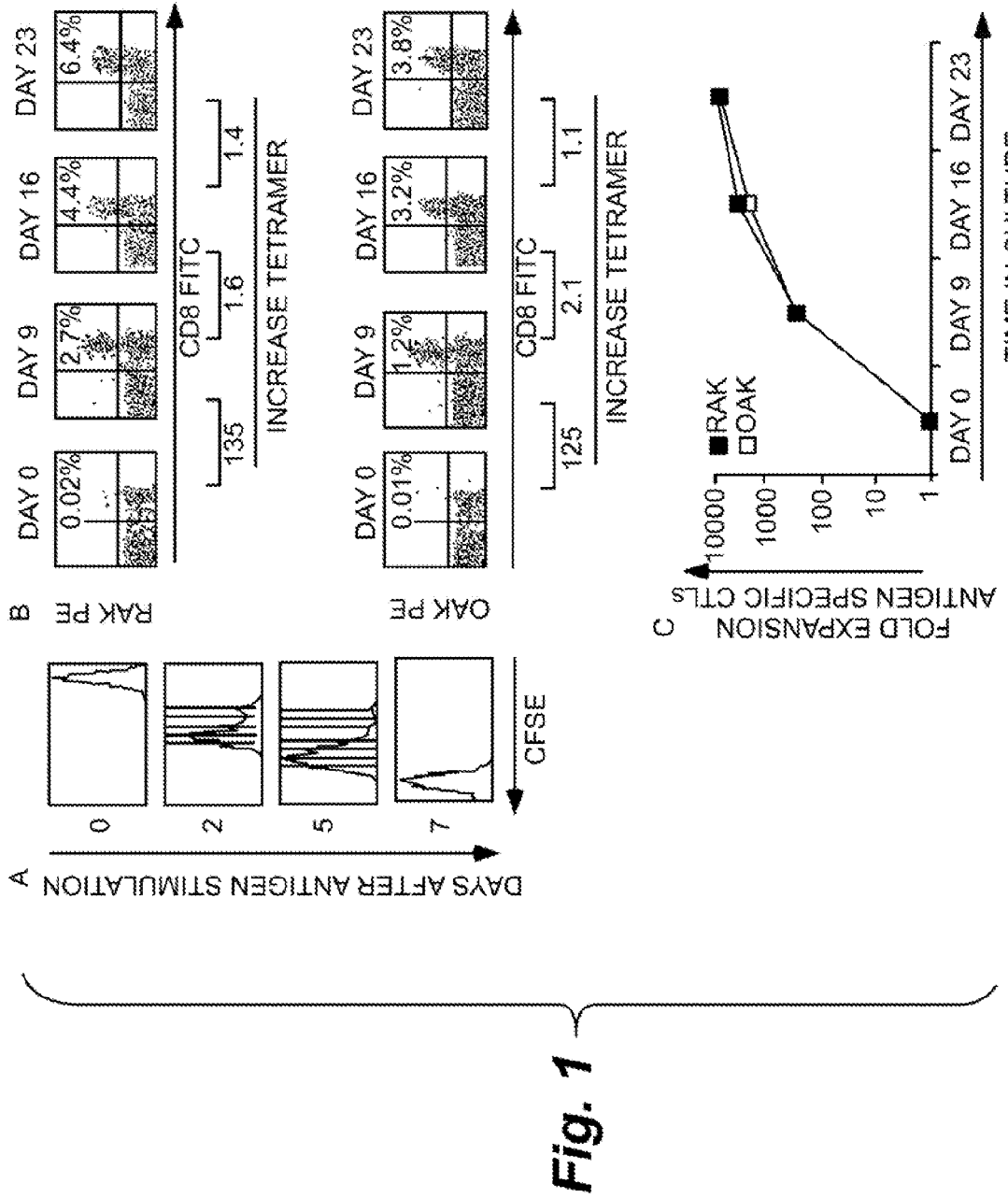

A
DAYS AFTER ANTIGEN STIMULATION
0
2
5
7
CFSE
B
RAK PE
OAK PE
DAY 0
DAY 9
DAY 16
DAY 23
0.02%
2.7%
4.4%
6.4%
0.01%
1.2%
3.2%
3.8%
CD8 FITC
135
1.6
1.4
125
2.1
1.1
INCREASE TETRAMER
C
FOLD EXPANSION
ANTIGEN SPECIFIC CTLs
10000
1000
100
10
1
RAK
OAK
DAY 0
DAY 9
DAY 16
DAY 23
TIME IN CULTURE

Fig. 1

Fig. 2

EXPECTED AND OBSERVED CTL EXPANSION

CELL DOUBLING	1	2	3	4	5	6	7
EXPECTED FOLD EXPANSION	2	4	8	16	32	64	128
OBSERVED FOLD EXPANSION (DAY 0 TO 9)							258 RANGE (48 TO 409)
OBSERVED FOLD EXPANSION (DAY 9 TO 16)							5.7 RANGE (2.2 TO 10.6)
OBSERVED FOLD EXPANSION (DAY 16 TO 23)							4.3 RANGE (4.1 TO 14.9)

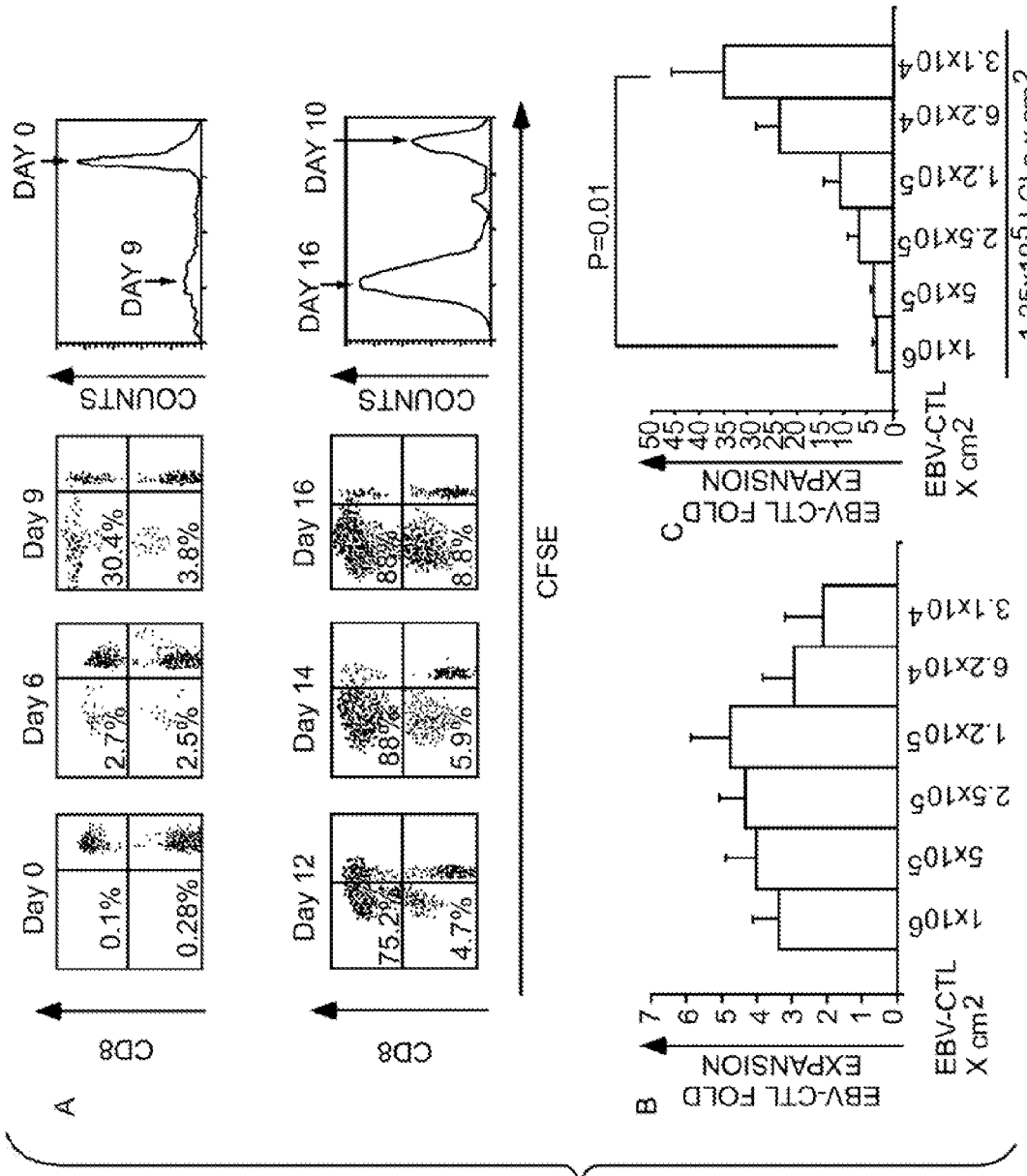
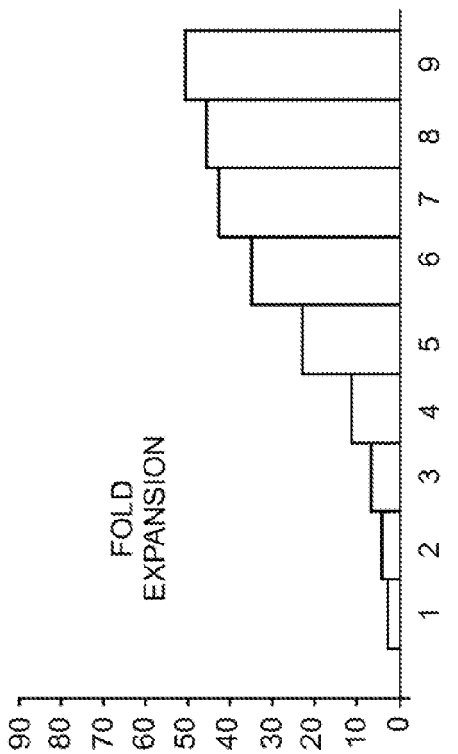

A
CD8
CFSE
COUNTS
Day 0
Day 6
Day 9
Day 12
Day 14
Day 16
DAY 0
DAY 9
DAY 10
DAY 16
0.1%
0.28%
2.7%
2.5%
30.4%
3.8%
75.2%
4.7%
88%
5.9%
88%
8.8%
B
EBV-CTL FOLD EXPANSION
EBV-CTL X cm2
1x10^6
5x10^5
2.5x10^5
1.2x10^5
6.2x10^4
3.1x10^4
C
EBV-CTL FOLD EXPANSION
EBV-CTL X cm2
P=0.01
1.25x10^5 LCLs x cm2

Fig. 3

Fig. 4

	CTL/cm2	LCL/cm2	TOTAL CTL + LCL	R:S RATIO
1	1,000,000	125,000	1,125,000	8:1
2	500,000	125,000	625,000	4:1
3	250,000	125,000	375,000	2:1
4	125,000	125,000	250,000	1:1
5	62,500	125,000	187,500	1:2
6	31,250	125,000	156,250	1:4
7	15,625	125,000	140,625	1:8
8	7,812	125,000	132,812	1:16
9	3,906	125,000	128,906	1:32



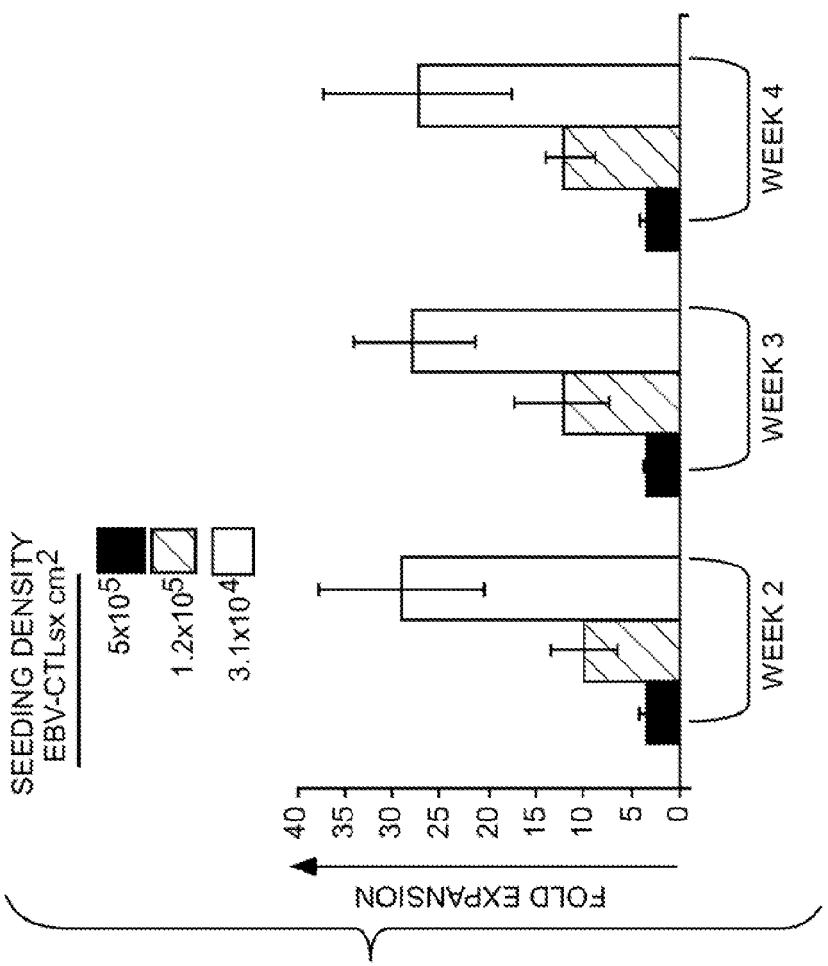

SEEDING DENSITY
EBV-CTLsx cm2
5x10^5
1.2x10^5
3.1x10^4
FOLD EXPANSION
0
5
10
15
20
25
30
35
40
WEEK 2
WEEK 3
WEEK 4

Fig. 5

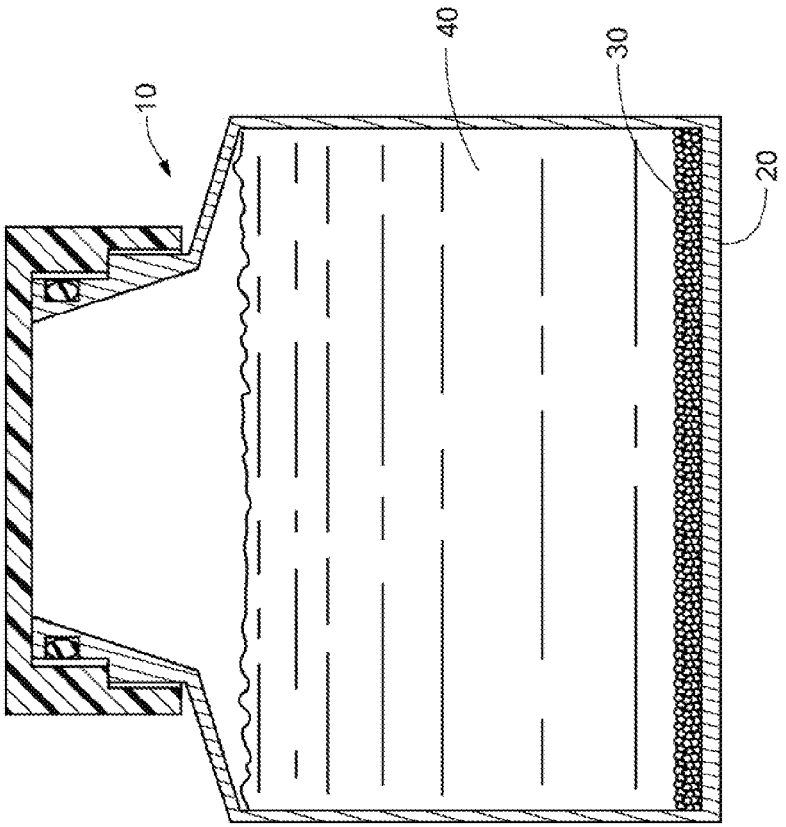


Fig. 6

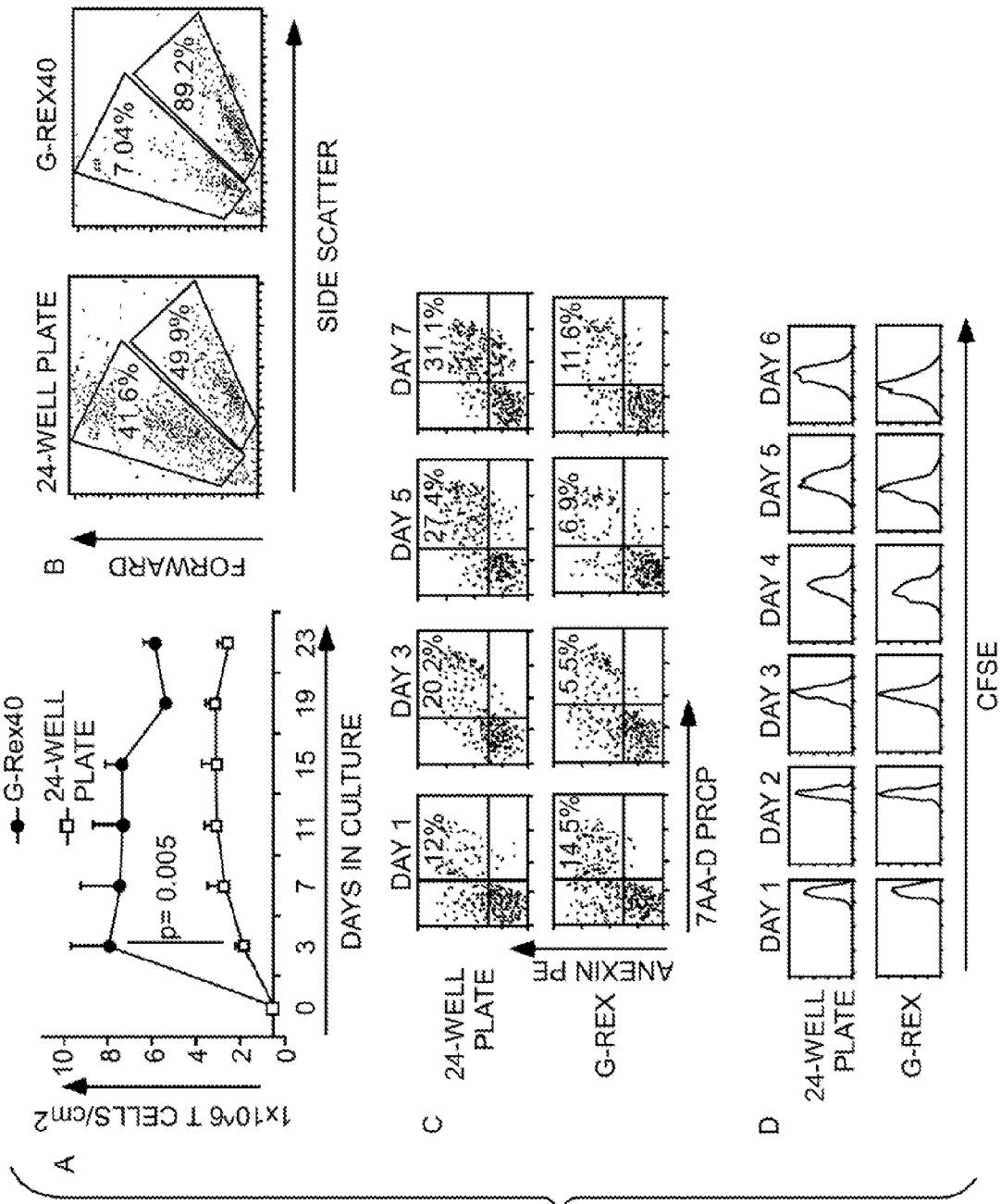

A
G-Rex40
24-WELL PLATE
p= 0.005
1x10^6 T CELLS/cm^2
DAYS IN CULTURE
B
FORWARD
24-WELL PLATE
41.6%
49.9%
G-REX40
7.04%
89.2%
SIDE SCATTER
C
24-WELL PLATE
G-REX
ANEXIN PE
7AA-D PRCP
DAY 1
DAY 3
DAY 5
DAY 7
12%
20.2%
27.4%
31.1%
14.5%
5.5%
6.9%
11.6%
D
24-WELL PLATE
G-REX
DAY 1
DAY 2
DAY 3
DAY 4
DAY 5
DAY 6
CFSE

Fig. 7

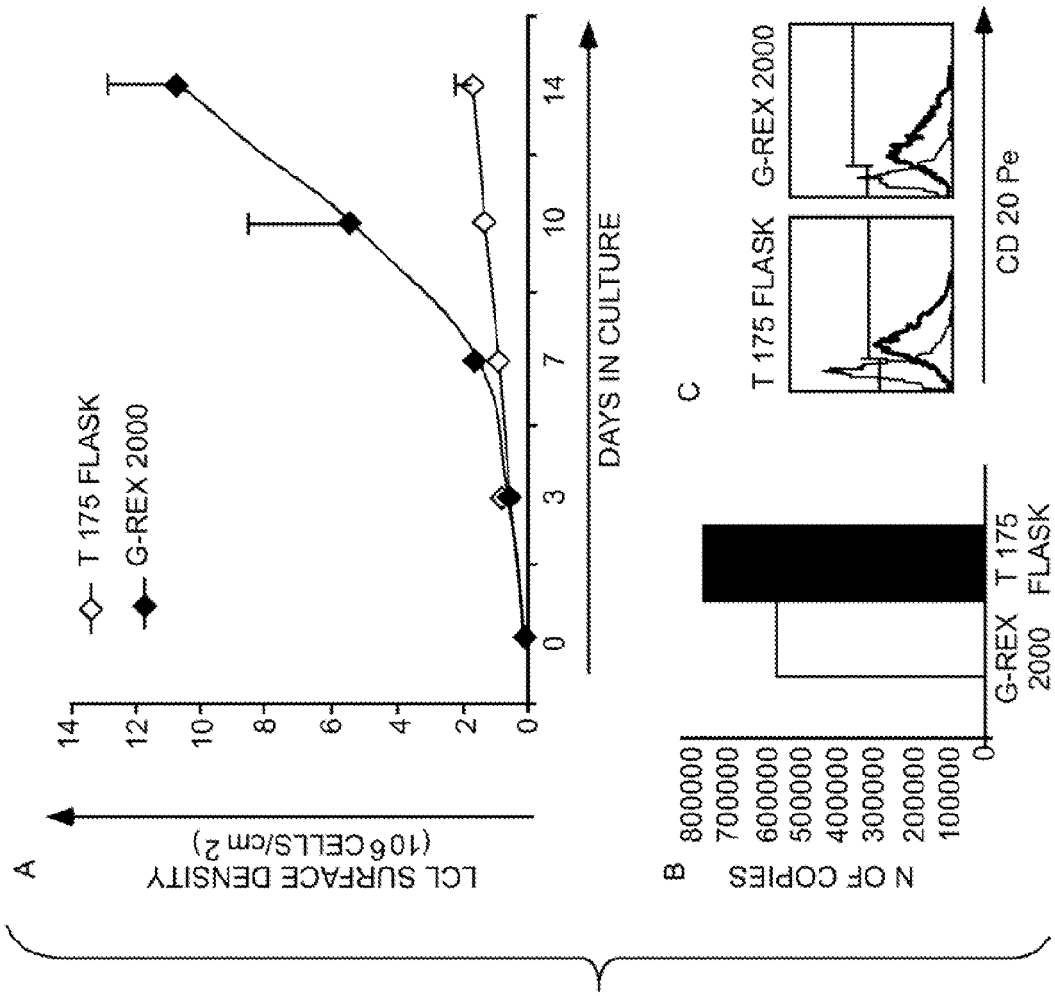
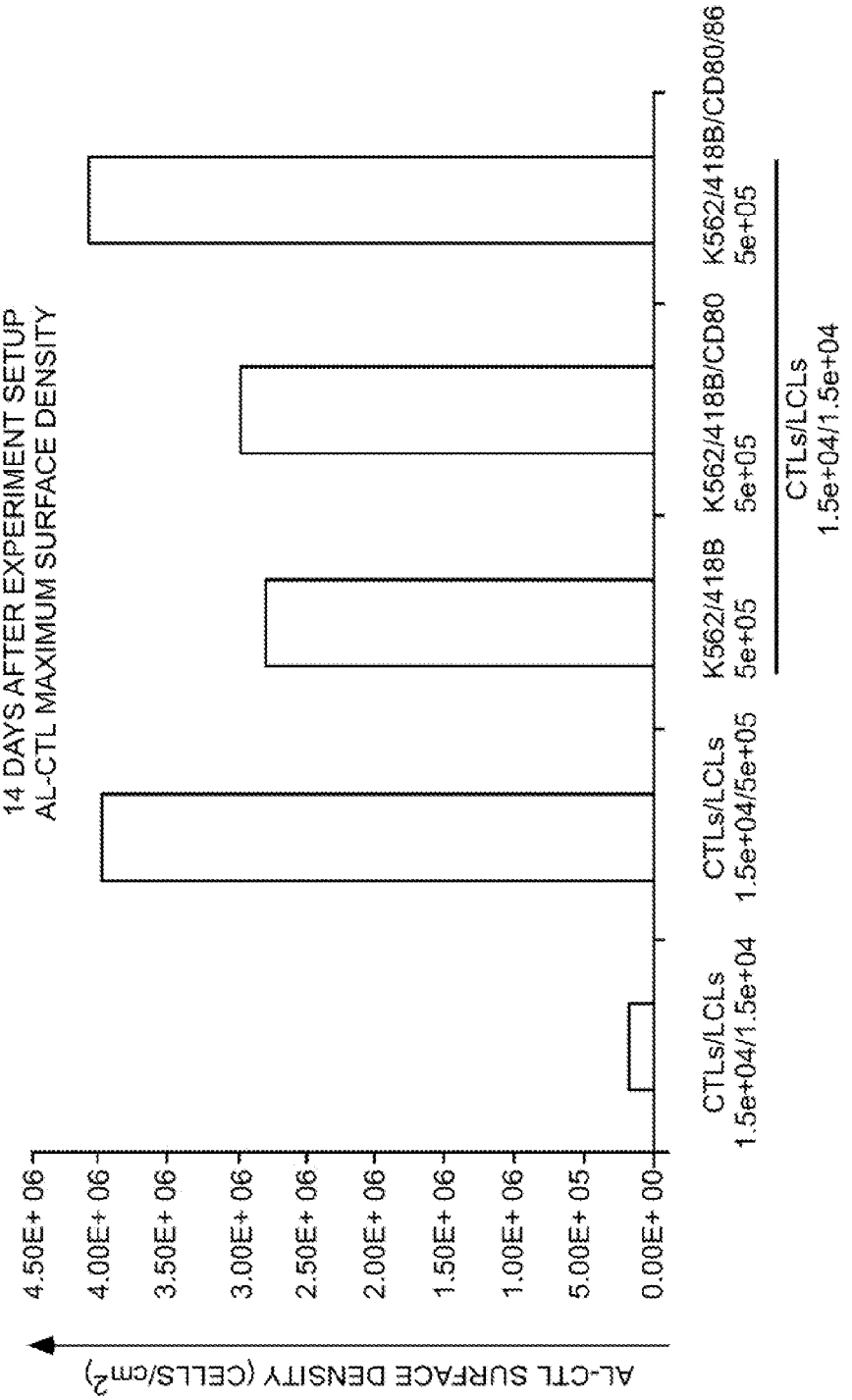

A
LCL SURFACE DENSITY
(10⁶ CELLS/cm²)
T 175 FLASK
G-REX 2000
DAYS IN CULTURE
B
N OF COPIES
G-REX 2000
T 175 FLASK
C
T 175 FLASK
G-REX 2000
CD 20 Pe

Fig. 8

Fig. 9


14 DAYS AFTER EXPERIMENT SETUP
AL-CTL MAXIMUM SURFACE DENSITY
AL-CTL SURFACE DENSITY (CELLS/cm2)
4.50E+ 06
4.00E+ 06
3.50E+ 06
3.00E+ 06
2.50E+ 06
2.00E+ 06
1.50E+ 06
1.00E+ 06
5.00E+ 05
0.00E+ 00
CTLs/LCLs
1.5e+04/1.5e+04
CTLs/LCLs
1.5e+04/5e+05
K562/4 18B
5e+05
K562/4 18B/CD80
5e+05
K562/4 18B/CD80/86
5e+05
CTLs/LCLs
1.5e+04/1.5e+04

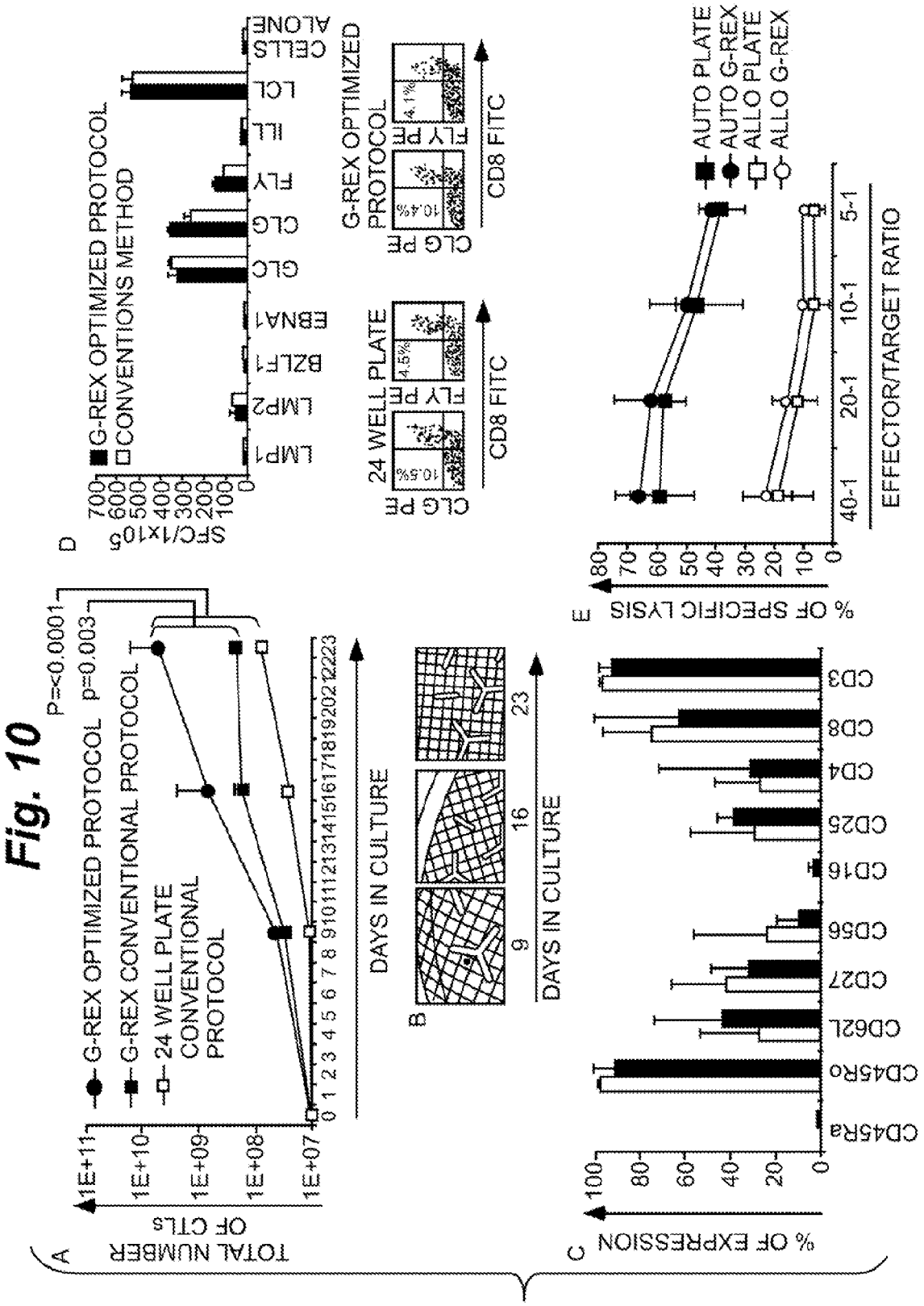

Fig. 10
A
TOTAL NUMBER OF CTLs
P=<0.0001
p=0.003
G-REX OPTIMIZED PROTOCOL
G-REX CONVENTIONAL PROTOCOL
24 WELL PLATE CONVENTIONAL PROTOCOL
1E+11
1E+10
1E+09
1E+08
1E+07
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
DAYS IN CULTURE
B
9
16
23
DAYS IN CULTURE
C
% OF EXPRESSION
100
80
60
40
20
0
CD45Ra
CD45Ro
CD62L
CD27
CD56
CD16
CD25
CD4
CD8
CD3
D
SFC/1x10^5
700
600
500
400
300
200
100
0
G-REX OPTIMIZED PROTOCOL
CONVENTIONS METHOD
LMP1
LMP2
BZLF1
EBNA1
GLC
CLG
FLY
ILL
LCL
CELLS ALONE
24 WELL PLATE
CLG PE
10.5%
FLY PE
4.5%
CD8 FITC
G-REX OPTIMIZED PROTOCOL
CLG PE
10.4%
FLY PE
4.1%
CD8 FITC
E
% OF SPECIFIC LYSIS
80
70
60
50
40
30
20
10
0
AUTO PLATE
AUTO G-REX
ALLO PLATE
ALLO G-REX
40-1
20-1
10-1
5-1
EFFECTOR/TARGET RATIO

Fig. 11

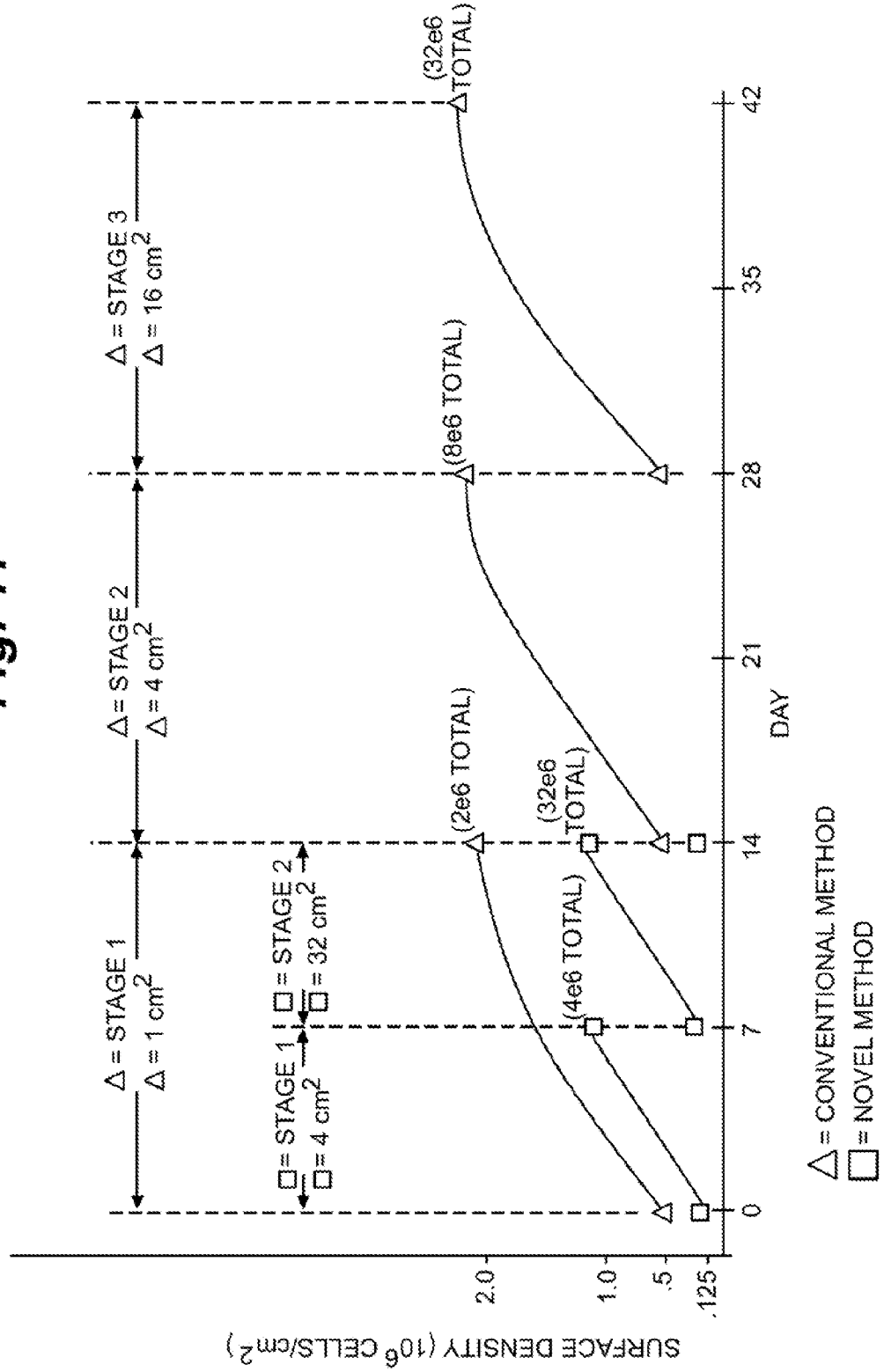

SURFACE DENSITY (10^6 CELLS/cm^2)
2.0
1.0
.5
.125
0
7
14
21
28
35
42
DAY
Δ = STAGE 1
Δ = 1 cm^2
Δ = STAGE 2
Δ = 4 cm^2
Δ = STAGE 3
Δ = 16 cm^2
□ = STAGE 1
□ = 4 cm^2
□ = STAGE 2
□ = 32 cm^2
(2e6 TOTAL)
(4e6 TOTAL)
(8e6 TOTAL)
(32e6 TOTAL)
(32e6 TOTAL)
Δ = CONVENTIONAL METHOD
□ = NOVEL METHOD

Fig. 12

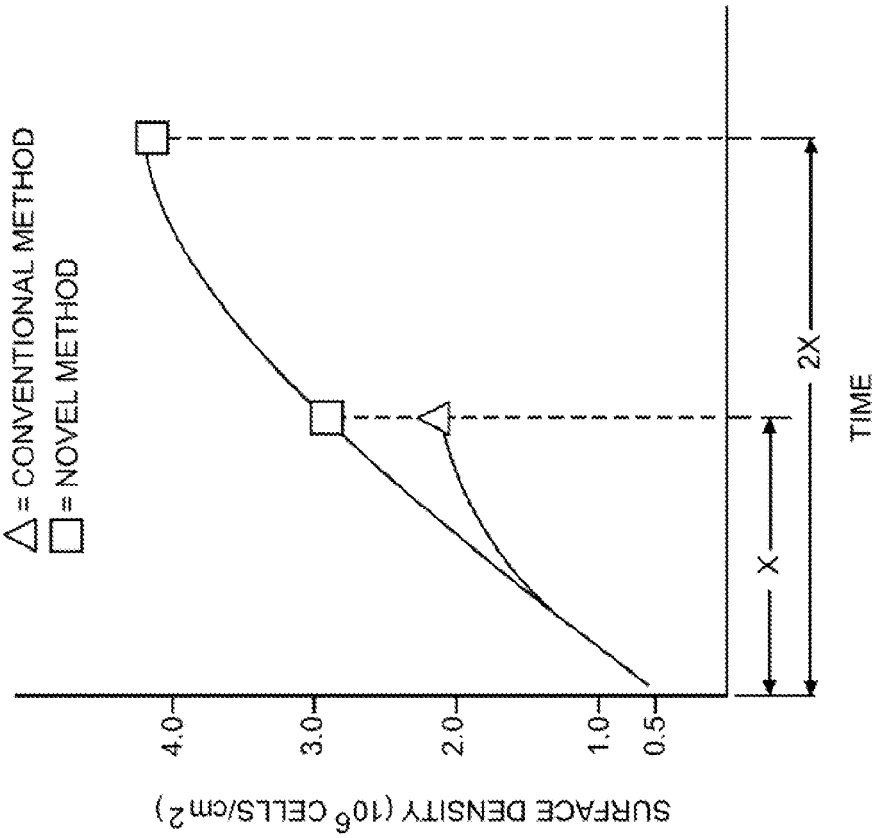

△ = CONVENTIONAL METHOD
□ = NOVEL METHOD
SURFACE DENSITY (10^6 CELLS/cm^2)
4.0
3.0
2.0
1.0
0.5
X
2X
TIME

Fig. 13

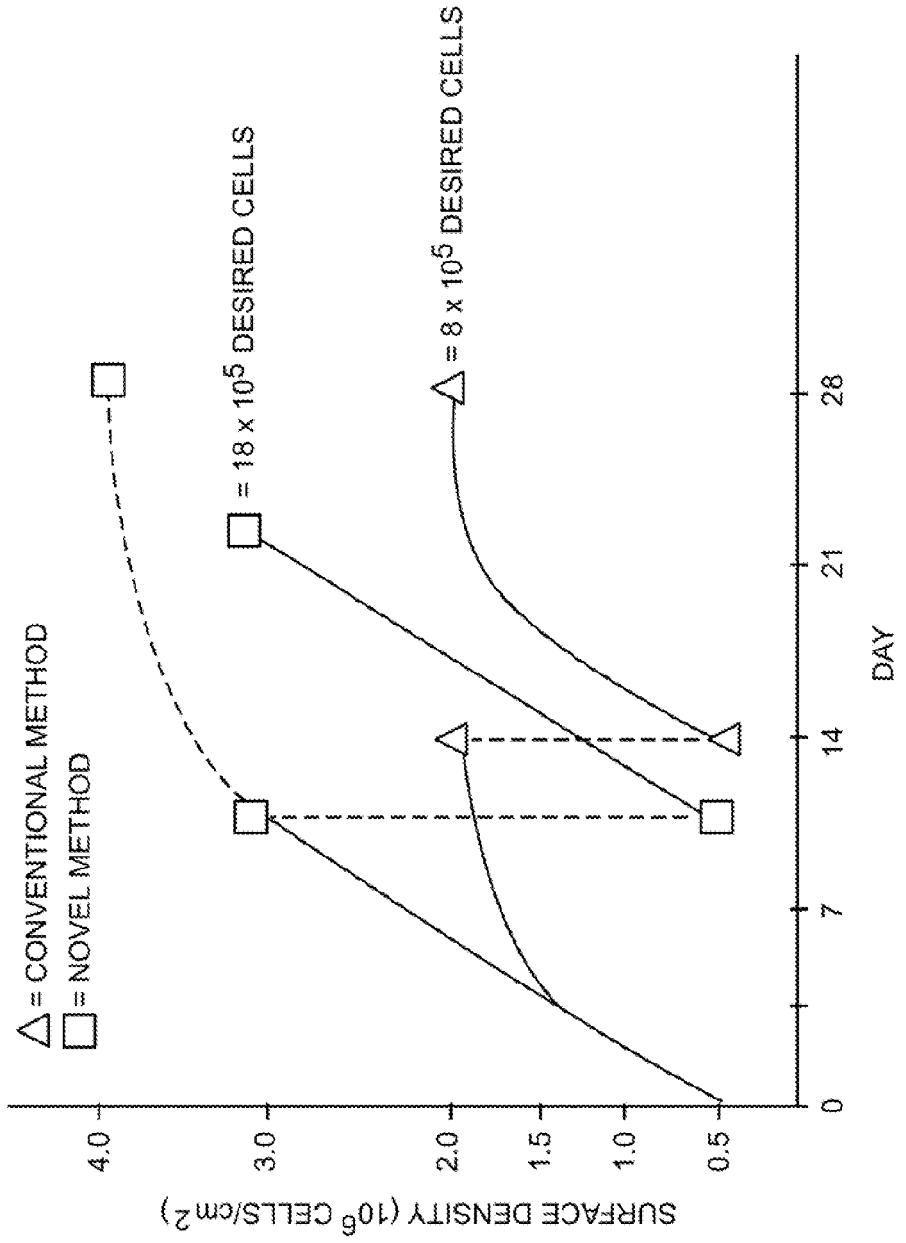

△ = CONVENTIONAL METHOD
□ = NOVEL METHOD
□ = 18 x 10^5 DESIRED CELLS
△ = 8 x 10^5 DESIRED CELLS
SURFACE DENSITY (10^6 CELLS/cm^2)
4.0
3.0
2.0
1.5
1.0
0.5
0
7
14
21
28
DAY

Fig. 14

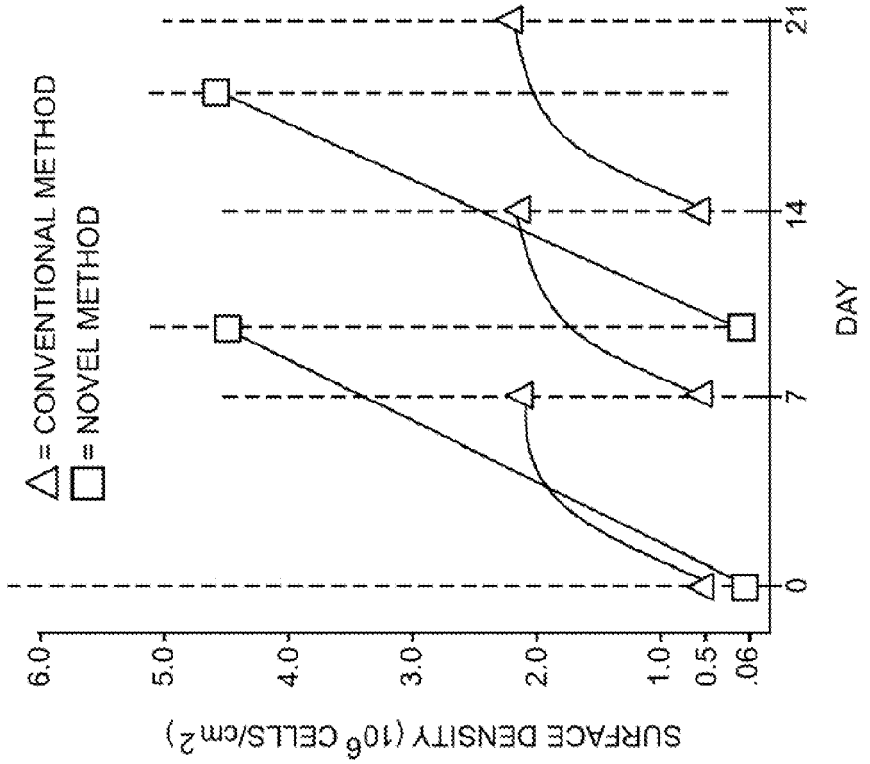

△ = CONVENTIONAL METHOD
□ = NOVEL METHOD
SURFACE DENSITY (10^6 CELLS/cm^2)
6.0
5.0
4.0
3.0
2.0
1.0
0.5
.06
0
7
14
21
DAY

Fig. 15

	CONVENTIONAL				
CYCLE	1	2	3	4	5
DAYS PER CYCLE	7	7	7	7	7
CUMULATIVE DAYS	7	14	21	28	35
SURFACE AREA (CM^2)	4	16	64	256	1024
STARTING SURFACE DENSITY ($CELLS/CM^2$)	1	1	1	1	1
FINAL SURFACE DENSITY ($CELLS/CM^2$)	2	2	2	2	2
CELLS PRODUCED ($x10^6$)	8	32	128	512	2048
	NOVEL				
CYCLE	1	2	3		
DAYS PER CYCLE (APPROX.)	9.5	9.5	15.0		
CUMULATIVE DAYS	9.5	19.0	34.0		
SURFACE AREA (CM^2)	8	625	46875		
STARTING SURFACE DENSITY ($CELLS/CM^2$)	0.06	0.06	0.06		
FINAL SURFACE DENSITY ($CELLS/CM^2$)	4.50	4.50	4.50		
CELLS PRODUCED ($x10^6$)	38	2813	210938		

Fig. 16

	CONVENTIONAL				
CYCLE	1	2	3	4	5
DAYS PER CYCLE	7	7	7	7	7
CUMULATIVE DAYS	7	14	21	28	35
SURFACE AREA (CM^2)	4	16	64	256	1024
STARTING SURFACE DENSITY ($CELLS/CM^2$)	1	1	1	1	1
FINAL SURFACE DENSITY ($CELLS/CM^2$)	2	2	2	2	2
CELLS PRODUCED ($x10^6$)	8	32	128	512	2048
	NOVEL				
CYCLE	1	2	3		
DAYS PER CYCLE (APPROX.)	9.5	9.5	15.0		
CUMULATIVE DAYS	9.5	19.0	34.0		
SURFACE AREA (CM^2)	8	625	4018		
STARTING SURFACE DENSITY ($CELLS/CM^2$)	0.06	0.06	0.70		
FINAL SURFACE DENSITY ($CELLS/CM^2$)	4.50	4.50	7.50		
CELLS PRODUCED ($x10^6$)	38	2813	30134		

Figure 17

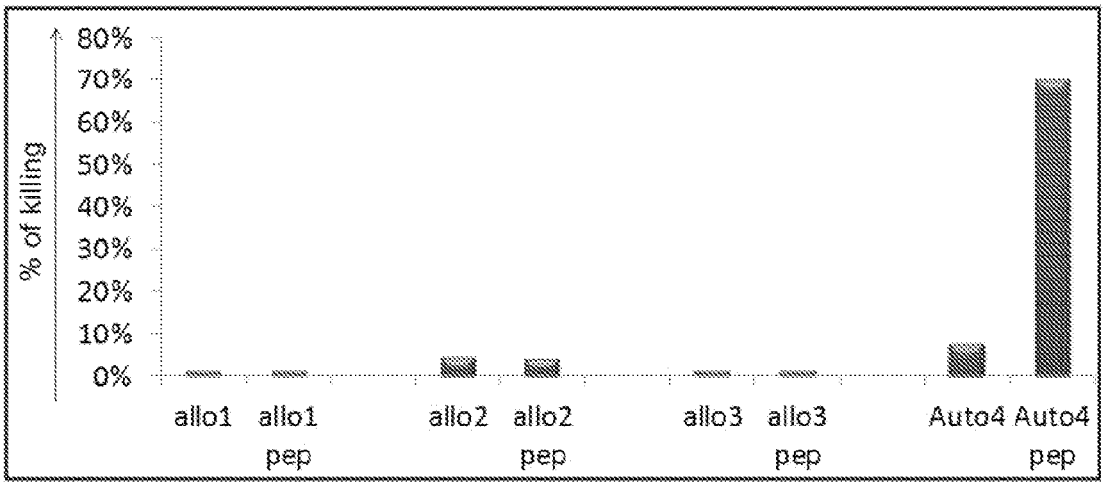
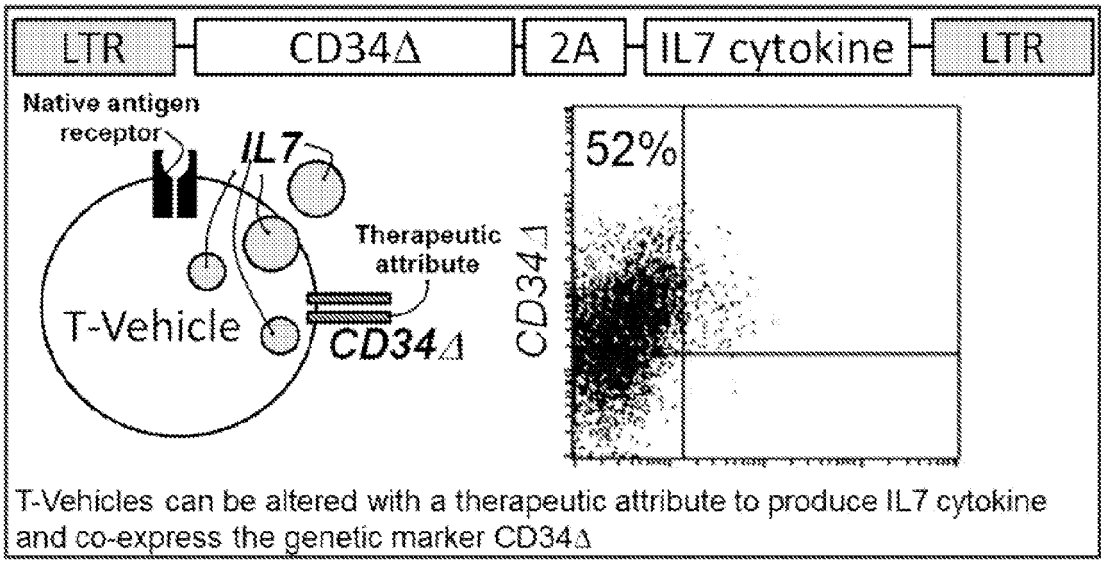


Figure 18



T-Vehicles can be altered with a therapeutic attribute to produce IL7 cytokine and co-express the genetic marker CD34Δ

Figure 19A

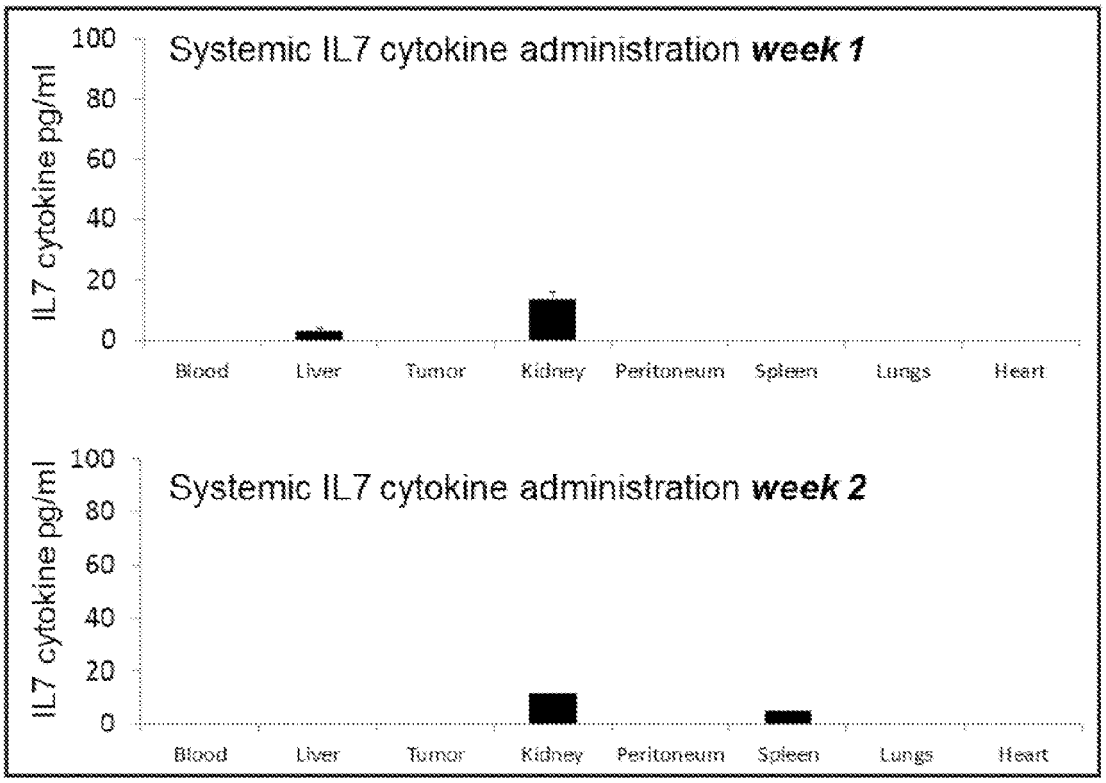

Systemic IL7 cytokine administration week 1
IL7 cytokine pg/ml
100
80
60
40
20
0
Blood
Liver
Tumor
Kidney
Peritoneum
Spleen
Lungs
Heart
Systemic IL7 cytokine administration week 2
IL7 cytokine pg/ml
100
80
60
40
20
0
Blood
Liver
Tumor
Kidney
Peritoneum
Spleen
Lungs
Heart

Figure 19B

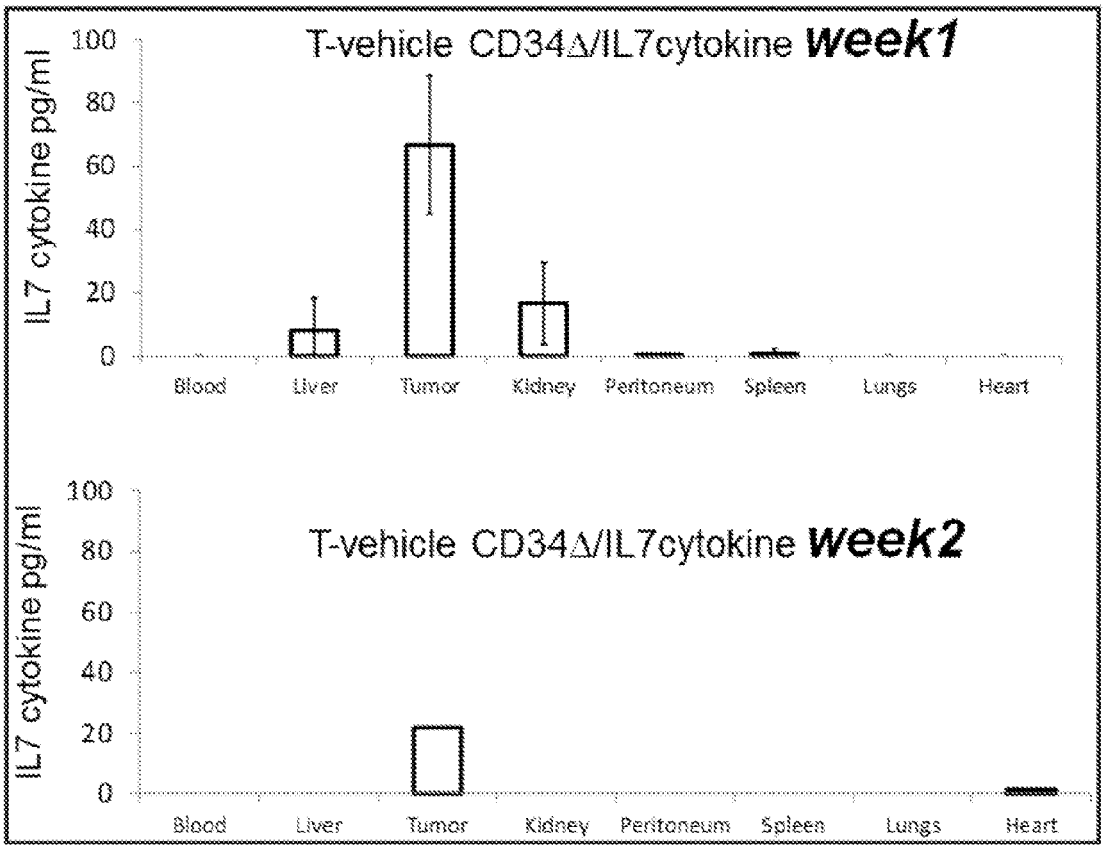

T-vehicle CD34Δ/IL7cytokine week1
IL7 cytokine pg/ml
100
80
60
40
20
0
Blood
Liver
Tumor
Kidney
Peritoneum
Spleen
Lungs
Heart
T-vehicle CD34Δ/IL7cytokine week2
IL7 cytokine pg/ml
100
80
60
40
20
0
Blood
Liver
Tumor
Kidney
Peritoneum
Spleen
Lungs
Heart

Figure 20

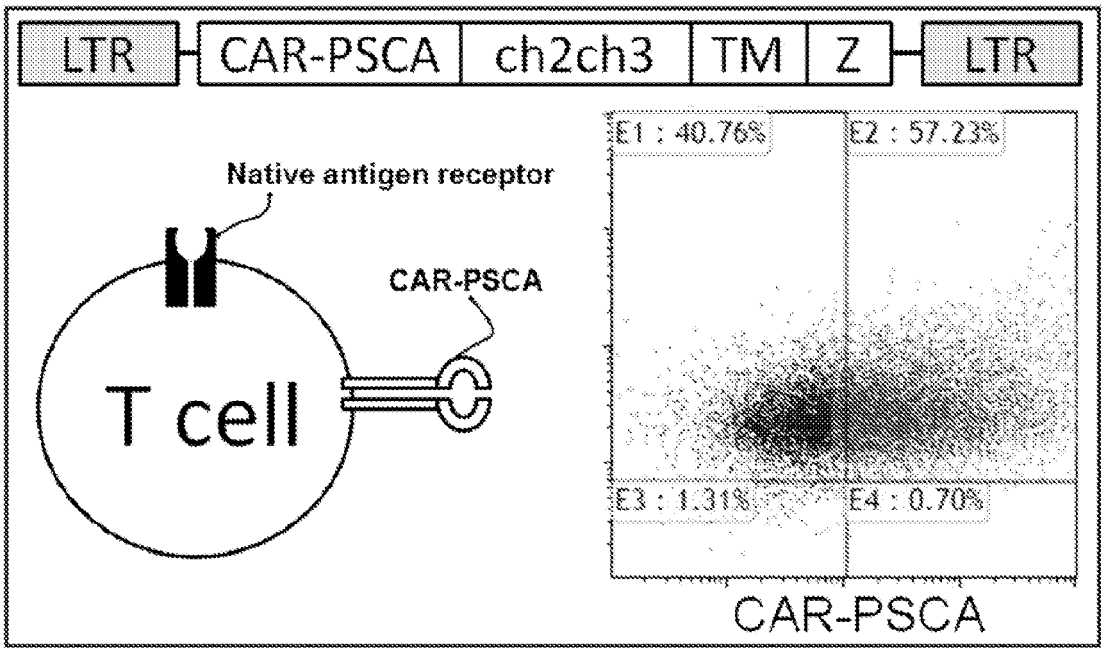


Figure 21

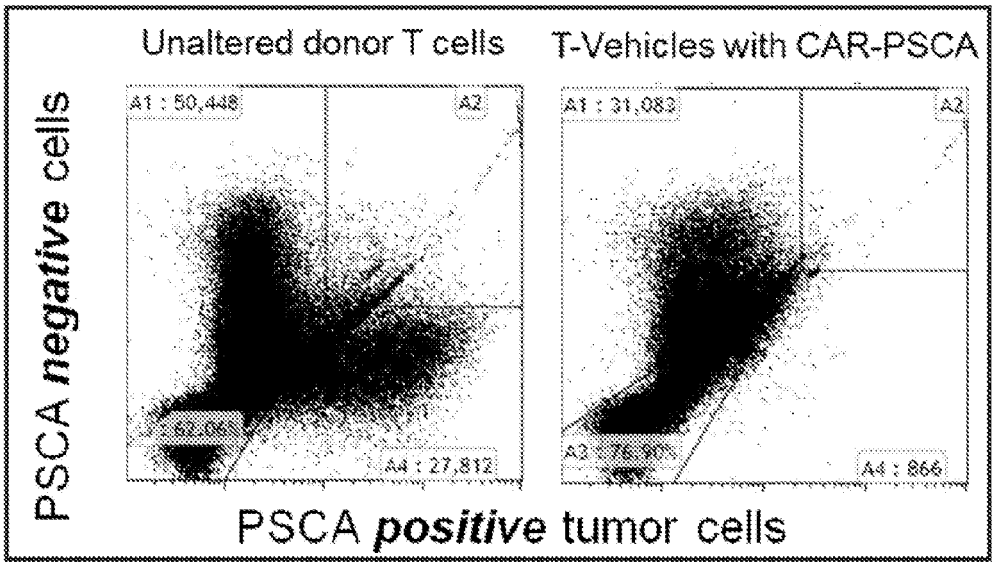


Figure 22A

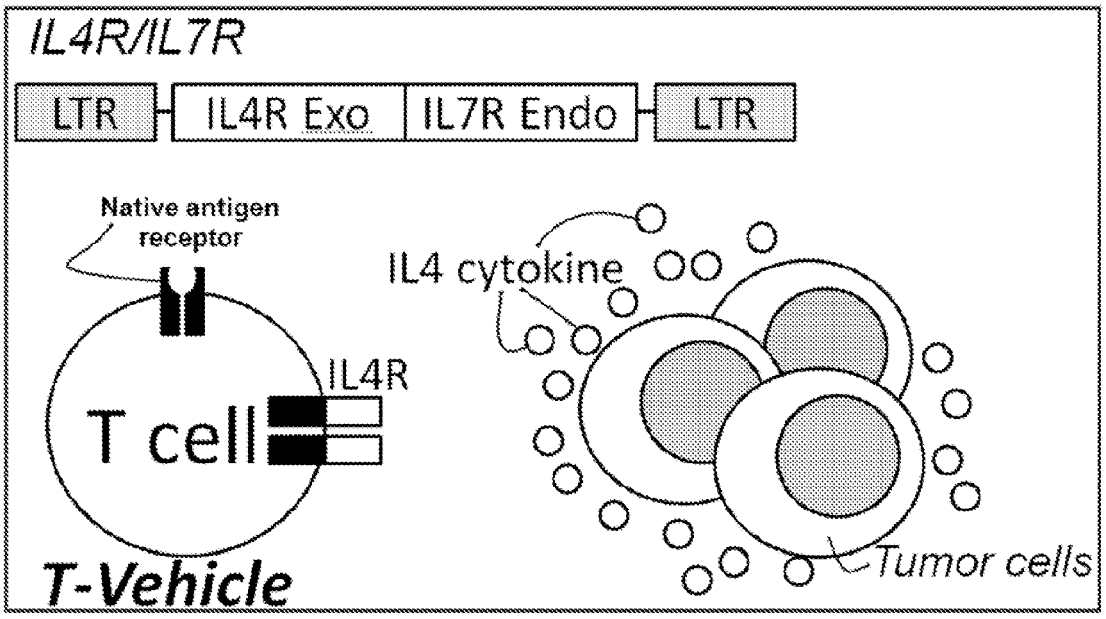


Figure 22B

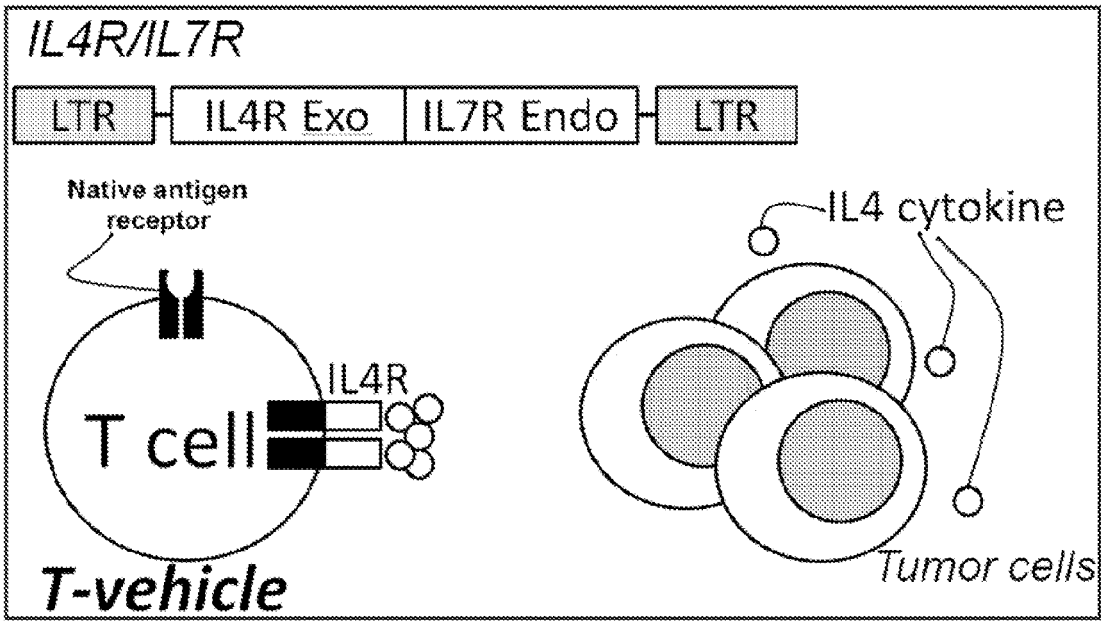


Figure 23

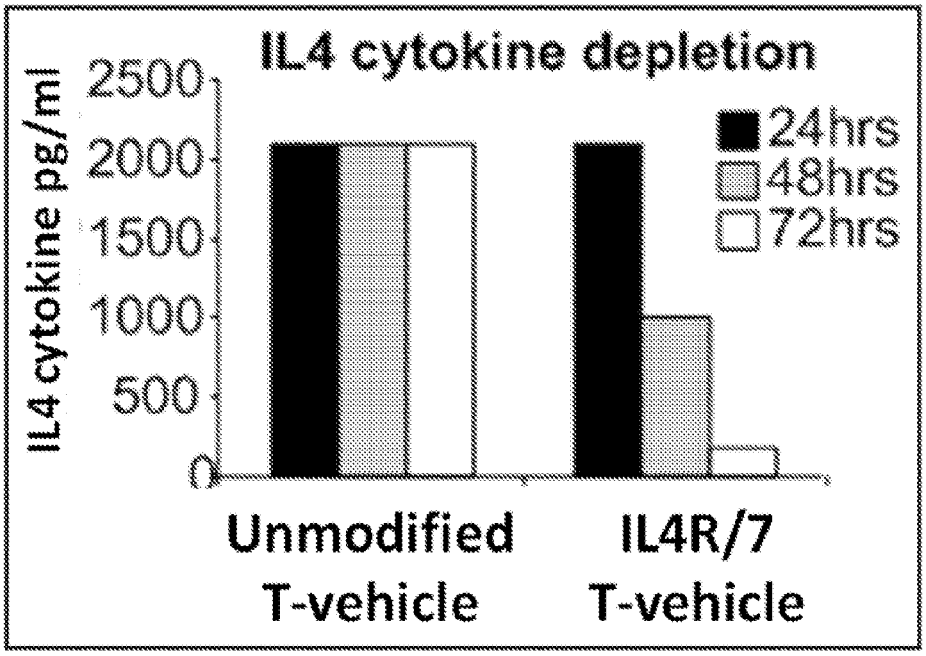


Figure 24A

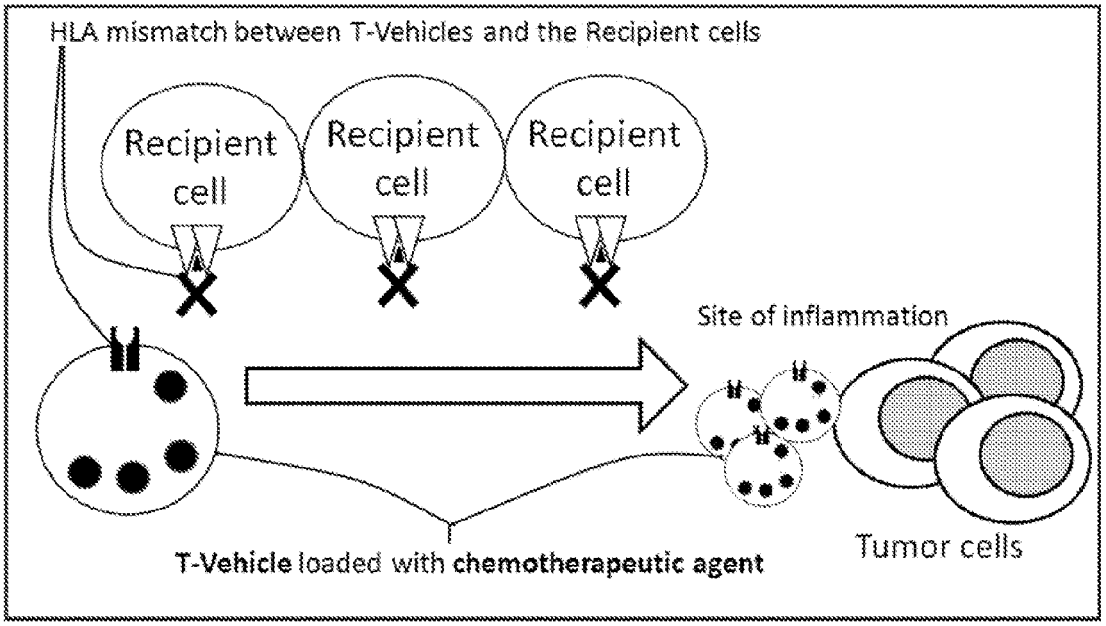


Figure 24B

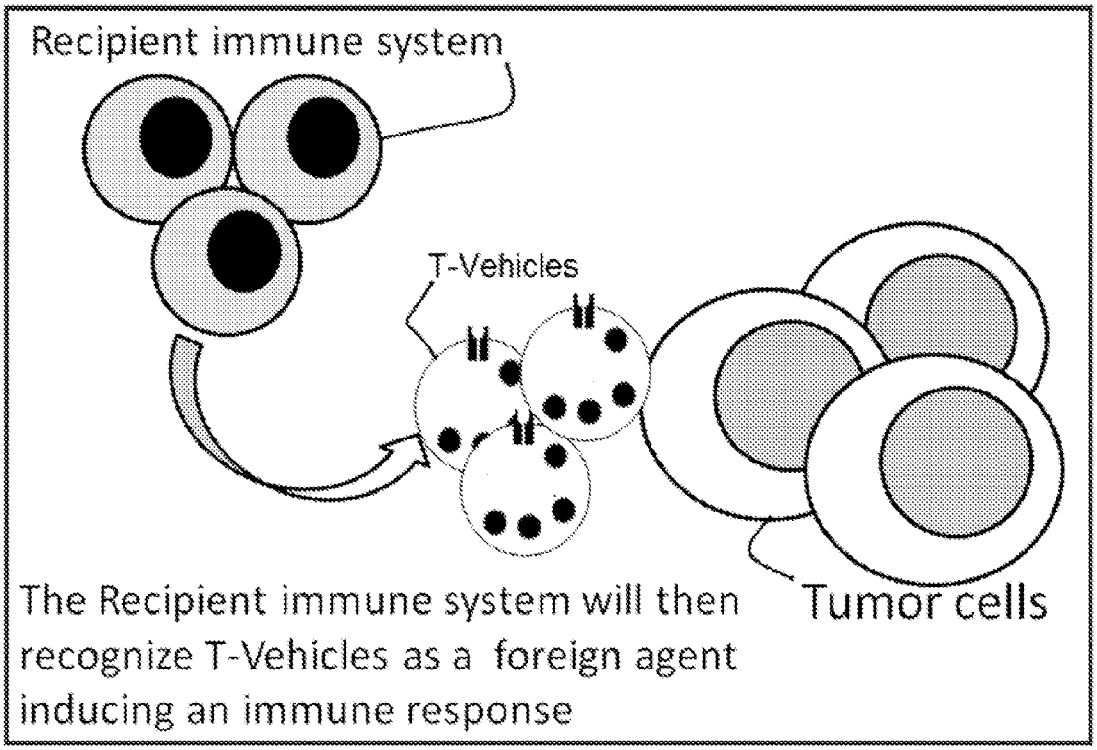


Figure 24C

