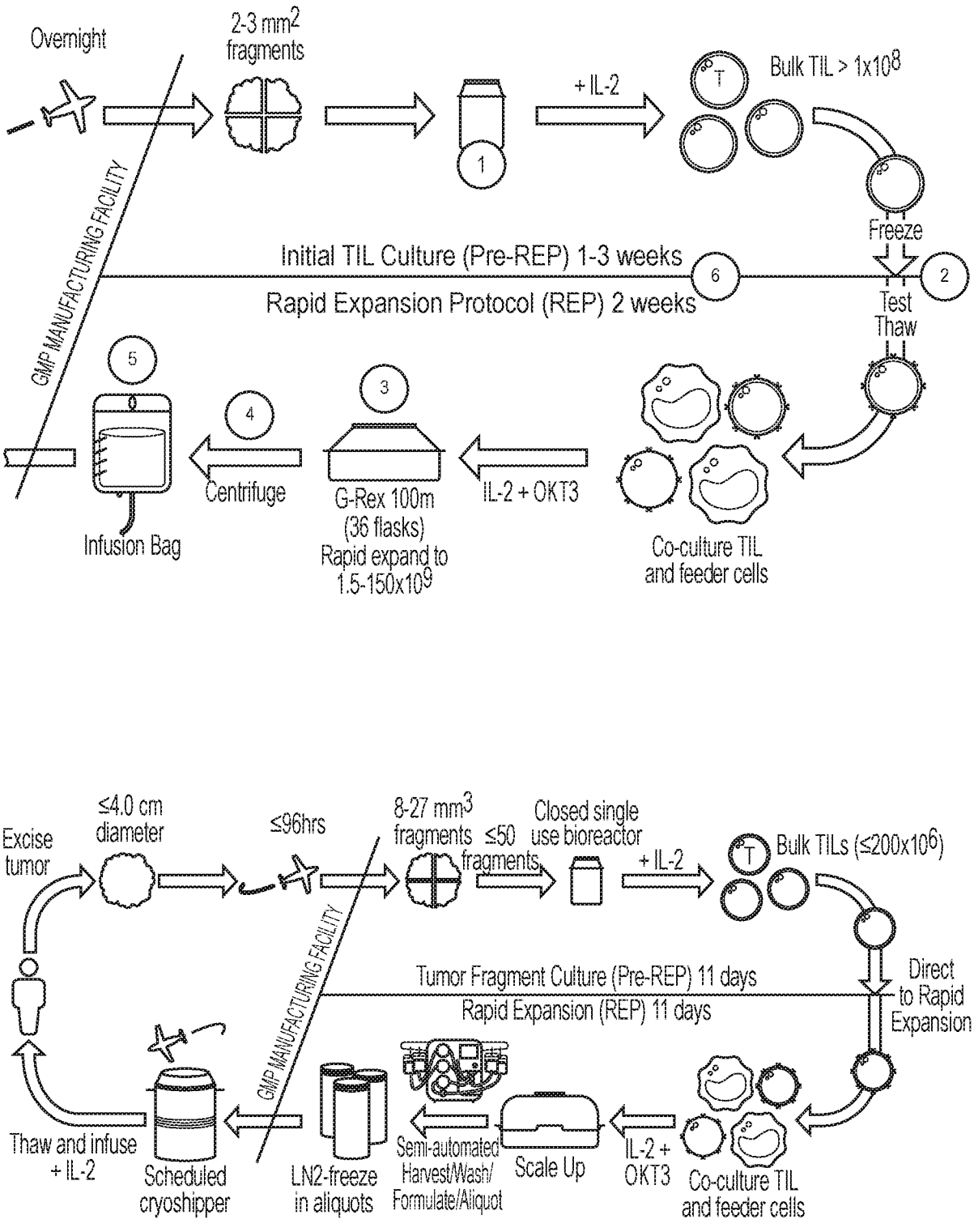

Overnight
2-3 mm2 fragments
+ IL-2
Bulk TIL > 1x10^8
Freeze
Initial TIL Culture (Pre-REP) 1-3 weeks
Rapid Expansion Protocol (REP) 2 weeks
Test Thaw
GMP MANUFACTURING FACILITY
Infusion Bag
Centrifuge
G-Rex 100m (36 flasks) Rapid expand to 1.5-150x10^9
IL-2 + OKT3
Co-culture TIL and feeder cells
Excise tumor
≤4.0 cm diameter
≤96hrs
8-27 mm3 fragments
≤50 fragments
Closed single use bioreactor
+ IL-2
Bulk TILs (≤200x10^6)
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
Direct to Rapid Expansion
Thaw and infuse + IL-2
Scheduled cryoshipper
LN2-freeze in aliquots
Semi-automated Harvest/Wash/ Formulate/Aliquot
Scale Up
IL-2 + OKT3
Co-culture TIL and feeder cells

FIG. 1

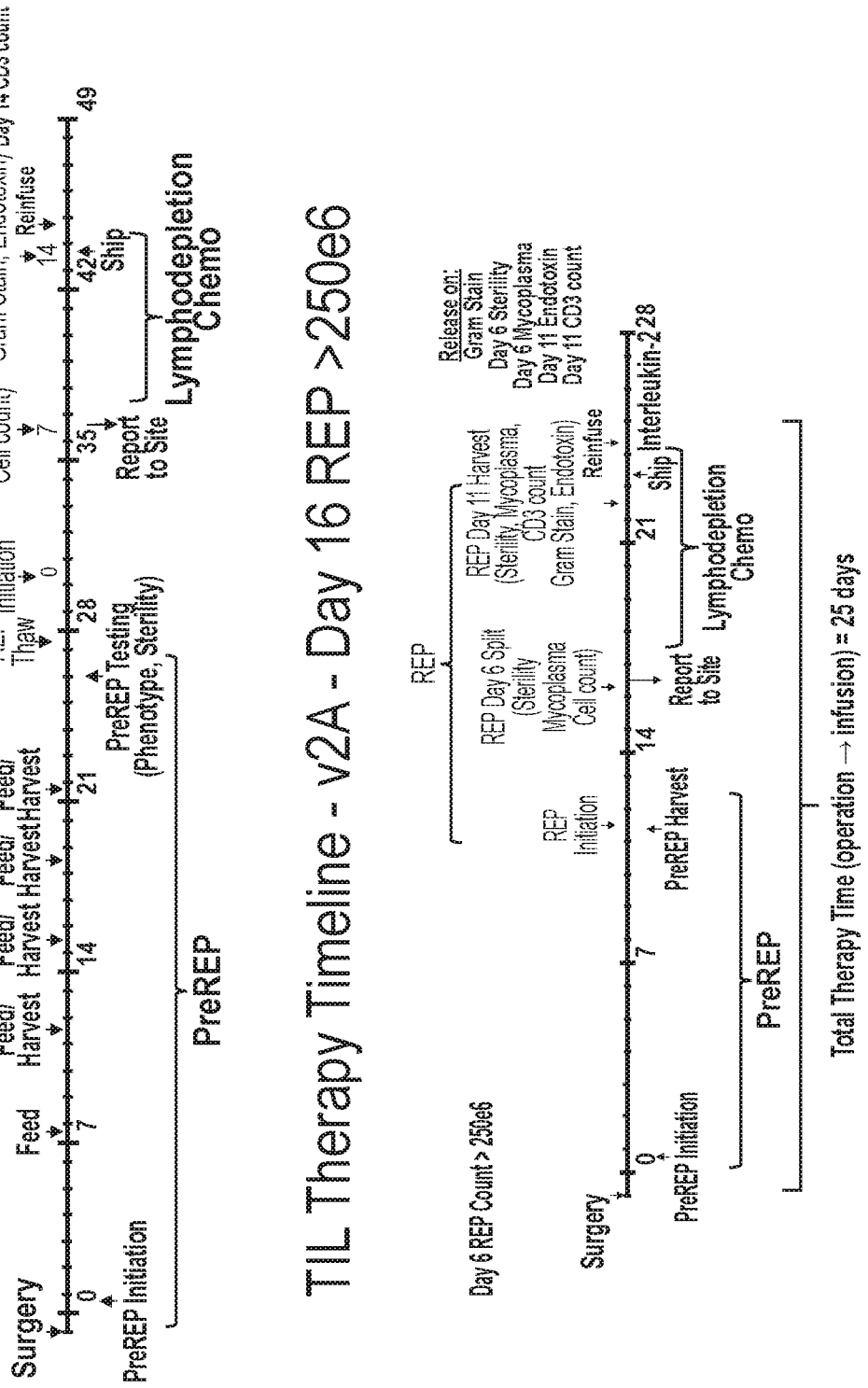

TIL Therapy Timeline - v2A - Day 16 REP >250e6
Surgery
PreREP Initiation
Feed
Feed/ Harvest
Feed/ Harvest
Feed/ Harvest
Feed/ Harvest
PreREP
PreREP Testing (Phenotype, Sterility)
REP Initiation Thaw
REP
REP Day 7 Split (Sterility Mycoplasma Cell count)
REP Day 14 Harvest (Sterility, Mycoplasma, CD3 count Gram Stain, Endotoxin)
Release on: Gram Stain Day 7 Sterility Day 7 Mycoplasma Day 14 Endotoxin Day 14 CD3 count
Reinfuse
Ship
Report to Site
Lymphodepletion Chemo
0
7
14
21
28
35
42
49
Day 6 REP Count > 250e6
PreREP Initiation
Surgery
PreREP Harvest
REP Initiation
REP Day 6 Split (Sterility Mycoplasma Cell count)
REP Day 11 Harvest (Sterility, Mycoplasma, CD3 count Gram Stain, Endotoxin)
Release on: Gram Stain Day 6 Sterility Day 6 Mycoplasma Day 11 Endotoxin Day 11 CD3 count
Reinfuse
Ship
Interleukin-2
Report to Site
Lymphodepletion Chemo
0
7
14
21
28
PreREP
REP
Total Therapy Time (operation → infusion) = 25 days

FIG. 2

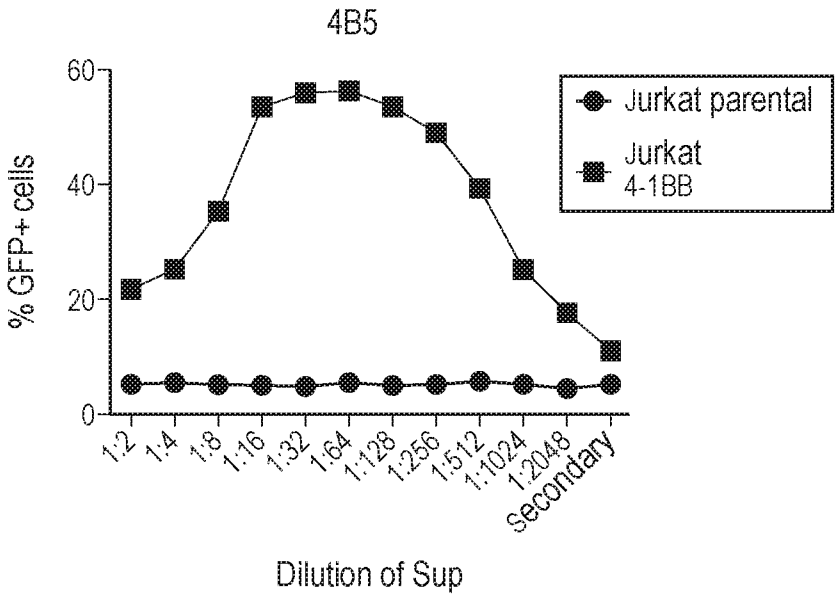

4B5
% GFP+ cells
60
40
20
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup
Jurkat parental
Jurkat
4-1BB

FIG. 3

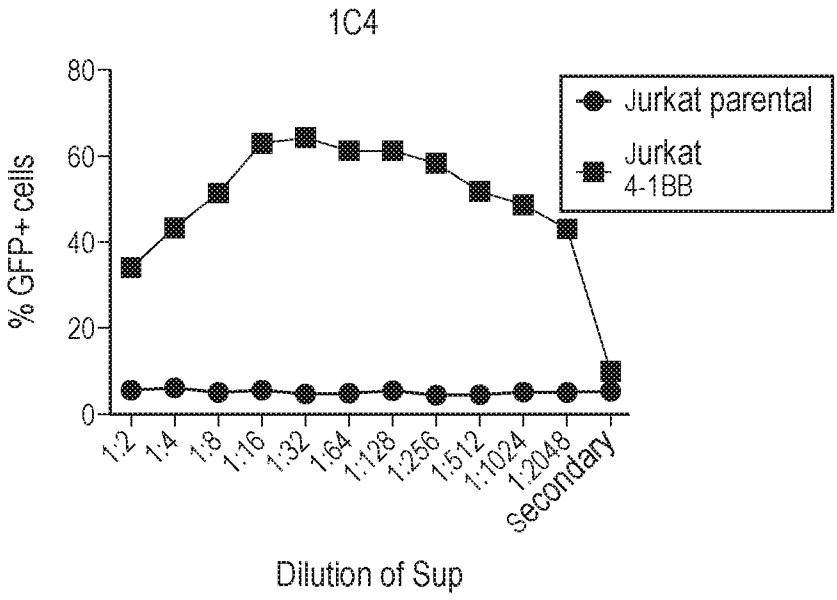

1C4
80
60
40
20
0
% GFP+ cells
Jurkat parental
Jurkat
4-1BB
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup

FIG. 4

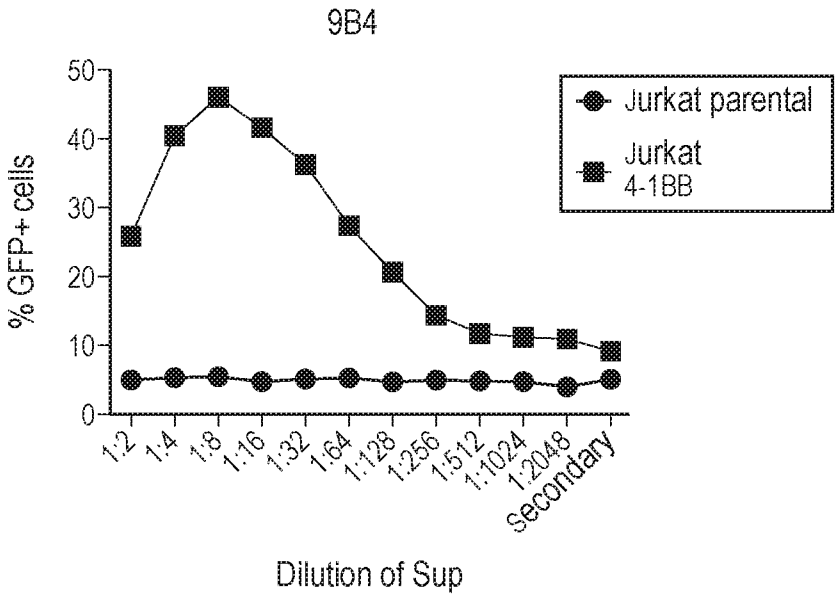

9B4
% GFP+ cells
50
40
30
20
10
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup
Jurkat parental
Jurkat
4-1BB

FIG. 5

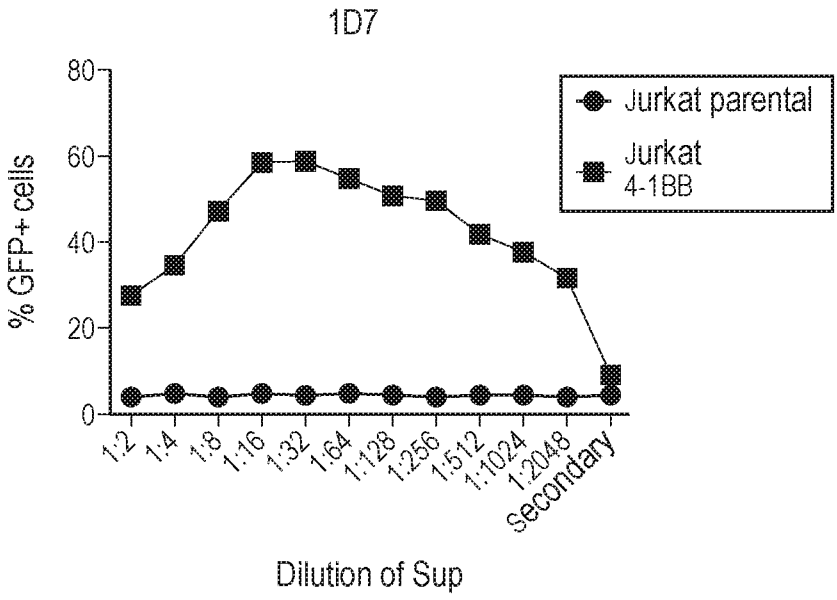

1D7
80
60
40
20
0
% GFP+ cells
Jurkat parental
Jurkat
4-1BB
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup

FIG. 6

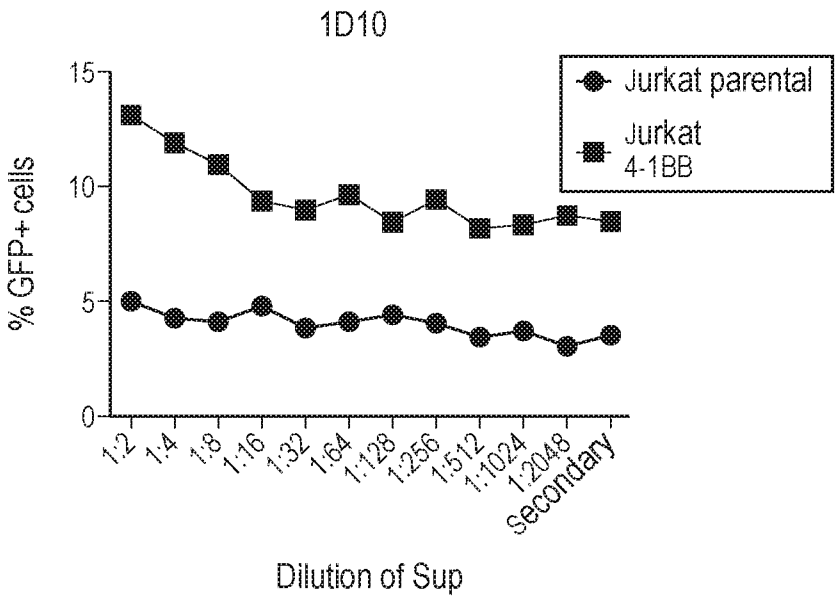

1D10
Jurkat parental
Jurkat
4-1BB
% GFP+ cells
15
10
5
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup

FIG. 7

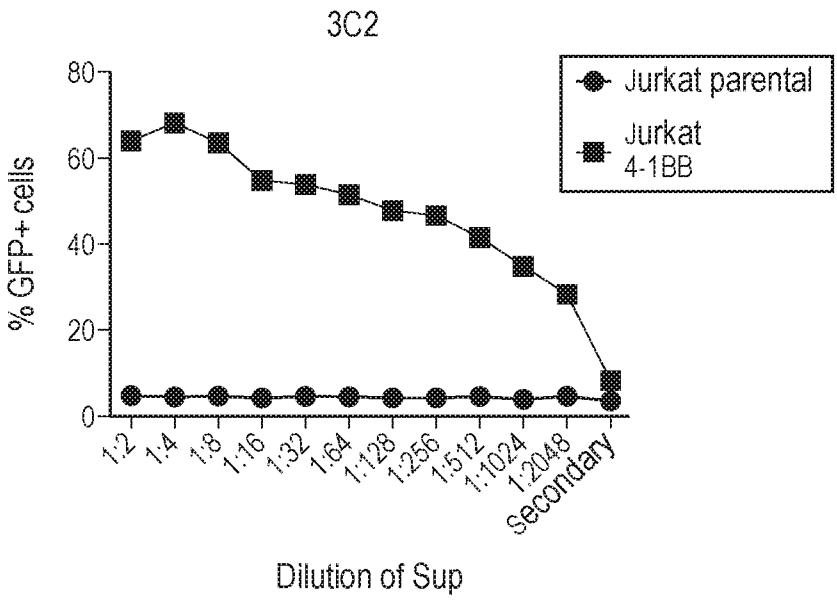

3C2
Jurkat parental
Jurkat
4-1BB
% GFP+ cells
80
60
40
20
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup

FIG. 8

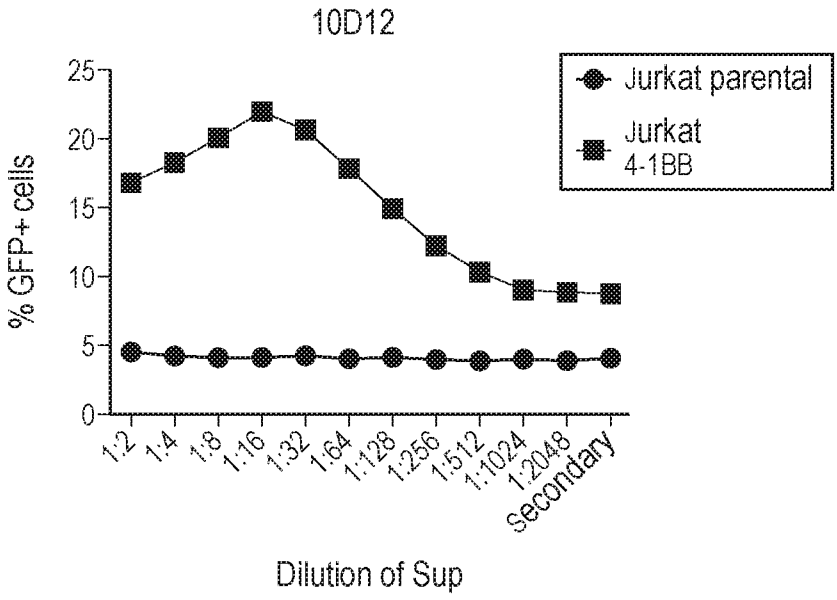

10D12
Jurkat parental
Jurkat
4-1BB
% GFP+ cells
25
20
15
10
5
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup

FIG. 9

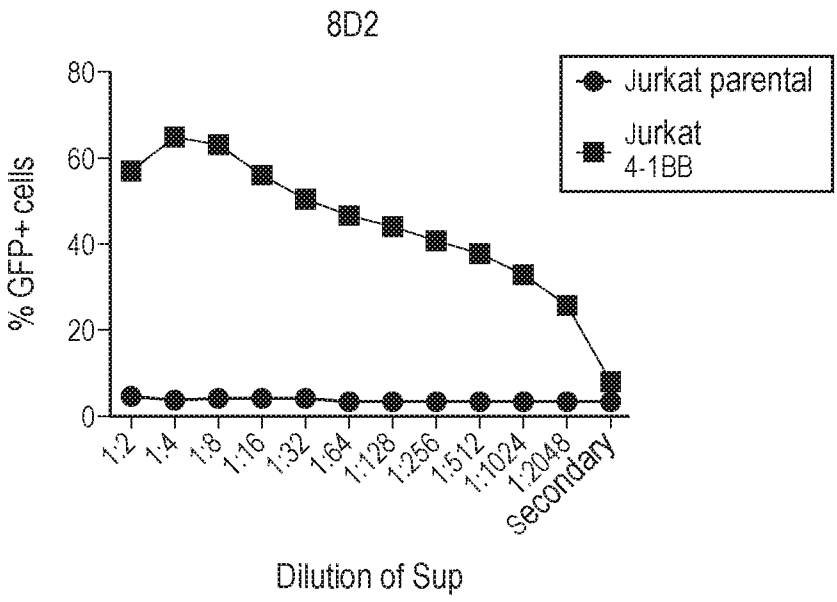

8D2
Jurkat parental
Jurkat
4-1BB
% GFP+ cells
80
60
40
20
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup

FIG. 10

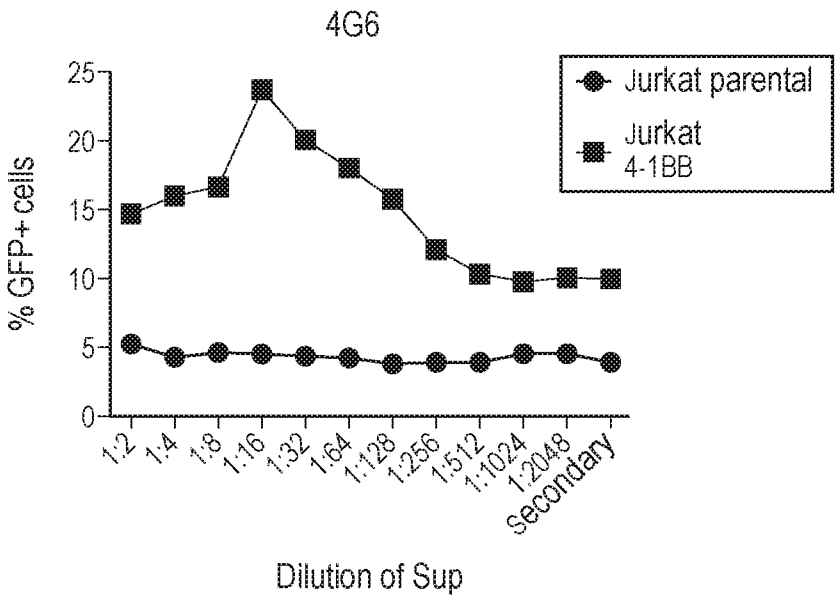

4G6
Jurkat parental
Jurkat
4-1BB
% GFP+ cells
25
20
15
10
5
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup

FIG. 11

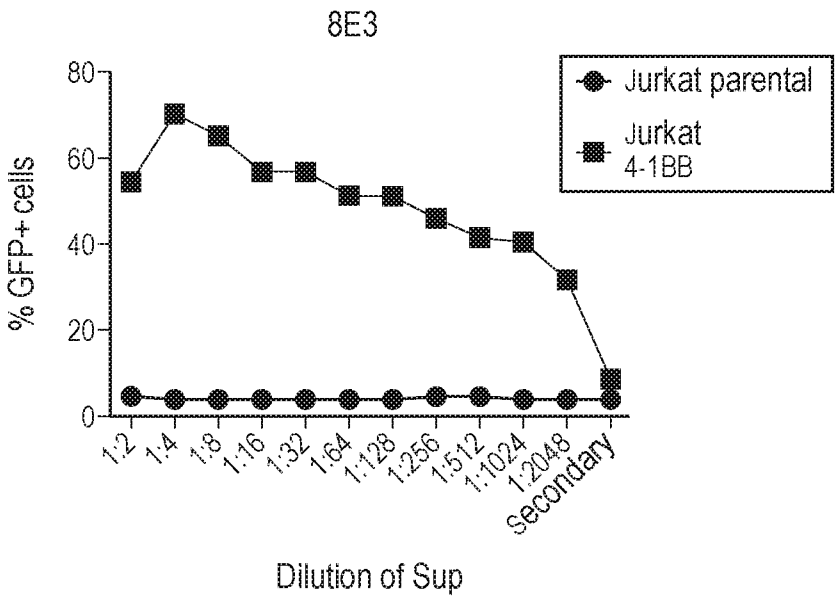

8E3
Jurkat parental
Jurkat
4-1BB
% GFP+ cells
80
60
40
20
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
secondary
Dilution of Sup

FIG. 12

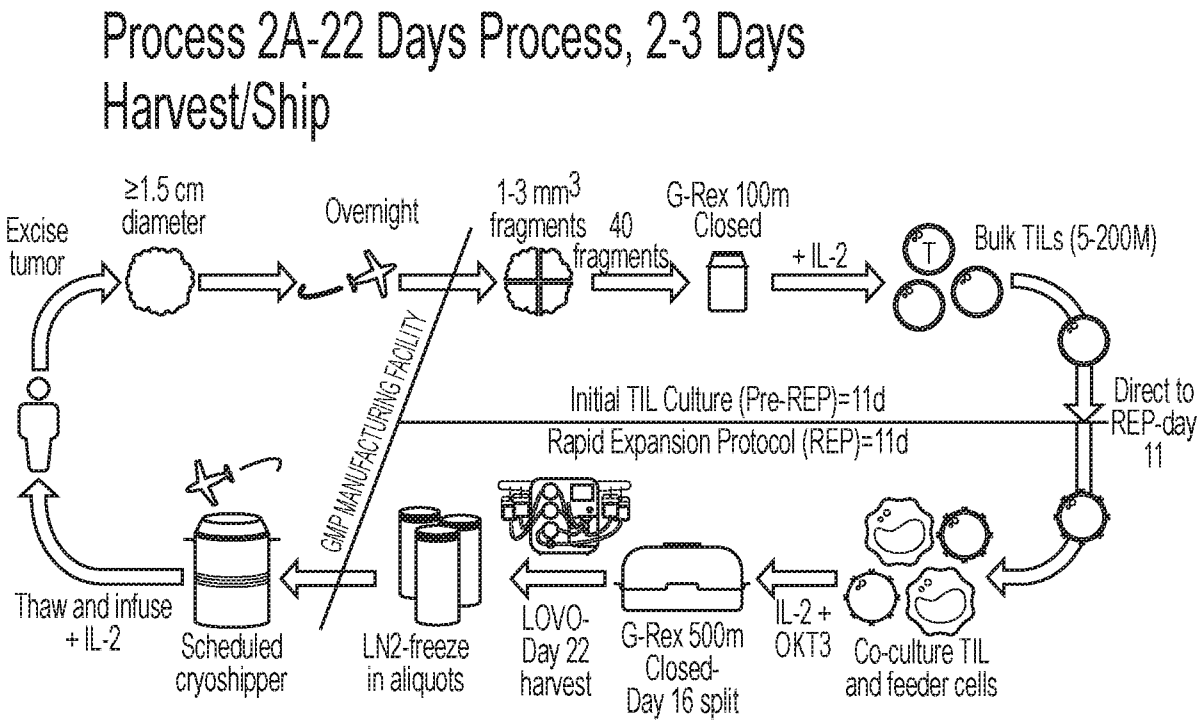

Process 2A-22 Days Process, 2-3 Days Harvest/Ship
Excise tumor
≥1.5 cm diameter
Overnight
1-3 mm3 fragments
40 fragments
G-Rex 100m Closed
+ IL-2
Bulk TILs (5-200M)
Direct to REP-day 11
Initial TIL Culture (Pre-REP)=11d
Rapid Expansion Protocol (REP)=11d
GMP MANUFACTURING FACILITY
Co-culture TIL and feeder cells
IL-2 + OKT3
G-Rex 500m Closed-Day 16 split
LOVO-Day 22 harvest
LN2-freeze in aliquots
Scheduled cryoshipper
Thaw and infuse + IL-2

FIG. 13

Process 2A: about 22 days from Steps A - E

1. **STEP A**

Obtain Patient Tumor Sample

2. **STEP B**

Fragmentation and First Expansion
3 days to 14 days

3. **STEP C**

First expansion to Second Expansion Transition
No Storage and Closed System

4. **STEP D**

Second Expansion
IL-2, OKT-3, and antigen-presenting feeder cells
Closed System

5. **STEP E**

Harvest TILS from Step D
Closed System

6. **STEP F**

Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

FIG. 14

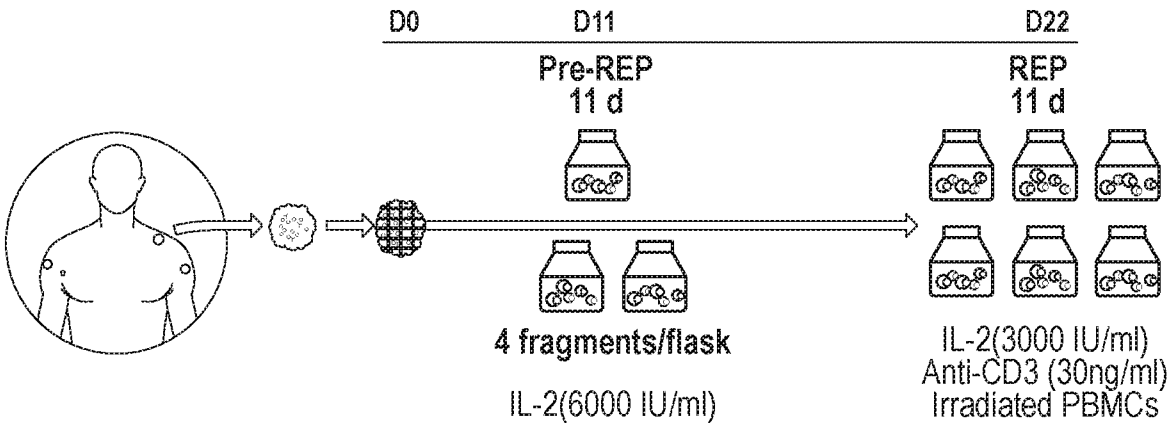

D0
D11
D22
Pre-REP
11 d
REP
11 d
4 fragments/flask
IL-2(6000 IU/ml)
IL-2(3000 IU/ml)
Anti-CD3 (30ng/ml)
Irradiated PBMCs

FIG. 15

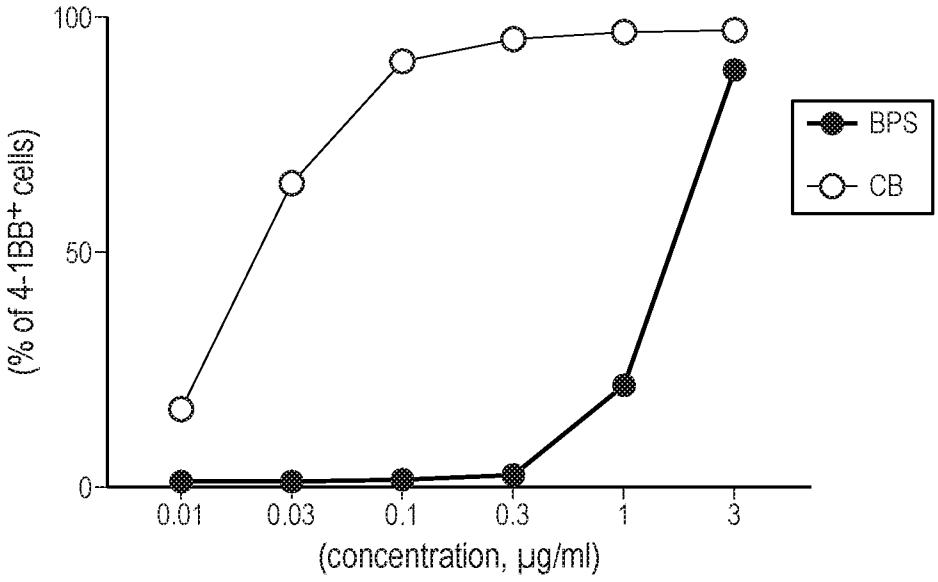

100
50
0
(% of 4-1BB+ cells)
0.01
0.03
0.1
0.3
1
3
(concentration, µg/ml)
BPS
CB

FIG. 16

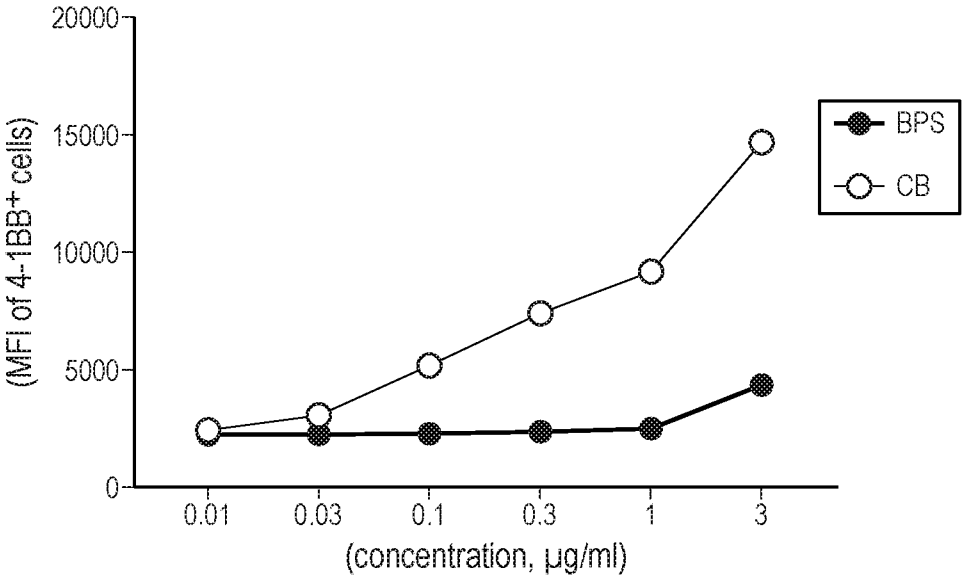

20000
15000
10000
5000
0
(MFI of 4-1BB+ cells)
0.01
0.03
0.1
0.3
1
3
(concentration, µg/ml)
BPS
CB

FIG. 17

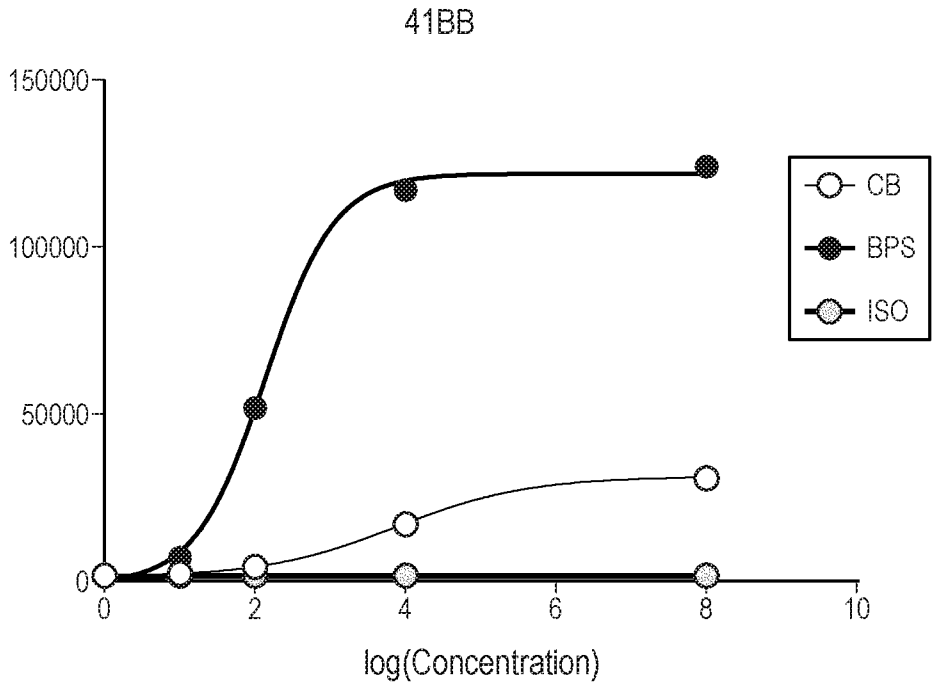

41BB
150000
100000
50000
0
0
2
4
6
8
10
log(Concentration)
CB
BPS
ISO

FIG. 18

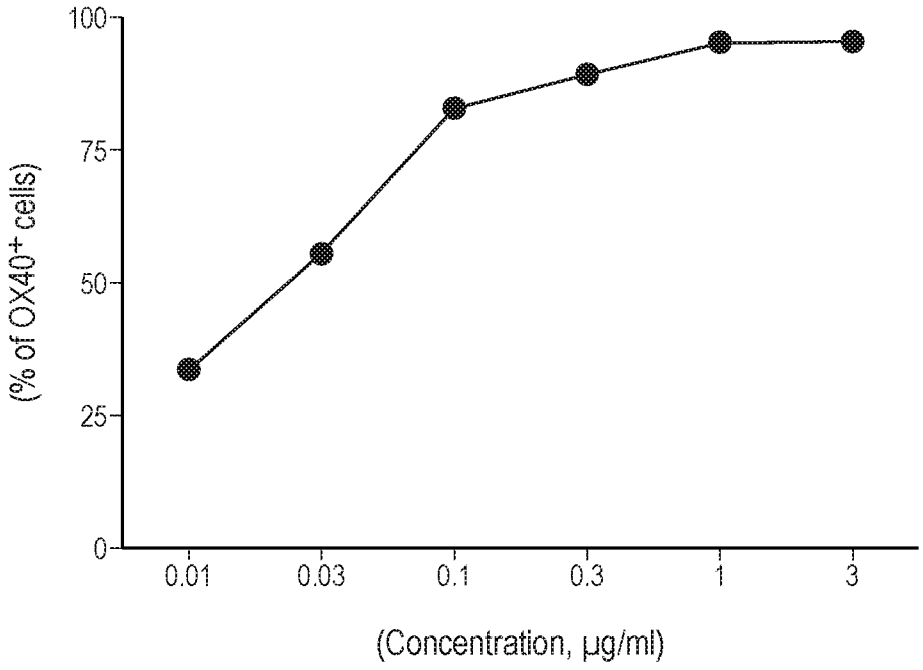

100
75
50
25
0
(% of OX40+ cells)
0.01
0.03
0.1
0.3
1
3
(Concentration, µg/ml)

FIG. 19

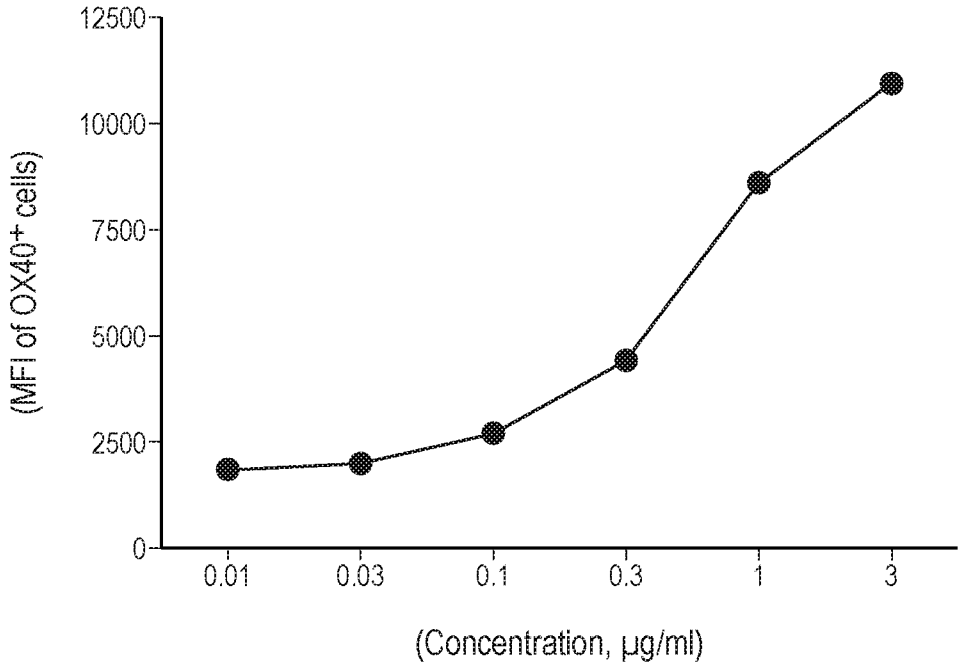

12500
10000
7500
5000
2500
0
(MFI of OX40+ cells)
0.01
0.03
0.1
0.3
1
3
(Concentration, µg/ml)

FIG. 20

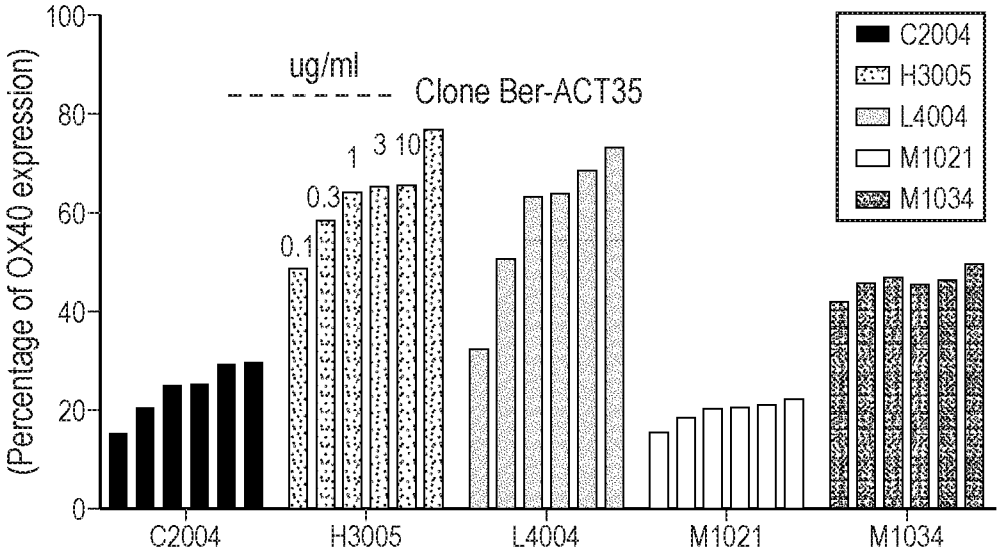

100
80
60
40
20
0
(Percentage of OX40 expression)
ug/ml
Clone Ber-ACT35
0.1
0.3
1
3
10
C2004
H3005
L4004
M1021
M1034
C2004
H3005
L4004
M1021
M1034

FIG. 21

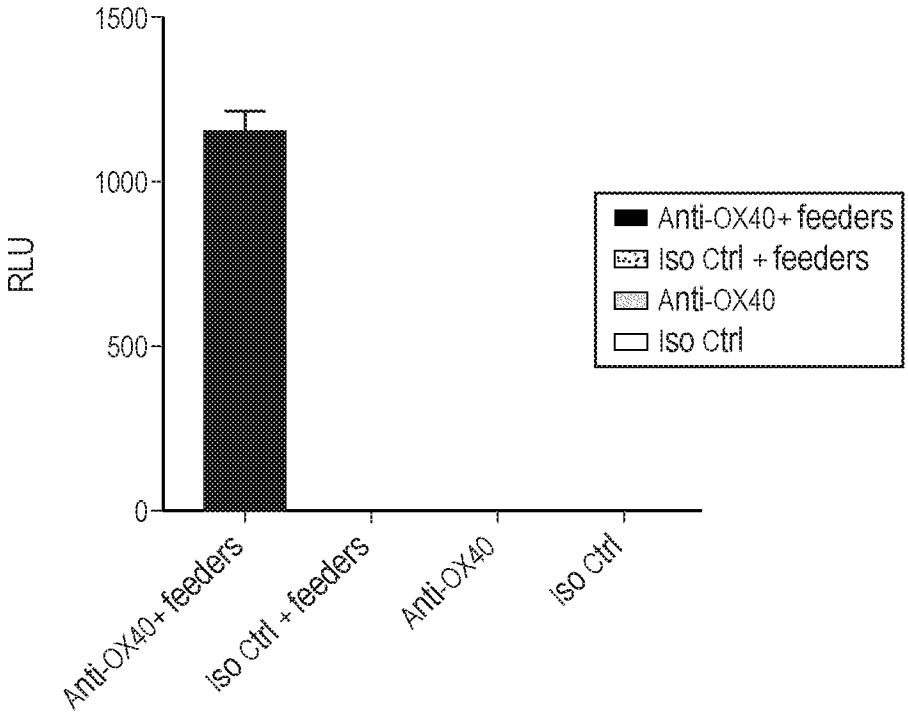

1500
1000
500
0
RLU
Anti-OX40+ feeders
Iso Ctrl + feeders
Anti-OX40
Iso Ctrl
Anti-OX40+ feeders
Iso Ctrl + feeders
Anti-OX40
Iso Ctrl

FIG. 22

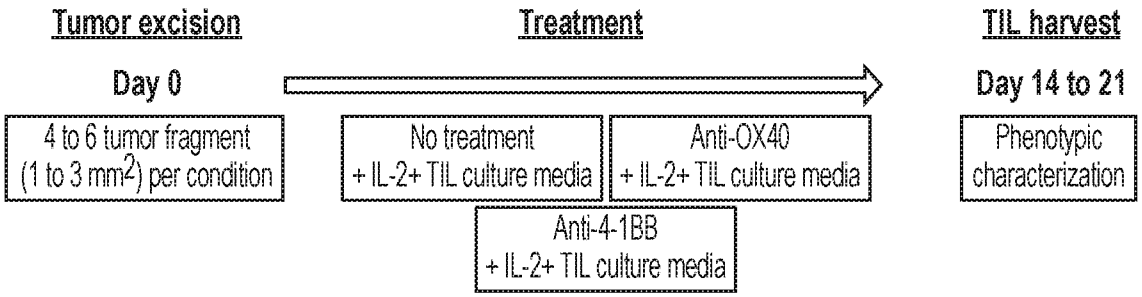

Tumor excision
Day 0
4 to 6 tumor fragment (1 to 3 mm2) per condition
Treatment
No treatment + IL-2+ TIL culture media
Anti-OX40 + IL-2+ TIL culture media
Anti-4-1BB + IL-2+ TIL culture media
TIL harvest
Day 14 to 21
Phenotypic characterization

FIG. 23

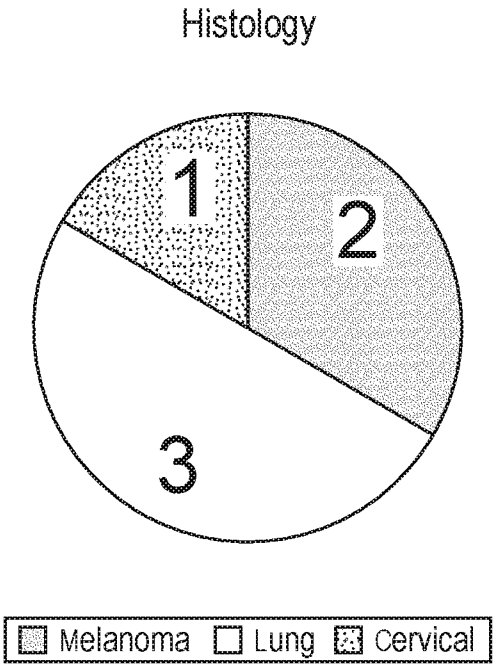


FIG. 24

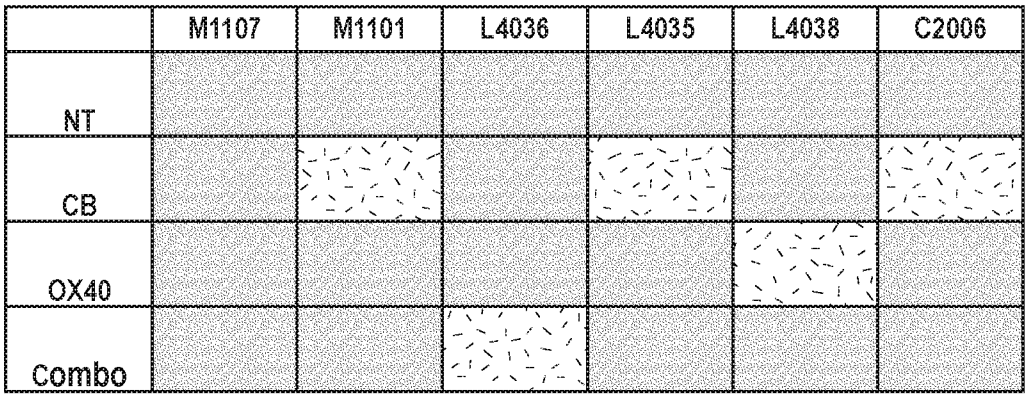


FIG. 25

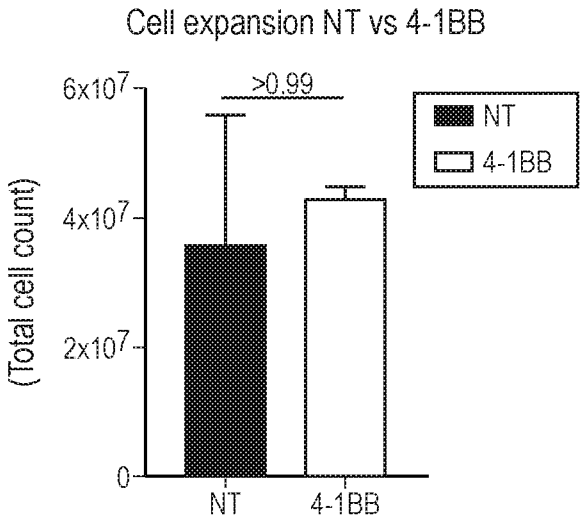

Cell expansion NT vs 4-1BB
(Total cell count)
6x10^7
4x10^7
2x10^7
0
>0.99
NT
4-1BB
NT
4-1BB

FIG. 26

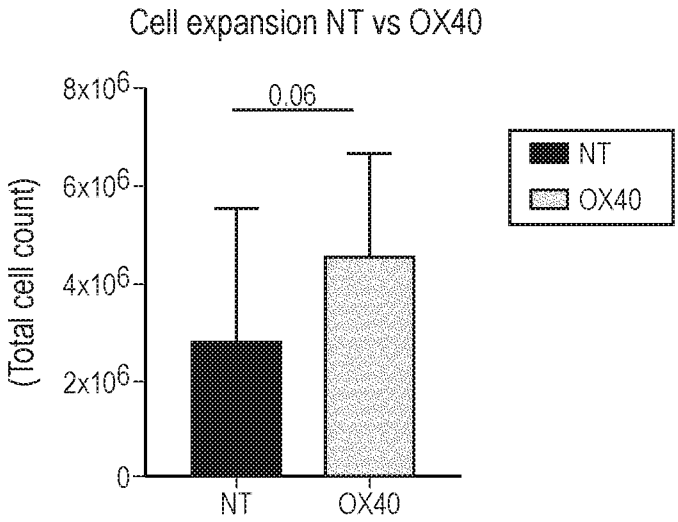

Cell expansion NT vs OX40
0.06
8x10^6
6x10^6
4x10^6
2x10^6
0
(Total cell count)
NT
OX40
NT
OX40

FIG. 27

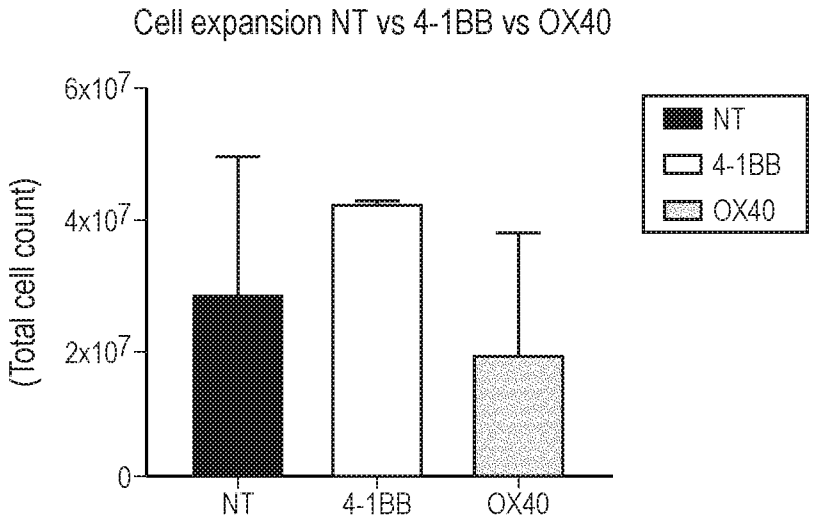

Cell expansion NT vs 4-1BB vs OX40
6x10^7
4x10^7
2x10^7
0
(Total cell count)
NT
4-1BB
OX40
NT
4-1BB
OX40

FIG. 28

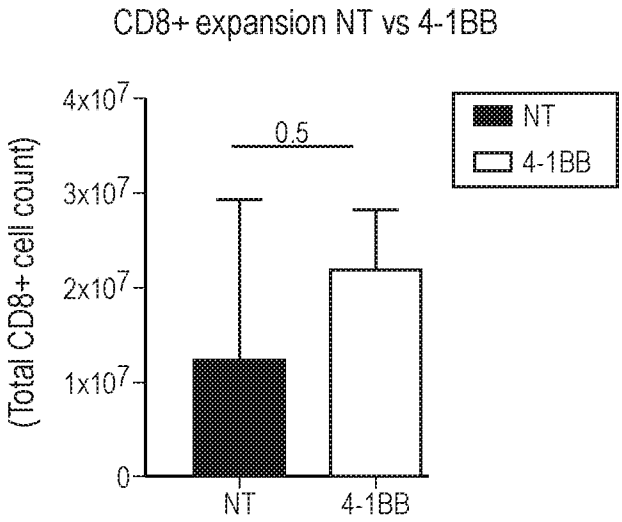

CD8+ expansion NT vs 4-1BB
(Total CD8+ cell count)
4x10^7
3x10^7
2x10^7
1x10^7
0
0.5
NT
4-1BB
NT
4-1BB

FIG. 29

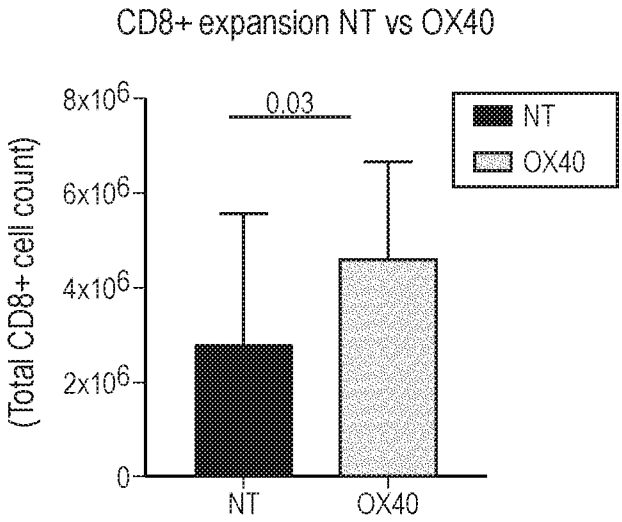

CD8+ expansion NT vs OX40
(Total CD8+ cell count)
8x10^6
6x10^6
4x10^6
2x10^6
0
0.03
NT
OX40
NT
OX40

FIG. 30

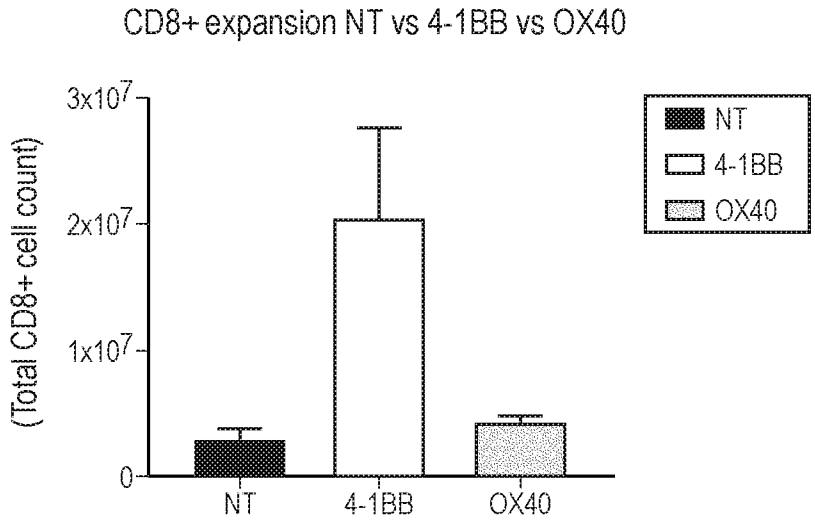

CD8+ expansion NT vs 4-1BB vs OX40
(Total CD8+ cell count)
3x10^7
2x10^7
1x10^7
0
NT
4-1BB
OX40
NT
4-1BB
OX40

FIG. 31

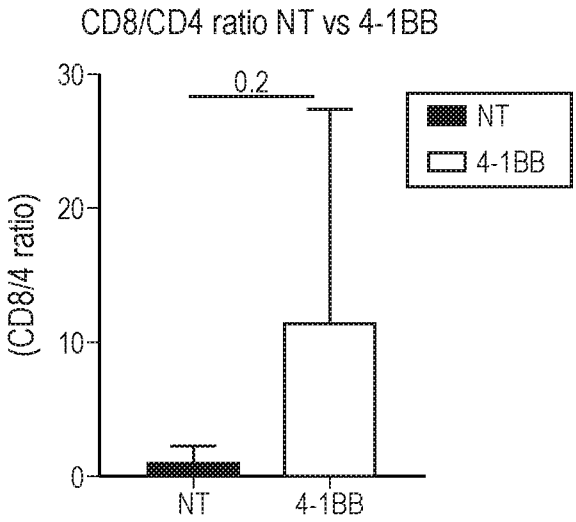


FIG. 32

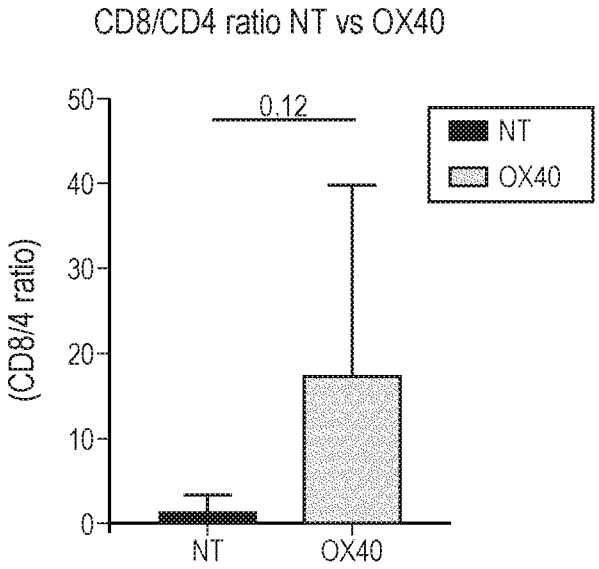

CD8/CD4 ratio NT vs OX40
(CD8/4 ratio)
50
40
30
20
10
0
0.12
NT
OX40
NT
OX40

FIG. 33

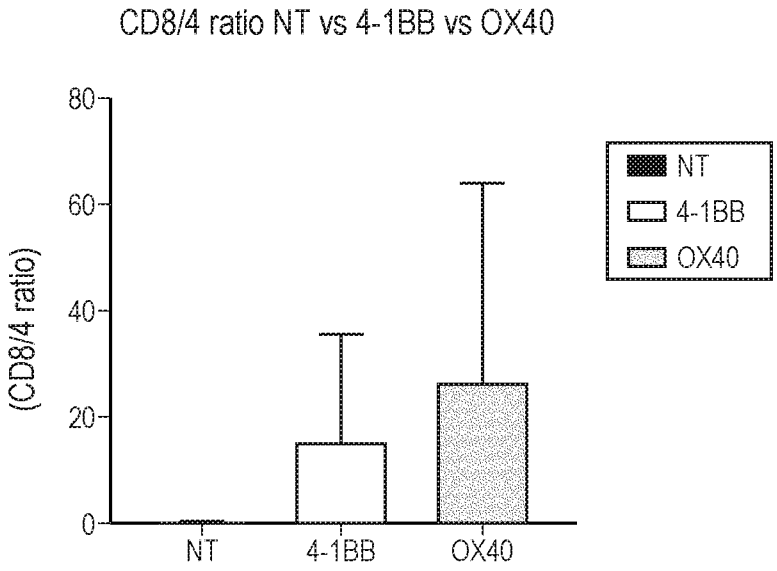

CD8/4 ratio NT vs 4-1BB vs OX40
(CD8/4 ratio)
80
60
40
20
0
NT
4-1BB
OX40
NT
4-1BB
OX40

FIG. 34


REP
D 0
No treatment
+ IL-2+ TIL culture media
Anti-4-1BB
+ IL-2+ TIL culture media
Anti-OX40
+ IL-2+ TIL culture media
TIL harvest
Day 11
Total cell count

FIG. 35

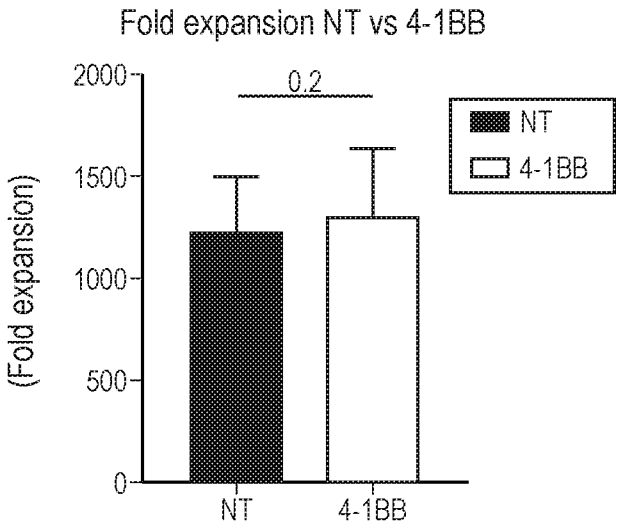

Fold expansion NT vs 4-1BB
0.2
NT
4-1BB
(Fold expansion)
2000
1500
1000
500
0
NT
4-1BB

FIG. 36

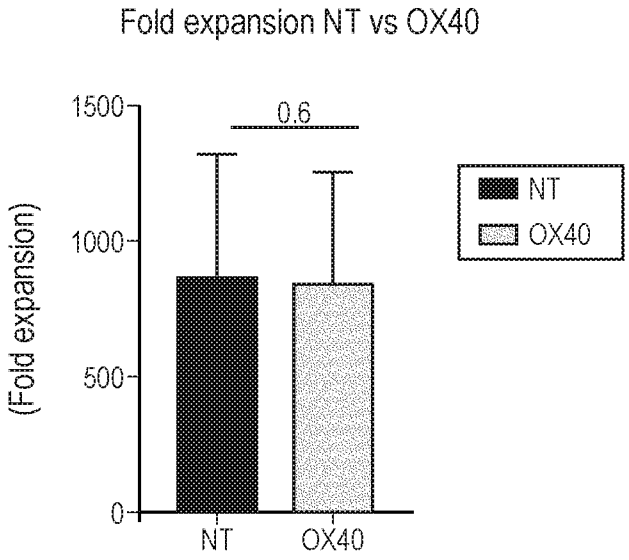

Fold expansion NT vs OX40
1500
1000
500
0
(Fold expansion)
0.6
NT
OX40
NT
OX40

FIG. 37

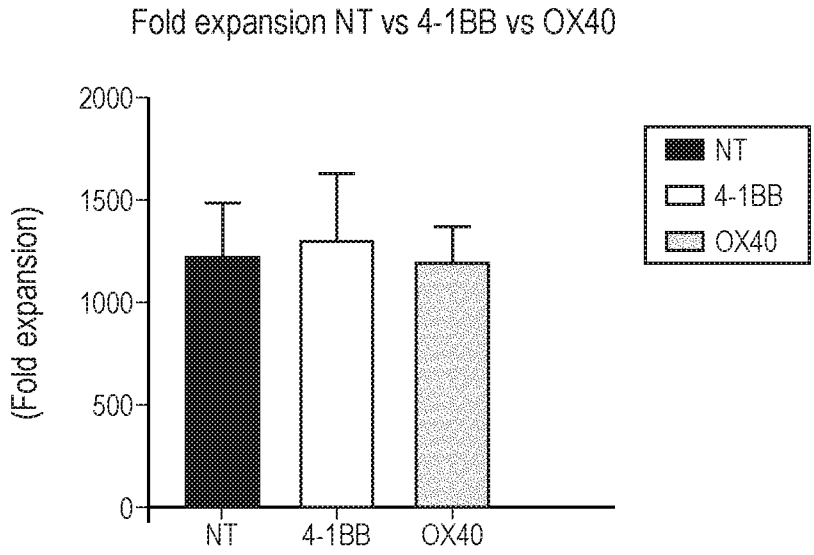

Fold expansion NT vs 4-1BB vs OX40
(Fold expansion)
2000
1500
1000
500
0
NT
4-1BB
OX40
NT
4-1BB
OX40

FIG. 38

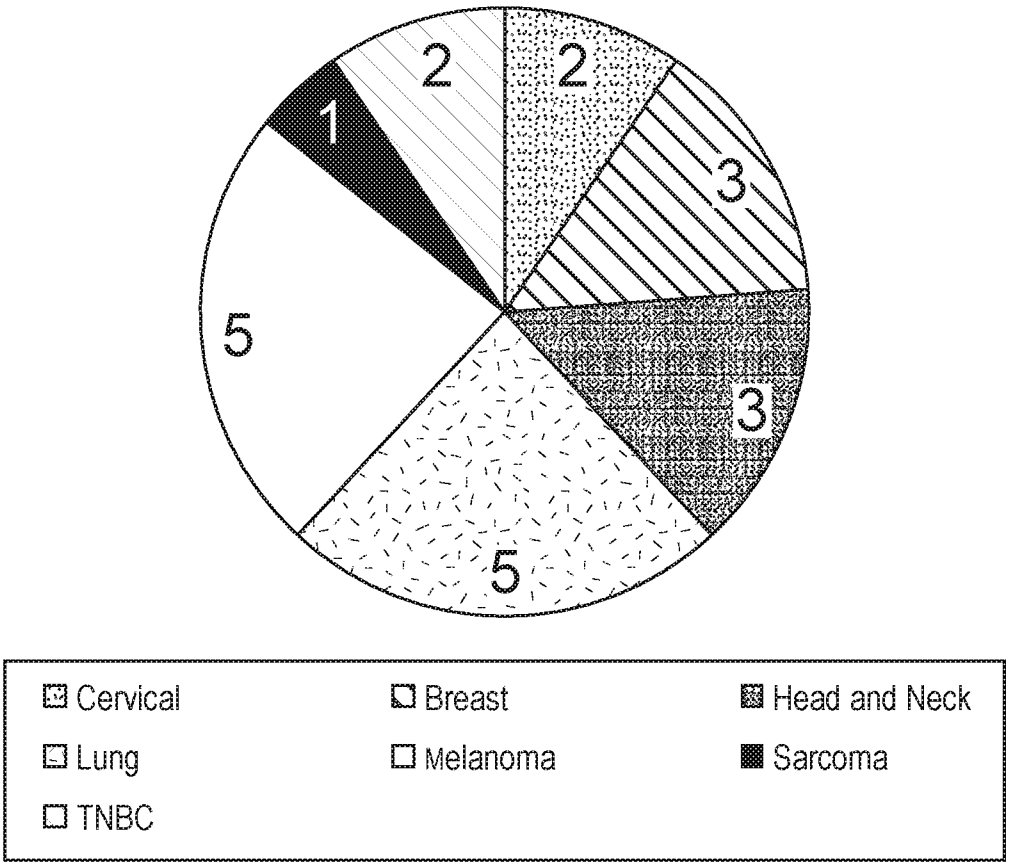

2
2
3
3
5
5
1
Cervical
Breast
Head and Neck
Lung
Melanoma
Sarcoma
TNBC

FIG. 39

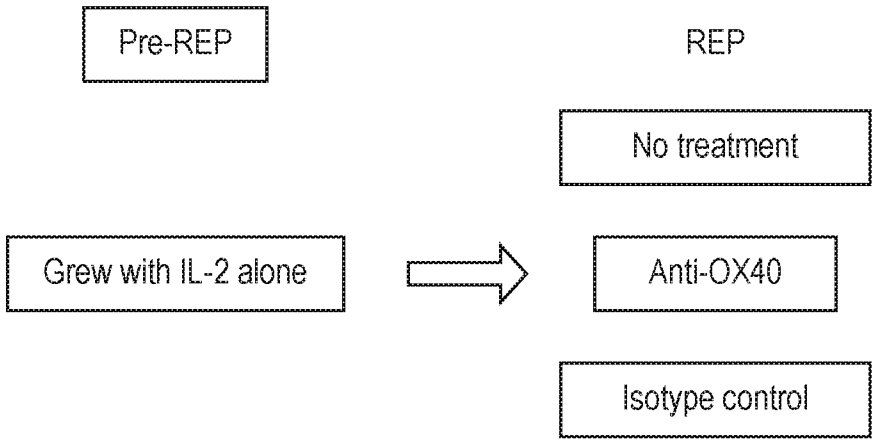

Pre-REP
REP
No treatment
Grew with IL-2 alone
Anti-OX40
Isotype control

FIG. 40

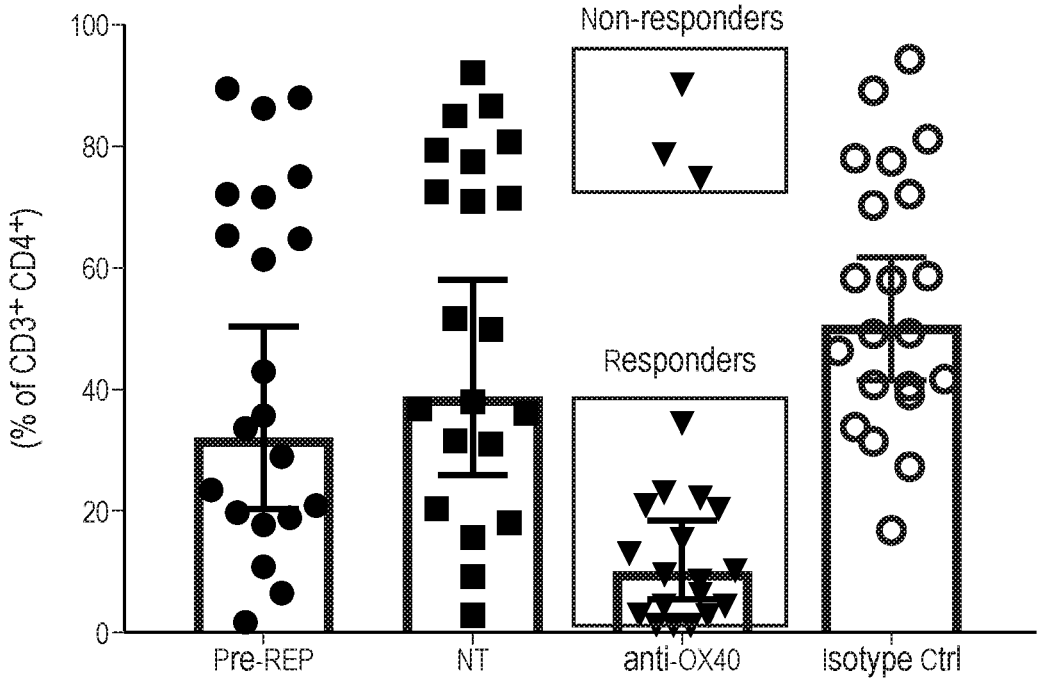

Non-responders
Responders
100
80
60
40
20
0
(% of CD3+ CD4+)
Pre-REP
NT
anti-OX40
Isotype Ctrl

FIG. 41

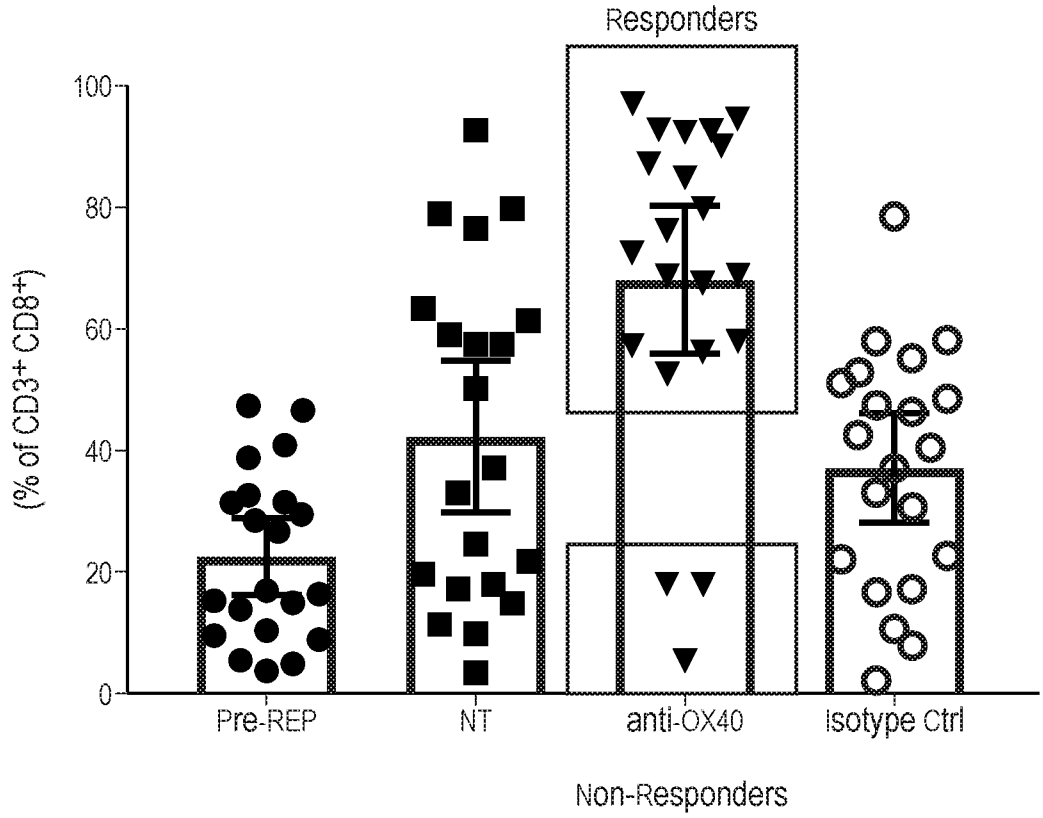

Responders
100
80
60
40
20
0
(% of CD3+ CD8+)
Pre-REP
NT
anti-OX40
Isotype Ctrl
Non-Responders

FIG. 42

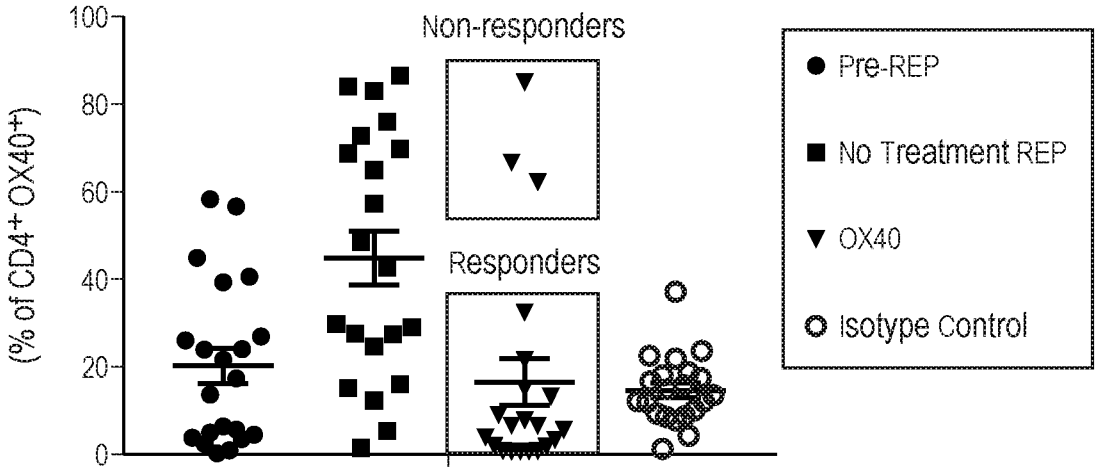

100
80
60
40
20
0
(% of CD4+ OX40+)
Non-responders
Responders
Pre-REP
No Treatment REP
OX40
Isotype Control

FIG. 43

	Anti-OX40 (ug/ml)						Isotype (ug/ml)
Responders	0.1	0.3	1	3	5	10	10
Non-responders	5	10	30				30

FIG. 44

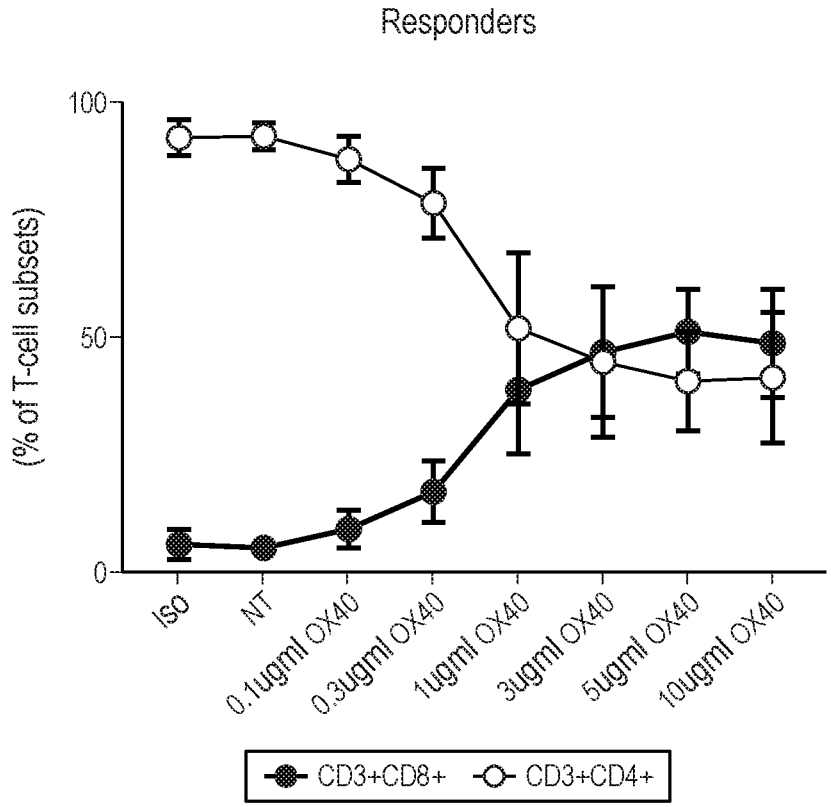

Responders
100
50
0
(% of T-cell subsets)
Iso
NT
0.1ugml OX40
0.3ugml OX40
1ugml OX40
3ugml OX40
5ugml OX40
10ugml OX40
CD3+CD8+
CD3+CD4+

FIG. 45

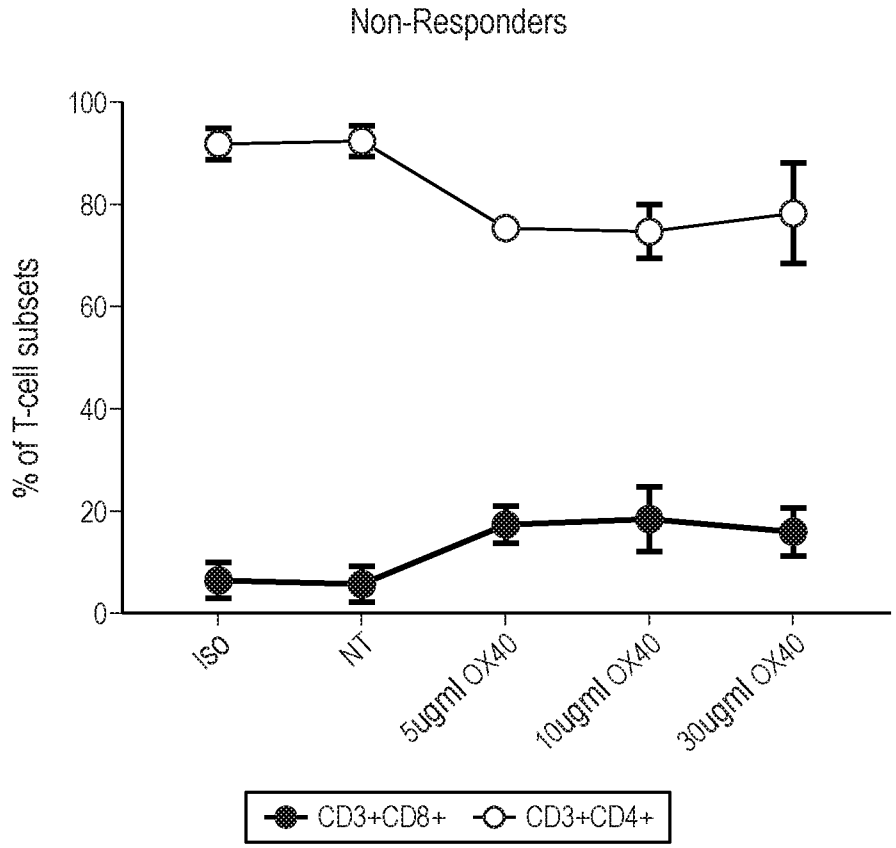

Non-Responders
% of T-cell subsets
100
80
60
40
20
0
Iso
NT
5ugml OX40
10ugml OX40
30ugml OX40
CD3+CD8+
CD3+CD4+

FIG. 46

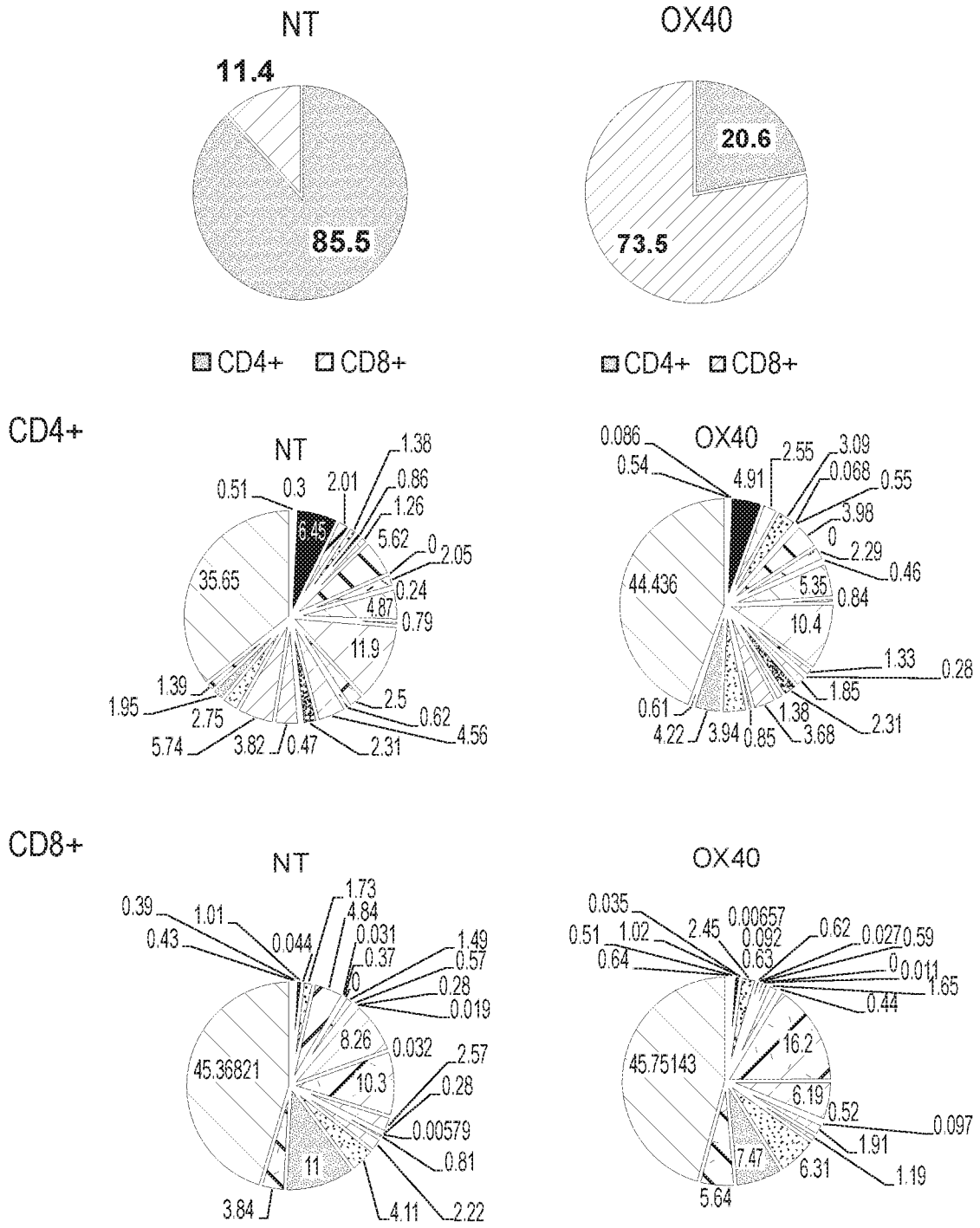

NT
11.4
85.5
OX40
20.6
73.5
CD4+ CD8+
CD4+ CD8+
CD4+
NT
1.38
0.86
1.26
5.62
0
2.05
0.24
0.79
11.9
2.5
0.62
4.56
2.31
0.47
3.82
5.74
1.95
2.75
1.39
0.51
0.3
2.01
4.87
35.65
OX40
0.086
0.54
4.91
2.55
3.09
0.068
0.55
3.98
0
2.29
0.46
0.84
5.35
10.4
1.33
0.28
1.85
2.31
1.38
3.68
0.85
3.94
4.22
0.61
44.436
CD8+
NT
1.73
4.84
0.031
0.37
0
1.49
0.57
0.28
0.019
0.032
2.57
0.28
0.00579
0.81
2.22
4.11
11
10.3
8.26
3.84
45.36821
0.39
1.01
0.43
0.044
OX40
0.035
0.51
1.02
2.45
0.00657
0.092
0.63
0.62
0.027
0.59
0.64
0
0.011
1.65
0.44
16.2
6.19
0.52
0.097
1.91
1.19
6.31
7.47
5.64
45.75143

FIG. 47

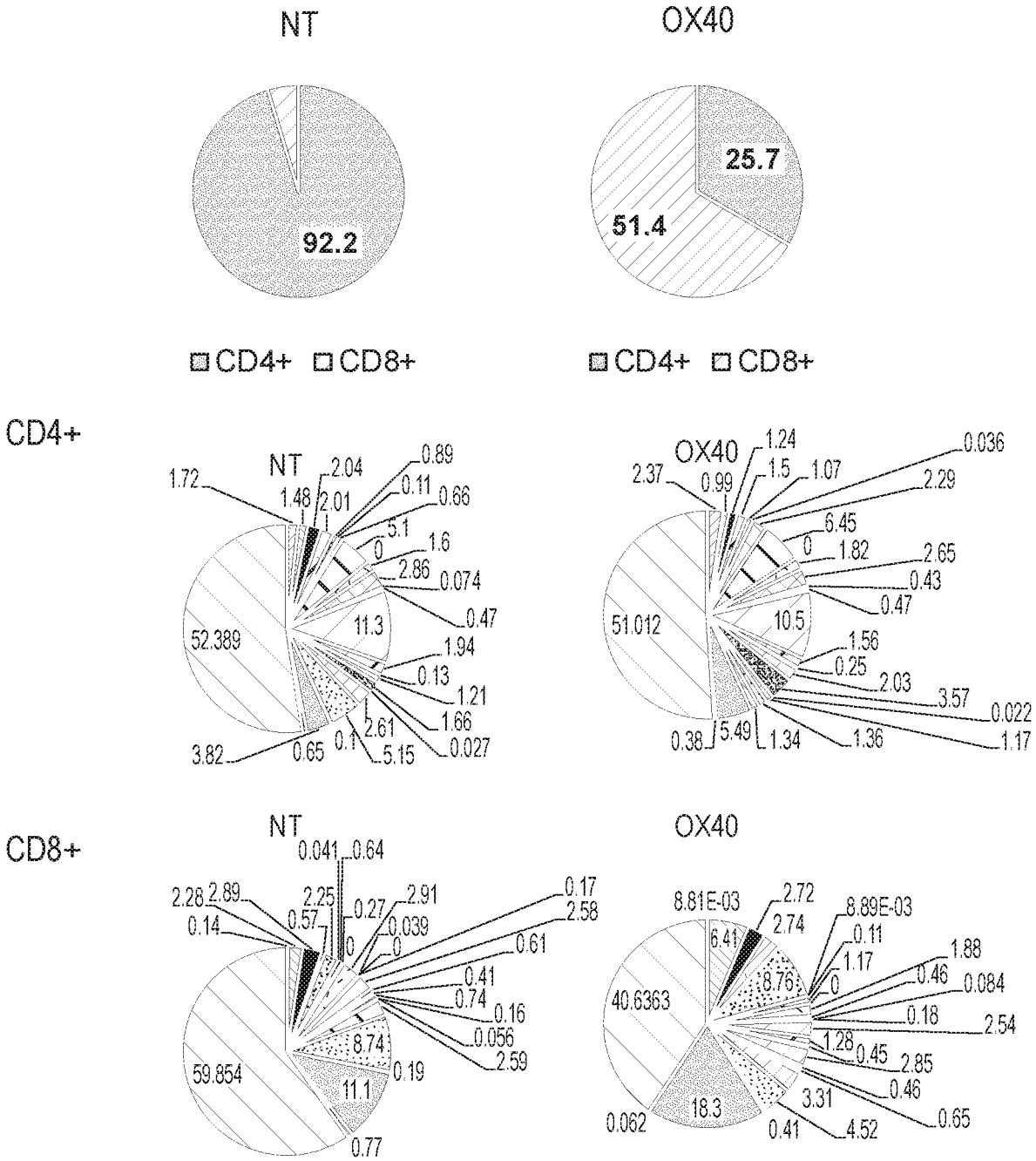

NT
OX40
92.2
25.7
51.4
CD4+ CD8+
CD4+ CD8+
CD4+
CD8+
NT
OX40
1.72
1.48
2.04
2.01
0.89
0.11
0.66
5.1
0
1.6
2.86
0.074
0.47
11.3
52.389
1.94
0.13
1.21
1.66
0.027
2.61
0.1
5.15
0.65
3.82
2.37
0.99
1.24
1.5
1.07
0.036
2.29
6.45
0
1.82
2.65
0.43
0.47
10.5
51.012
1.56
0.25
2.03
3.57
0.022
1.17
0.38
5.49
1.34
1.36
NT
OX40
0.041
0.64
2.28
2.89
0.14
0.57
2.25
0.27
2.91
0.039
0
0
0.17
2.58
0.61
0.41
0.74
0.16
0.056
2.59
8.74
0.19
59.854
11.1
0.77
8.81E-03
2.72
6.41
2.74
8.89E-03
0.11
1.17
0
8.76
1.88
0.46
0.084
0.18
2.54
40.6363
1.28
0.45
2.85
0.46
3.31
0.65
18.3
0.062
0.41
4.52

FIG. 48

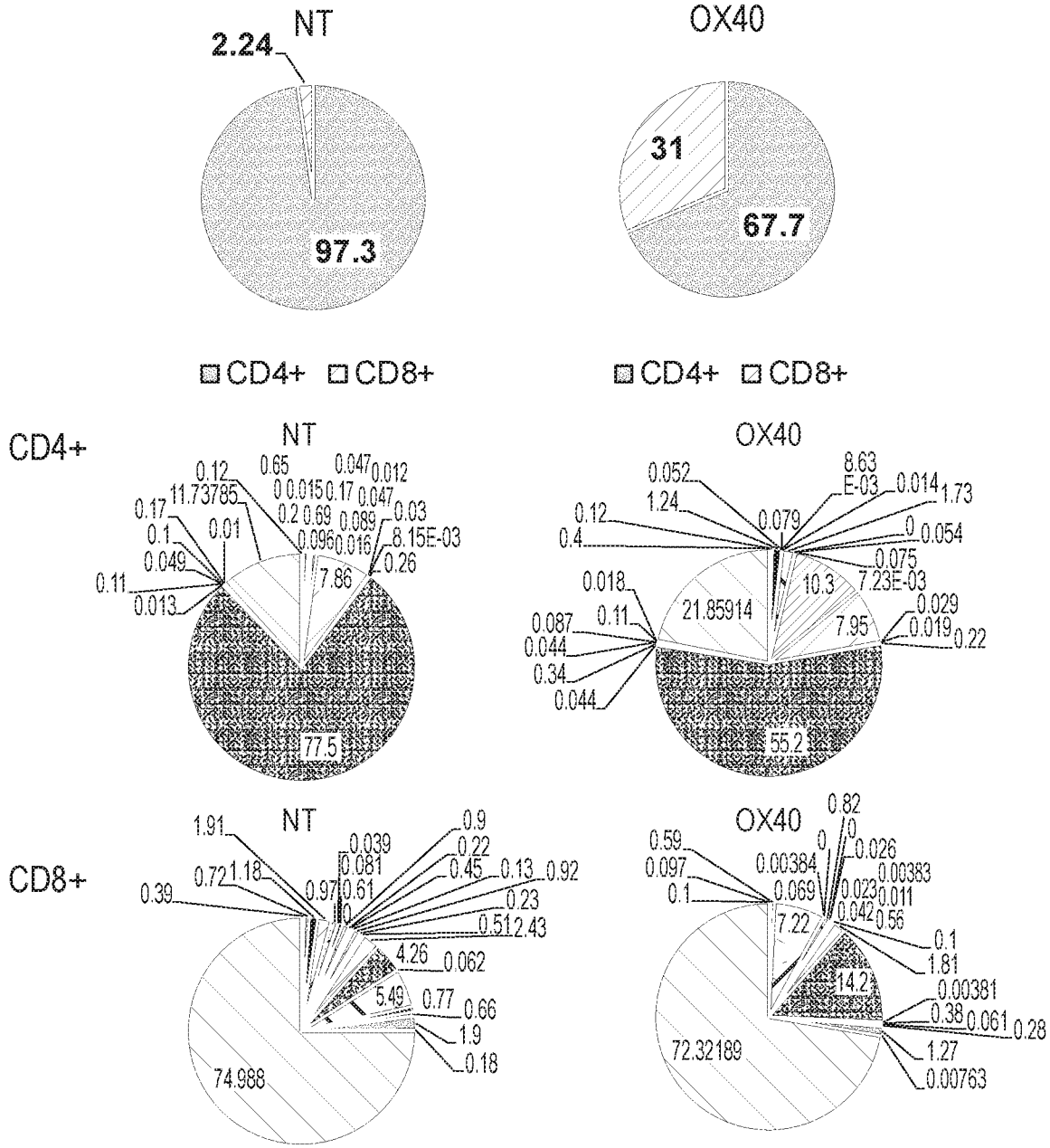

NT
2.24
97.3
OX40
31
67.7
CD4+ CD8+
CD4+ CD8+
CD4+
NT
0.12
0.65
0.047
0.012
11.73785
0.015
0.17
0.047
0.17
0.2
0.69
0.089
0.03
0.1
0.01
0.095
0.016
8.15E-03
0.049
0.26
0.11
7.86
0.013
77.5
OX40
0.052
8.63
E-03
0.014
1.73
1.24
0.12
0.079
0.4
0
0.054
0.075
0.018
7.23E-03
10.3
0.029
0.087
0.11
21.85914
7.95
0.019
0.22
0.044
0.34
0.044
55.2
CD8+
NT
1.91
0.9
0.039
0.22
0.72
1.18
0.081
0.45
0.13
0.92
0.39
0.97
0.61
0
0.23
0.51
2.43
4.26
0.062
5.49
0.77
0.66
1.9
0.18
74.988
OX40
0.82
0.59
0
0
0.026
0.00384
0.00383
0.097
0.069
0.023
0.011
0.1
0.042
0.56
7.22
0.1
1.81
14.2
0.00381
0.38
0.061
0.28
72.32189
1.27
0.00763

FIG. 49