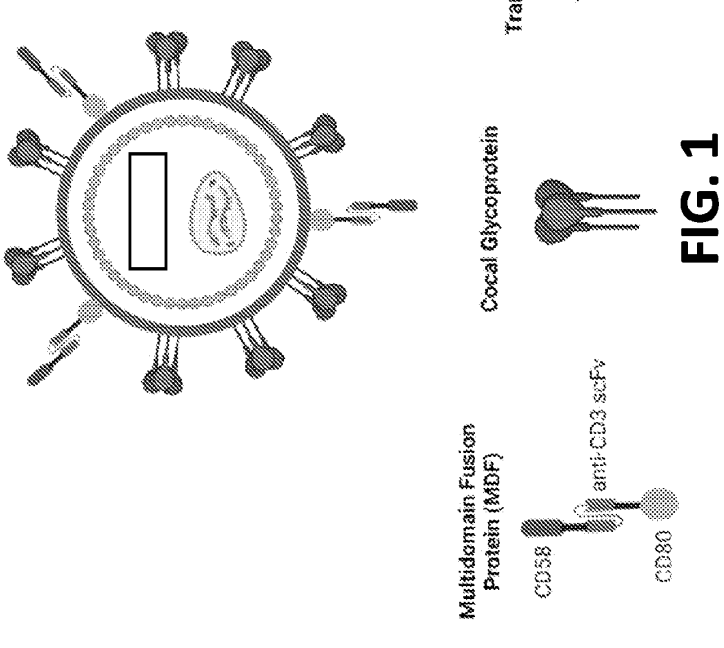

Multidomain Fusion Protein (MDF)
CD58
anti-CD3 scFv
CD80
Cocal Glycoprotein
CD22 CAR Transgene Payload

FIG. 1

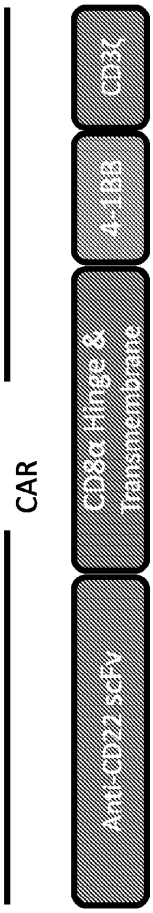

CD22 CAR
Anti-CD22 scFv
CD8α Hinge & Transmembrane
4-1BB
CD3ζ

FIG. 2

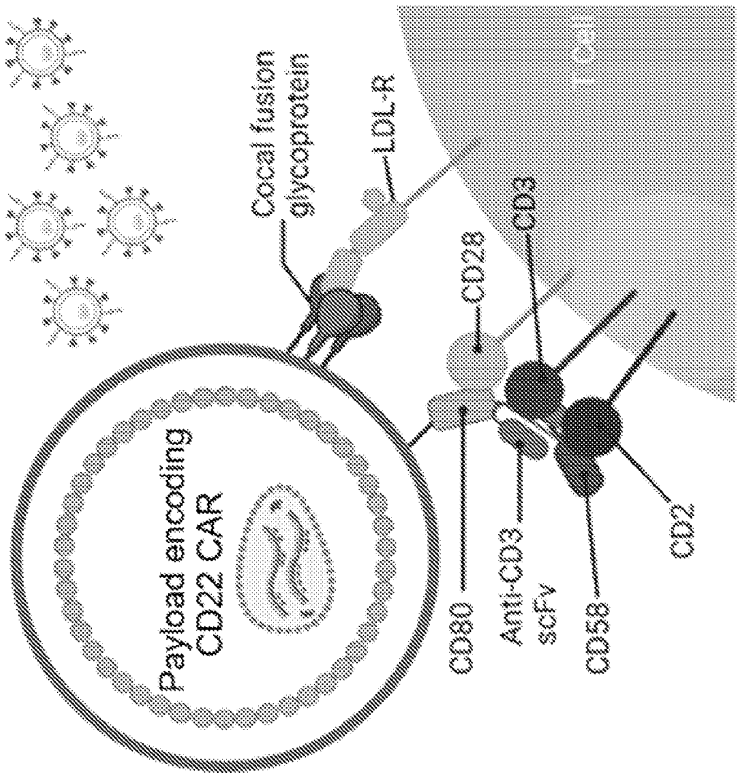

Payload encoding
CD22 CAR
Cocal fusion
glycoprotein
LDL-R
CD80
CD28
CD3
Anti-CD3
scFv
CD58
CD2

FIG. 3A

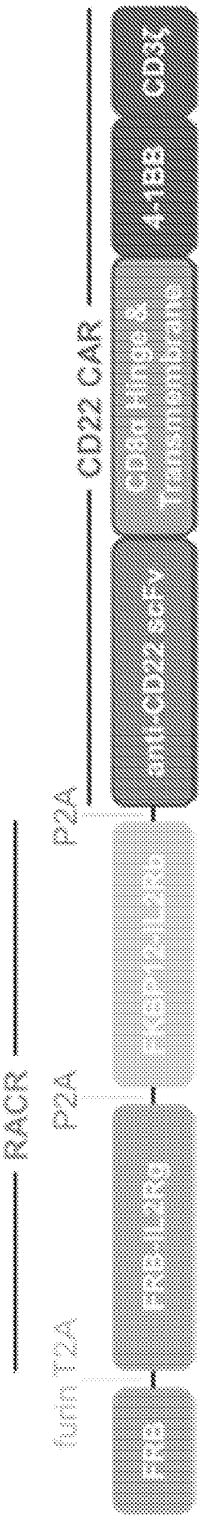

RACR
furin T2A
P2A
FRB
FRB-L-FKBP-FCRL3
P2A
CD22 CAR
anti-CD22 scFv
CD8α Hinge & Transmembrane
4-1BB
CD3ζ

FIG. 3B

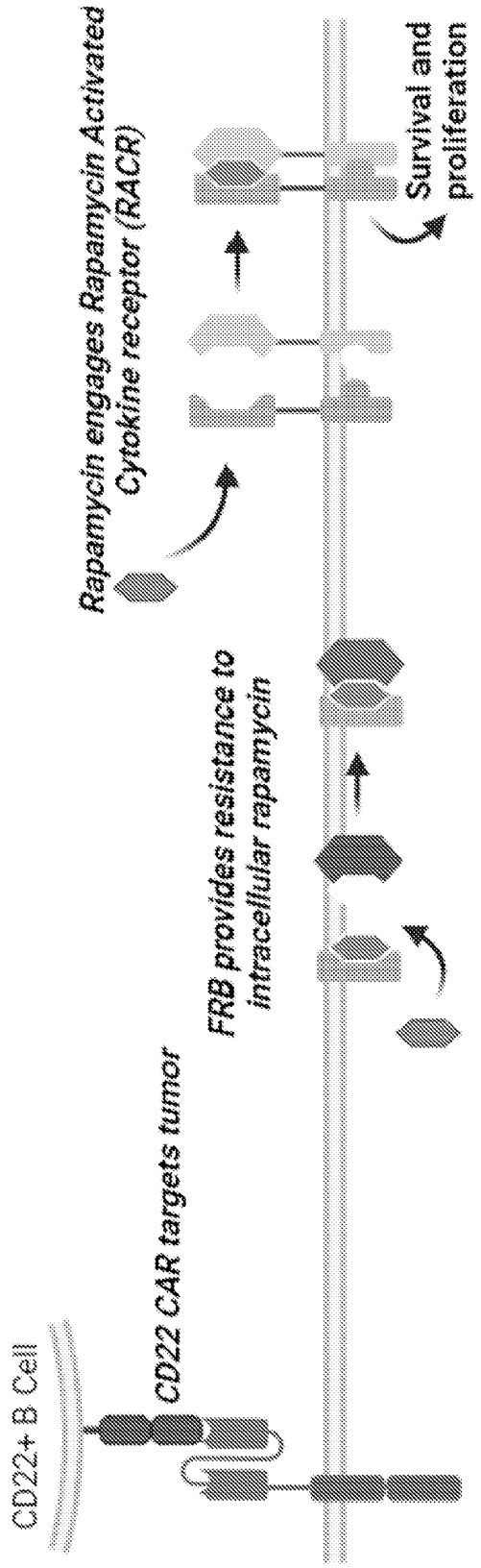

Rapamycin engages Rapamycin Activated Cytokine receptor (RACR)
Survival and proliferation
FRB provides resistance to intracellular rapamycin
CD22 CAR targets tumor
CD22+ B Cell
CAR T Cell

FIG. 3C

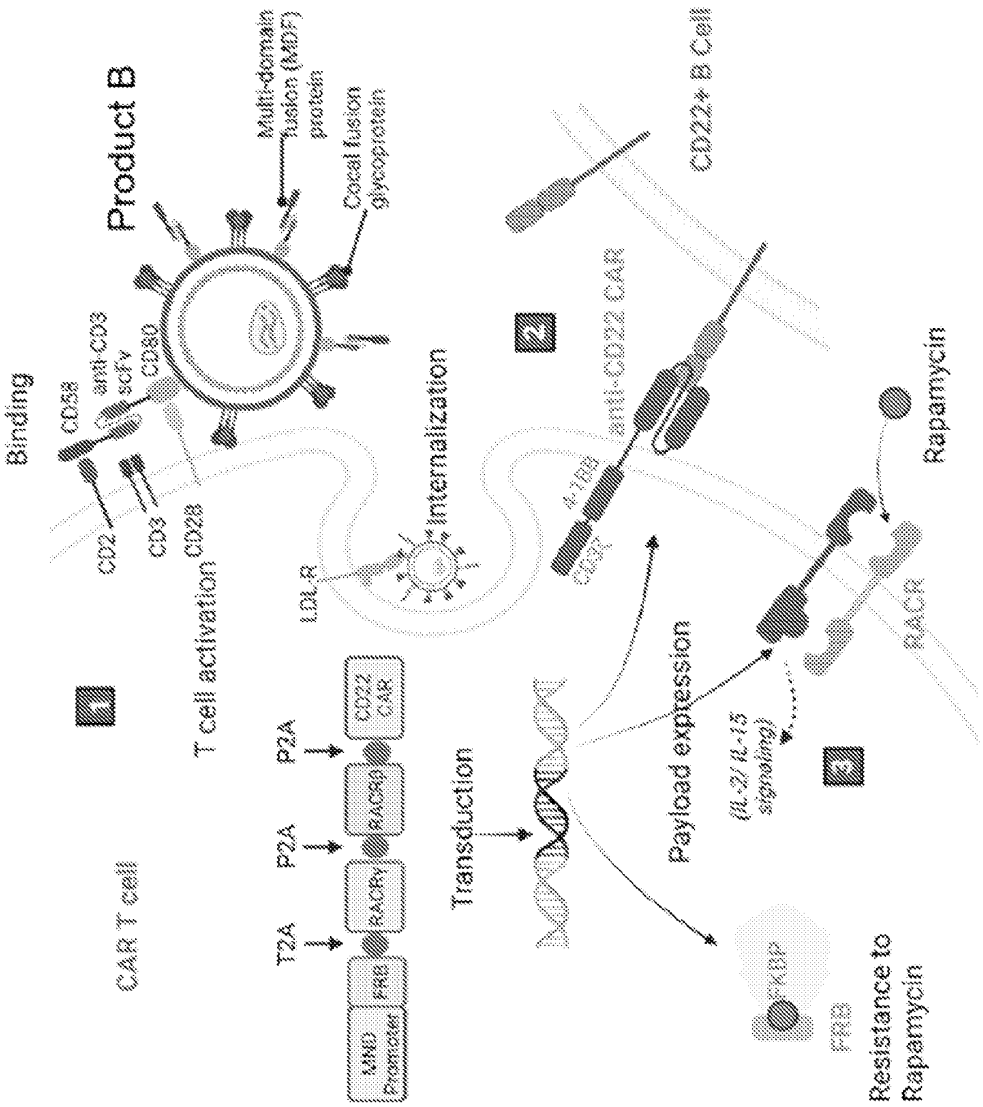
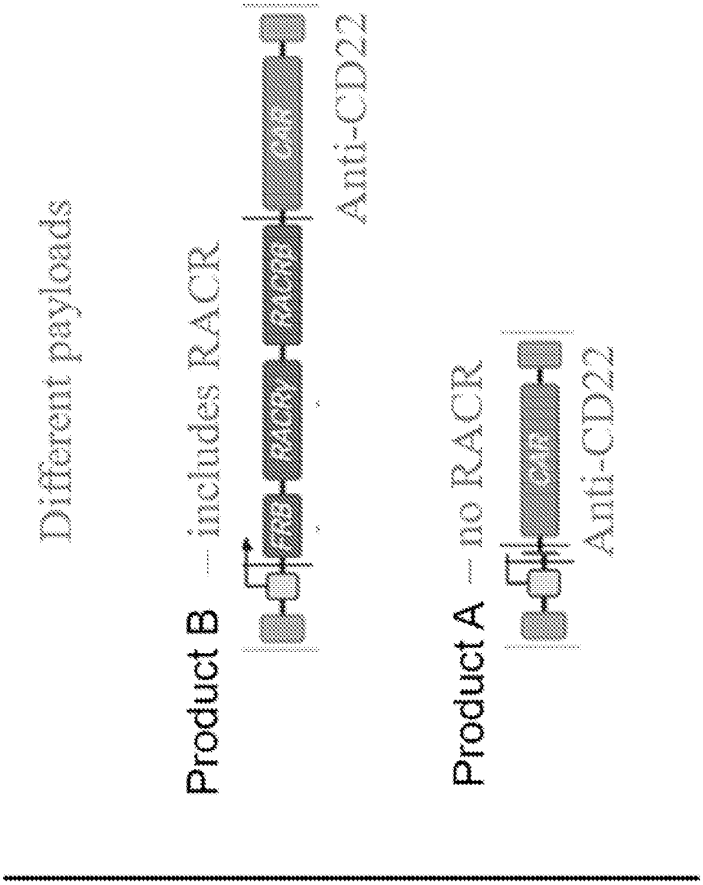

Product B
Binding
CD58
anti-CD3 scFv
CD80
CD2
CD3
CD28
T cell activation
Multi-domain fusion (MDF) protein
Cocal fusion glycoprotein
LDLR
Internalization
CAR T cell
MND Promoter
FRB
RACR
T2A
P2A
RACR
P2A
CD22 CAR
Transduction
4-1BB
CD3ζ
anti-CD22 CAR
CD22+ B Cell
Payload expression
(IL-2/ IL-15 signaling)
FKBP
FRB
Resistance to Rapamycin
RACR
Rapamycin

FIG. 4


Different payloads
Product B – includes RACR
Anti-CD22
Product A – no RACR
Anti-CD22

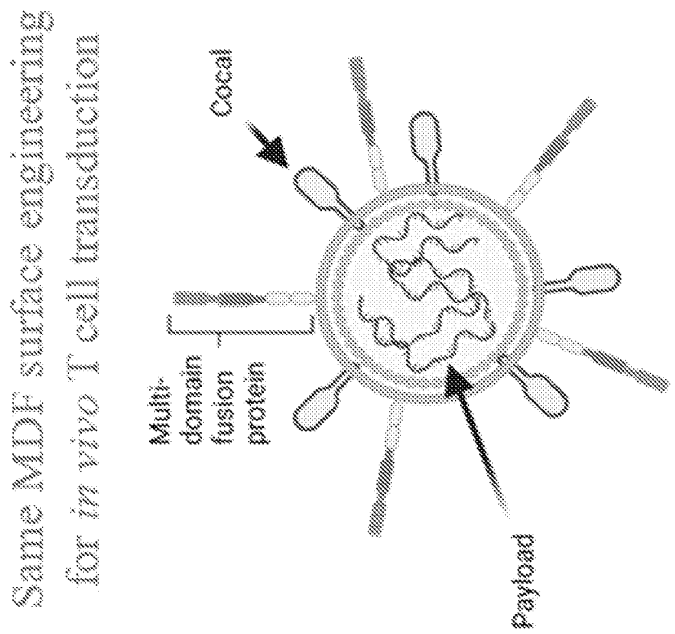

Same MDF surface engineering
for in vivo T cell transduction
Multi-domain fusion protein
Cocal
Payload

FIG. 5

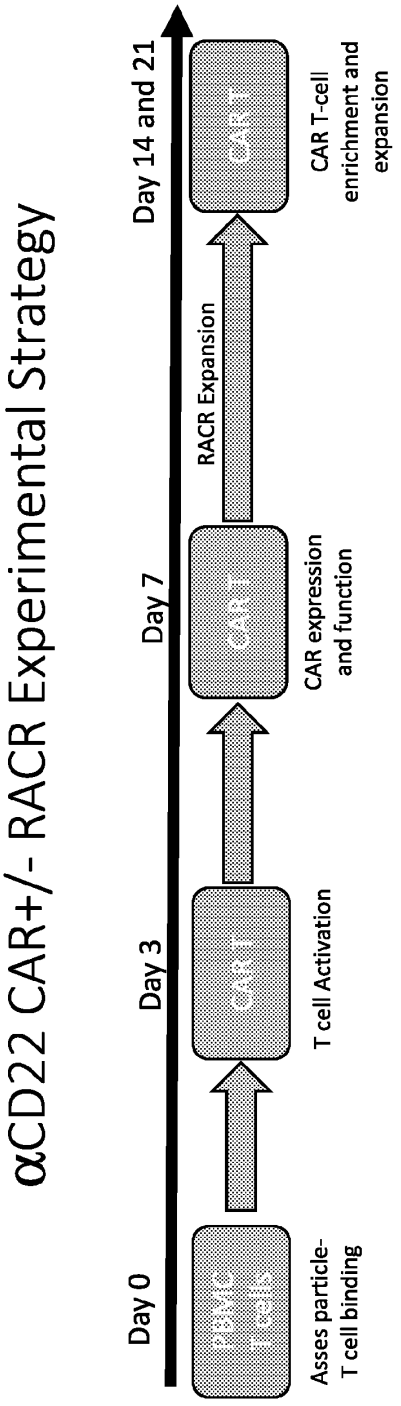

αCD22 CAR+/- RACR Experimental Strategy
Day 0
Day 3
Day 7
Day 14 and 21
PBMC
T cells
CAR T
CAR T
CAR T
Asses particle-
T cell binding
T cell Activation
CAR expression
and function
RACR Expansion
CAR T-cell
enrichment and
expansion

FIG. 6

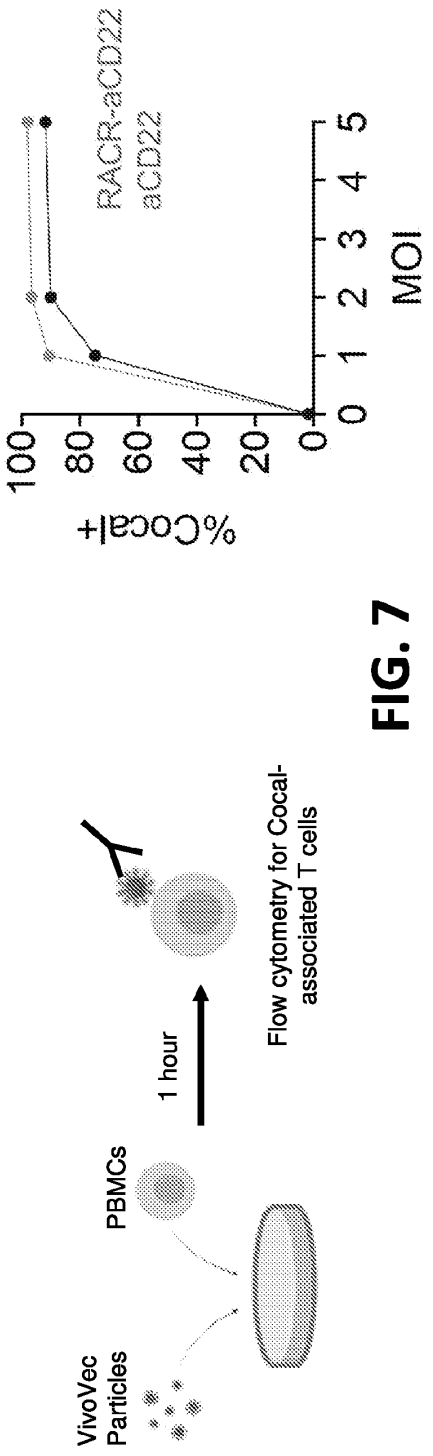

CD3 T cells
%Cocal+
100
80
60
40
20
0
0 1 2 3 4 5
MOI
RACR-aCD22
aCD22
VivoVec
Particles
PBMCs
1 hour
Flow cytometry for Cocal-
associated T cells

FIG. 7

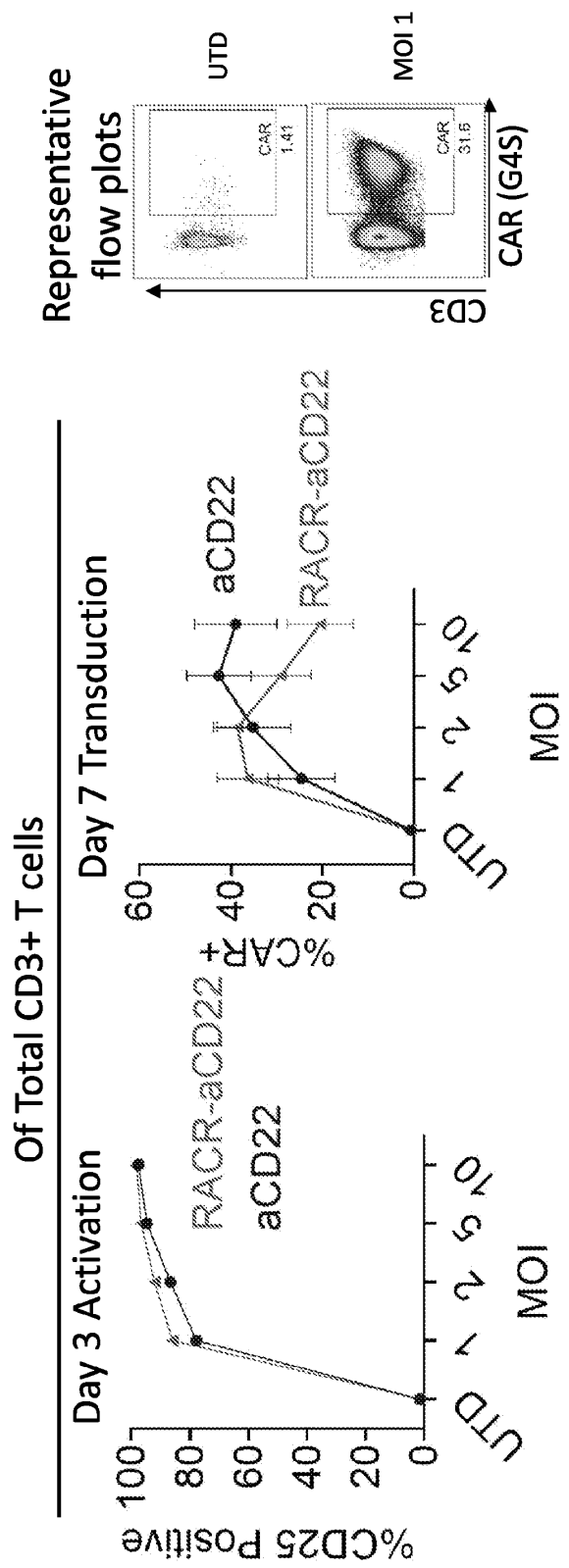

Representative flow plots
UTD
MOI 1
CAR 1.41
CAR 31.6
CD3
CAR (G4S)
Of Total CD3+ T cells
Day 3 Activation
%CD25 Positive
RACR-aCD22
aCD22
UTD 1 2 5 10
MOI
Day 7 Transduction
%CAR+
aCD22
RACR-aCD22
UTD 1 2 5 10
MOI

FIG. 8

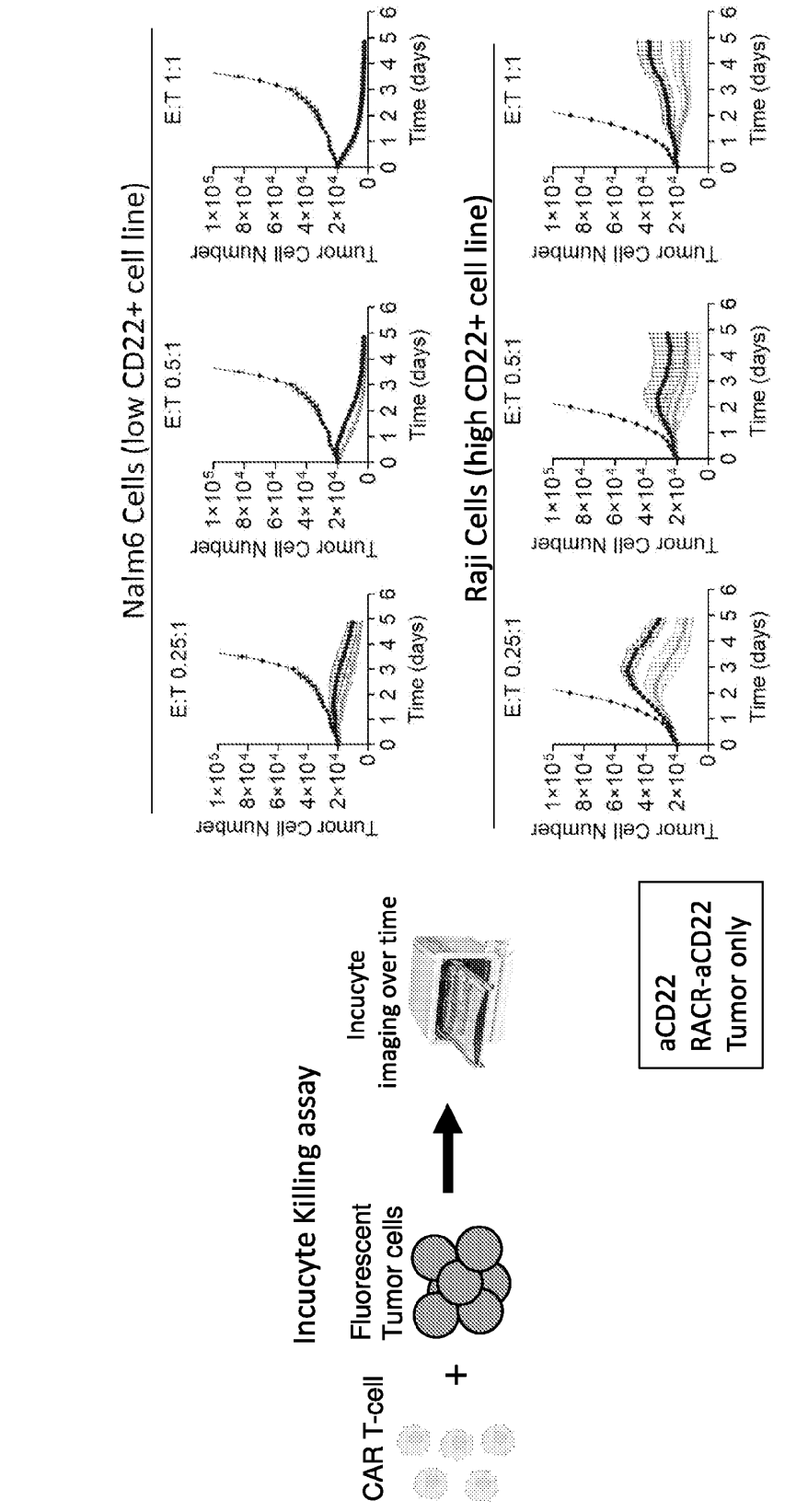

Nalm6 Cells (low CD22+ cell line)
Raji Cells (high CD22+ cell line)
E:T 0.25:1
E:T 0.5:1
E:T 1:1
Tumor Cell Number
Time (days)
Incucyte Killing assay
CAR T-cell
Fluorescent Tumor cells
Incucyte imaging over time
aCD22 RACR-aCD22 Tumor only

FIG. 9

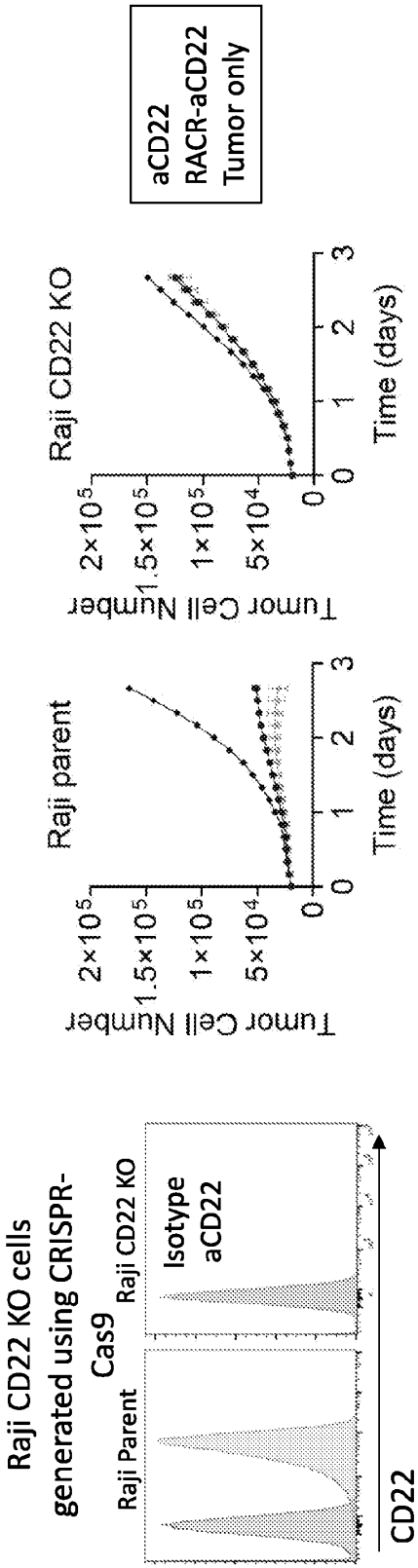


FIG. 10A

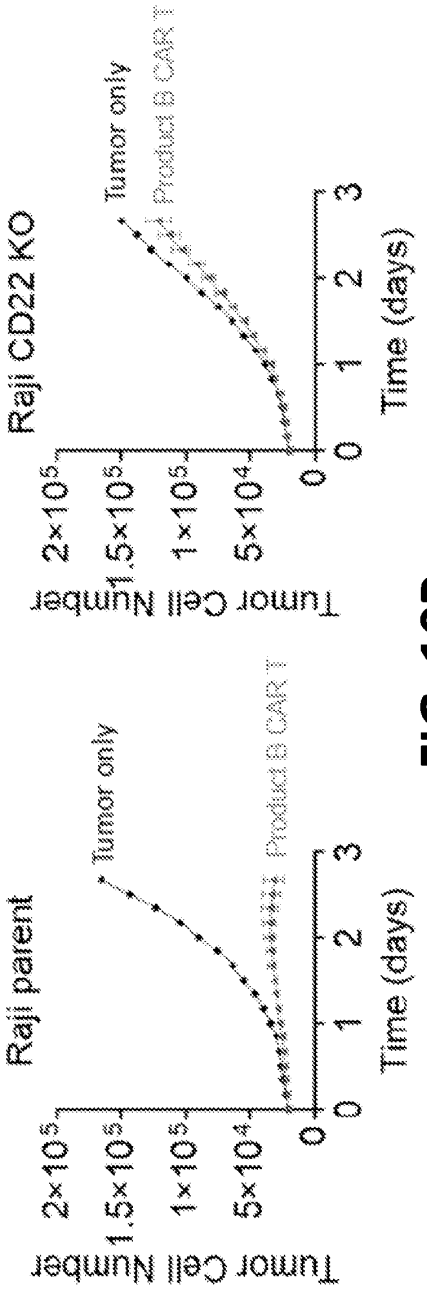


FIG. 10B

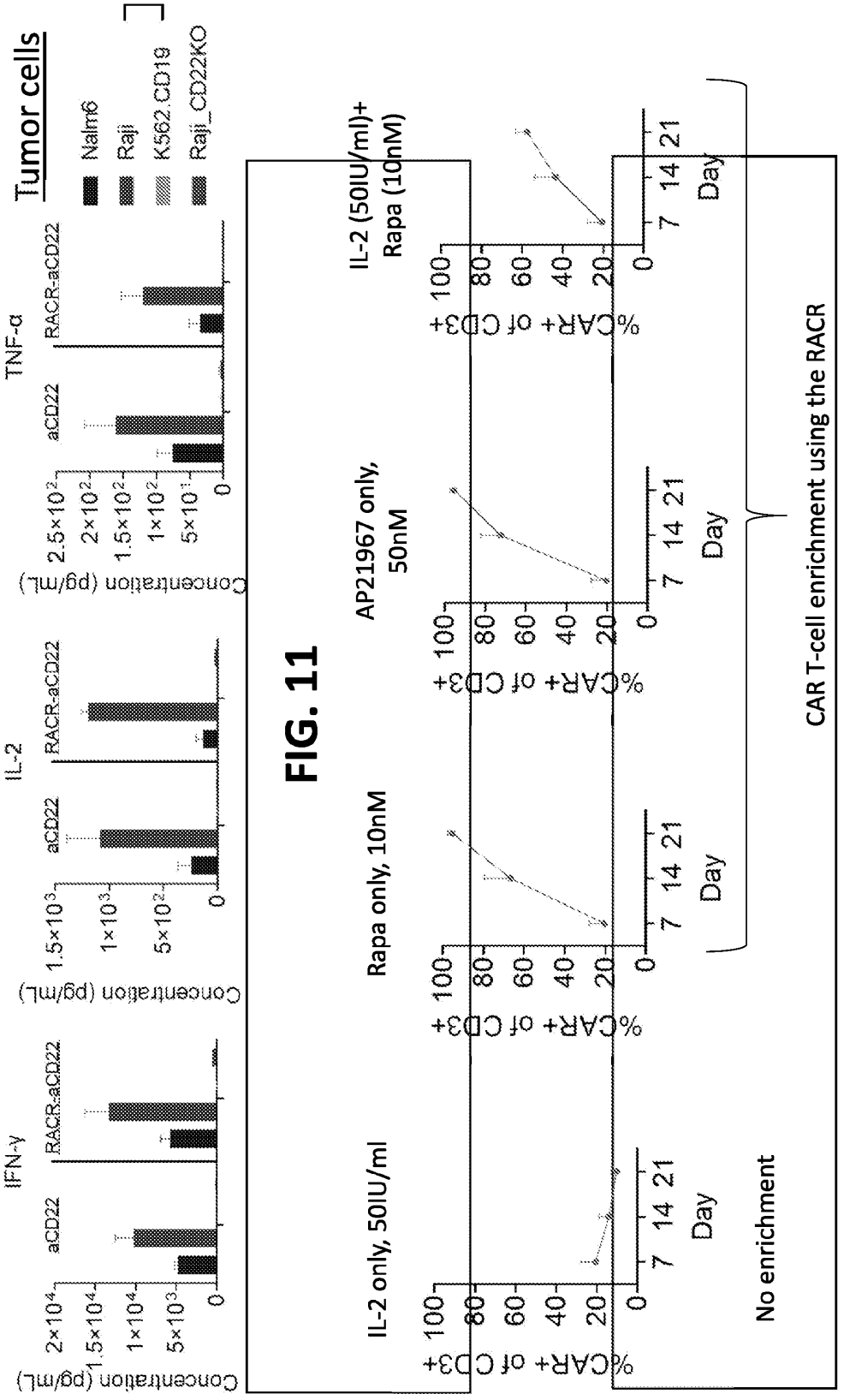

Tumor cells
Nalm6
Raji
K562.CD19
Raji_CD22KO
IFN-γ
TNF-α
IL-2
Concentration (pg/mL)
aCD22
RACR-aCD22
IL-2 only, 50IU/ml
Rapa only, 10nM
AP21967 only, 50nM
IL-2 (50IU/ml)+ Rapa (10nM)
%CAR+ of CD3+
Day
No enrichment
CAR T-cell enrichment using the RACR

FIG. 11

FIG. 12

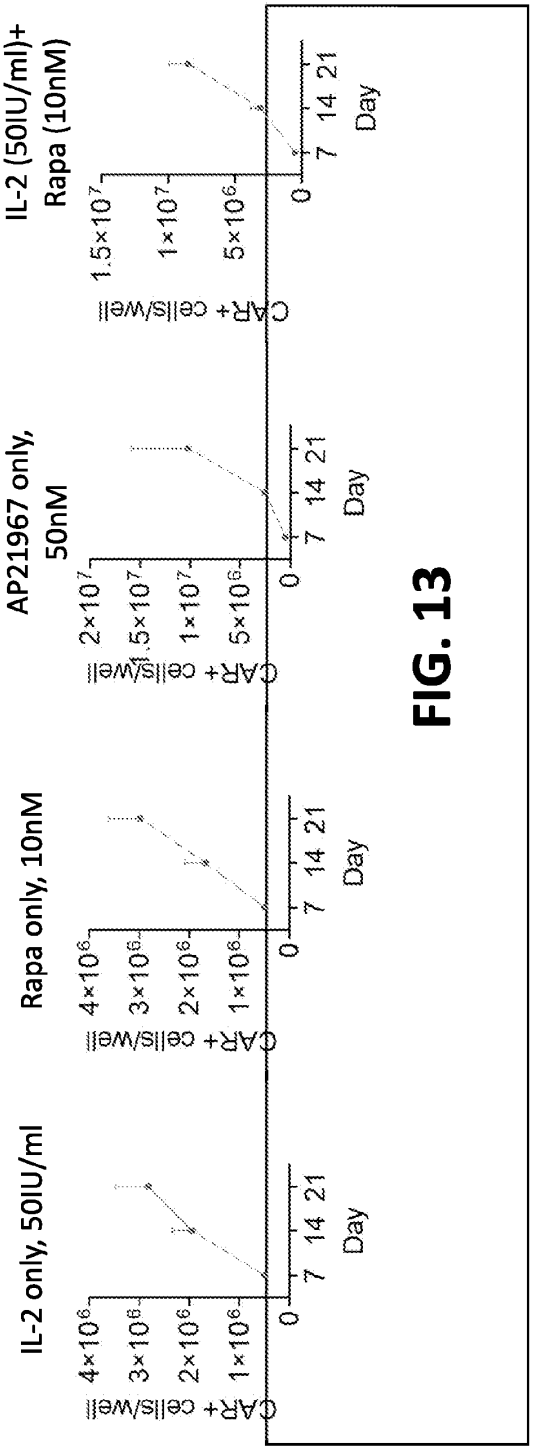


FIG. 13



FIG. 14A

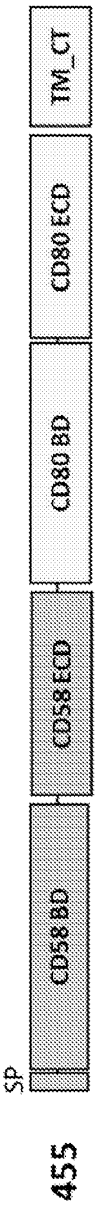


FIG. 14B

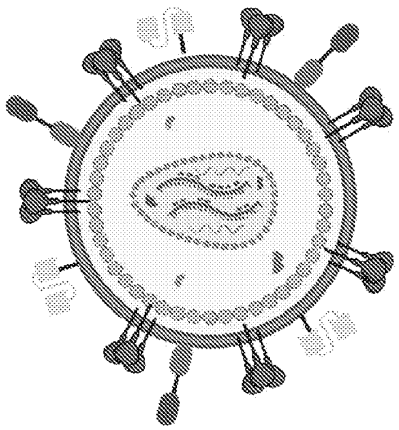


FIG. 15A

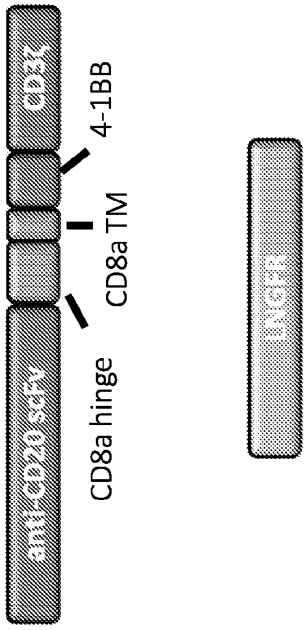
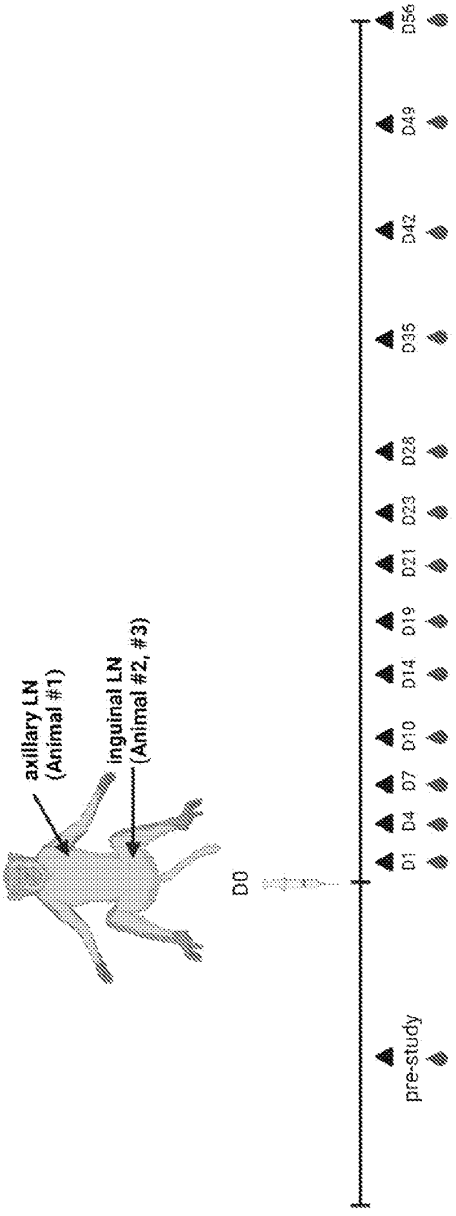


FIG. 15B

Animal	Sex	Age (years)	Weight (kg)*	Surface Engineering	Payload	Dose (TUs)
#1	F	3	3.92	Dual Fusion	αCD20-CAR-LNGFR	1E+08
#2	F	15	7.08	Dual Fusion	αCD20-CAR-LNGFR	5E+08
#3	F	16	9.73	Dual Fusion	αCD20-CAR-LNGFR	5E+08

FIG. 15C



* Difficulty injecting inguinal LN due to small size of animal

FIG. 16

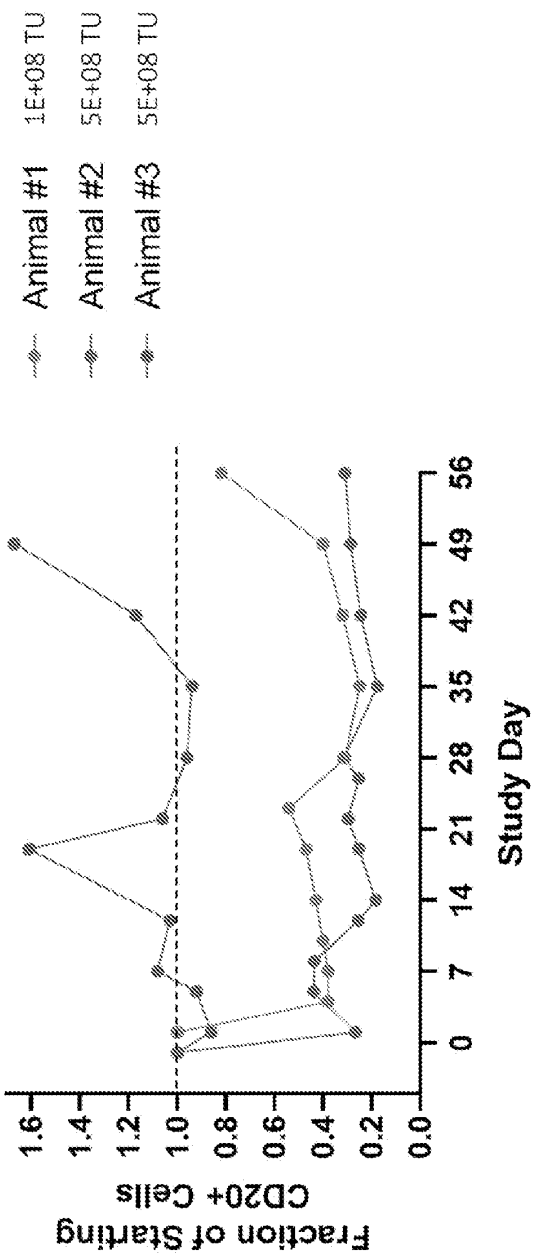

Animal #1 1E+08 TU
Animal #2 5E+08 TU
Animal #3 5E+08 TU
Fraction of Starting CD20+ Cells
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
7
14
21
28
35
42
49
56
Study Day

FIG. 17

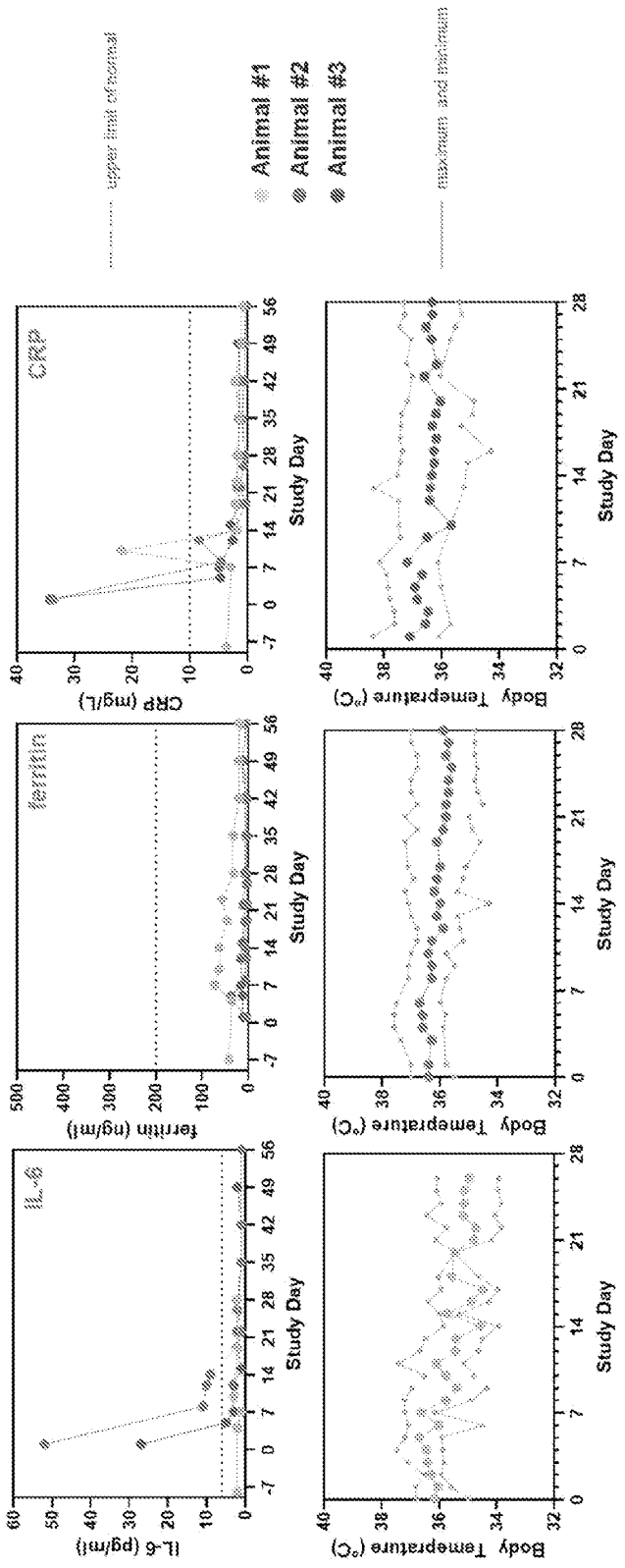

upper limit of normal
Animal #1
Animal #2
Animal #3
maximum and minimum
IL-6
IL-6 (pg/ml)
ferritin
ferritin (ng/ml)
CRP
CRP (mg/L)
Body Temperature (°C)
Study Day

FIG. 18

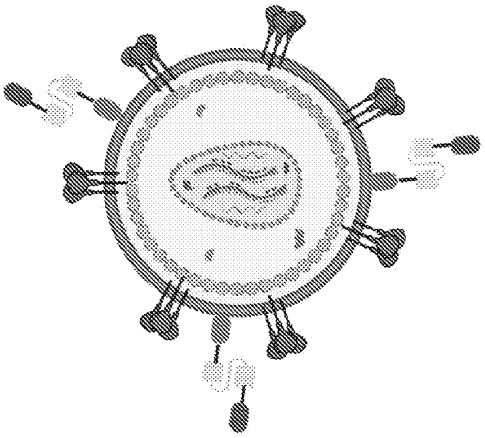


FIG. 19A

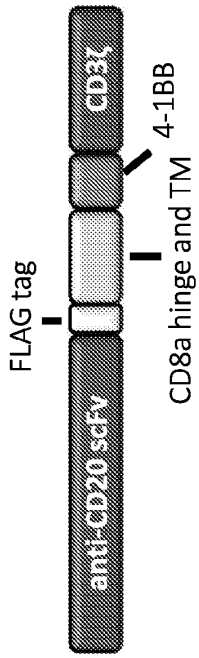


FIG. 19B

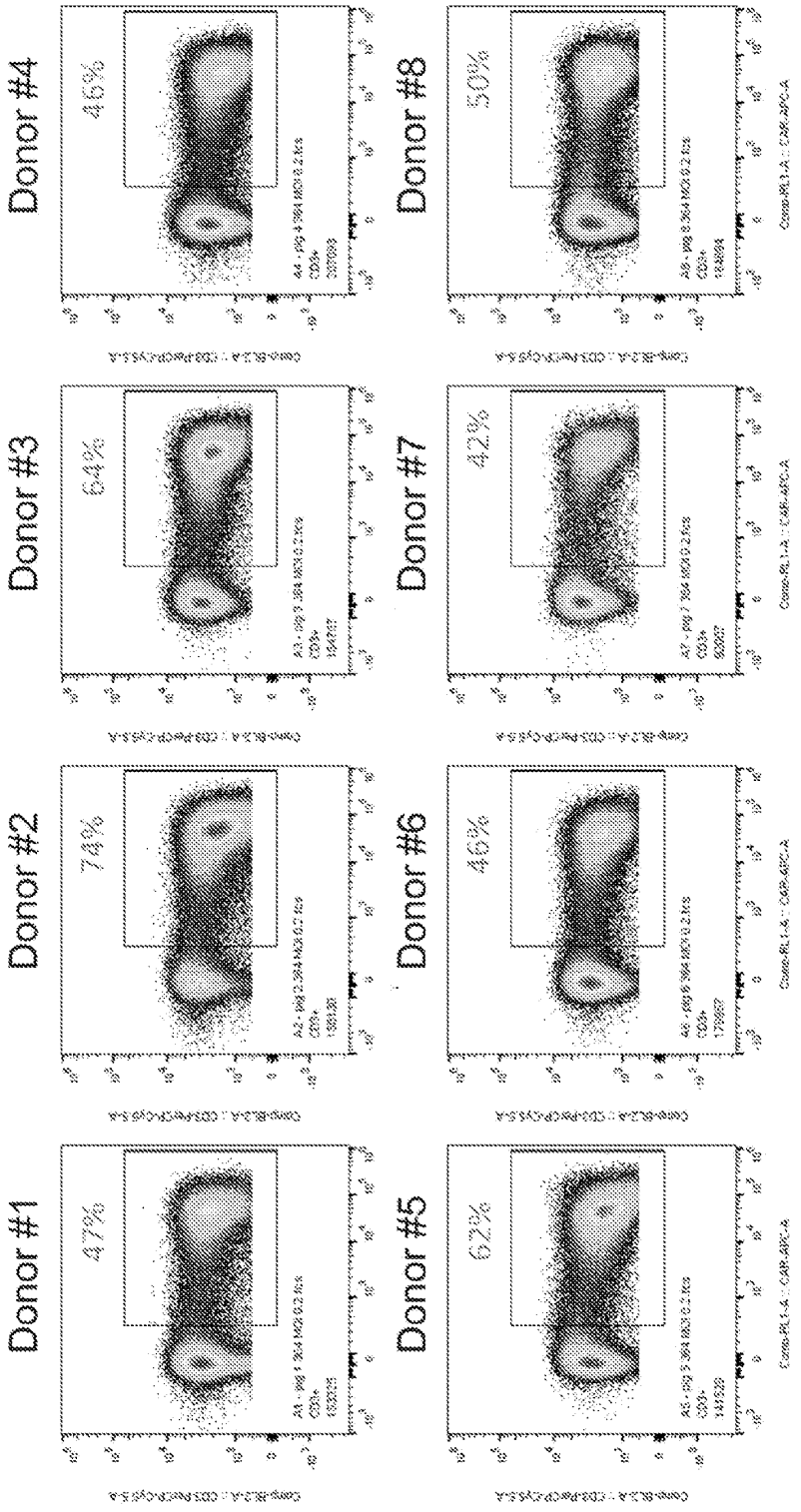

Donor #1
47%
Donor #2
74%
Donor #3
64%
Donor #4
46%
Donor #5
62%
Donor #6
46%
Donor #7
42%
Donor #8
50%

FIG. 20

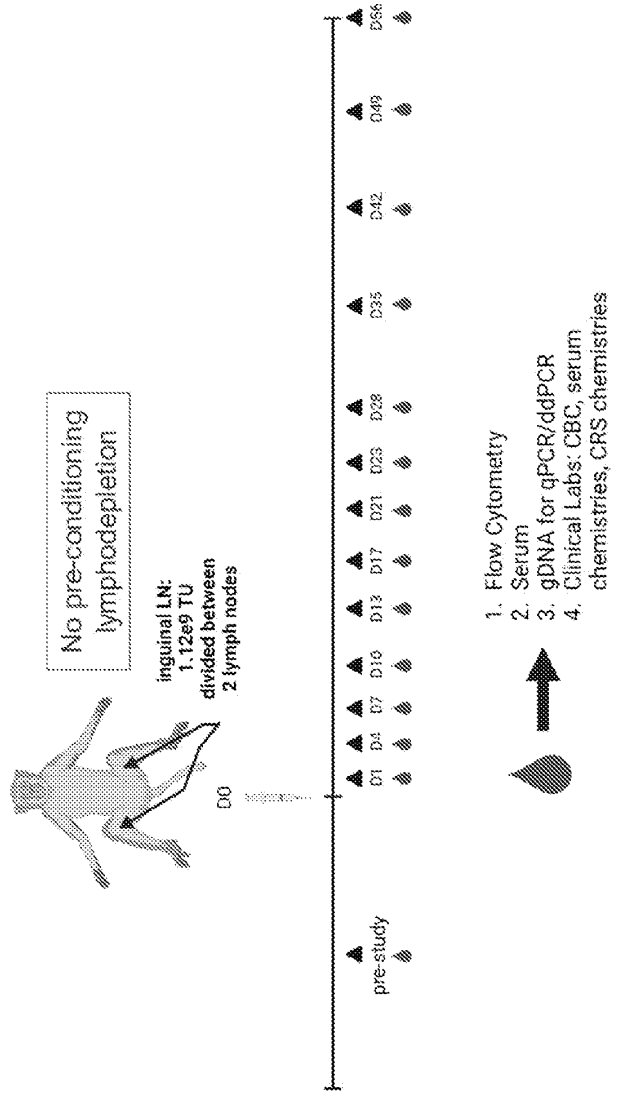

No pre-conditioning lymphodepletion
inguinal LN:
1.12e9 TU
divided between
2 lymph nodes
D0
pre-study
D1
D4
D7
D10
D14
D17
D21
D28
D35
D42
D49
D56
1. Flow Cytometry
2. Serum
3. gDNA for qPCR/ddPCR
4. Clinical Labs: CBC, serum chemistries, CRS chemistries

FIG. 21A

Animal	Sex	Age (years)	Weight (kg)	Dose (Total TU)	Follow Up (days)
MDF #1	M	3	4.47	1.1E9	139**
MDF #2	F	3	4.12	3.2E8	84
MDF #3	M	4	7.16	1.8E9	35
MDF #4	M	4	7.46	1.9E9	17

FIG. 21B

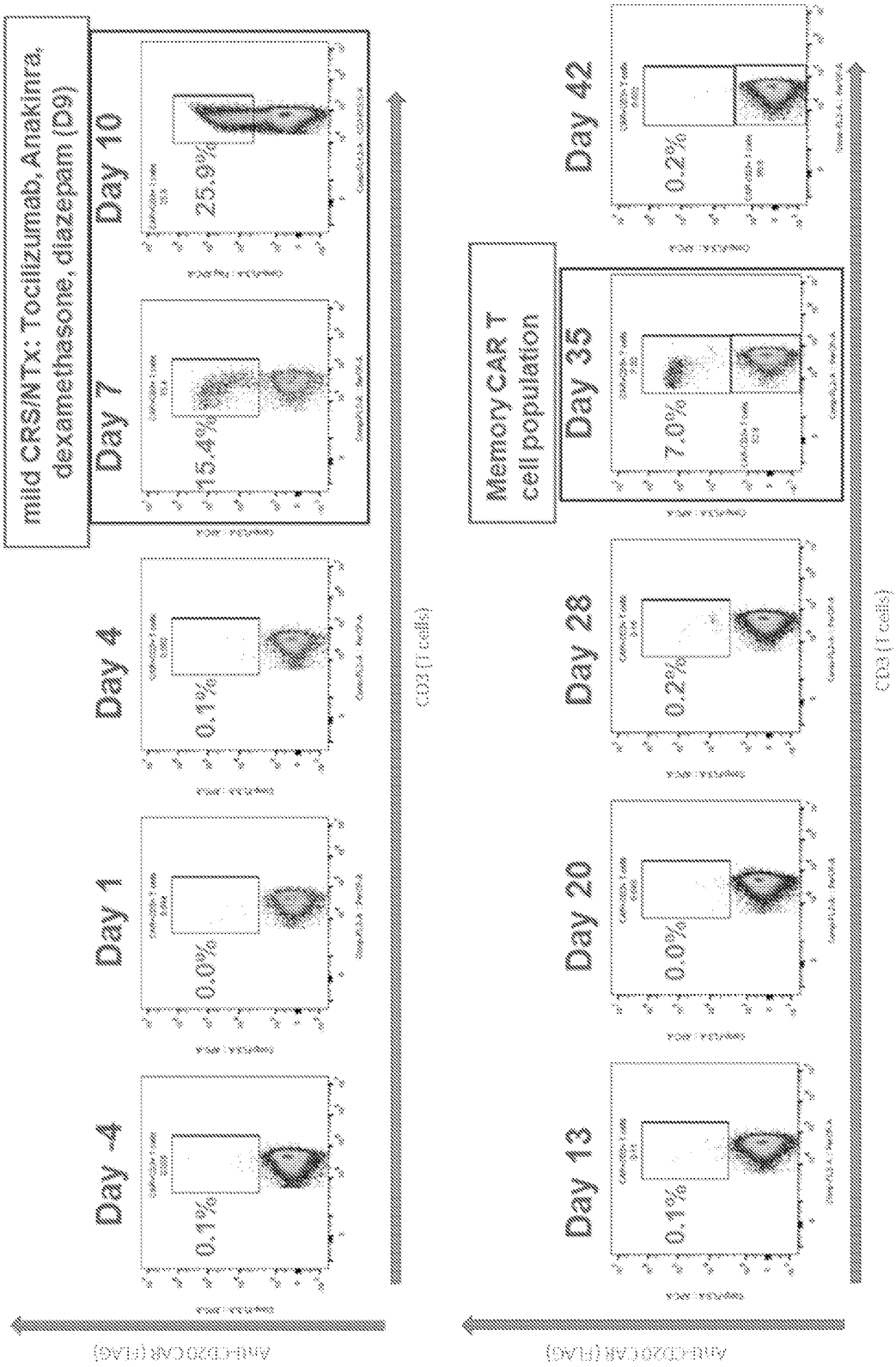

mild CRS/NTx: Tocilizumab, Anakinra, dexamethasone, diazepam (D9)
Day -4
Day 1
Day 4
Day 7
Day 10
Day 13
Day 20
Day 28
Day 35
Day 42
Memory CAR T cell population
0.1%
0.0%
0.1%
15.4%
25.9%
0.1%
0.0%
0.2%
7.0%
0.2%
anti-CD20 CAR (FLAG)
CD3 (T cells)

FIG. 22A

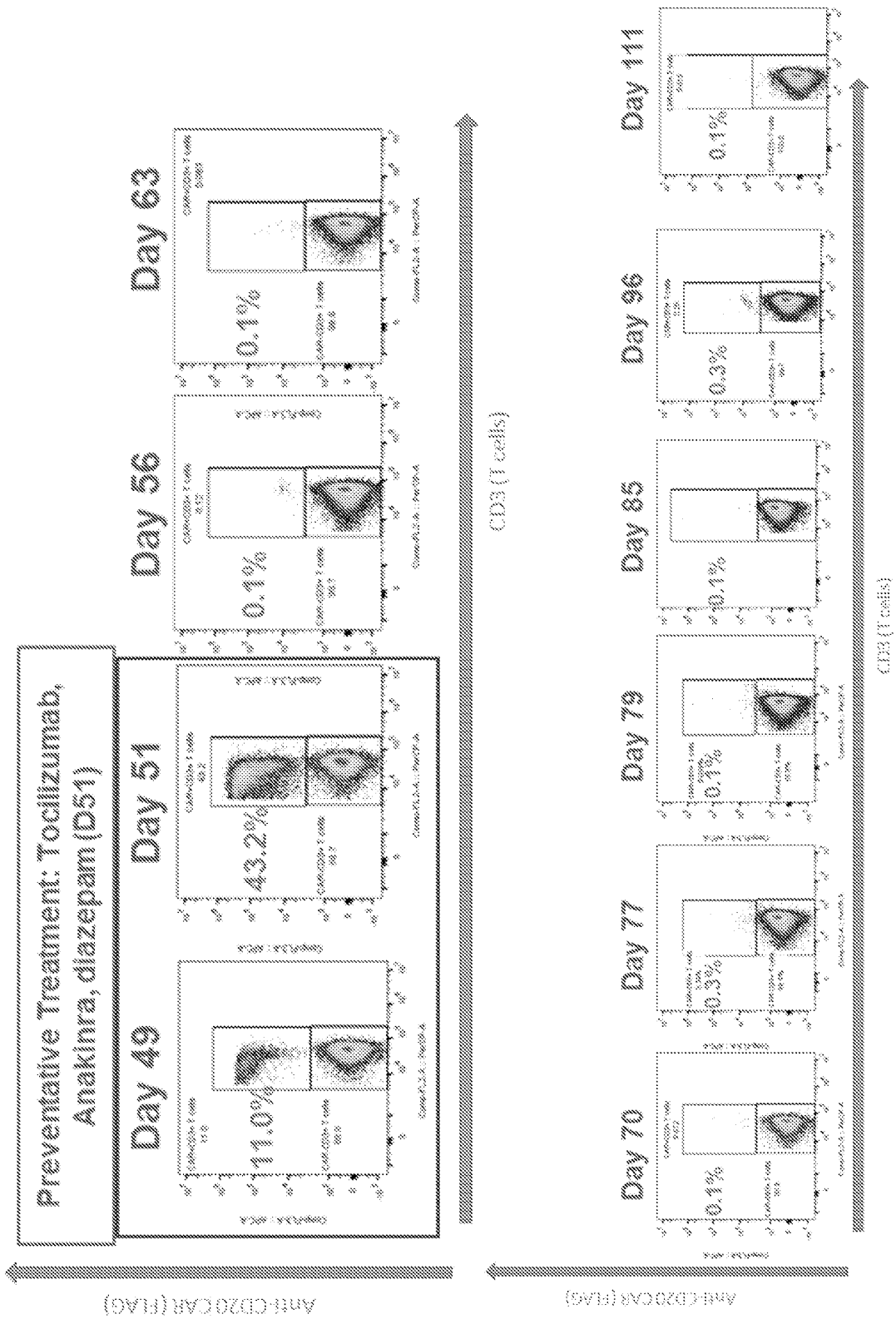

Preventative Treatment: Toclizumab, Anakinra, diazepam (D51)
Day 49
Day 51
Day 56
Day 63
Day 70
Day 77
Day 79
Day 85
Day 96
Day 111
11.0%
43.2%
0.1%
0.1%
0.1%
0.3%
0.1%
0.1%
0.3%
0.1%
Anti-CD20 CAR (FLAG)
CD3 (T cells)

FIG. 22B

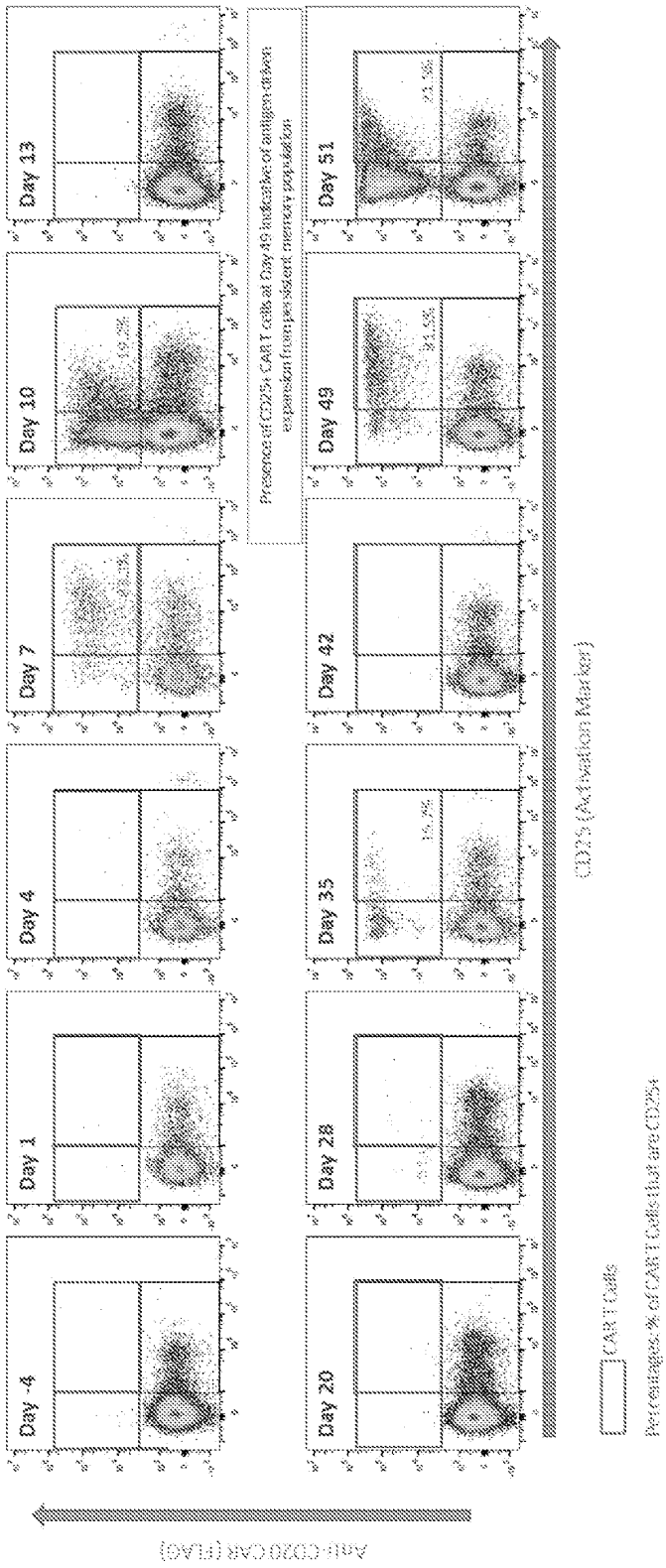

Day -4
Day 1
Day 4
Day 7
Day 10
Day 13
Day 20
Day 28
Day 35
Day 42
Day 49
Day 51
Presence of CD25+ CAR T cells at Day 49 indicative of antigen driven expansion from persistent memory population
Anti-CD20 CAR (FLAG)
CD25 (Activation Marker)
CAR T Cells
Percentages: % of CAR T Cells that are CD25+

FIG. 23

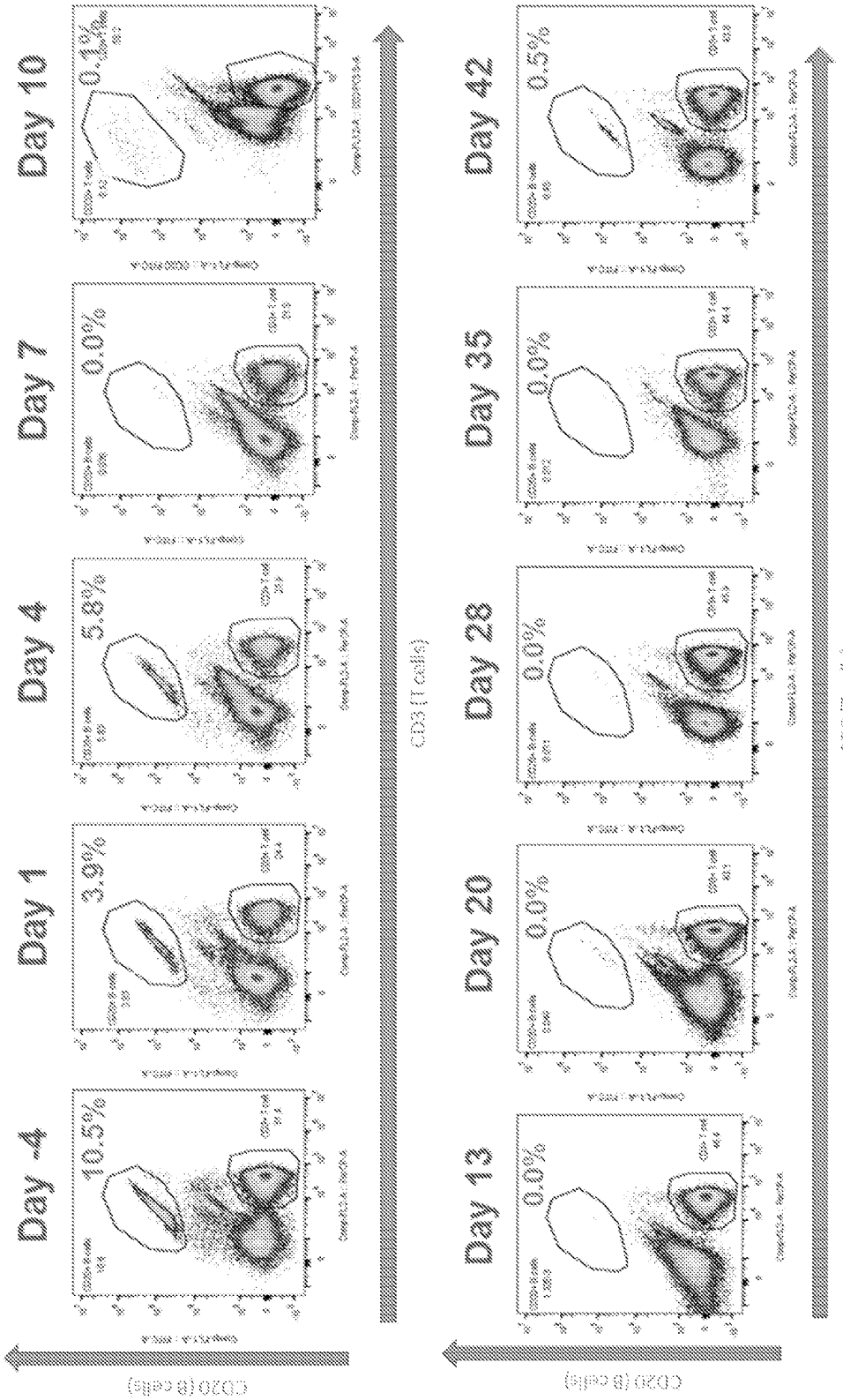

Day -4
Day 1
Day 4
Day 7
Day 10
Day 13
Day 20
Day 28
Day 35
Day 42
10.5%
3.9%
5.8%
0.0%
0.1%
0.0%
0.0%
0.0%
0.0%
0.5%
CD20 (B cells)
CD3 (T cells)

FIG. 24A

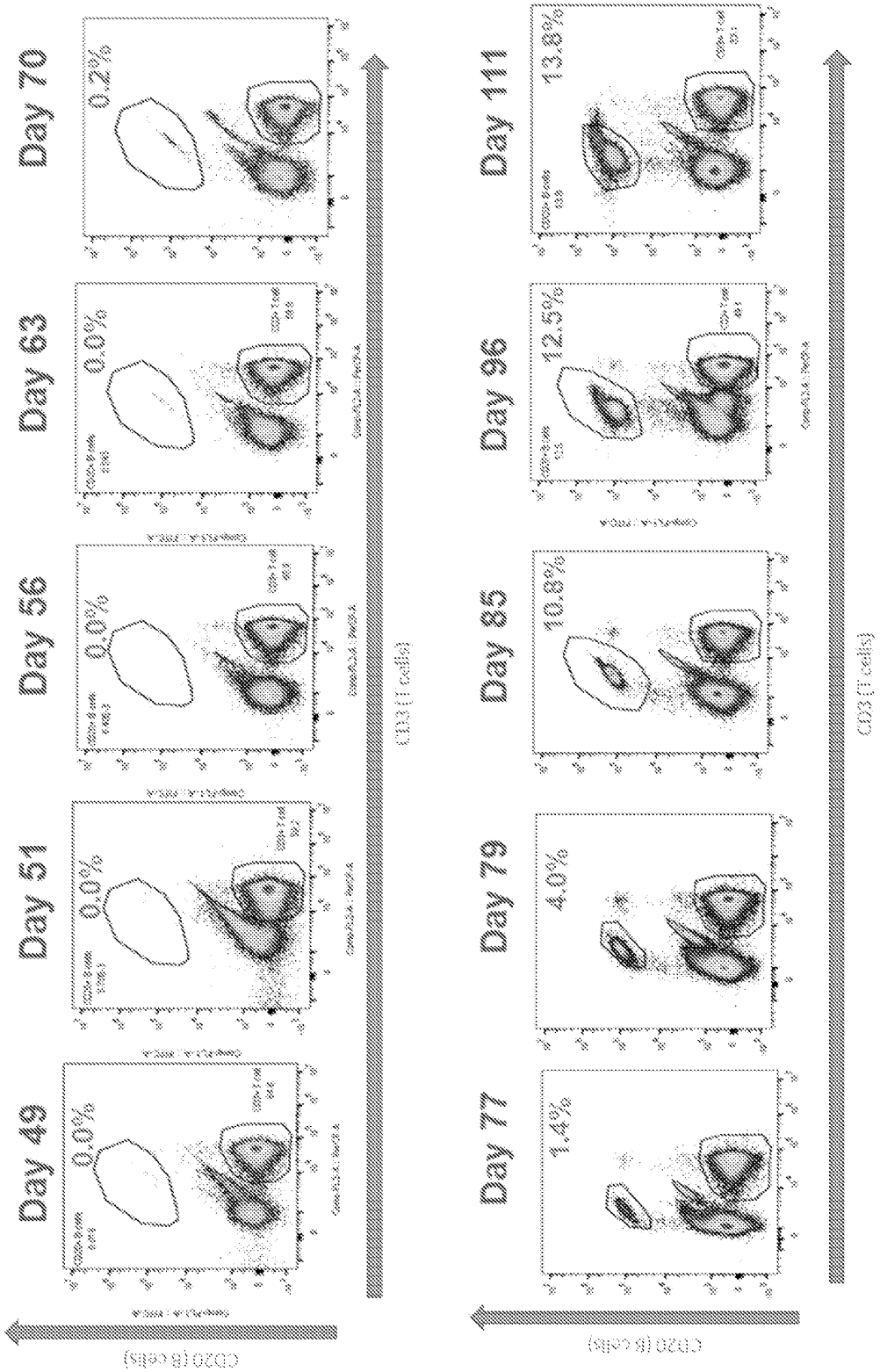

Day 49
0.0%
Day 51
0.0%
Day 56
0.0%
Day 63
0.0%
Day 70
0.2%
Day 77
1.4%
Day 79
4.0%
Day 85
10.8%
Day 96
12.5%
Day 111
13.8%
CD20 (B cells)
CD3 (T cells)

FIG. 24B

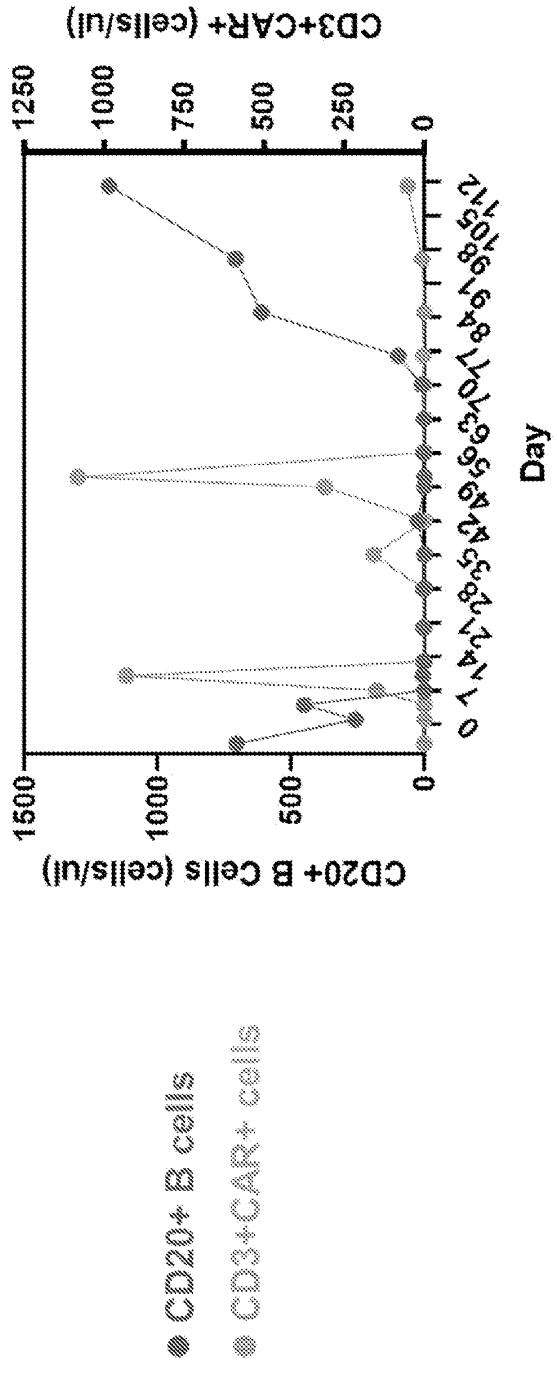

CD20+ B cells (cells/ul)
1500
1000
500
0
CD3+CAR+ (cells/ul)
1250
1000
750
500
250
0
Day
0
7
14
21
28
35
42
49
56
63
70
77
84
91
98
105
112
CD20+ B cells
CD3+CAR+ cells

FIG. 25

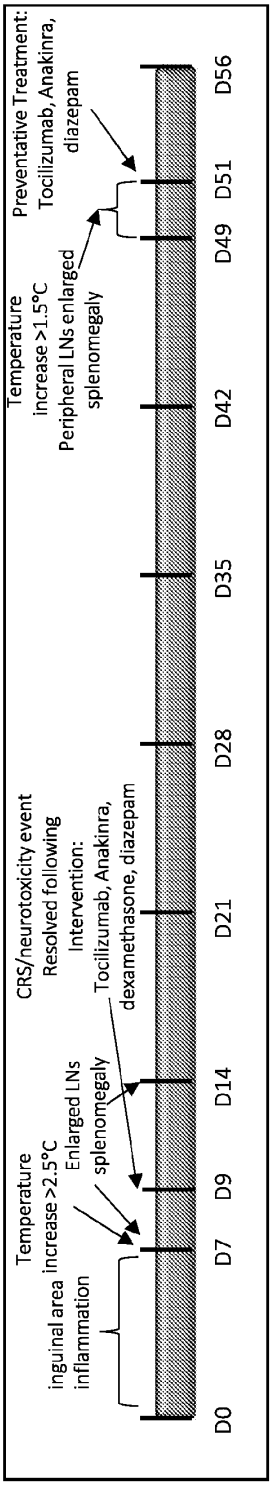


FIG. 26A

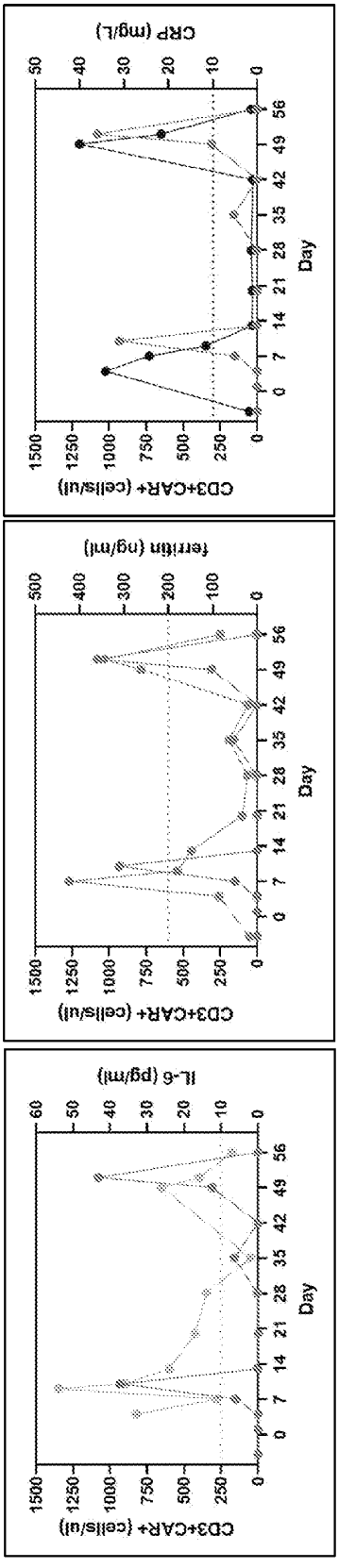


FIG. 26B

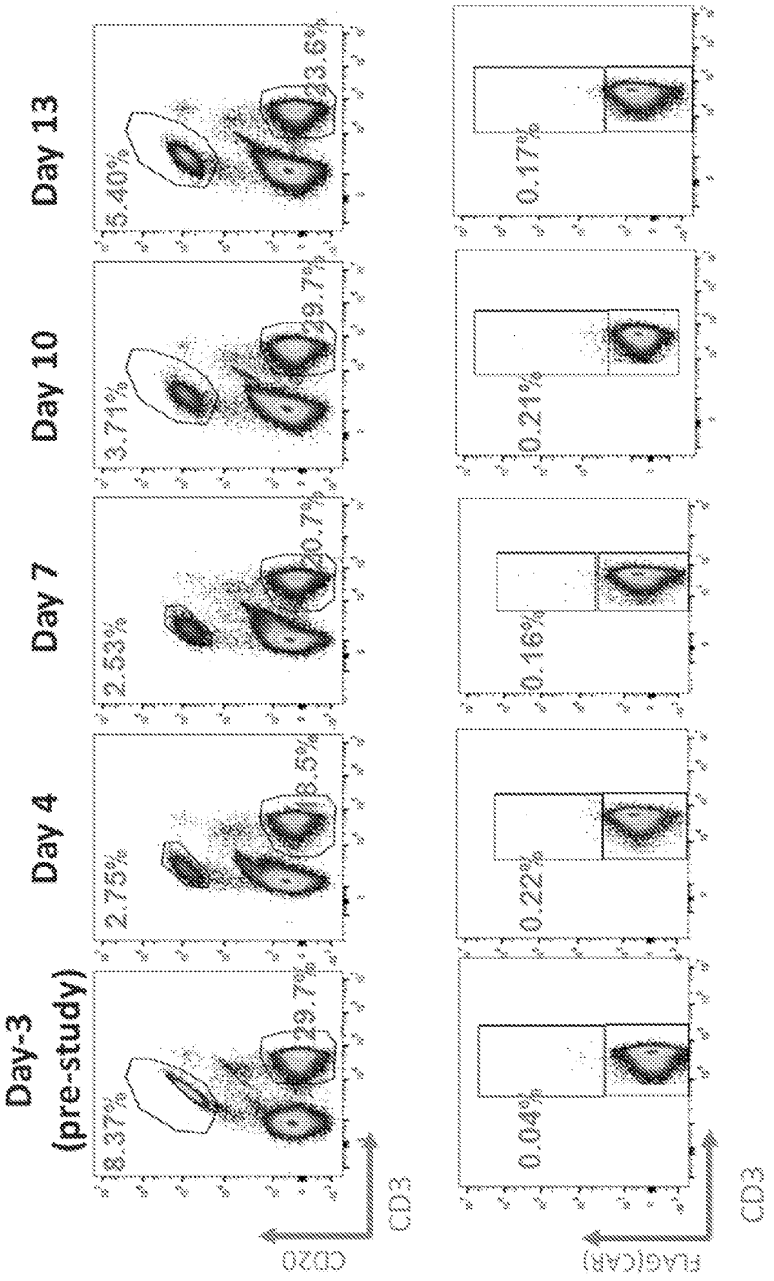

Day-3
(pre-study)
Day 4
Day 7
Day 10
Day 13
18.37%
49.7%
2.75%
28.5%
2.53%
30.7%
3.71%
29.7%
5.40%
33.6%
0.04%
0.22%
0.16%
0.21%
0.17%
CD20
CD3
FLAG(CAR)
CD3

FIG. 27A

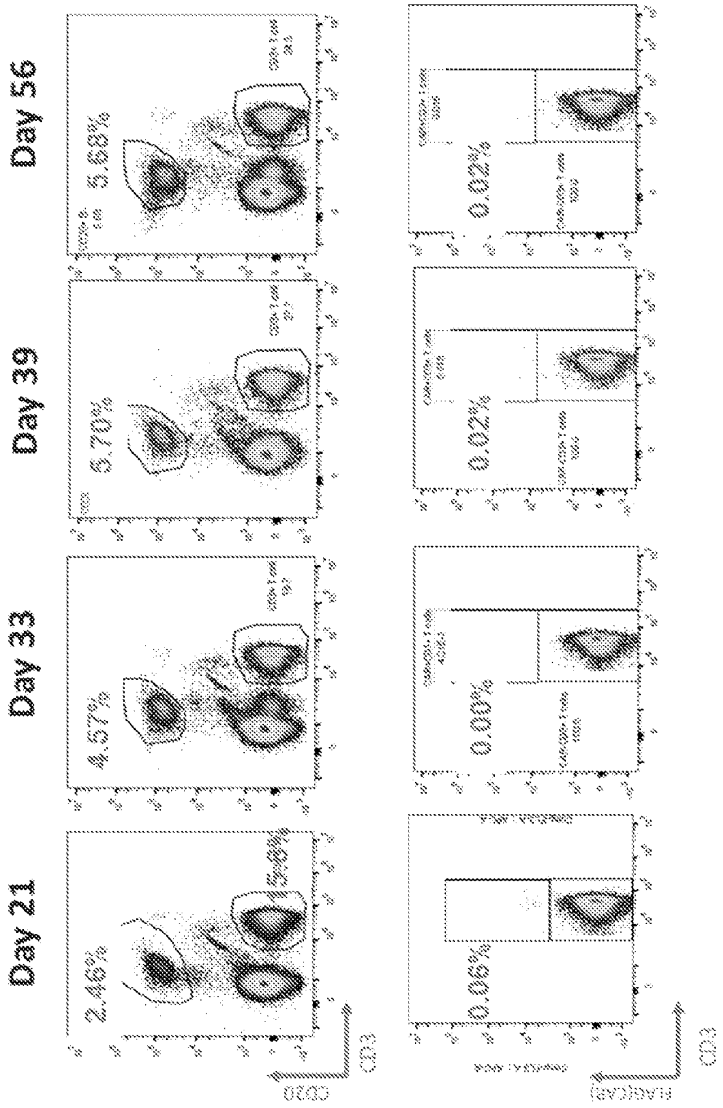

Day 21
Day 33
Day 39
Day 56
2.46%
4.57%
6.70%
5.68%
0.06%
0.00%
0.02%
0.02%
CD3
CD3

FIG. 27B

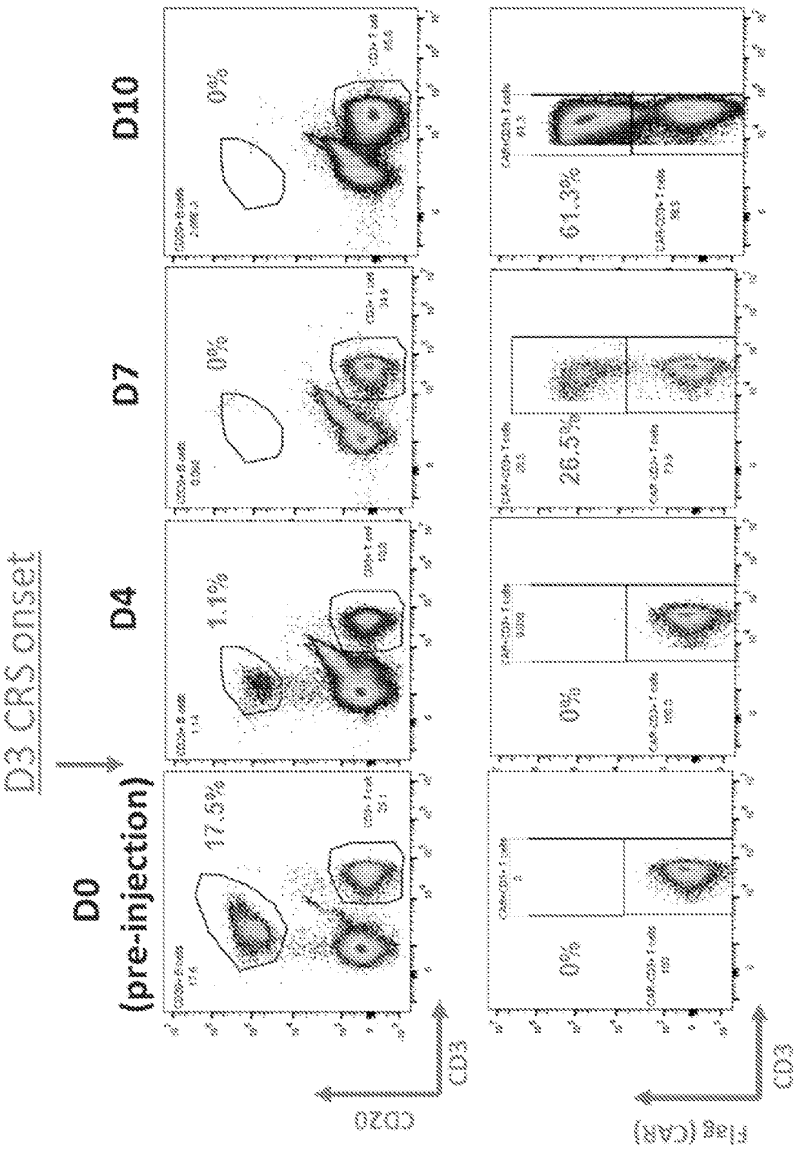

D3 CRS onset
D0
(pre-injection)
D4
D7
D10
17.5%
1.1%
0%
0%
0%
61.3%
26.3%
0%
0%
CD20
CD3
Flag (CAR)
CD3

FIG. 28A

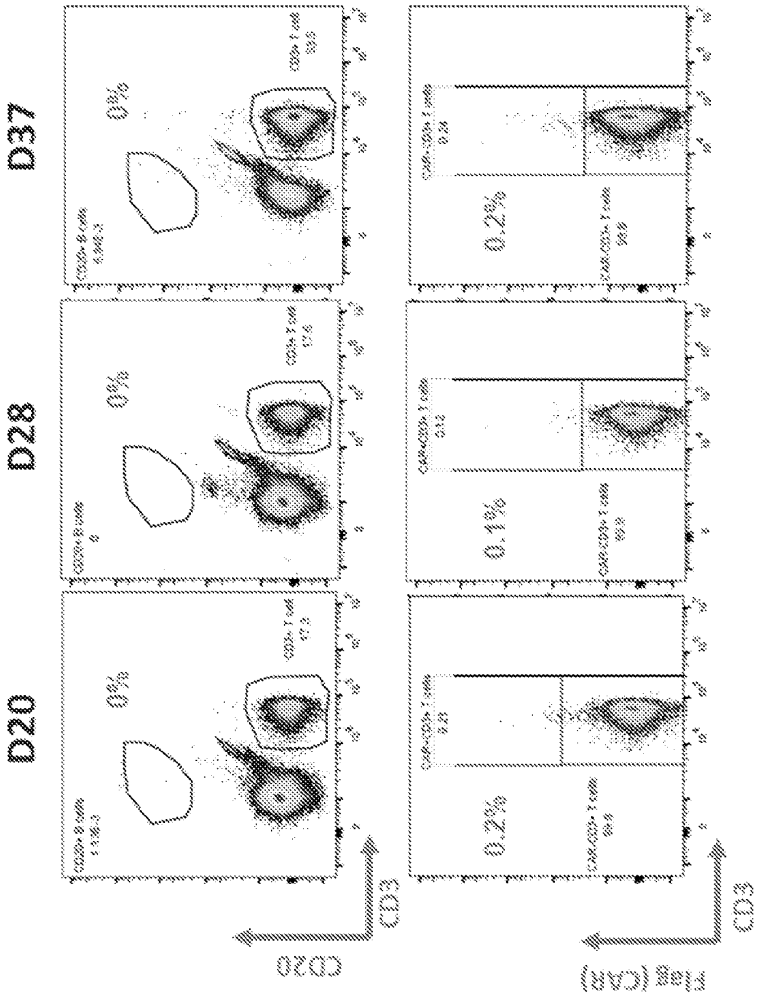


FIG. 28B

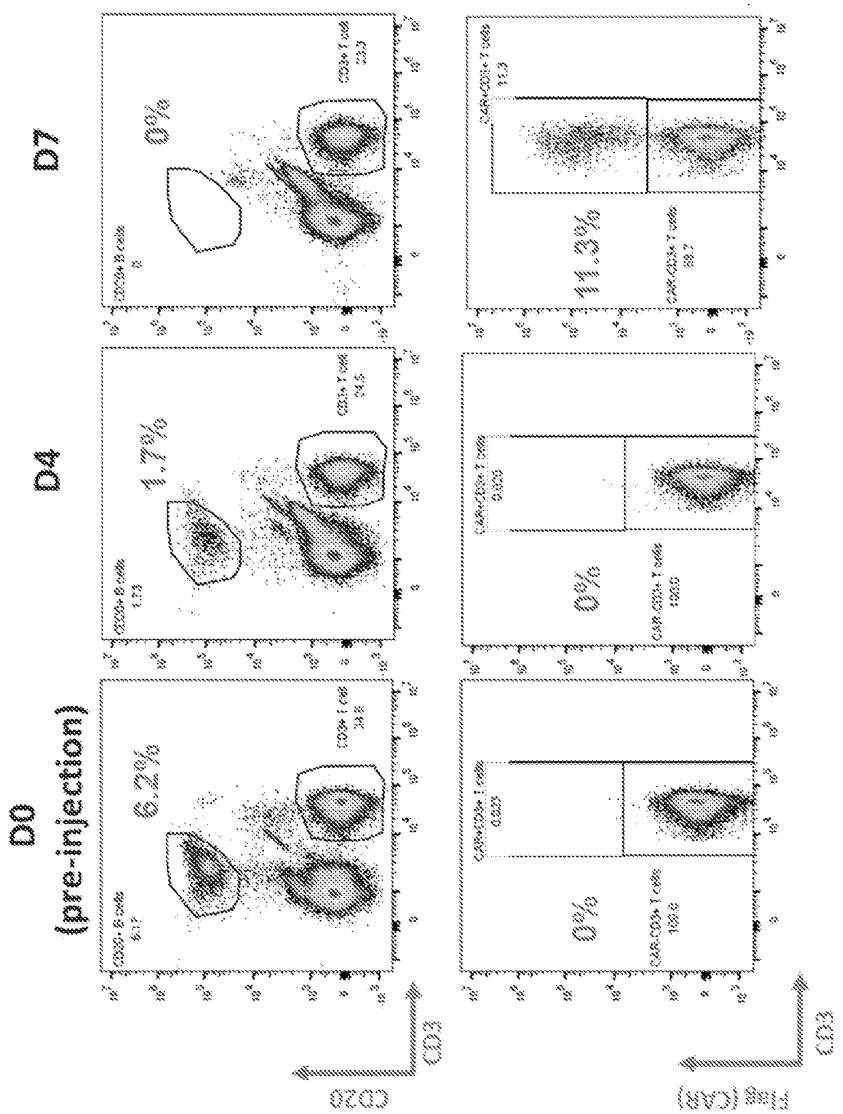


FIG. 29A

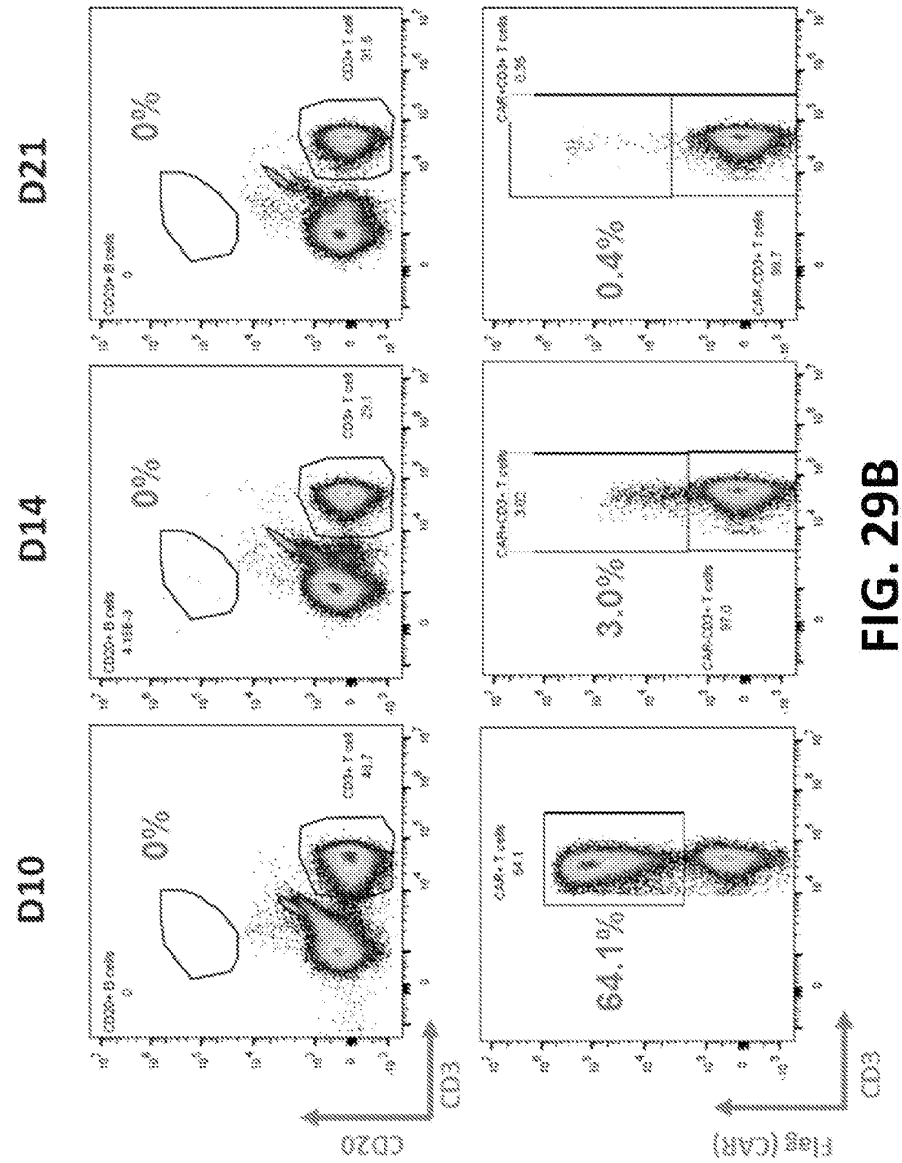


FIG. 29B

FIG. 30A

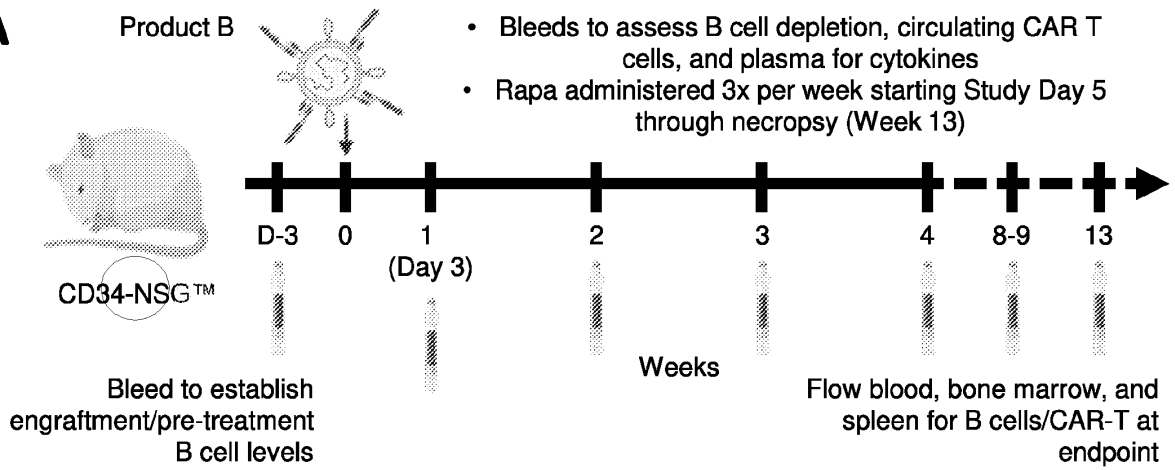


FIG. 30B

Treatment Group	n (Donor 1/ Donor 2)	UB-VV400 Dose (TU/animal)	ROA	Rapamycin (0.5 mg/kg)	Necropsy (Week)
1	3/3	0 (Form. Buf.)	IV	NO	13
2	3/3	$6x10^6$ (Low)	IV	NO	13
3	3/3	$40x10^6$ (Mid)	IV	NO	13
4	3/3	$140x10^6$ (Max feasible)	IV	NO	13
5	3/3	0 (Form. Buf.)	IV	YES	13
6	3/3	$6x10^6$ (Low)	IV	YES	13
7	3/3	$40x10^6$ (Mid)	IV	YES	13
8	3/3	$140x10^6$ (Max feasible)	IV	YES	13

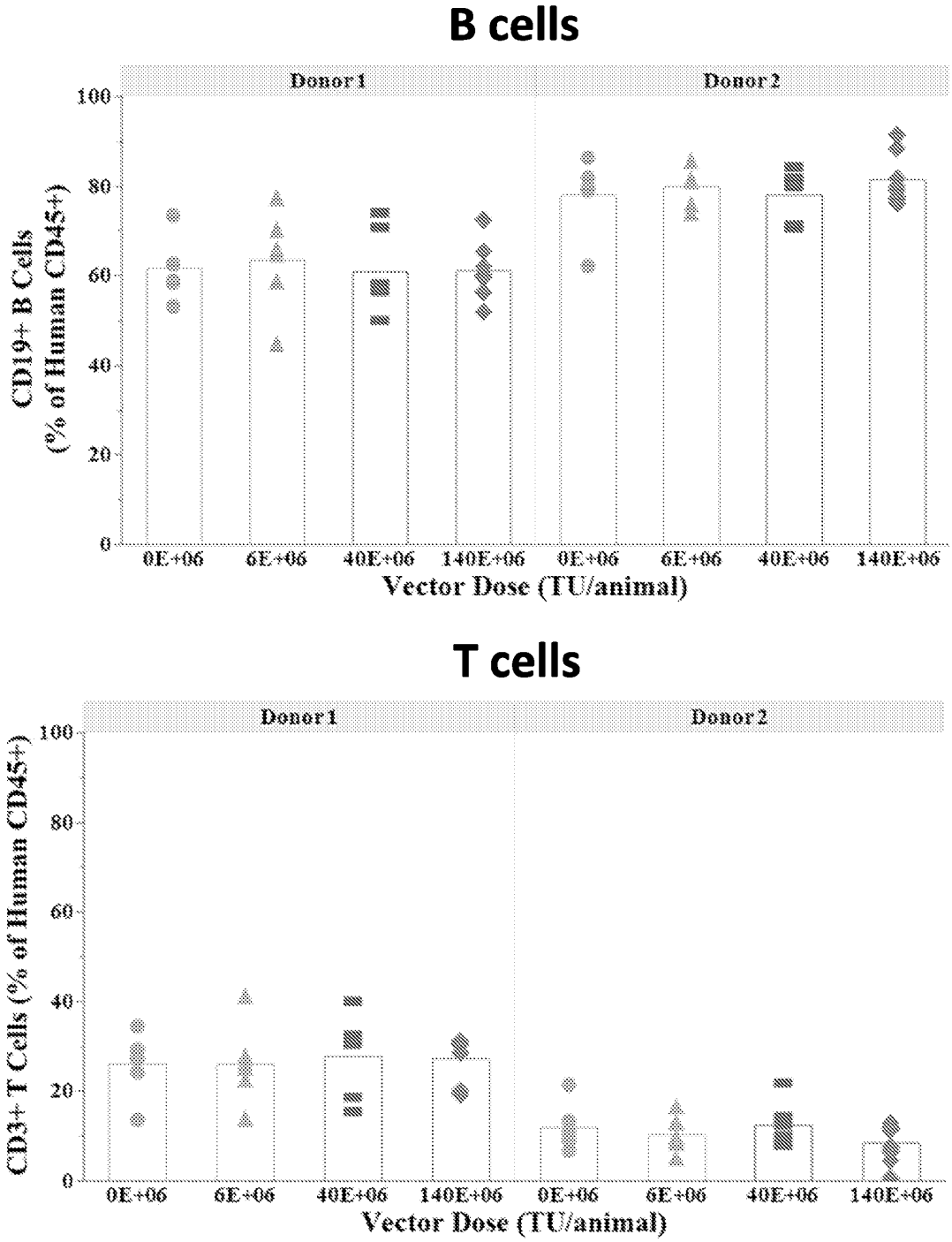
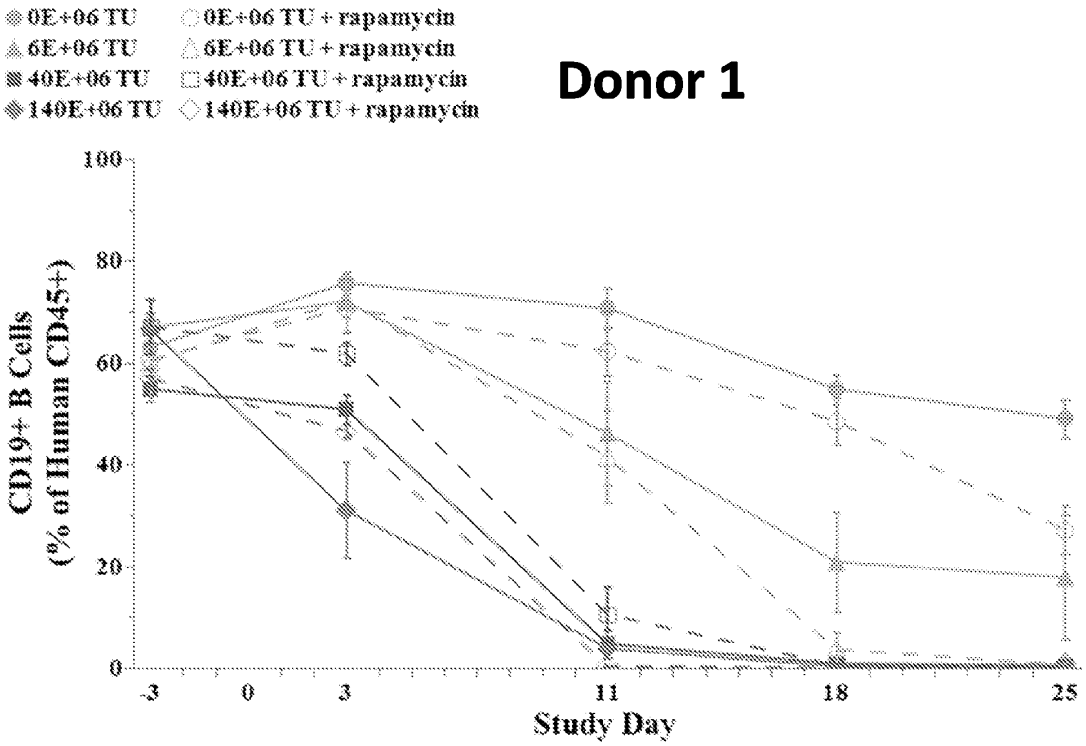

B cells
Donor 1
Donor 2
CD19+ B Cells
(% of Human CD45+)
100
80
60
40
20
0
0E+06
6E+06
40E+06
140E+06
0E+06
6E+06
40E+06
140E+06
Vector Dose (TU/animal)
T cells
Donor 1
Donor 2
CD3+ T Cells (% of Human CD45+)
100
80
60
40
20
0
0E+06
6E+06
40E+06
140E+06
0E+06
6E+06
40E+06
140E+06
Vector Dose (TU/animal)

FIG. 31


0E+06 TU
0E+06 TU + rapamycin
6E+06 TU
6E+06 TU + rapamycin
40E+06 TU
40E+06 TU + rapamycin
140E+06 TU
140E+06 TU + rapamycin
Donor 1
CD19+ B Cells
(% of Human CD45+)
100
80
60
40
20
0
-3
0
3
11
18
25
Study Day

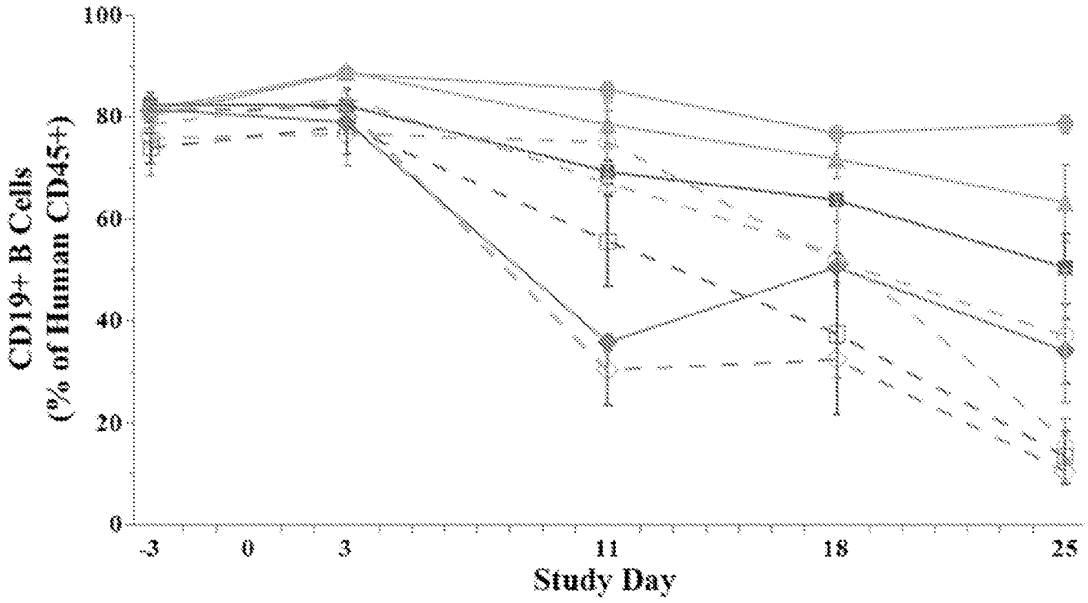
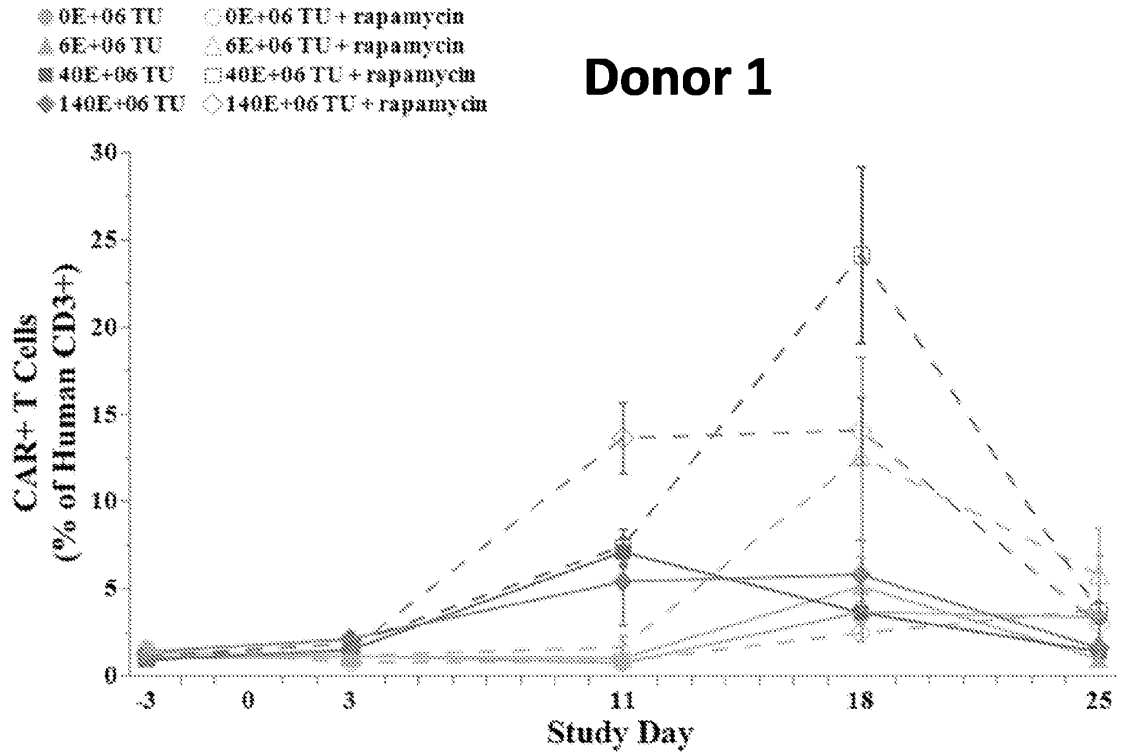

Donor 2
CD19+ B Cells
(% of Human CD45+)
100
80
60
40
20
0
-3
0
3
11
18
25
Study Day

FIG. 32


0E+06 TU
0E+06 TU + rapamycin
6E+06 TU
6E+06 TU + rapamycin
40E+06 TU
40E+06 TU + rapamycin
140E+06 TU
140E+06 TU + rapamycin
Donor 1
CAR+ T Cells
(% of Human CD3+)
30
25
20
15
10
5
0
-3
0
3
11
18
25
Study Day

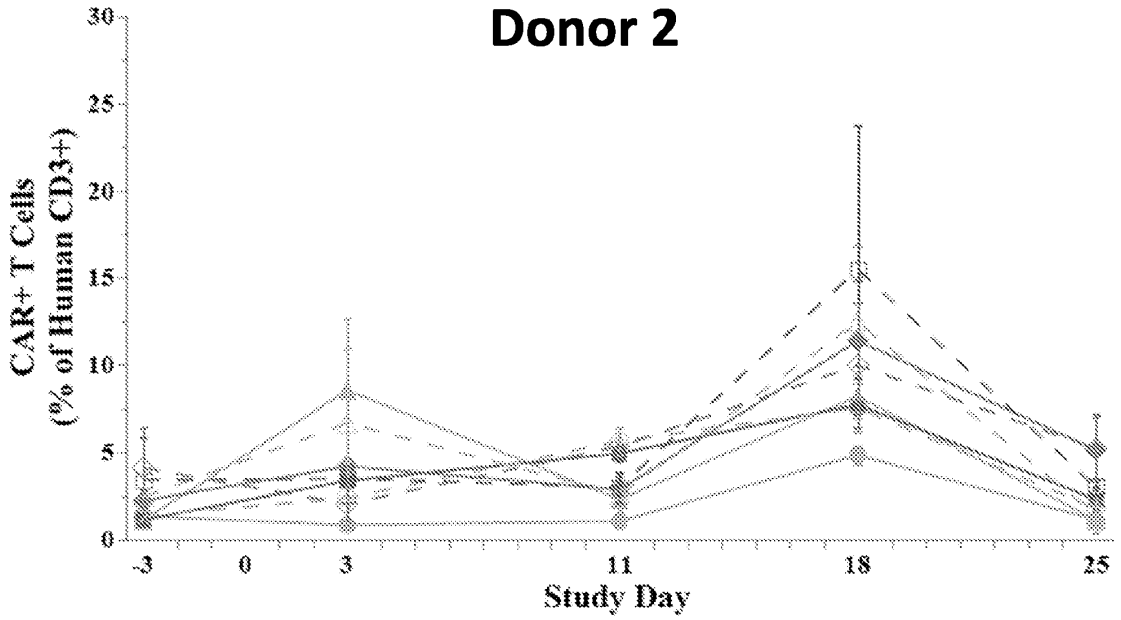
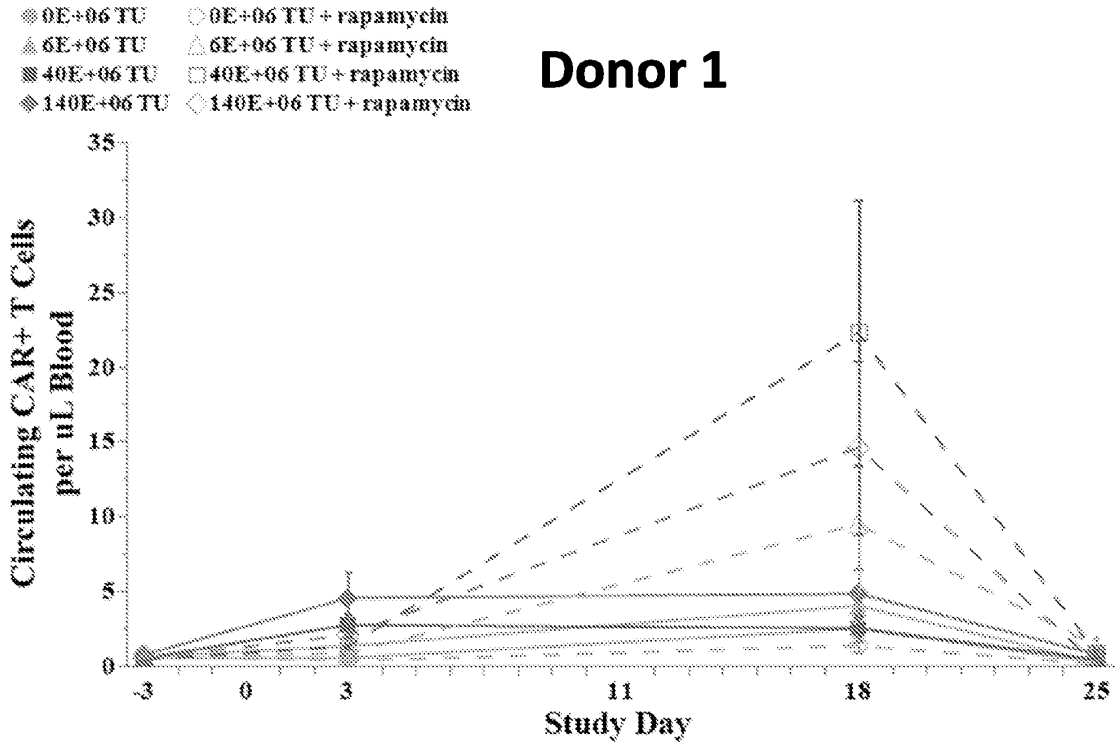

Donor 2
CAR+ T Cells
(% of Human CD3+)
30
25
20
15
10
5
0
-3
0
3
11
18
25
Study Day

FIG. 33


0E+06 TU
6E+06 TU
40E+06 TU
140E+06 TU
0E+06 TU + rapamycin
6E+06 TU + rapamycin
40E+06 TU + rapamycin
140E+06 TU + rapamycin
Donor 1
Circulating CAR+ T Cells per uL Blood
35
30
25
20
15
10
5
0
-3
0
3
11
18
25
Study Day

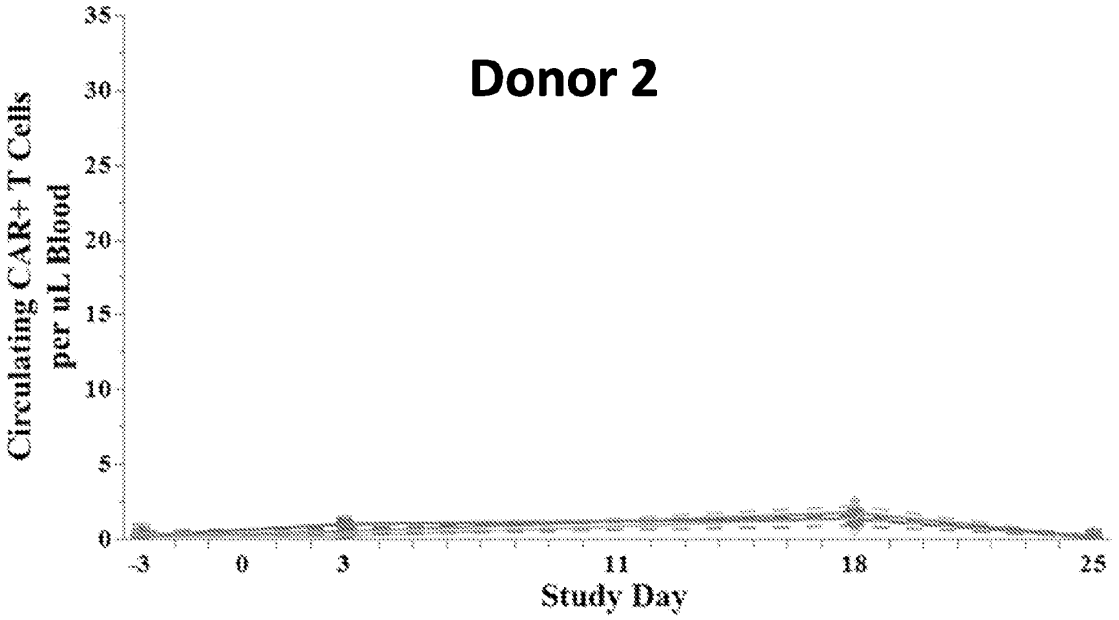

Donor 2
Circulating CAR+ T Cells per uL Blood
35
30
25
20
15
10
5
0
-3
0
3
11
18
25
Study Day

FIG. 34

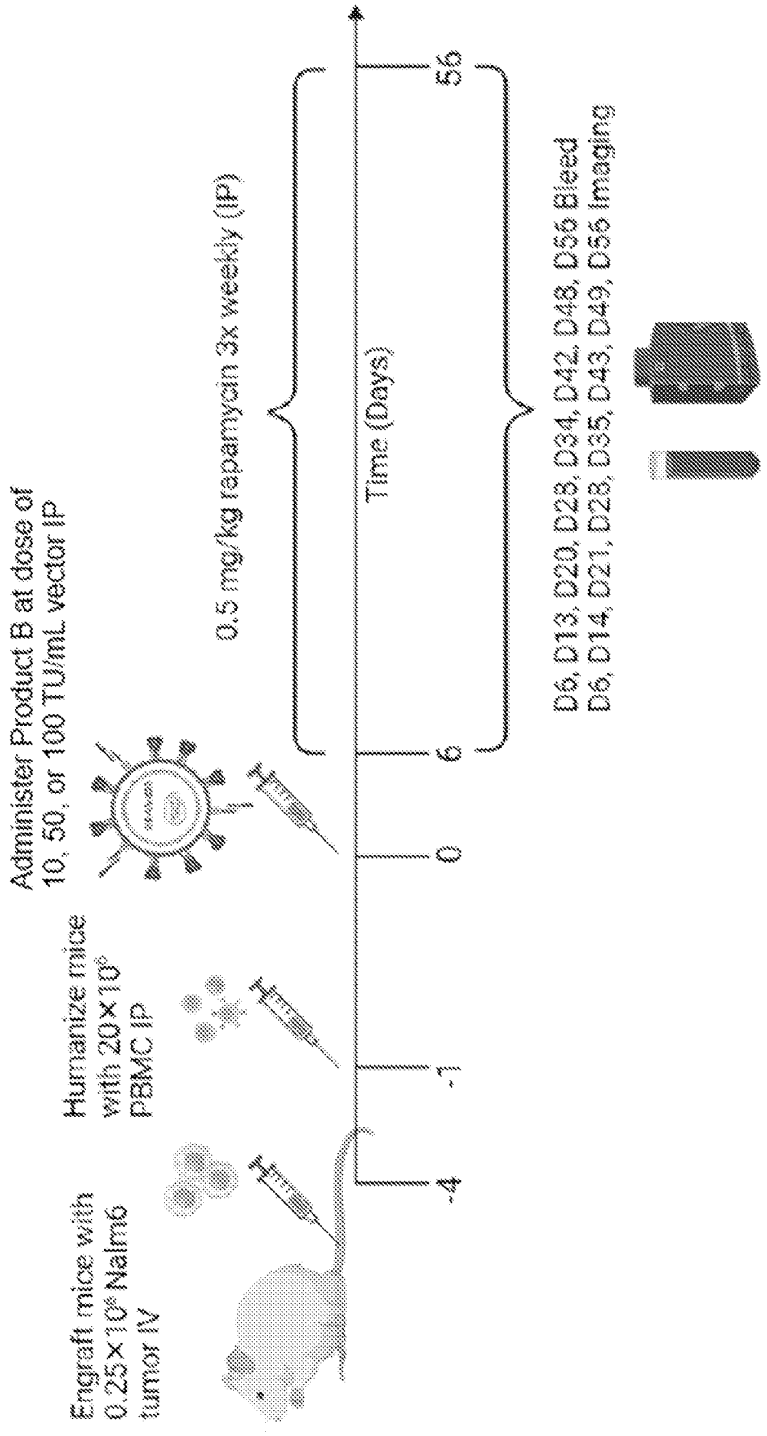

Engraft mice with
0.25×10⁶ Nalm6
tumor IV
Humanize mice
with 20×10⁶
PBMC IP
Administer Product B at dose of
10, 50, or 100 TU/mL vector IP
0.5 mg/kg rapamycin 3x weekly (IP)
Time (Days)
-4
-1
0
6
56
D6, D13, D20, D28, D34, D42, D48, D56 Bleed
D6, D14, D21, D28, D35, D43, D49, D56 Imaging

FIG. 35

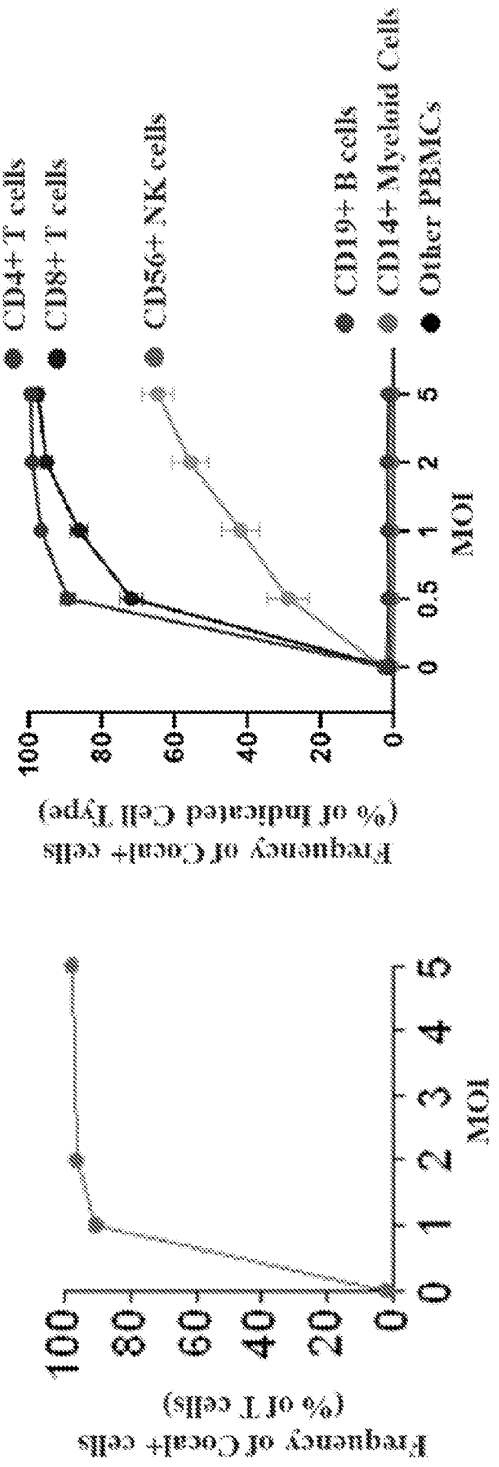

Frequency of Cocal+ cells
(% of T cells)
100
80
60
40
20
0
0 1 2 3 4 5
MOI
Frequency of Cocal+ cells
(% of Indicated Cell Type)
100
80
60
40
20
0
0 0.5 1 2 5
MOI
CD4+ T cells
CD8+ T cells
CD56+ NK cells
CD19+ B cells
CD14+ Myeloid Cells
Other PBMCs

FIG. 36A

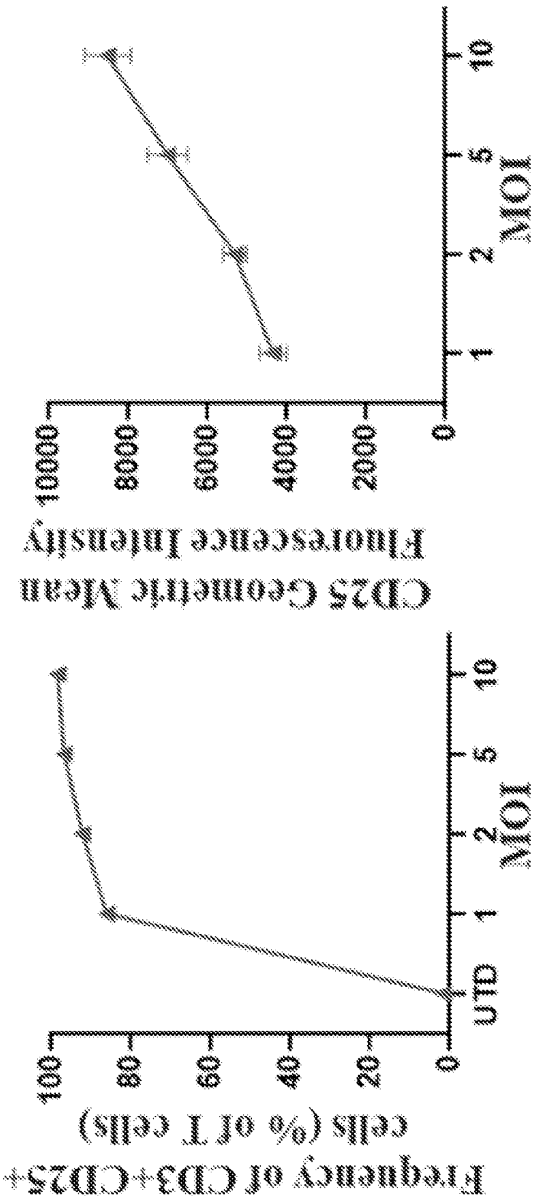

Frequency of CD3+CD25+ cells (% of T cells)
100
80
60
40
20
0
UTD
1
2
5
10
MOI
CD25 Geometric Mean Fluorescence Intensity
10000
8000
6000
4000
2000
0
1
2
5
10
MOI

FIG. 36B

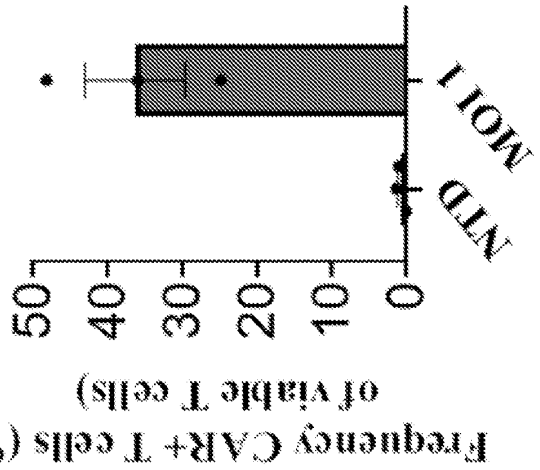

Frequency CAR+ T cells (% of viable T cells)
50
40
30
20
10
0
NTD
MOI 1

FIG. 36C

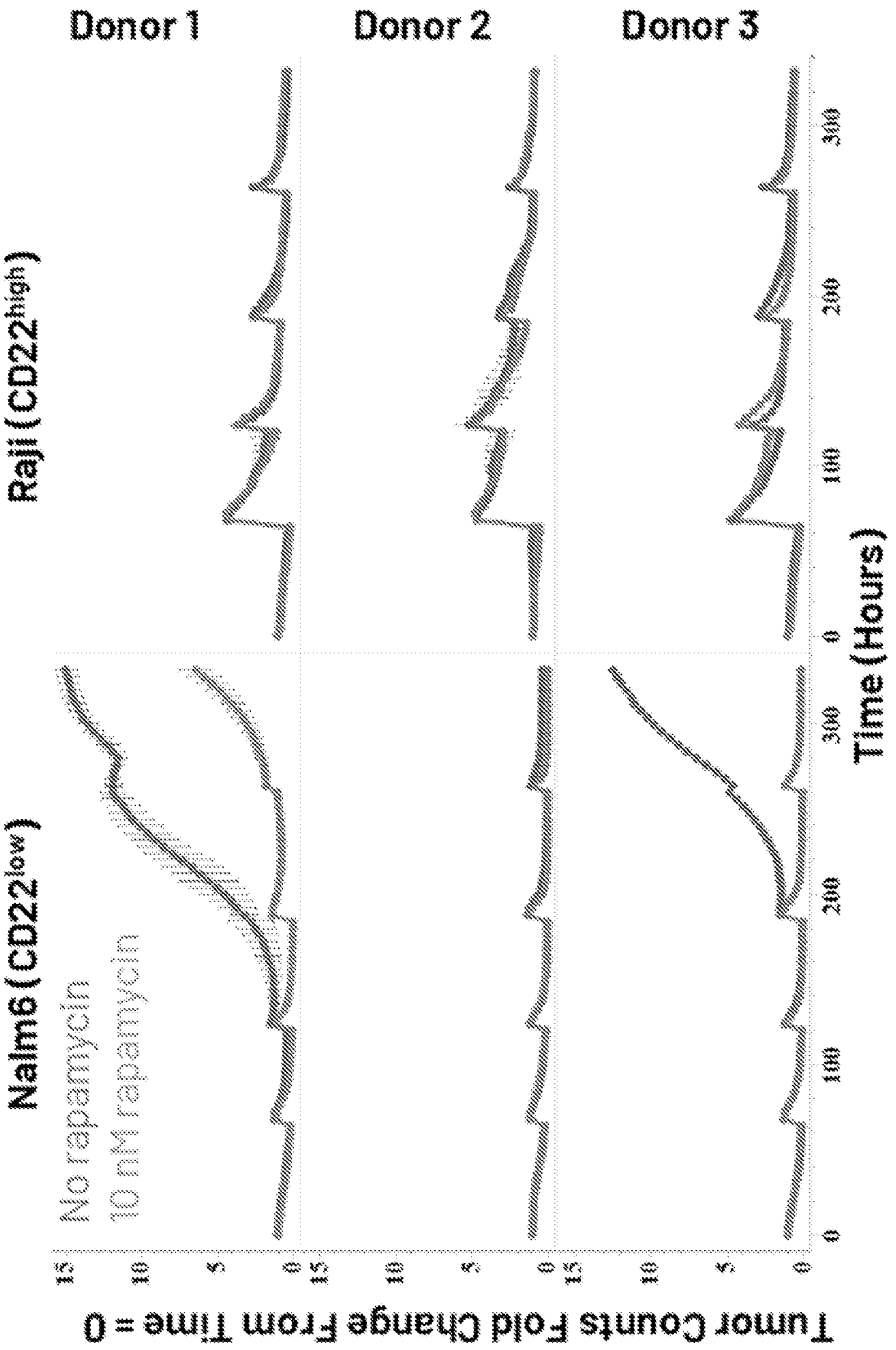

Donor 1
Donor 2
Donor 3
Raji (CD22high)
Nalm6 (CD22low)
No rapamycin
10 nM rapamycin
Tumor Counts Fold Change From Time = 0
Time (Hours)
0
5
10
15
100
200
300

FIG. 37

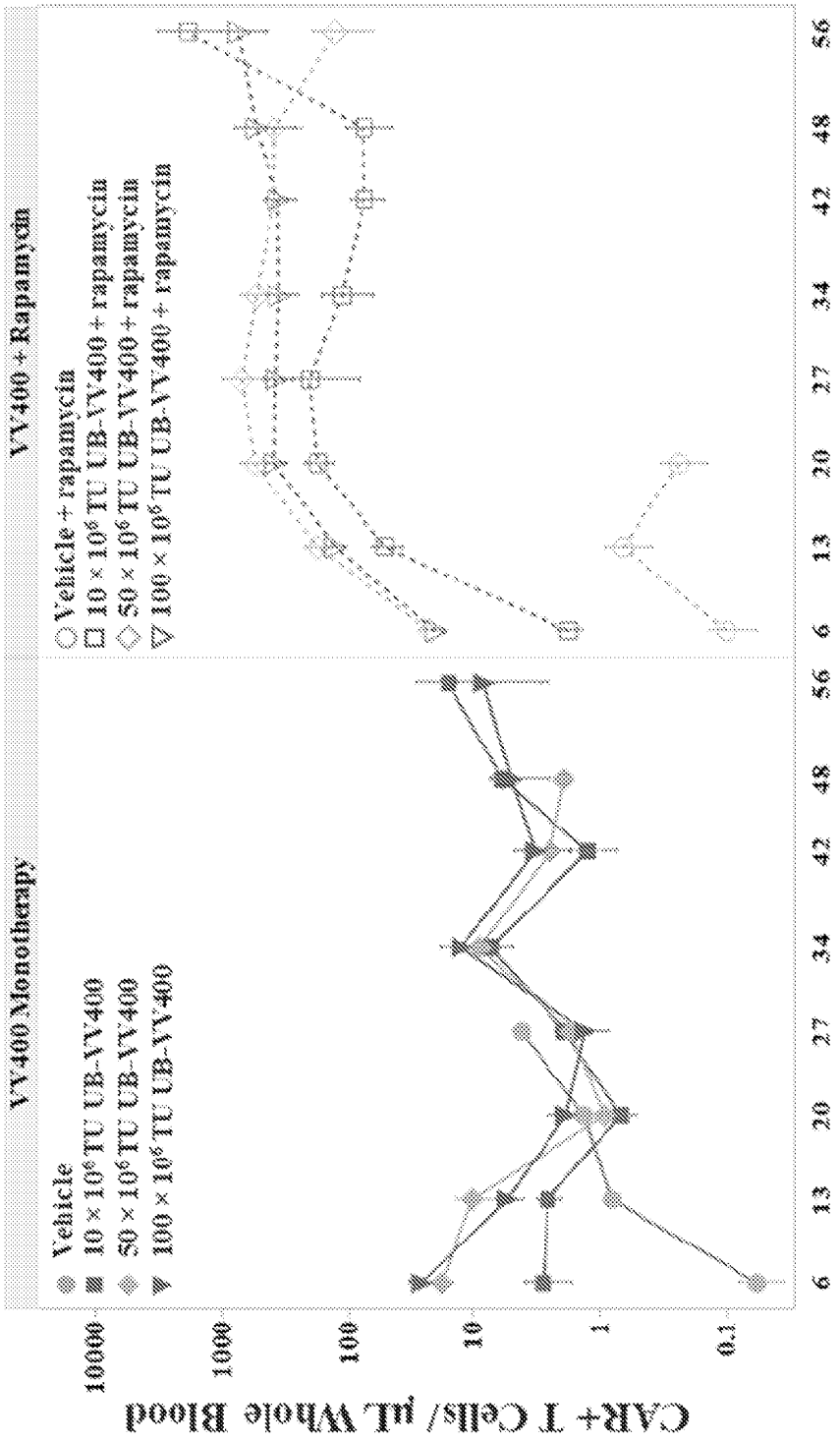

VV400 Monotherapy
Vehicle
10 × 10⁶ TU UB-VV400
50 × 10⁶ TU UB-VV400
100 × 10⁶ TU UB-VV400
VV400 + Rapamycin
Vehicle + rapamycin
10 × 10⁶ TU UB-VV400 + rapamycin
50 × 10⁶ TU UB-VV400 + rapamycin
100 × 10⁶ TU UB-VV400 + rapamycin
CAR+ T Cells/ µL Whole Blood
10000
1000
100
10
1
0.1
6
13
20
27
34
42
48
56
Study Day

FIG. 38

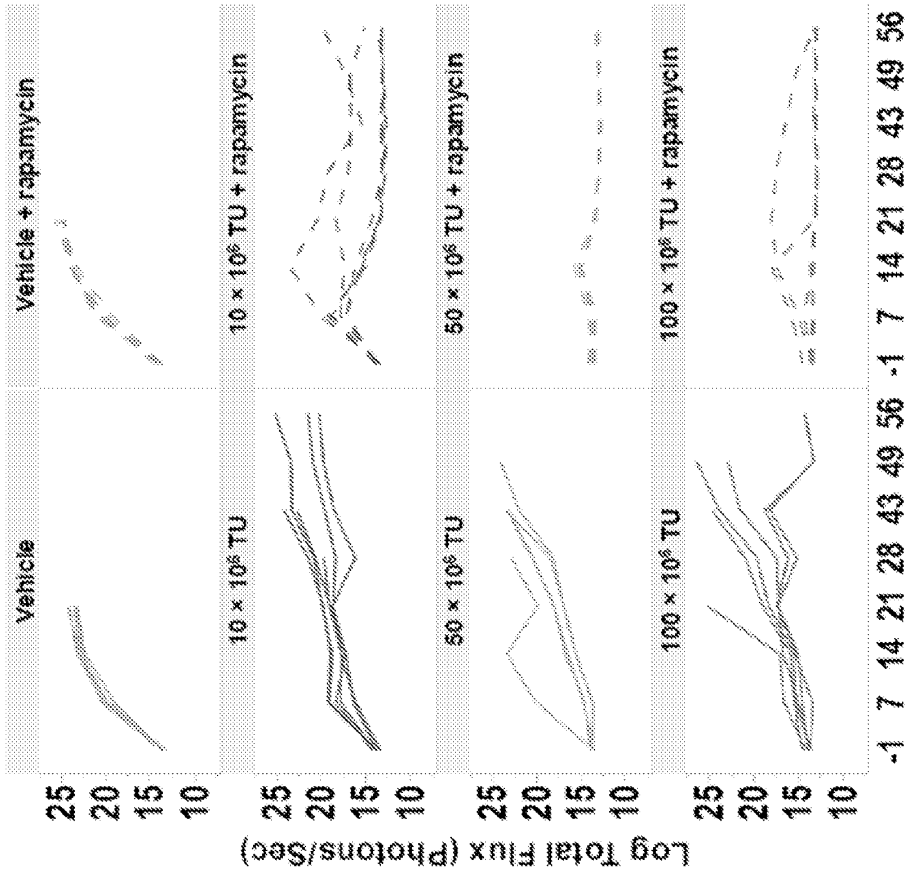

Vehicle
Vehicle + rapamycin
10 × 10⁶ TU
10 × 10⁶ TU + rapamycin
50 × 10⁶ TU
50 × 10⁶ TU + rapamycin
100 × 10⁶ TU
100 × 10⁶ TU + rapamycin
Log Total Flux (Photons/Sec)
25
20
15
10
-1 7 14 21 28 43 49 56
Study Day

FIG. 39A

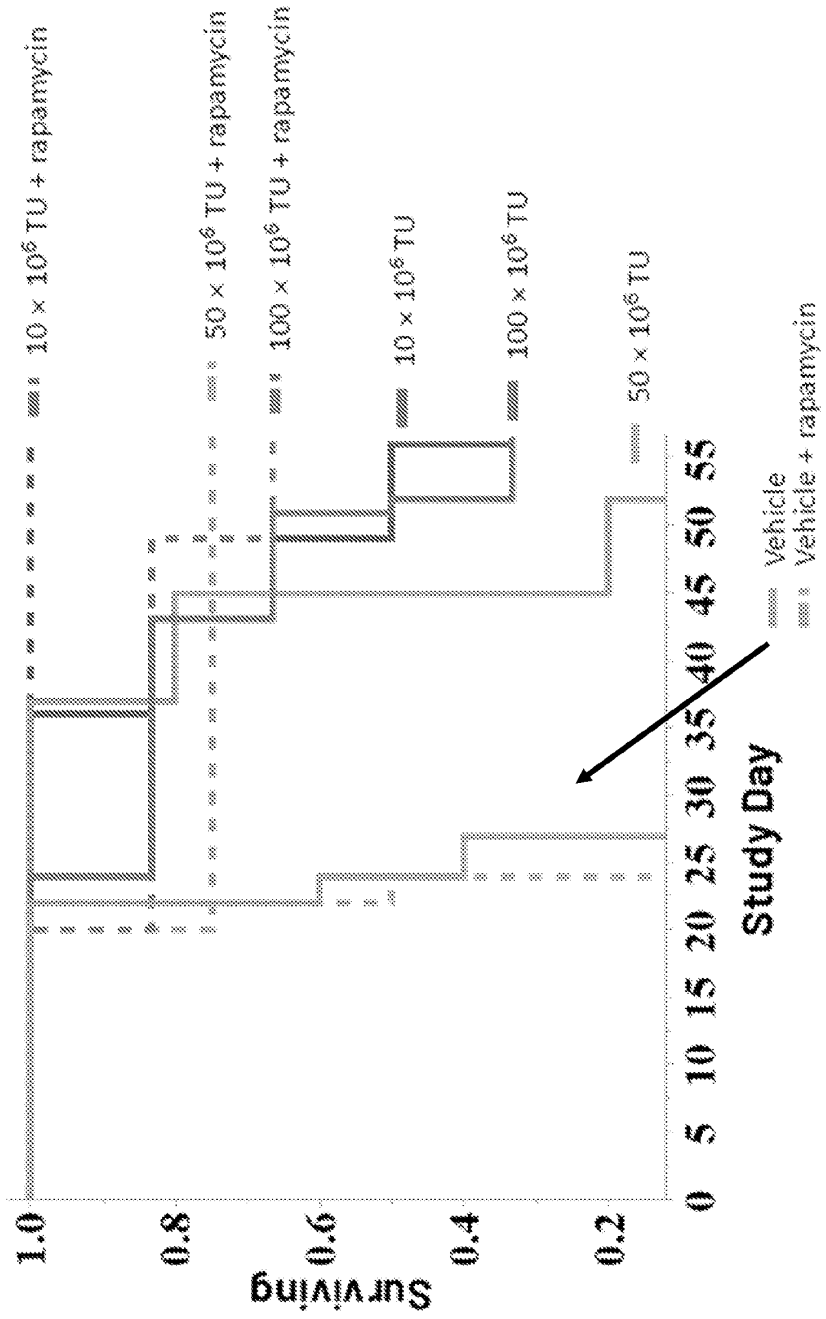

Surviving
1.0
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
40
45
50
55
Study Day
Vehicle
Vehicle + rapamycin
50 × 10^6 TU
100 × 10^6 TU
10 × 10^6 TU
100 × 10^6 TU + rapamycin
50 × 10^6 TU + rapamycin
10 × 10^6 TU + rapamycin

FIG. 39B