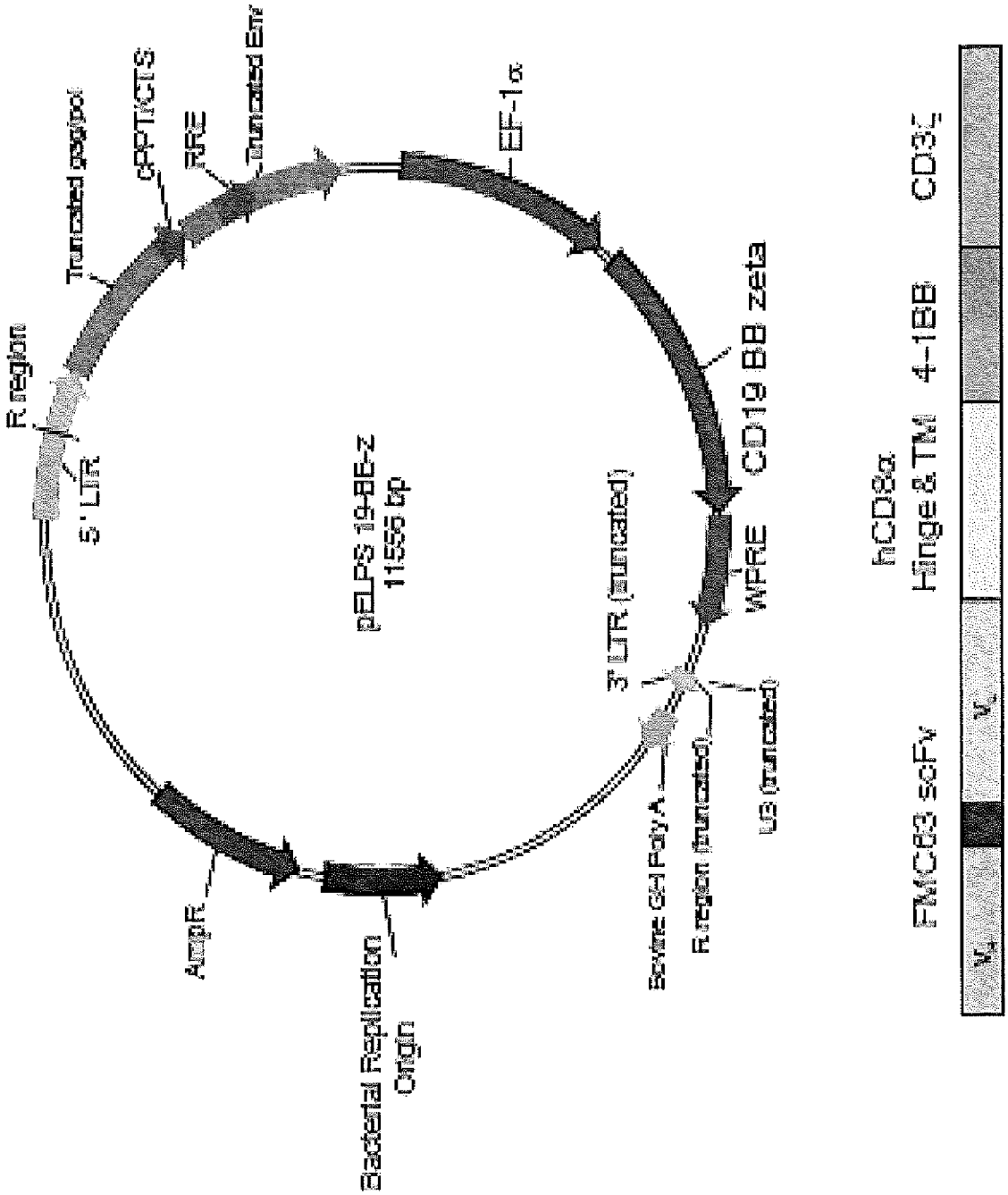

pELPS 19-BB-z
11555 bp
R region
5' LTR
Truncated gag/pol
cPPT/CTS
RRE
Truncated Env
EF-1α
CD19 BB zeta
WPRE
3' LTR (truncated)
Bovine GH Poly A
R region (truncated)
U3 (truncated)
AmpR
Bacterial Replication Origin
hCD8α
Hinge & TM
4-1BB
CD3ζ
FMC63 scFv

Figure 1A

Day 0
Day 0-1
Day 3
Day 5
Harvest Day
(10 ± 2)
Leukocyte Apheresis
CD3/28 positive selection of T-cells with anti-CD3/anti-CD28 mAb coated magnetic beads
Seed in gas permeable bags. Transduction w/ αCD19-41BBζ vector
Vector wash out Culture in gas permeable bags
Culture in Wave Bioreactor
Remove Beads
Harvest, Wash, Concentrate
Cryopreserve Final Product in Infusible Cryomedia

Figure 1B

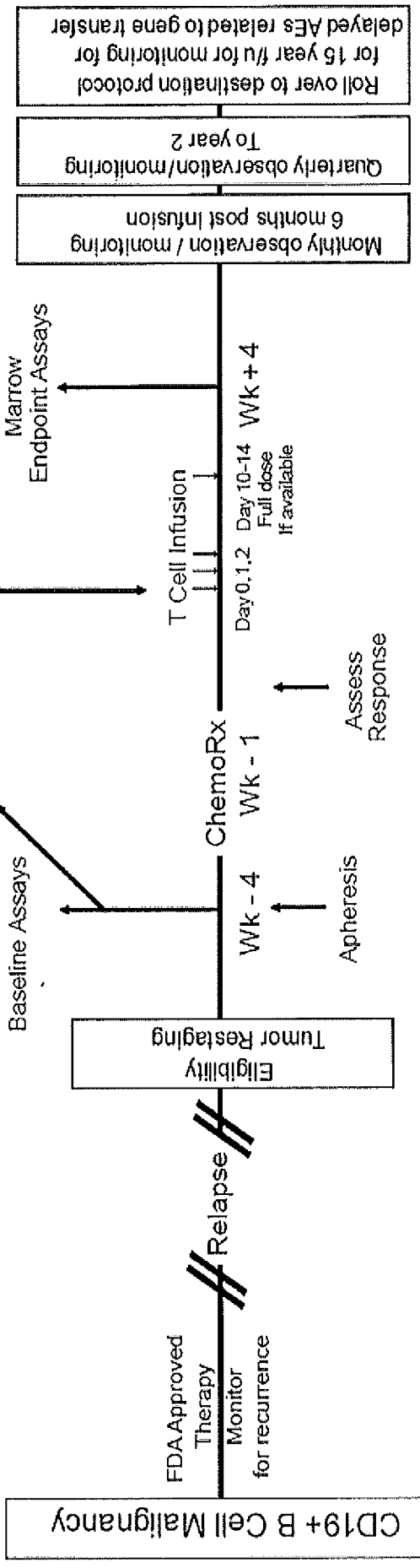
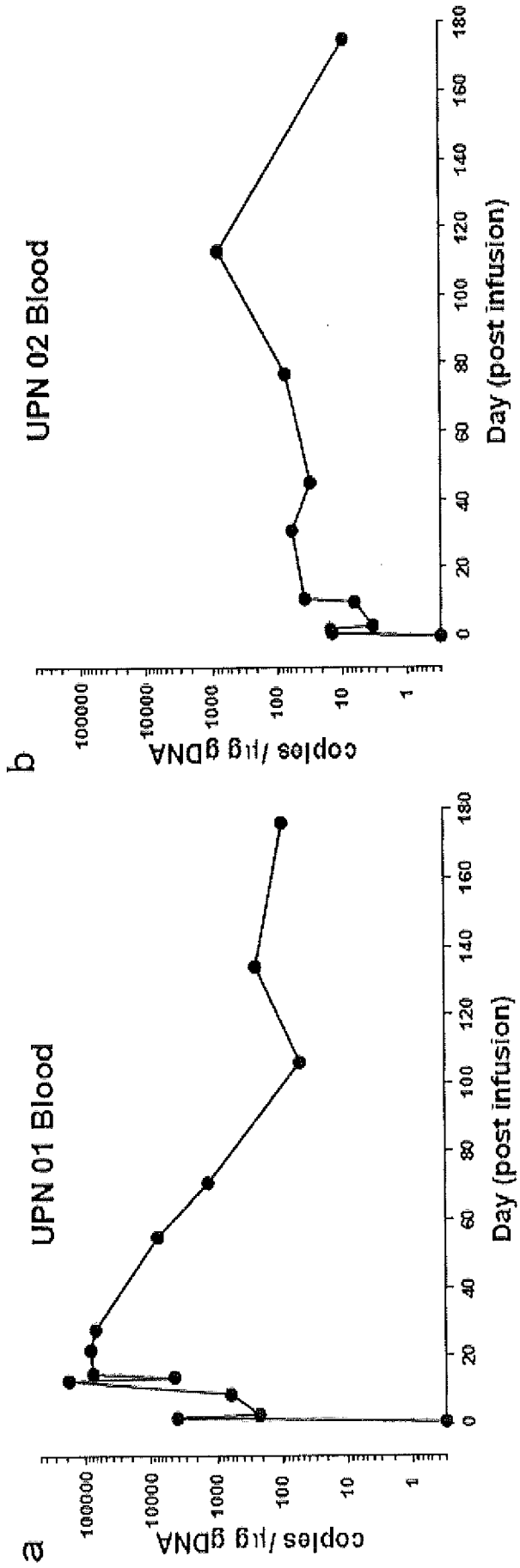
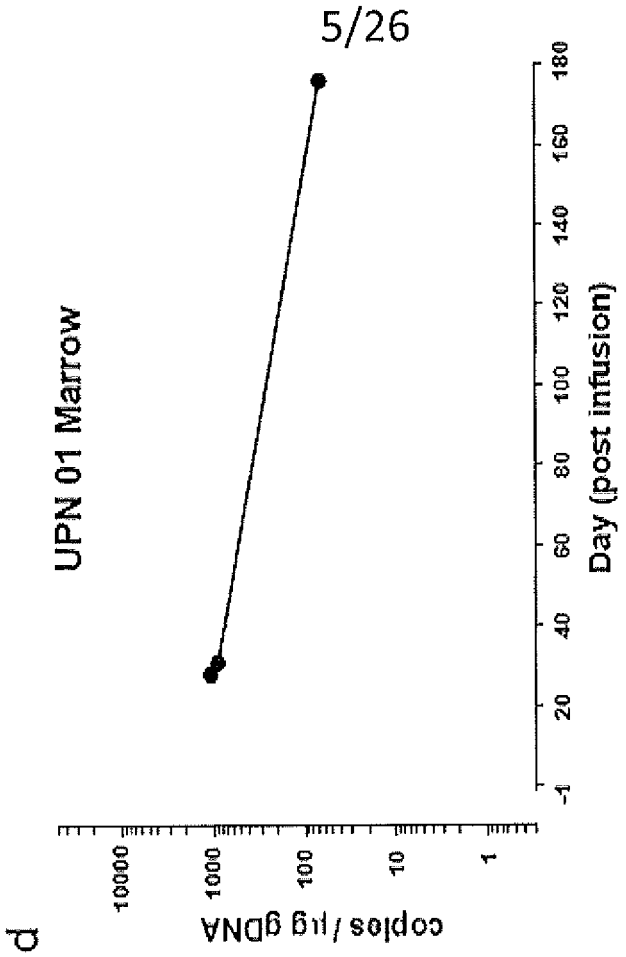
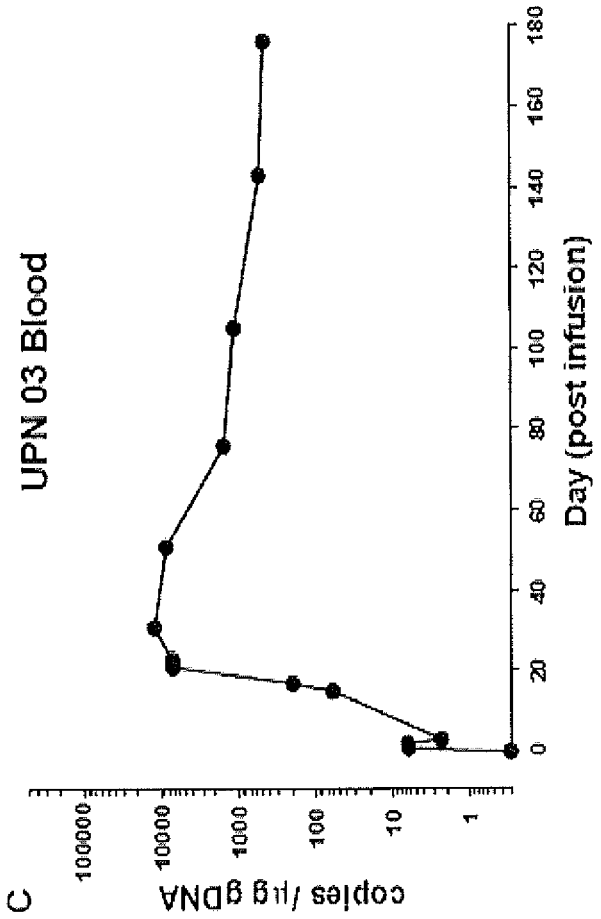

CD19+ B Cell Malignancy
FDA Approved Therapy
Monitor for recurrence
Relapse
Eligibility
Tumor Restaging
PBMC
Baseline Assays
Manufacture / Cryopreservation
Apheresis
Wk - 4
ChemoRx
Wk - 1
Assess Response
T Cell Infusion
Day 0,1,2
Day 10-14
Full dose
If available
PBMC
Marrow
Endpoint Assays
Wk + 4
Monthly observation / monitoring
6 months post Infusion
Quarterly observation/monitoring
To year 2
Roll over to destination protocol
for 15 year f/u for monitoring for
delayed AEs related to gene transfer

Figure 1C

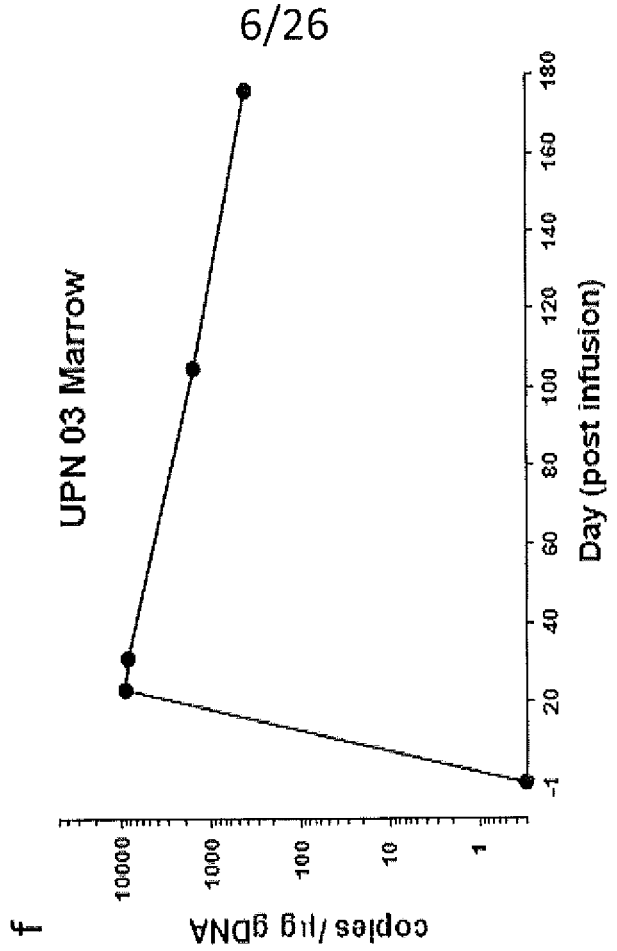


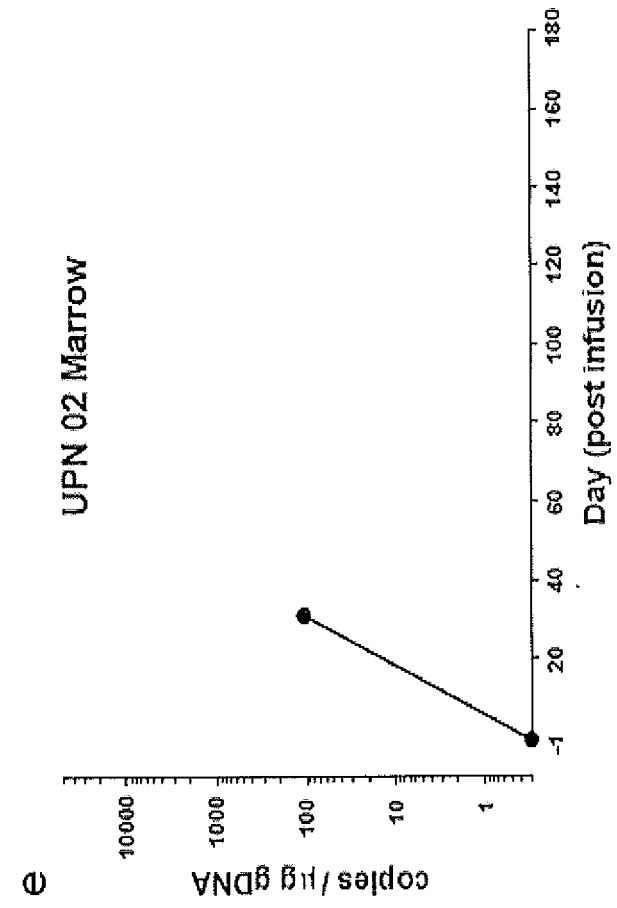
Figures 2A-2B



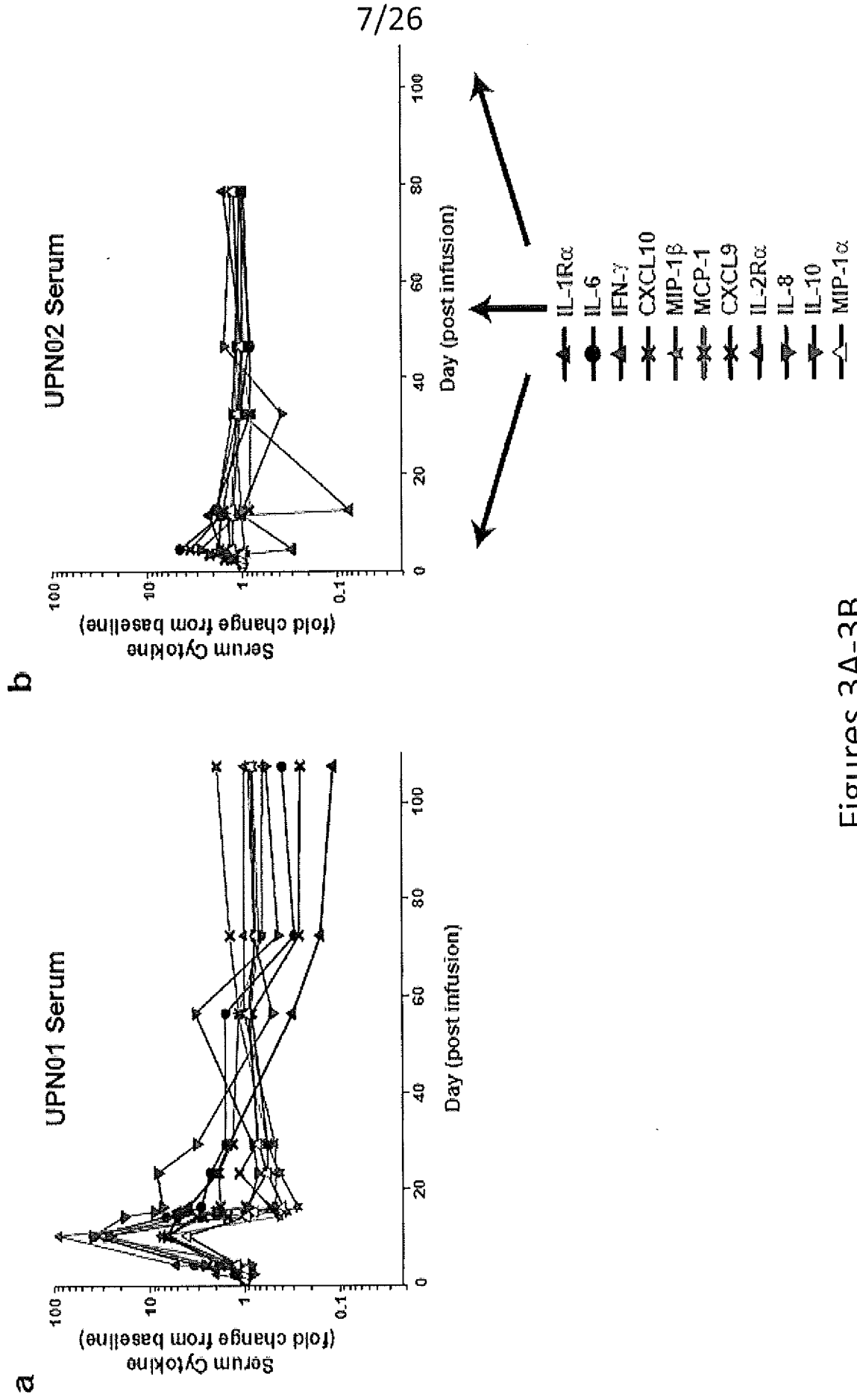


Figures 2C-2D

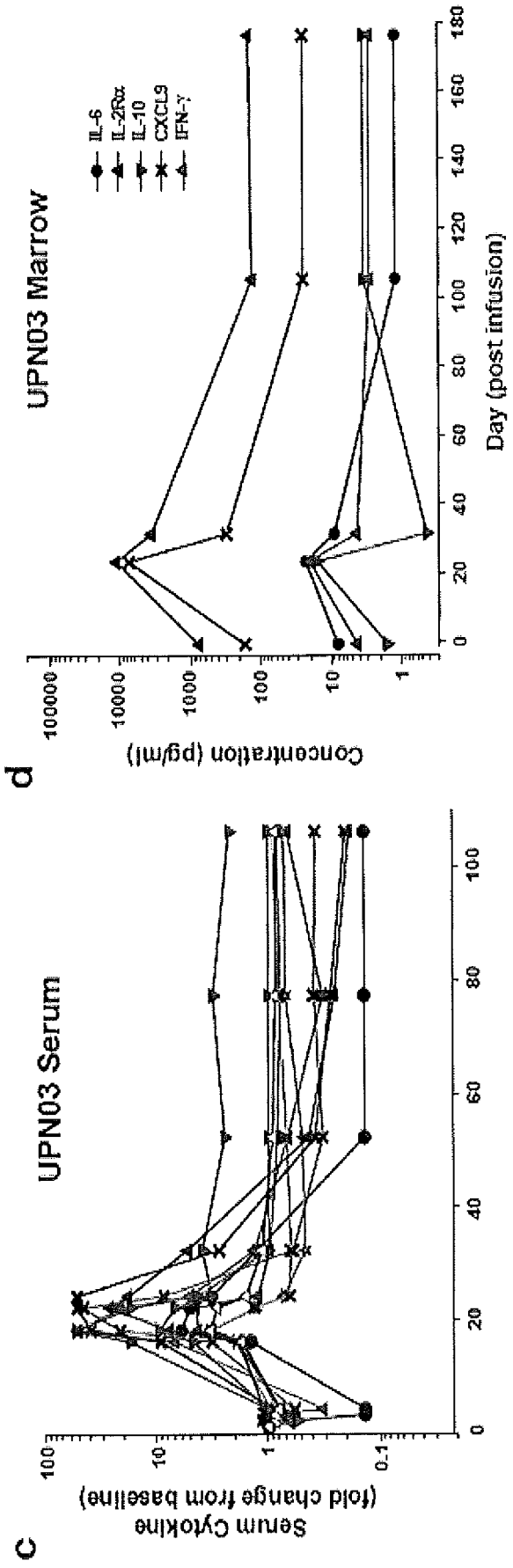




Figures 2E-2F



Figures 3A-3B



Figures 3C-3D

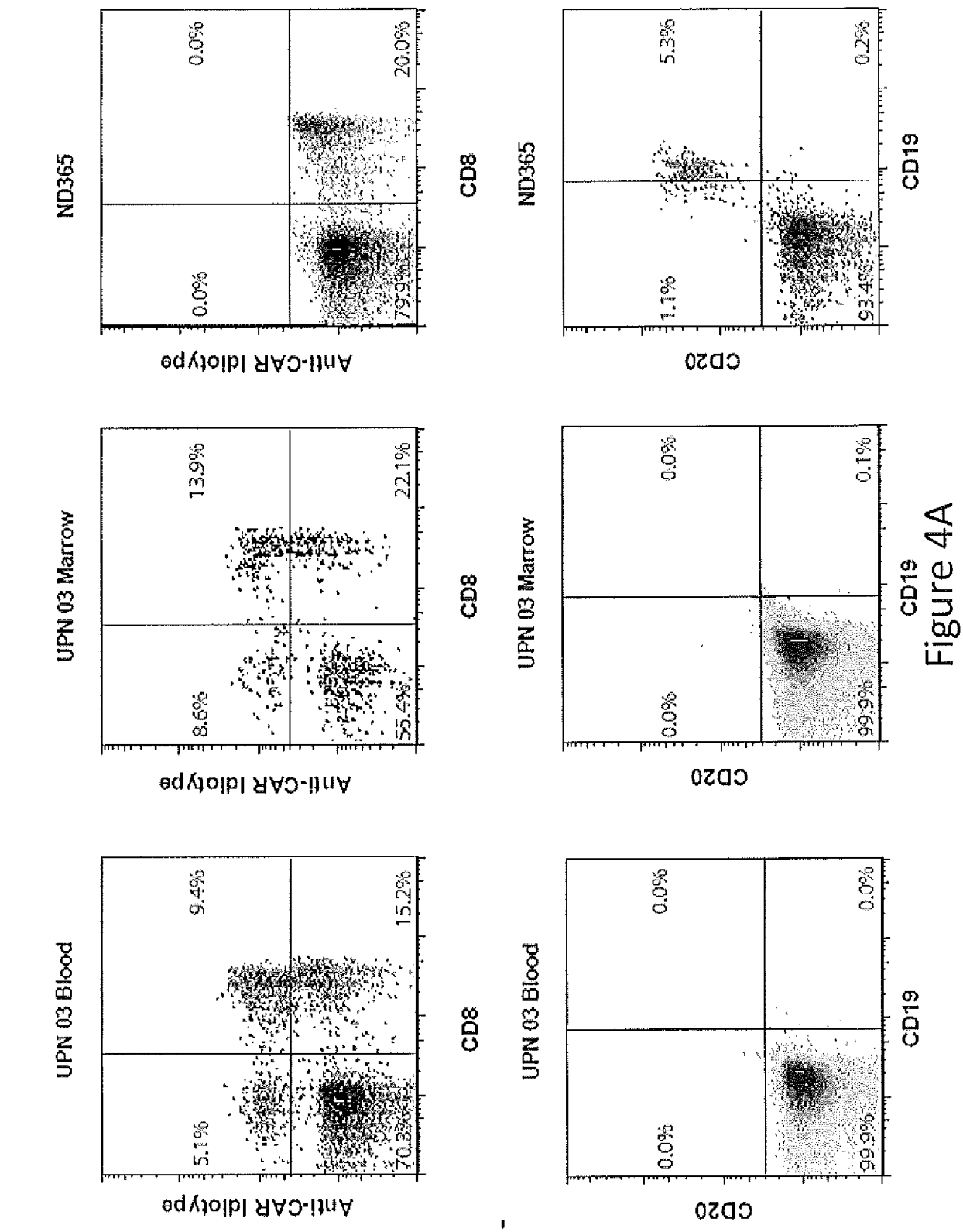

a
CD3+ Gate
CD3-, CD14 - Gate
UPN 03 Blood
UPN 03 Marrow
ND365
Anti-CAR Idiotype
CD8
CD20
CD19
5.1%
9.4%
70.3%
15.2%
8.6%
13.9%
55.4%
22.1%
0.0%
0.0%
99.9%
0.1%
1.1%
5.3%
93.4%
0.2%
79.9%
0.0%
20.0%

Figure 4A

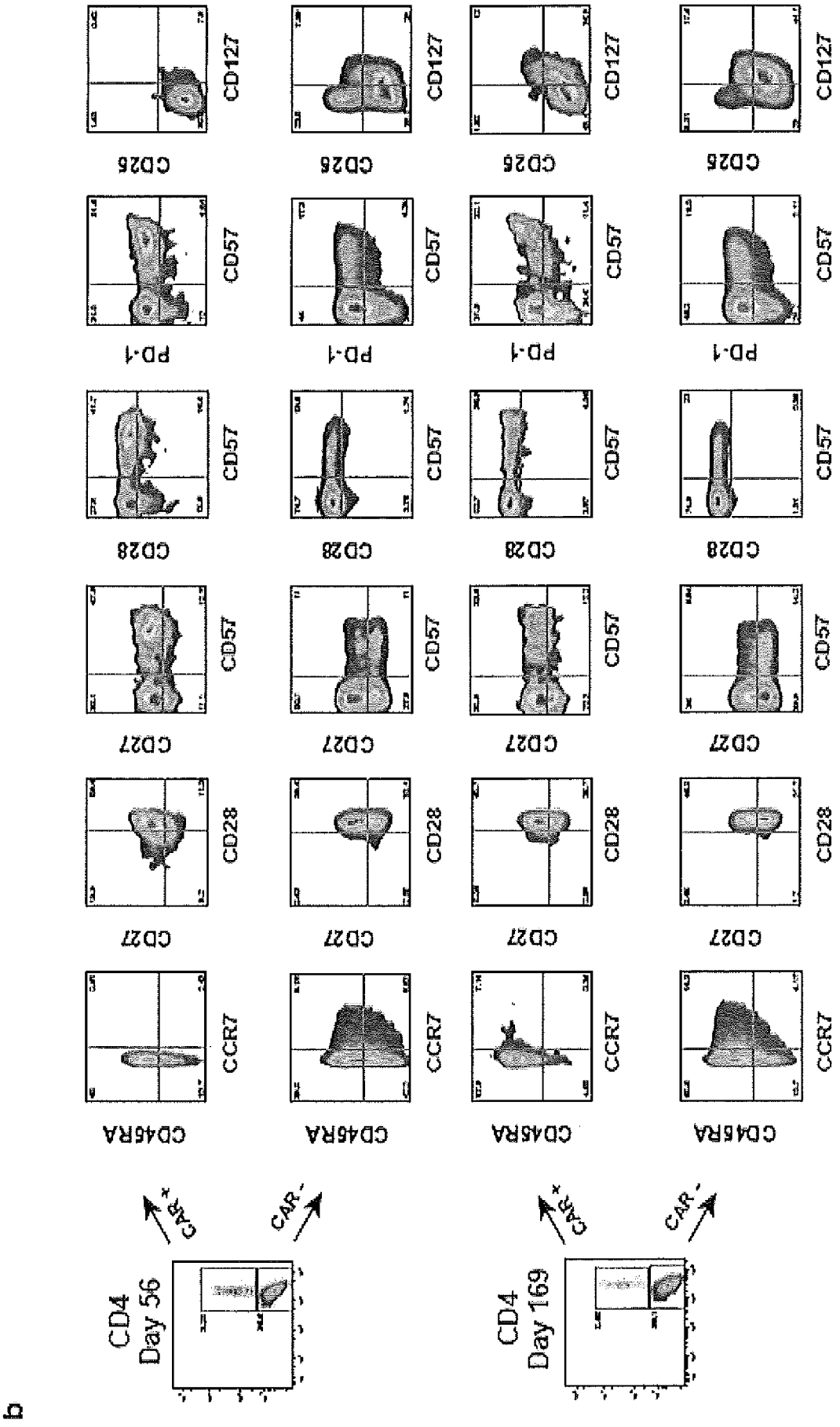

b
CD4
Day 56
CAR+
CAR-
CD4
Day 169
CAR+
CAR-
CD45RA
CCR7
CD27
CD28
CD27
CD57
CD28
CD57
PD-1
CD57
CD26
CD127

Figure 4B

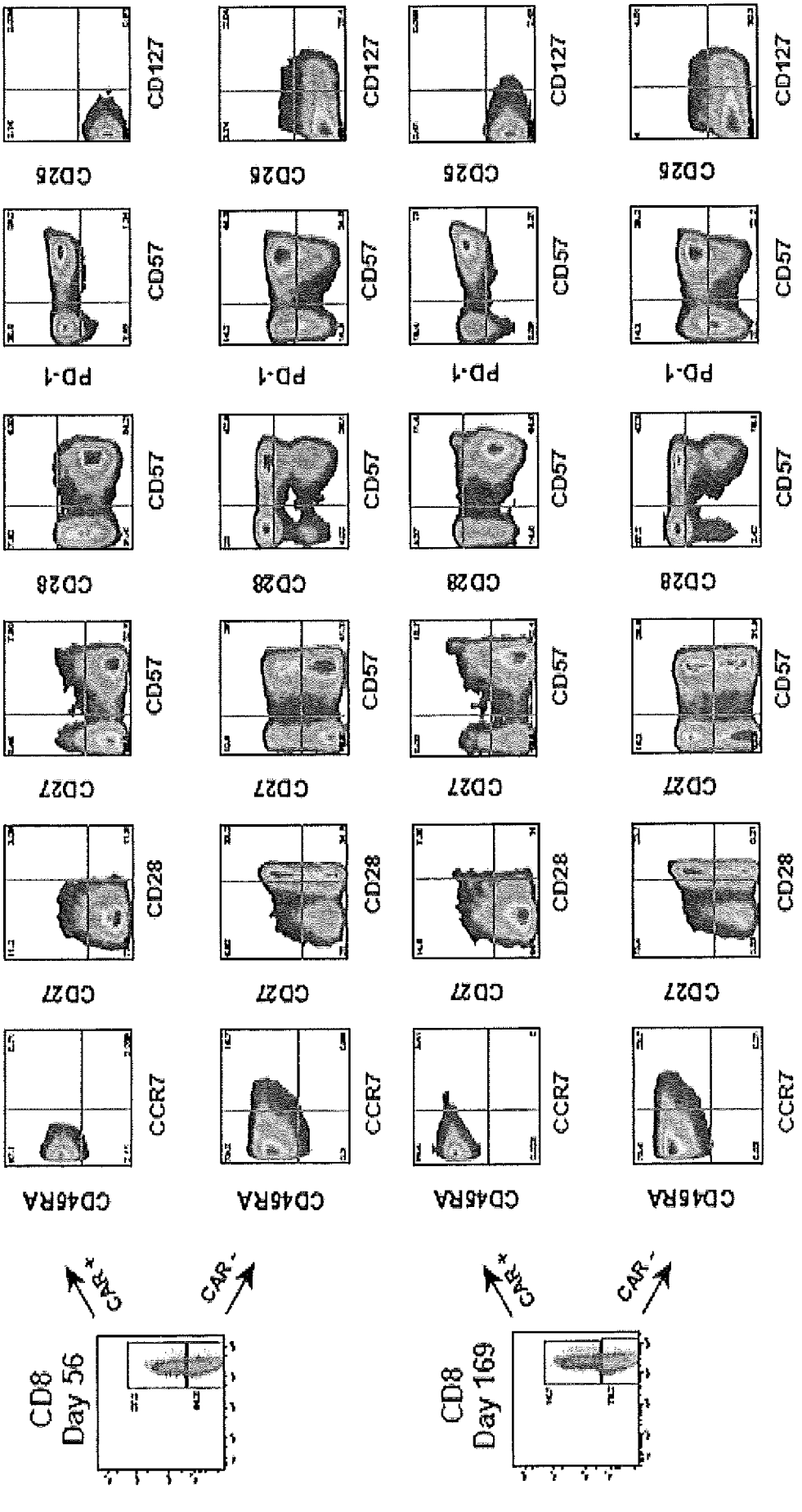

c
CD8
Day 56
CAR+
CAR-
CD8
Day 169
CAR+
CAR-
CD45RA
CCR7
CD27
CD28
CD27
CD57
CD28
CD57
PD-1
CD57
CD26
CD127

Figure 4C

d

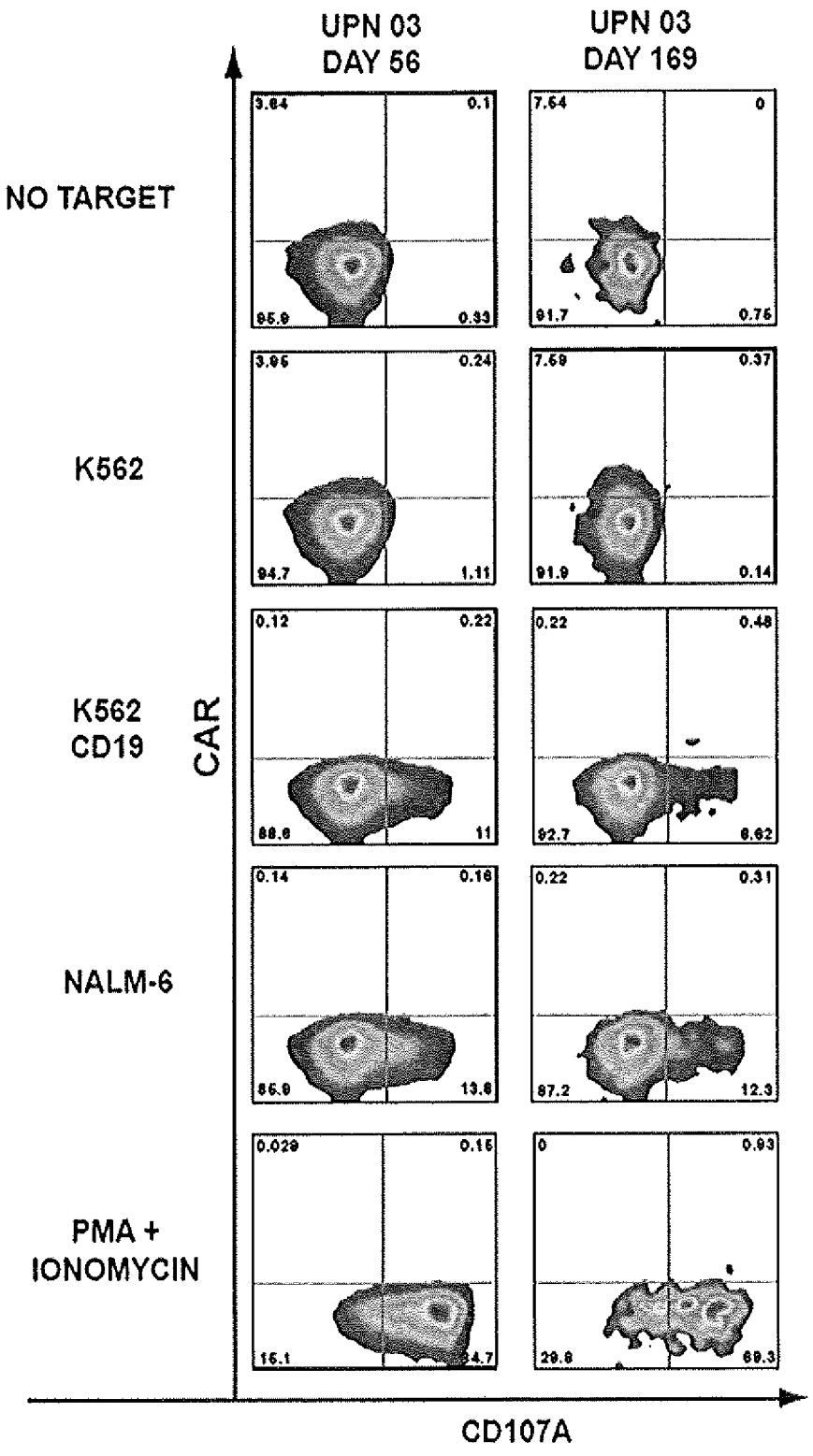

UPN 03
DAY 56
UPN 03
DAY 169
NO TARGET
K562
K562
CD19
NALM-6
PMA +
IONOMYCIN
CAR
CD107A
3.64
0.1
95.9
0.33
7.64
0
91.7
0.76
3.96
0.24
94.7
1.11
7.69
0.37
91.9
0.14
0.12
0.22
88.6
11
0.22
0.48
92.7
6.62
0.14
0.16
86.9
13.6
0.22
0.31
87.2
12.3
0.029
0.16
15.1
84.7
0
0.93
29.8
69.3

Figure 4D

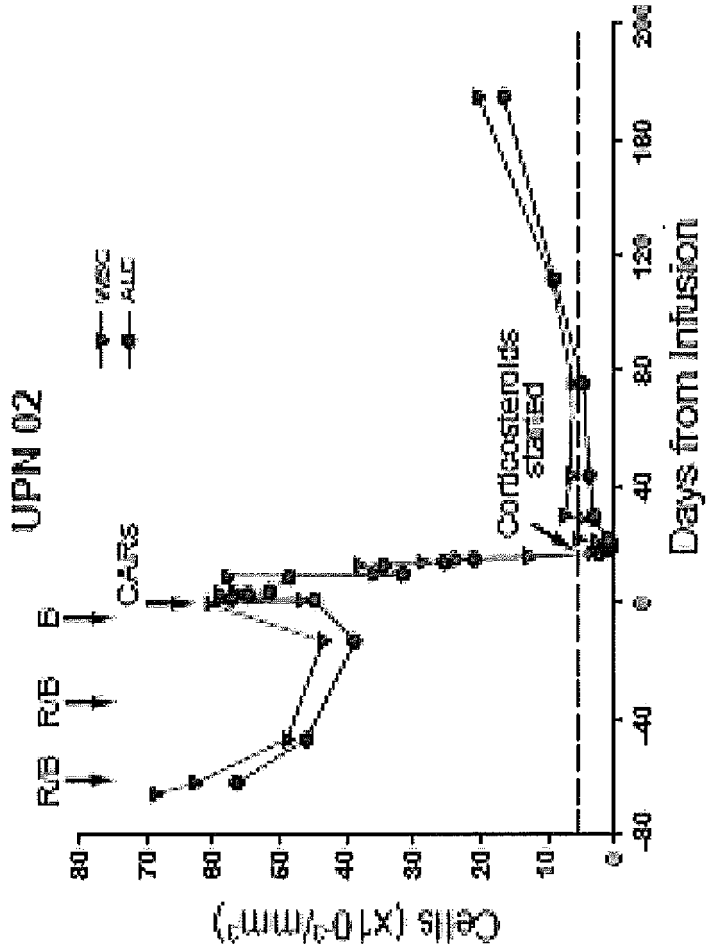
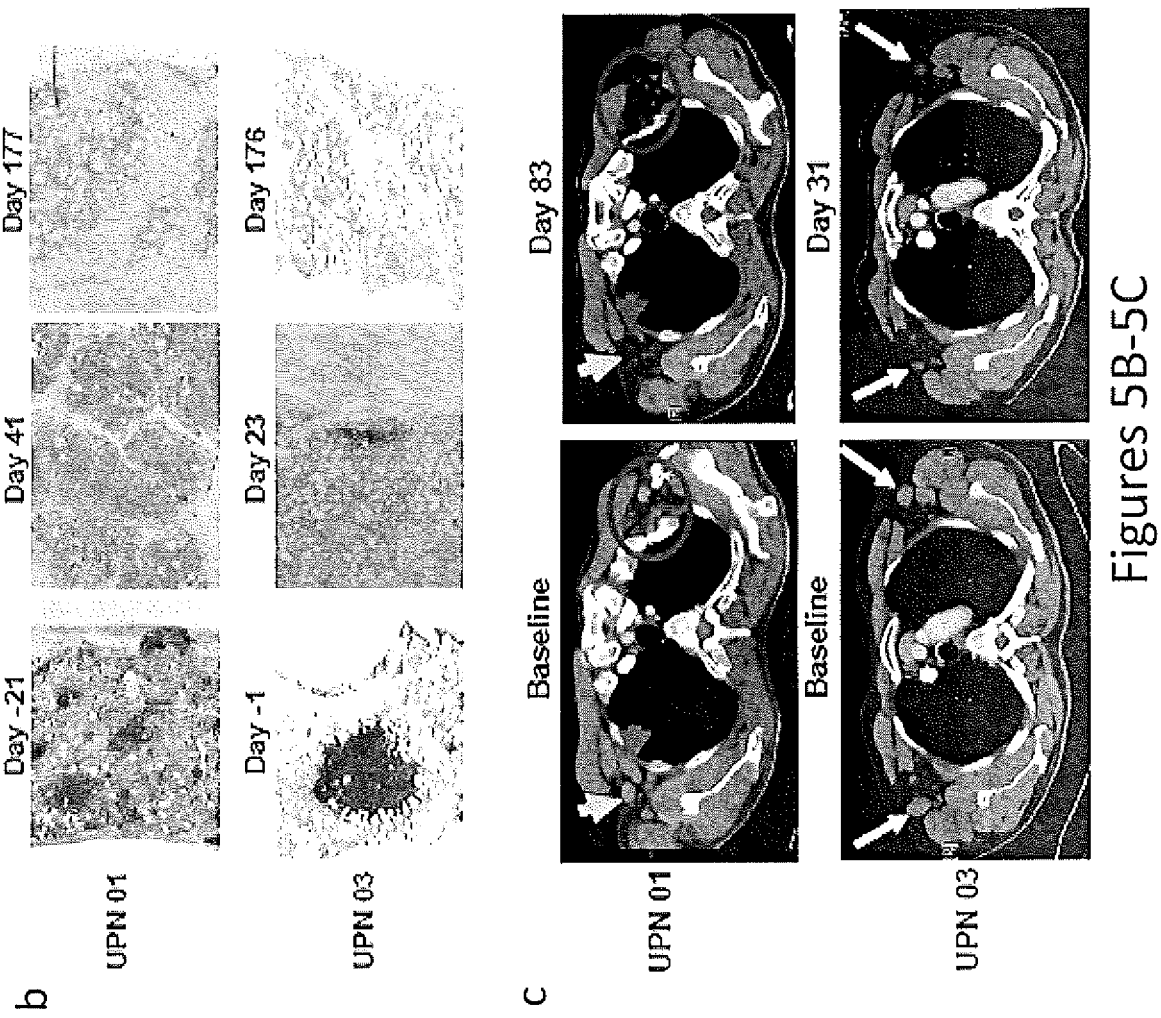

UPN 02
R/B
R/B
B
CARs
Corticosteroids started
ANC
ALC
Cells (x10⁻³/mm³)
Days from Infusion
80
70
60
50
40
30
20
10
0
-80
-40
0
40
80
120
180
200

Figure 5A



Figures 5B-5C

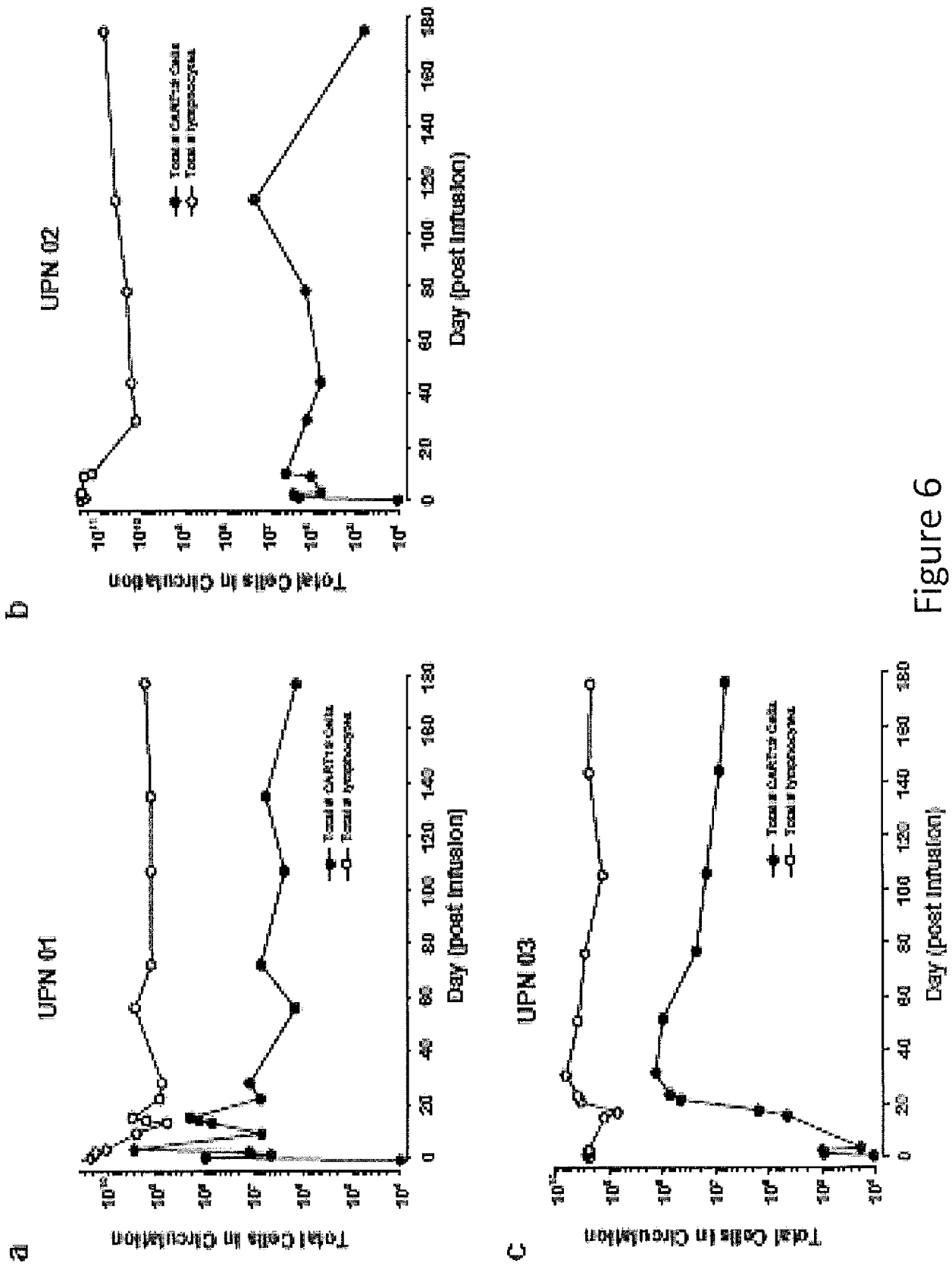

a
UPN 01
b
UPN 02
c
UPN 03
Total Cells in Circulation
Day (post Infusion)

Figure 6

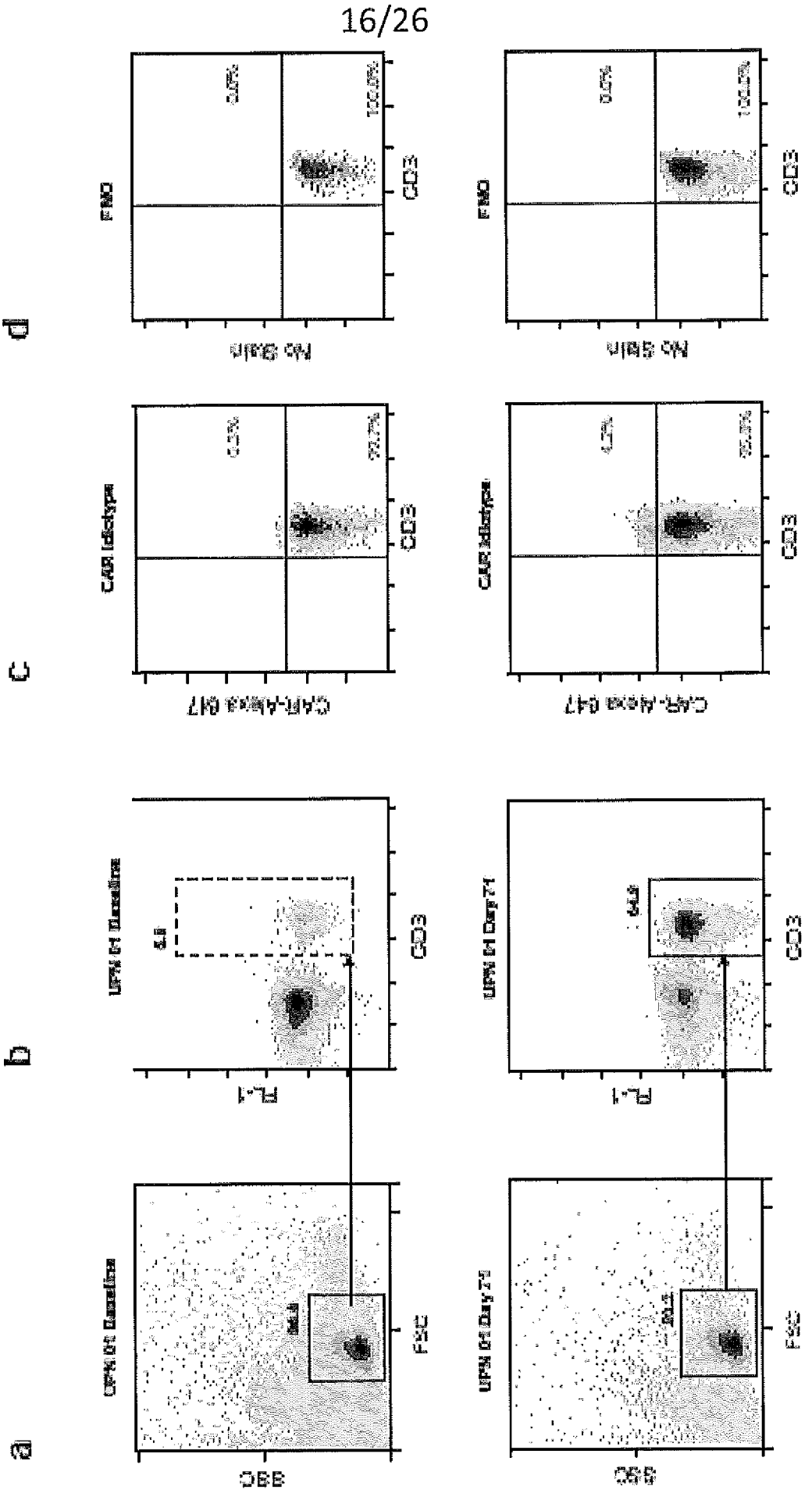

a
b
c
d
SSC
FSC
UPN 01 Baseline
UPN 01 Day 71
FL-1
CD3
CAR Idiotype
CAR-Alexa 647
FMO
No Stain

Figure 7

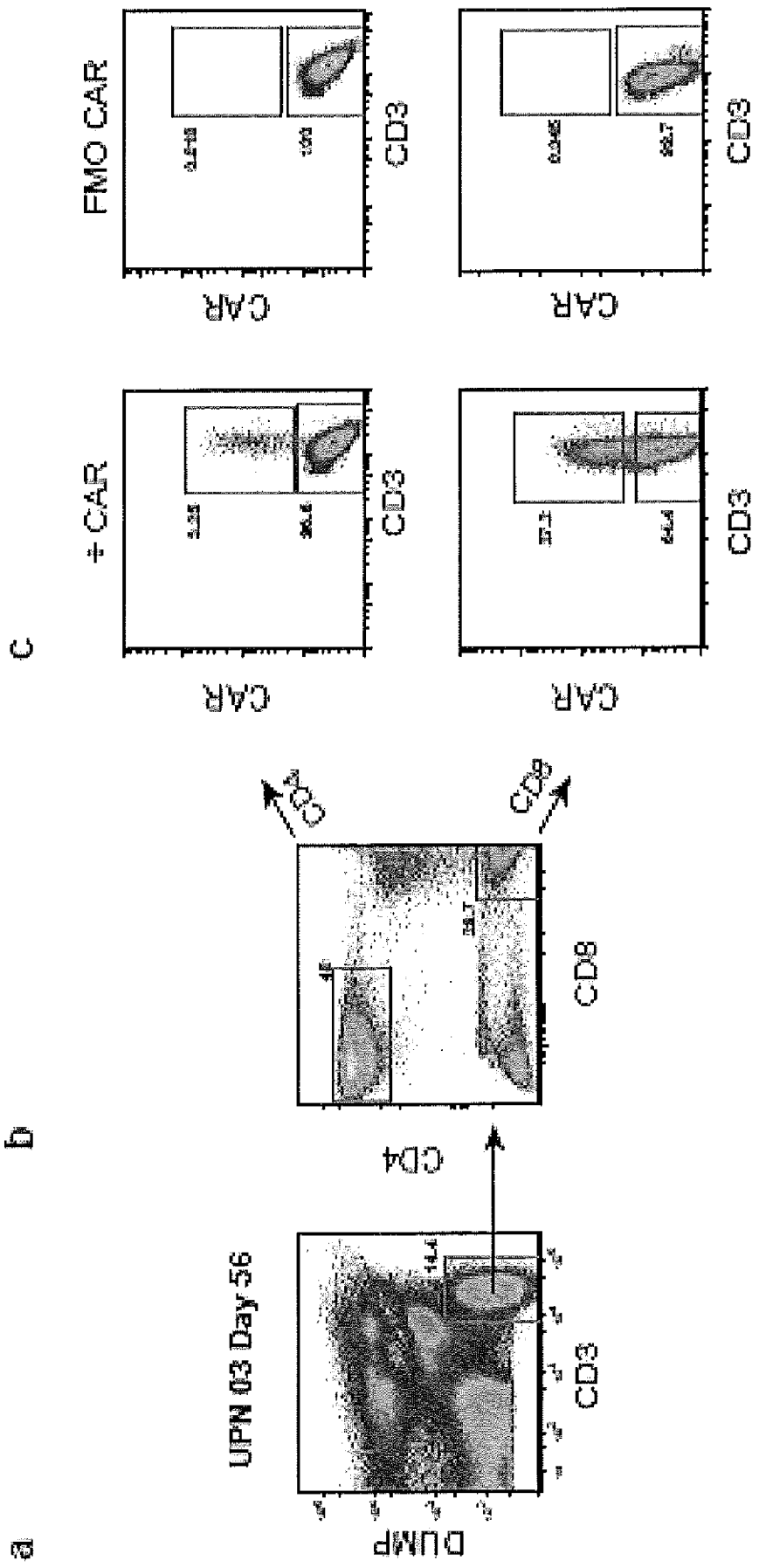


Figure 8

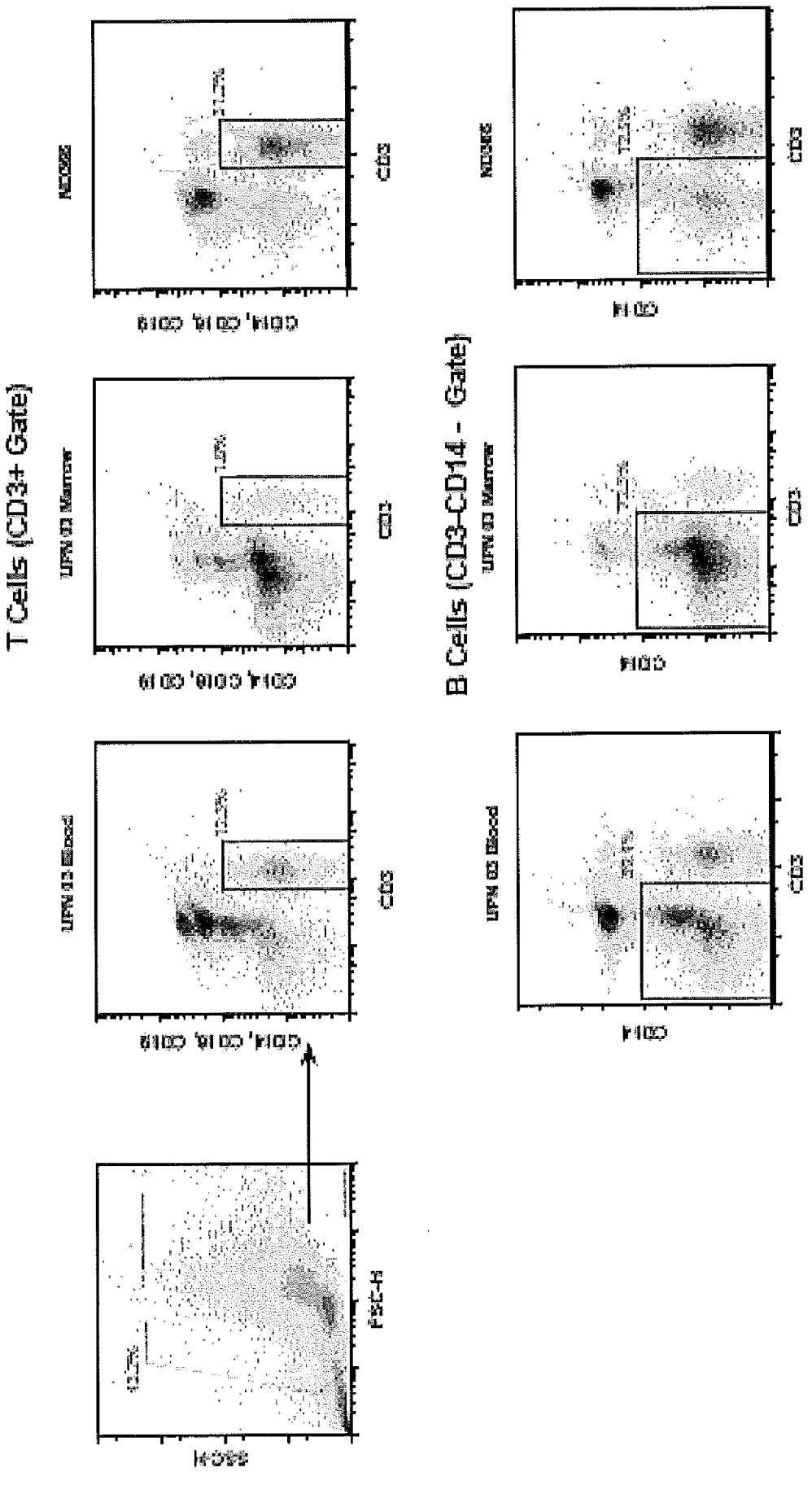

T Cells (CD3+ Gate)
B Cells (CD3-CD14 - Gate)
CD3
CD14, CD16, CD19
SSC-H
FSC-H

Figure 9

Subject UPN	Age/sex Karyotype	Previous therapies	CLL Tumor Burden at Baseline			Total Dose of CART19 (cells/kg)	Response D +30 (Duration)
			Bone marrow[3] (Study Day)	Blood[3] (Study Day)	Nodes/spleen[3] (Study Day)		
01	65/M normal	Fludarabine x 4 cycles (2002) Rituximab/fludarabine x 4 cycles (2005) Alemtuzumab x 12 wks (2006) Rituximab (2 courses 2008-2009) R-CVP x 2 cycles (2009) Lenalidomide (2009) PCR x 2 cycles (5/18-6/18/2010) Bendamustine x 1 cycle (7/31-8/1/10) pre-CART19	Hypercellular 70% CLL $2.4x10^{12}$ CLL cells (Day -14) $1.7x10^{12}$ CLL cells (Day -1)	N/A	$6.2x10^{11}$ - $1.0x10^{12}$ CLL cells (Day -37)	$1.1x10^{9}$ ($1.5x10^{7}$/kg)	CR (8+ months)
02	77/M del(17)(p13)[1]	Alemtuzumab x 16 wks (6/2007) Alemtuzumab x 18 wks (3/2009) Bendamustine/ Rituximab: 7/1/2010 (cycle 1) 7/28/2010 (cycle 2) 8/26/2010 (cycle 3) pre-CART19	Hypercellular >95% CLL $3.2x10^{12}$ CLL cells (Day -47)	$2.75 x 10^{11}$ CLL Cells (Day -1)	$1.2x10^{12}$ -$2.0x10^{12}$ CLL cells (Day -24)	$5.8x10^{8}$ ($1.0x10^{7}$/kg)	PR (5 months)
03	64/M del(17)(p13)[2]	R- Fludarabine x 2 cycles (2002) R-Fludarabine x 4 cycles (10/06-1/07) R- Bendamustine x1 cycle (2/09) Bendamustine x 3 cycles (3-5/09) Alemtuzumab x 11 wks (12/09-3/10) Pentastatin/cyclophosphamide (9/10/10) pre-CART19	Hypercellular 40% CLL $8.8x10^{11}$ CLL cells (Day -1)	N/A	$3.3x10^{11}$ - $5.5x10^{11}$ CLL cells (Day -10)	$1.4x10^{7}$ ($1.46x10^{6}$/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),add(9)(p22),?del(13)(q14q34),-14,del(17)(p13)[cp24]

2. UPN 03 Karyotype (ISCN Nomenclature): 46,XY,del(17)(p12)[18]/44~46,idem,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 10

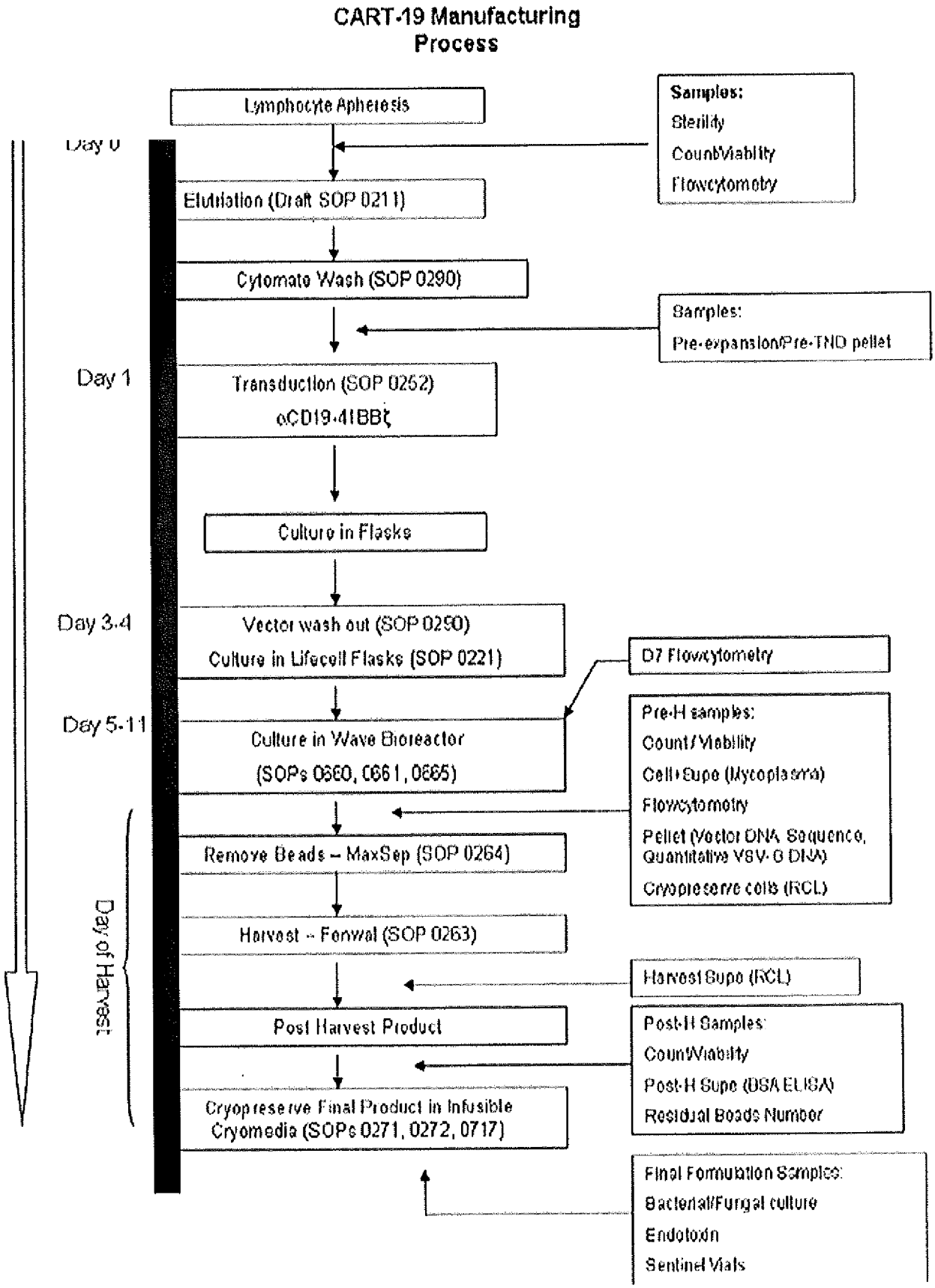
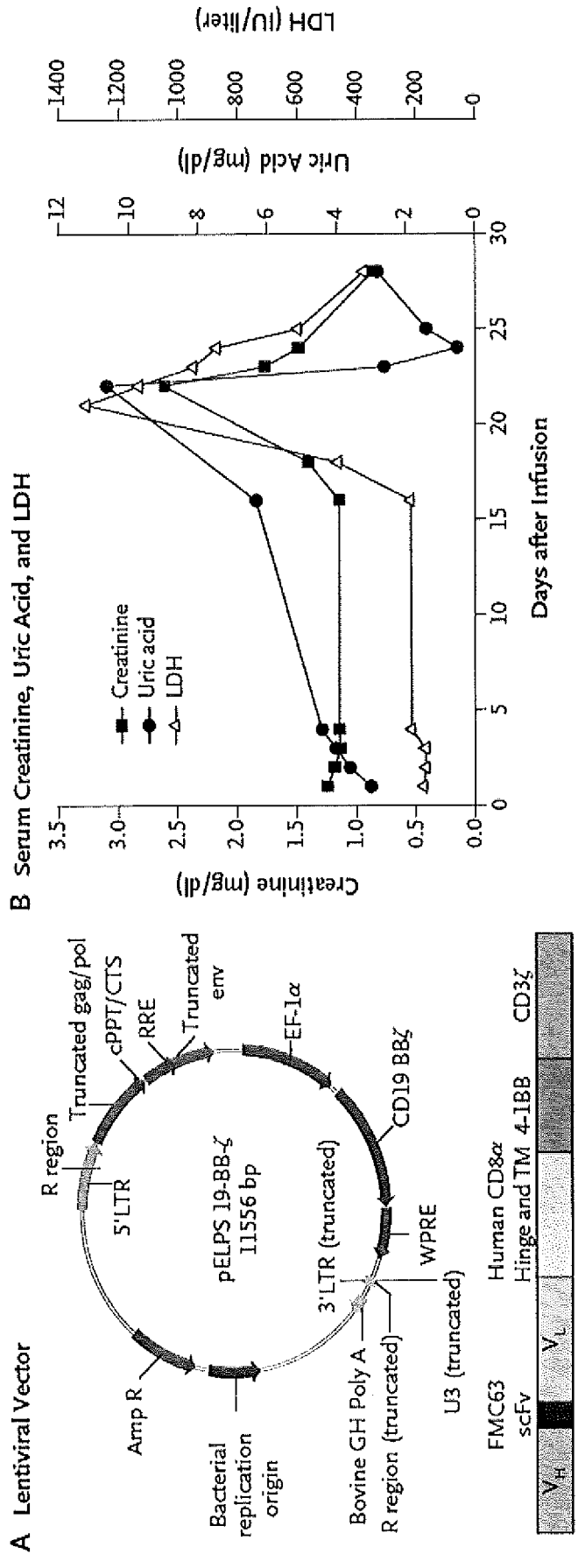
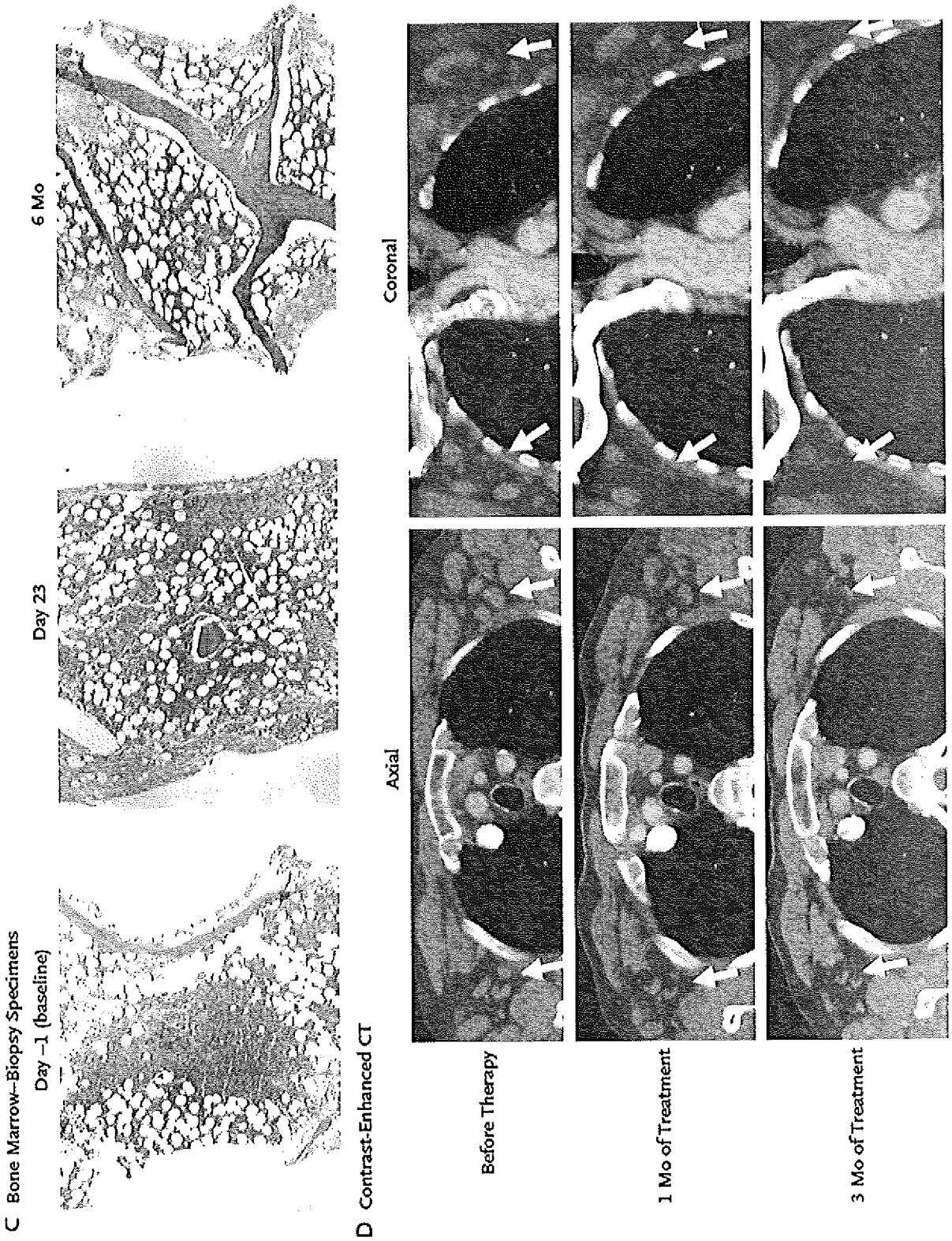

CART-19 Manufacturing Process
Lymphocyte Apheresis
Samples:
Sterility
Count/Viability
Flowcytometry
Day 0
Elutriation (Draft SOP 0211)
Cytomate Wash (SOP 0290)
Samples:
Pre-expansion/Pre-TND pellet
Day 1
Transduction (SOP 0252)
αCD19-41BBζ
Culture in Flasks
Day 3-4
Vector wash out (SOP 0290)
Culture in Lifecell Flasks (SOP 0221)
D7 Flowcytometry
Day 5-11
Culture in Wave Bioreactor
(SOPs 0660, 0661, 0665)
Pre-H samples:
Count/Viability
Cell+Supe (Mycoplasma)
Flowcytometry
Pellet (Vector DNA Sequence, Quantitative VSV-G DNA)
Cryopreserve cells (RCL)
Remove Beads – MaxSep (SOP 0264)
Harvest – Fenwal (SOP 0263)
Harvest Supe (RCL)
Day of Harvest
Post Harvest Product
Post-H Samples:
Count/Viability
Post-H Supe (BSA ELISA)
Residual Beads Number
Cryopreserve Final Product in Infusible Cryomedia (SOPs 0271, 0272, 0717)
Final Formulation Samples:
Bacterial/Fungal culture
Endotoxin
Sentinel Vials

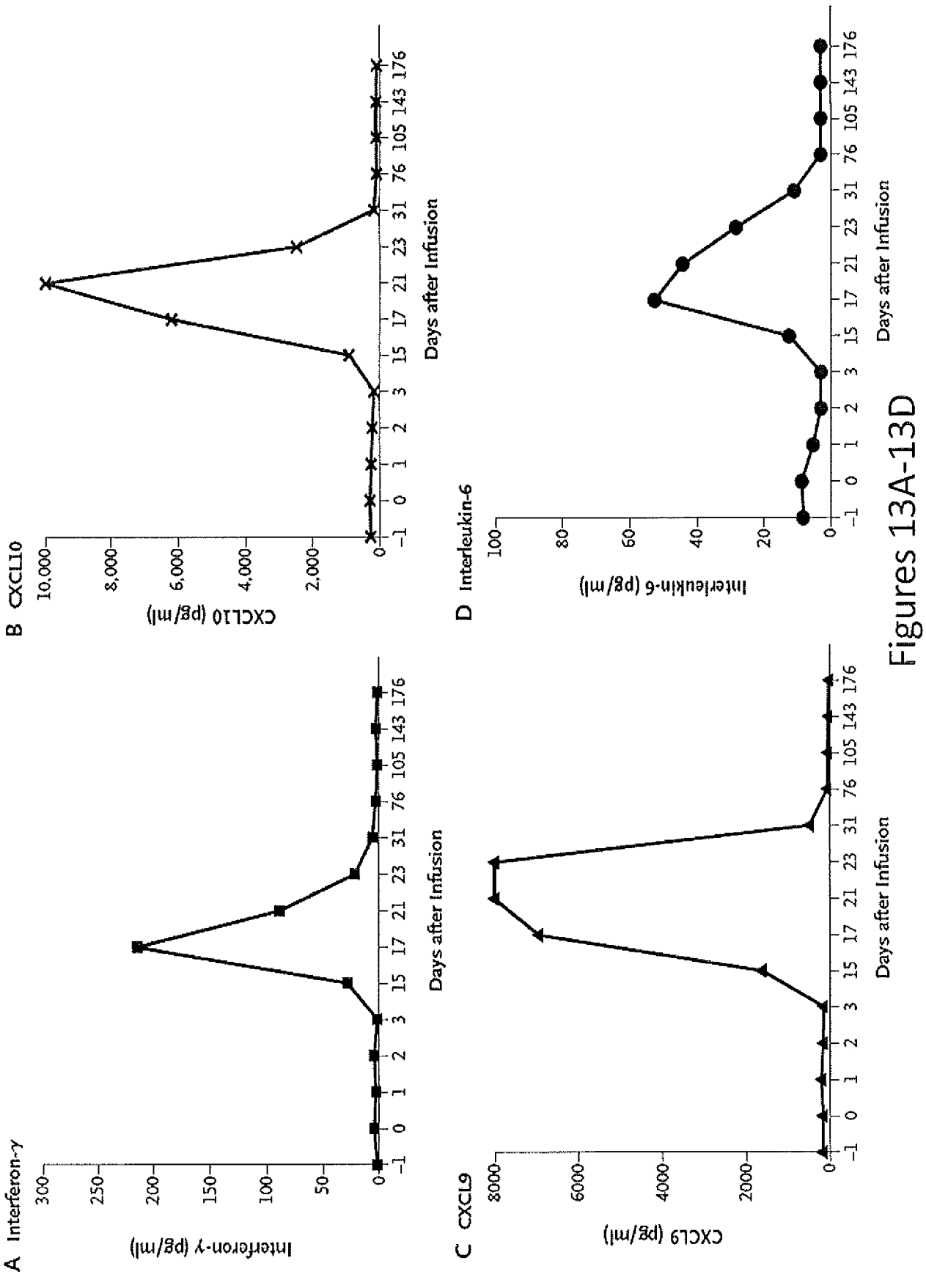
Figure 11



Figures 12A-12B



Figures 12C-12D



Figures 13A-13D

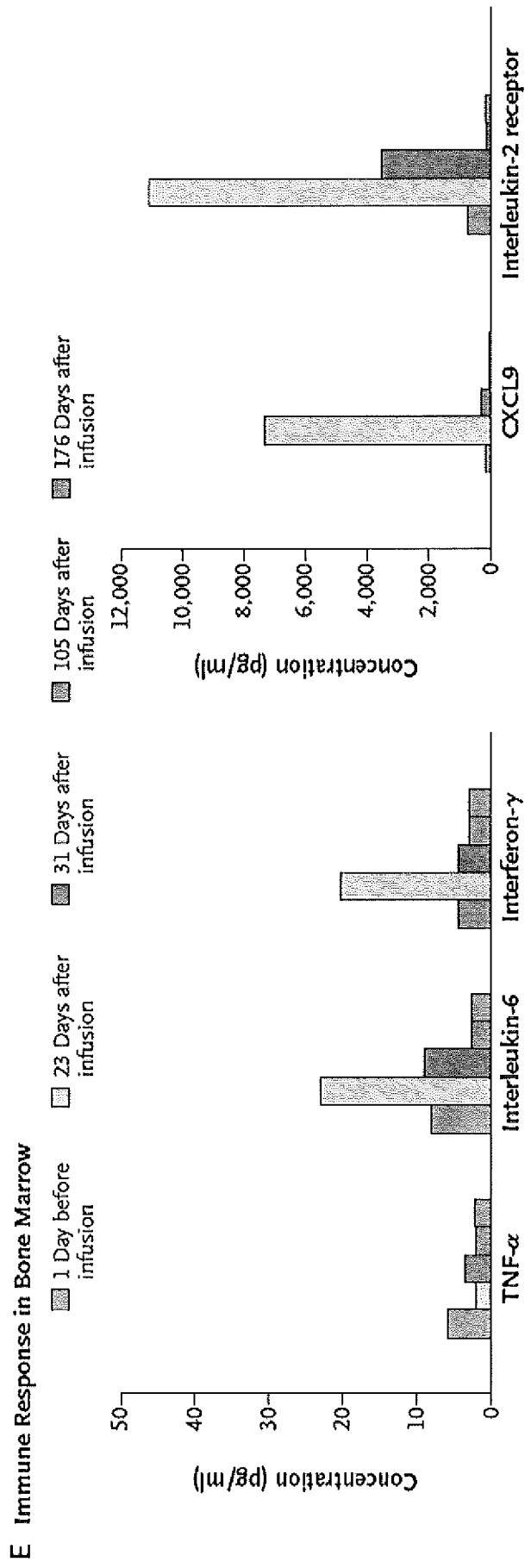
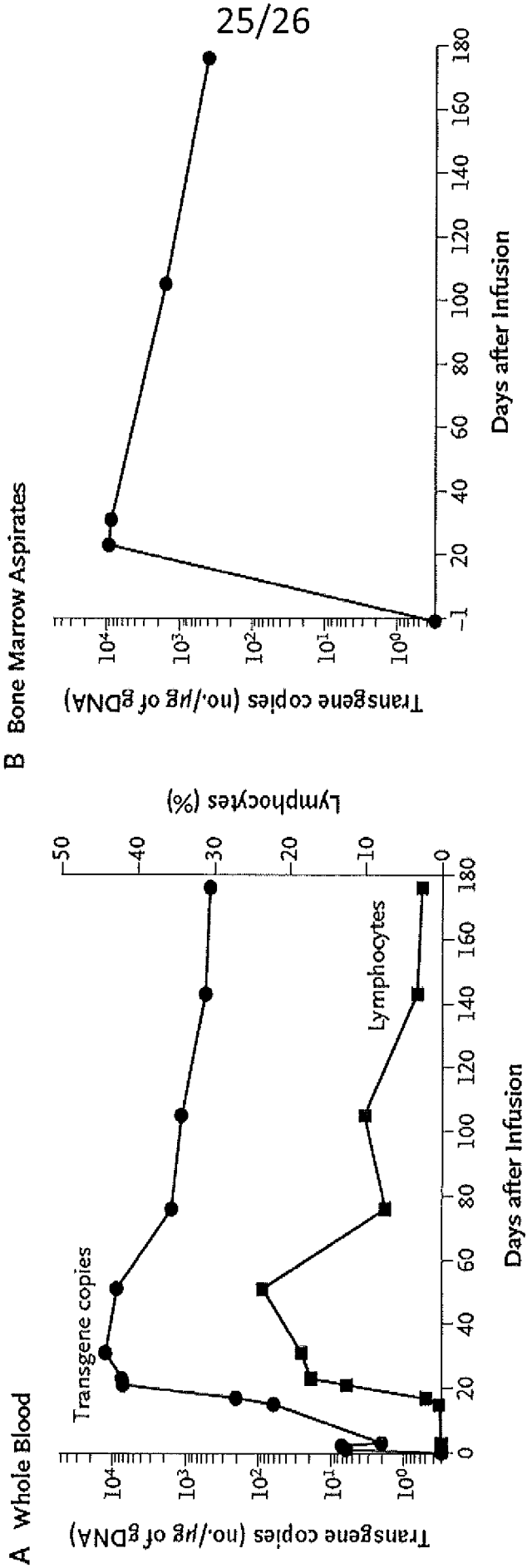

E Immune Response in Bone Marrow
1 Day before infusion
23 Days after infusion
31 Days after infusion
105 Days after infusion
176 Days after infusion
Concentration (pg/ml)
0
10
20
30
40
50
TNF-α
Interleukin-6
Interferon-γ
Concentration (pg/ml)
0
2,000
4,000
6,000
8,000
10,000
12,000
CXCL9
Interleukin-2 receptor

Figure 13E



Figures 14A-14B

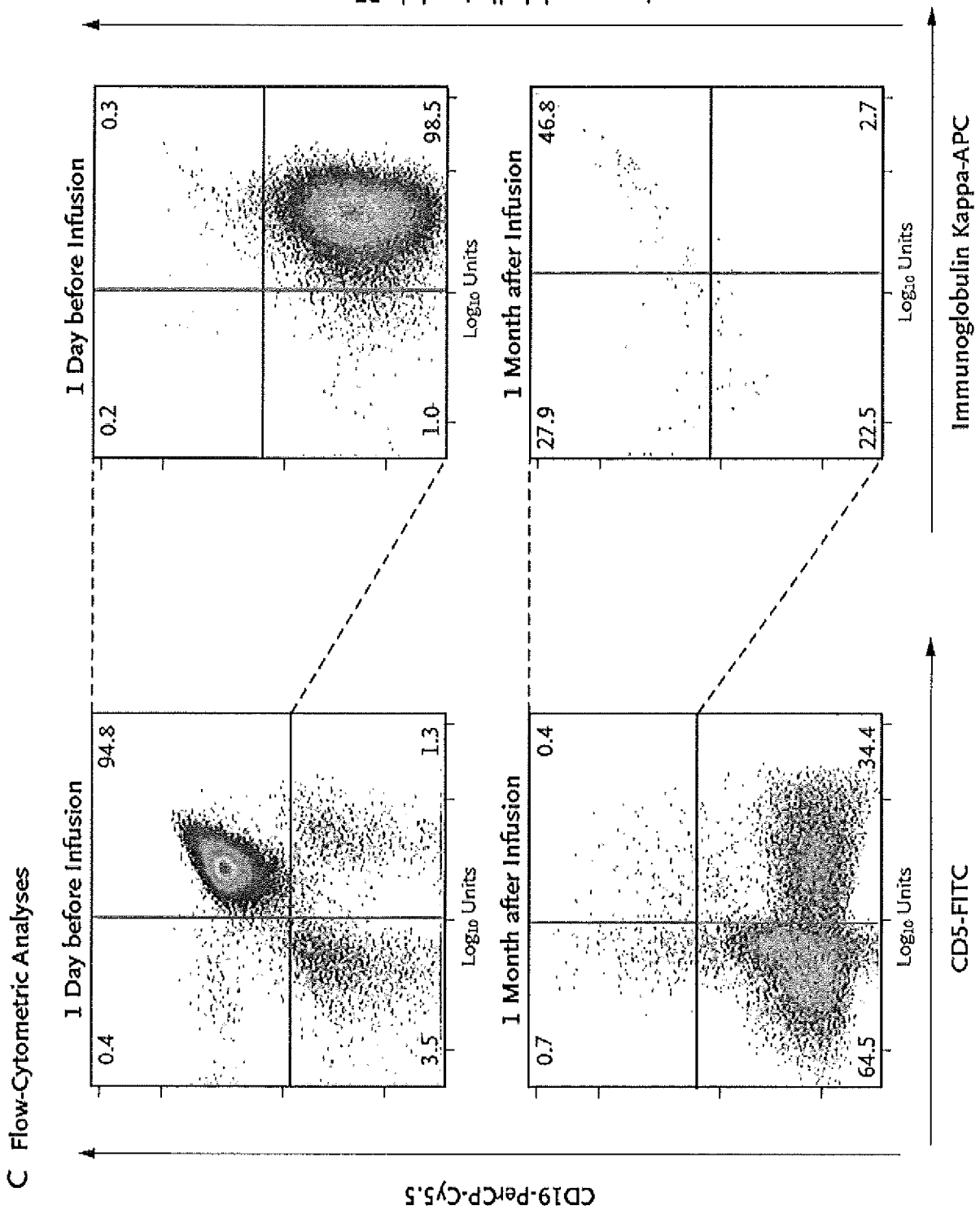

C Flow-Cytometric Analyses
1 Day before Infusion
1 Month after Infusion
CD19-PerCP-Cy5.5
CD5-FITC
Immunoglobulin Lambda-PE
Immunoglobulin Kappa-APC
Log10 Units
0.4
94.8
3.5
1.3
0.2
0.3
1.0
98.5
0.7
0.4
64.5
34.4
27.9
46.8
22.5
2.7

Figure 14C