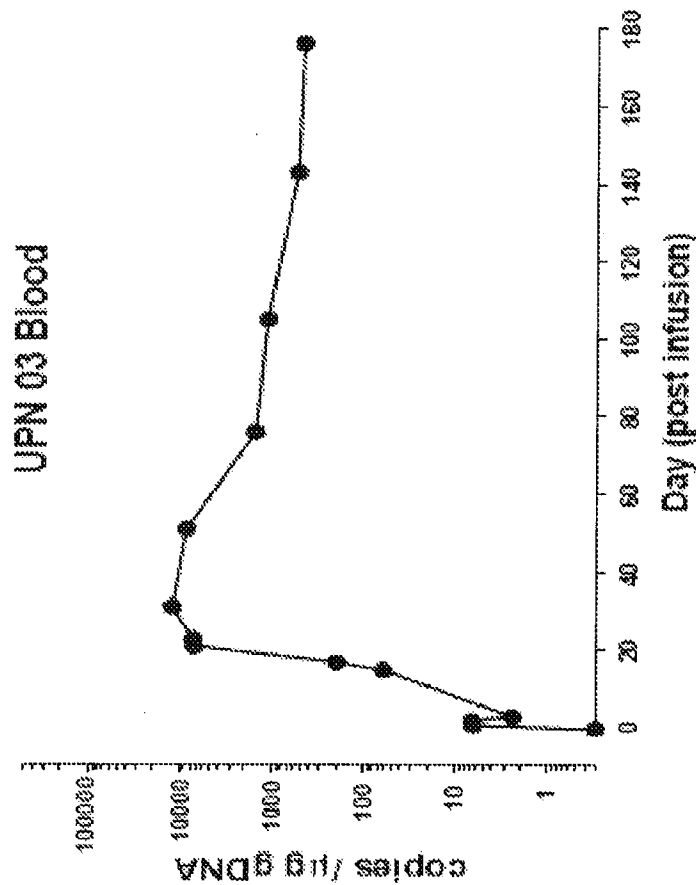


Figure 1E

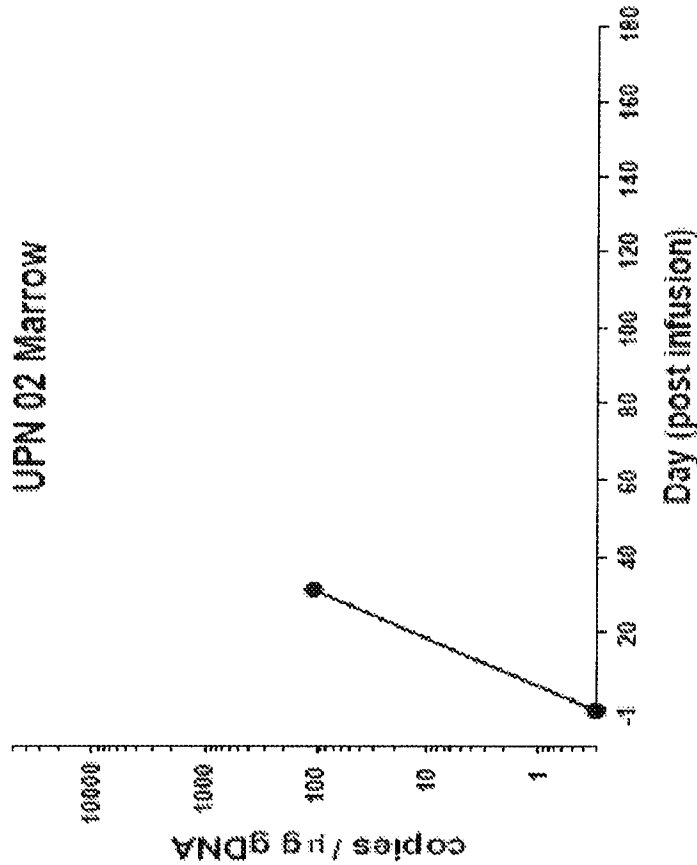
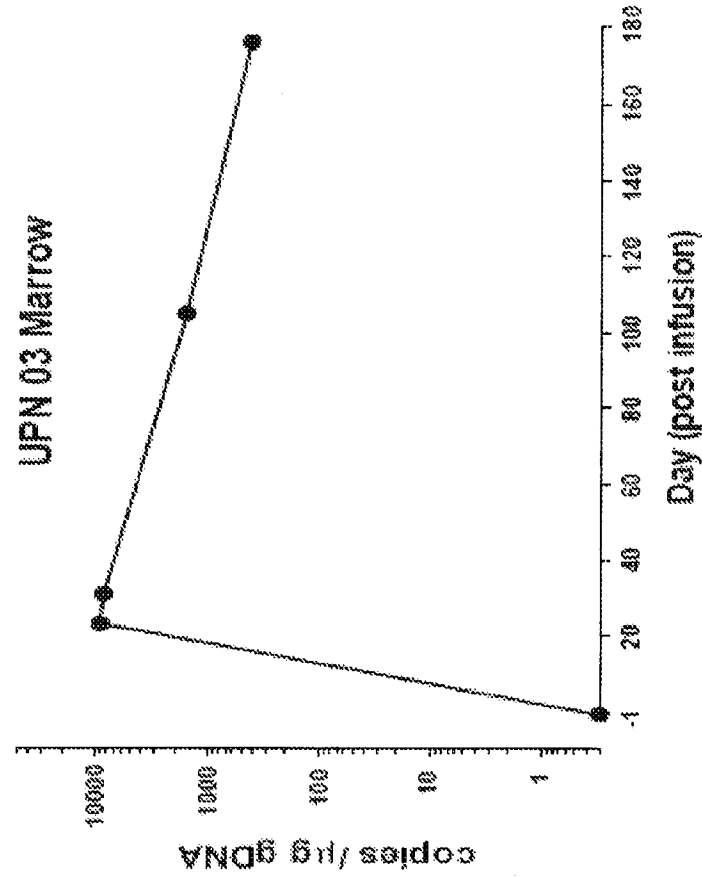


Figure 1F



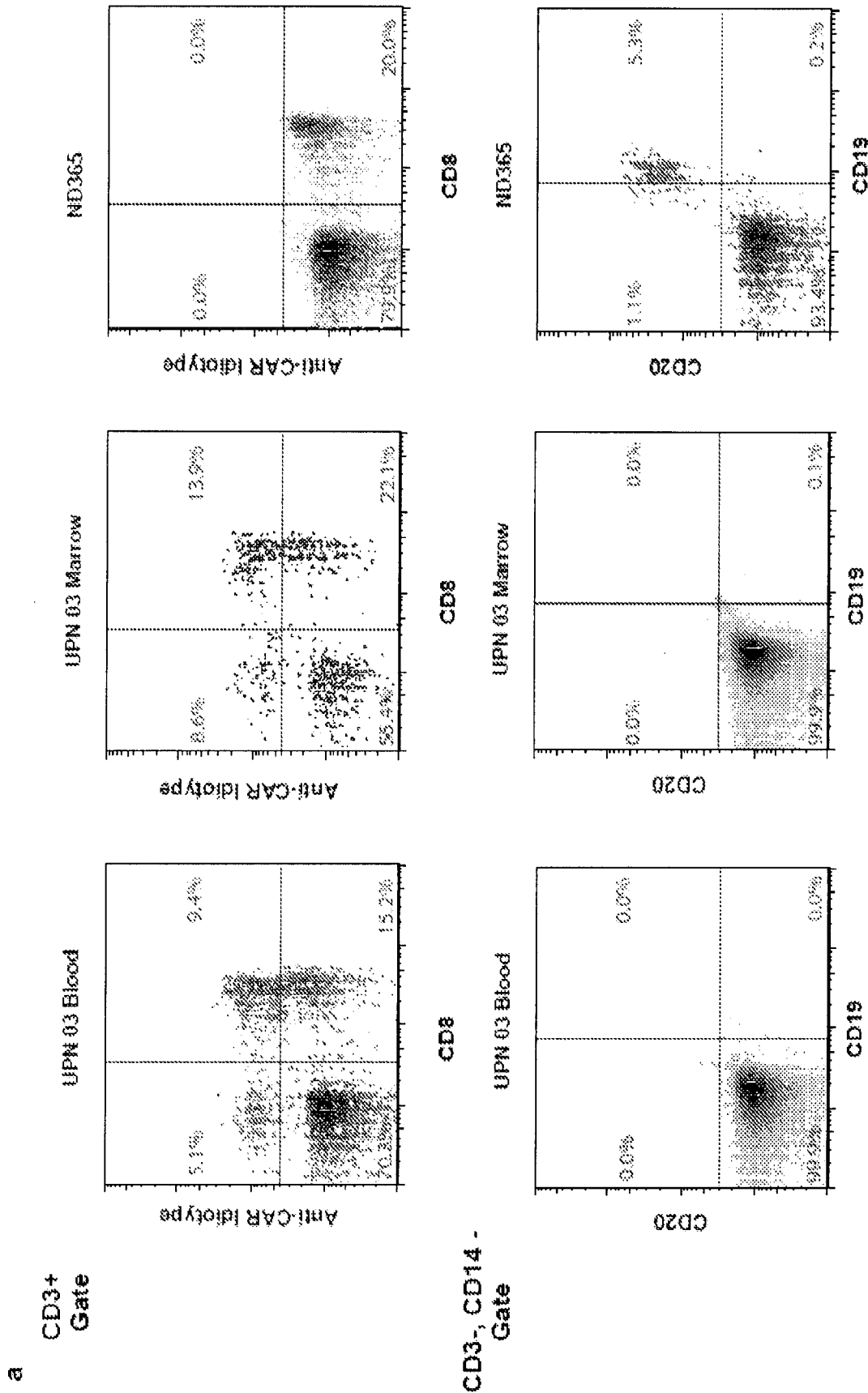


Figure 2A

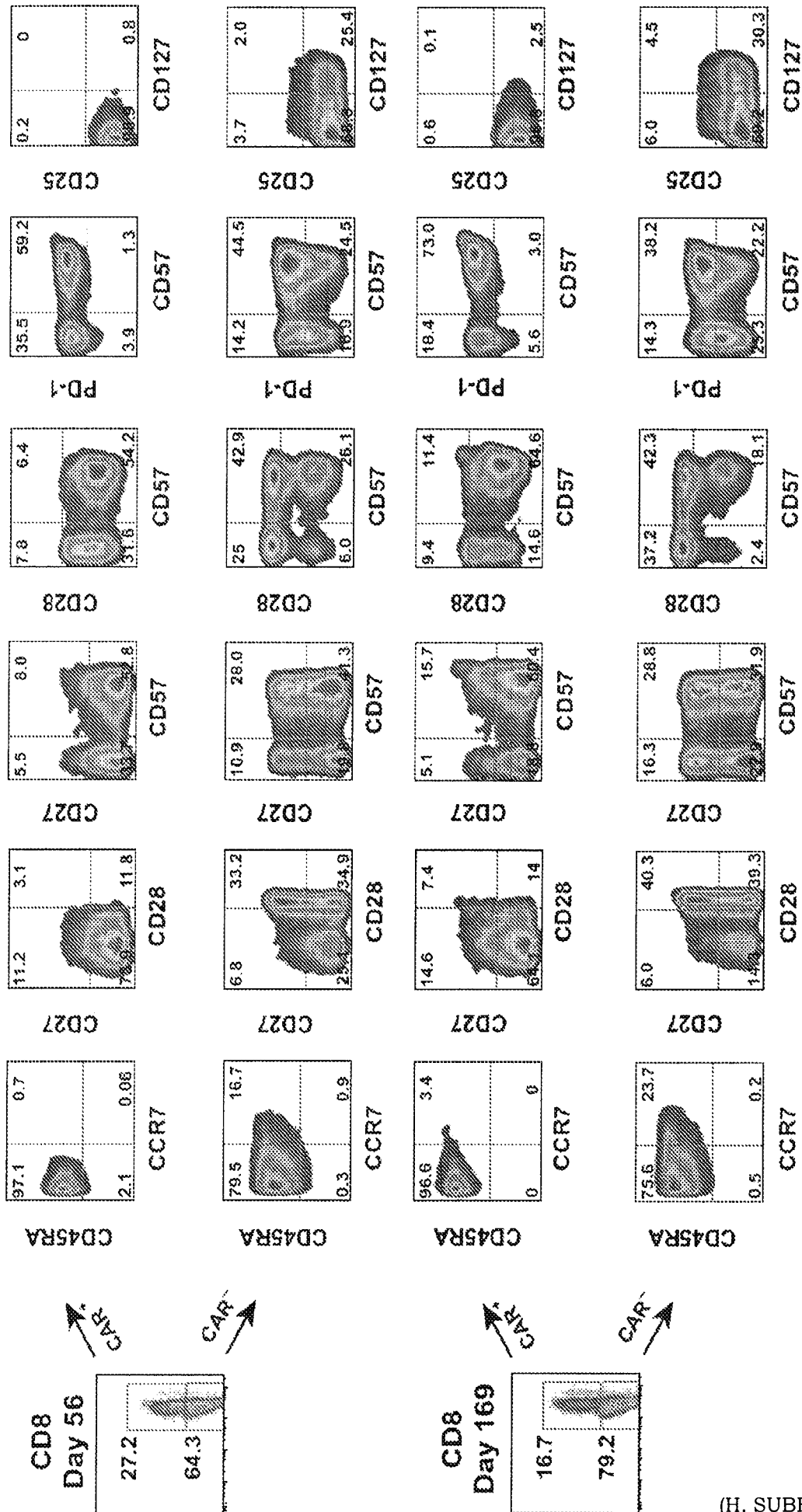


Figure 2C

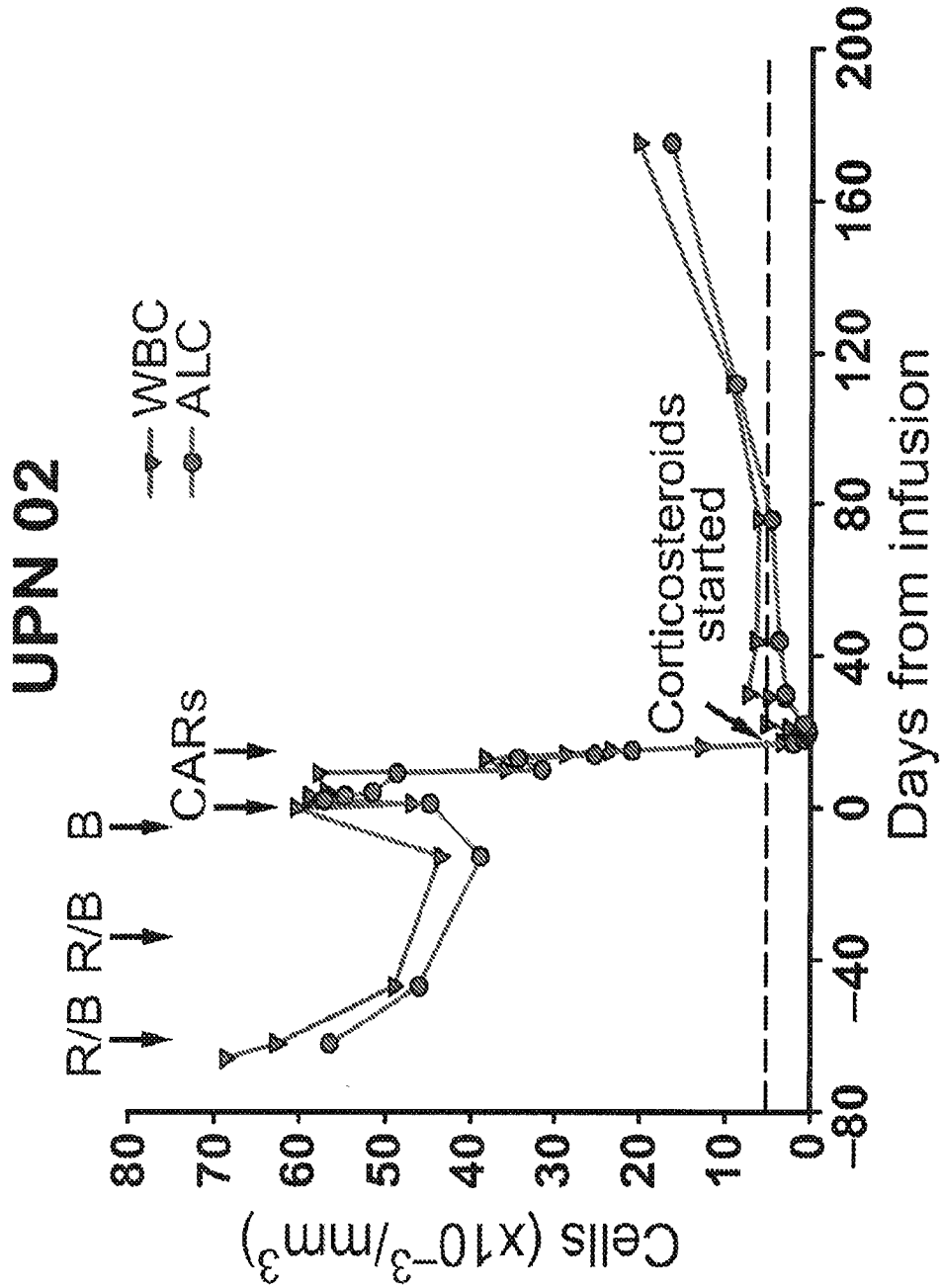


Figure 3A

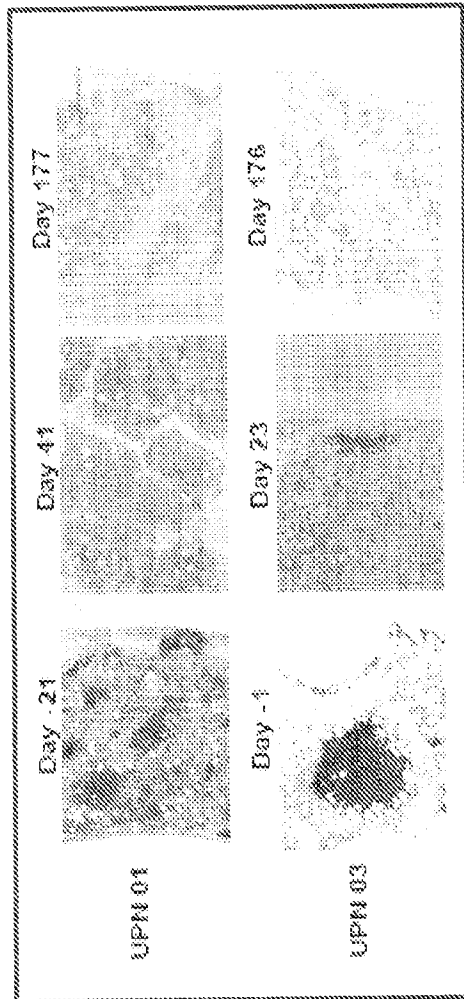


Figure 3B

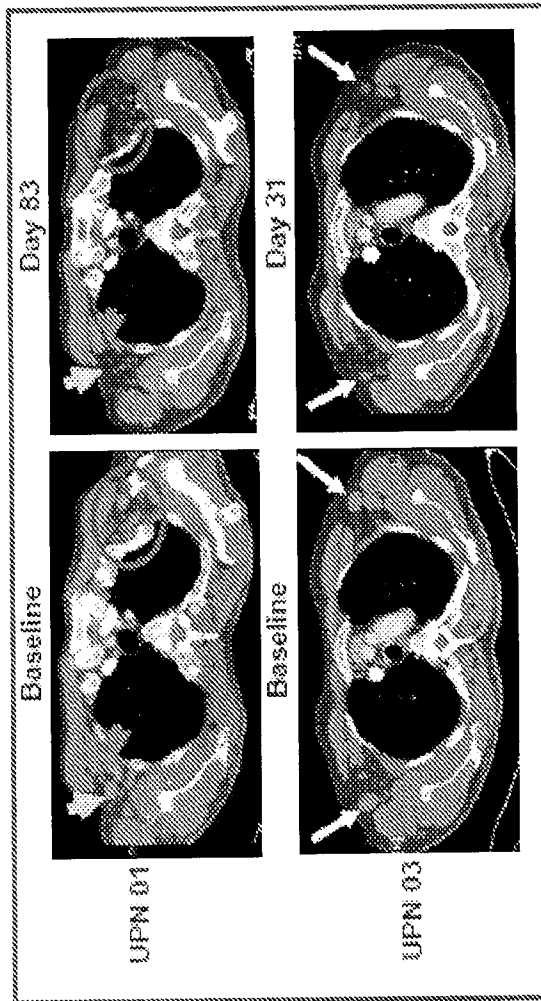


Figure 3C

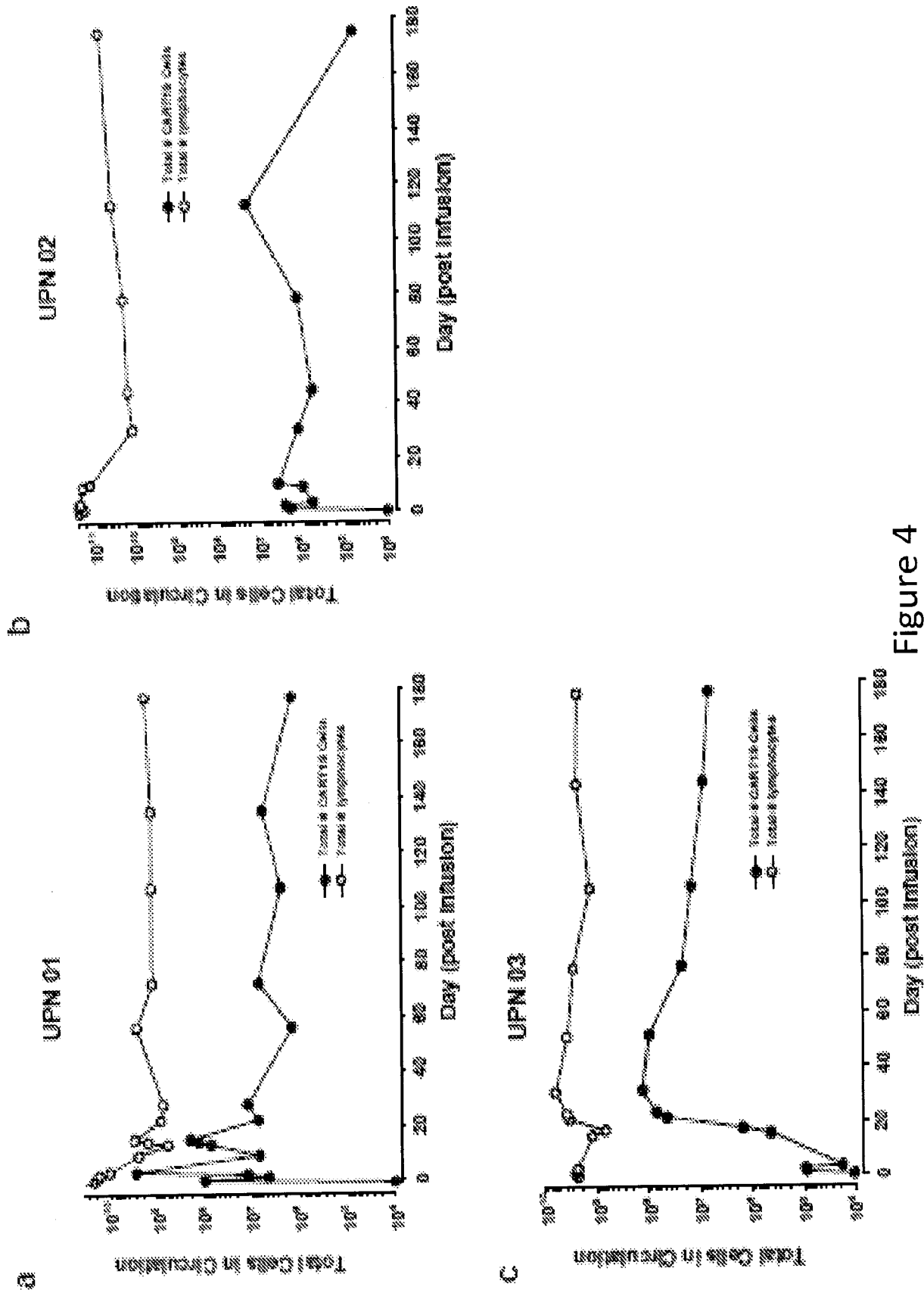


Figure 4

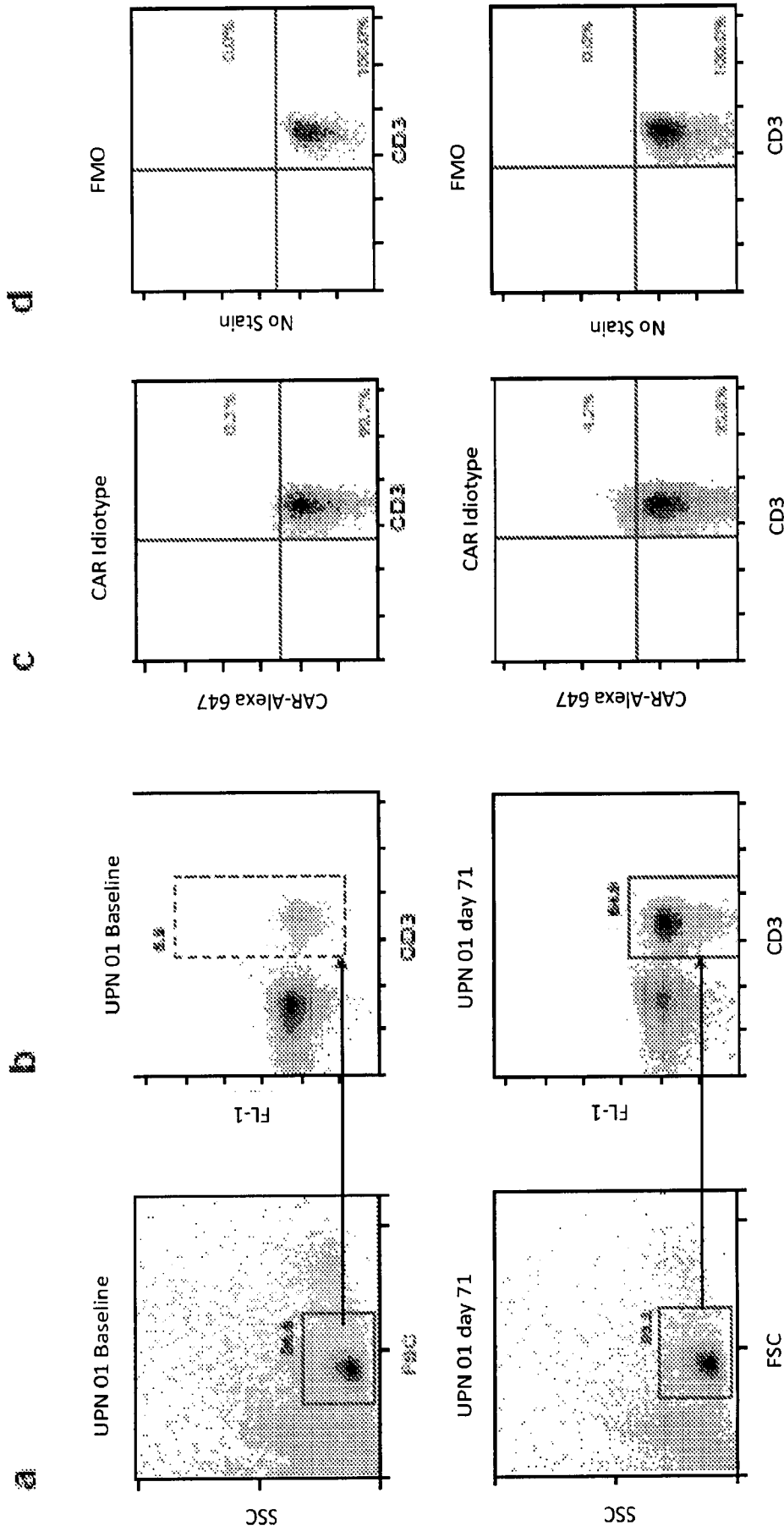


Figure 5

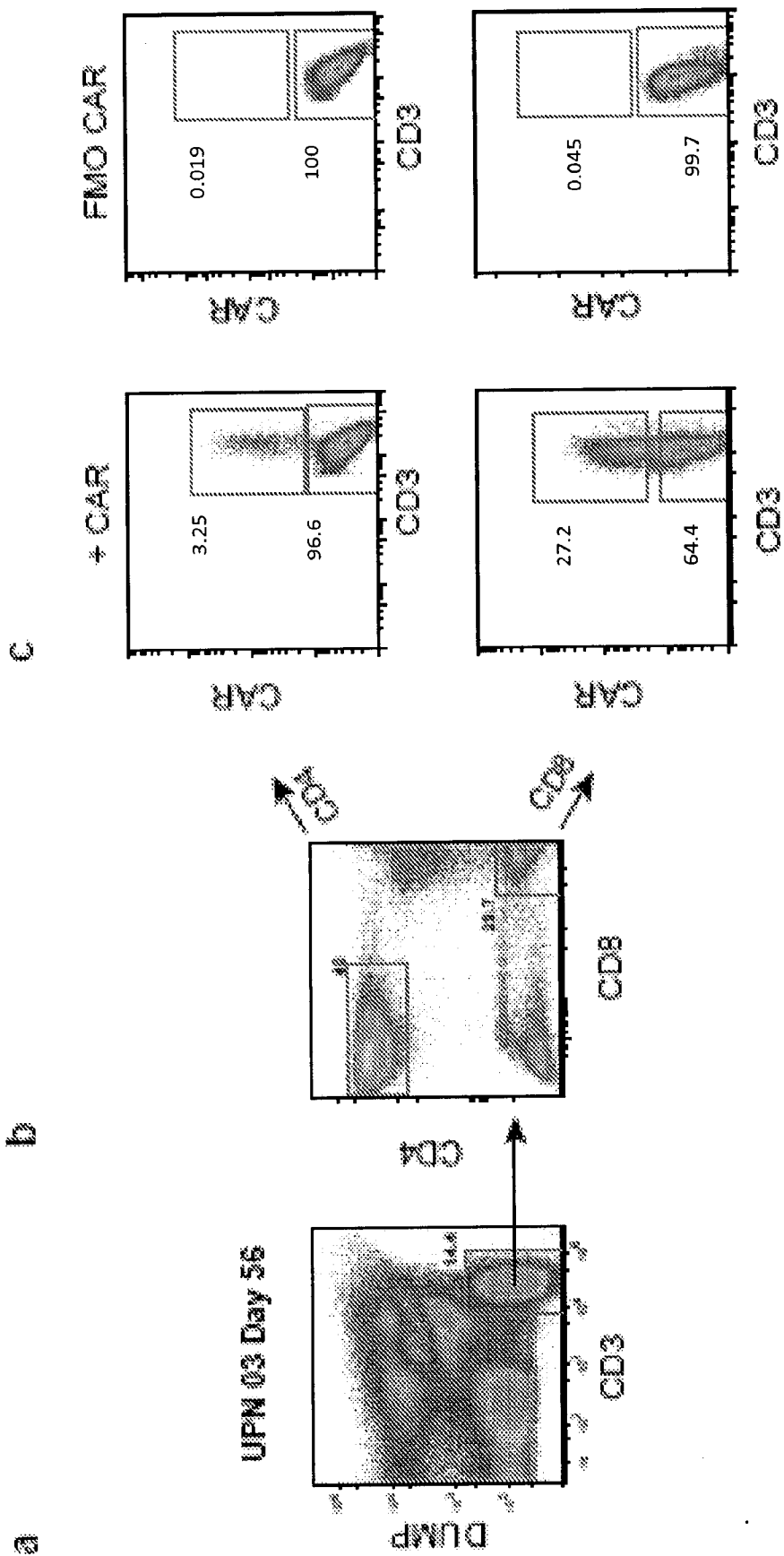


Figure 6

Subject UPN	Age/sex Karyotype	Previous therapies	CLL Tumor Burden at Baseline			Total Dose of CART19 (cells/kg)	Response D +30 (Duration)
			Bone marrow (Study Day)	Blood (Study Day)	Nodes/spleen (Study Day)		
01	63M normal	Fluorouracil x 4 cycles (2002) Rituximab/Fluorouracil x 4 cycles (2003) Alectuzumab x 12 wks (2006) Rituximab (2 courses 2008-2009) R-CHP x 2 cycles (2009) Lenalidomide (2008) PCR x 2 cycles (5/08-6/18/2010) Benadumab x 1 cycle (7/11-8/1/10) pre-CART19	Hypocellular 70% CLL 2.4x10 ⁶ CLL cells (Day -14) 1.7x10 ⁶ CLL cells (Day -1)	N/A	6.2x10 ¹¹ - 1.0x10 ¹² CLL cells (Day -37)	1.1x10 ⁷ (1.6x10 ⁷ /kg)	CR (8-months)
02	77M del(17)(p13)	Alectuzumab x 12 wks (6/2007) Alectuzumab x 18 wks (3/2009) Benadumab/Rituximab: 7/1/2010 (cycle 1) 7/28/2010 (cycle 2) 8/26/2010 (cycle 3) pre-CART19	Hypocellular >90% CLL 3.2x10 ⁶ CLL cells (Day -47)	2.75 x 10 ¹¹ CLL Cells (Day -1)	1.2x10 ¹¹ - 2.0x10 ¹¹ CLL cells (Day -34)	3.8x10 ⁷ (1.0x10 ⁷ /kg)	PR (5 months)
03	64M del(17)(p13)	R-Fluorouracil x 2 cycles (2002) R-Fluorouracil x 4 cycles (1006-1007) R- Benadumab x 1 cycle (2008) Benadumab x 3 cycles (3-8/09) Alectuzumab x 11 wks (12/09-3/10) Pentostatin/cyclophosphamide (9/10/10) pre-CART19	Hypocellular 40% CLL 8.8x10 ⁶ CLL cells (Day -1)	N/A	3.5x10 ¹¹ - 5.5x10 ¹¹ CLL cells (Day -10)	1.4x10 ⁷ (1.46x10 ⁷ /kg)	CR (7+ months)

1. UPN 02 Karyotype (ISCN Nomenclature): 45,XY,del(1)(q25),+del(1)(p13),t(2;20)(p13;q11.2),t(3;5)(p13;q35),adcf9)(p22),?del(13)(q14q34),-14,del(17)(p13)[cp24]

2. UPN 03 Karyotype (ISCN Nomenclature): 46,XY,del(17)(p12)[18]44-46,iden,der(17)t(17;21)(p11.2;q11.2)[cp4]40-45,XY,-17[cp3]

3. See Supplementary Materials for methods of tumor burden determination.

Figure 7

Sustained Detection of CART19 Cells 18 Months Post-Infusion

UPN03

UPN01

Blood

Bone Marrow

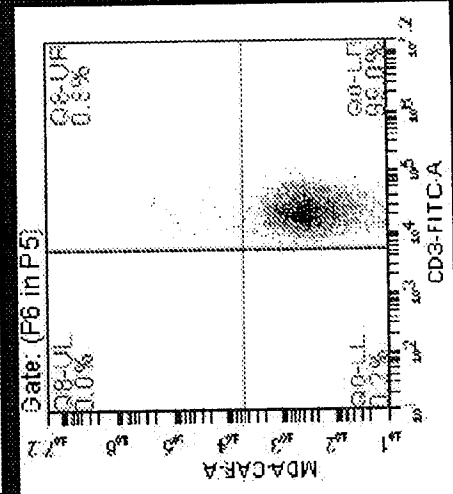
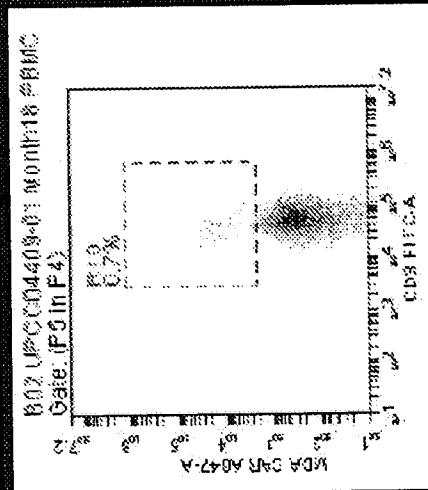
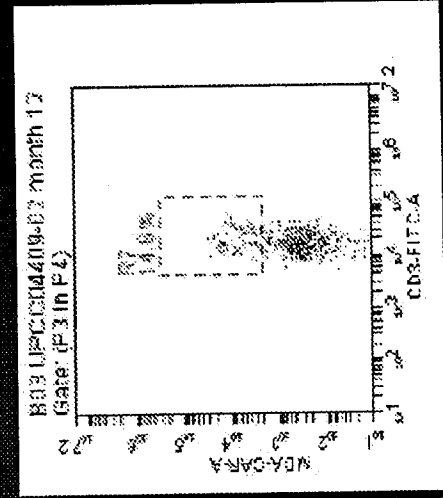
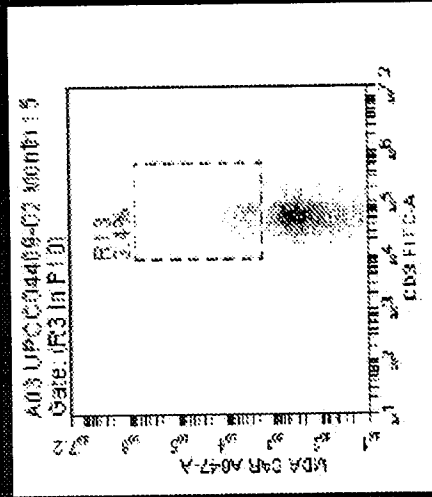


Figure 8

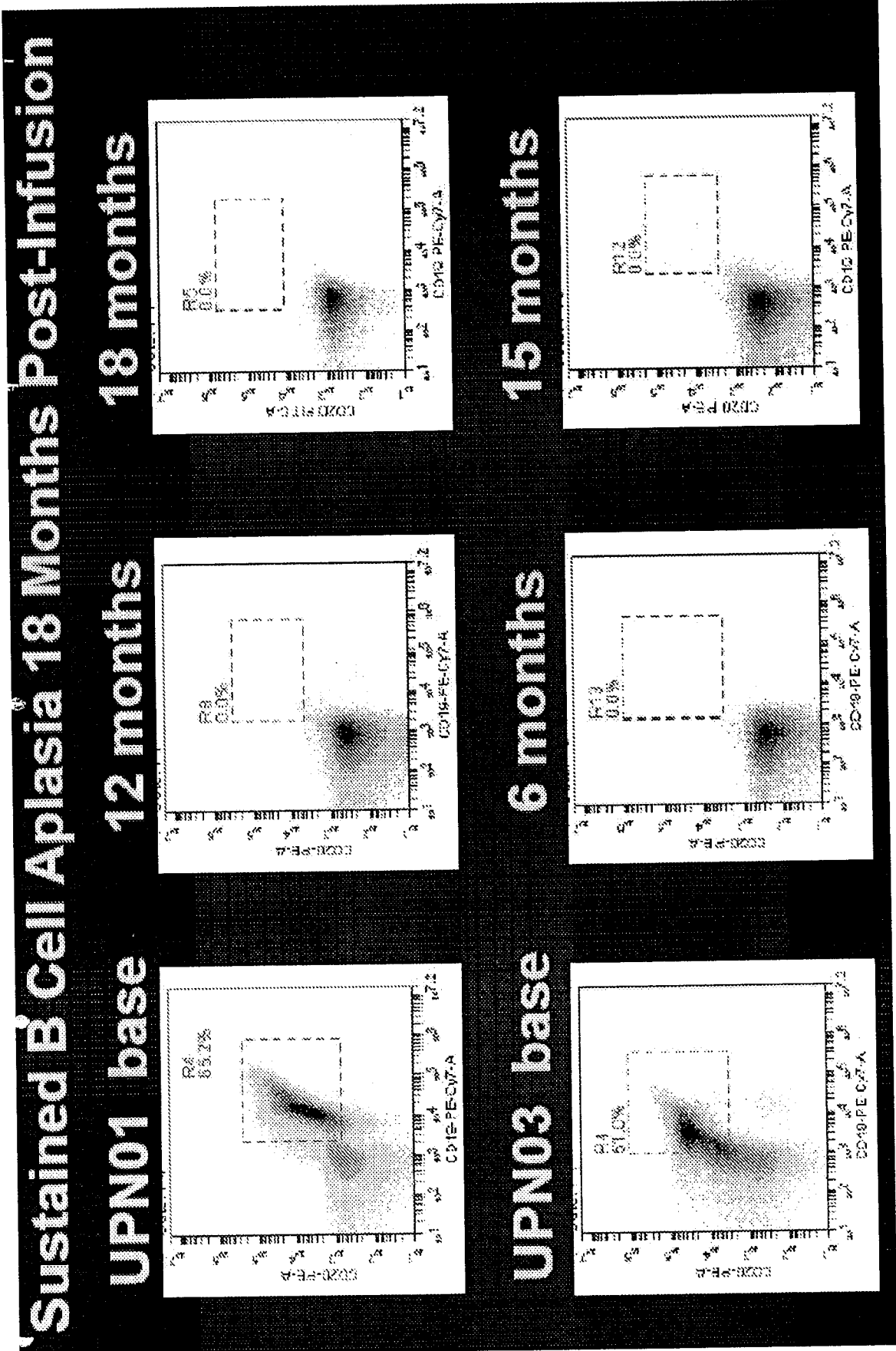


Figure 9A

Deep sequencing to detect residual CLL and B cells

Patient ID	Time Point	Amount of DNA in PCR (ng)	Cell equivalents	Total Productive Sequence Reads	Total Productive Unique Clonotypes	Dominant Clone Reads	Clone Frequency (% Productives)
UPN03	Pre- Infusion	2,000	317,460	22,074,912	68,234	19,948,508	90.4
03	Day -1	386	61,270	1,385,340	4,544	1,231,018	88.9
03	D+31	1,000	158,730	0	0	0	0.000
03	D+176	2,000	317,460	0	0	0	0.000
UPN01	Pre- Infusion	1,000	158,730	184,786	24	184,256	99.7
01	Day -1	1,000	158,730	408,579	48	407,592	99.9
01	D+28	1,000	158,730	0	0	0	0.000
01	D+176	600	79,366	285,305	7,362	0	0.000

Figure 9B

Reduction of plasma cells after CART19 Therapy

1. 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 marrow.

Patient	Days after T cell infusion	% plasma cell (CD138 ⁺)
UPN 01	-23	1%
	+29	0%
	+116	0%
	+174	0%
UPN 02	-45	2.3%
	-3	2.3%
	+128	1%
UPN 03	-3	3.5%
	+21	1.2%
	+174	1.2%

Figure 10