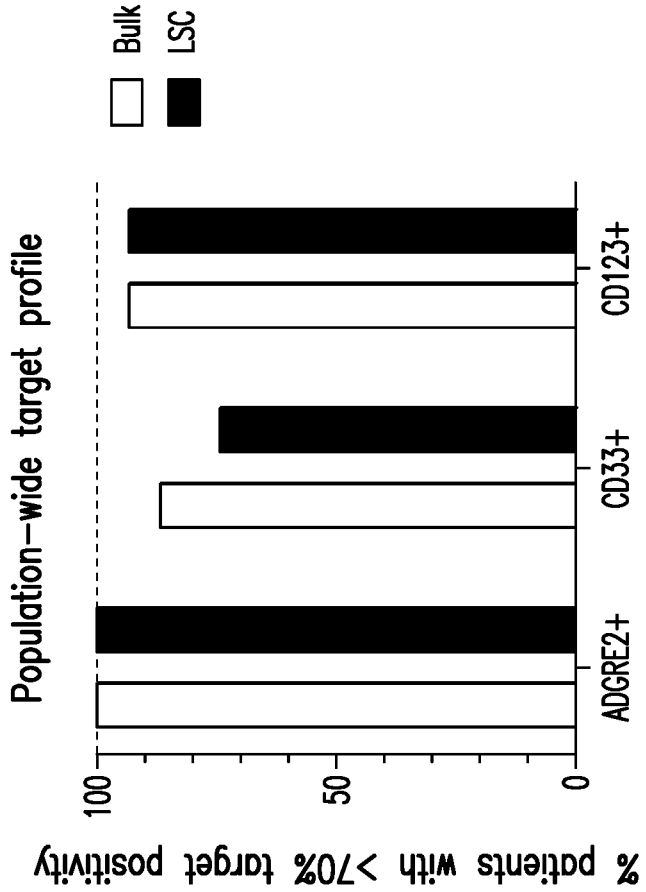


FIGURE 1B

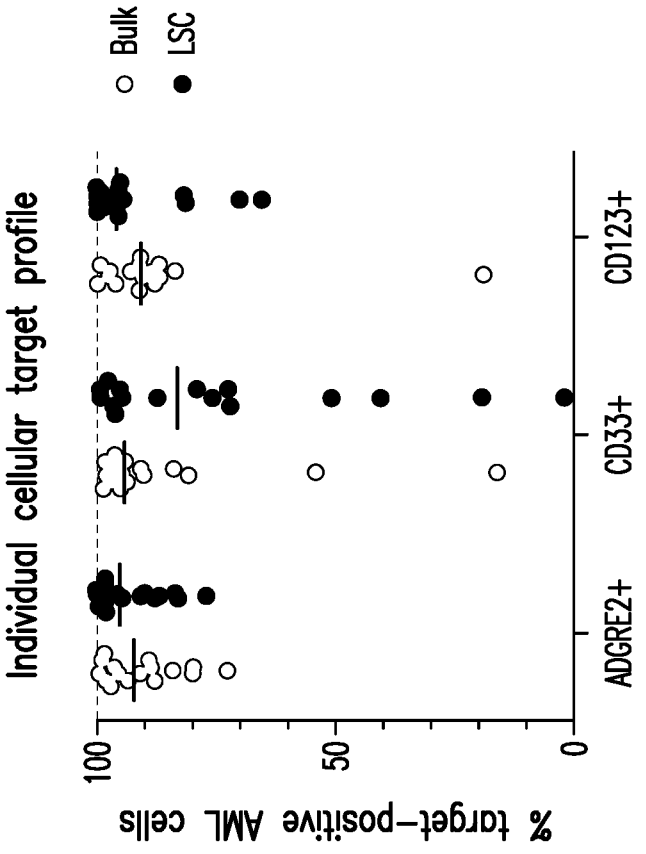


FIGURE 1A

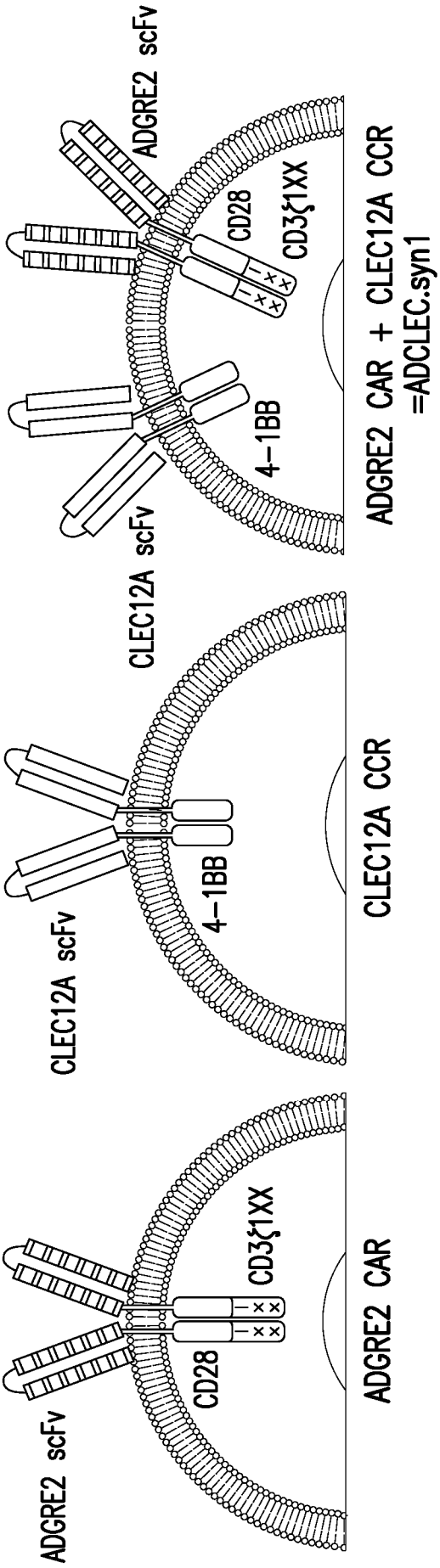

ADGRE2 scFv
CD28
CD3ζ1XX
4–1BB
CLEC12A scFv
ADGRE2 CAR + CLEC12A CCR
=ADCLEC.syn1
CLEC12A scFv
4–1BB
CLEC12A CCR
ADGRE2 scFv
CD28
CD3ζ1XX
ADGRE2 CAR

FIGURE 2

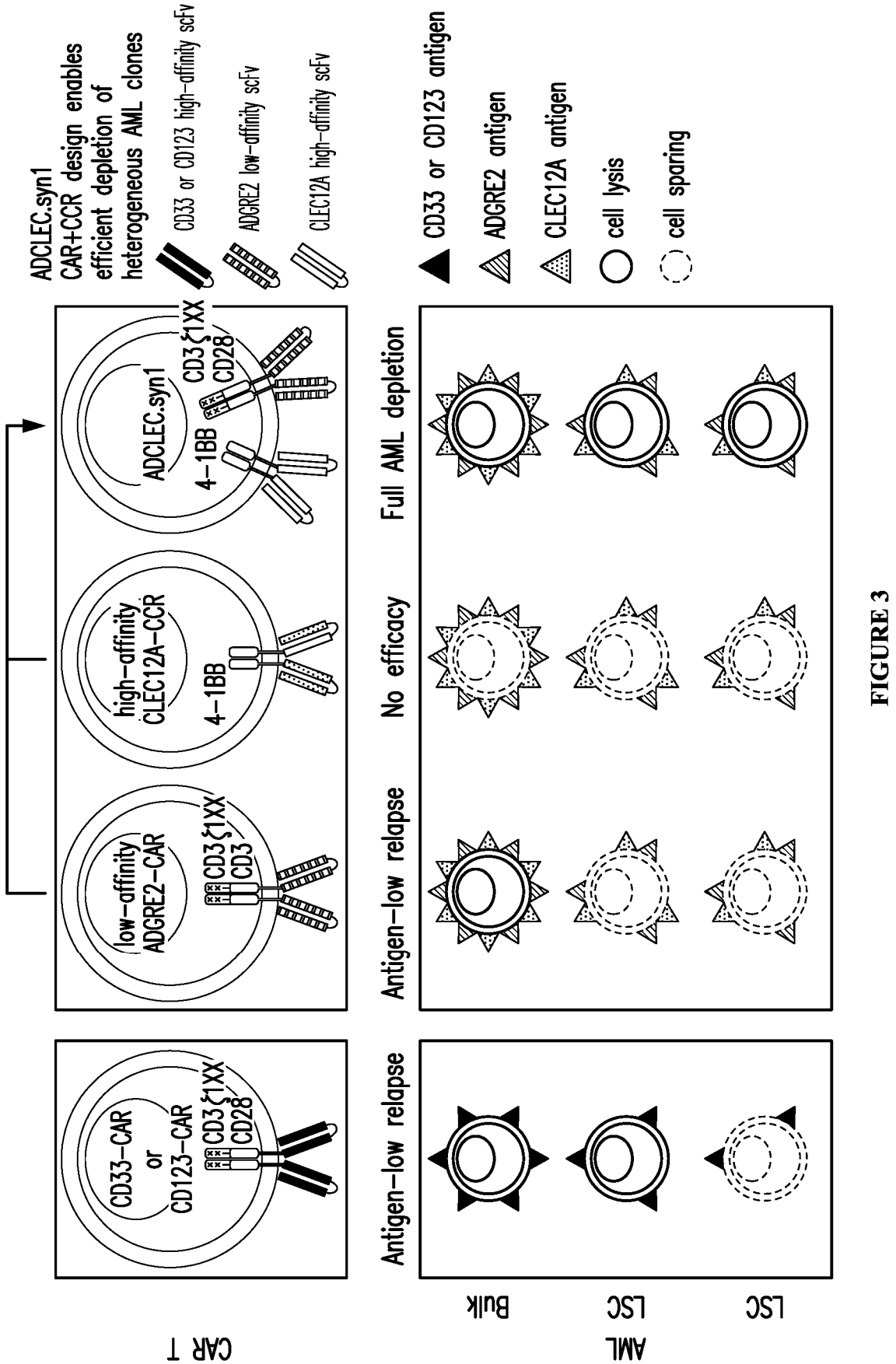

ADCLEC.syn1
CAR+CCR design enables
efficient depletion of
heterogeneous AML clones
CD33 or CD123 high-affinity scFv
ADGRE2 low-affinity scFv
CLEC12A high-affinity scFv
CD33 or CD123 antigen
ADGRE2 antigen
CLEC12A antigen
cell lysis
cell sparing
CAR T
CD33-CAR or CD123-CAR
CD3ζ1XX
CD28
low-affinity ADGRE2-CAR
CD3
high-affinity CLEC12A-CCR
4-1BB
ADCLEC.syn1
Antigen-low relapse
No efficacy
Full AML depletion
AML
Bulk
LSC
LSC

FIGURE 3

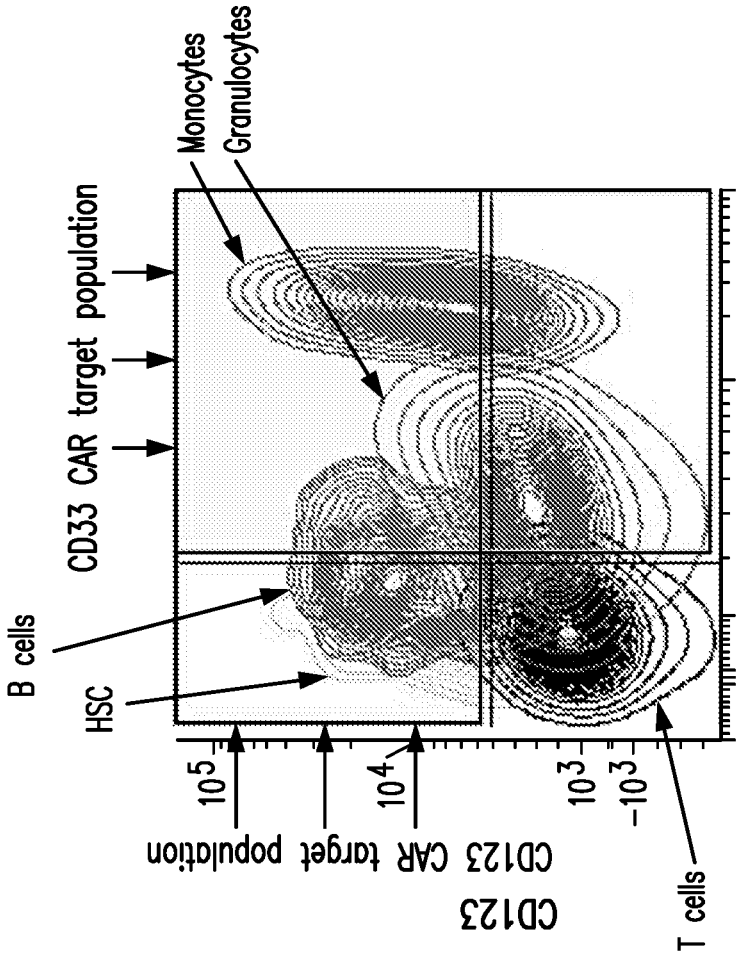


FIGURE 4B

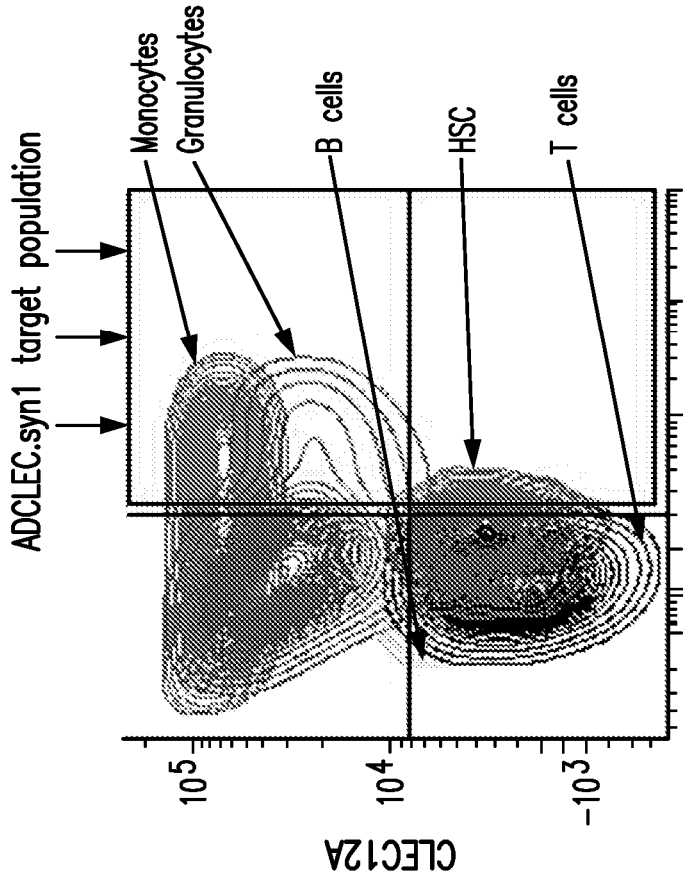


FIGURE 4A

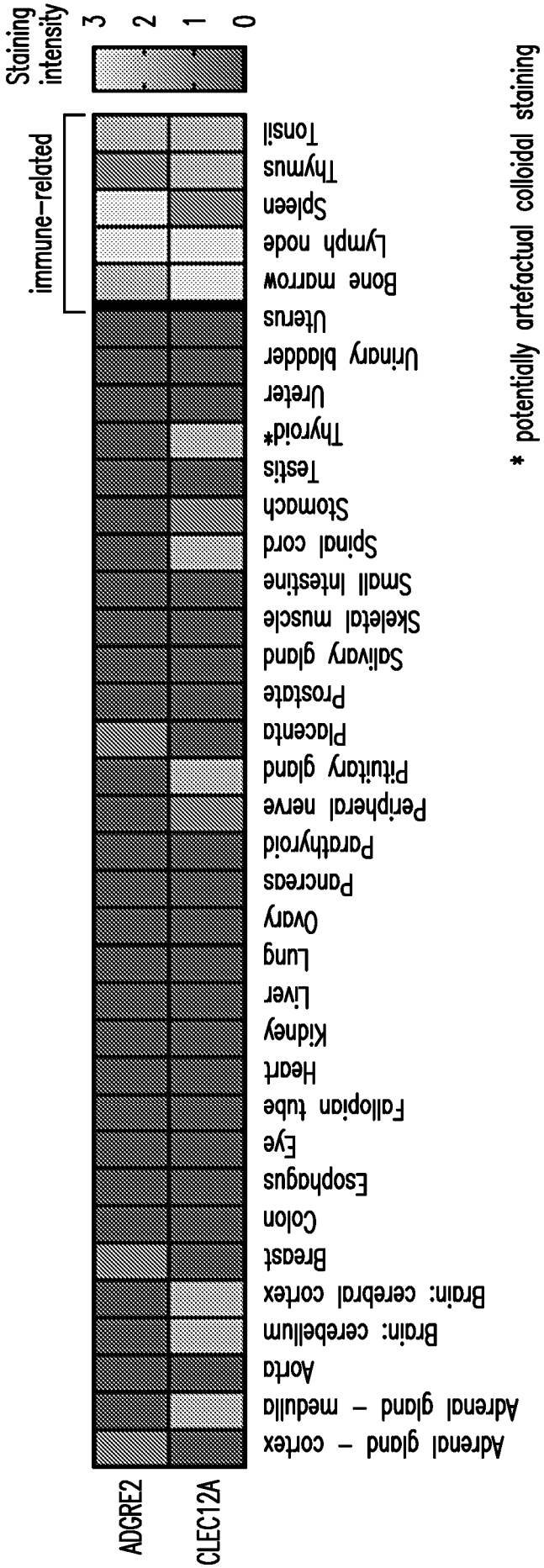

Staining intensity
3
2
1
0
immune-related
ADGRE2
CLEC12A
Adrenal gland – cortex
Adrenal gland – medulla
Aorta
Brain: cerebellum
Brain: cerebral cortex
Breast
Colon
Esophagus
Eye
Fallopian tube
Heart
Kidney
Liver
Lung
Ovary
Pancreas
Parathyroid
Peripheral nerve
Pituitary gland
Placenta
Prostate
Salivary gland
Skeletal muscle
Small Intestine
Spinal cord
Stomach
Testis
Thyroid*
Ureter
Urinary bladder
Uterus
Bone marrow
Lymph node
Spleen
Thymus
Tonsil
* potentially artefactual colloidal staining

FIGURE 4C

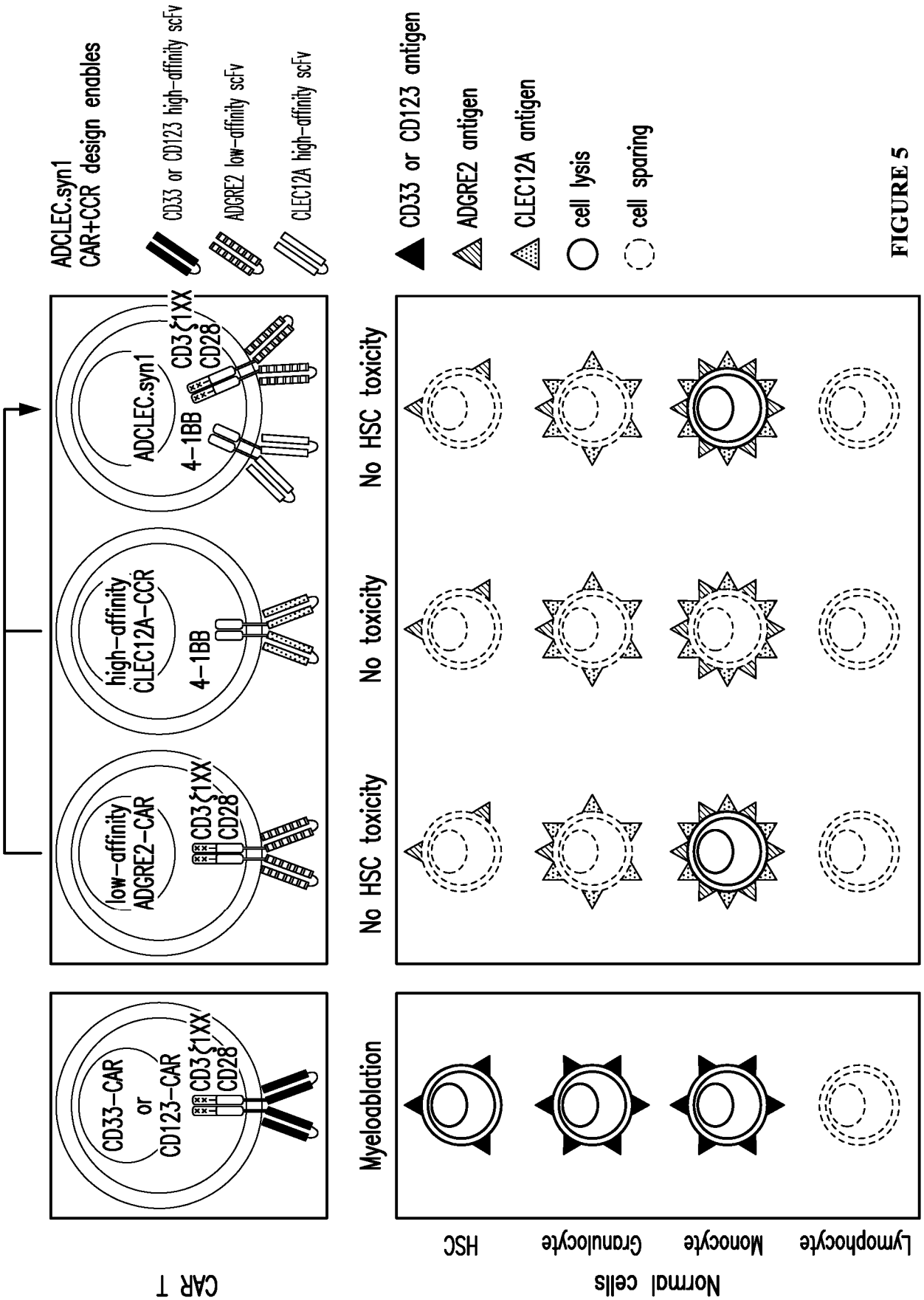

ADCLEC.syn1
CAR+CCR design enables
CD33 or CD123 high-affinity scFv
ADGRE2 low-affinity scFv
CLEC12A high-affinity scFv
CD33 or CD123 antigen
ADGRE2 antigen
CLEC12A antigen
cell lysis
cell sparing
CAR T
CD33-CAR or CD123-CAR
CD3ζ1XX
CD28
low-affinity ADGRE2-CAR
high-affinity CLEC12A-CCR
4-1BB
ADCLEC.syn1
Myeloablation
No HSC toxicity
No toxicity
No HSC toxicity
Normal cells
HSC
Granulocyte
Monocyte
Lymphocyte

FIGURE 5

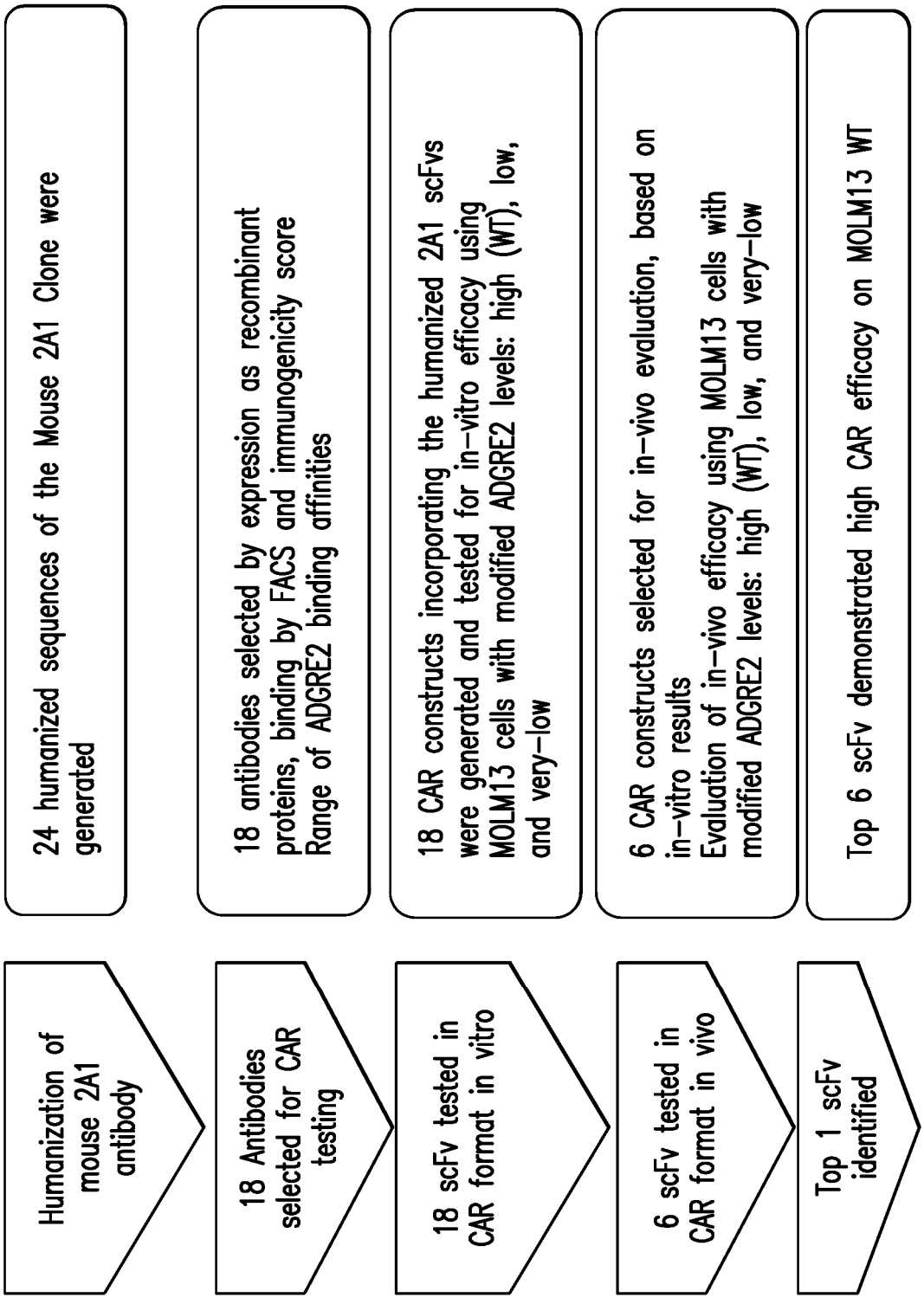

Humanization of mouse 2A1 antibody
24 humanized sequences of the Mouse 2A1 Clone were generated
18 Antibodies selected for CAR testing
18 antibodies selected by expression as recombinant proteins, binding by FACS and immunogenicity score
Range of ADGRE2 binding affinities
18 scFv tested in CAR format in vitro
18 CAR constructs incorporating the humanized 2A1 scFvs were generated and tested for in-vitro efficacy using MOLM13 cells with modified ADGRE2 levels: high (WT), low, and very-low
6 scFv tested in CAR format in vivo
6 CAR constructs selected for in-vivo evaluation, based on in-vitro results
Evaluation of in-vivo efficacy using MOLM13 cells with modified ADGRE2 levels: high (WT), low, and very-low
Top 1 scFv identified
Top 6 scFv demonstrated high CAR efficacy on MOLM13 WT

FIGURE 6

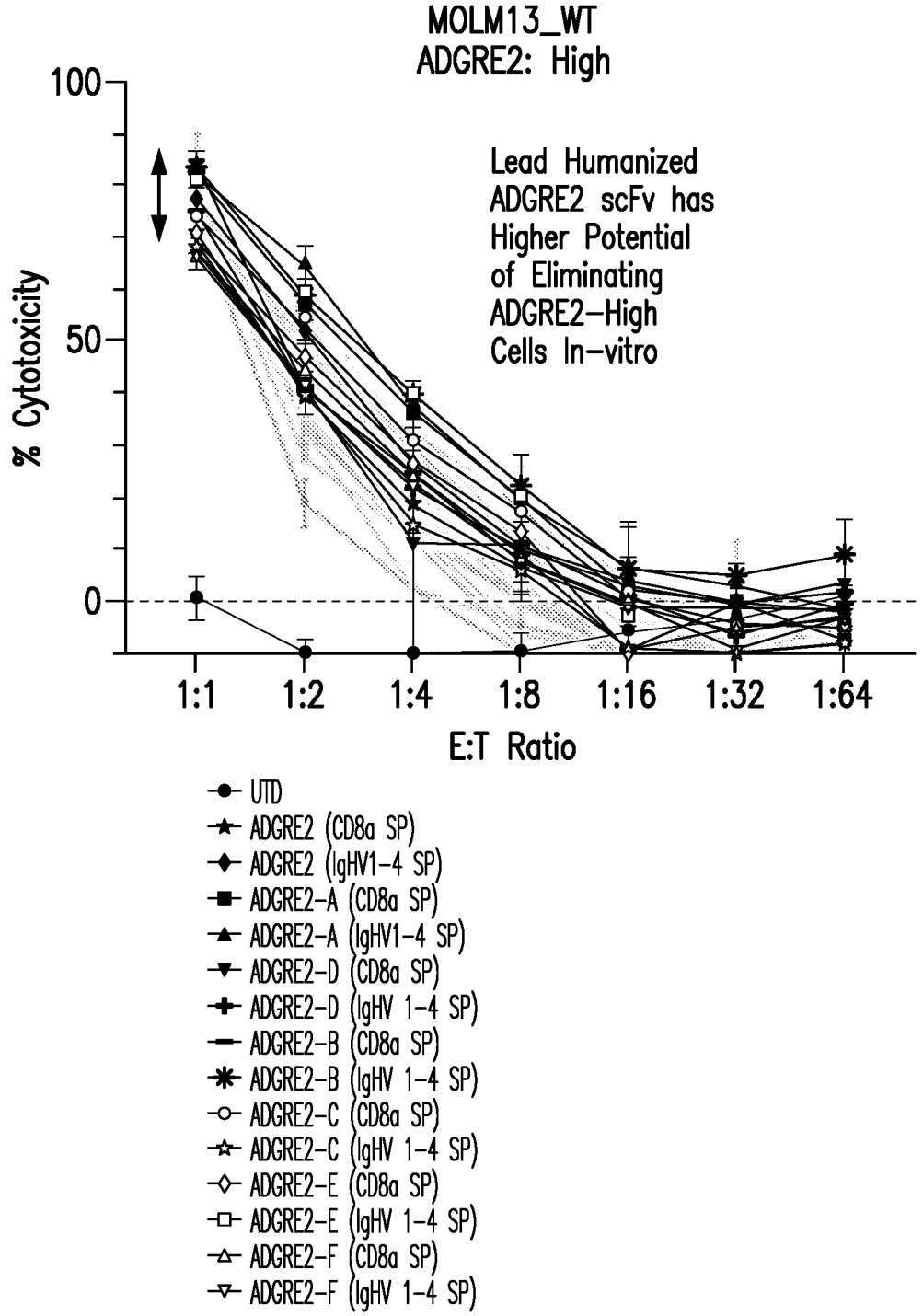

MOLM13_WT
ADGRE2: High
Lead Humanized
ADGRE2 scFv has
Higher Potential
of Eliminating
ADGRE2–High
Cells In–vitro
% Cytotoxicity
100
50
0
1:1
1:2
1:4
1:8
1:16
1:32
1:64
E:T Ratio
UTD
ADGRE2 (CD8α SP)
ADGRE2 (IgHV1–4 SP)
ADGRE2–A (CD8α SP)
ADGRE2–A (IgHV1–4 SP)
ADGRE2–D (CD8α SP)
ADGRE2–D (IgHV 1–4 SP)
ADGRE2–B (CD8α SP)
ADGRE2–B (IgHV 1–4 SP)
ADGRE2–C (CD8α SP)
ADGRE2–C (IgHV 1–4 SP)
ADGRE2–E (CD8α SP)
ADGRE2–E (IgHV 1–4 SP)
ADGRE2–F (CD8α SP)
ADGRE2–F (IgHV 1–4 SP)

FIGURE 7A

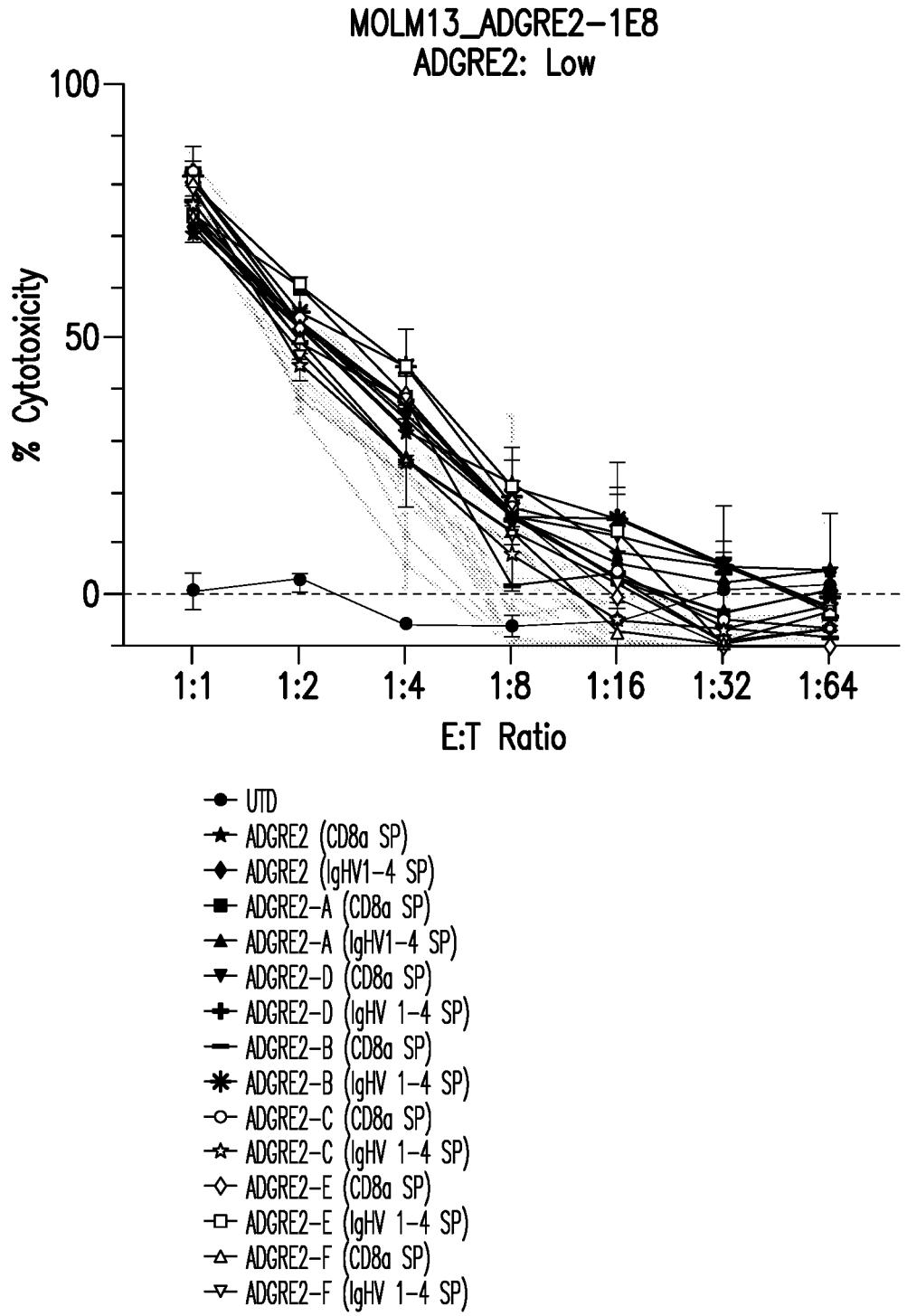

MOLM13_ADGRE2-1E8
ADGRE2: Low
100
50
0
% Cytotoxicity
1:1
1:2
1:4
1:8
1:16
1:32
1:64
E:T Ratio
UTD
ADGRE2 (CD8α SP)
ADGRE2 (IgHV1-4 SP)
ADGRE2-A (CD8α SP)
ADGRE2-A (IgHV1-4 SP)
ADGRE2-D (CD8α SP)
ADGRE2-D (IgHV 1-4 SP)
ADGRE2-B (CD8α SP)
ADGRE2-B (IgHV 1-4 SP)
ADGRE2-C (CD8α SP)
ADGRE2-C (IgHV 1-4 SP)
ADGRE2-E (CD8α SP)
ADGRE2-E (IgHV 1-4 SP)
ADGRE2-F (CD8α SP)
ADGRE2-F (IgHV 1-4 SP)

FIGURE 7B

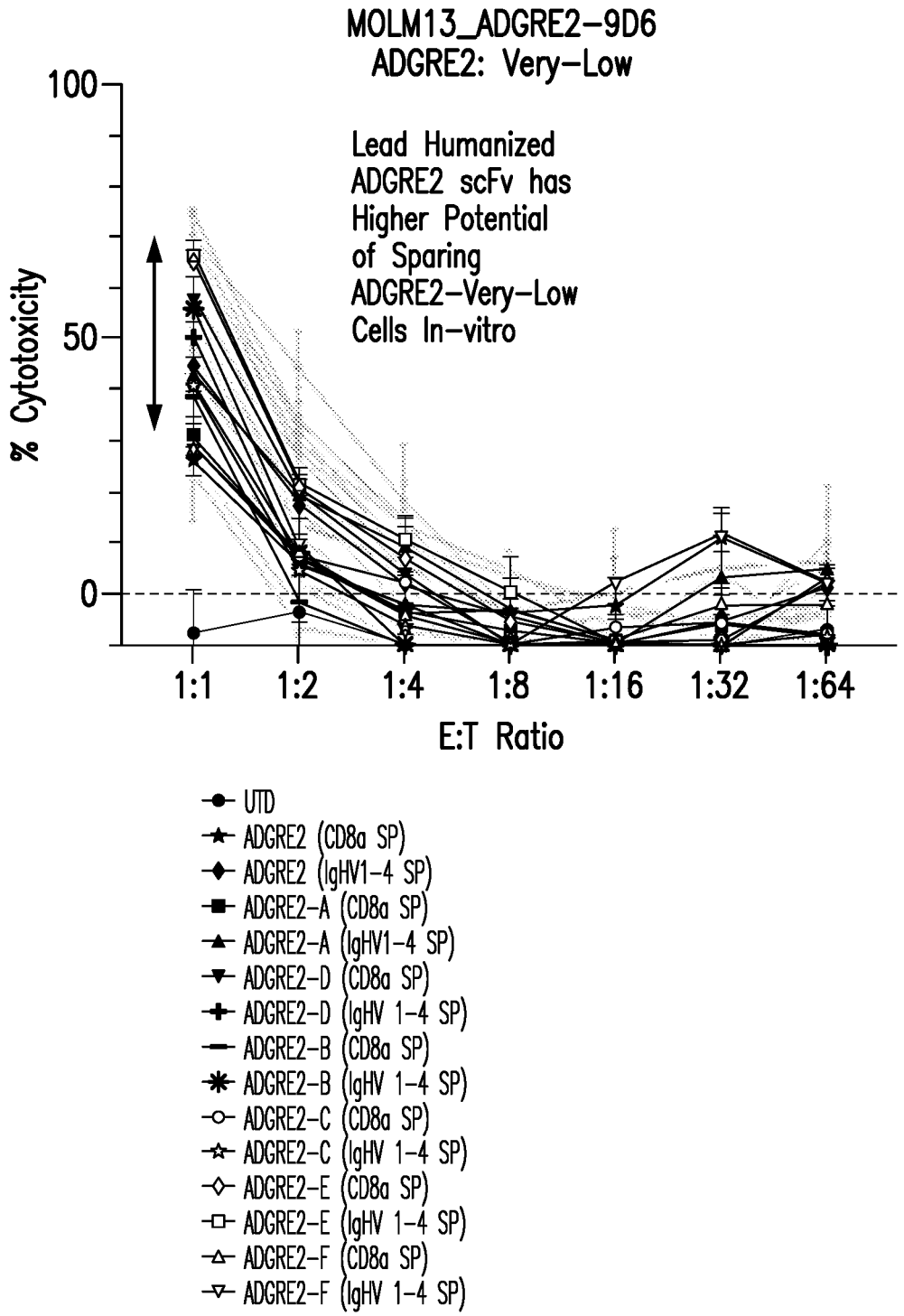

MOLM13_ADGRE2-9D6
ADGRE2: Very-Low
Lead Humanized ADGRE2 scFv has Higher Potential of Sparing ADGRE2-Very-Low Cells In-vitro
% Cytotoxicity
100
50
0
1:1
1:2
1:4
1:8
1:16
1:32
1:64
E:T Ratio
UTD
ADGRE2 (CD8α SP)
ADGRE2 (IgHV1-4 SP)
ADGRE2-A (CD8α SP)
ADGRE2-A (IgHV1-4 SP)
ADGRE2-D (CD8α SP)
ADGRE2-D (IgHV 1-4 SP)
ADGRE2-B (CD8α SP)
ADGRE2-B (IgHV 1-4 SP)
ADGRE2-C (CD8α SP)
ADGRE2-C (IgHV 1-4 SP)
ADGRE2-E (CD8α SP)
ADGRE2-E (IgHV 1-4 SP)
ADGRE2-F (CD8α SP)
ADGRE2-F (IgHV 1-4 SP)

FIGURE 7C

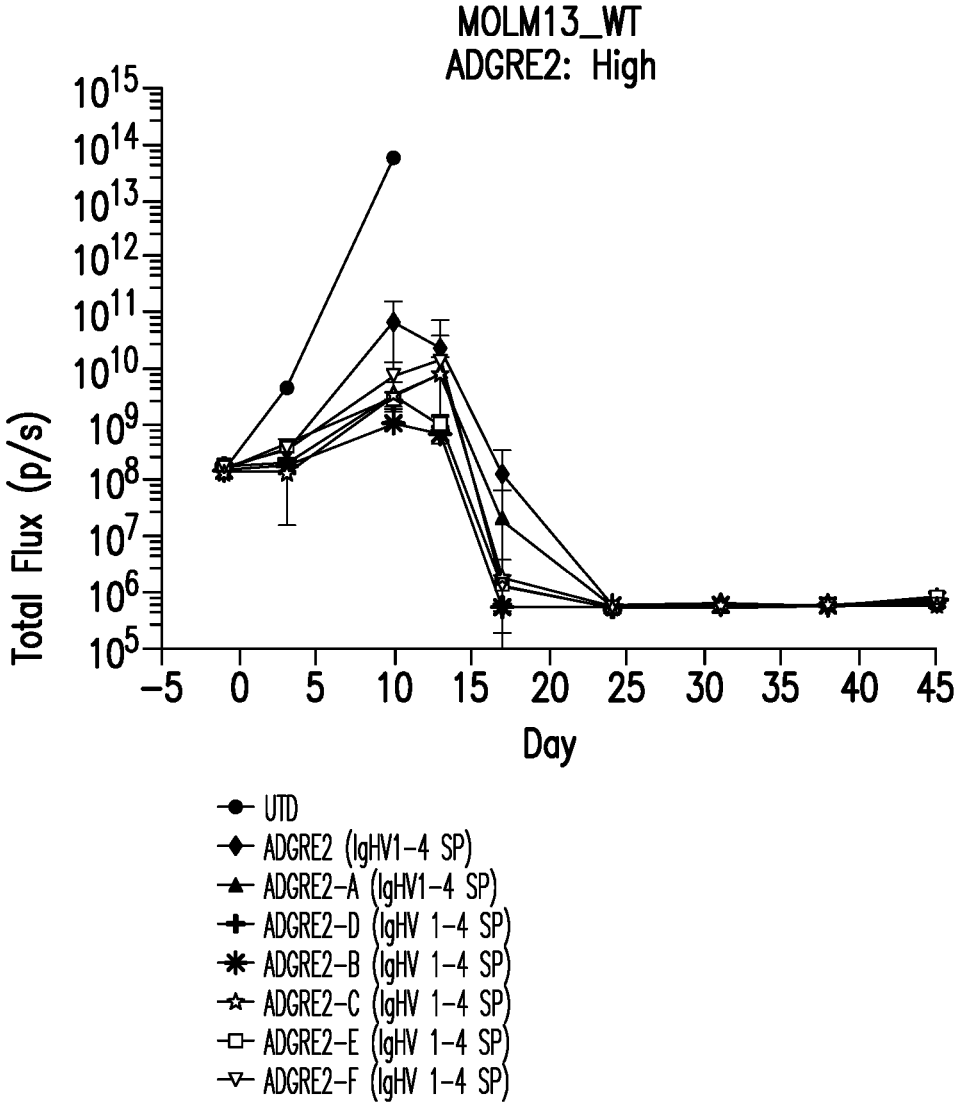

MOLM13_WT
ADGRE2: High
Total Flux (p/s)
10^{15}
10^{14}
10^{13}
10^{12}
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
−5 0 5 10 15 20 25 30 35 40 45
Day
UTD
ADGRE2 (IgHV1-4 SP)
ADGRE2-A (IgHV1-4 SP)
ADGRE2-D (IgHV 1-4 SP)
ADGRE2-B (IgHV 1-4 SP)
ADGRE2-C (IgHV 1-4 SP)
ADGRE2-E (IgHV 1-4 SP)
ADGRE2-F (IgHV 1-4 SP)

FIGURE 8

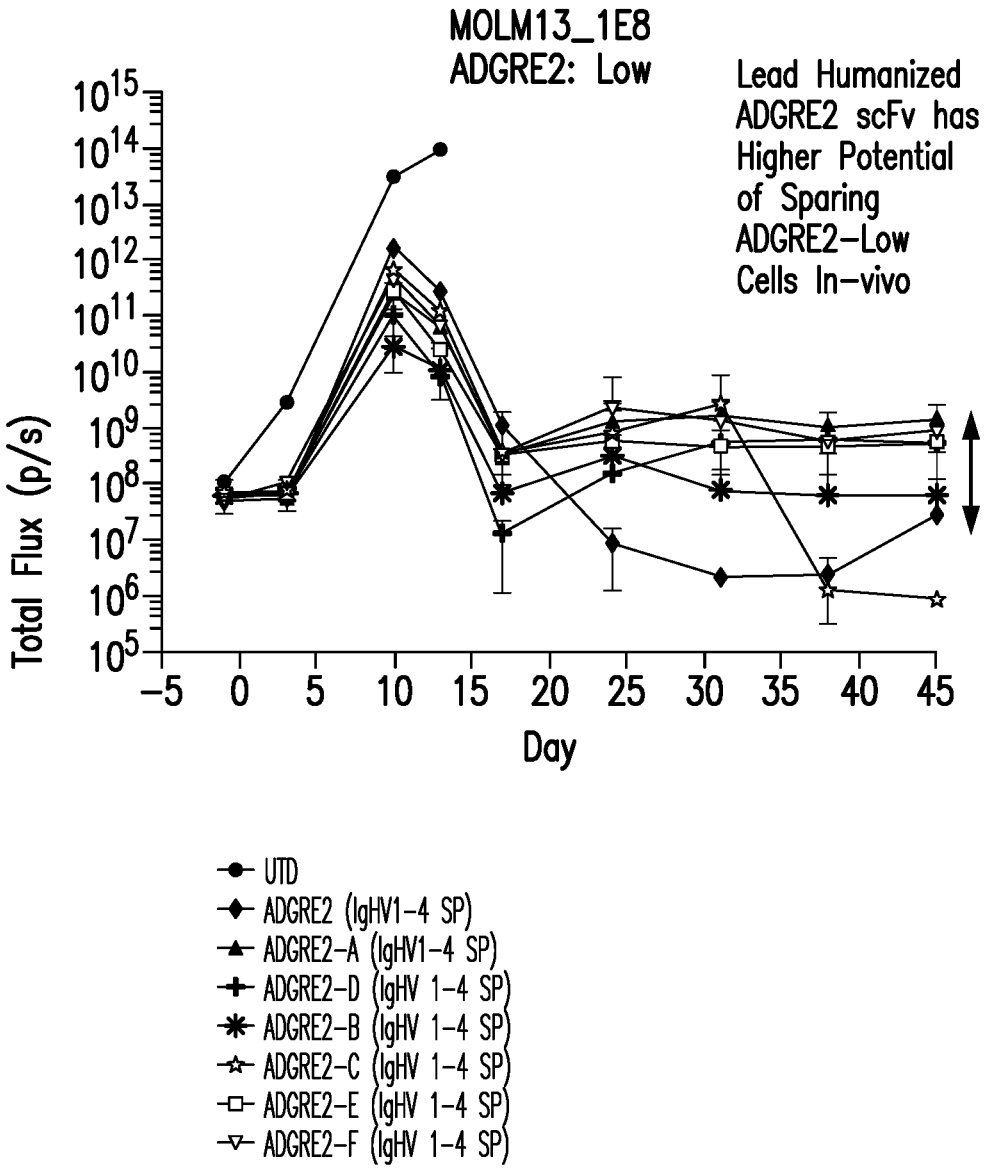


FIGURE 8 Continued

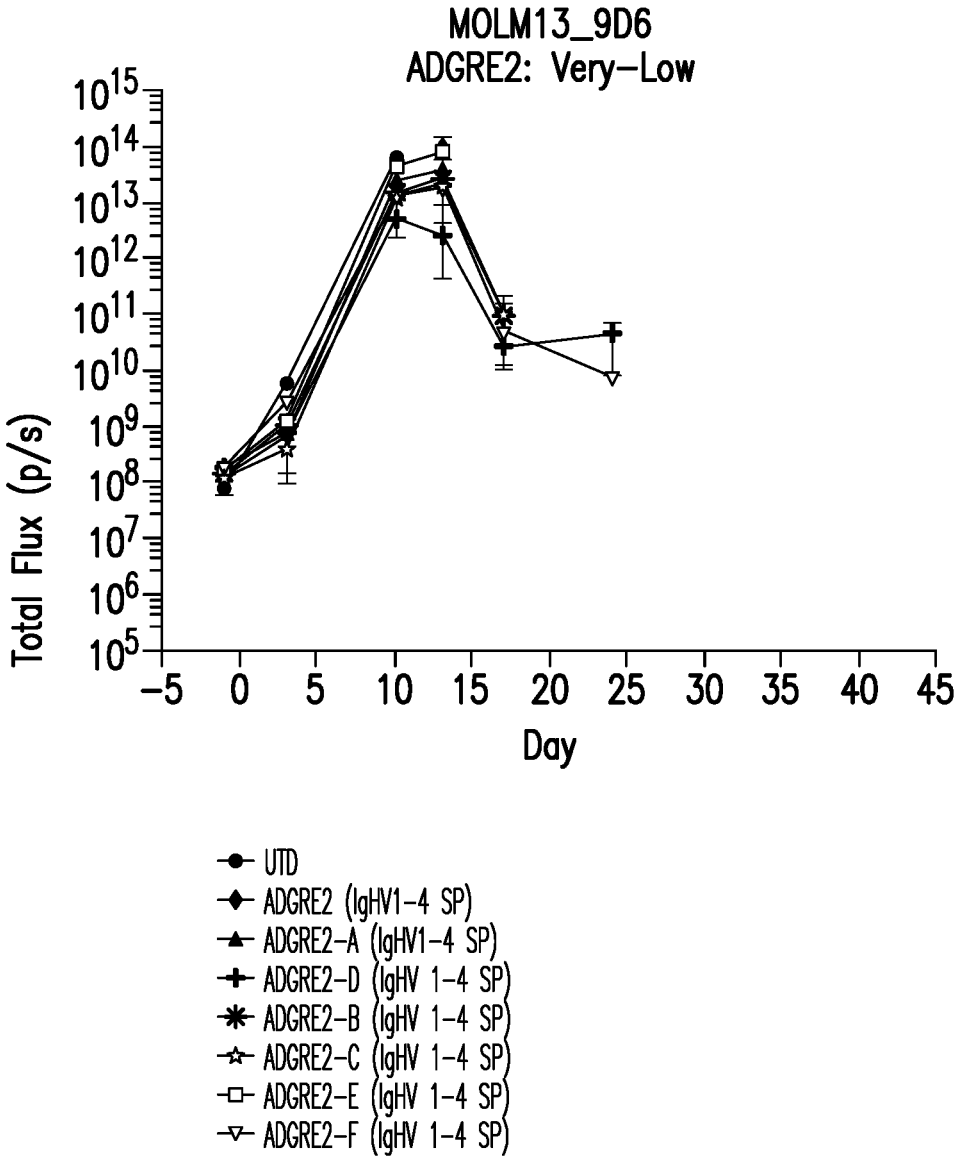


FIGURE 8 Continued

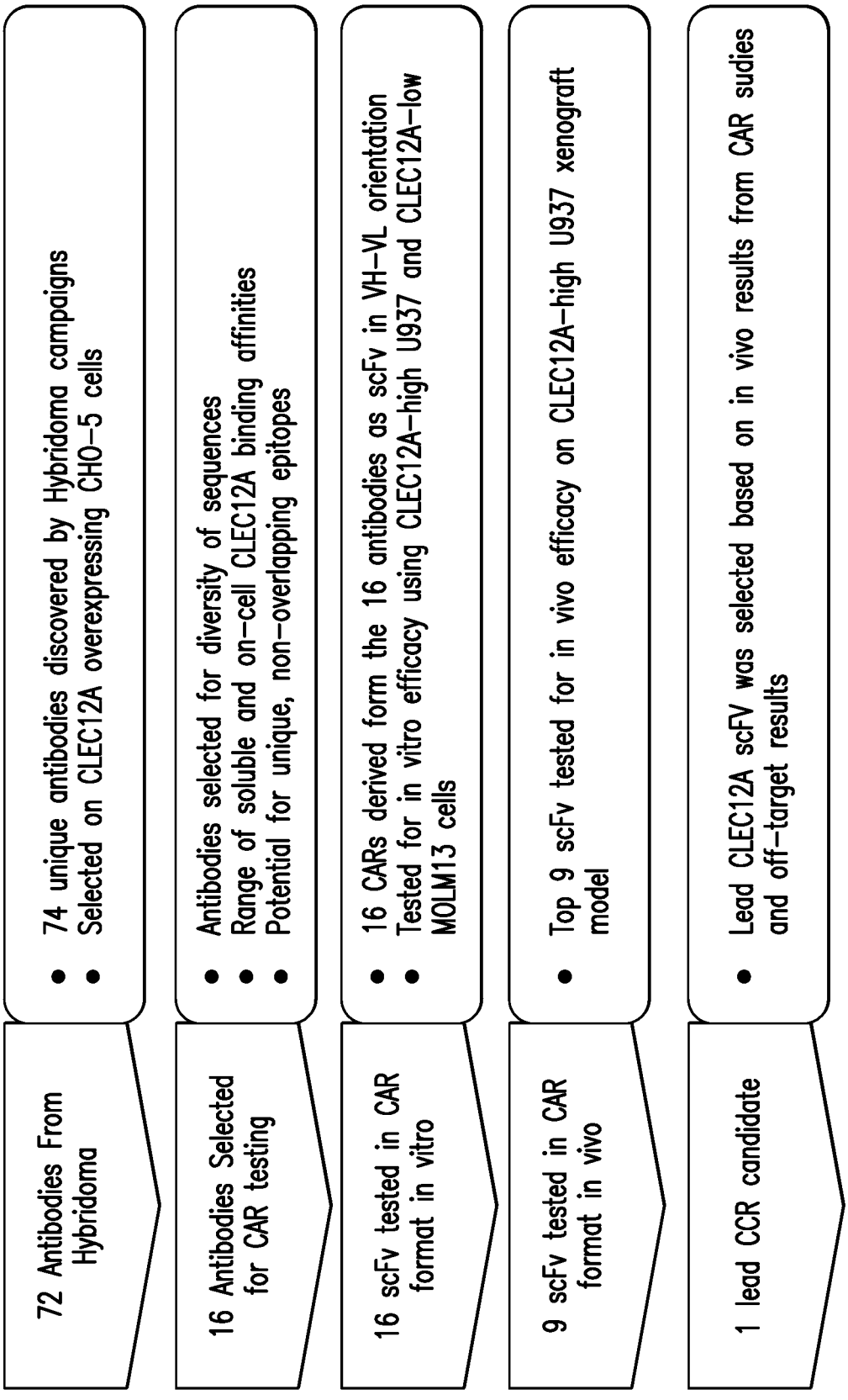

72 Antibodies From Hybridoma
74 unique antibodies discovered by Hybridoma campaigns
Selected on CLEC12A overexpressing CHO-5 cells
16 Antibodies Selected for CAR testing
Antibodies selected for diversity of sequences
Range of soluble and on-cell CLEC12A binding affinities
Potential for unique, non-overlapping epitopes
16 scFv tested in CAR format in vitro
16 CARs derived form the 16 antibodies as scFv in VH-VL orientation
Tested for in vitro efficacy using CLEC12A-high U937 and CLEC12A-low MOLM13 cells
9 scFv tested in CAR format in vivo
Top 9 scFv tested for in vivo efficacy on CLEC12A-high U937 xenograft model
1 lead CCR candidate
Lead CLEC12A scFV was selected based on in vivo results from CAR sudies and off-target results

FIGURE 9

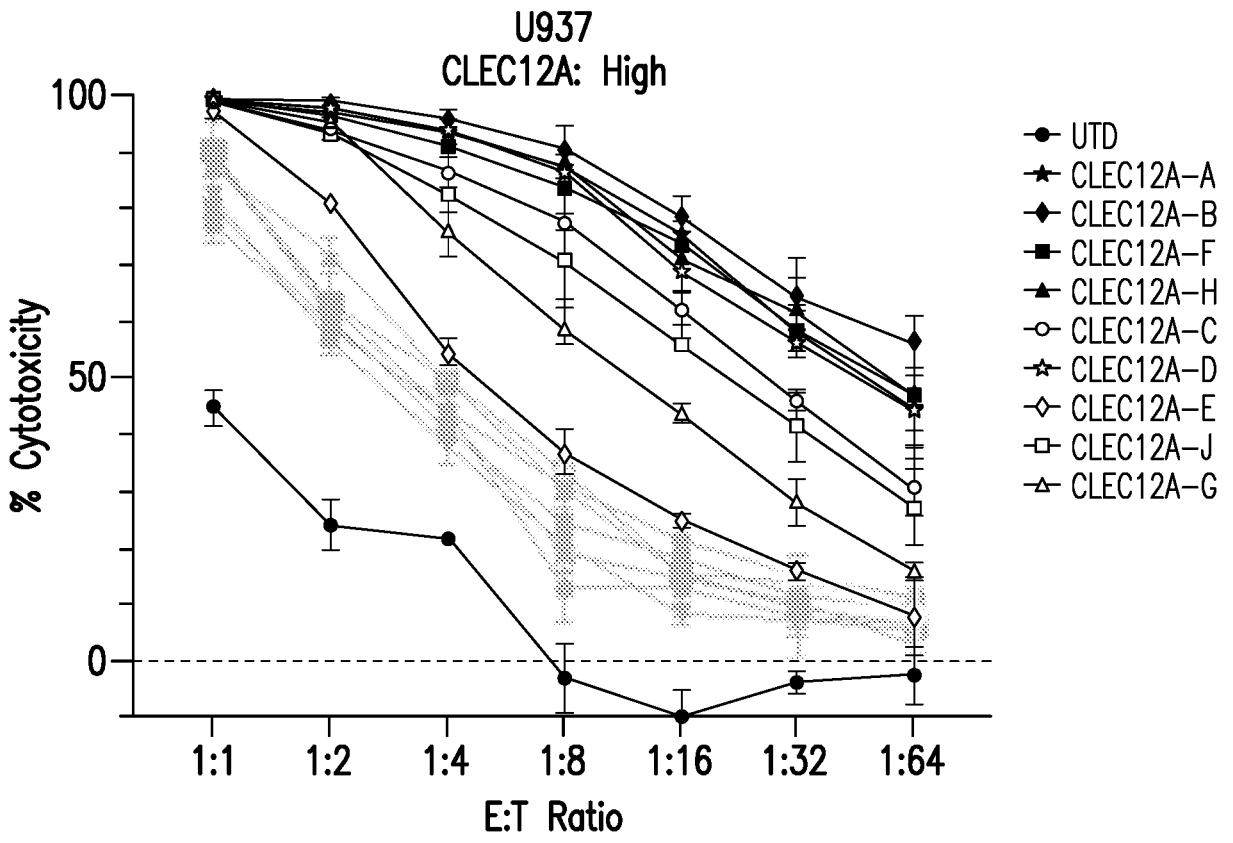


FIGURE 10A

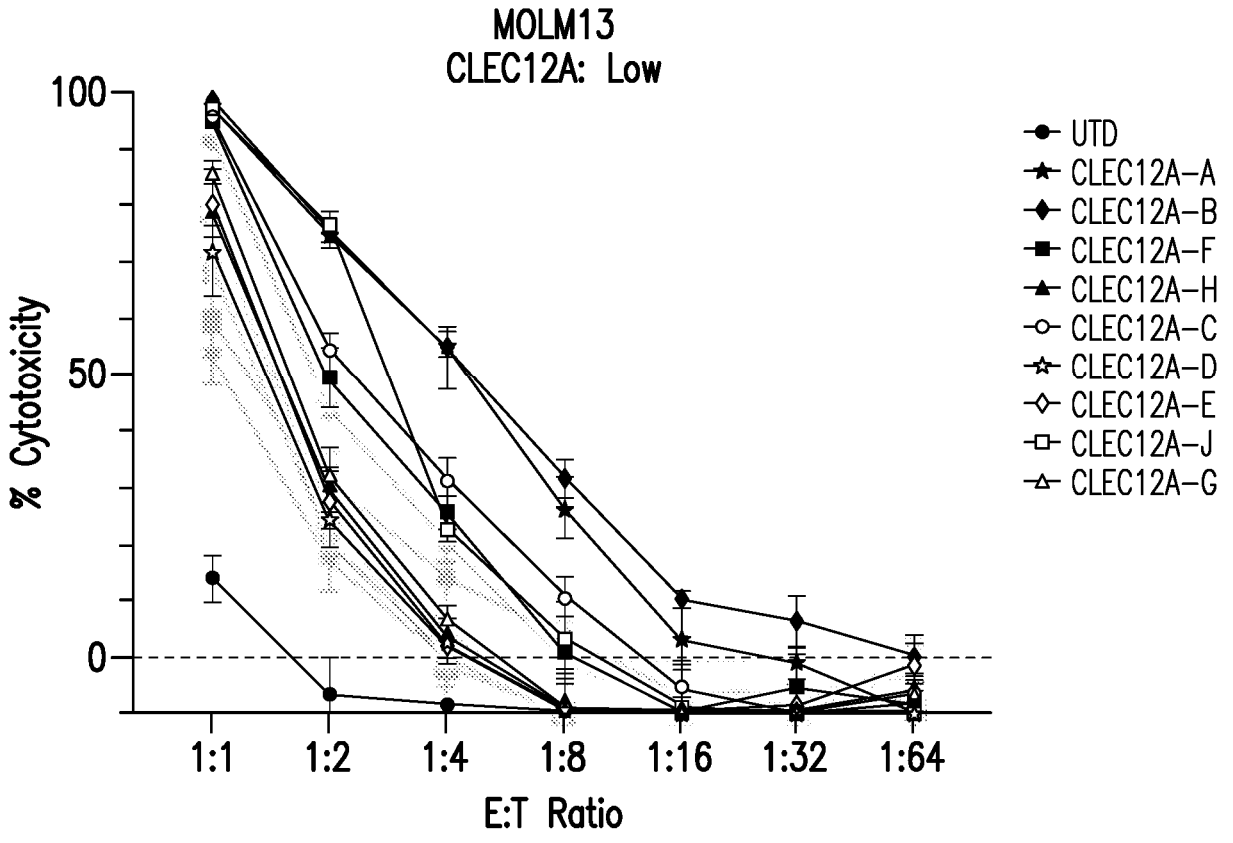


FIGURE 10B

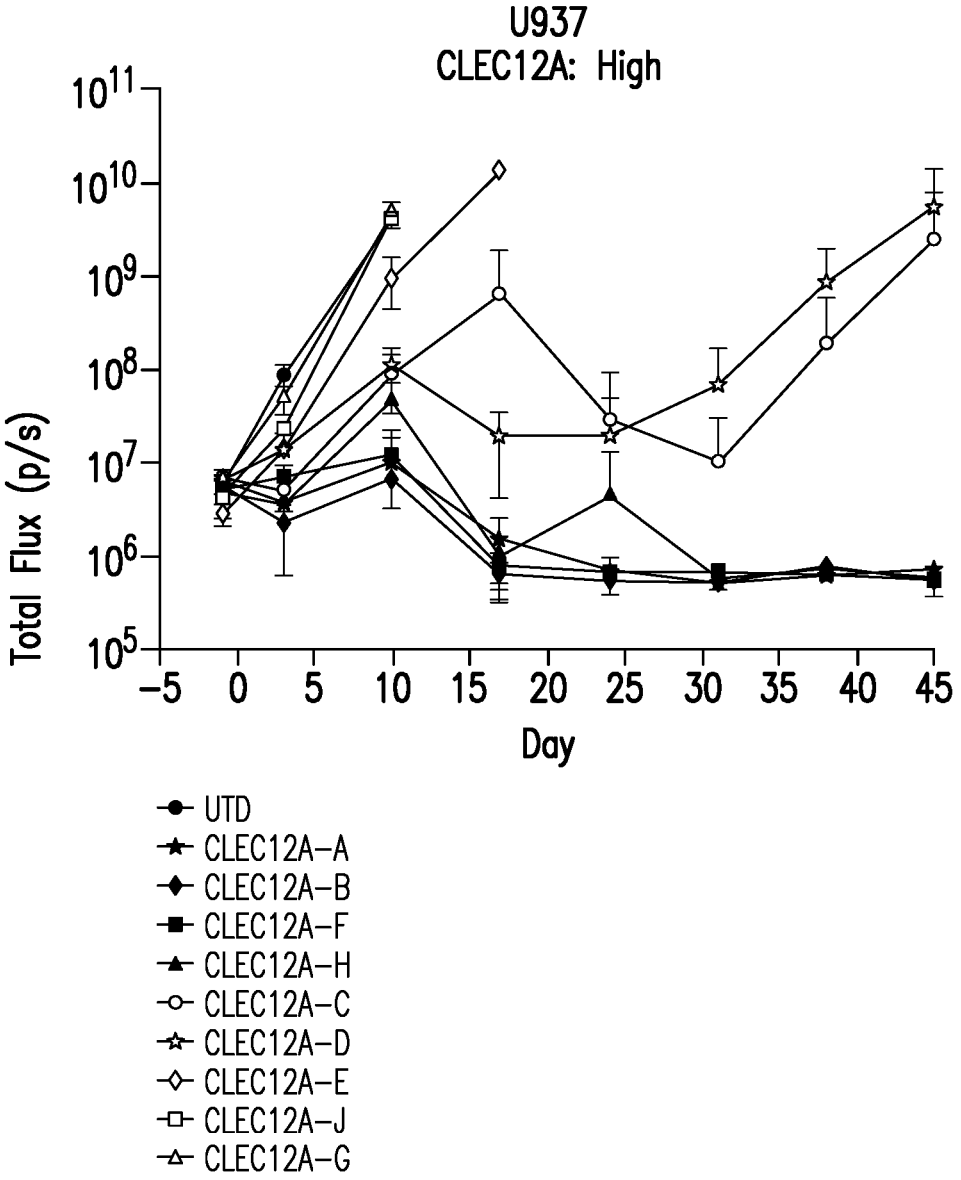
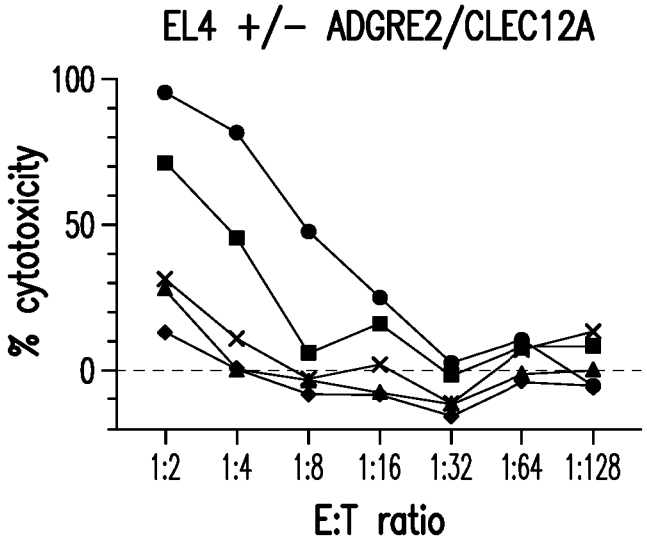

U937
CLEC12A: High
Total Flux (p/s)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
−5 0 5 10 15 20 25 30 35 40 45
Day
UTD
CLEC12A–A
CLEC12A–B
CLEC12A–F
CLEC12A–H
CLEC12A–C
CLEC12A–D
CLEC12A–E
CLEC12A–J
CLEC12A–G

FIGURE 11



CAR construct	Target cell line	Scenario
ADCLEC.syn1	ADGRE2-/CLEC12A-	No target antigen
ADCLEC.syn1	ADGRE2+/CLEC12A-	Only CAR target antigen
ADCLEC.syn1	ADGRE2-/CLEC12A+	Only CCR target antigen
ADCLEC.syn1	ADGRE2+/CLEC12A+	CAR and CCR target antigens
1928z1XX	CD19-	No target antigen

FIGURE 12A

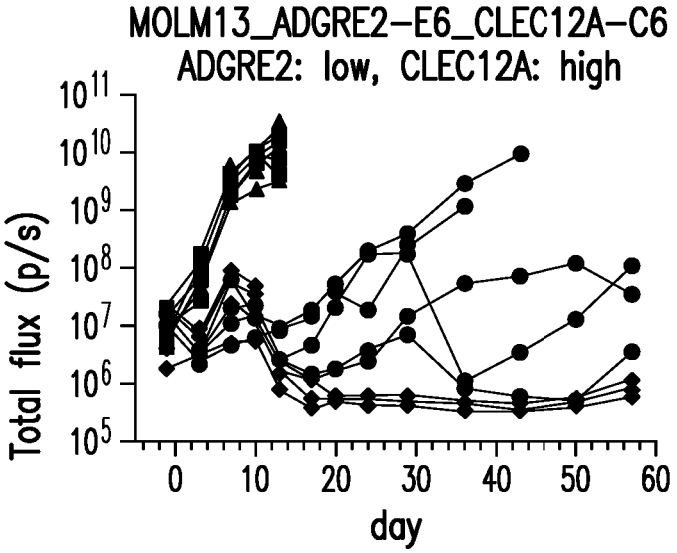


FIGURE 12B

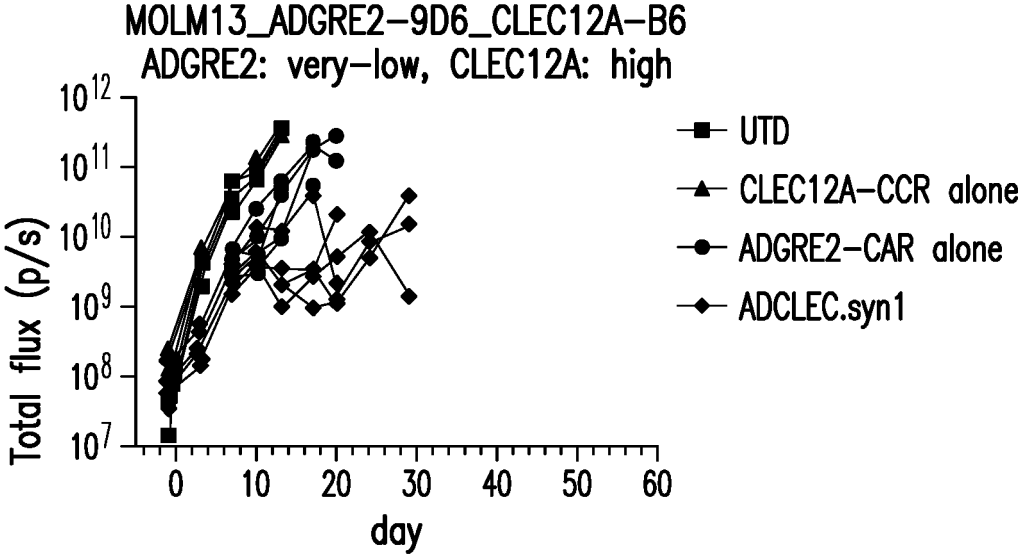


FIGURE 12C

SUBSTITUTE SHEET (RULE 26)

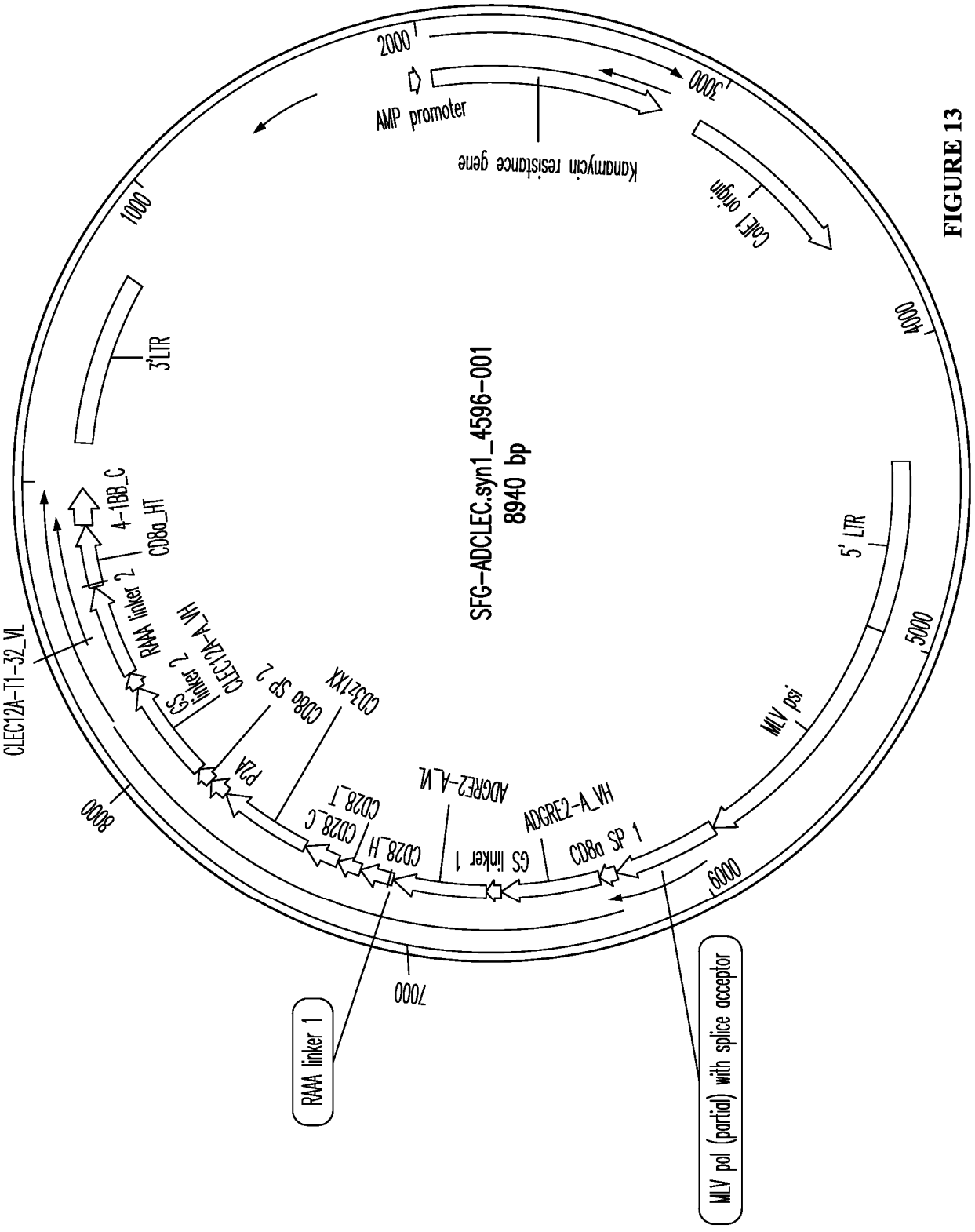

SFG-ADCLEC.syn1_4596-001
8940 bp
2000
3000
AMP promoter
Kanamycin resistance gene
ColE1 origin
1000
4000
3'LTR
5' LTR
5000
MLV psi
6000
CD8a SP 1
ADGRE2-A_VH
GS linker 1
ADGRE2-A_VL
CD28_H
CD28_C
CD28_I
7000
P2A
CD8a SP 2
CD32-1XX
GS linker 2
CLEC12A-A_VH
RAAA linker 2
CD8a_HT
4-1BB_C
8000
CLEC12A-T1-32_VL
RAAA linker 1
MLV pol (partial) with splice acceptor

FIGURE 13

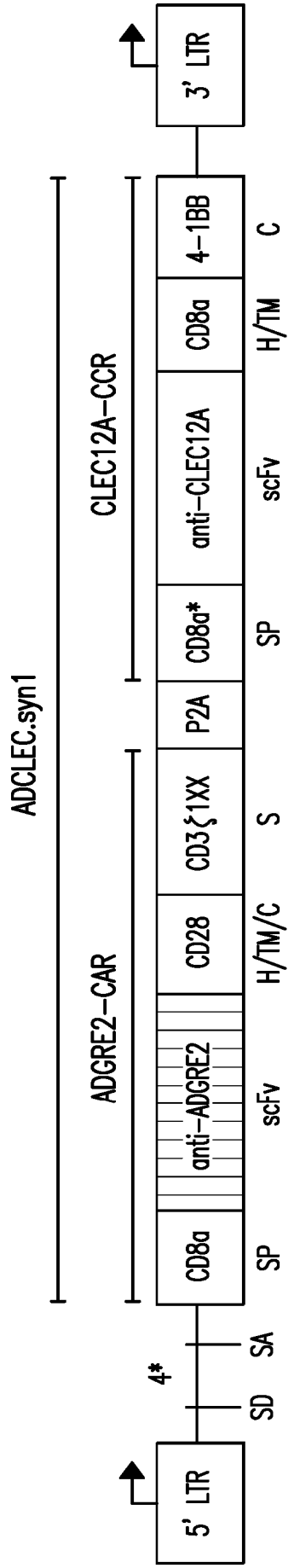

5' LTR
4*
SD
SA
CD8α
SP
anti-ADGRE2
scFv
CD28
H/TM/C
CD3ζ1XX
S
P2A
CD8α*
SP
anti-CLEC12A
scFv
CD8α
H/TM
4-1BB
C
3' LTR
ADCLEC.syn1
ADGRE2-CAR
CLEC12A-CCR

FIGURE 14

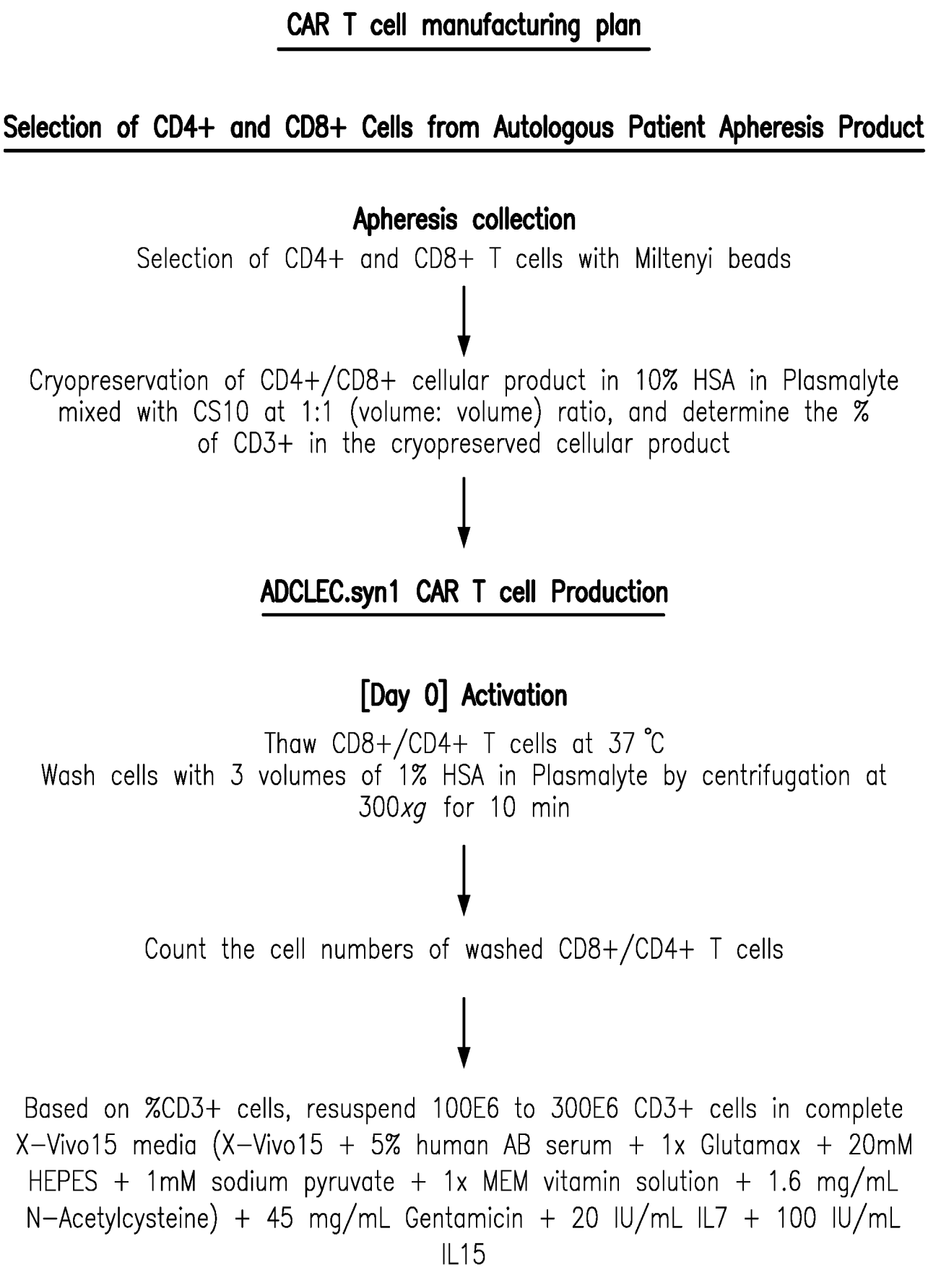

CAR T cell manufacturing plan
Selection of CD4+ and CD8+ Cells from Autologous Patient Apheresis Product
Apheresis collection
Selection of CD4+ and CD8+ T cells with Miltenyi beads
Cryopreservation of CD4+/CD8+ cellular product in 10% HSA in Plasmalyte mixed with CS10 at 1:1 (volume: volume) ratio, and determine the % of CD3+ in the cryopreserved cellular product
ADCLEC.syn1 CAR T cell Production
[Day 0] Activation
Thaw CD8+/CD4+ T cells at 37 °C
Wash cells with 3 volumes of 1% HSA in Plasmalyte by centrifugation at 300xg for 10 min
Count the cell numbers of washed CD8+/CD4+ T cells
Based on %CD3+ cells, resuspend 100E6 to 300E6 CD3+ cells in complete X-Vivo15 media (X-Vivo15 + 5% human AB serum + 1x Glutamax + 20mM HEPES + 1mM sodium pyruvate + 1x MEM vitamin solution + 1.6 mg/mL N-Acetylcysteine) + 45 mg/mL Gentamicin + 20 IU/mL IL7 + 100 IU/mL IL15

FIGURE 15



Activate T cells with Dynabeads® ClinEx Vivo CD3/CD28 using a bead to T cell ratio of 1:1 for approximately 48hr in a 37 ℃ incubator with 5% CO2



[Day 2] Transduction

Transduce the activated T cells with ADCLEC.syn1 vector stocks in PL72/PL240 bag with overwrap pouch/seal. Spin at 1200 rpm for 60 min at RT at 0.5E6 cells/mL



[Day 3]

Transfer the transduced cells to the G-Rex bioreactor and grow the cells in complete X-Vivo15 media w/o gentamicin



[Day 7 and Day 10]

Spike IL7 and IL15 into the medium to maintain 20 IU/mL IL7 + 100 IU/mL IL15



[Day ≤ 14]

When the target number of cells is reached, wash the cells with 1%HSA in 1x Plasmalyte A using a closed system cell washer & debead using a ClinExVivo magnet



FIGURE 15 Continued

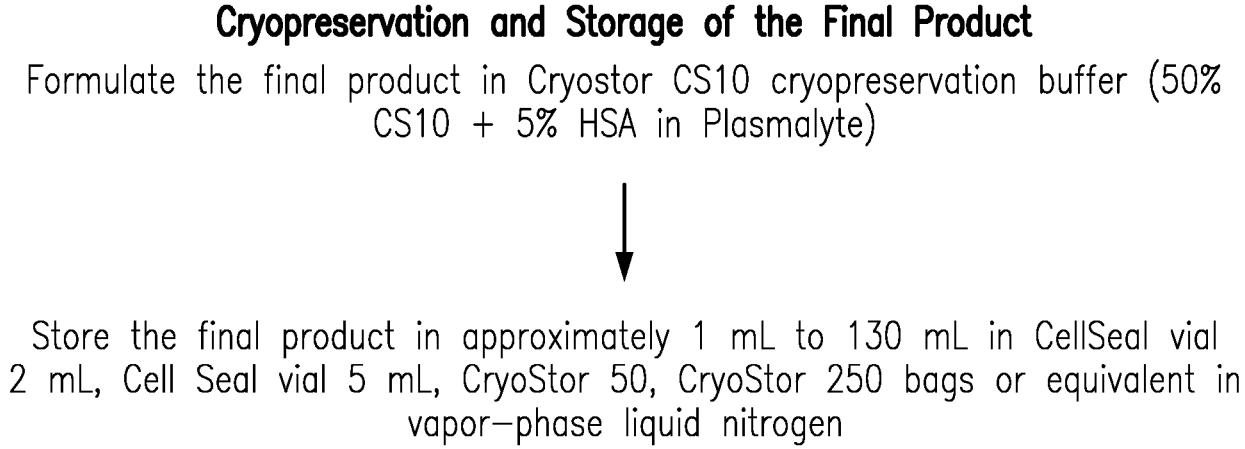
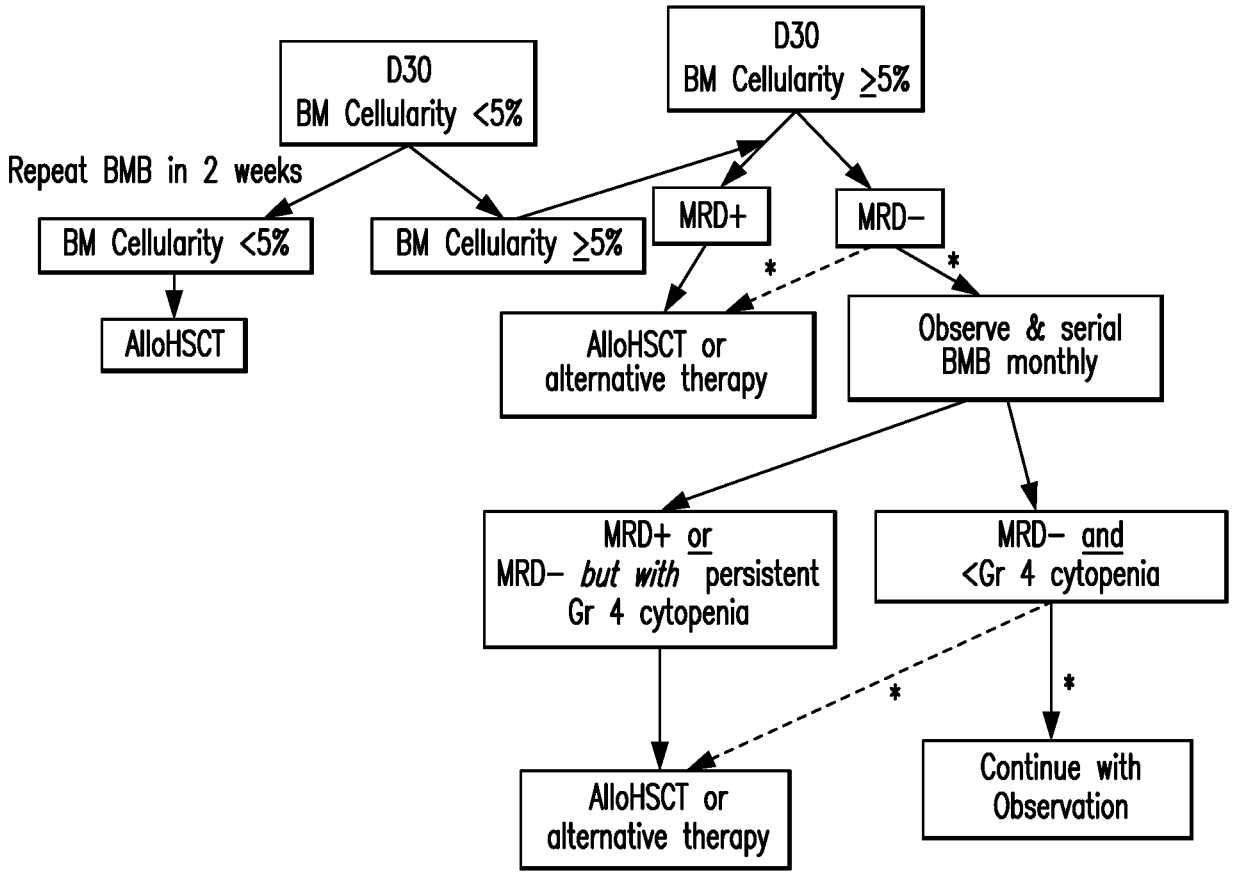


FIGURE 15 Continued



BM: bone marrow
BMB: bone marrow biopsy
MRD: measurable residual disease as assessed by flow cytometry or NGS
AlloHSCT: allogeneic hematopoietic stem cell transplant

*Decision b/w observation and alloHSCT is at the treating physician discretion and after discussion with the study PI based on the patient's prior treatments, goals of therapy and others.

FIGURE 16

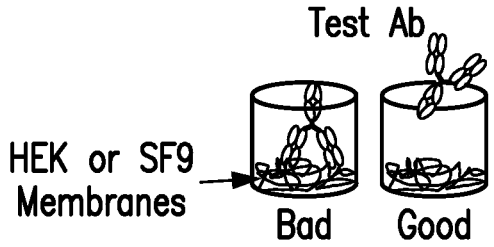


FIGURE 17A

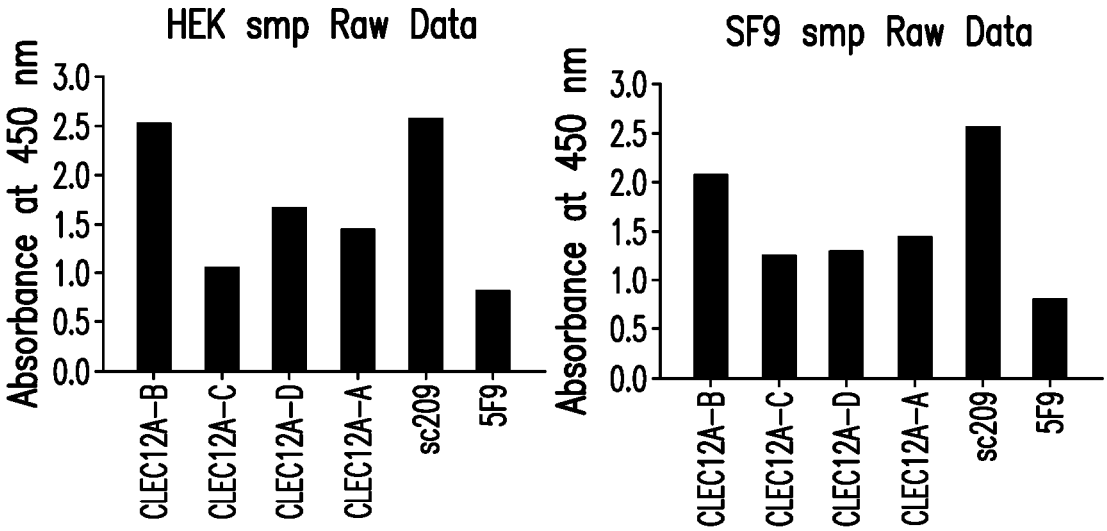


FIGURE 17B

OR–GATE
"Requires antigen A or B"
Overcome target heterogeneity
2nd–gen. CAR + 2nd–gen. CAR
Tandem CAR
CD3ζ
costim
Tumor clone 1
Tumor clone 2
Dual CAR
CD3ζ
costim
Tumor clone 1
Tumor clone 2
AND–GATE
"Requires antigen A and B"
Increases tumor selectivity
1st–gen. CAR + CCR
CD3ζ
costim
Site 1
Healthy tissue 1
Site 2
Tumor
Site 3
Healthy tissue 2
Antigen A
Antigen B

FIGURE 18A

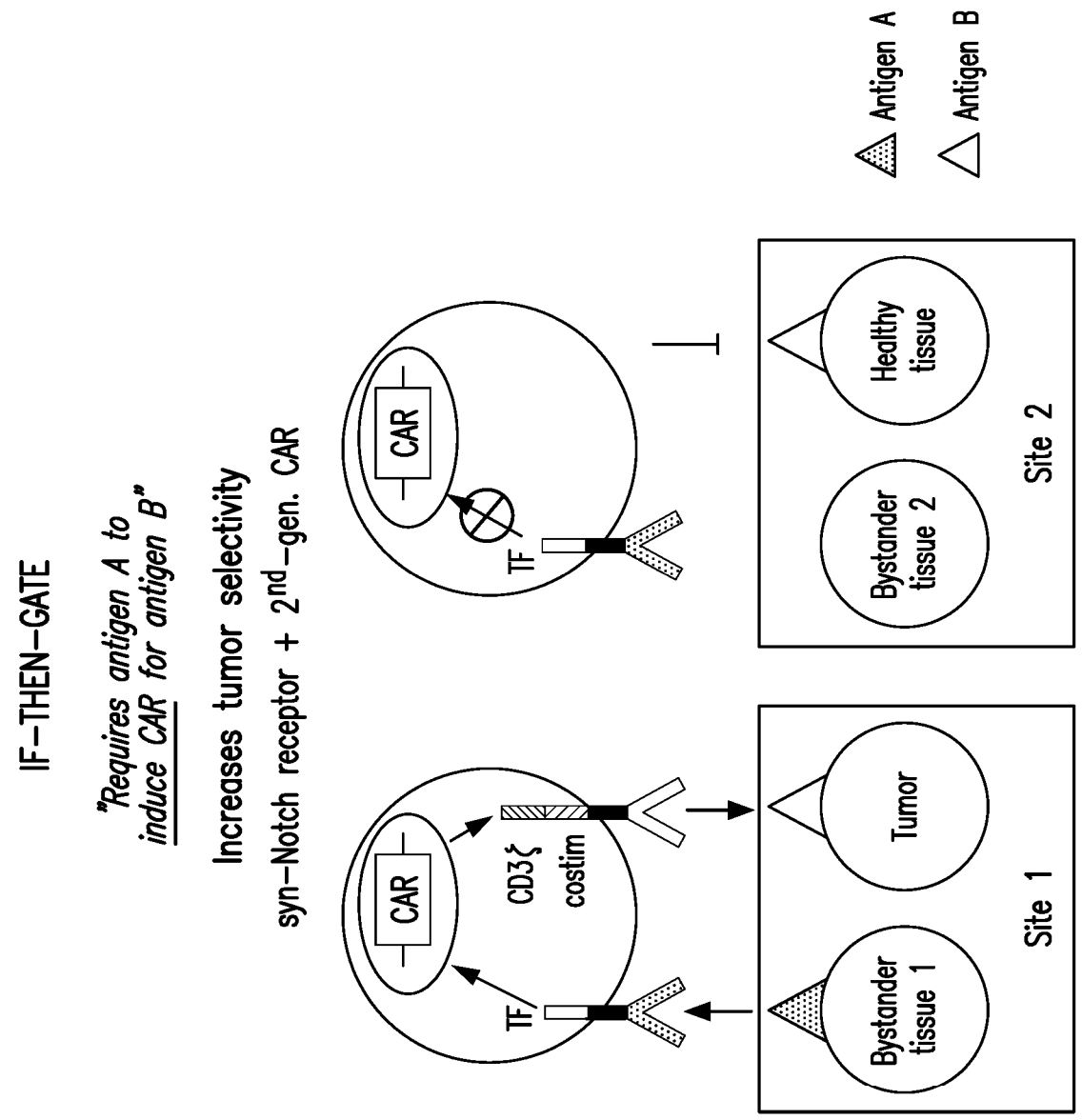


FIGURE 18A Continued

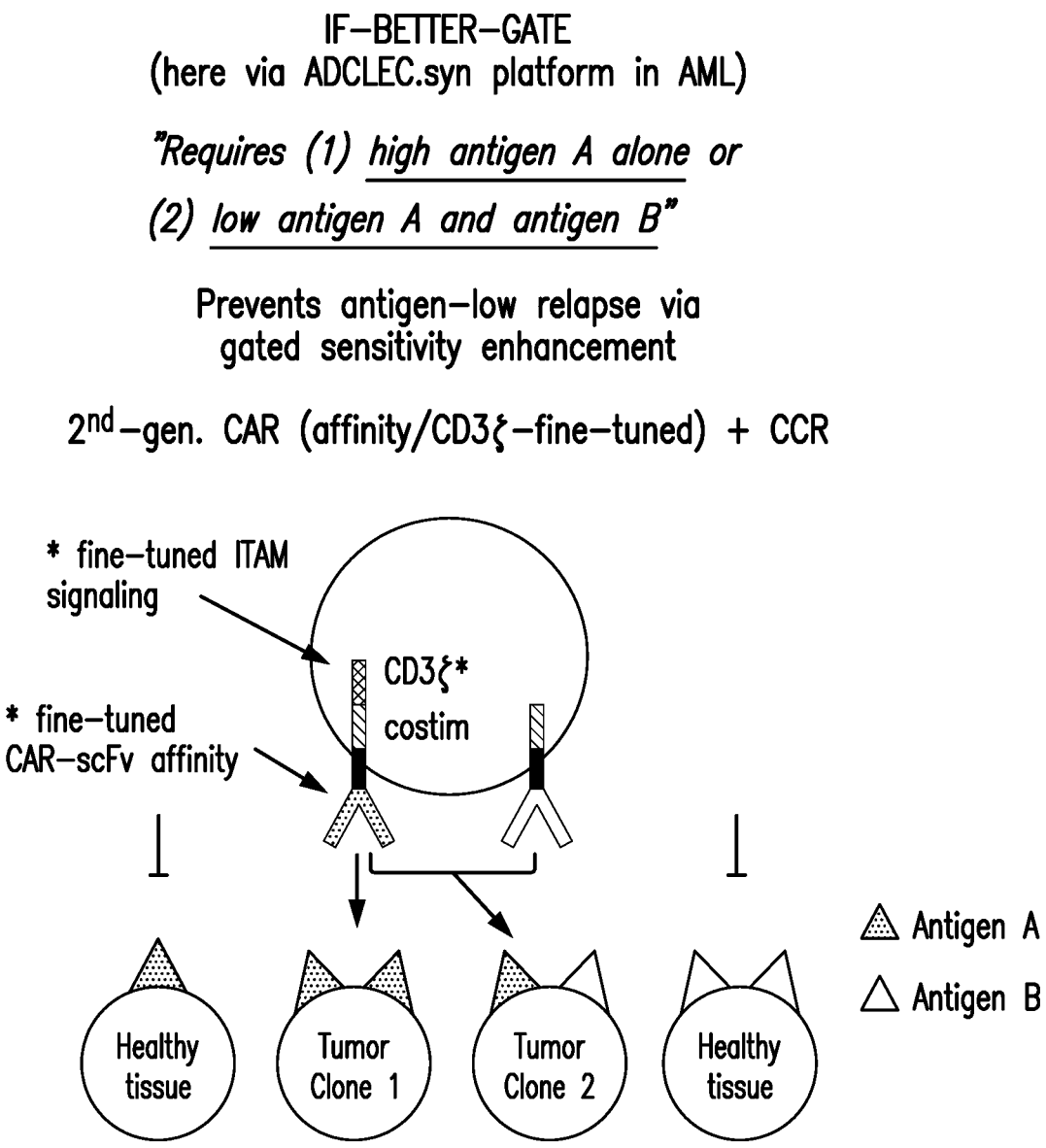

IF–BETTER–GATE
(here via ADCLEC.syn platform in AML)
"Requires (1) high antigen A alone or
(2) low antigen A and antigen B"
Prevents antigen–low relapse via
gated sensitivity enhancement
2nd–gen. CAR (affinity/CD3ζ–fine–tuned) + CCR
* fine–tuned ITAM
signaling
* fine–tuned
CAR–scFv affinity
CD3ζ*
costim
Antigen A
Antigen B
Healthy
tissue
Tumor
Clone 1
Tumor
Clone 2
Healthy
tissue

FIGURE 18B

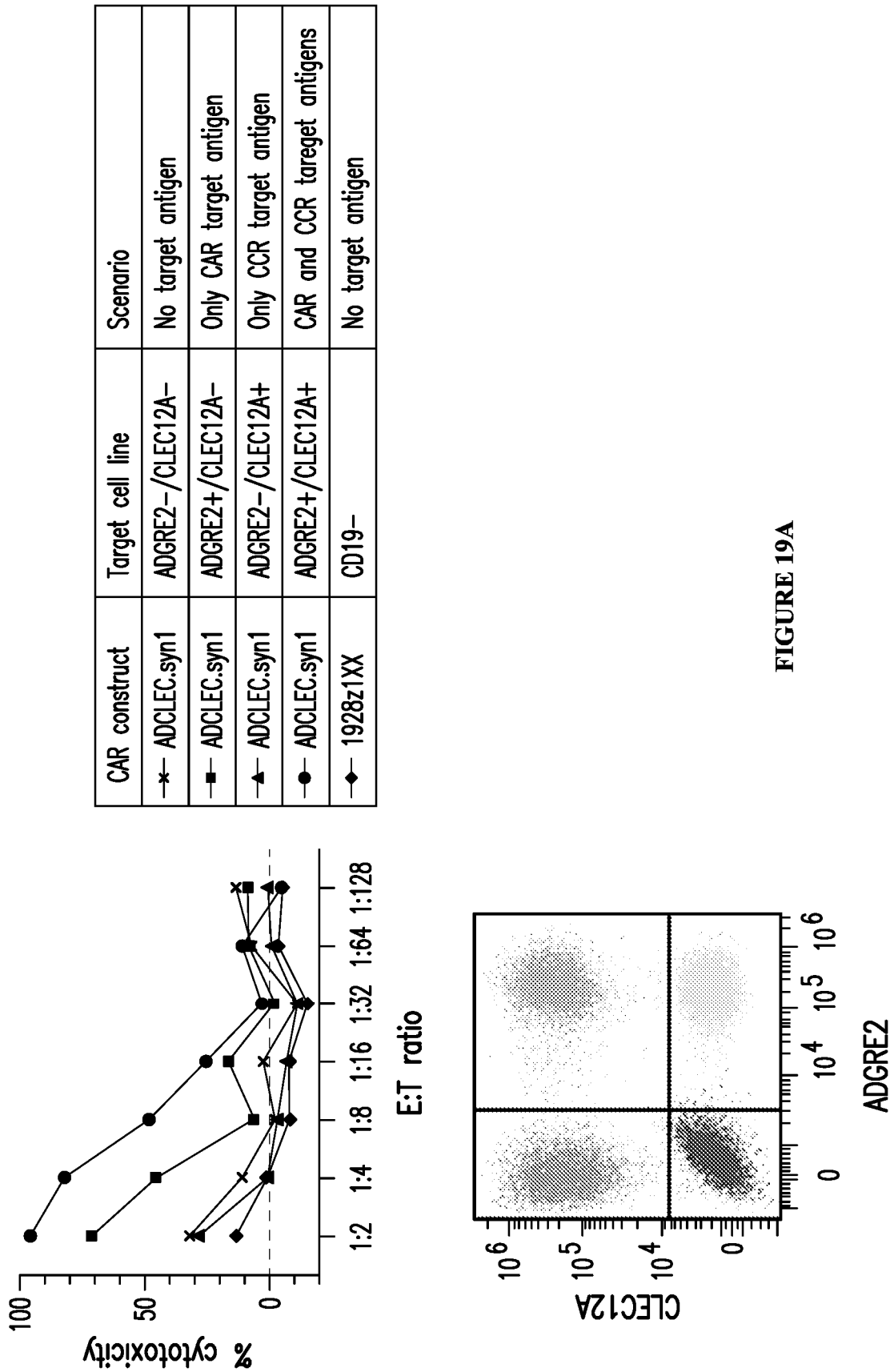

CAR construct
Target cell line
Scenario
ADCLEC.syn1
ADGRE2−/CLEC12A−
No target antigen
ADCLEC.syn1
ADGRE2+/CLEC12A−
Only CAR target antigen
ADCLEC.syn1
ADGRE2−/CLEC12A+
Only CCR target antigen
ADCLEC.syn1
ADGRE2+/CLEC12A+
CAR and CCR tareget antigens
1928z1XX
CD19−
No target antigen
% cytotoxicity
100
50
0
1:2
1:4
1:8
1:16
1:32
1:64
1:128
E:T ratio
CLEC12A
ADGRE2
0
10^4
10^5
10^6

FIGURE 19A

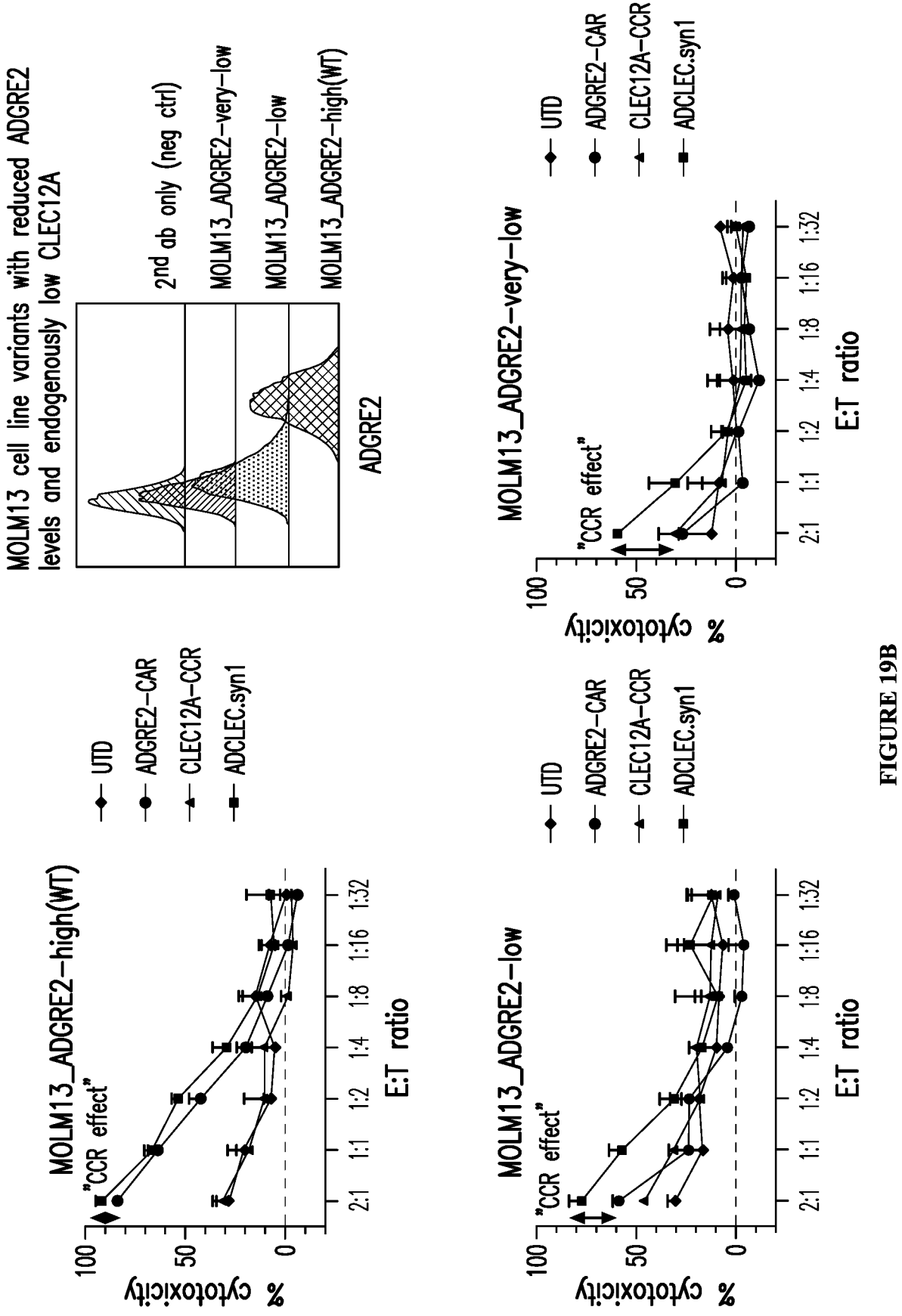

MOLM13 cell line variants with reduced ADGRE2 levels and endogenously low CLEC12A
2nd ab only (neg ctrl)
MOLM13_ADGRE2-very-low
MOLM13_ADGRE2-low
MOLM13_ADGRE2-high(WT)
ADGRE2
UTD
ADGRE2-CAR
CLEC12A-CCR
ADCLEC.syn1
MOLM13_ADGRE2-high(WT)
"CCR effect"
% cytotoxicity
E:T ratio
MOLM13_ADGRE2-very-low
MOLM13_ADGRE2-low
100
50
0
2:1
1:1
1:2
1:4
1:8
1:16
1:32

FIGURE 19B

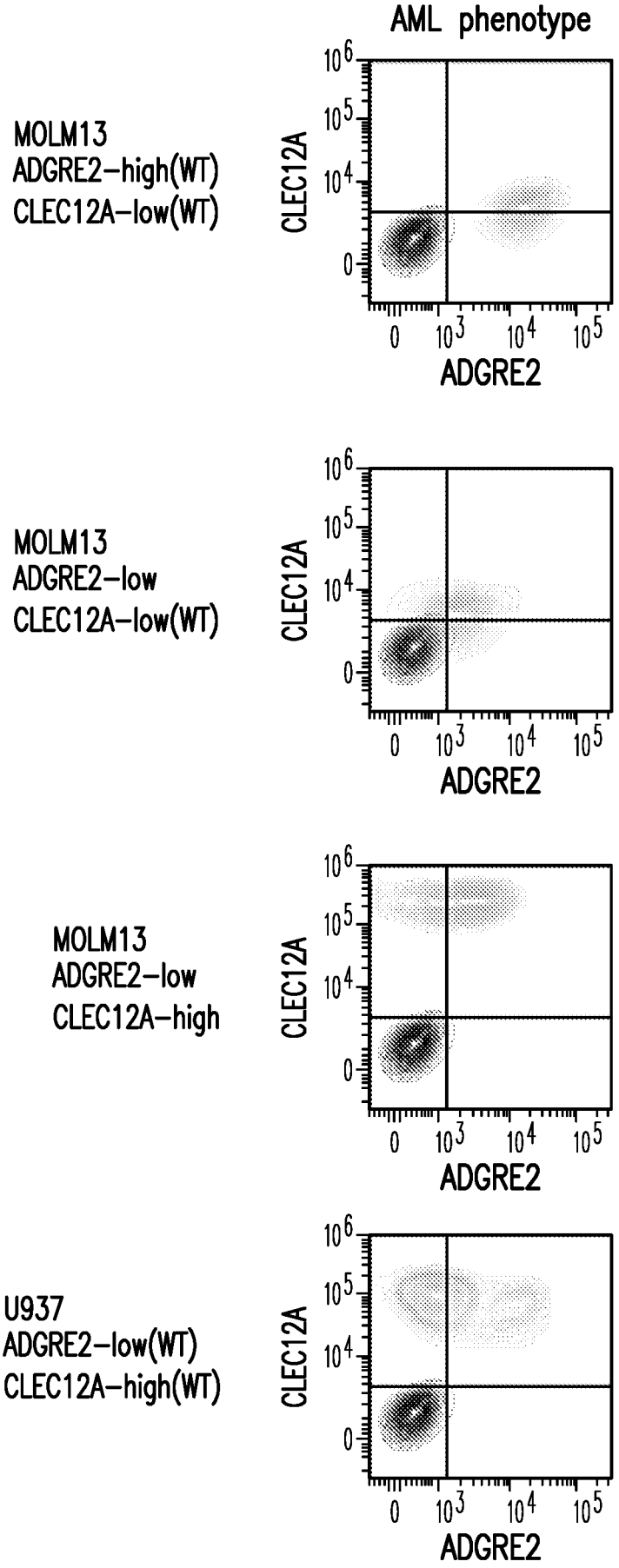

AML phenotype
MOLM13
ADGRE2–high(WT)
CLEC12A–low(WT)
MOLM13
ADGRE2–low
CLEC12A–low(WT)
MOLM13
ADGRE2–low
CLEC12A–high
U937
ADGRE2–low(WT)
CLEC12A–high(WT)
CLEC12A
ADGRE2
0
10^3
10^4
10^5
10^6

FIGURE 20

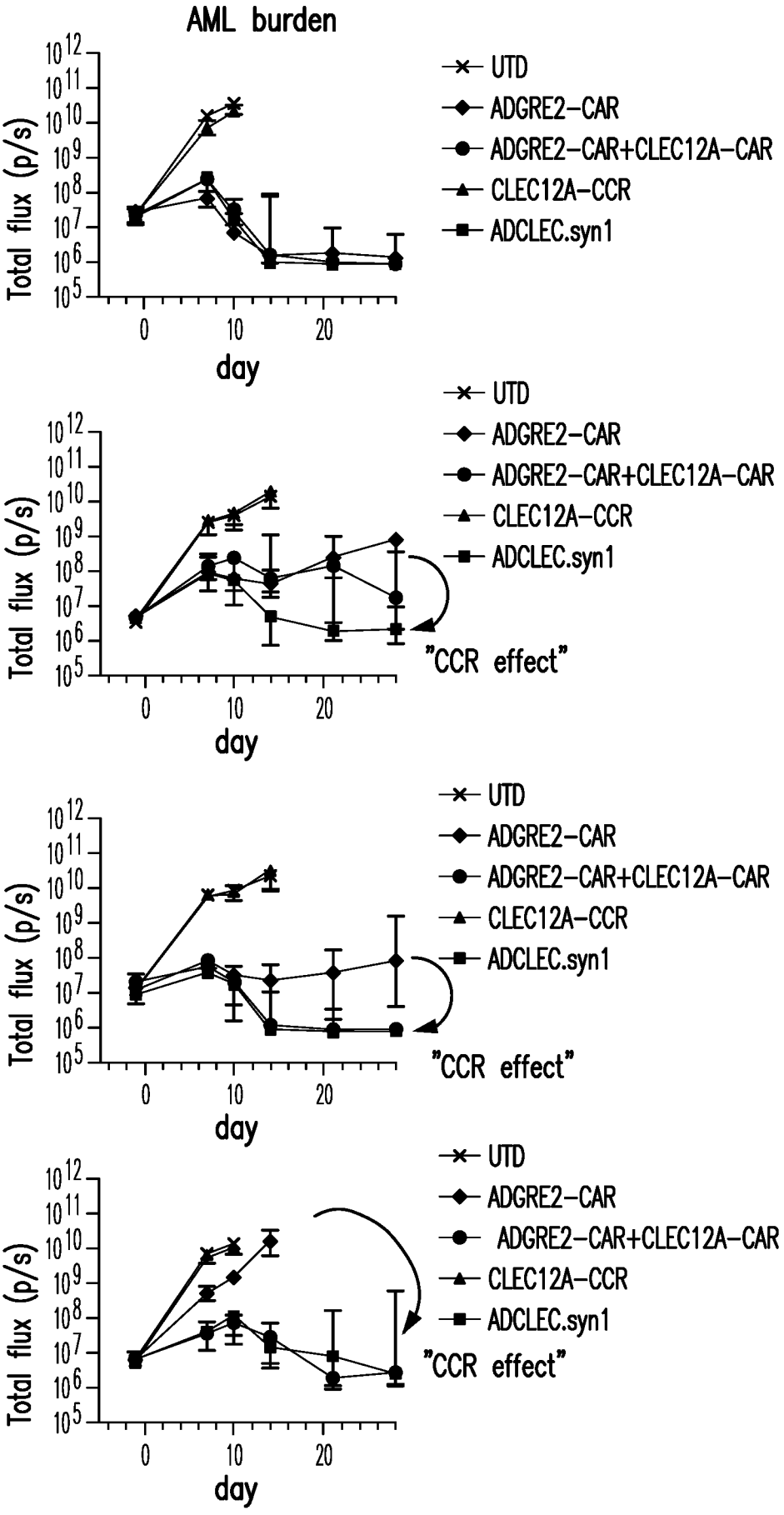


FIGURE 20 Continued

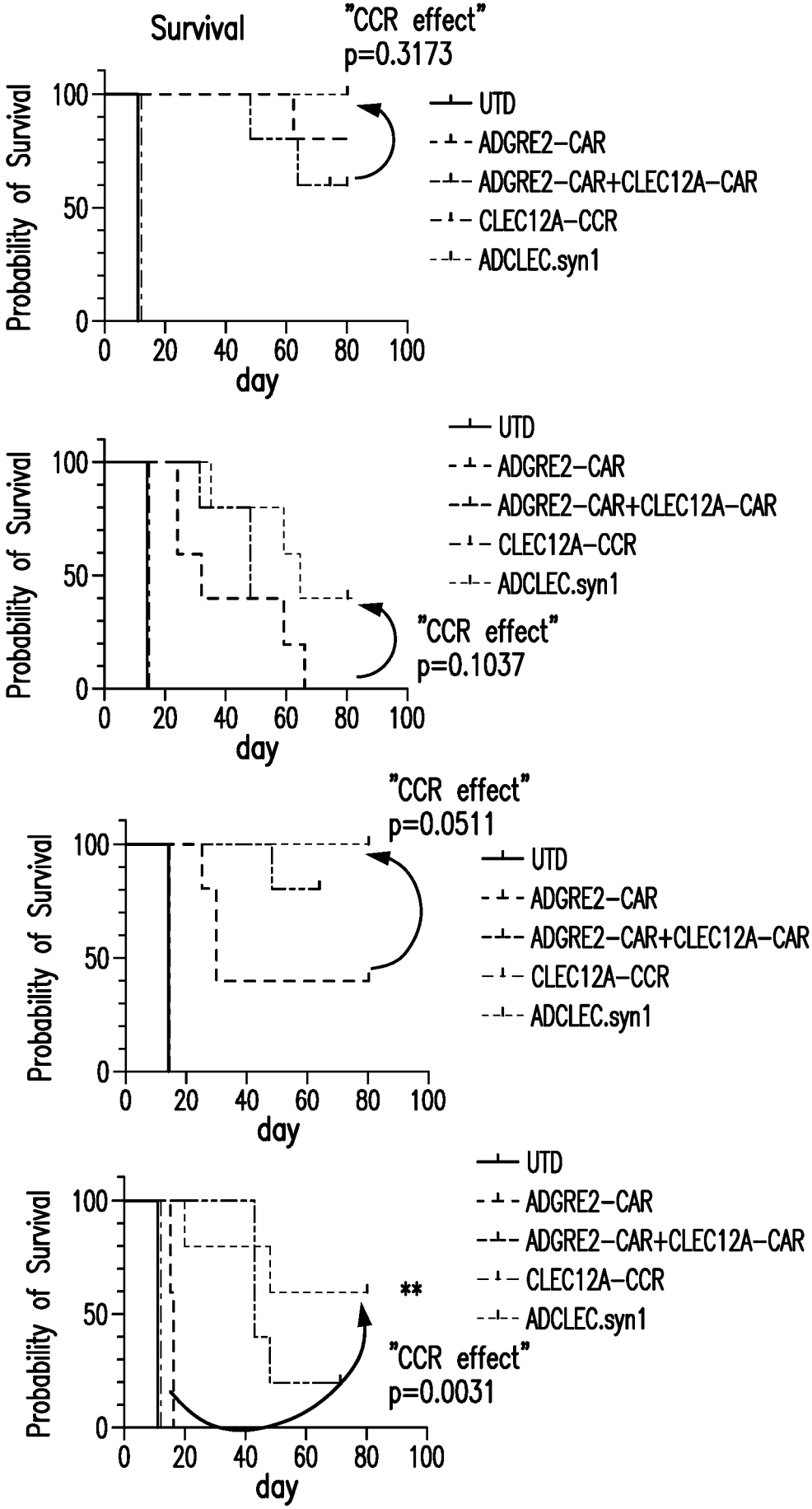
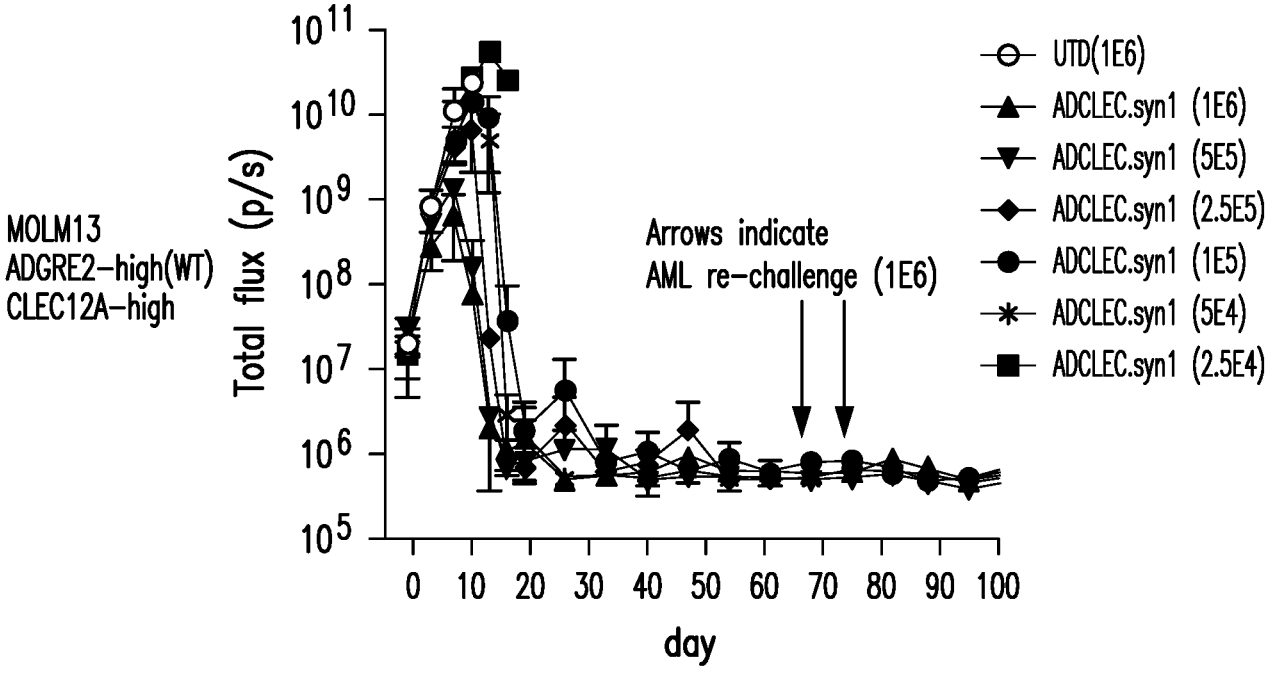


FIGURE 20 Continued


MOLM13
ADGRE2-high(WT)
CLEC12A-high
Total flux (p/s)
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
Arrows indicate
AML re-challenge (1E6)
0 10 20 30 40 50 60 70 80 90 100
day
UTD(1E6)
ADCLEC.syn1 (1E6)
ADCLEC.syn1 (5E5)
ADCLEC.syn1 (2.5E5)
ADCLEC.syn1 (1E5)
ADCLEC.syn1 (5E4)
ADCLEC.syn1 (2.5E4)

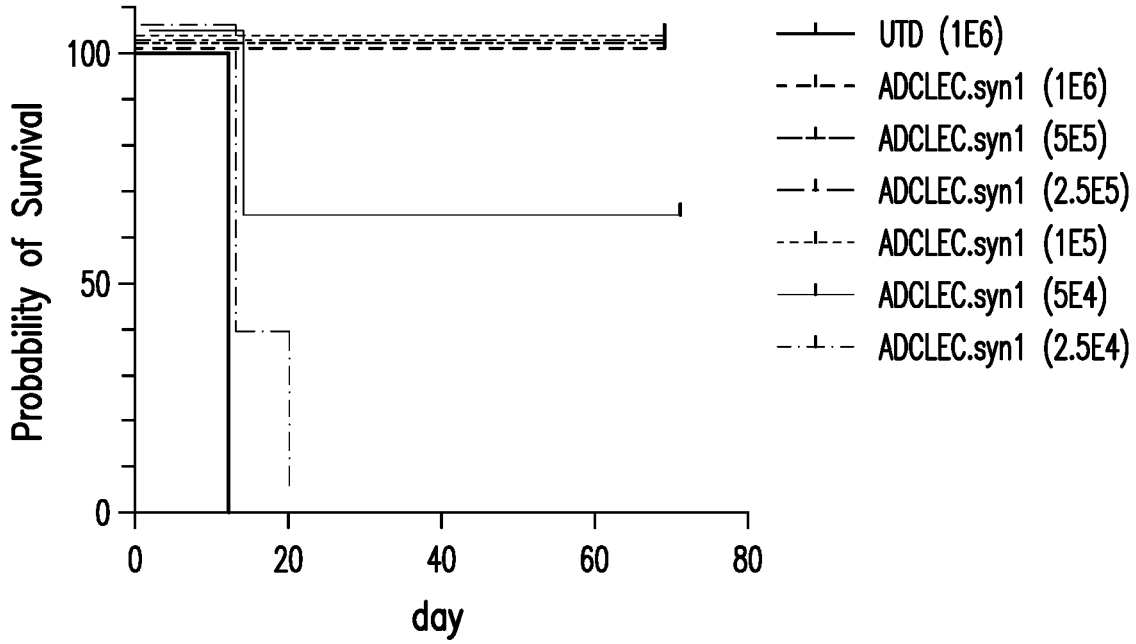

Probability of Survival
100
50
0
0 20 40 60 80
day
UTD (1E6)
ADCLEC.syn1 (1E6)
ADCLEC.syn1 (5E5)
ADCLEC.syn1 (2.5E5)
ADCLEC.syn1 (1E5)
ADCLEC.syn1 (5E4)
ADCLEC.syn1 (2.5E4)

FIGURE 21