FIG. 1

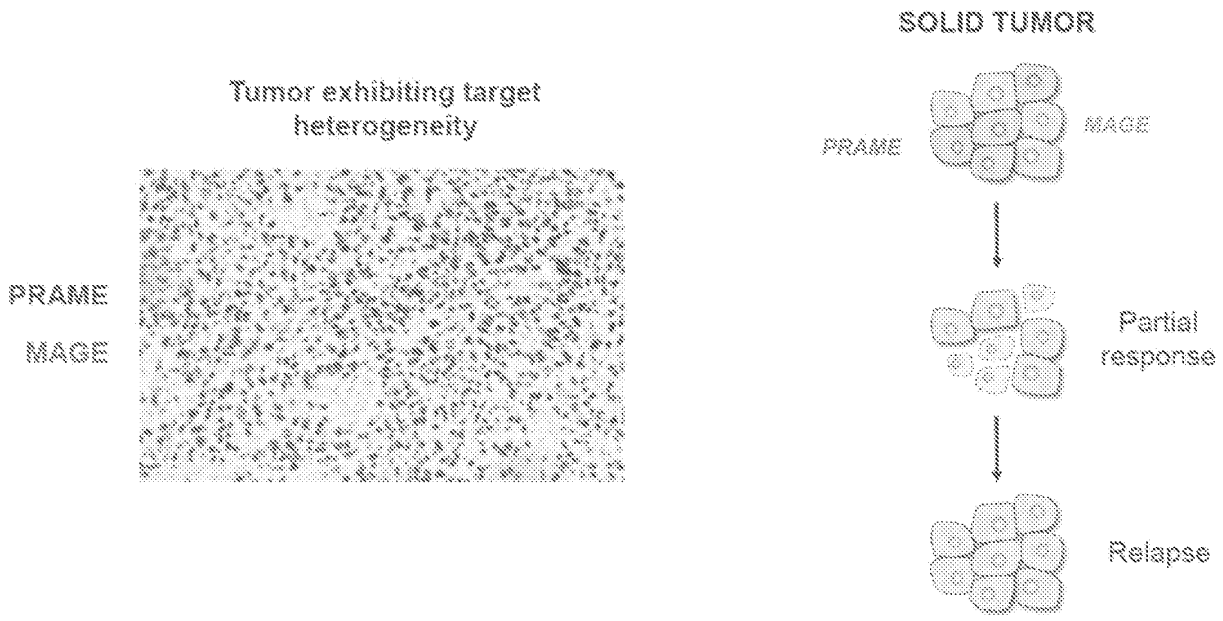
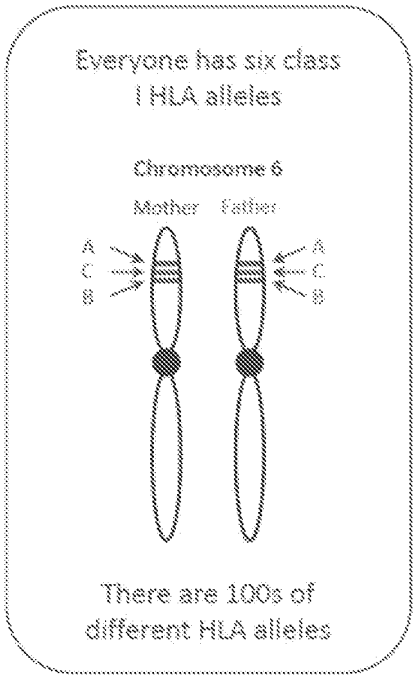

Tumor exhibiting target heterogeneity
PRAME
MAGE
SOLID TUMOR
PRAME
MAGE
Partial response
Relapse

FIG. 2



~90% of people in the U.S. are positive for at least one of the top 6 HLA types

	% people positive for each HLA type		
HLA type	United States	Europe	Asia
A*02:01	42	47	19
A*01:01	24	26	14
A*03:01	22	25	7.0
B*07:02	20	21	8.1
C*07:02	24	23	24
A*24:02	17	19	37

FIG. 3

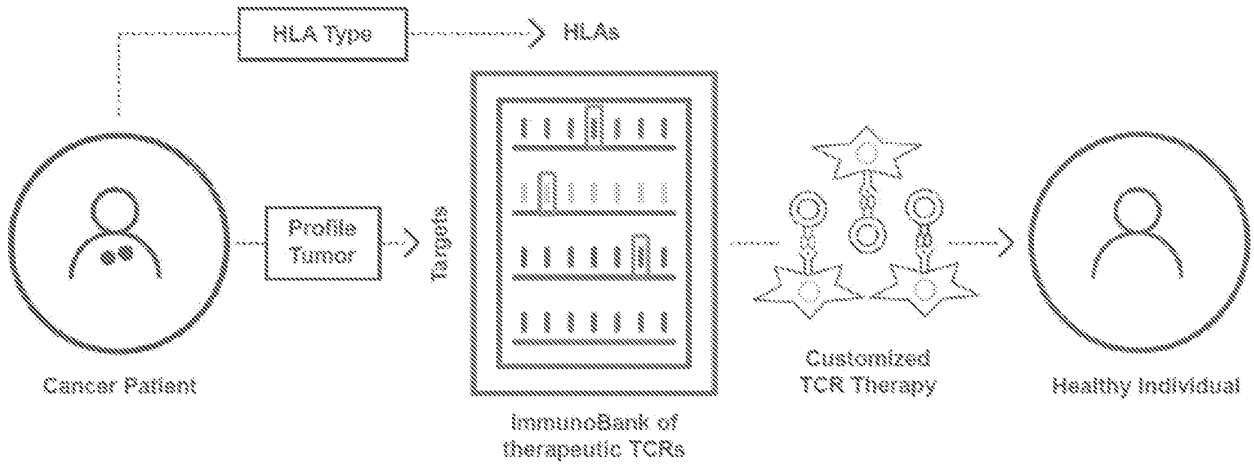

HLA Type
HLAs
Profile Tumor
Targets
Cancer Patient
ImmunoBank of therapeutic TCRs
Customized TCR Therapy
Healthy Individual

FIG. 4

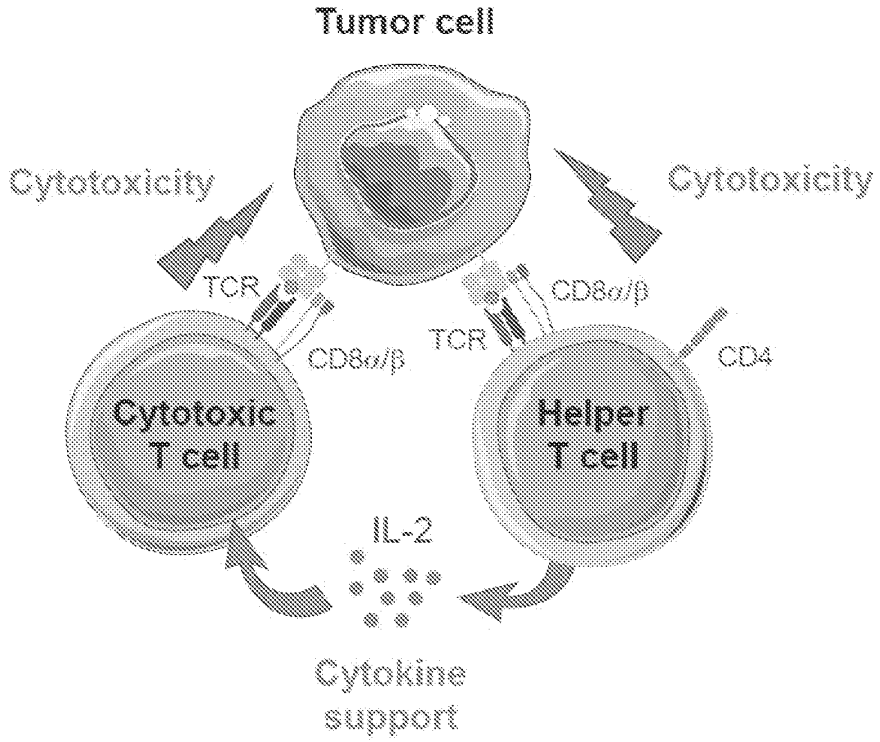

Tumor cell
Cytotoxicity
Cytotoxicity
TCR
CD8α/β
TCR
CD8α/β
CD4
Cytotoxic T cell
Helper T cell
IL-2
Cytokine support

FIG. 5

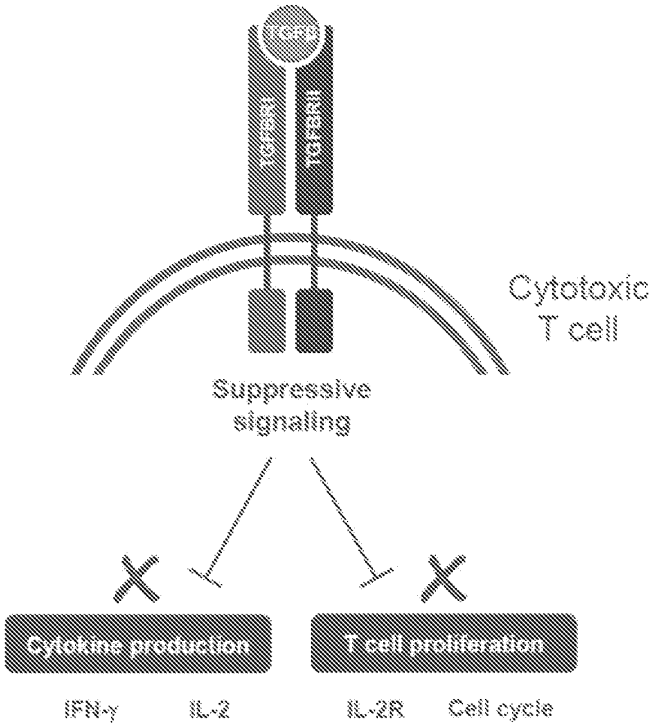

TCR
TCR
TCR
Cytotoxic
T cell
Suppressive
signaling
Cytokine production
T cell proliferation
IFN-γ
IL-2
IL-2R
Cell cycle

FIG. 6

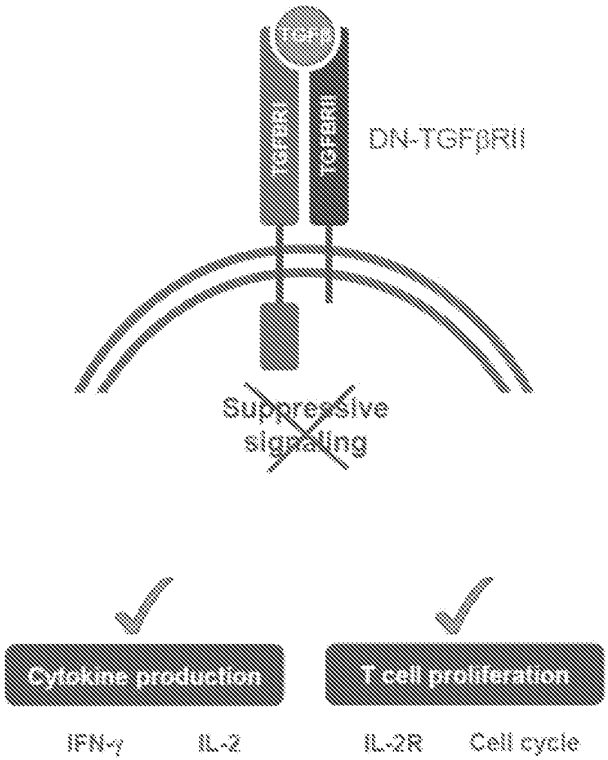

TGFβ
TGFβRII
TGFβRII
DN-TGFβRII
Suppressive signaling
Cytokine production
T cell proliferation
IFN-γ
IL-2
IL-2R
Cell cycle

FIG. 7

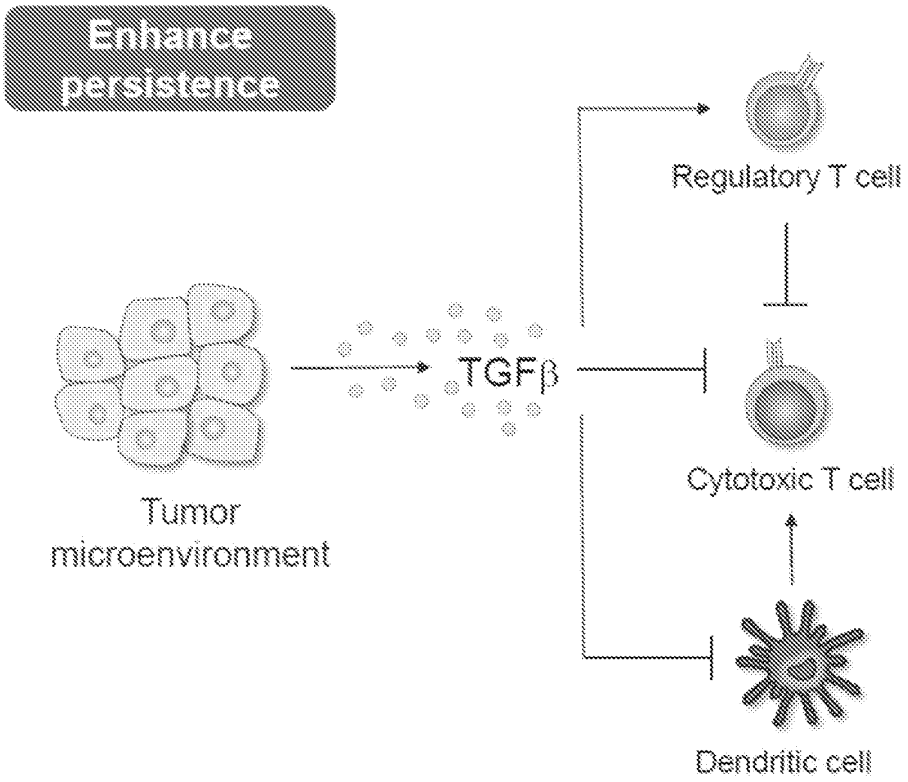

Enhance persistence
Regulatory T cell
TGFβ
Cytotoxic T cell
Tumor microenvironment
Dendritic cell

FIG. 8

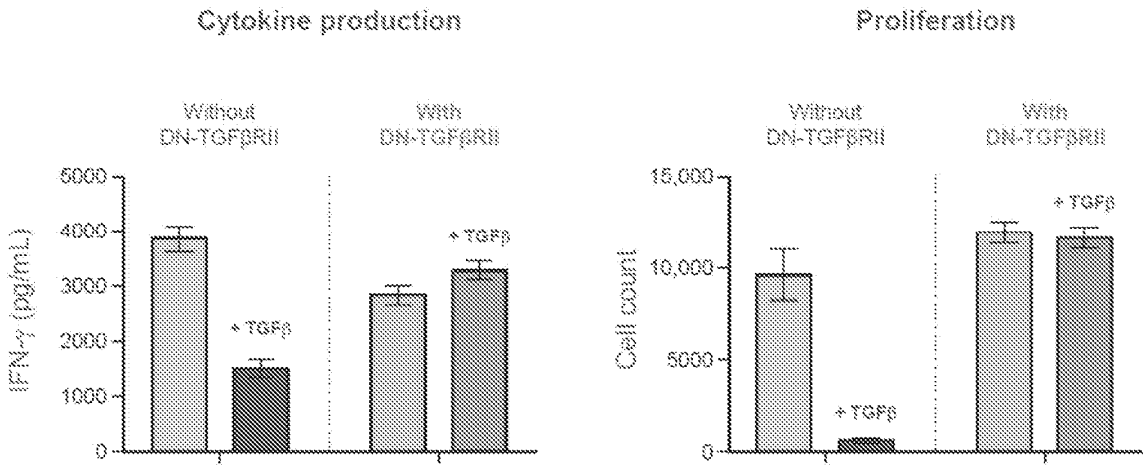

Cytokine production
Without DN-TGFβRII
With DN-TGFβRII
IFN-γ (pg/mL)
5000
4000
3000
2000
1000
0
+ TGFβ
+ TGFβ
Proliferation
Without DN-TGFβRII
With DN-TGFβRII
Cell count
15,000
10,000
5000
0
+ TGFβ
+ TGFβ

FIG. 9

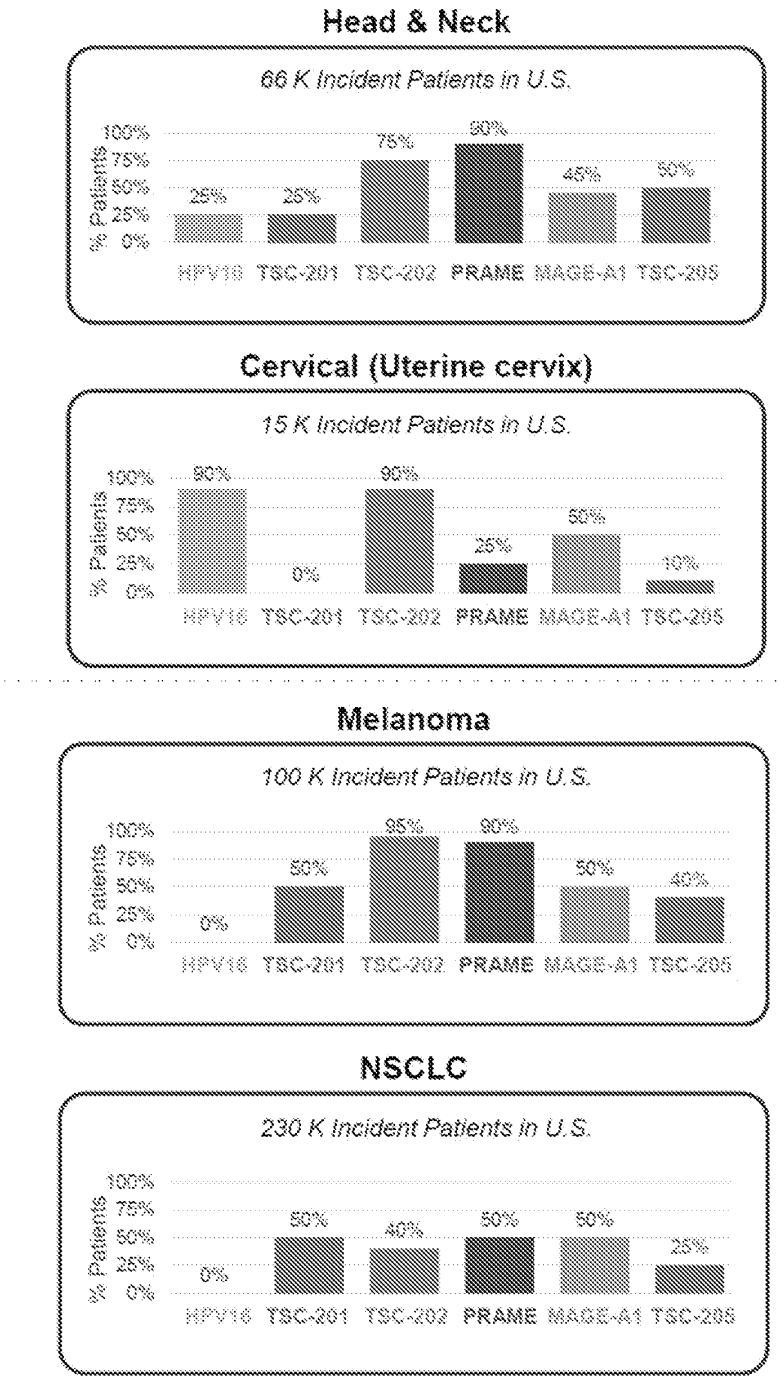
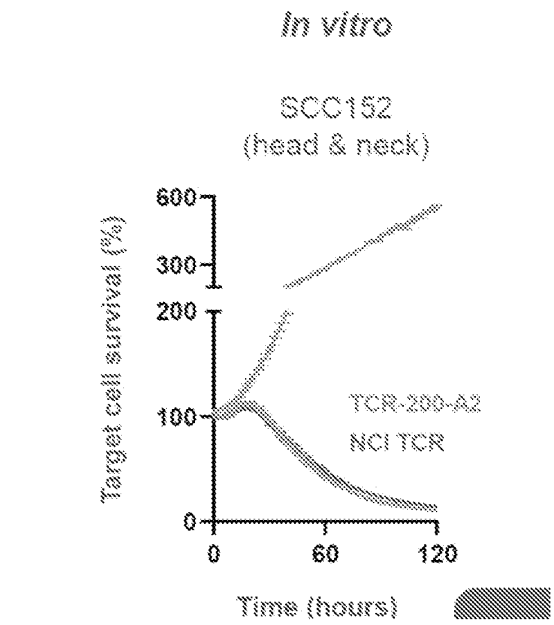

Head & Neck
66 K Incident Patients in U.S.
% Patients
100%
75%
50%
25%
0%
25%
25%
75%
90%
45%
50%
HPV16
TSC-201
TSC-202
PRAME
MAGE-A1
TSC-205
Cervical (Uterine cervix)
15 K Incident Patients in U.S.
% Patients
100%
75%
50%
25%
0%
90%
0%
90%
25%
50%
10%
HPV16
TSC-201
TSC-202
PRAME
MAGE-A1
TSC-205
Melanoma
100 K Incident Patients in U.S.
% Patients
100%
75%
50%
25%
0%
0%
50%
95%
90%
50%
40%
HPV16
TSC-201
TSC-202
PRAME
MAGE-A1
TSC-205
NSCLC
230 K Incident Patients in U.S.
% Patients
100%
75%
50%
25%
0%
0%
50%
40%
50%
50%
25%
HPV16
TSC-201
TSC-202
PRAME
MAGE-A1
TSC-205

FIG. 10


In vitro
SCC152
(head & neck)
Target cell survival (%)
600
300
200
100
0
TCR-200-A2
NCI TCR
0
60
120
Time (hours)

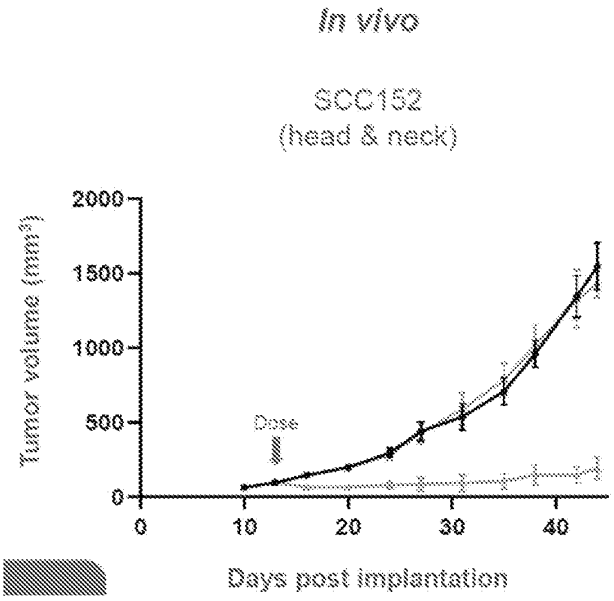
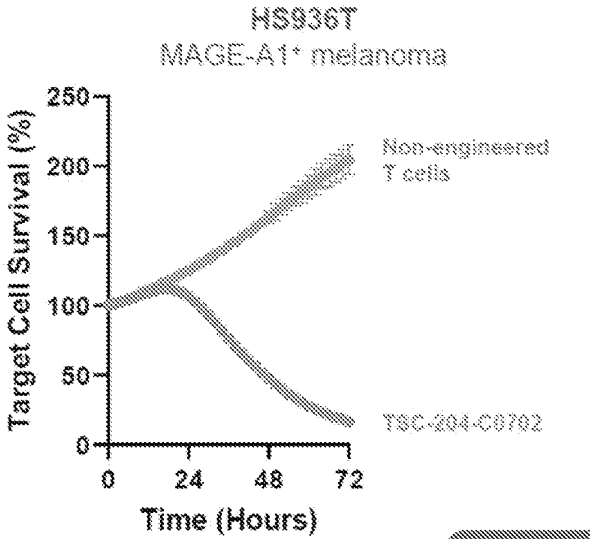

In vivo
SCC152
(head & neck)
Tumor volume (mm³)
2000
1500
1000
500
0
Dose
0
10
20
30
40
Days post implantation

FIG. 11


HS936T
MAGE-A1* melanoma
Target Cell Survival (%)
250
200
150
100
50
0
0
24
48
72
Time (Hours)
Non-engineered T cells
TSC-204-C0702

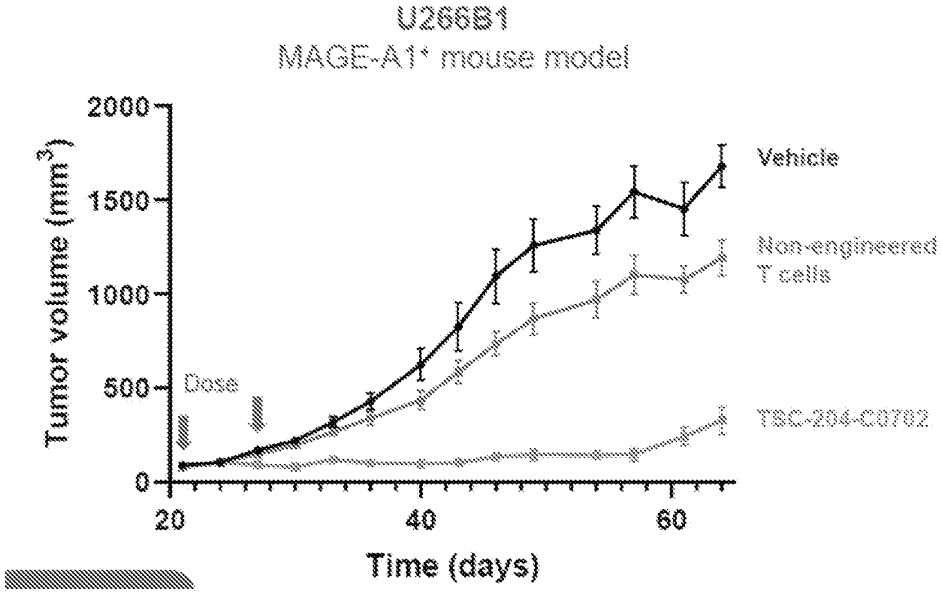
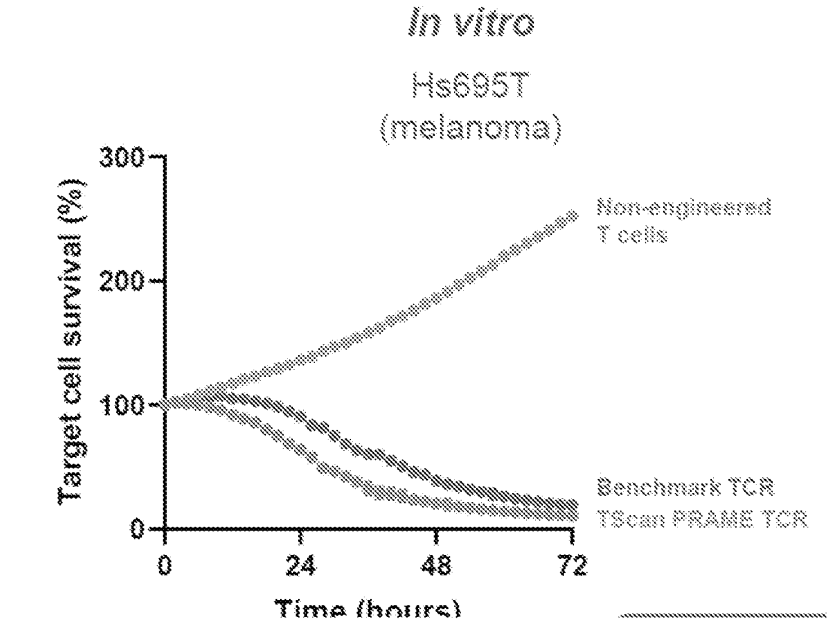

U266B1
MAGE-A1* mouse model
Tumor volume (mm3)
2000
1500
1000
500
0
Dose
20
40
60
Time (days)
Vehicle
Non-engineered T cells
TSC-204-C0702

FIG. 12


In vitro
Hs695T
(melanoma)
Target cell survival (%)
300
200
100
0
0
24
48
72
Time (hours)
Non-engineered
T cells
Benchmark TCR
TScan PRAME TCR

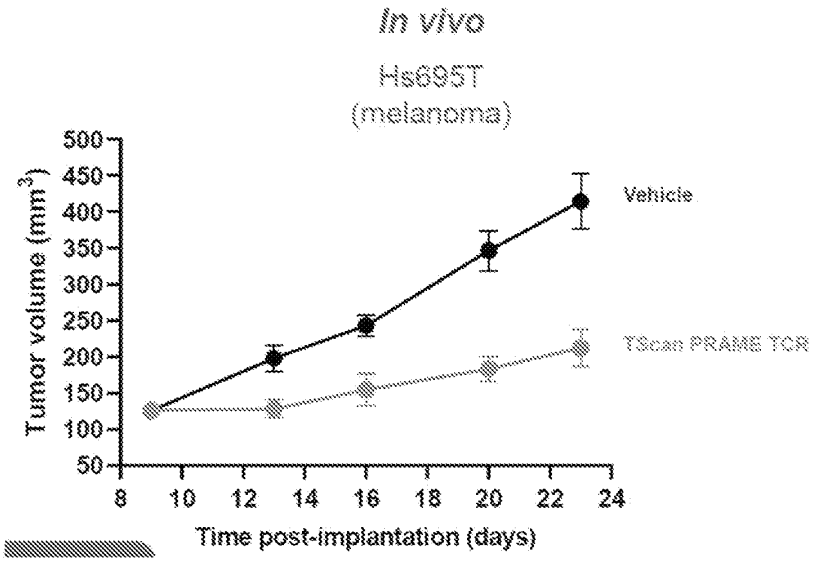

In vivo
Hs695T
(melanoma)
Tumor volume (mm3)
500
450
400
350
300
250
200
150
100
50
8
10
12
14
16
18
20
22
24
Time post-implantation (days)
Vehicle
TScan PRAME TCR

FIG. 13

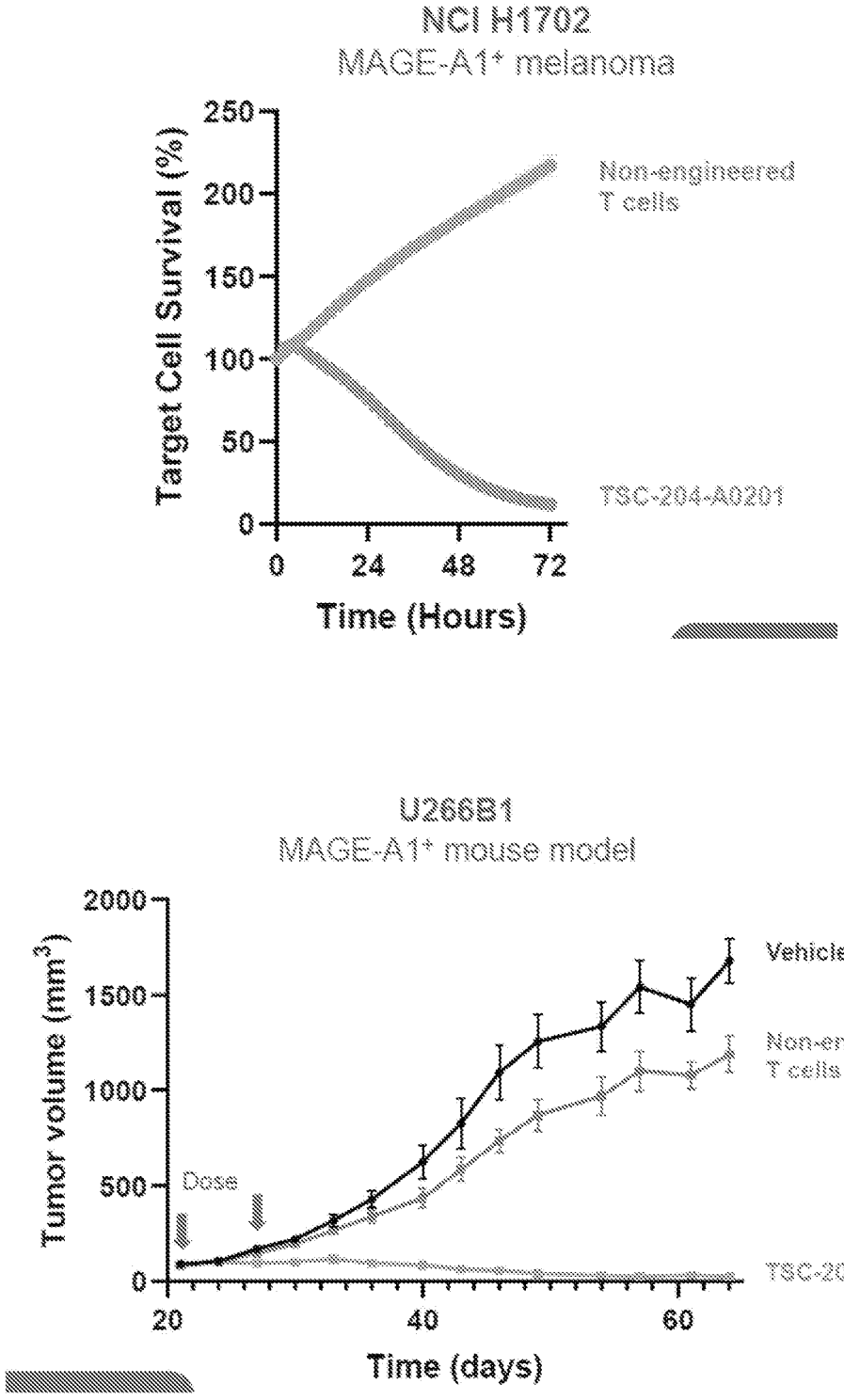

NCI H1702
MAGE-A1+ melanoma
Target Cell Survival (%)
250
200
150
100
50
0
0
24
48
72
Time (Hours)
Non-engineered T cells
TSC-204-A0201
U266B1
MAGE-A1+ mouse model
Tumor volume (mm3)
2000
1500
1000
500
0
Dose
20
40
60
Time (days)
Vehicle
Non-engineered T cells
TSC-204-A0201

FIG. 14

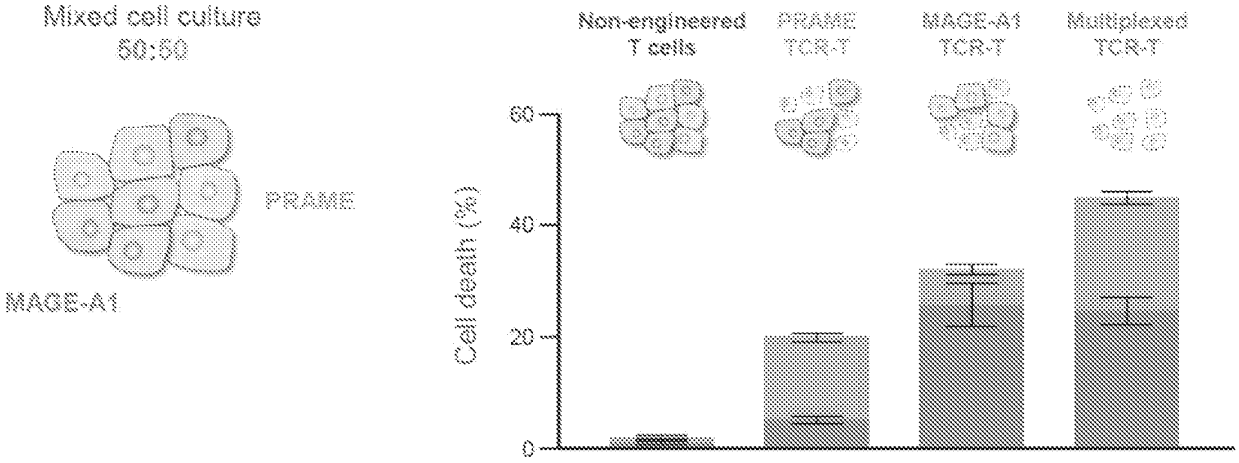

Mixed cell culture
50:50
PRAME
MAGE-A1
Non-engineered T cells
PRAME TCR-T
MAGE-A1 TCR-T
Multiplexed TCR-T
Cell death (%)
60
40
20
0

FIG. 15

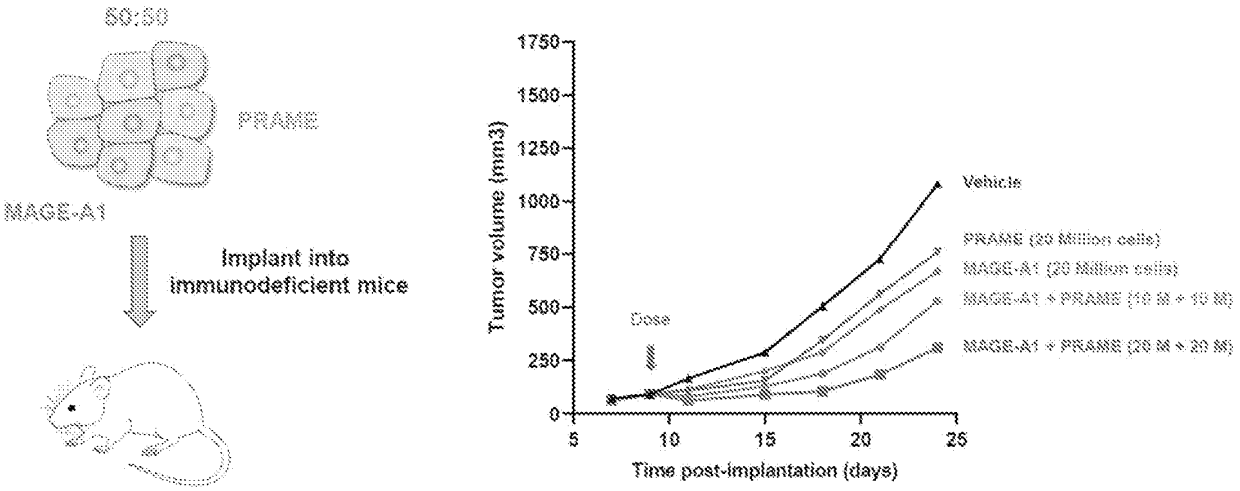

50:50
PRAME
MAGE-A1
Implant into
immunodeficient mice
Tumor volume (mm3)
1750
1500
1250
1000
750
500
250
0
Dose
5
10
15
20
25
Time post-implantation (days)
Vehicle
PRAME (20 Million cells)
MAGE-A1 (20 Million cells)
MAGE-A1 + PRAME (10 M + 10 M)
MAGE-A1 + PRAME (20 M + 20 M)

FIG. 16

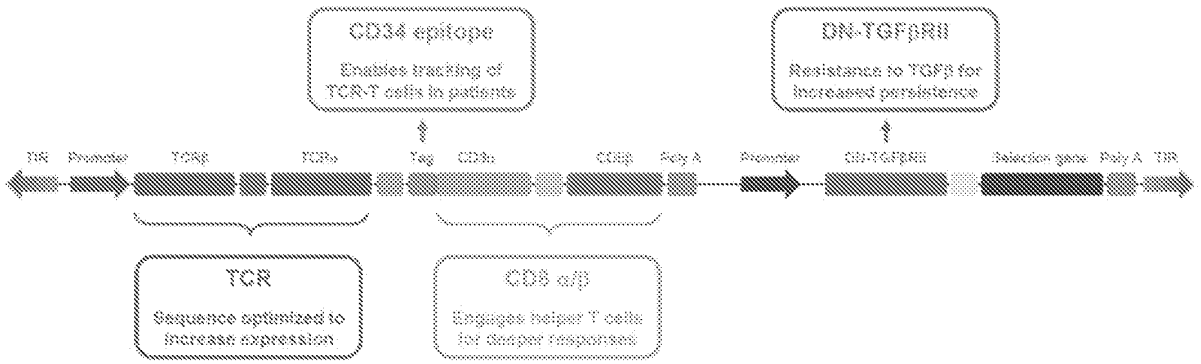

CD34 epitope
Enables tracking of TCR-T cells in patients
DN-TGFβRII
Resistance to TGFβ for increased persistence
ITR
Promoter
TCRβ
TCRα
Tag
CD8α
CD8β
Poly A
Promoter
DN-TGFβRII
Selection gene
Poly A
ITR
TCR
Sequence optimized to increase expression
CD8 α/β
Engages helper T cells for deeper responses

FIG. 17

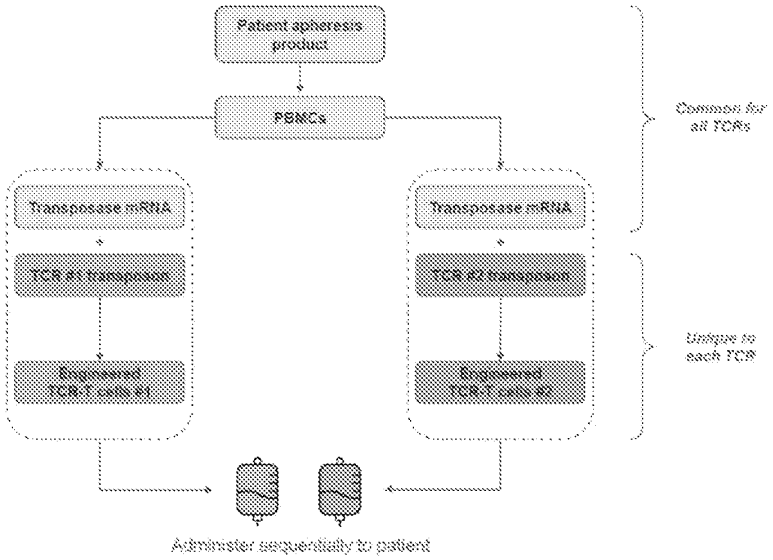
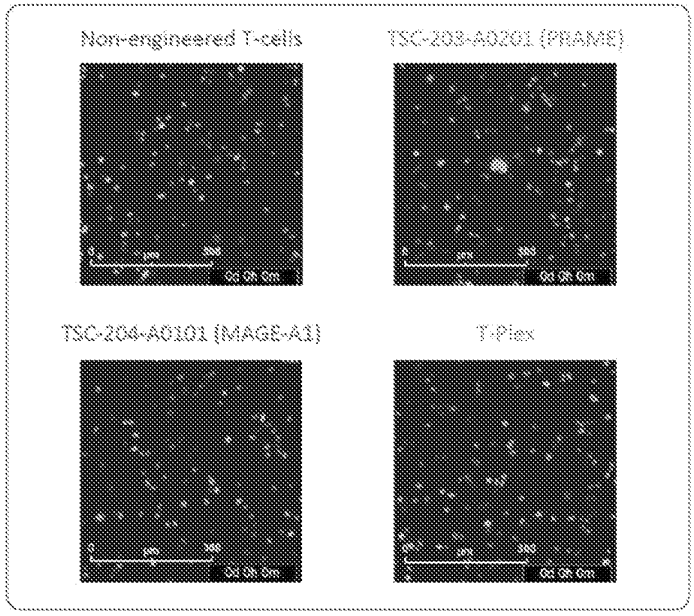

Patient apheresis product
PBMCs
Transposase mRNA
TCR #1 transposon
Engineered TCR-T cells #1
Transposase mRNA
TCR #2 transposon
Engineered TCR-T cells #2
Common for all TCRs
Unique to each TCR
Administer sequentially to patient

FIG. 18



Green cells: SKMEL5 (PRAME-positive)
Red cells: A101D (MAGE-A1-positive)

FIG. 19

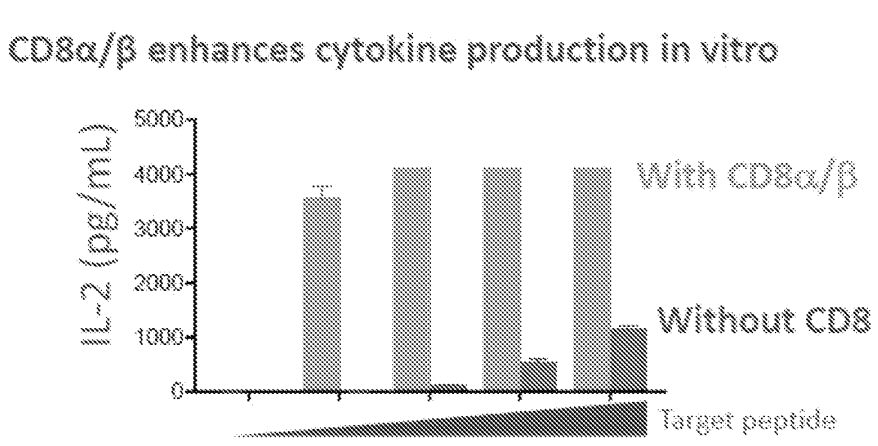

CD8α/β enhances cytokine production in vitro
IL-2 (pg/mL)
5000
4000
3000
2000
1000
0
With CD8α/β
Without CD8
Target peptide

FIG. 20

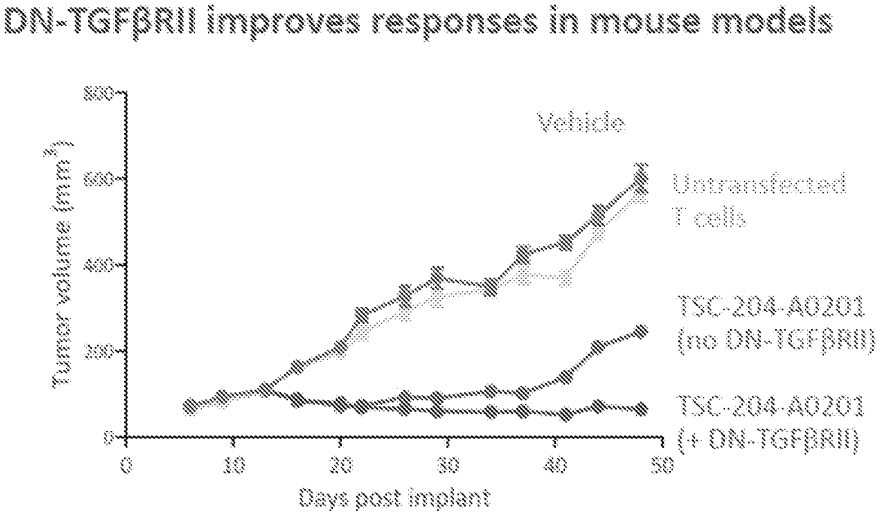

DN-TGFβRII improves responses in mouse models
Tumor volume (mm3)
800
600
400
200
0
0
10
20
30
40
50
Days post implant
Vehicle
Untransfected T cells
TSC-204-A0201 (no DN-TGFβRII)
TSC-204-A0201 (+ DN-TGFβRII)

FIG. 21

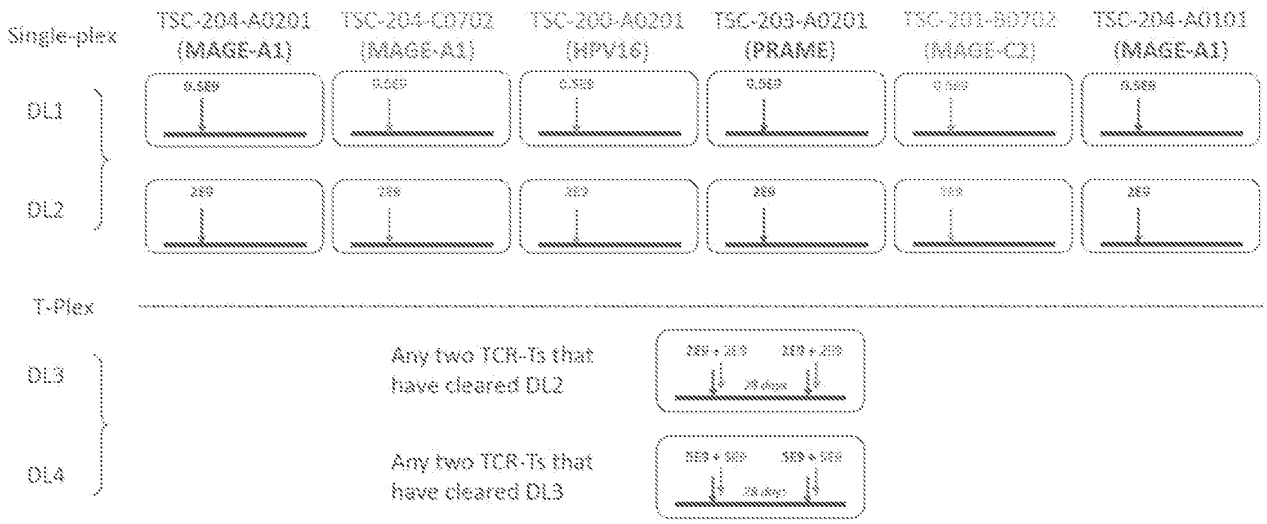

Single-plex
TSC-204-A0201 (MAGE-A1)
TSC-204-C0702 (MAGE-A1)
TSC-200-A0201 (HPV16)
TSC-203-A0201 (PRAME)
TSC-201-B0702 (MAGE-C2)
TSC-204-A0101 (MAGE-A1)
DL1
DL2
T-Plex
DL3
DL4
Any two TCR-Ts that have cleared DL2
Any two TCR-Ts that have cleared DL3

FIG. 22

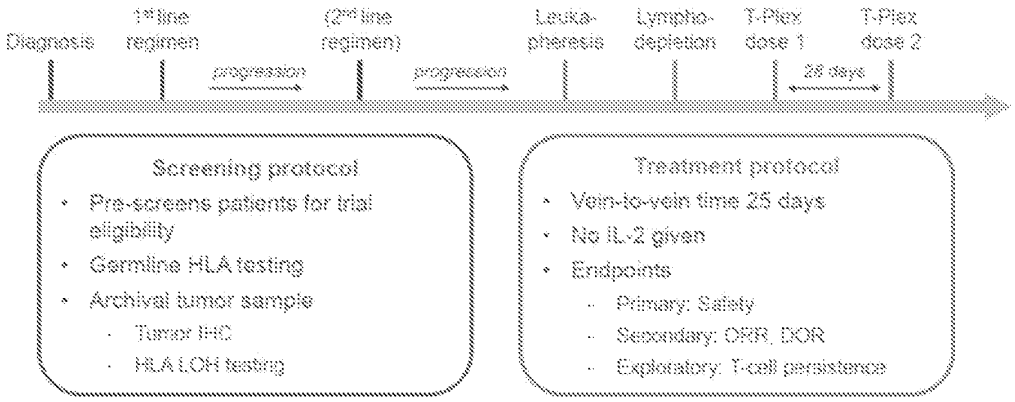

Diagnosis
1st line regimen
progression
(2nd line regimen)
progression
Leuka-pheresis
Lympho-depletion
T-Plex dose 1
28 days
T-Plex dose 2
Screening protocol
Pre-screens patients for trial eligibility
Germline HLA testing
Archival tumor sample
Tumor IHC
HLA LOH testing
Treatment protocol
Vein-to-vein time 25 days
No IL-2 given
Endpoints
Primary: Safety
Secondary: ORR, DOR
Exploratory: T-cell persistence

FIG. 23

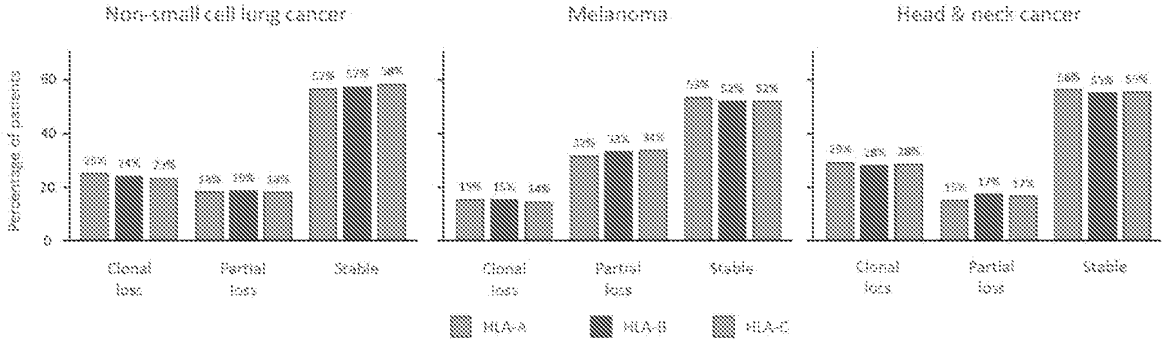

Non-small cell lung cancer
Melanoma
Head & neck cancer
Percentage of patients
0
20
40
60
Clonal loss
Partial loss
Stable
HLA-A
HLA-B
HLA-C

FIG. 24

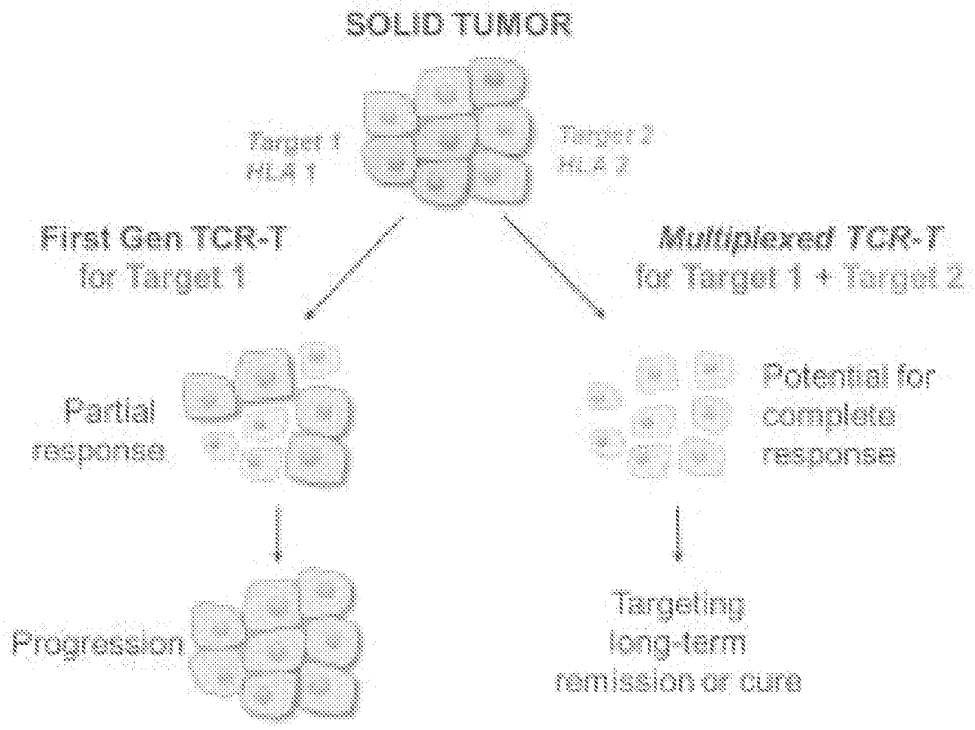

SOLID TUMOR
Target 1
HLA 1
Target 2
HLA 2
First Gen TCR-T
for Target 1
Multiplexed TCR-T
for Target 1 + Target 2
Partial
response
Potential for
complete
response
Progression
Targeting
long-term
remission or cure

FIG. 25

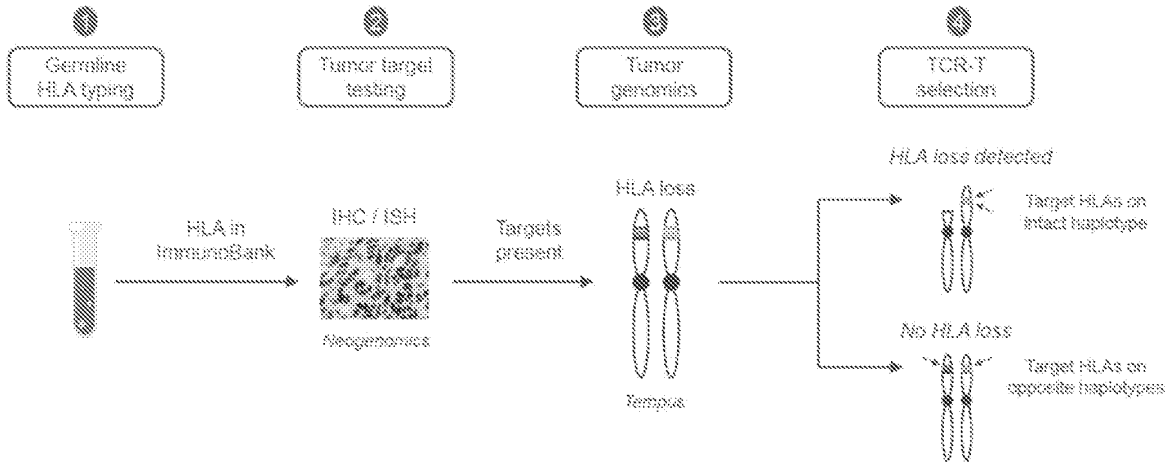

Germline HLA typing
Tumor target testing
Tumor genomics
TCR-T selection
HLA in ImmunoBank
IHC / ISH
Neogenomics
Targets present
HLA loss
Tempus
HLA loss detected
Target HLAs on intact haplotype
No HLA loss
Target HLAs on opposite haplotypes

FIG. 26

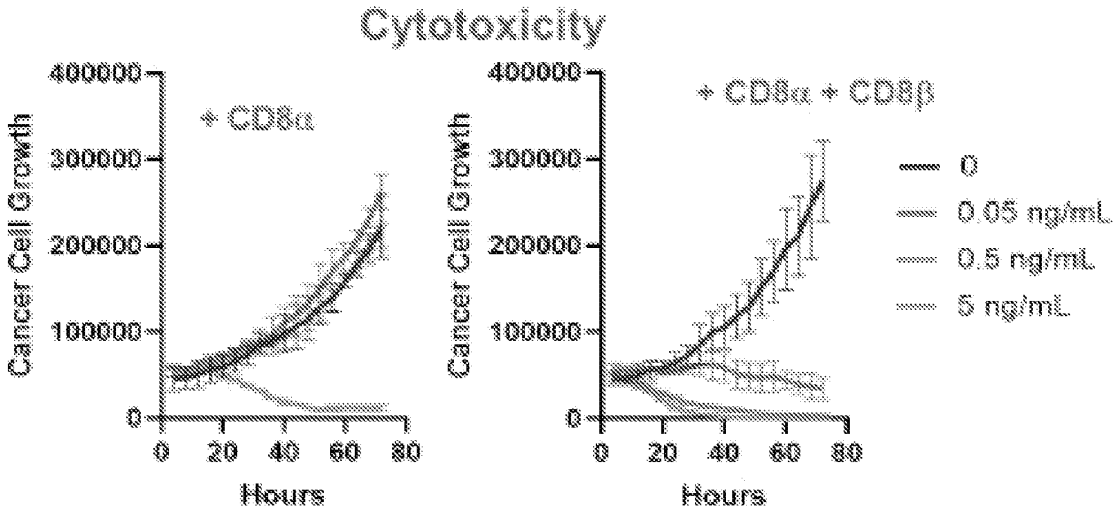
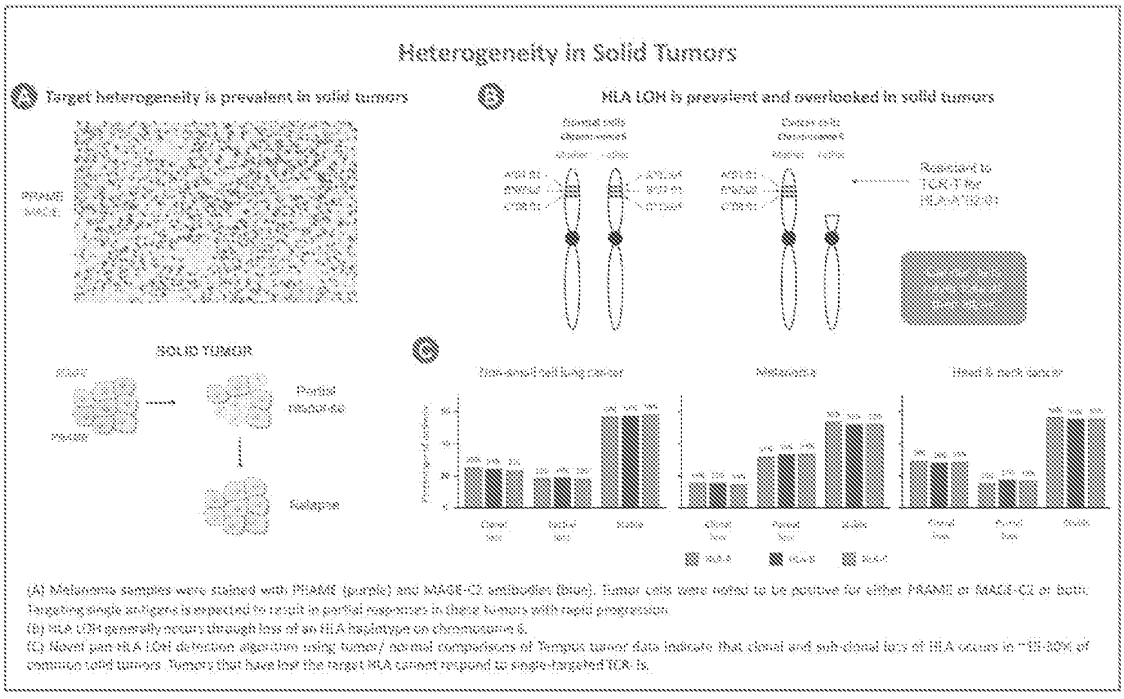

Cytotoxicity
+ CD8α
+ CD8α + CD8β
Cancer Cell Growth
400000
300000
200000
100000
0
0
20
40
60
80
Hours
0
0.05 ng/mL
0.5 ng/mL
5 ng/mL

FIG. 27

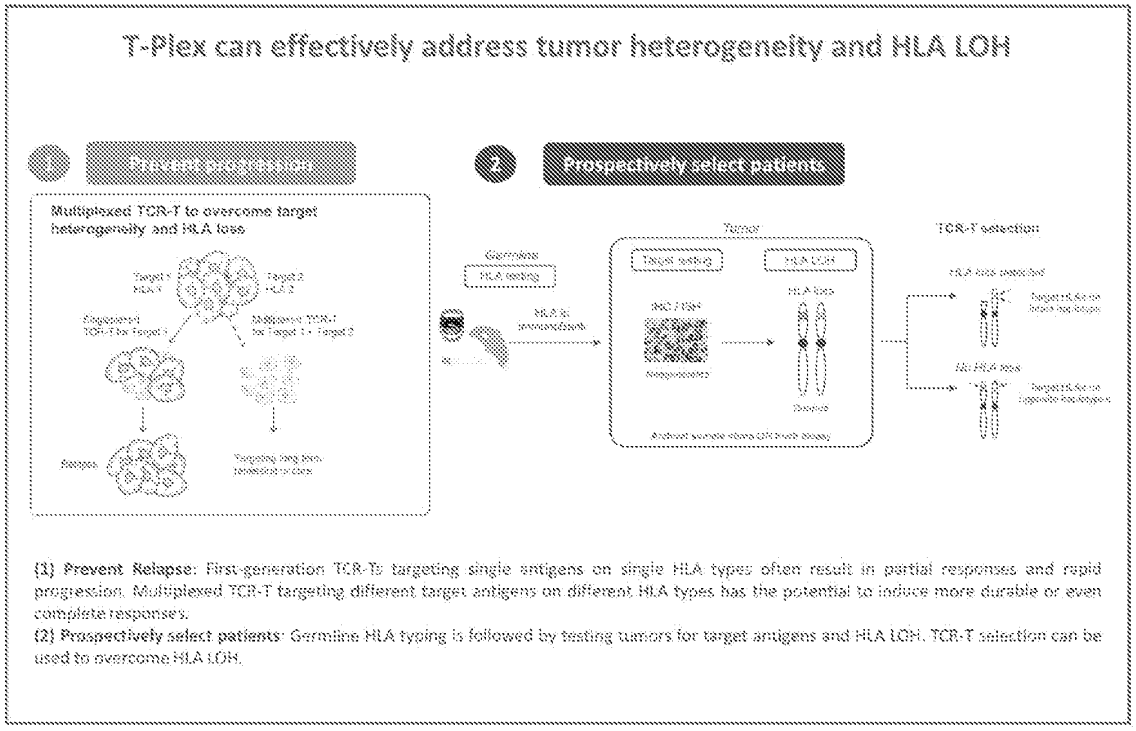
Background and Rationale

- Most patients fail checkpoint immunotherapy due to lack of sufficient endogenous anti-tumor T cells
- A potential solution is to engineer T cells with exogenous T cell receptors (TCRs) that target tumor specific antigens
- However, solid tumors are notoriously heterogenous with variable target antigen expression
- Solid tumors have also been recently recognized to have HLA loss of heterozygosity (LOH) in up to 40% of tumors
- First-generation TCR-Ts targeting single antigens had limited response rates (30-50%) and short durations of response (3-4 months)
- TScan's solution is to develop multiplexed TCR-Ts targeting different antigens on different HLA types (T-Plex)
- TScan's TCR-Ts also have genetic enhancements to enable potent tumor killing and long-term persistence



(A) Melanoma samples were stained with PRAME (purple) and MAGE-C2 antibodies (blue). Tumor cells were noted to be positive for either PRAME or MAGE-C2 or both. Targeting single antigens is expected to result in partial responses in these tumors with rapid progression.
(B) HLA LOH generally occurs through loss of an HLA haplotype on chromosome 6.
(C) Novel pan-HLA LOH detection algorithm using tumor/ normal comparisons of Tempus tumor data indicate that clonal and sub-clonal loss of HLA occurs in ~18-30% of common solid tumors. Tumors that have lost the target HLA cannot respond to single-targeted TCR-Ts.

FIG. 28


T-Plex can effectively address tumor heterogeneity and HLA LOH
1 Prevent relapse
2 Prospectively select patients
Multiplexed TCR-T to overcome target heterogeneity and HLA loss
Tumor
Germline HLA typing
HLA LOH
TCR-T selection
(1) Prevent Relapse: First-generation TCR-Ts targeting single antigens on single HLA types often result in partial responses and rapid progression. Multiplexed TCR-T targeting different target antigens on different HLA types has the potential to induce more durable or even complete responses.
(2) Prospectively select patients: Germline HLA typing is followed by testing tumors for target antigens and HLA LOH. TCR-T selection can be used to overcome HLA LOH.

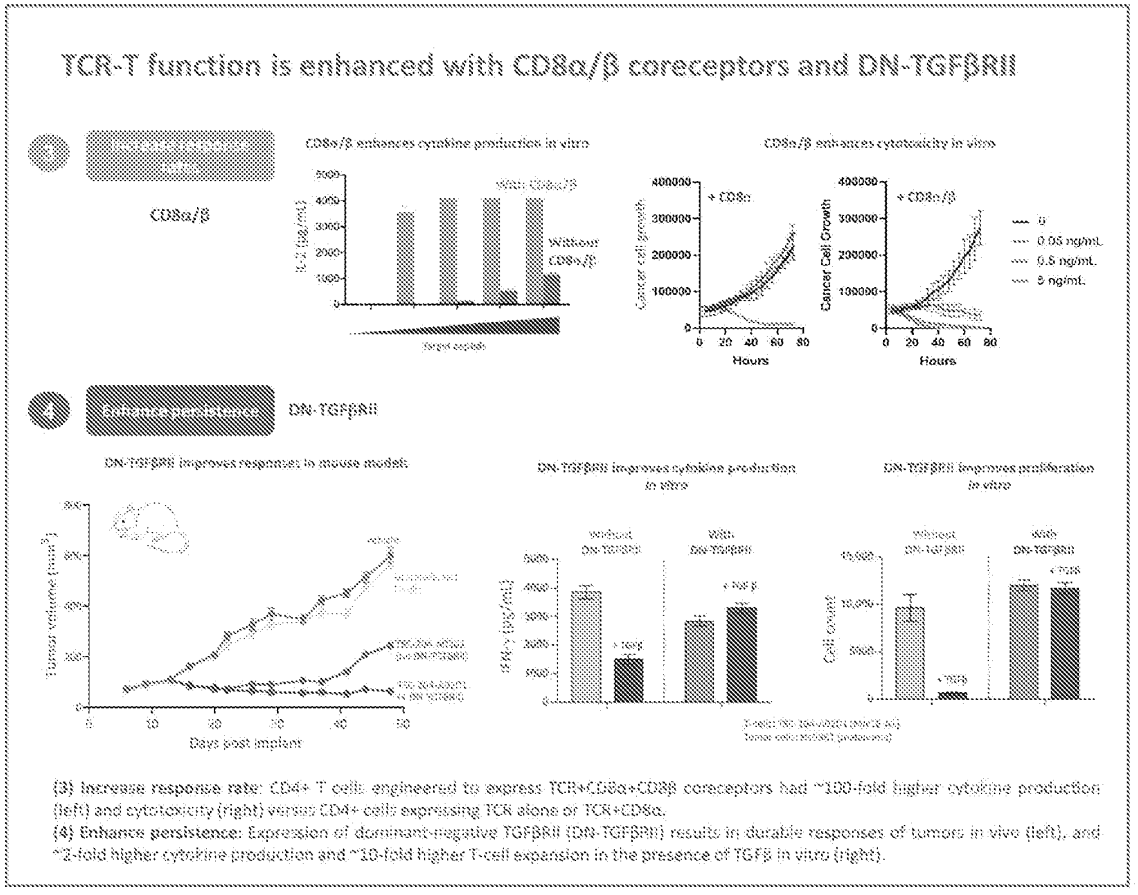
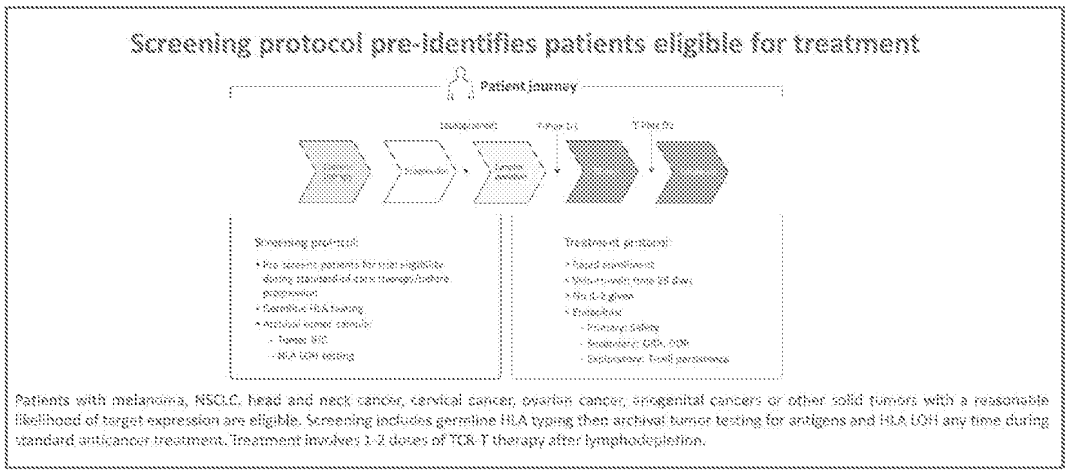
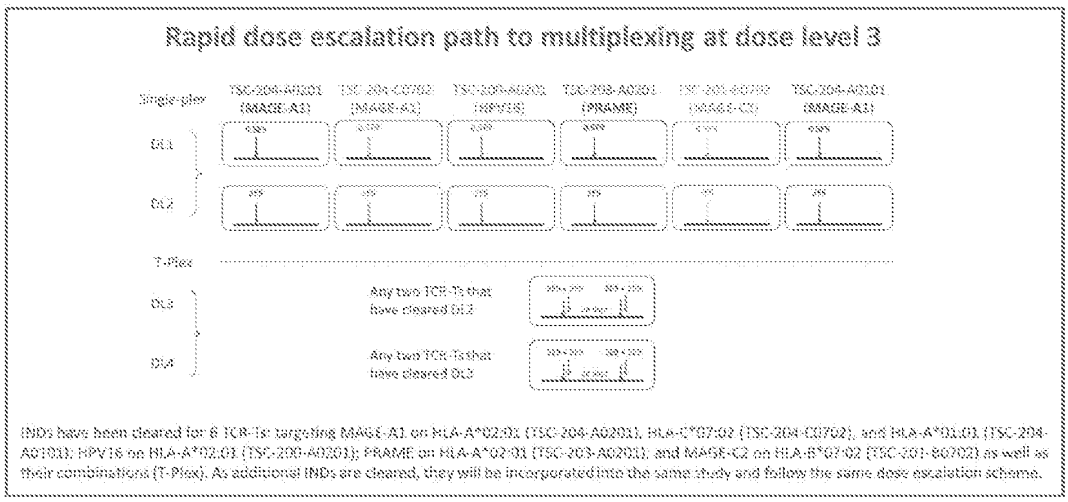

TCR-T function is enhanced with CD8α/β coreceptors and DN-TGFβRII
3 Increase response rate
CD8α/β
CD8α enhances cytokine production in vitro
CD8α/β enhances cytotoxicity in vitro
With CD8α/β
Without CD8α/β
+ CD8α
+ CD8α/β
0
0.05 ng/mL
0.5 ng/mL
5 ng/mL
Hours
4 Enhance persistence
DN-TGFβRII
DN-TGFβRII improves responses in mouse model
DN-TGFβRII improves cytokine production in vitro
DN-TGFβRII improves proliferation in vitro
Days post implant
(3) Increase response rate: CD4+ T cells engineered to express TCR+CD8α+CD8β coreceptors had ~100-fold higher cytokine production (left) and cytotoxicity (right) versus CD4+ cells expressing TCR alone or TCR+CD8α.
(4) Enhance persistence: Expression of dominant-negative TGFβRII (DN-TGFβRII) results in durable responses of tumors in vivo (left), and ~2-fold higher cytokine production and ~10-fold higher T-cell expansion in the presence of TGFβ in vitro (right).

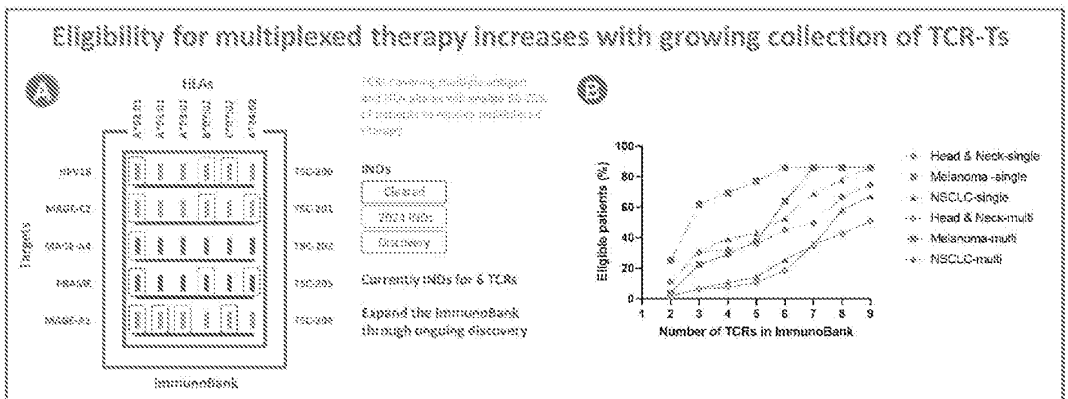
FIG. 29



Patients with melanoma, NSCLC, head and neck cancer, cervical cancer, ovarian cancer, urogenital cancers or other solid tumors with a reasonable likelihood of target expression are eligible. Screening includes germline HLA typing then archival tumor testing for antigens and HLA LOH any time during standard anticancer treatment. Treatment involves 1-2 doses of TCR-T therapy after lymphodepletion.



INDs have been cleared for 6 TCR-Ts targeting MAGE-A1 on HLA-A*02:01 (TSC-204-A0201), HLA-C*07:02 (TSC-204-C0702), and HLA-A*01:01 (TSC-204-A0101); HPV16 on HLA-A*02:01 (TSC-203-A0201); PRAME on HLA-A*02:01 (TSC-203-A0201), and MAGE-C2 on HLA-B*07:02 (TSC-201-B0702) as well as their combinations (T-Plex). As additional INDs are cleared, they will be incorporated into the same study and follow the same dose escalation scheme.



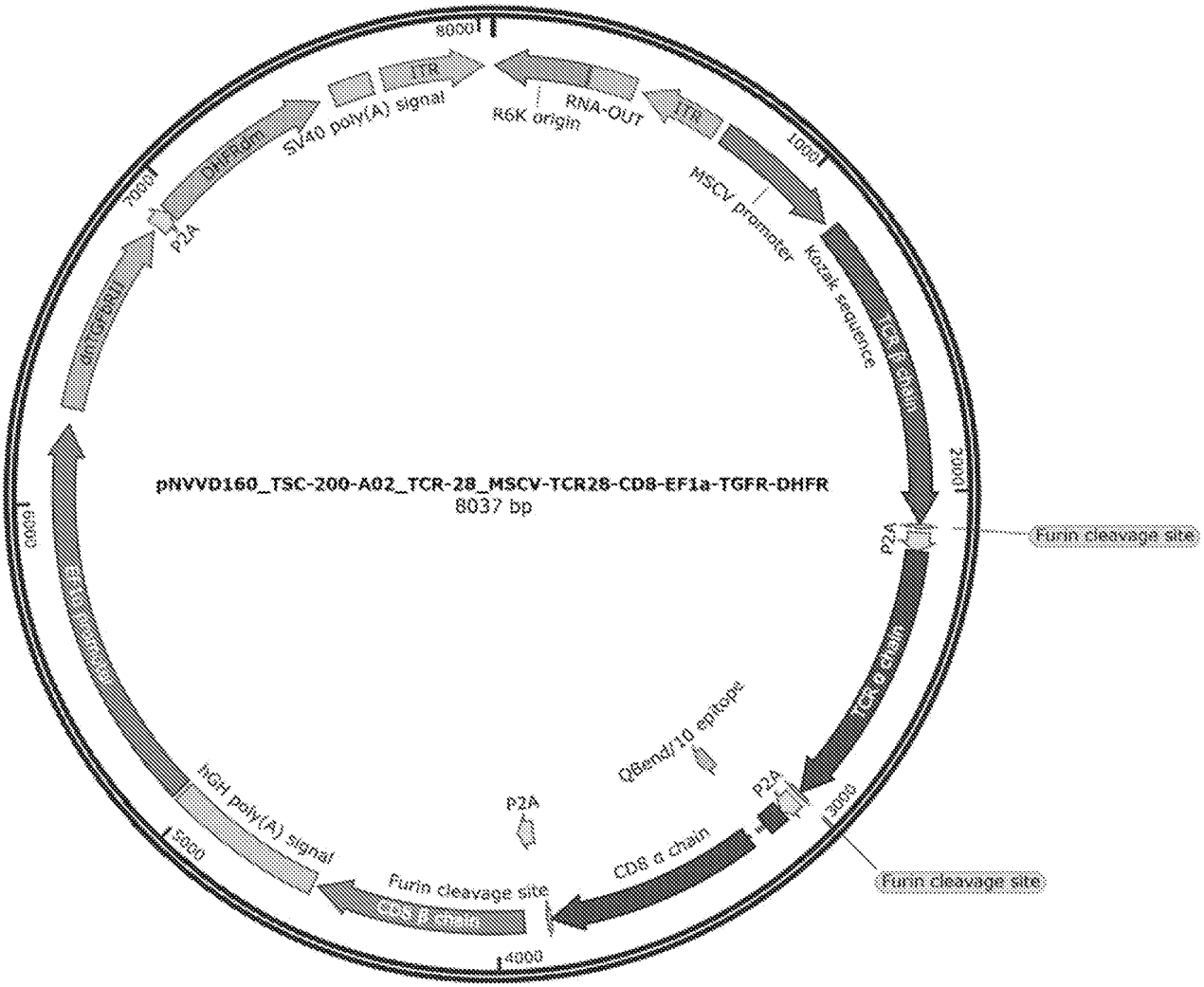
(A) The ImmunoBank is the collection of TCR-Ts from which 1-2 therapies for individual patients are chosen. INDs for 6 TCR-Ts and the T-Plex combination have been cleared. Two additional INDs are on track to be submitted by end-2025. (B) As the number of TCR-T choices grows, the number of solid tumor patients eligible for single/plexed therapy (dotted lines) or multiplexed therapy (solid lines) increases.

FIG. 30



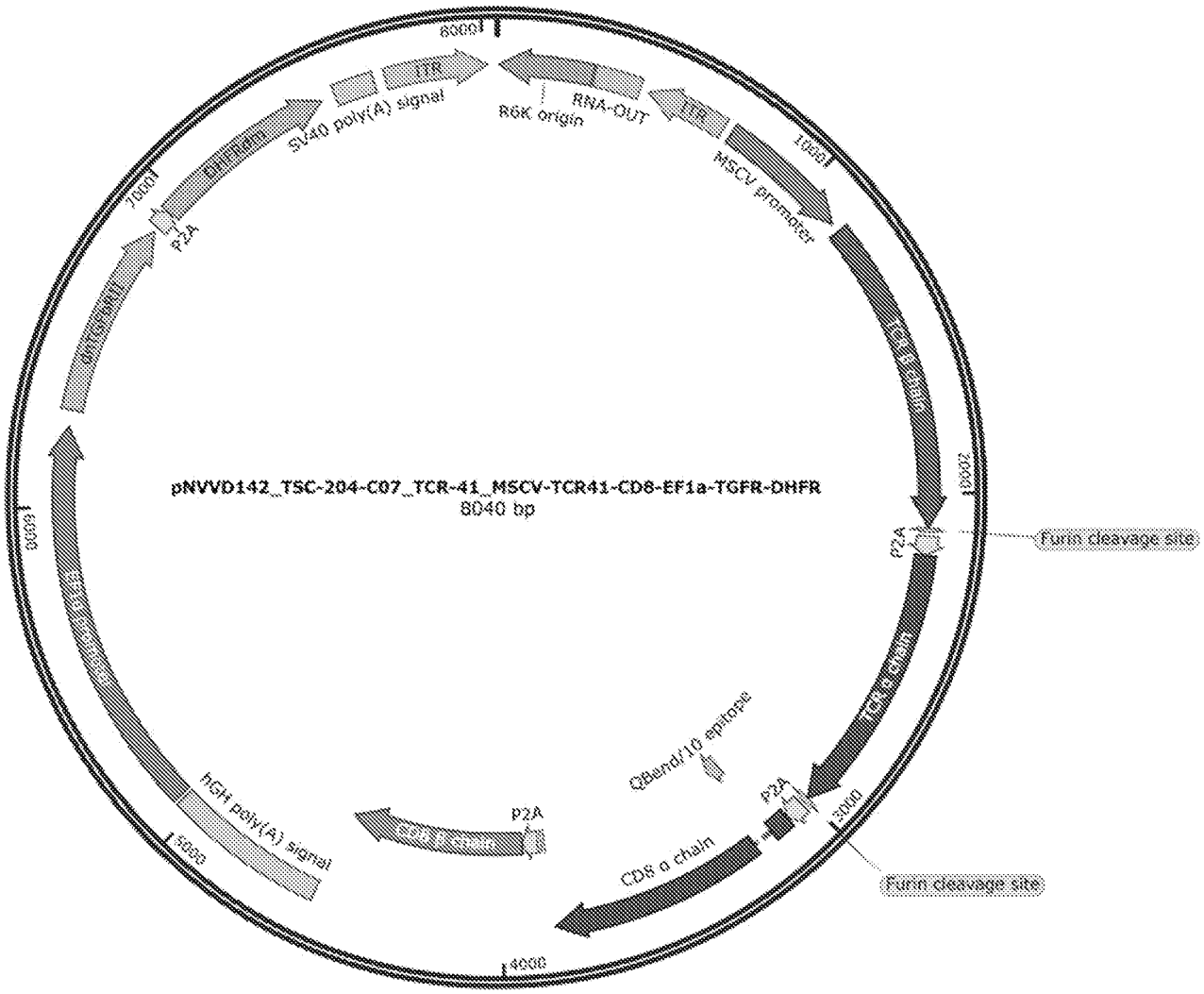
Key: CD: cluster of differentiation RNA-OUT: anti-sense RNA against the bacterial levansucrase encoded by sacB. SV: simian virus TCR: T Cell Receptor, ITR: inverted terminal repeat, QBend: Mouse anti Human CD34 antibody, dnTGFbRII: Dominant-negative TGF beta Receptor II, DHFR: Dihydrofolate reductase selection marker

FIG. 31



Key: CD: cluster of differentiation RNA-OUT: anti-sense RNA against the bacterial levansucrase encoded by sacB. SV: simian virus TCR: T Cell Receptor, ITR: inverted terminal repeat, QBend: Mouse anti Human CD34 antibody, dnTGFbRII: Dominant-negative TGF beta Receptor II, DHFR: Dihydrofolate reductase selection marker

FIG. 32



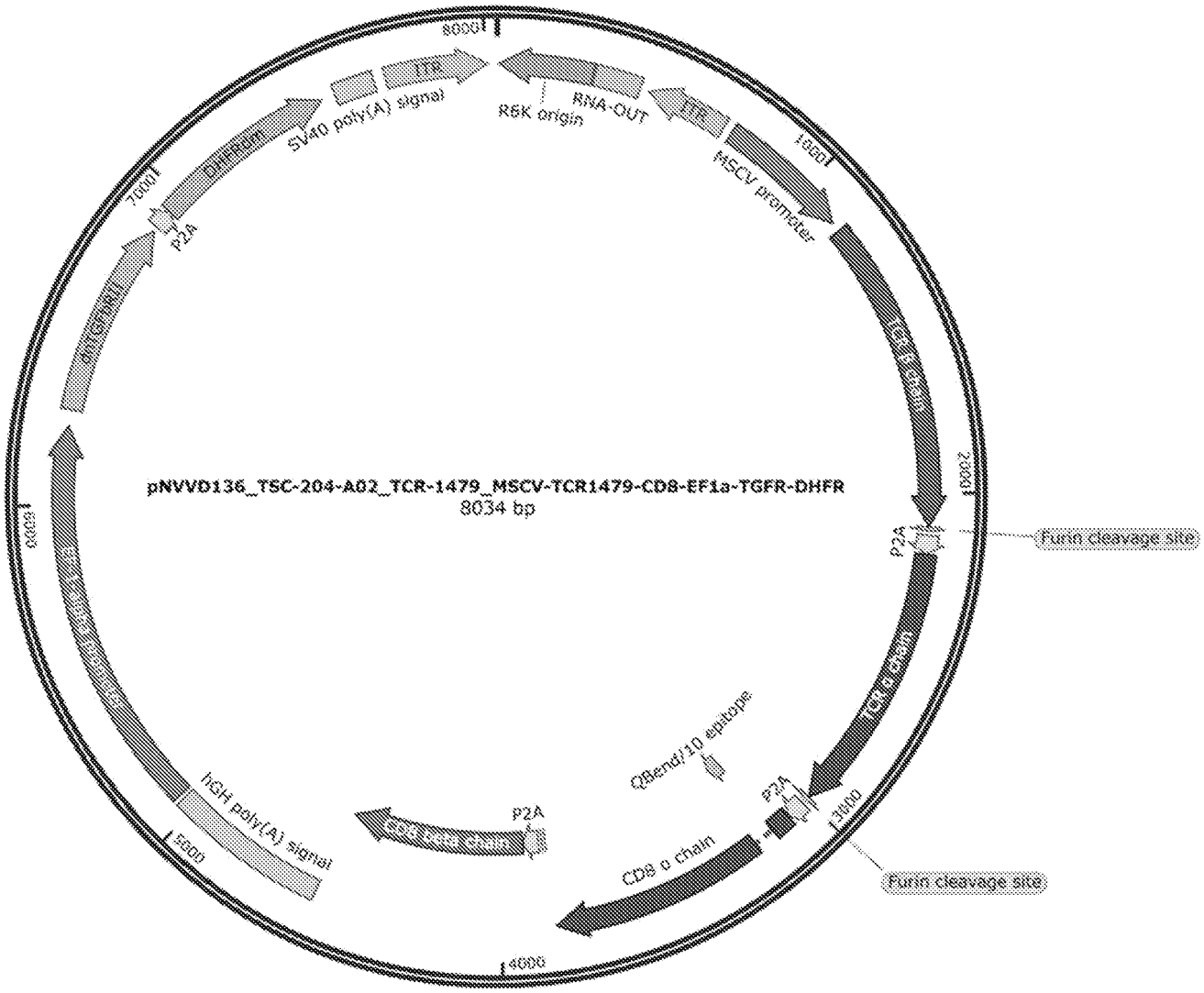
Key: CD: cluster of differentiation RNA-OUT: anti-sense RNA against the bacterial levansucrase encoded by sacB. SV: simian virus TCR: T Cell Receptor, ITR: inverted terminal repeat, QBend: Mouse anti Human CD34 antibody, dnTGFbRII: Dominant-negative TGF beta Receptor II, DHFR: Dihydrofolate reductase selection marker

FIG. 33



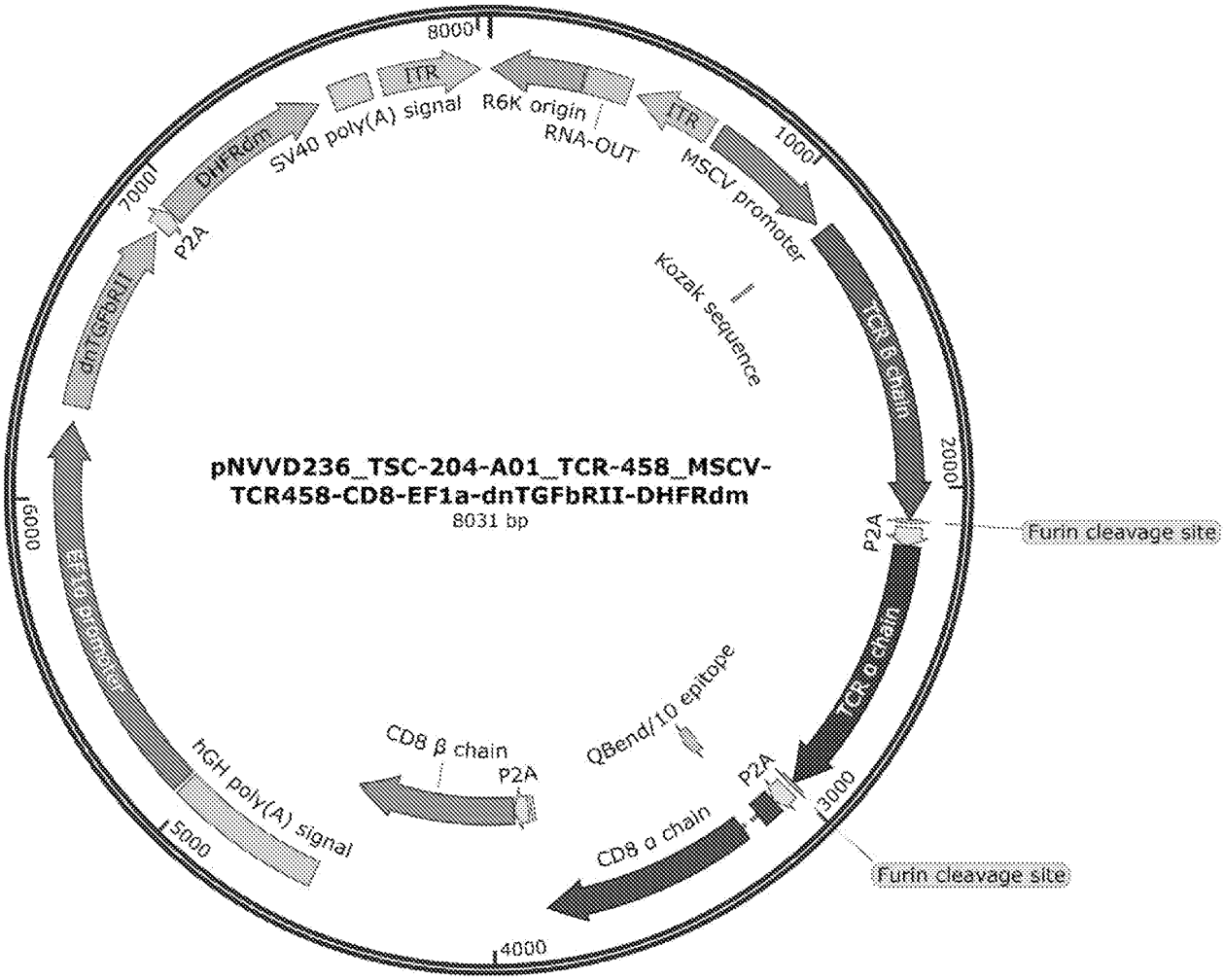
Key: CD: cluster of differentiation RNA-OUT: anti-sense RNA against the bacterial levansucrase encoded by sacB. SV: simian virus TCR: T Cell Receptor, ITR: inverted terminal repeat, QBend: Mouse anti Human CD34 antibody, dnTGFbRII: Dominant-negative TGF beta Receptor II, DHFR: Dihydrofolate reductase selection marker.

FIG. 34



CD: cluster of differentiation RNA-OUT: anti-sense RNA against the bacterial levansucrase encoded by sacB. SV: simian virus TCR: T Cell Receptor, ITR: inverted terminal repeat, QBend: Mouse anti Human CD34 antibody, dnTGFbRII: Dominant-negative TGF beta Receptor II, DHFR: Dihydrofolate reductase selection marker.

FIG. 35

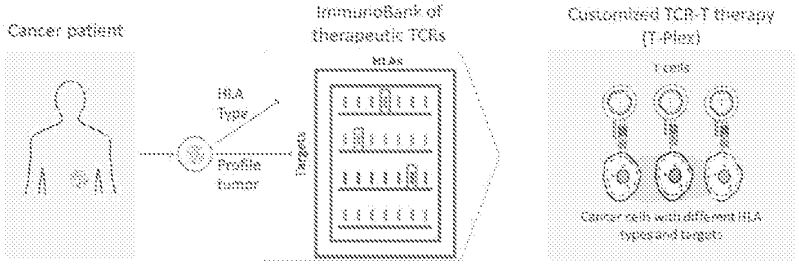


CD: cluster of differentiation, RNA-OUT: anti-sense RNA against the bacterial levansucrase encoded by sacB, SV40: simian virus 40, TCR: T Cell Receptor, ITR: inverted terminal repeat, QBend: Mouse anti Human CD34 antibody, dnTGFbRII: Dominant-negative TGF beta Receptor II, DHFR: Dihydrofolate reductase selection marker.

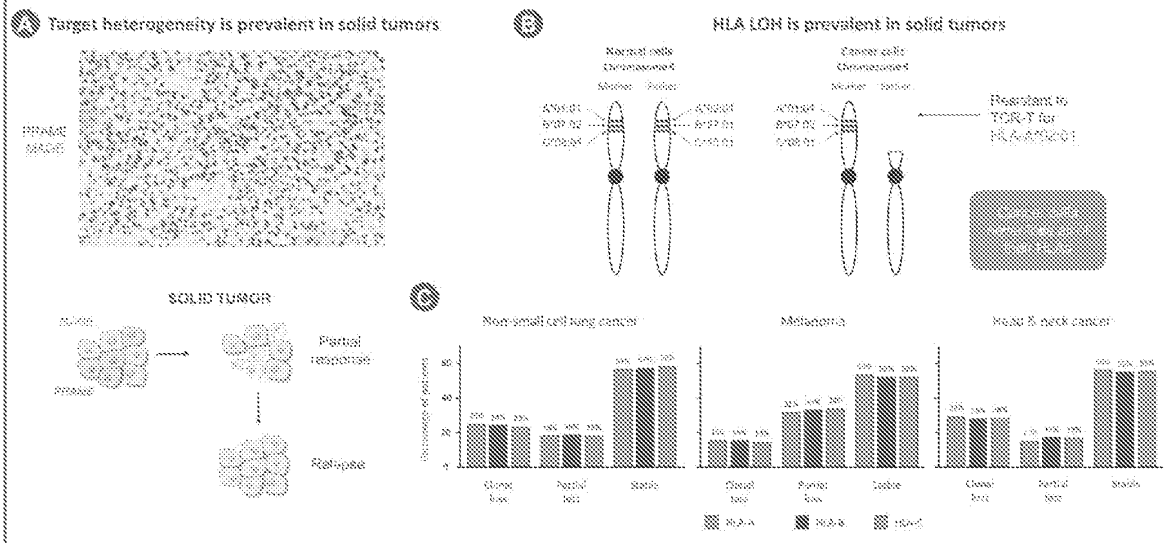
FIG. 36

Background and Rationale

- Advanced solid tumor malignancies are difficult to conquer due to complex biology
- Lack of sufficient endogenous anti-tumor T cells hinders the immune response, even in the presence of checkpoint inhibitors
- T cells engineered with exogenous T cell receptors (TCR-Ts) can target tumor specific antigens presented on HLAs to kill tumor cells
- However, solid tumors are notoriously heterogenous with variable target antigen expression, and may also have HLA loss of heterozygosity (LOH) in up to 40% of tumors, a common resistance mechanism to avoid immune detection
- First-generation TCR-Ts targeting single antigens had limited response rates (30-50%) and short durations of response (3-4 months), possibly due to tumor target heterogeneity and HLA LOH, an immuno-suppressive tumor microenvironment, poor TCR-T persistence and/or TCR-T exhaustion.
- Our solution is to administer multiple enhanced TCR-Ts targeting different shared antigens commonly expressed in solid tumors presented on diverse and frequent HLA types (T-Plex).
- TCR-Ts are enhanced with co-delivery of CD8α/β and a dominant negative TGFβ receptor to enable potent tumor killing and long-term persistence

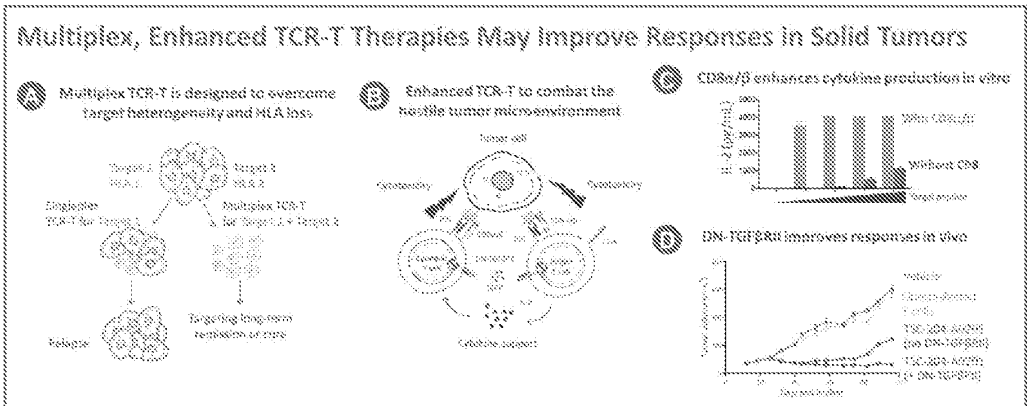


Heterogeneity in Solid Tumors Limits the Efficacy of Singleplex Therapies

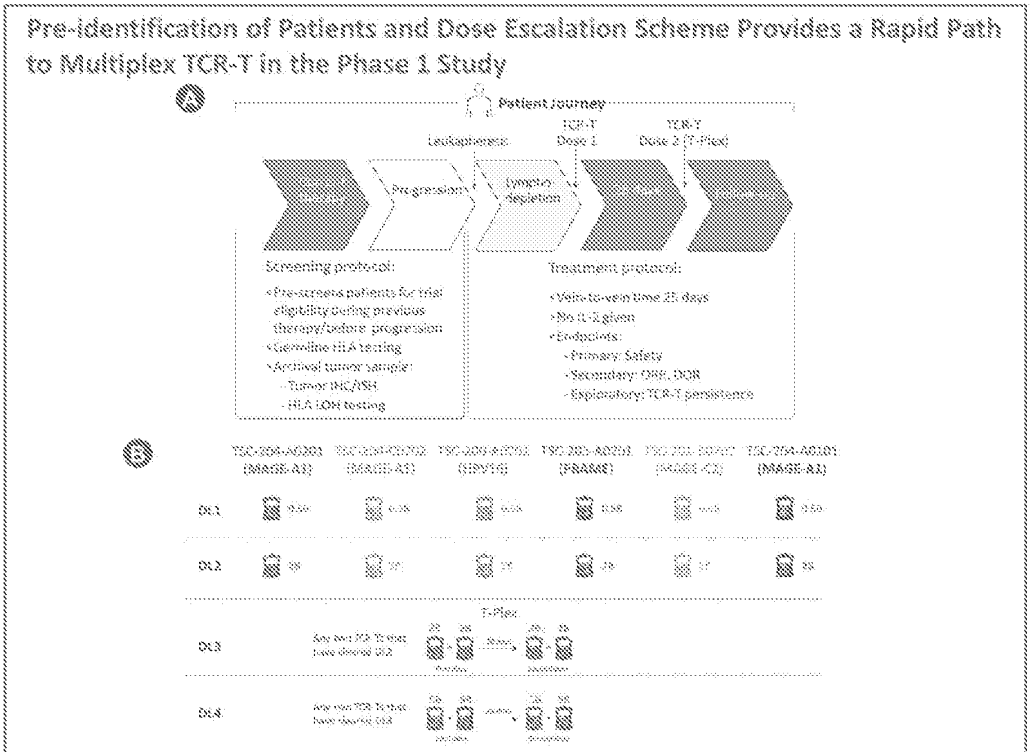


(A) Melanoma samples were stained with PRAME (purple) and MAGE-C2 (blue) antibodies. Tumor cells were noted to be positive for either PRAME or MAGE-C2 or both. Targeting single antigens, for example PRAME, could result in partial responses followed by eventual disease progression. (B) HLA LOH generally occurs through loss of an HLA haplotype on chromosome 6. (C) Novel pan-HLA LOH detection algorithm using tumor/normal comparisons of Tempus tumor data indicate that clonal and subclonal loss of HLA occurs in ~15-50% of common solid tumors (Mayor et al SITC 2023). Tumors that have lost the target HLA cannot respond to TCR-T therapy, however cancer cells never lose all their HLAs. Prospective screening for LOH allows for selection of TCR-Ts targeting intact HLAs only. Administration of multiplex TCR-Ts, with targets on HLAs from both haplotypes, may prevent tumor escape due to the emergence of LOH during treatment.

FIG. 37



(A) First-generation TCR-Ts targeting single antigens on single HLA types often result in partial responses and rapid progression. Administration of multiplexed TCR-T targeting different target antigens on different HLA types has the potential to induce more durable or even complete responses. (B) TCR-Ts are enhanced with co-delivery of CD8α/β to engage helper T cells and a dominant negative TGFβ receptor (DN-TGFβRII) to enhance T cell expansion/persistence. (C) CD4+ T cells engineered to express TCRs+CD8α+CD8β receptors had ~1700-fold higher cytokine production versus CD4+ cells expressing TCR only. (D) Expression of dominant-negative TGFβRII (DN-TGFβRII) in a xenograft mouse model demonstrates more durable responses *in vivo*. NOG mice implanted with Human U266B1 tumors were randomized and received two doses of TSC-204-A0201 TCR-T cells ± DN-TGFβRII, untransfected control T cells, or vehicle control (PBS). Tumor volumes were measured twice weekly.



(A) Patients with melanoma, NSCLC, head and neck cancer, cervical cancer, ovarian cancer, anogenital cancers or other solid tumors with a reasonable likelihood of target expression are eligible. Screening includes germline HLA typing then archival tumor testing for antigens and HLA LOH any time during standard antecedent treatment. Treatment involves one to two doses of TCR-T therapy after lymphodepletion.

(B) INDs have been cleared for six TCR-Ts targeting: MAGE-A1 on HLA-A*02:01 (TSC-204-A0201), HLA-C*07:02 (TSC-204-CD792), and HLA-A*01:01 (TSC-204-A0101); HPV16 on HLA-A*02:01 (TSC-200-A0201); PRAME on HLA-A*02:01 (TSC-203-A0201), and MAGE-C2 on HLA-B*07:02 (TSC-201-B0702) and their combinations (T-Plex). As additional IND applications are cleared, they will be incorporated into the same study and follow the same dose escalation scheme.

FIG. 38

Key Inclusion/Exclusion Criteria and Specifications for Study NCT05973487:

Inclusion Criteria

- Age ≥ 18, all genders
- Diagnosis of advanced melanoma, non-small cell lung cancer, head and neck cancer, ovarian cancer, cervical or anogenital cancers, or other solid tumors with reasonable chance of expressing 1 or more relevant targets
- Failure of standard of care, including any relevant targeted therapy or checkpoint inhibitor
- At least 1 HLA with a TCR-T match and no LOH of that HLA in the tumor
- At least 1 target match
- At least 1 measurable lesion per modified RECIST v1.1
- ECOG PS 0-1 with adequate organ function

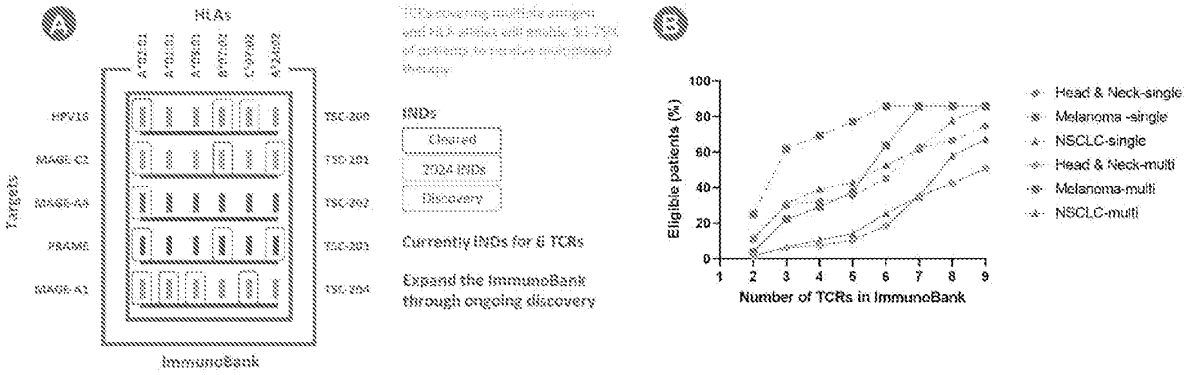
Exclusion Criteria

- CNS metastases that are symptomatic or in need of treatment; carcinomatous meningitis
- Major cardiac pathology or stroke/TIA within 12 months of enrollment
- Systemic corticosteroids within 7 days of enrollment
- Concurrent therapy that has not yet met washout requirements
- Hypersensitivity to fludarabine, cyclophosphamide, or study drug components
- Presence of an HLA type that may interfere with the HLA type being targeted

Protocol Specifications

- Lymphodepletion: Cyclophosphamide (60 mg/kg on Days -7 and -6); fludarabine (30 mg/m2 on Days -7 to -3)
- Hospitalization of 1st three singleplex and 1st three T-Plex patients for 3-7 days post cell infusion

Eligibility for Multiplex Therapy Increases with Growing Collection of TCR-Ts



(A) The ImmunoBank is the collection of TCR-Ts from which one to two therapies for individual patients are chosen. IND applications for six TCR-Ts and the T-Plex combination have been cleared. Two additional IND applications are on track to be submitted by end-2024. (B) As the number of TCR-T choices grows, the number of solid tumor patients eligible for singleplex therapy (dotted lines) or multiplex therapy (solid lines) increases

FIG. 39A

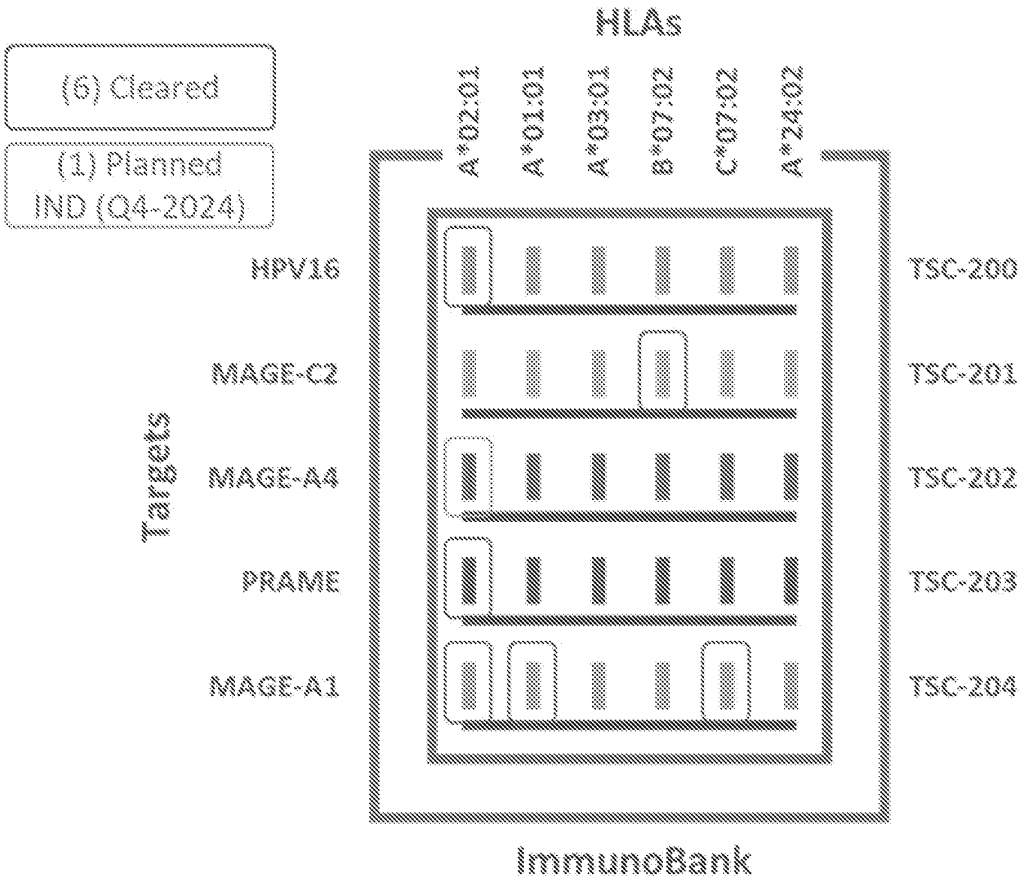

(6) Cleared
(1) Planned IND (Q4-2024)
HLAs
A*02:01
A*01:01
A*03:01
B*07:02
C*07:02
A*24:02
Targets
HPV16
MAGE-C2
MAGE-A4
PRAME
MAGE-A1
TSC-200
TSC-201
TSC-202
TSC-203
TSC-204
ImmunoBank

FIG. 39B

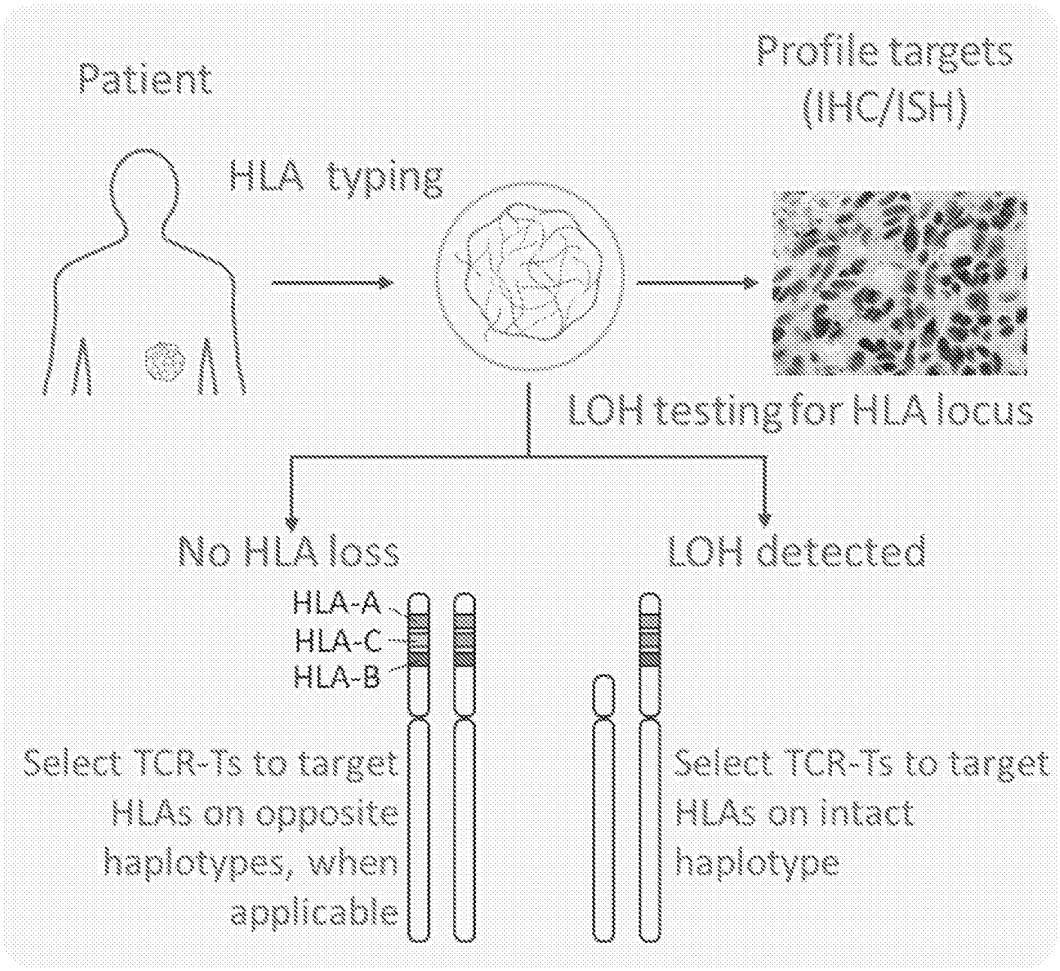

Patient
HLA typing
Profile targets
(IHC/ISH)
LOH testing for HLA locus
No HLA loss
LOH detected
HLA-A
HLA-C
HLA-B
Select TCR-Ts to target HLAs on opposite haplotypes, when applicable
Select TCR-Ts to target HLAs on intact haplotype

FIG. 39C

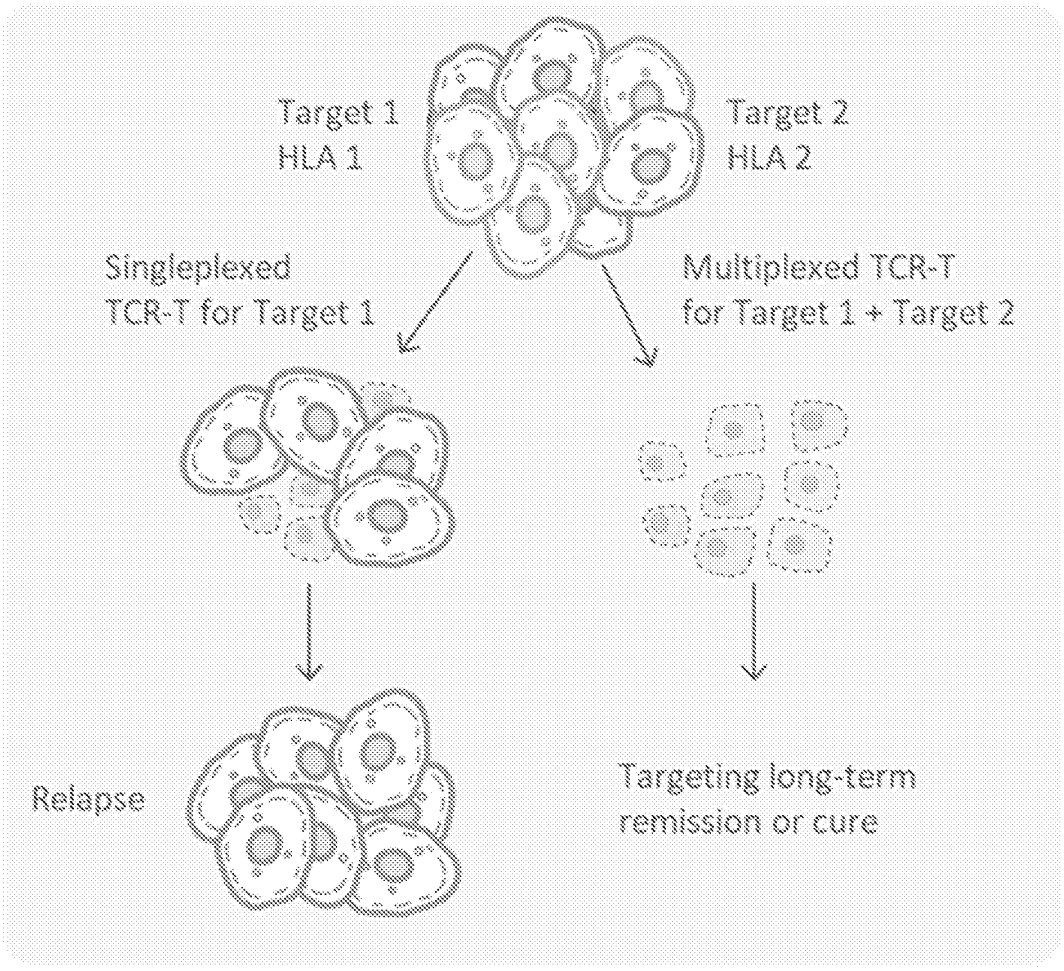

Target 1
HLA 1
Target 2
HLA 2
Singleplexed
TCR-T for Target 1
Multiplexed TCR-T
for Target 1 + Target 2
Relapse
Targeting long-term
remission or cure

FIG. 40A

INDICATION	Both PRAME and MAGE-C2	Both MAGE-A1 and MAGE-C2	Both PRAME and MAGE-A1	Both MAGE-A4 and PRAME	Both MAGE-A4 and MAGE-A1	Both MAGE-A4 and MAGE-C2
Skin Cutaneous Melanoma (N=457)	40.9%	18.4%	27.1%	21.7%	15.8%	14.9%
Lung Squamous Cell Carcinoma (N=427)	11.0%	7.0%	33.7%	58.5%	27.2%	8.9%
Ovarian Serous Cystadenocarcinoma (N=413)	7.7%	1.2%	5.6%	27.1%	3.9%	2.7%
Head & Neck Squamous Cell Carcinoma (N=268)	6.3%	4.5%	19.8%	42.5%	19.0%	7.5%
Cervical & Endocervical Cancer (N=122)	3.3%	1.6%	11.5%	19.7%	10.7%	2.5%

FIG. 40B Melanoma

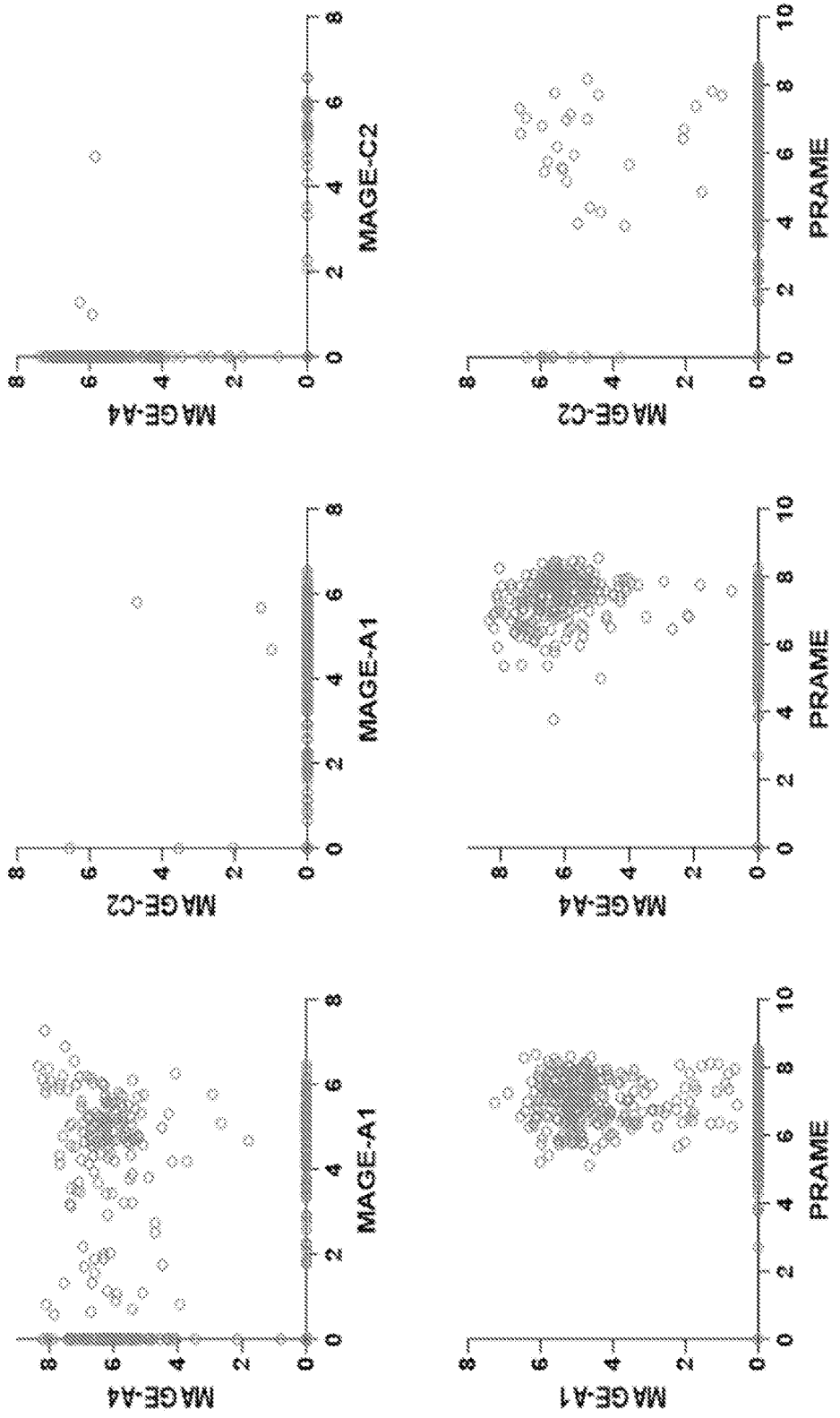


FIG. 40B (continued)

NSCLC

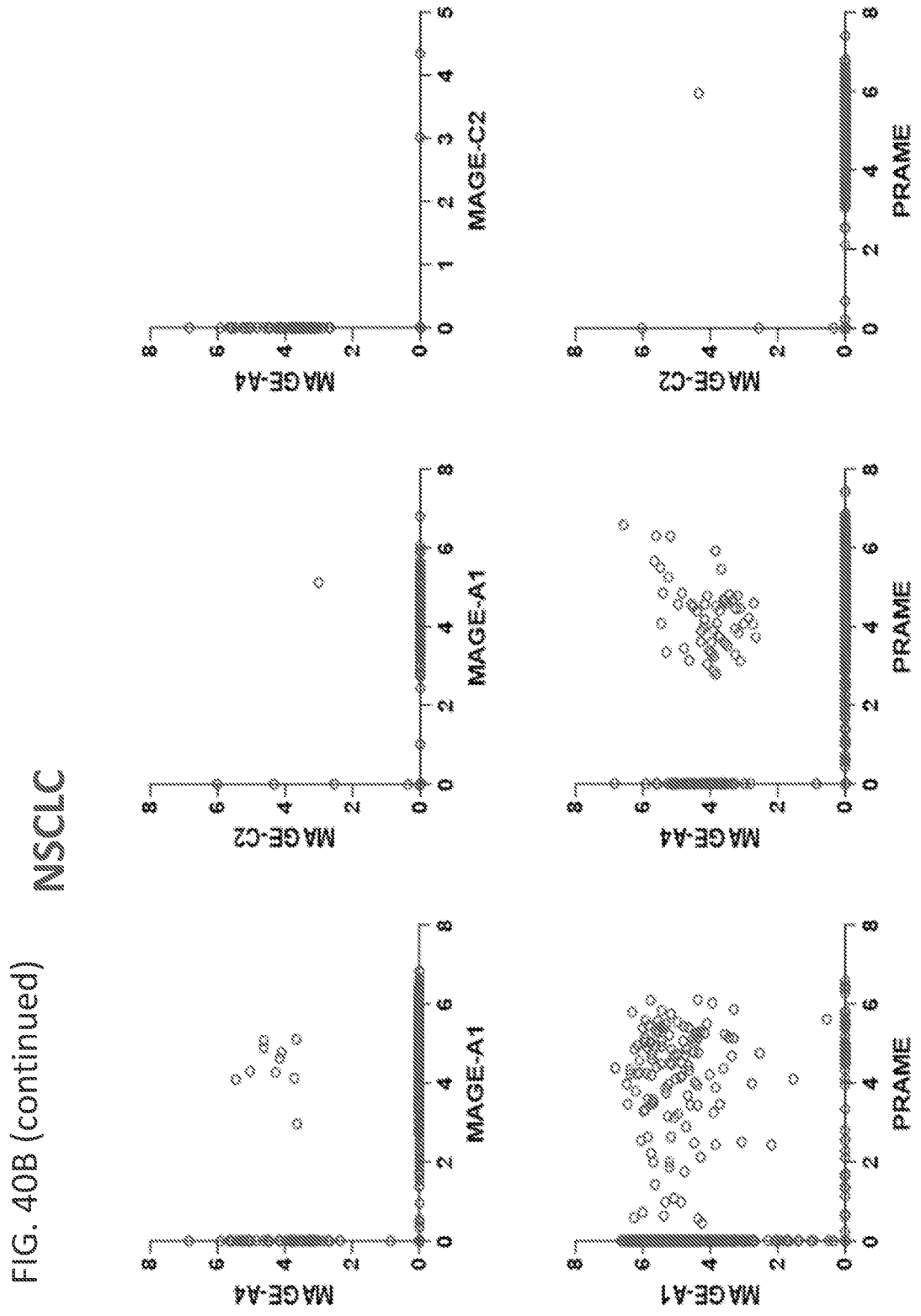


FIG. 40C

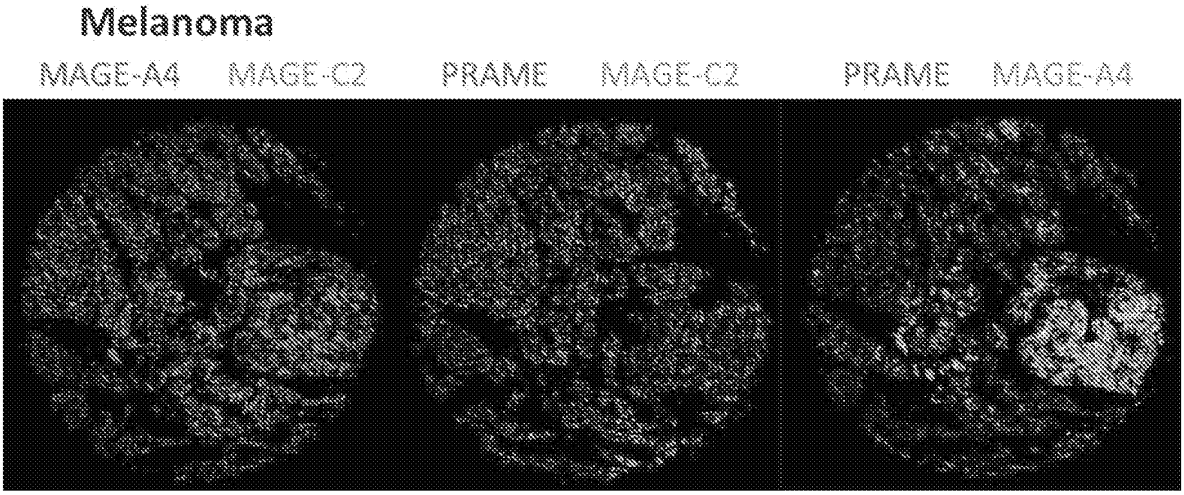


FIG. 40D

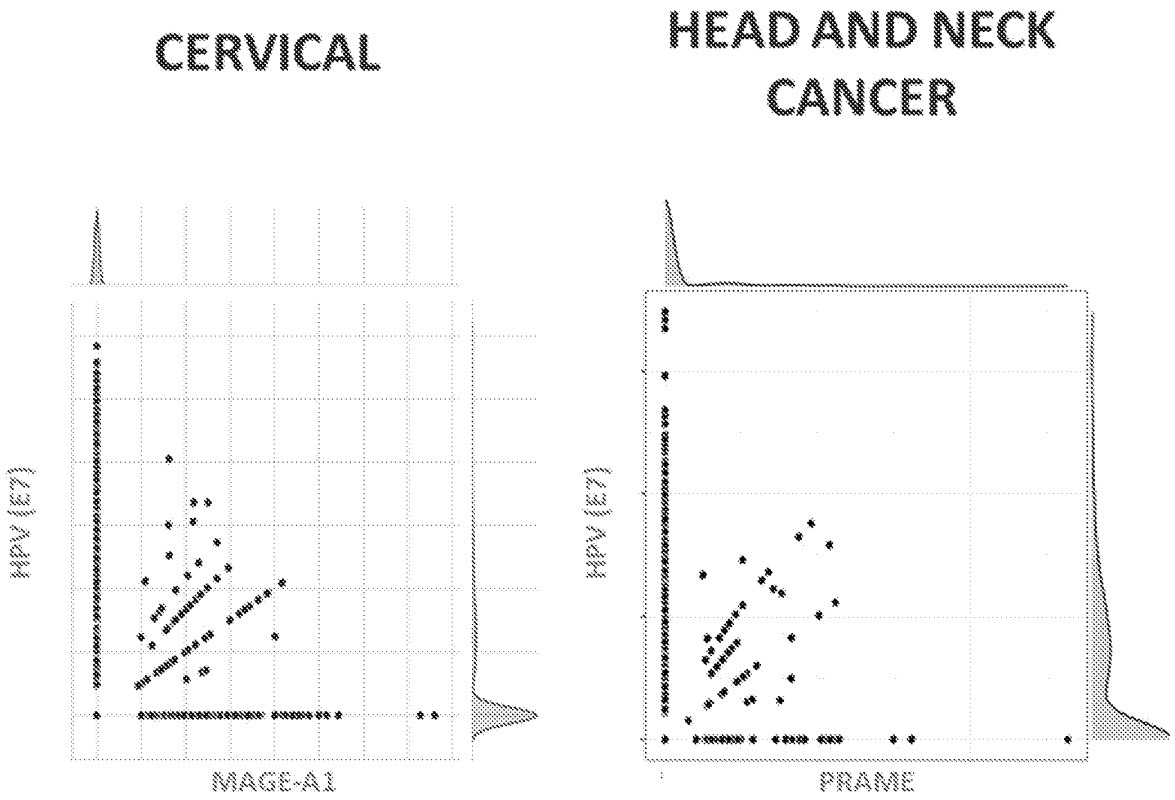
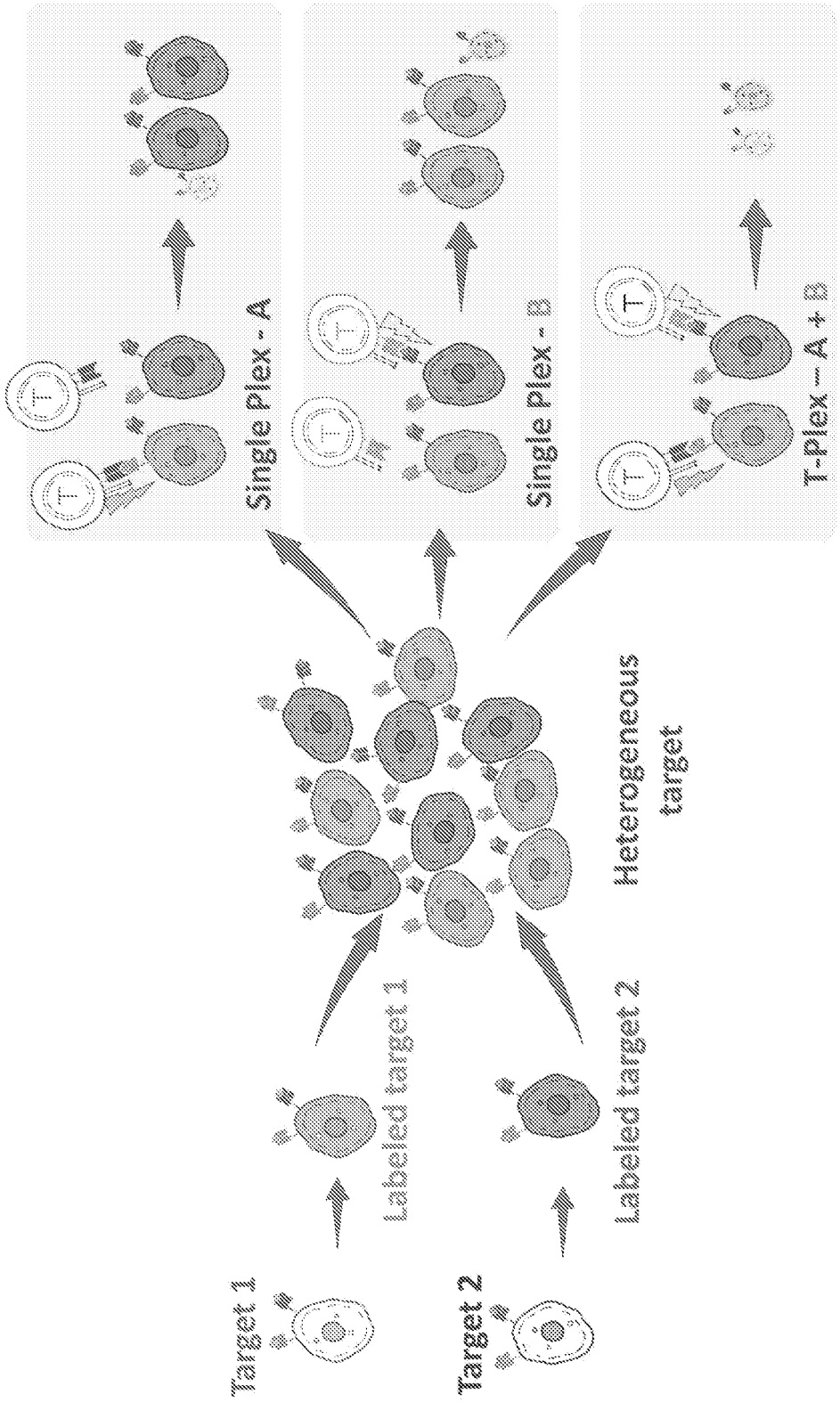


FIG. 41A


Target 1
Target 2
Labeled target 1
Labeled target 2
Heterogeneous target
Single Plex - A
Single Plex - B
T-Plex – A + B

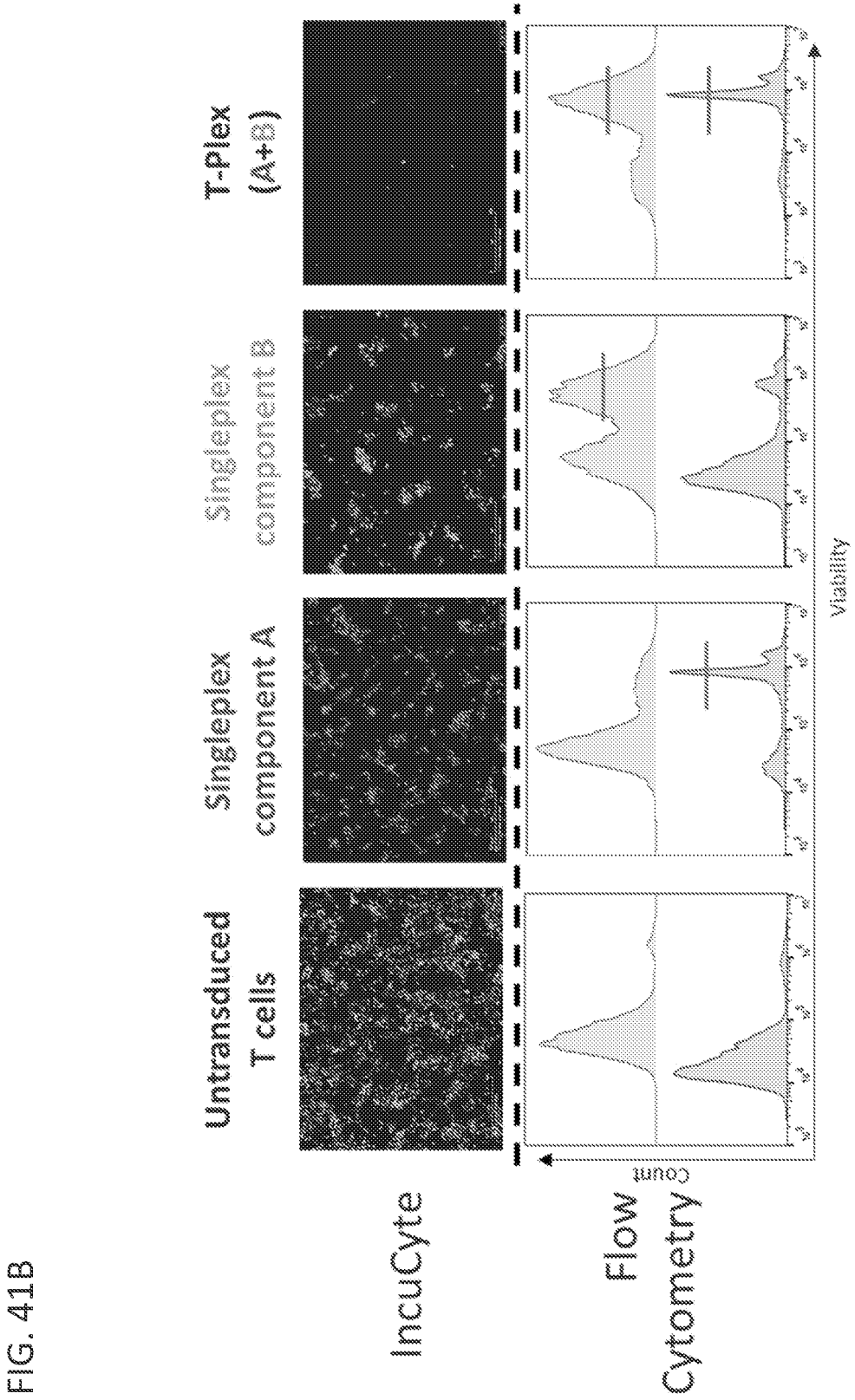
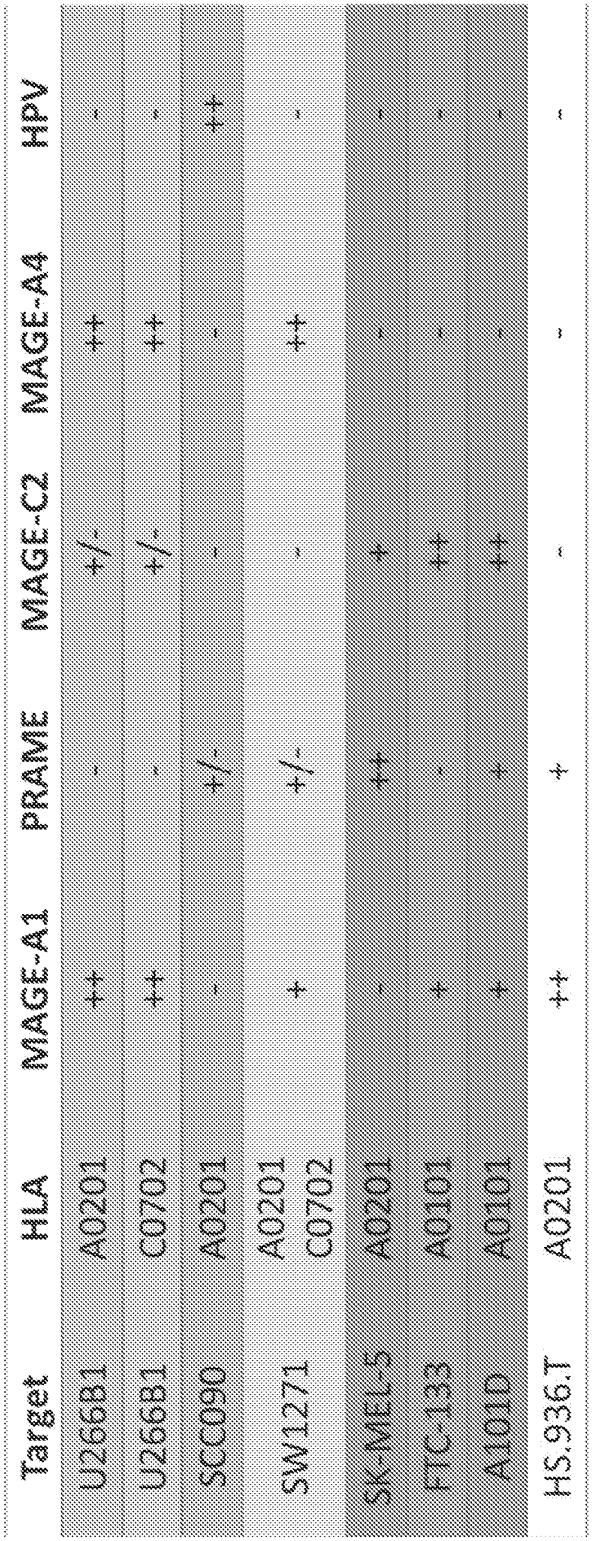

Untransduced T cells
Singleplex component A
Singleplex component B
T-Plex (A+B)
IncuCyte
Flow Cytometry
Count
Viability

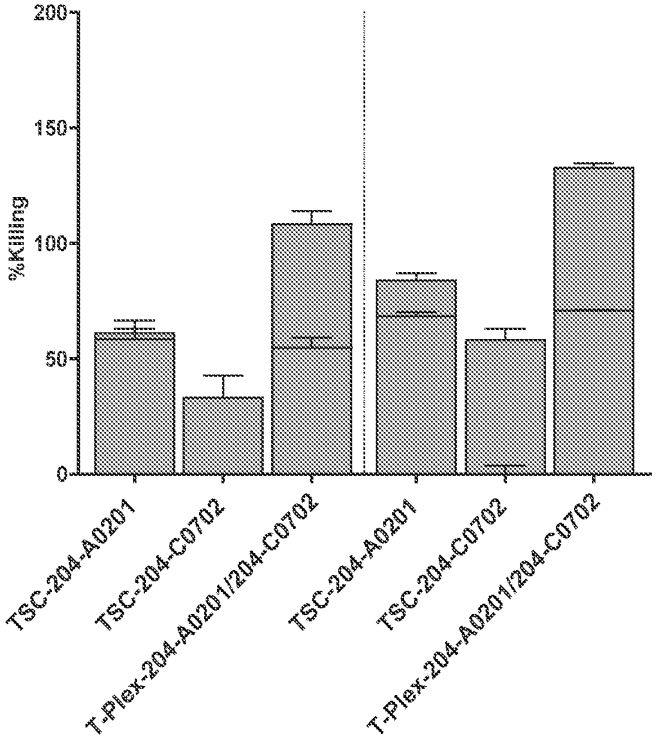
FIG. 41B

FIG. 41C



Target	HLA	MAGE-A1	PRAME	MAGE-C2	MAGE-A4	HPV
U266B1	A0201	++	-	+/-	++	-
U266B1	C0702	++	-	+/-	++	-
SCC090	A0201	-	+/-	-	-	++
SW1271	A0201	+	+/-	-	++	-
SW1271	C0702					
SK-MEL-5	A0201	-	++	+	-	-
FTC-133	A0101	+	-	++	-	-
A101D	A0101	+	+	++	-	-
HS.936.T	A0201	++	+	-	-	-

FIG. 41D


200
150
100
50
0
%Killing
TSC-204-A0201
TSC-204-C0702
T-Plex-204-A0201/204-C0702
TSC-204-A0201
TSC-204-C0702
T-Plex-204-A0201/204-C0702

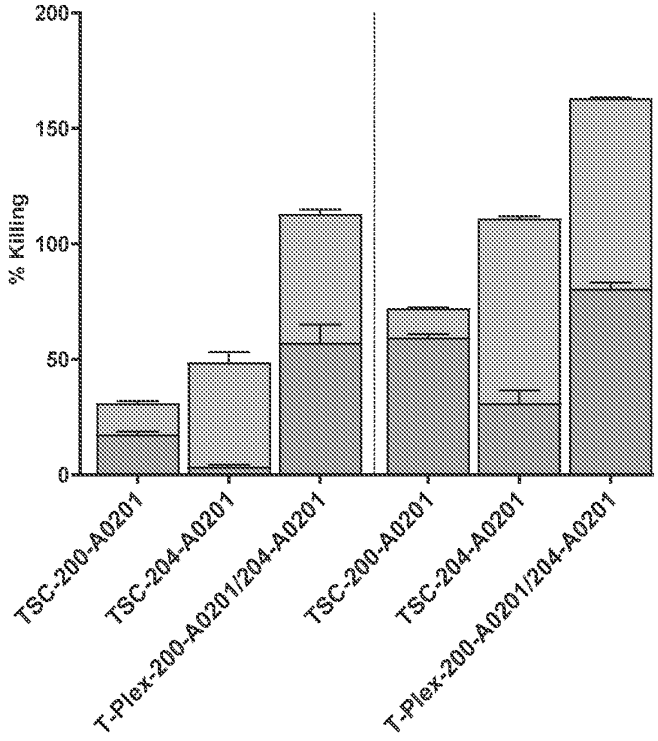
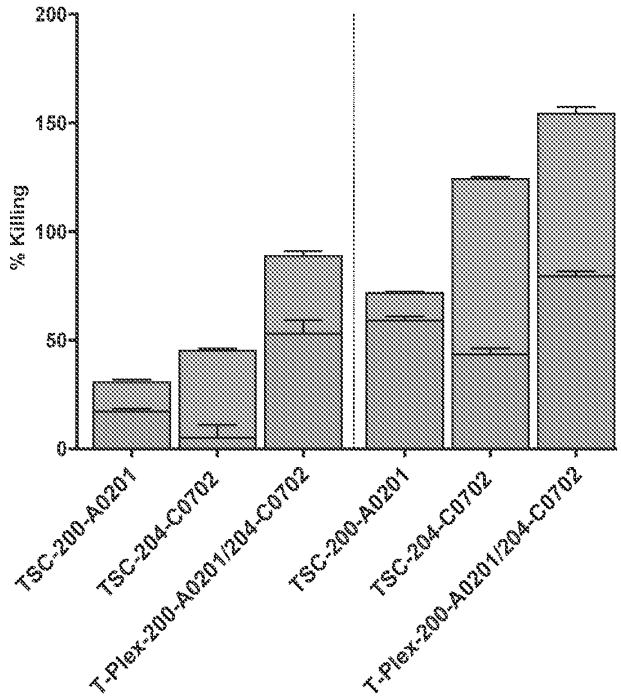

200
150
100
50
0
% Killing
TSC-200-A0201
TSC-204-A0201
T-Plex-200-A0201/204-A0201
TSC-200-A0201
TSC-204-A0201
T-Plex-200-A0201/204-A0201

FIG. 41D (continued)



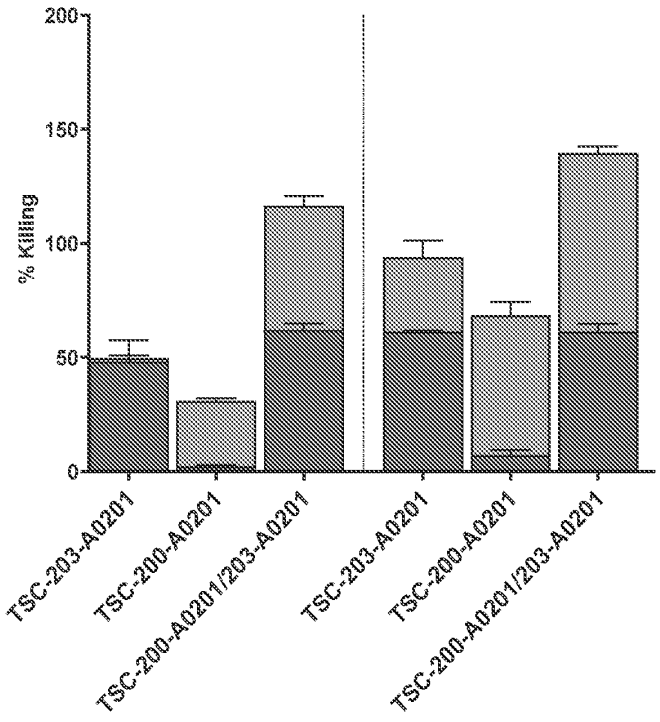
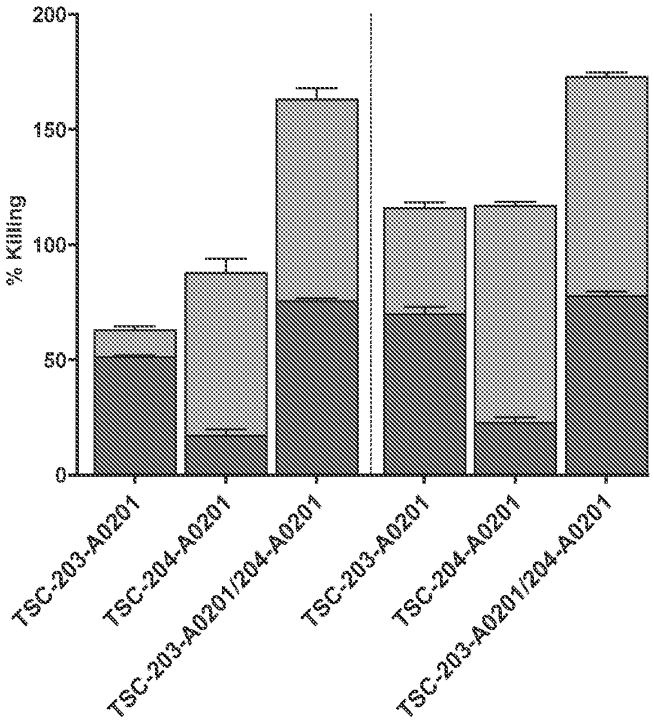


FIG. 41D (continued)



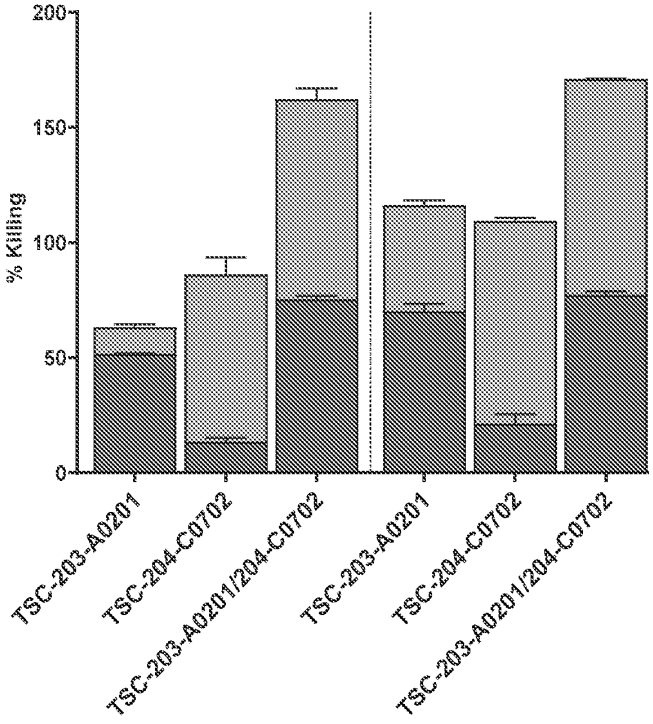
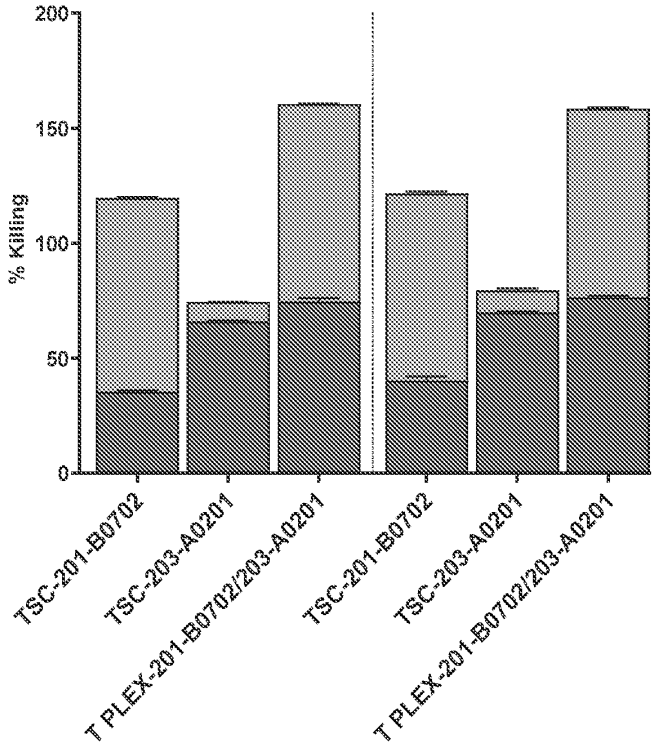


FIG. 41D (continued)



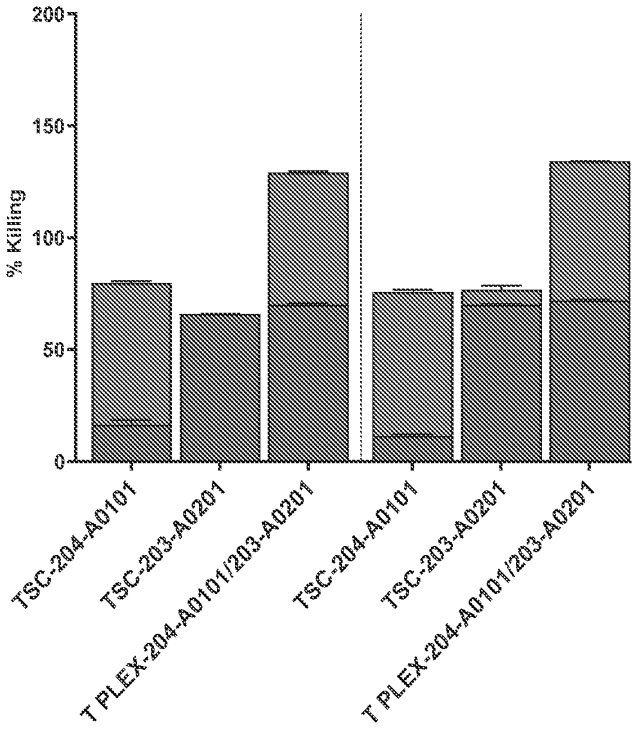


FIG. 41D (continued)

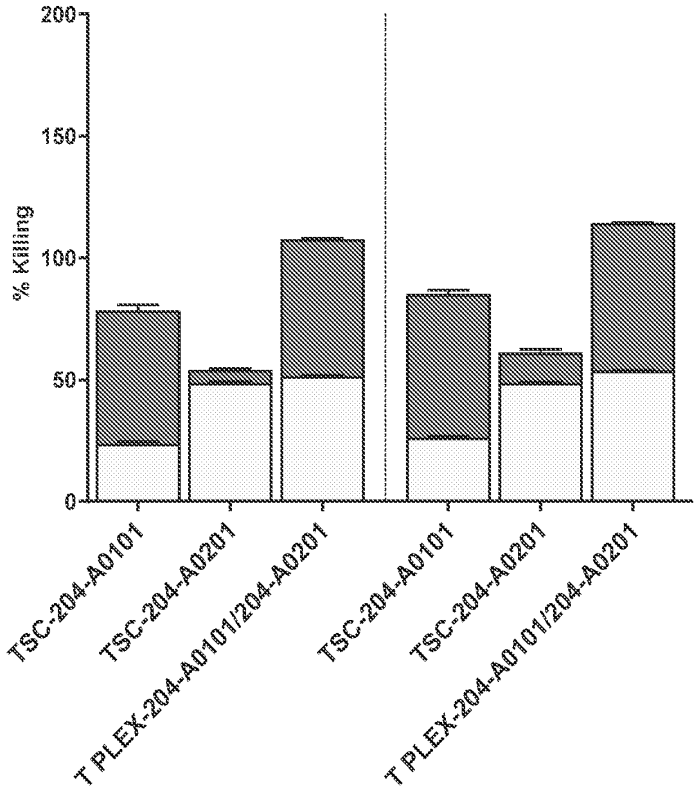


FIG. 42A

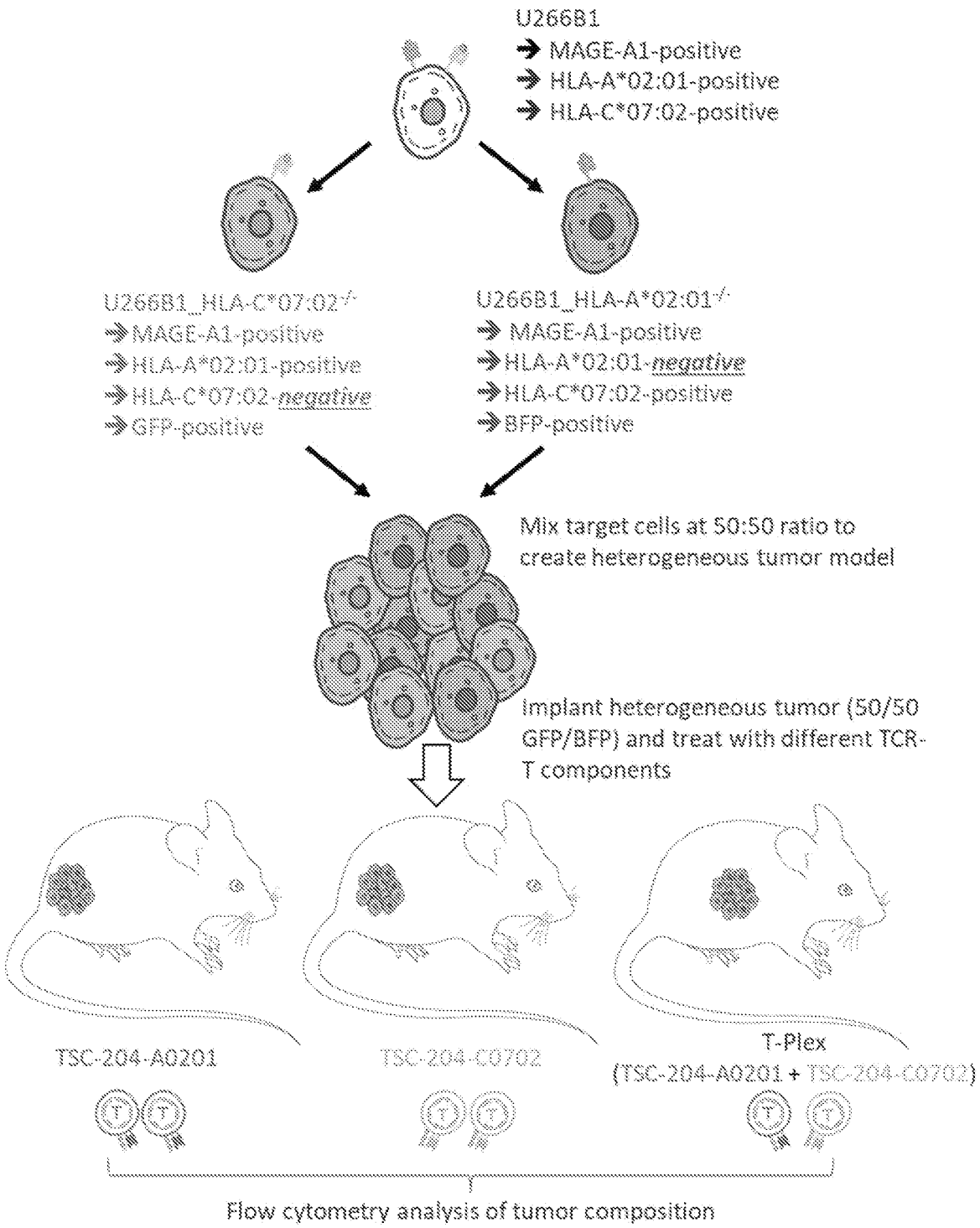

U266B1
MAGE-A1-positive
HLA-A*02:01-positive
HLA-C*07:02-positive
U266B1_HLA-C*07:02-/-
MAGE-A1-positive
HLA-A*02:01-positive
HLA-C*07:02-negative
GFP-positive
U266B1_HLA-A*02:01-/-
MAGE-A1-positive
HLA-A*02:01-negative
HLA-C*07:02-positive
BFP-positive
Mix target cells at 50:50 ratio to create heterogeneous tumor model
Implant heterogeneous tumor (50/50 GFP/BFP) and treat with different TCR-T components
TSC-204-A0201
TSC-204-C0702
T-Plex
(TSC-204-A0201 + TSC-204-C0702)
Flow cytometry analysis of tumor composition

FIG. 42B

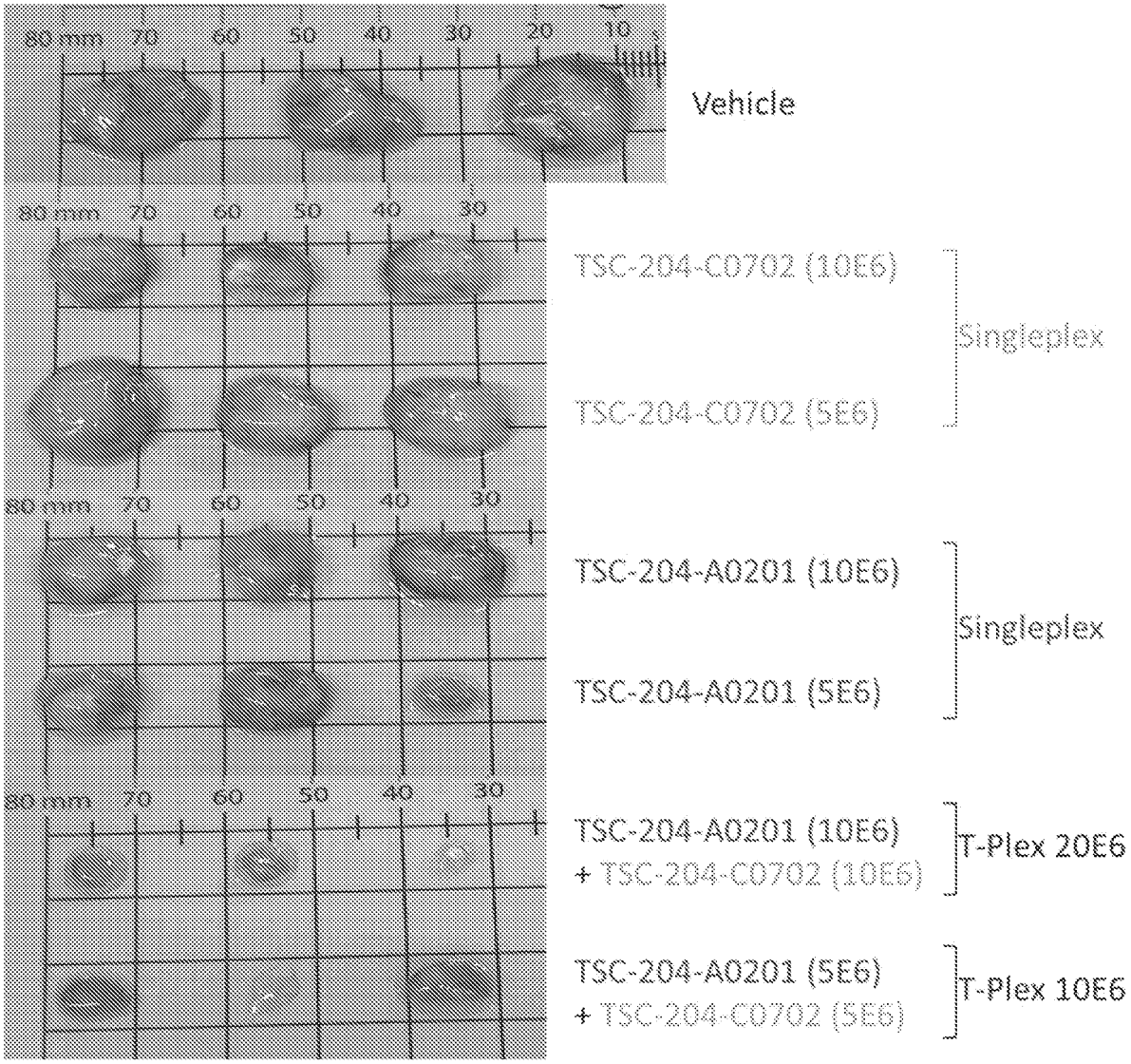

80 mm 70 60 50 40 30 20 10
Vehicle
80 mm 70 60 50 40 30
TSC-204-C07O2 (10E6)
TSC-204-C07O2 (5E6)
Singleplex
80 mm 70 60 50 40 30
TSC-204-A0201 (10E6)
TSC-204-A0201 (5E6)
Singleplex
80 mm 70 60 50 40 30
TSC-204-A0201 (10E6)
+ TSC-204-C07O2 (10E6)
T-Plex 20E6
TSC-204-A0201 (5E6)
+ TSC-204-C07O2 (5E6)
T-Plex 10E6

FIG. 42C

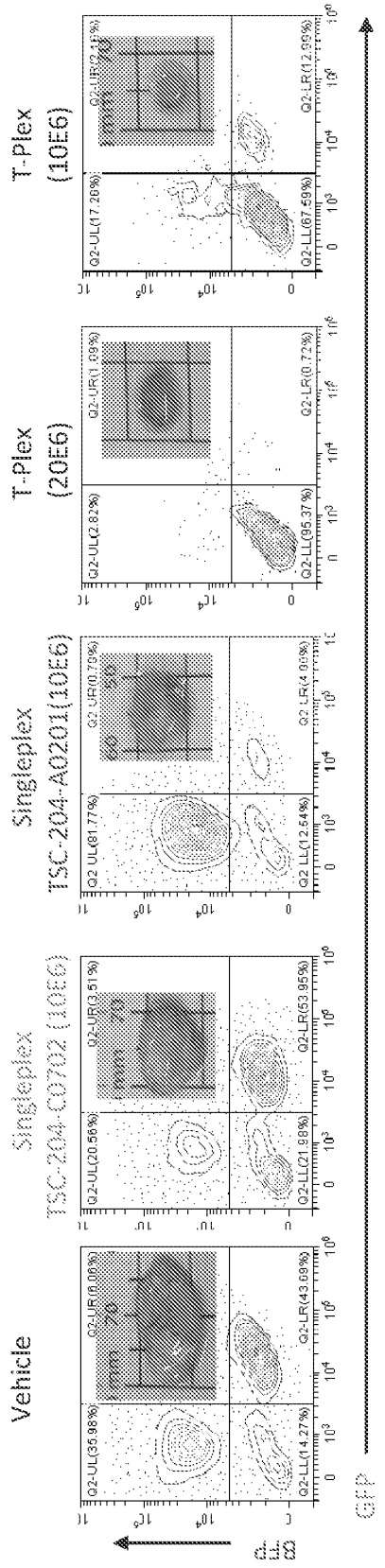

Vehicle
Singleplex
TSC-204-C0702 (10E6)
Singleplex
TSC-204-A0201(10E6)
T-Plex
(20E6)
T-Plex
(10E6)
Q2-UL(35.98%)
Q2-UR(0.00%)
Q2-LL(14.27%)
Q2-LR(43.69%)
Q2-UL(20.56%)
Q2-UR(3.51%)
Q2-LL(21.98%)
Q2-LR(53.95%)
Q2 UL(81.77%)
Q2 UR(0.79%)
Q2 LL(12.54%)
Q2 LR(4.89%)
Q2-UL(2.82%)
Q2-UR(1.09%)
Q2-LL(95.37%)
Q2-LR(0.72%)
Q2-UL(17.26%)
Q2-UR(2.11%)
Q2-LL(67.59%)
Q2-LR(12.99%)
CD8
GFP

FIG. 42D

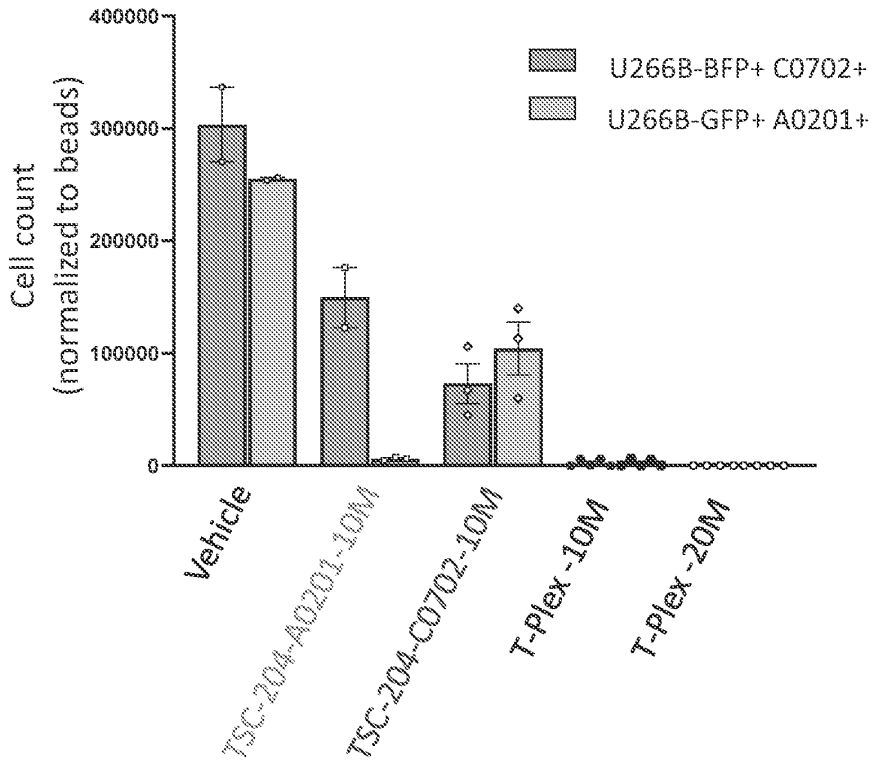
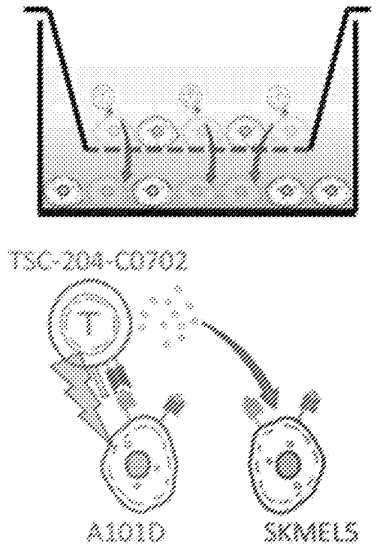

400000
300000
200000
100000
0
Cell count
(normalized to beads)
U266B-BFP+ C0702+
U266B-GFP+ A0201+
Vehicle
TSC-204-A0201-10M
TSC-204-C0702-10M
T-Plex -10M
T-Plex -20M

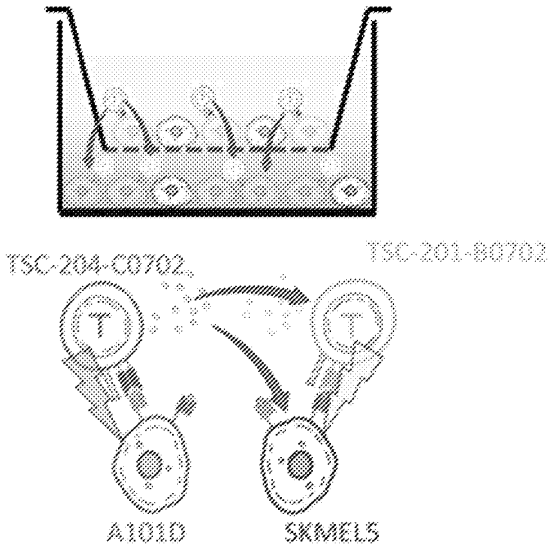
FIG. 43A

MAGE-A1
HLA-C*07:02
A101D
SKMEL5
MAGE-C2
HLA-B*07:02
TSC-201-B0702
A101D
SKMEL5
Weak engagement of TSC-201-B0702 against SK-MEL-5, due to low HLA and target expression, resulting in poor killing

FIG. 43A (continued)



Engagement of
TSC-204-C0702 against A101D (top well)
influences SK-MEL-5 in the bottom well
(HLA expression and bystander killing)



Engagement of TSC-204-C0702 on A101D
(top well) maximizes TSC-201-B0702
engagement in the bottom well resulting
in enhanced killing of SK-MEL-5

FIG. 43B

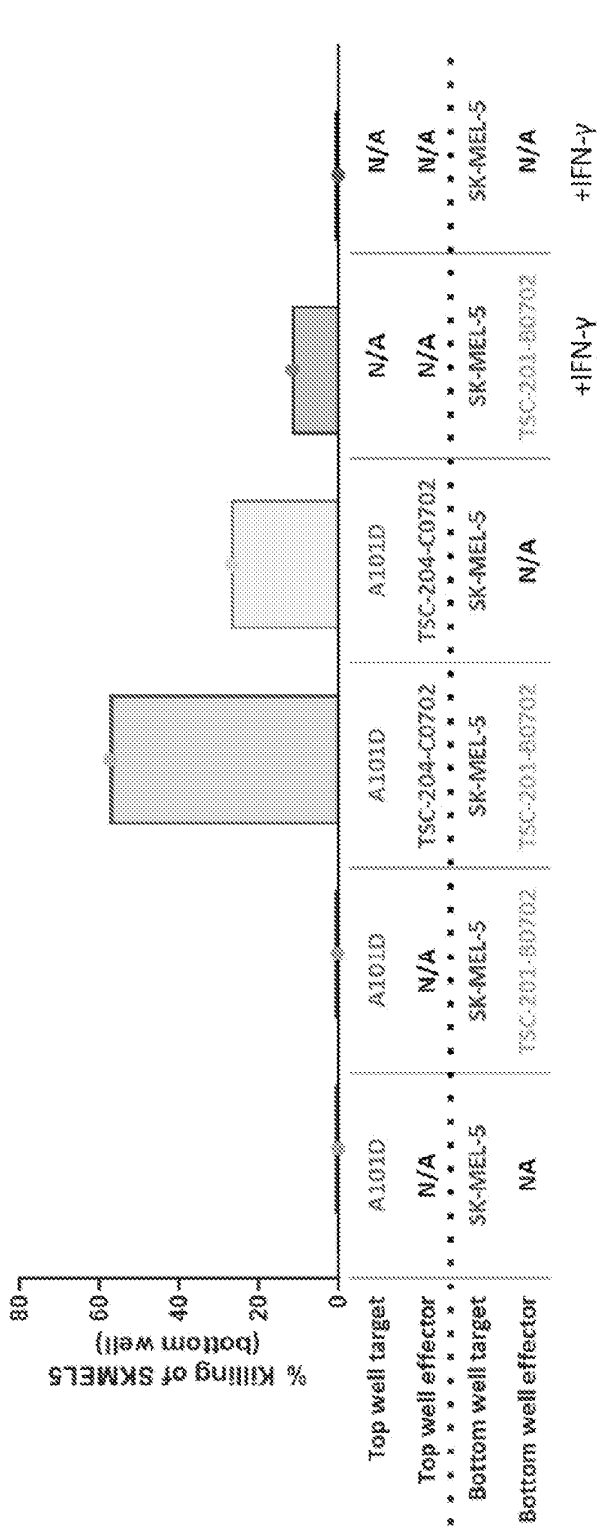

T-Plex supports enhanced direct and indirect killing of target cells
% Killing of SKMEL5 (bottom well)
0
20
40
60
80
Top well target
Top well effector
Bottom well target
Bottom well effector
A101D
N/A
SK-MEL-5
NA
A101D
N/A
SK-MEL-5
TSC-201-A0702
A101D
TSC-204-C0702
SK-MEL-5
TSC-201-A0702
A101D
TSC-204-C0702
SK-MEL-5
N/A
N/A
N/A
SK-MEL-5
TSC-201-A0702
+IFN-γ
N/A
N/A
SK-MEL-5
N/A
+IFN-γ

FIG. 43B (continued)

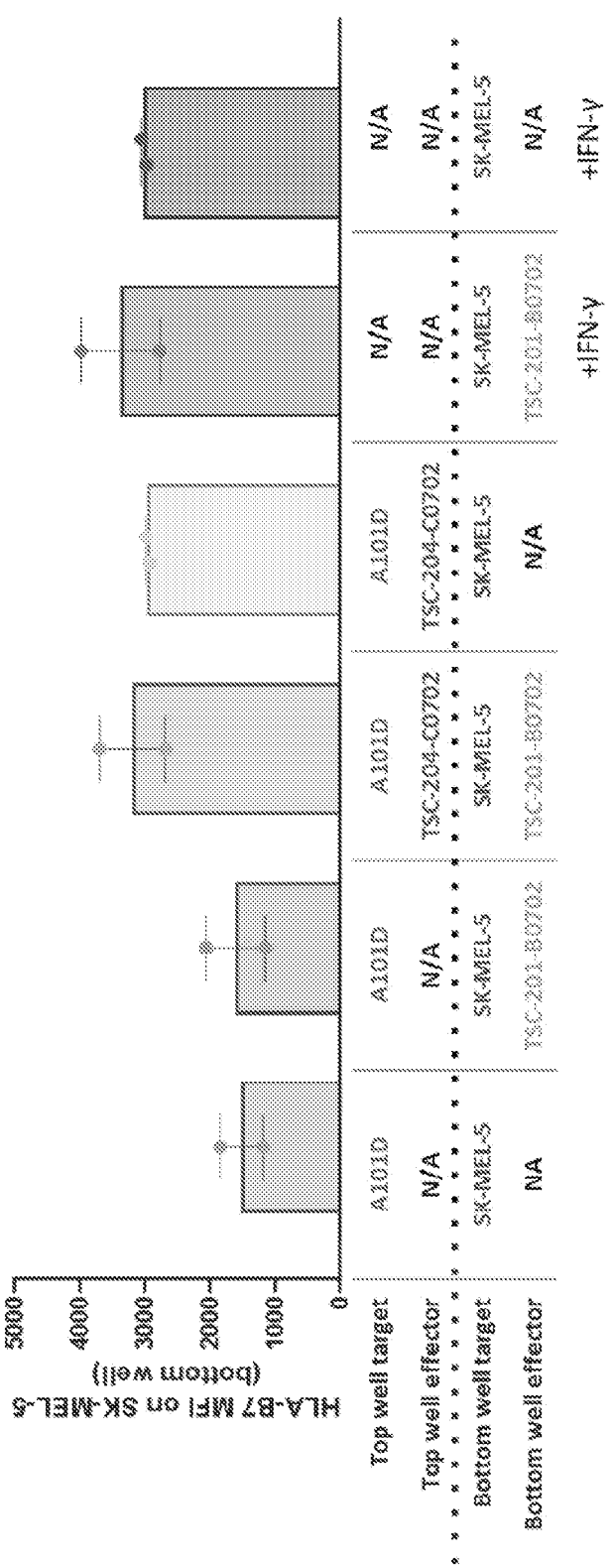


FIG. 43C

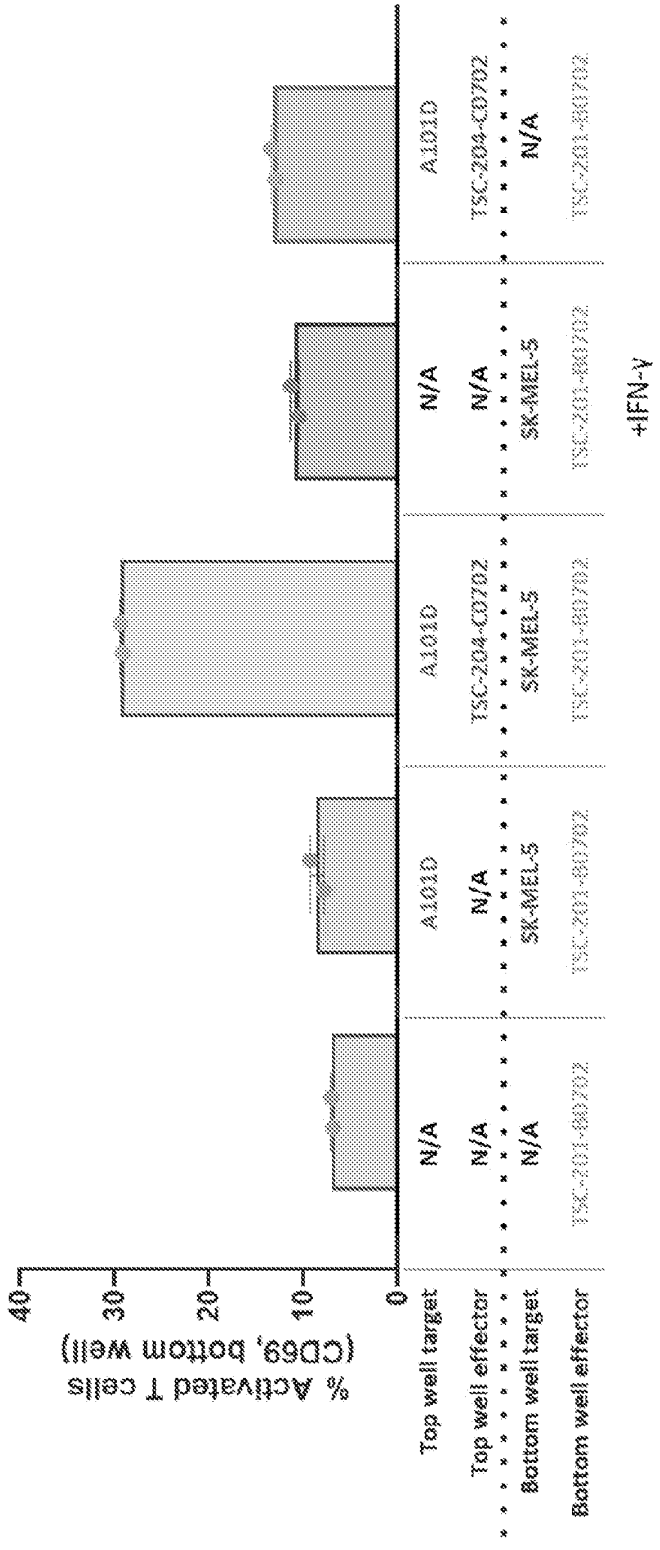

T-Plex supports target-dependent T cell activation
% Activated T cells (CD69, bottom well)
0
10
20
30
40
Top well target
Top well effector
Bottom well target
Bottom well effector
N/A
N/A
N/A
TSC-201-00702
A101D
N/A
SK-MEL-5
TSC-201-00702
A101D
TSC-204-00702
SK-MEL-5
TSC-201-00702
N/A
N/A
SK-MEL-5
TSC-201-00702
A101D
TSC-204-00702
N/A
TSC-201-00702
+IFN-γ

FIG. 43C (continued)

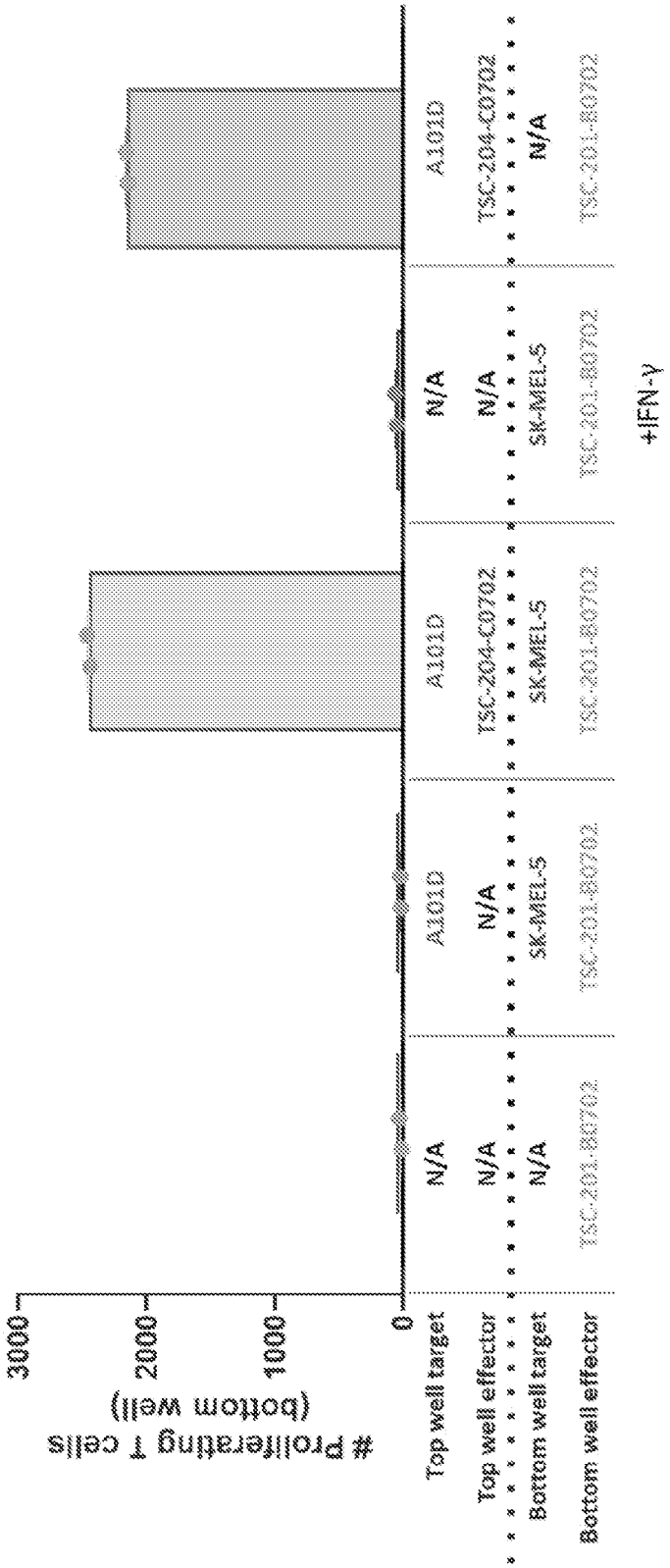


FIG. 43D

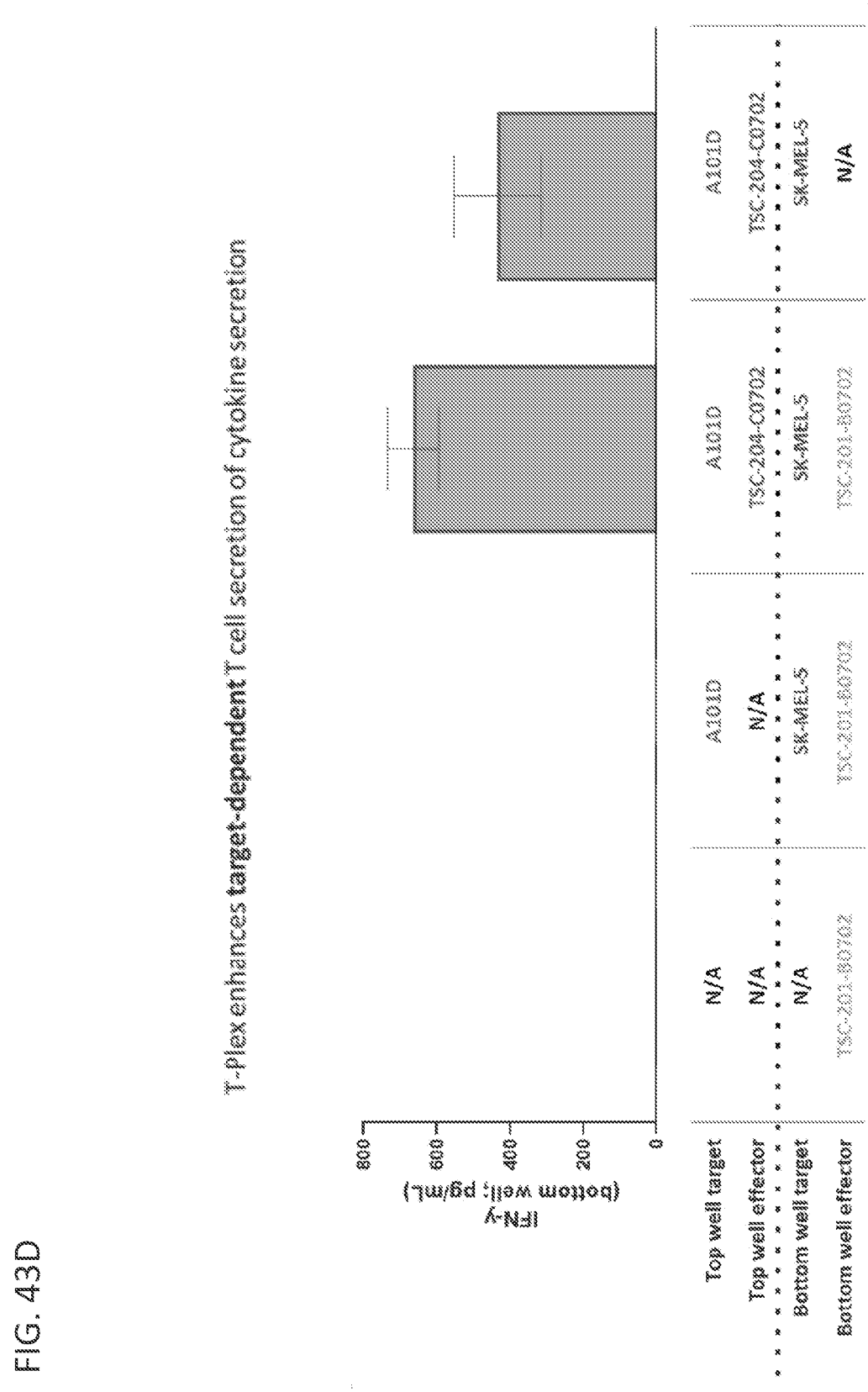
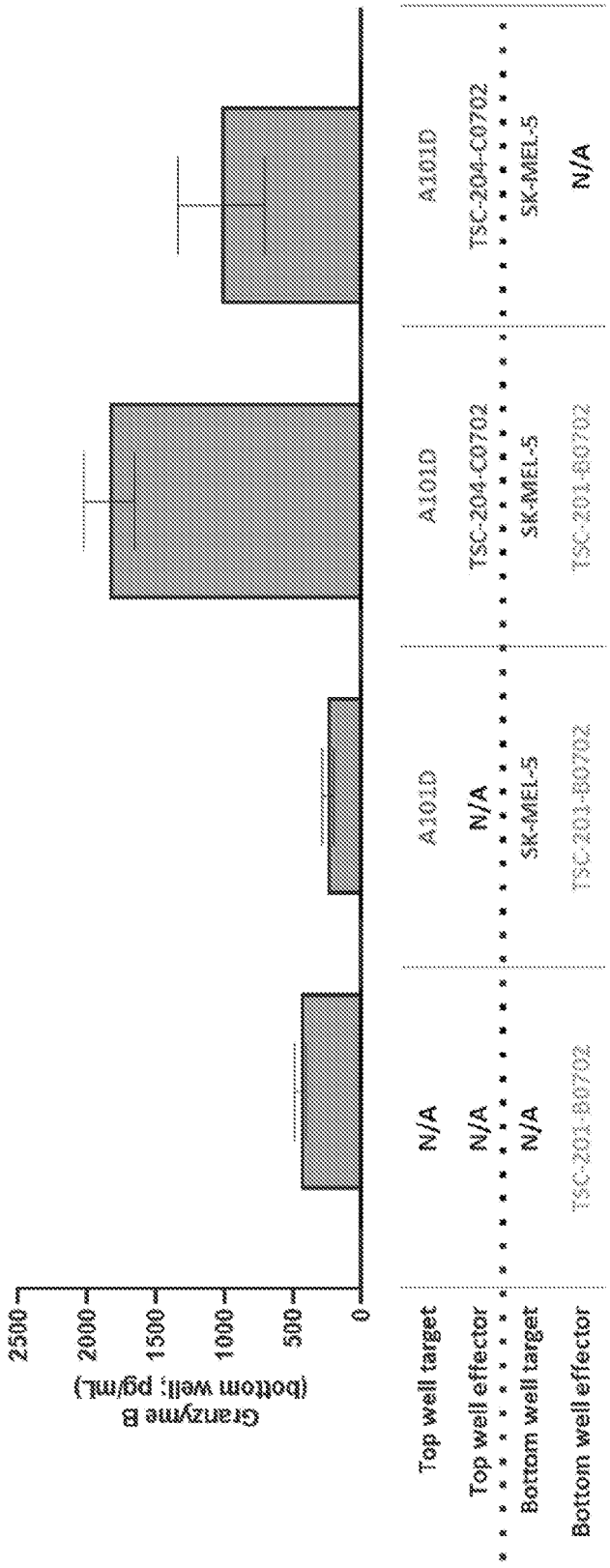

T-Plex enhances target-dependent T cell secretion of cytokine secretion
IFN-γ (bottom well; pg/mL)
800
600
400
200
0
Top well target
Top well effector
Bottom well target
Bottom well effector
N/A
N/A
N/A
TSC-201-80702
A101D
N/A
SK-MEL-5
TSC-201-80702
A101D
TSC-204-C0702
SK-MEL-5
TSC-201-80702
A101D
TSC-204-C0702
SK-MEL-5
N/A

FIG. 43D (continued)



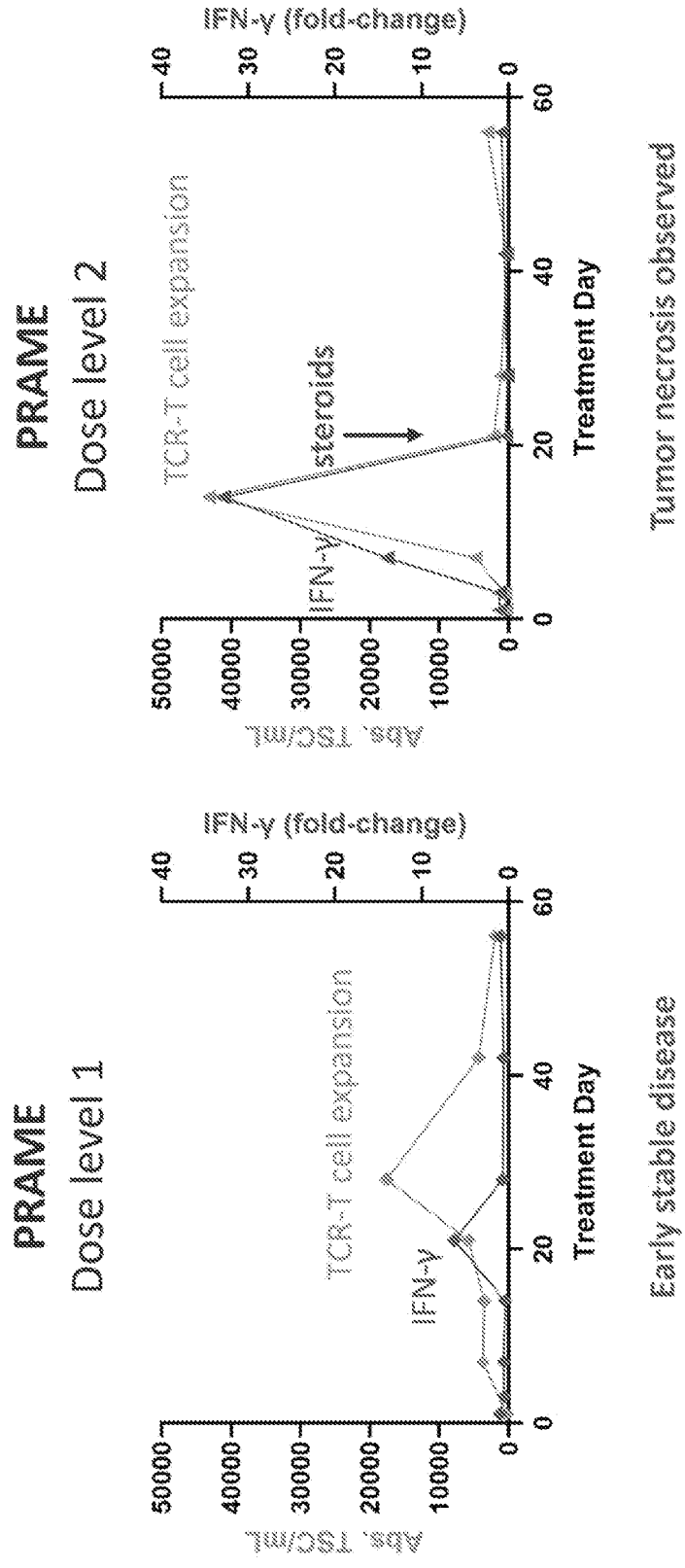

PRAME
Dose level 2
IFN-γ (fold-change)
TCR-T cell expansion
steroids
IFN-γ
Abs. TSC/mL
Treatment Day
Tumor necrosis observed
PRAME
Dose level 1
IFN-γ (fold-change)
TCR-T cell expansion
IFN-γ
Abs. TSC/mL
Treatment Day
Early stable disease

FIG. 44