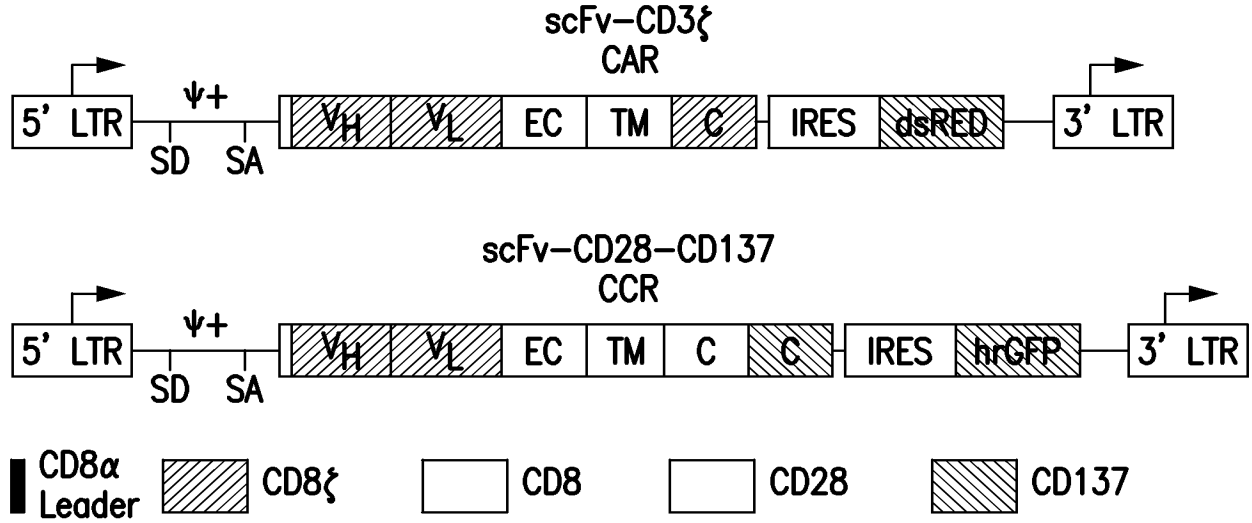

scFv-CD3ζ
CAR
5' LTR
Ψ+
SD
SA
VH
VL
EC
TM
C
IRES
dsRED
3' LTR
scFv-CD28-CD137
CCR
5' LTR
Ψ+
SD
SA
VH
VL
EC
TM
C
C
IRES
hrGFP
3' LTR
CD8α Leader
CD8ζ
CD8
CD28
CD137

FIG. 1A

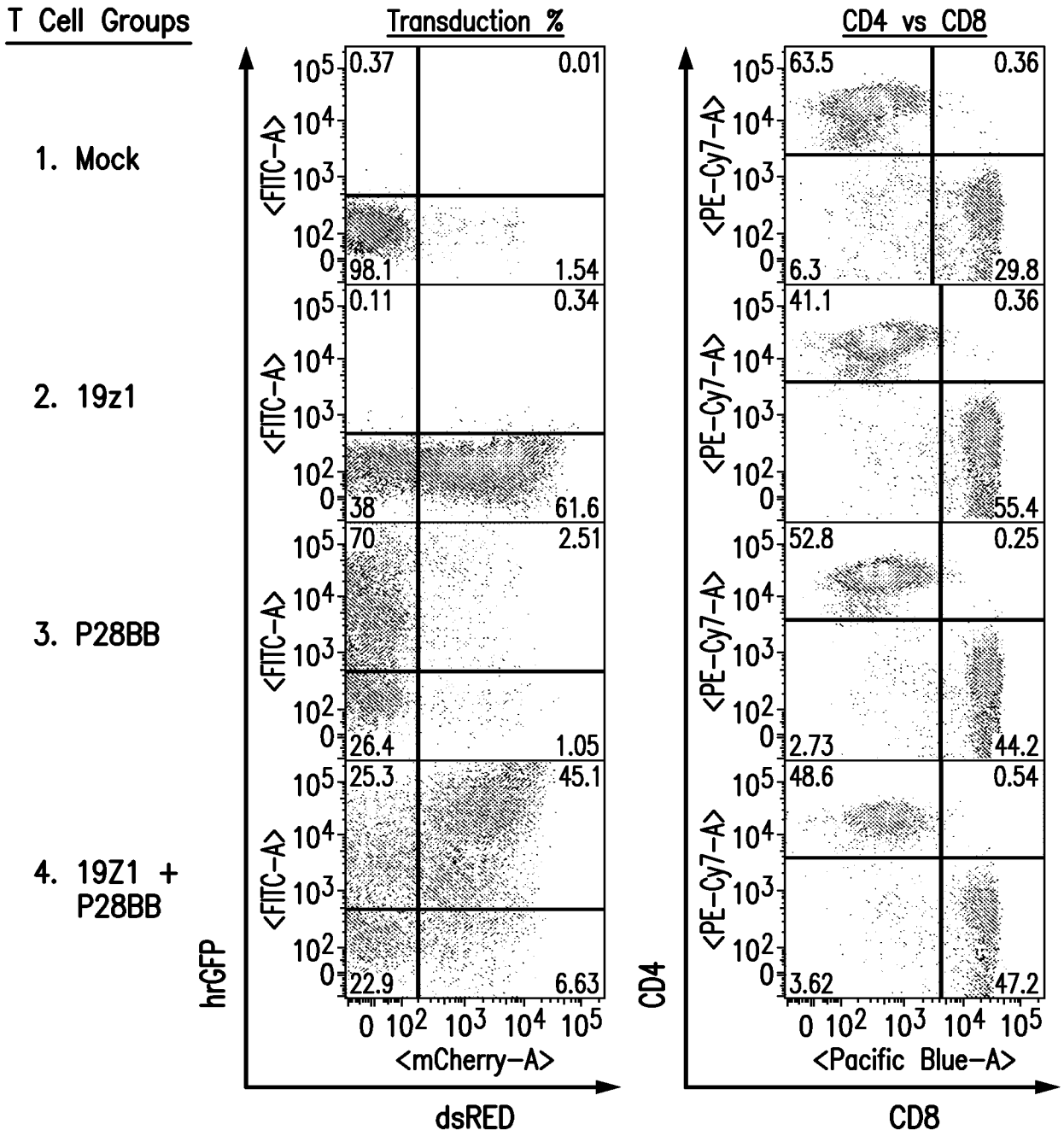

T Cell Groups
1. Mock
2. 19z1
3. P28BB
4. 19Z1 + P28BB
Transduction %
CD4 vs CD8
<FITC-A>
<PE-Cy7-A>
hrGFP
CD4
0.37
0.01
98.1
1.54
0.11
0.34
38
61.6
70
2.51
26.4
1.05
25.3
45.1
22.9
6.63
63.5
0.36
6.3
29.8
41.1
0.36
55.4
52.8
0.25
2.73
44.2
48.6
0.54
3.62
47.2
0 10^2 10^3 10^4 10^5
<mCherry-A>
dsRED
<Pacific Blue-A>
CD8

FIG. 1B

Transduction of CTLs with Different and Multiple CARs

T Cell Groups	SFG Retroviral Transduction		
1. Mock	N/A		
2. 19z1	α–CD19 – CD8–CD3zeta	IRES	dsRED
3. P28BB	α–PSMA – CD28 – 4–1BB	IRES	hrGFP
4. 19z1 + P28BB	α–CD19 – CD8–CD3zeta	IRES	dsRED
	α–PSA – CD28 – 4–1BB	IRES	hrGFP

FIG. 1C

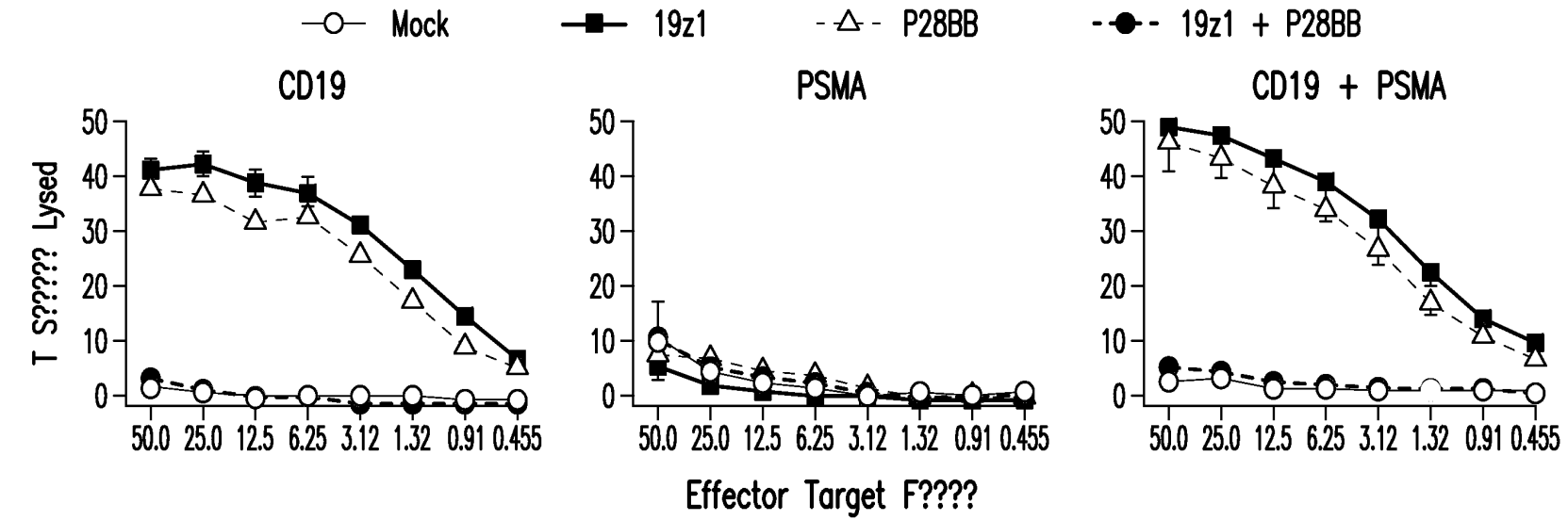

Mock
19z1
P28BB
19z1 + P28BB
CD19
PSMA
CD19 + PSMA
T S????? Lysed
50
40
30
20
10
0
50.0 25.0 12.5 6.25 3.12 1.32 0.91 0.455
Effector Target F????

FIG. 2A

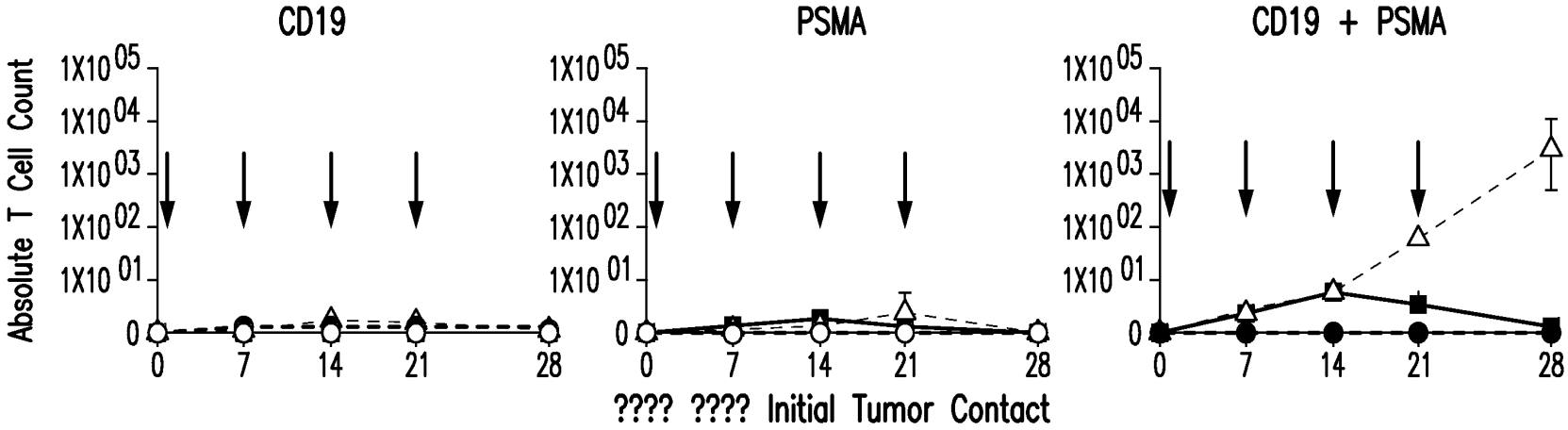

CD19
PSMA
CD19 + PSMA
Absolute T Cell Count
1X10 05
1X10 04
1X10 03
1X10 02
1X10 01
0
0 7 14 21 28
???? ???? Initial Tumor Contact

FIG. 2B

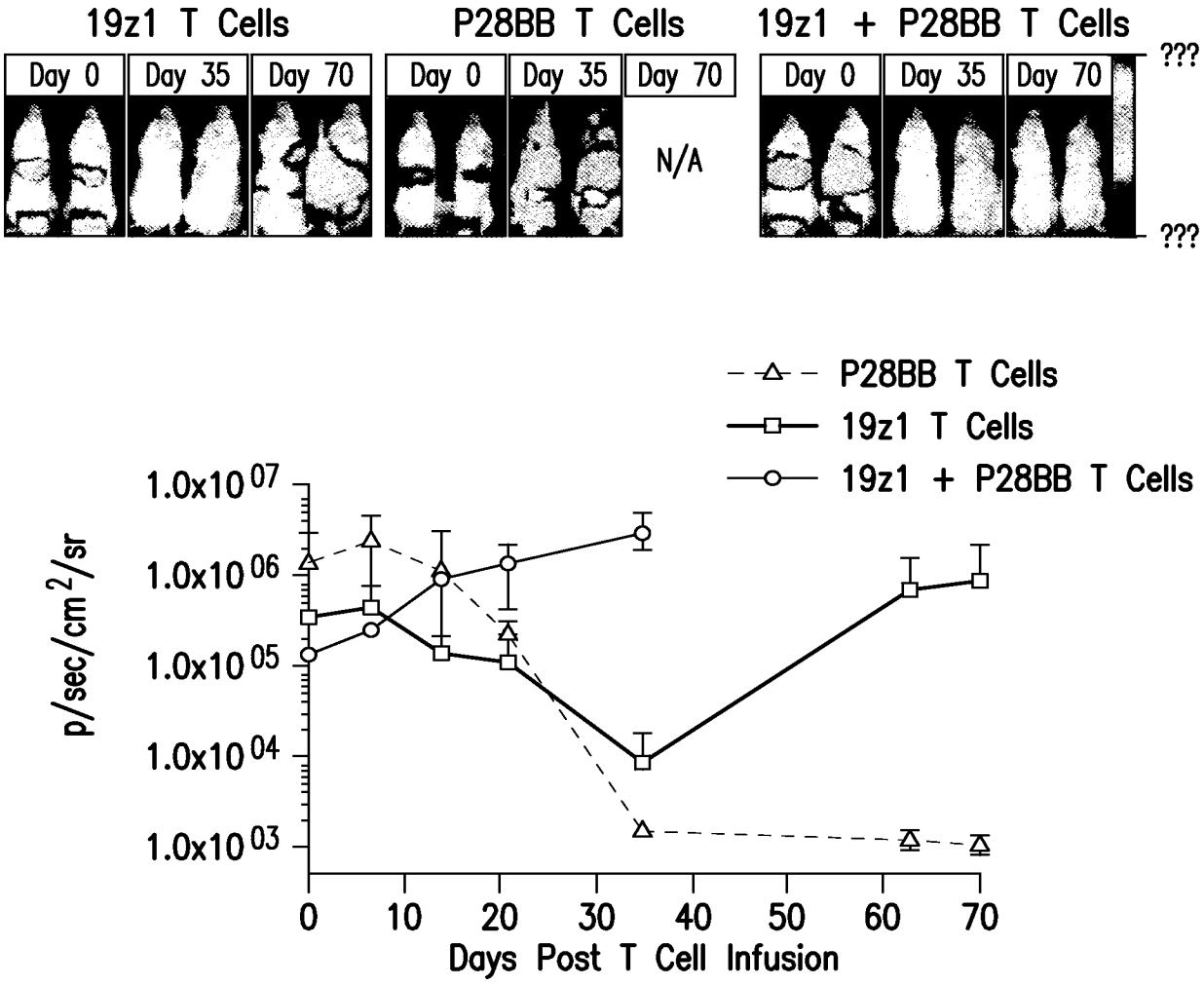

19z1 T Cells
P28BB T Cells
19z1 + P28BB T Cells
Day 0
Day 35
Day 70
N/A
???
???
P28BB T Cells
19z1 T Cells
19z1 + P28BB T Cells
p/sec/cm 2/sr
1.0x10 07
1.0x10 06
1.0x10 05
1.0x10 04
1.0x10 03
0
10
20
30
40
50
60
70
Days Post T Cell Infusion

FIG. 2C

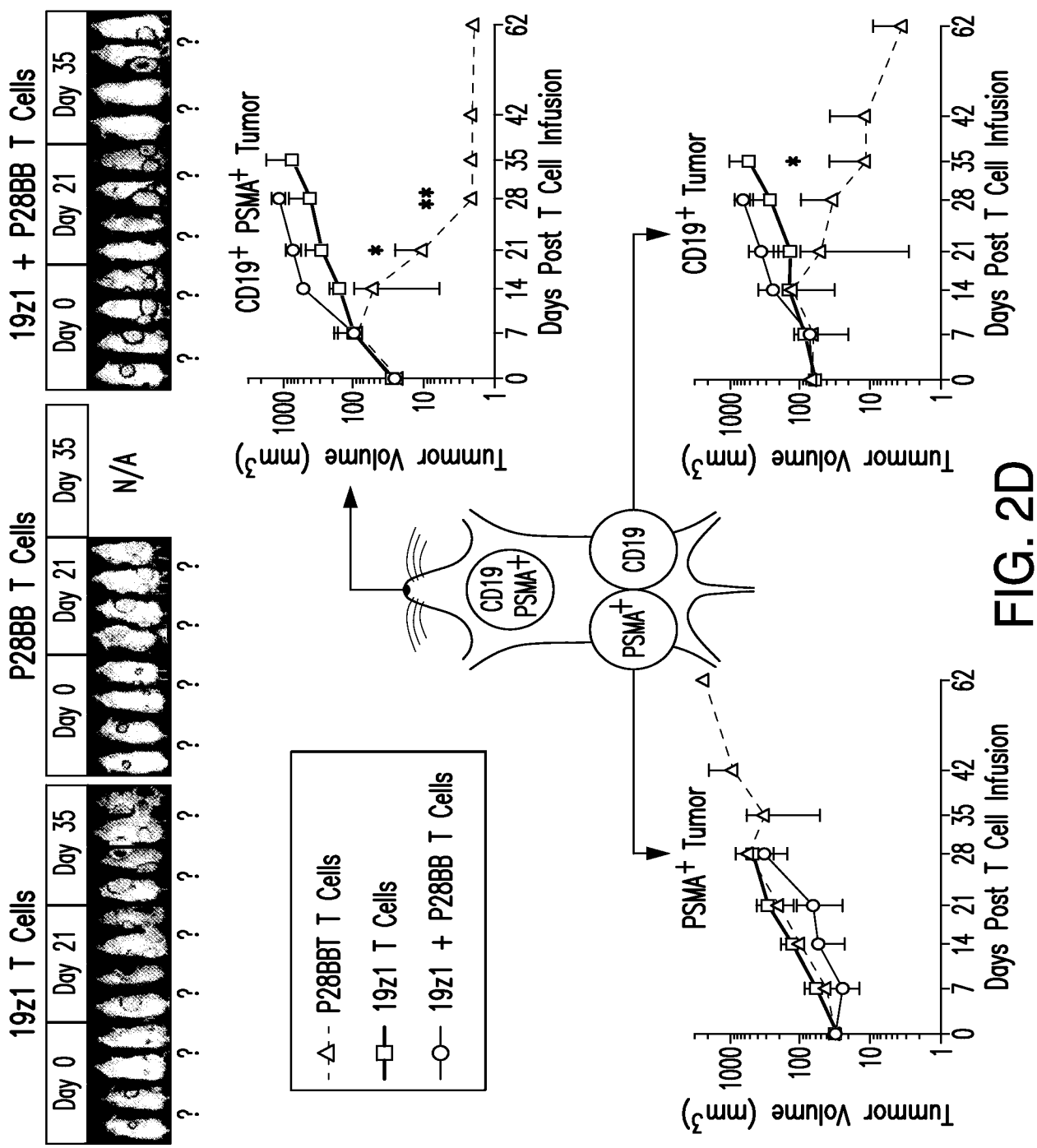

19z1 T Cells
P28BB T Cells
19z1 + P28BB T Cells
Day 0
Day 21
Day 35
N/A
P28BBT T Cells
19z1 T Cells
19z1 + P28BB T Cells
CD19+ PSMA+ Tumor
PSMA+ Tumor
CD19+ Tumor
Tumor Volume (mm3)
Days Post T Cell Infusion
CD19 PSMA+
PSMA+ CD19
*
**

FIG. 2D

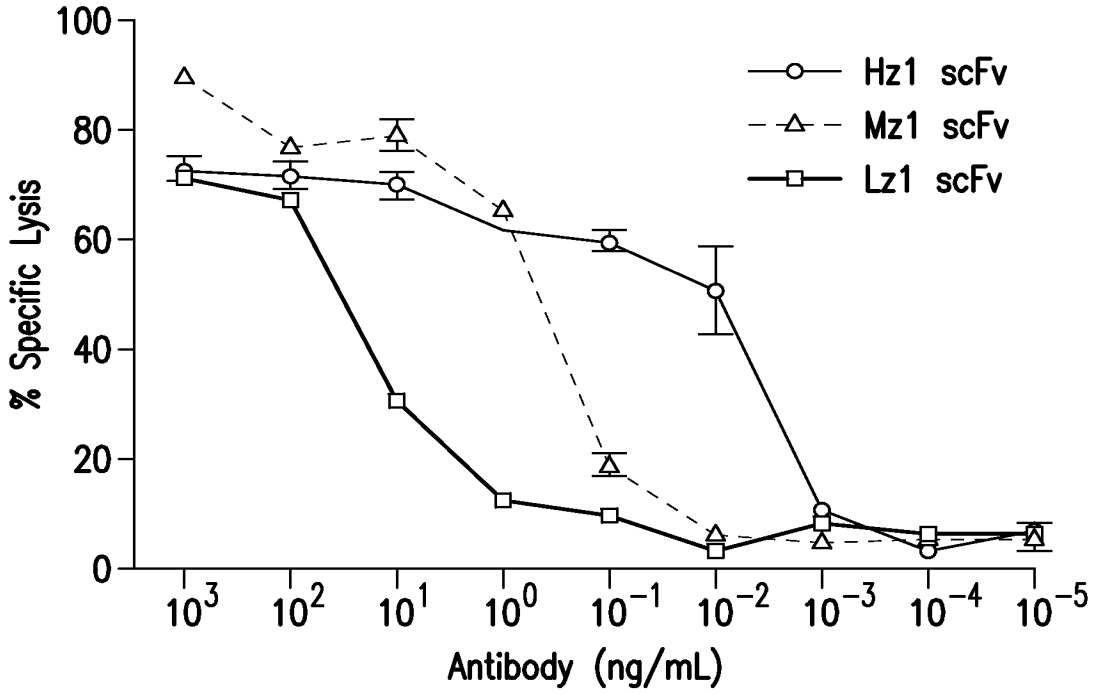

100
80
60
40
20
0
% Specific Lysis
Hz1 scFv
Mz1 scFv
Lz1 scFv
10^3
10^2
10^1
10^0
10^-1
10^-2
10^-3
10^-4
10^-5
Antibody (ng/mL)

FIG. 3A

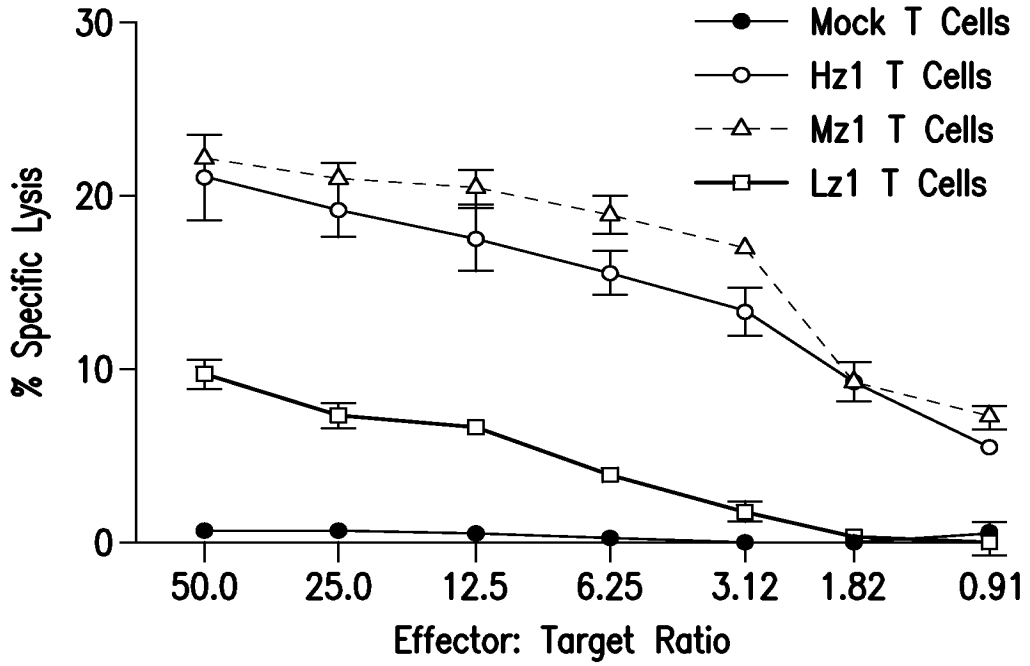

30
20
10
0
% Specific Lysis
Mock T Cells
Hz1 T Cells
Mz1 T Cells
Lz1 T Cells
50.0
25.0
12.5
6.25
3.12
1.82
0.91
Effector: Target Ratio

FIG. 3B

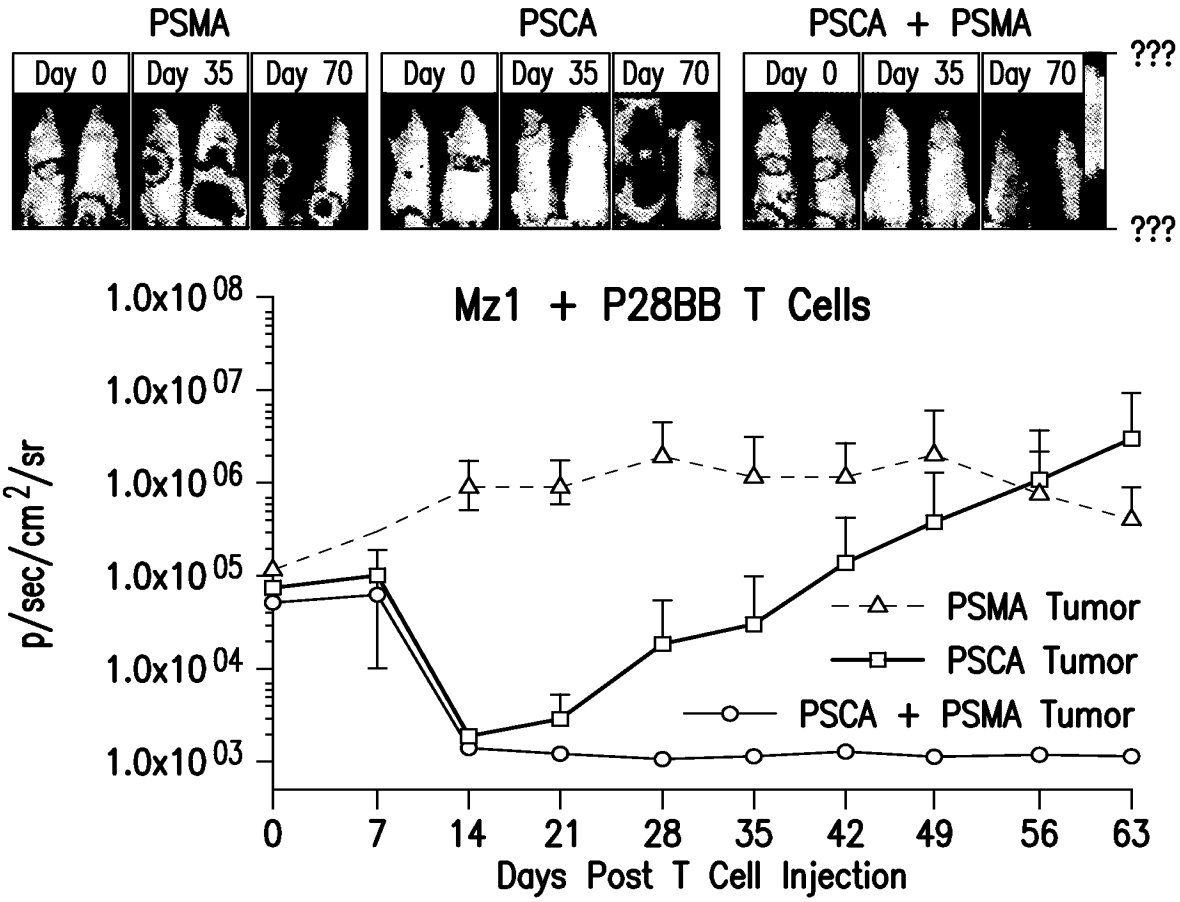

PSMA
PSCA
PSCA + PSMA
Day 0
Day 35
Day 70
???
???
Mz1 + P28BB T Cells
p/sec/cm2/sr
1.0x10 08
1.0x10 07
1.0x10 06
1.0x10 05
1.0x10 04
1.0x10 03
0
7
14
21
28
35
42
49
56
63
Days Post T Cell Injection
PSMA Tumor
PSCA Tumor
PSCA + PSMA Tumor

FIG. 3C

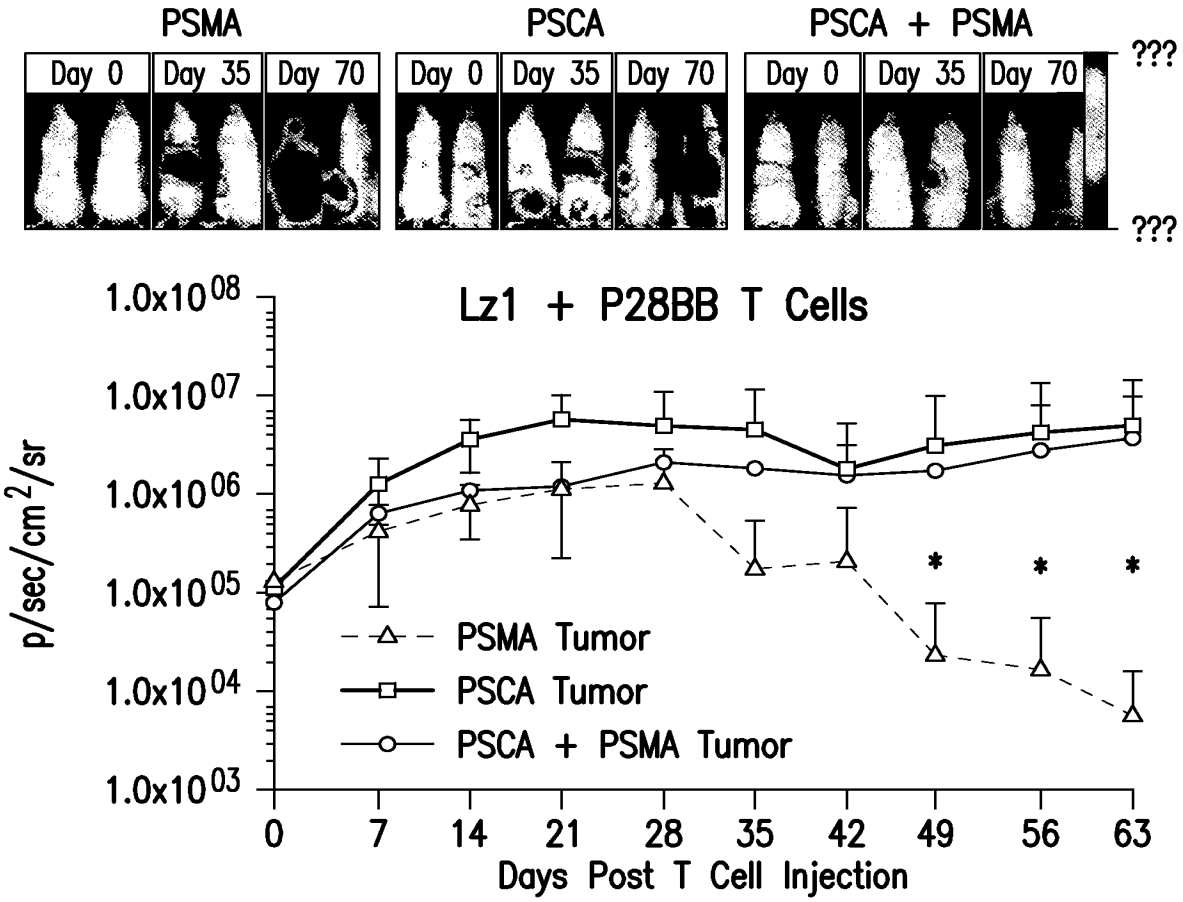

PSMA
PSCA
PSCA + PSMA
Day 0
Day 35
Day 70
???
???
Lz1 + P28BB T Cells
p/sec/cm2/sr
1.0x10 08
1.0x10 07
1.0x10 06
1.0x10 05
1.0x10 04
1.0x10 03
PSMA Tumor
PSCA Tumor
PSCA + PSMA Tumor
0
7
14
21
28
35
42
49
56
63
Days Post T Cell Injection

FIG. 3D

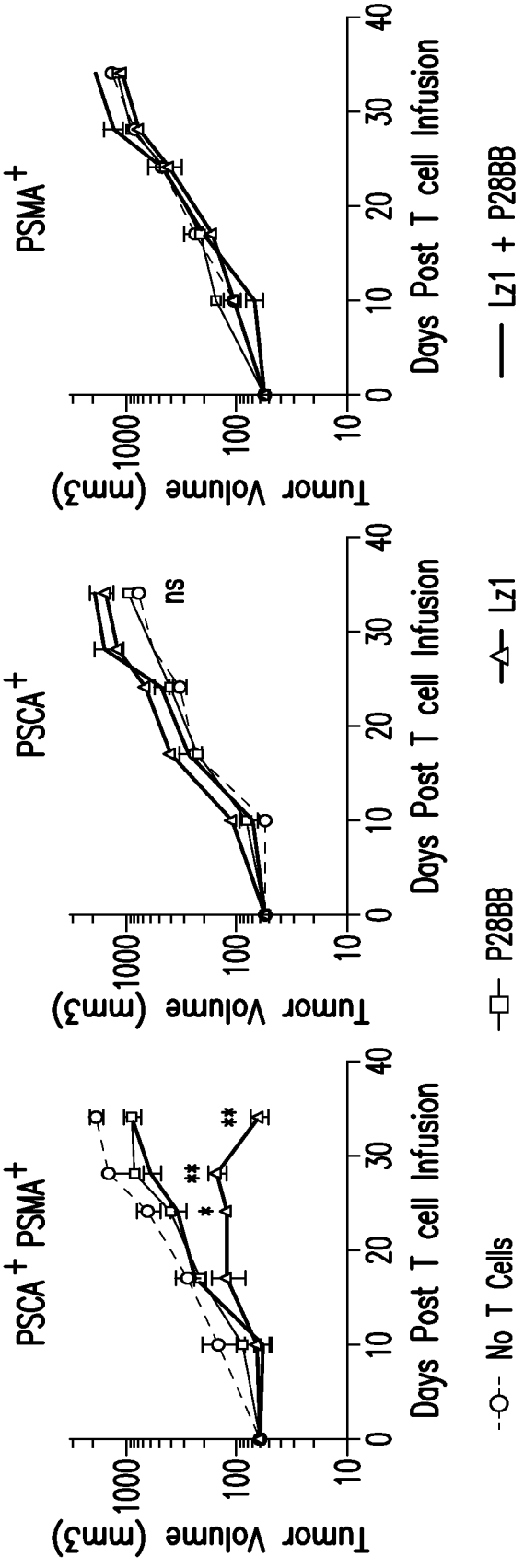

PSCA+ PSMA+
PSCA+
PSMA+
Tumor Volume (mm3)
1000
100
10
0
10
20
30
40
Days Post T cell Infusion
ns
*
**
No T Cells
P28BB
Lz1
Lz1 + P28BB

FIG. 3E

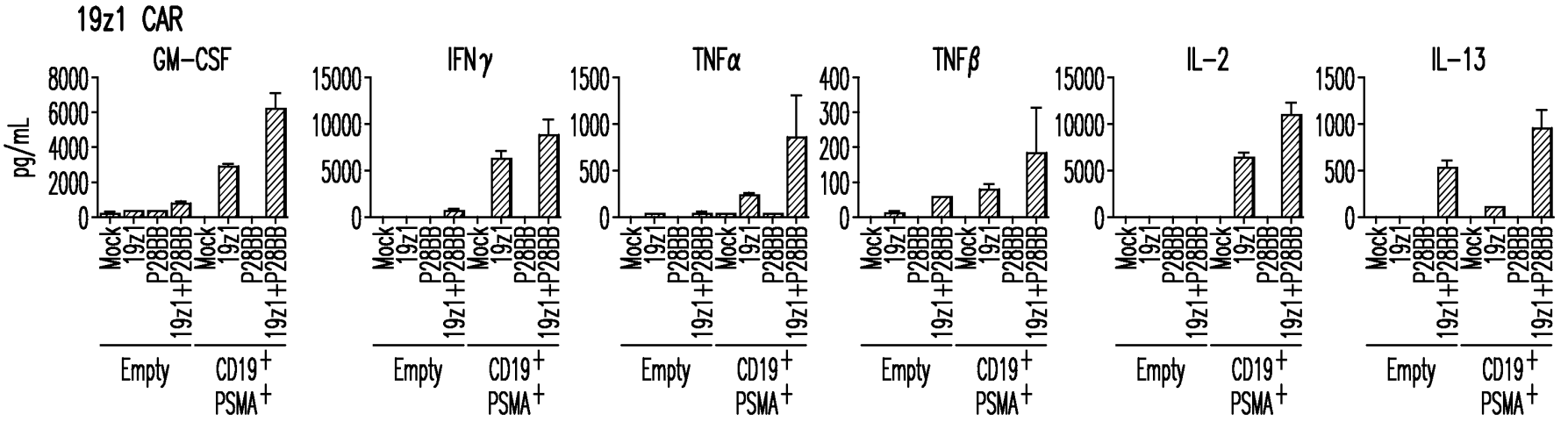

19z1 CAR
GM-CSF
IFNγ
TNFα
TNFβ
IL-2
IL-13
pg/mL
Mock
19z1
P28BB
19z1+P28BB
Empty
CD19+ PSMA+

FIG. 4A

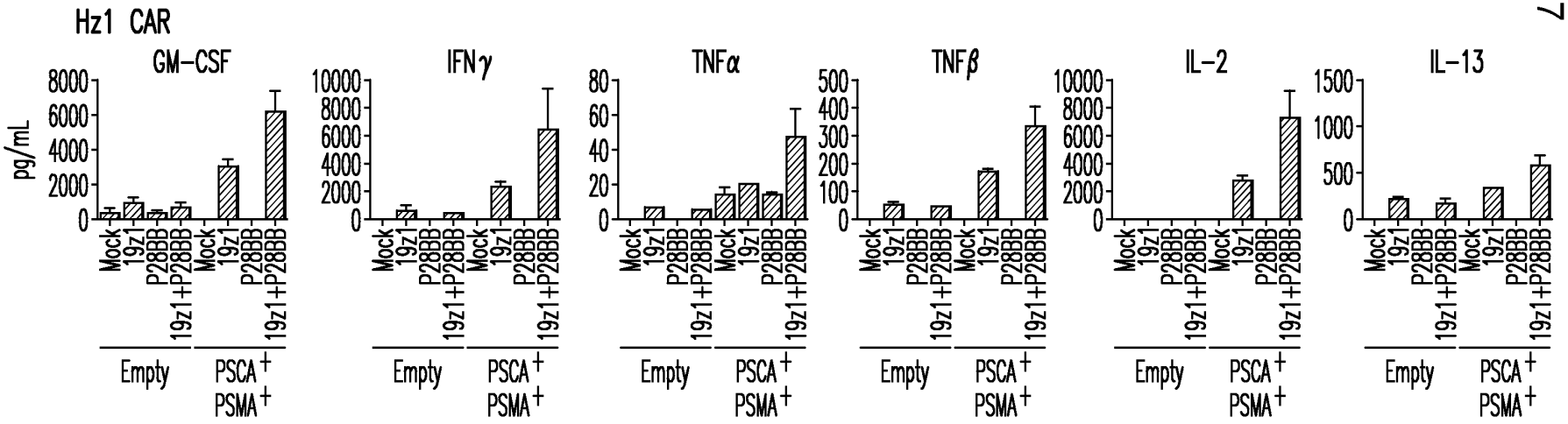

Hz1 CAR
GM-CSF
IFNγ
TNFα
TNFβ
IL-2
IL-13
pg/mL
Mock
19z1
P28BB
19z1+P28BB
Empty
PSCA+ PSMA+

FIG. 4B

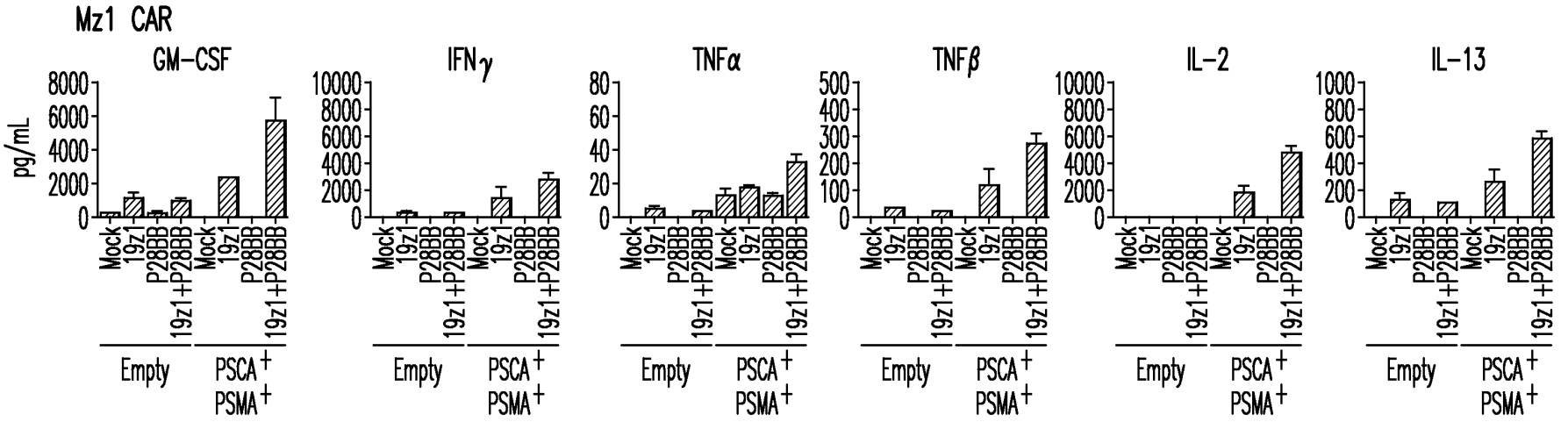

Mz1 CAR
GM-CSF
IFNγ
TNFα
TNFβ
IL-2
IL-13
pg/mL
Mock
19z1
P28BB
19z1+P28BB
Empty
PSCA+ PSMA+

FIG. 4C

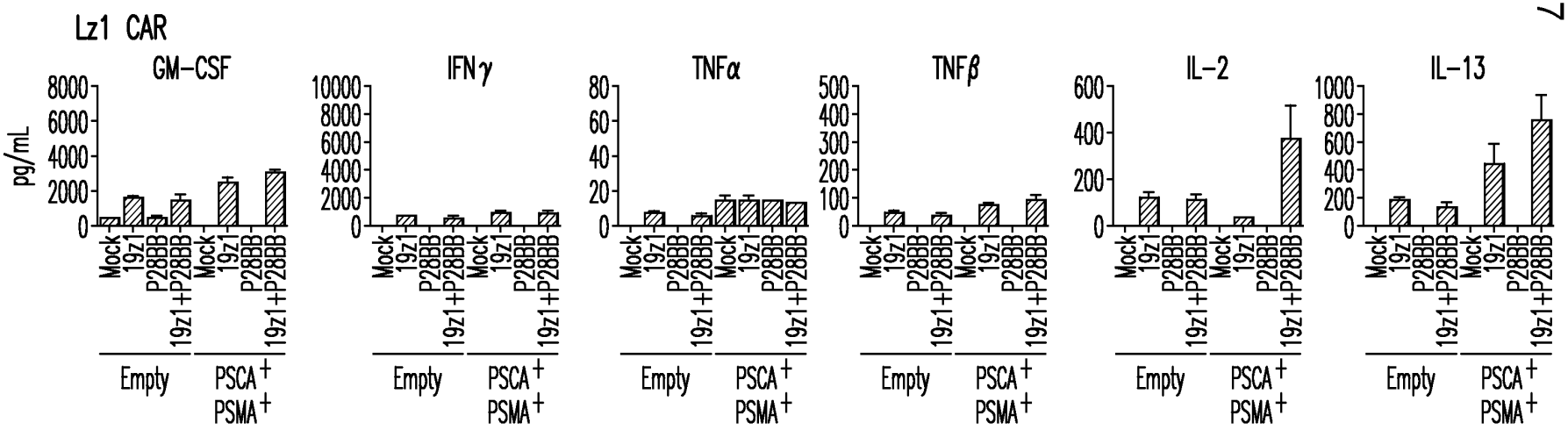

Lz1 CAR
GM-CSF
IFNγ
TNFα
TNFβ
IL-2
IL-13
pg/mL
Mock
19z1
P28BB
19z1+P28BB
Empty
PSCA+ PSMA+

FIG. 4D

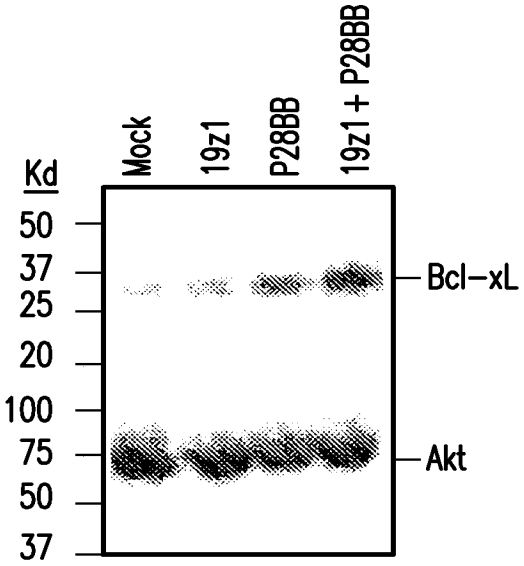


FIG. 4E

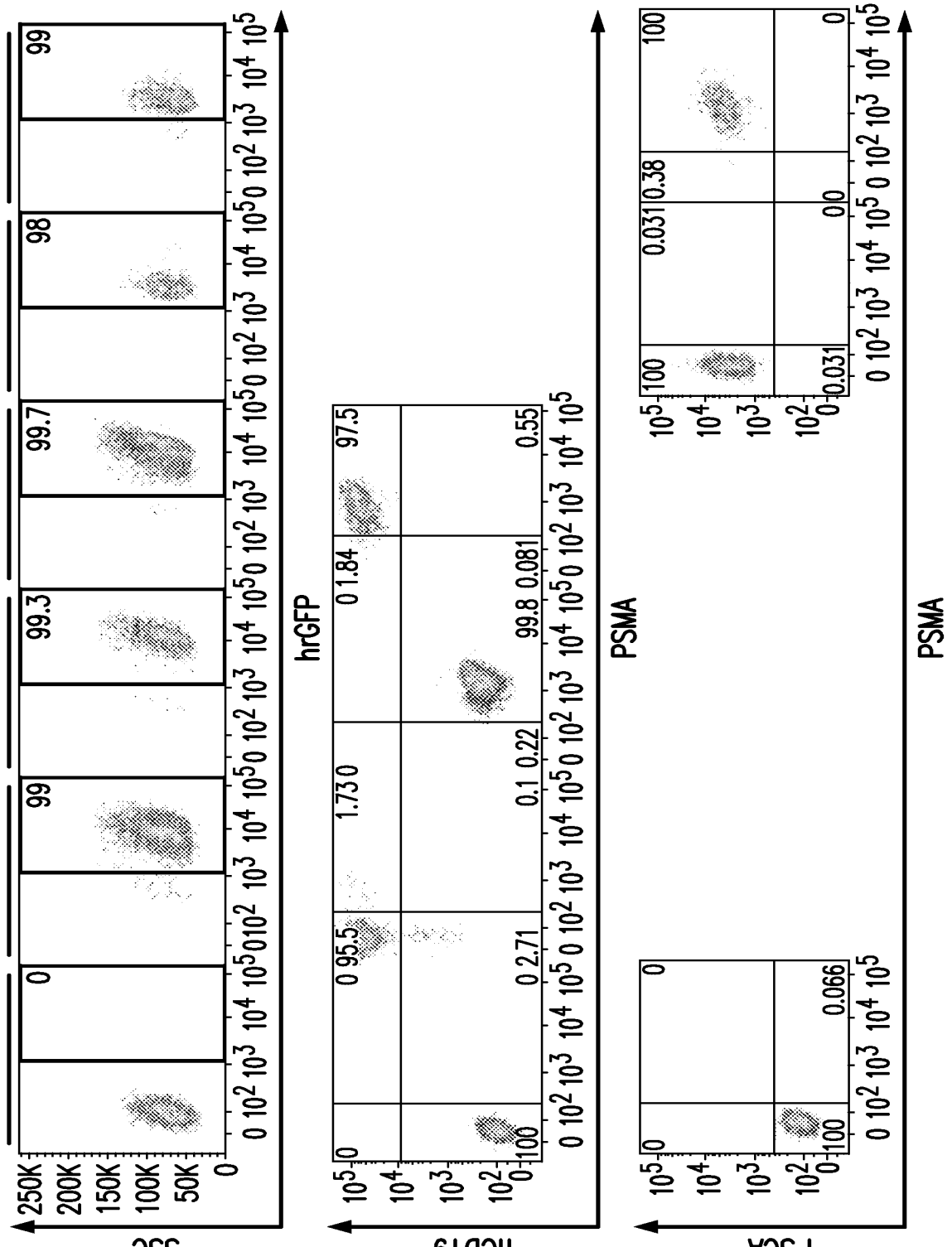

PC3-Empty
PC3-CD19-GFP/Luc
PC3-PSMA-GFP/Luc
PC3-PSMA-CD19-GFP/Luc
PC3-PSCA-GFP/Luc
PC3-PSCA-PSMA-GFP/Luc
SSC
hrGFP
hCD19
PSMA
PSCA
PSMA

FIG. 5

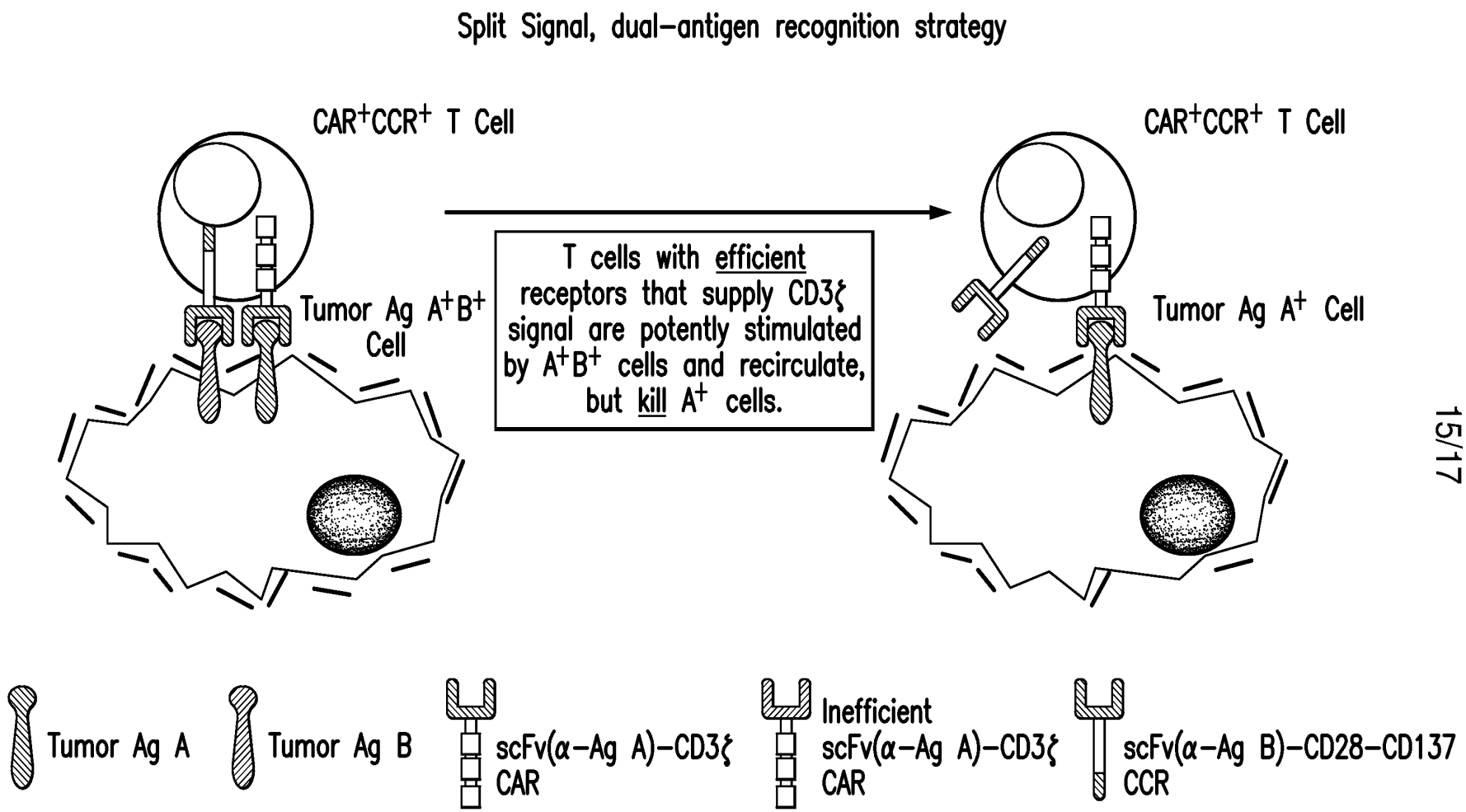

Split Signal, dual-antigen recognition strategy
CAR+CCR+ T Cell
Tumor Ag A+B+ Cell
T cells with efficient receptors that supply CD3ζ signal are potently stimulated by A+B+ cells and recirculate, but kill A+ cells.
CAR+CCR+ T Cell
Tumor Ag A+ Cell
Tumor Ag A
Tumor Ag B
scFv(α-Ag A)-CD3ζ CAR
Inefficient scFv(α-Ag A)-CD3ζ CAR
scFv(α-Ag B)-CD28-CD137 CCR

FIG. 6A

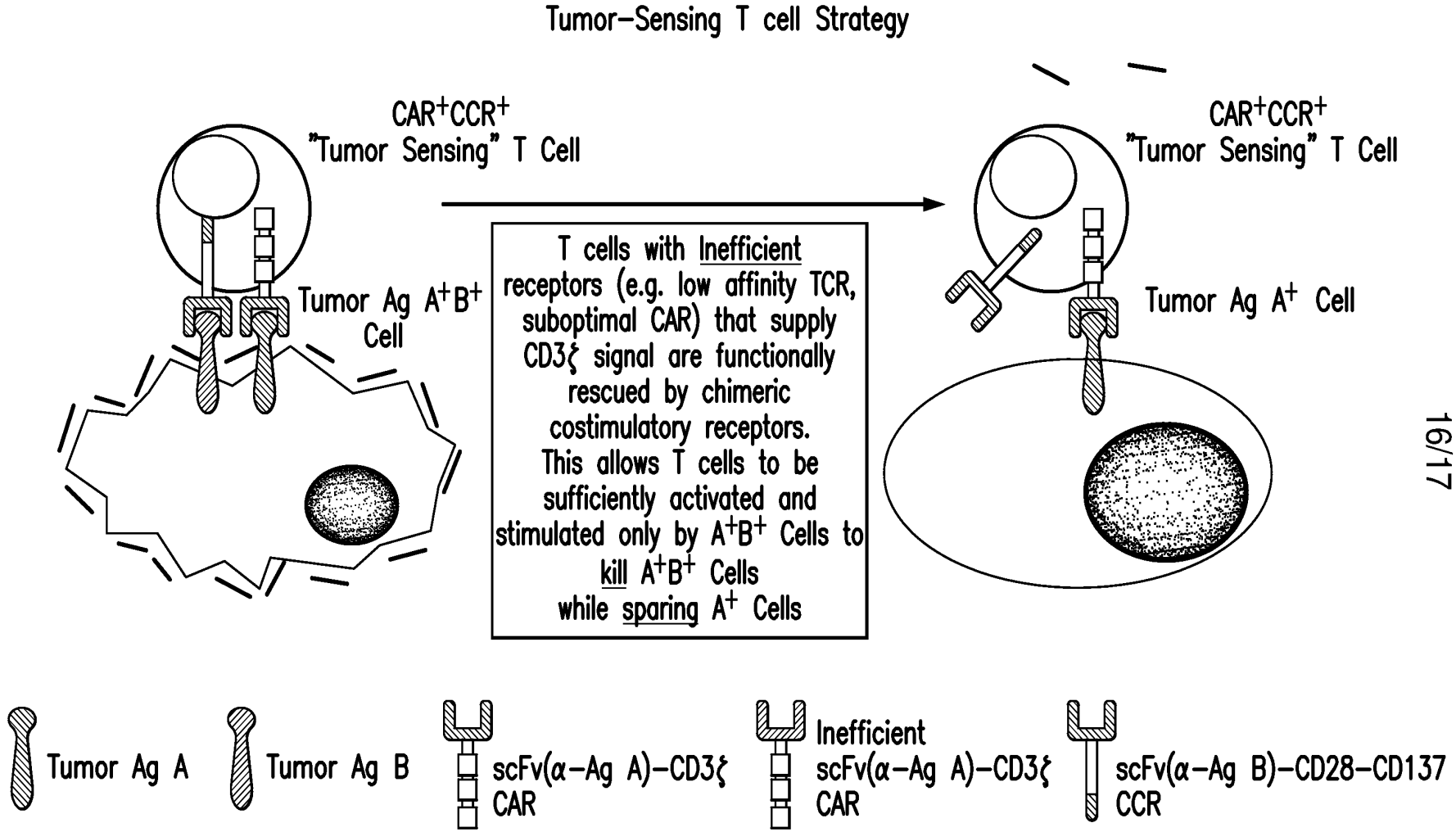

Tumor–Sensing T cell Strategy
CAR+CCR+
"Tumor Sensing" T Cell
Tumor Ag A+B+
Cell
T cells with Inefficient receptors (e.g. low affinity TCR, suboptimal CAR) that supply CD3ζ signal are functionally rescued by chimeric costimulatory receptors. This allows T cells to be sufficiently activated and stimulated only by A+B+ Cells to kill A+B+ Cells while sparing A+ Cells
CAR+CCR+
"Tumor Sensing" T Cell
Tumor Ag A+ Cell
Tumor Ag A
Tumor Ag B
scFv(α–Ag A)–CD3ζ
CAR
Inefficient
scFv(α–Ag A)–CD3ζ
CAR
scFv(α–Ag B)–CD28–CD137
CCR

FIG. 6B

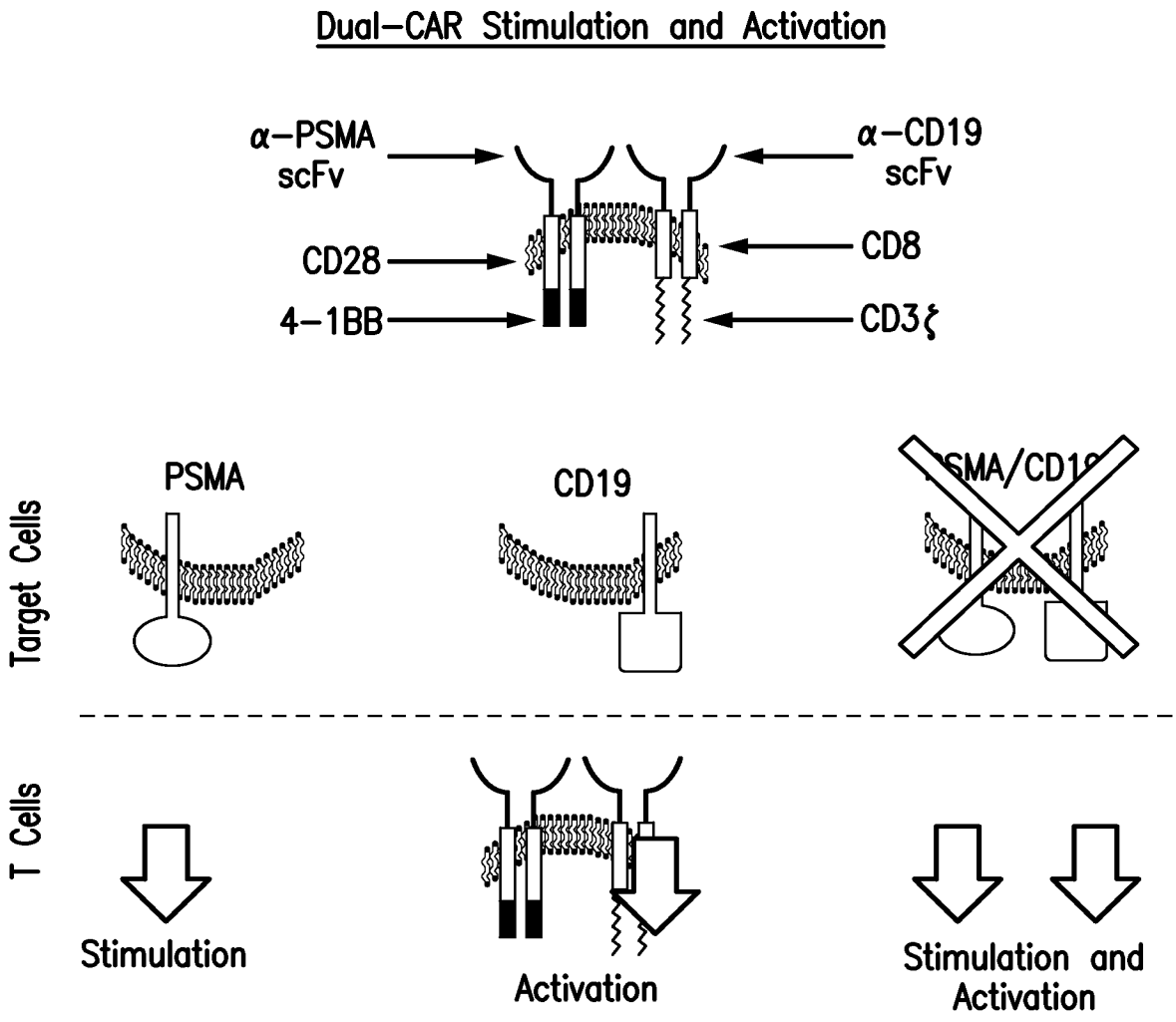

Dual-CAR Stimulation and Activation
α-PSMA scFv
α-CD19 scFv
CD28
CD8
4-1BB
CD3ζ
Target Cells
PSMA
CD19
T Cells
Stimulation
Activation
Stimulation and Activation

FIG. 6C