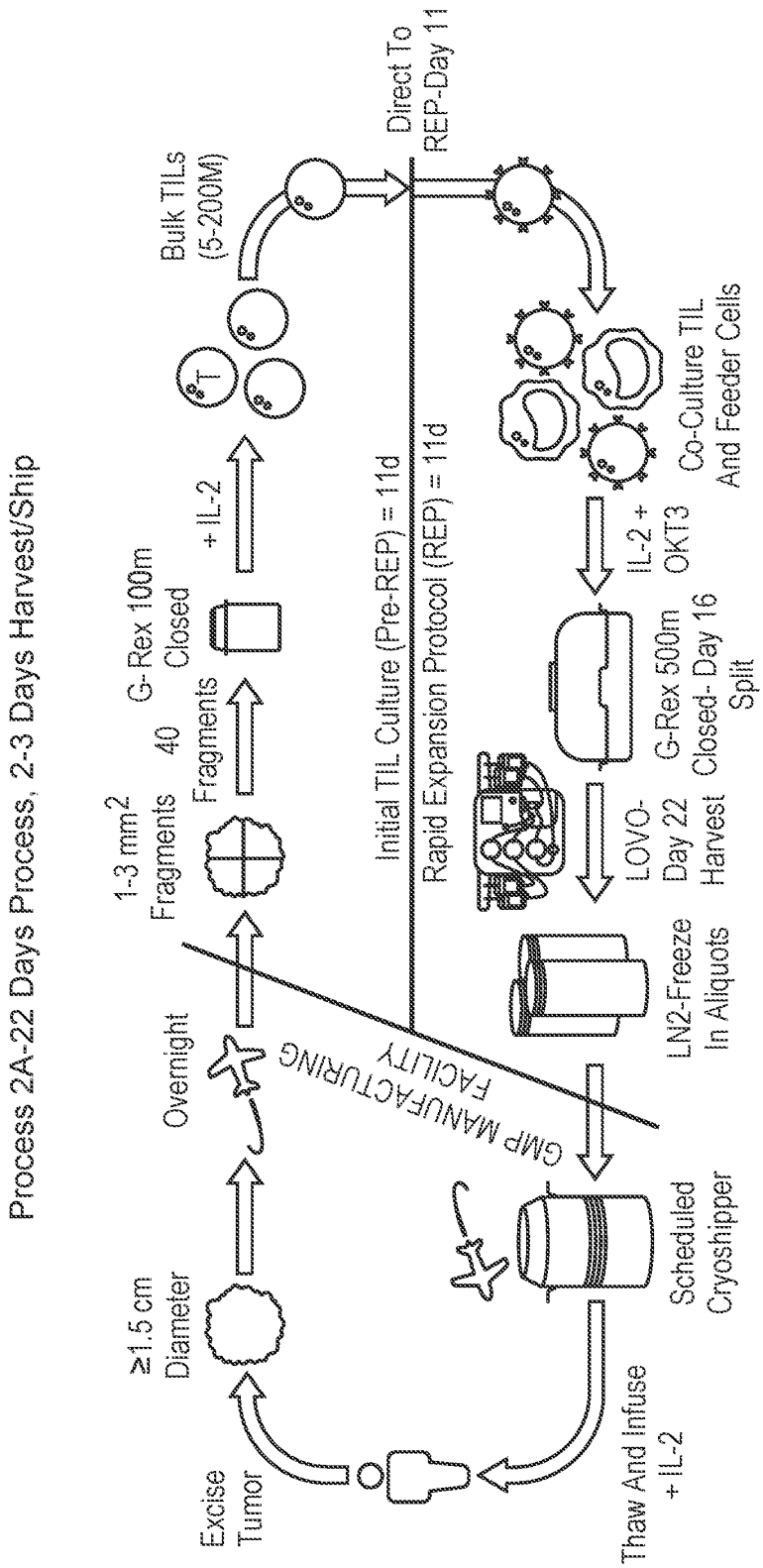

Process 2A-22 Days Process, 2-3 Days Harvest/Ship
Excise Tumor
≥1.5 cm Diameter
Overnight
1-3 mm2 Fragments
40 Fragments
G- Rex 100m Closed
+ IL-2
Bulk TILs (5-200M)
Direct To REP-Day 11
Initial TIL Culture (Pre-REP) = 11d
Rapid Expansion Protocol (REP) = 11d
GMP MANUFACTURING FACILITY
Co-Culture TIL 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

Figure 1

Process Development

Step	Current Process-1C	New Process-2A	Impact
1	4 Fragments/10 G-Rex 10-21 Days	40 Fragments/1-G-Rex 100 CS- (x2?) 11 Days	Increases Tumor Sample/Container, Shortens Culture, Reduces Steps, Amenable To Closed System
2	PreREP Freeze-> Testing -> Thaw- ~Day 27- >40e6TIL	Direct To REP- Day 11- <200e6	Shorten Process, Reduces Steps, Eliminates Testing
3	36 G-Rex 100-~Day 30 >5e6TIL - Split ~Day 36	4-5 G-Rex 500CS-TIL- Split Day 16	Reduces Steps, Closed System, Shorter REP
4	Harvest Day ~43+ Harvesting By Centrifugation	Harvest Day 22 LOVO-Automated Cell Washer	Reduces Steps, Automated, Closed System
5	Fresh Product-Hypothermosol-Single Infusion Bag	Cryopreserved Product-CS10 In LN_2 , Multiple Aliquots	Shipping Flexibility, Patient Scheduling, Easier Release Testing, Global Trials
6	43+ Day Process Time	22 Day Process Time	Turnaround To Patient, Clean Room Throughput, COGs

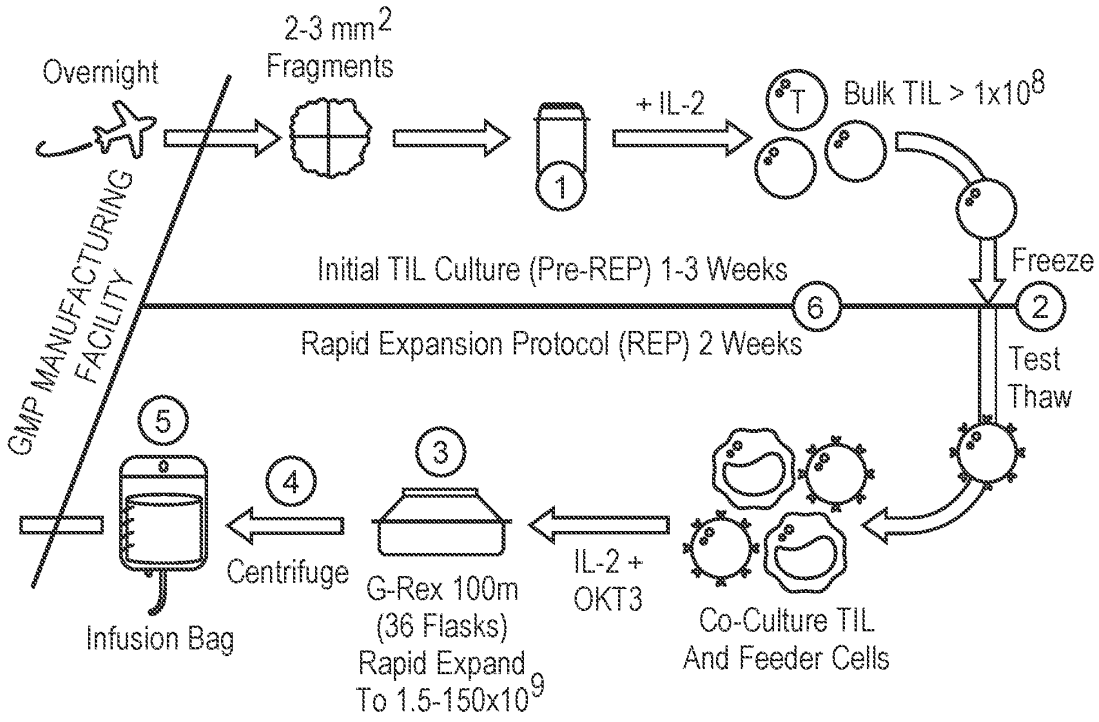


Figure 2

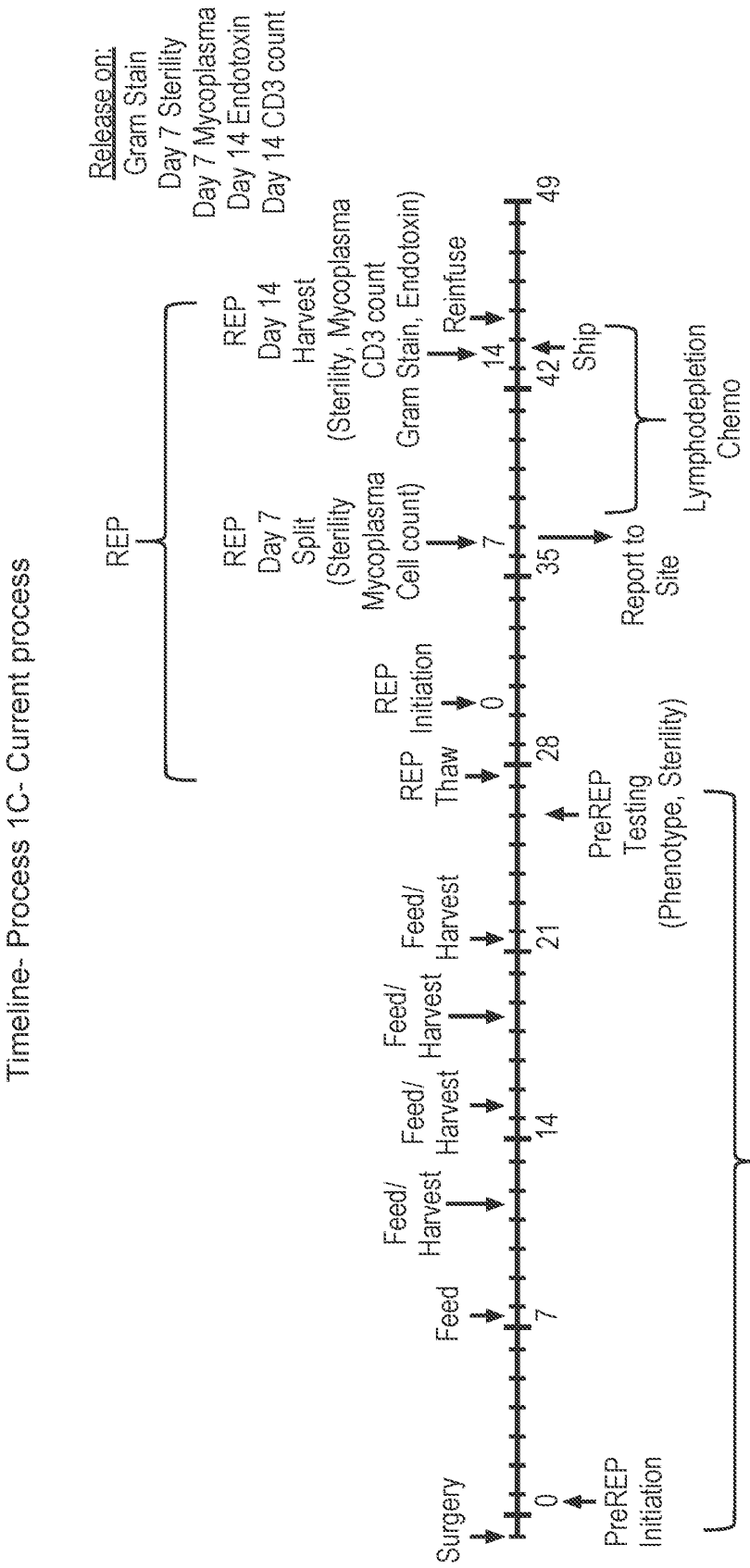

Timeline- Process 1C- Current process
Release on:
Gram Stain
Day 7 Sterility
Day 7 Mycoplasma
Day 14 Endotoxin
Day 14 CD3 count
REP
REP
Day 14
Harvest
(Sterility, Mycoplasma
CD3 count
Gram Stain, Endotoxin)
REP
Day 7
Split
(Sterility
Mycoplasma
Cell count)
Reinfuse
Ship
Report to
Site
Lymphodepletion
Chemo
REP
Initiation
REP
Thaw
PreREP
Testing
(Phenotype, Sterility)
Feed/
Harvest
Feed
Surgery
PreREP
Initiation
PreREP
0
7
14
21
28
35
42
49
14

Figure 3

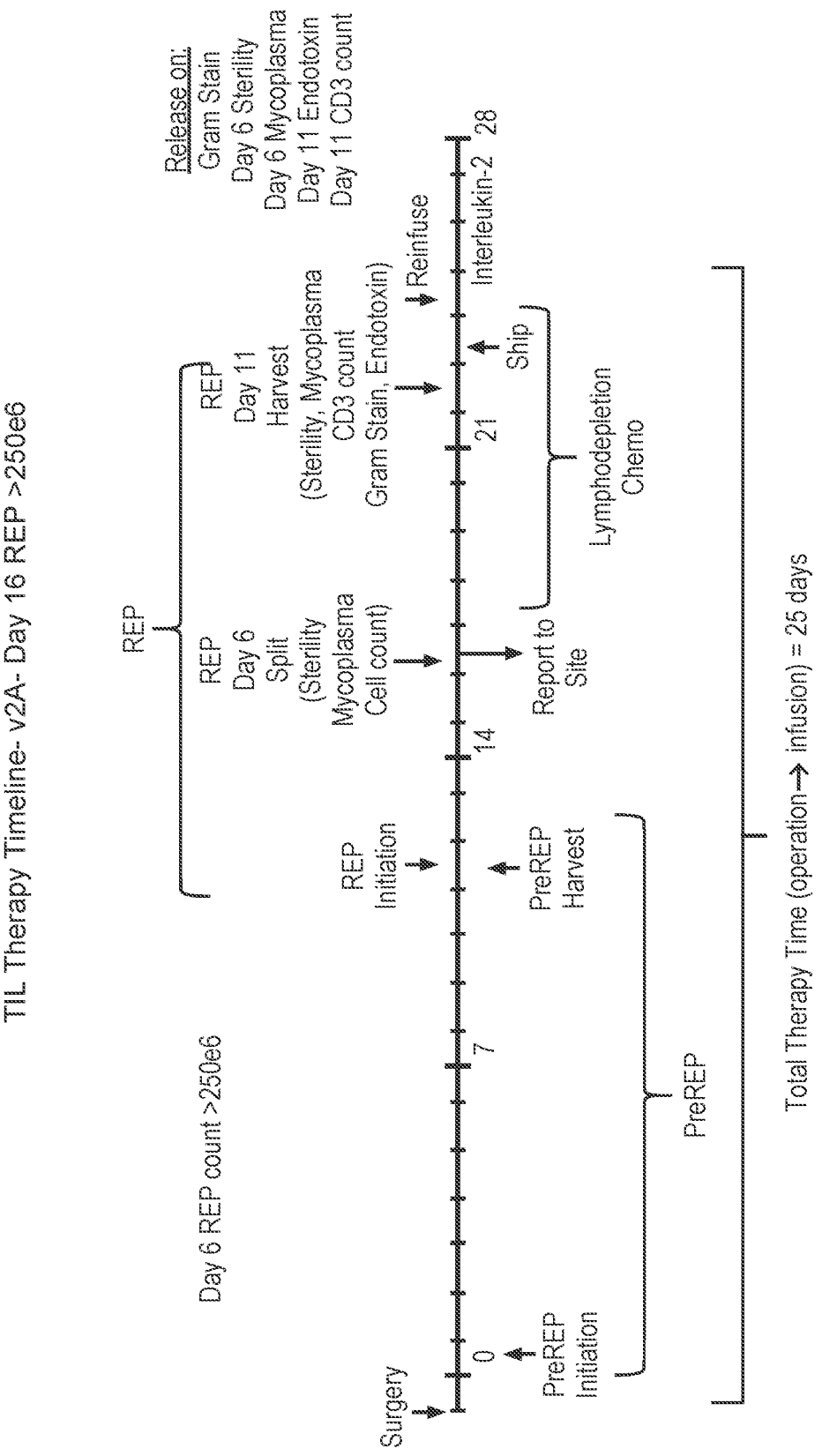

TIL Therapy Timeline- v2A- Day 16 REP >250e6
Surgery
0
PreREP Initiation
7
PreREP Harvest
14
REP Initiation
Day 6 REP count >250e6
REP
REP Day 6 Split (Sterility Mycoplasma Cell count)
REP Day 11 Harvest (Sterility, Mycoplasma CD3 count Gram Stain, Endotoxin)
Release on:
Gram Stain
Day 6 Sterility
Day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

Figure 4

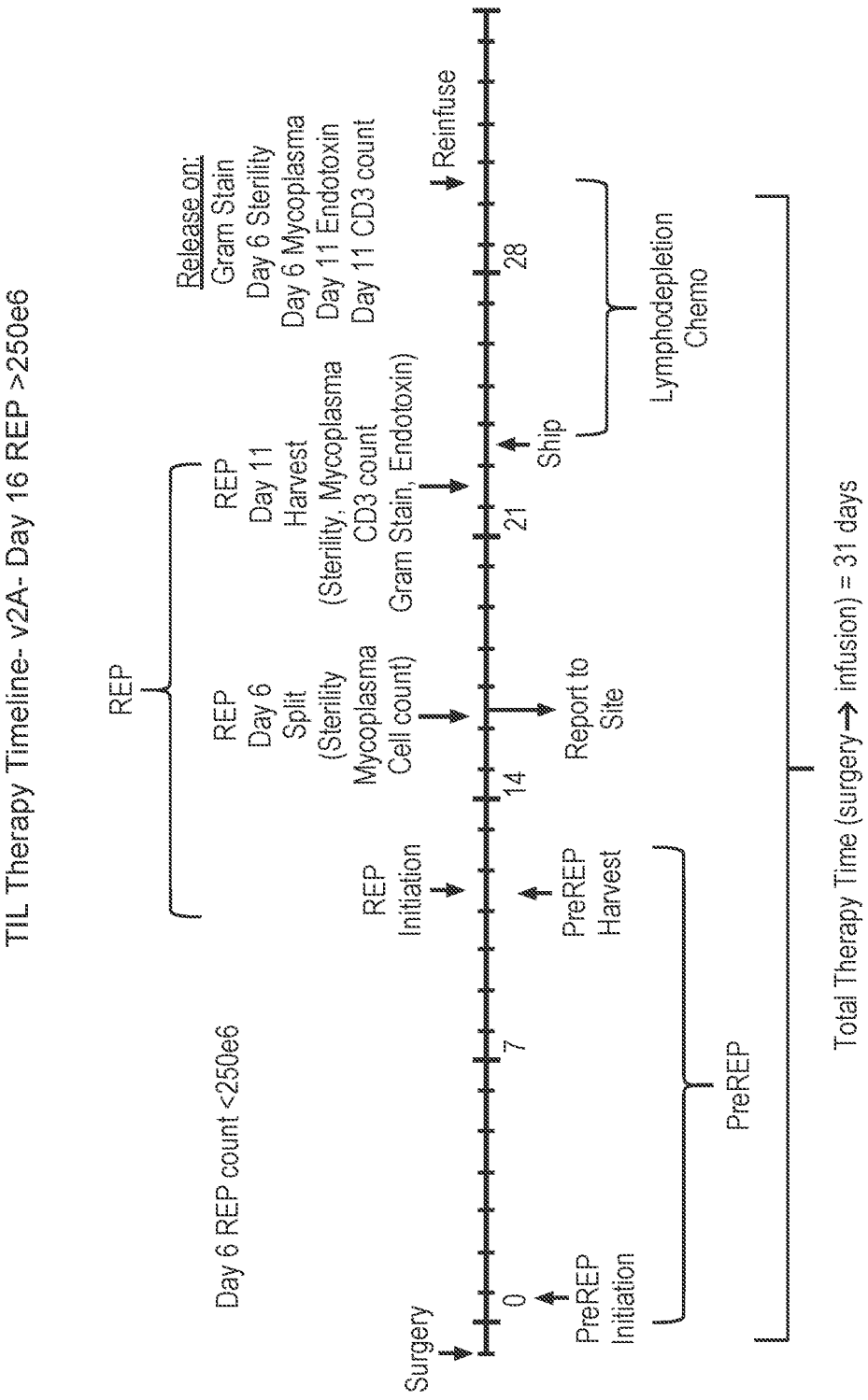

TIL Therapy Timeline- v2A- Day 16 REP >250e6
Surgery
PreREP Initiation
REP Initiation
PreREP Harvest
Report to Site
Ship
Reinfuse
Lymphodepletion Chemo
0
7
14
21
28
REP
Day 6 REP count <250e6
REP
Day 6 Split (Sterility Mycoplasma Cell count)
REP
Day 11 Harvest (Sterility, Mycoplasma CD3 count Gram Stain, Endotoxin)
Release on:
Gram Stain
Day 6 Sterility
Day 6 Mycoplasma
Day 11 Endotoxin
Day 11 CD3 count
PreREP
Total Therapy Time (surgery→ infusion) = 31 days

Figure 5

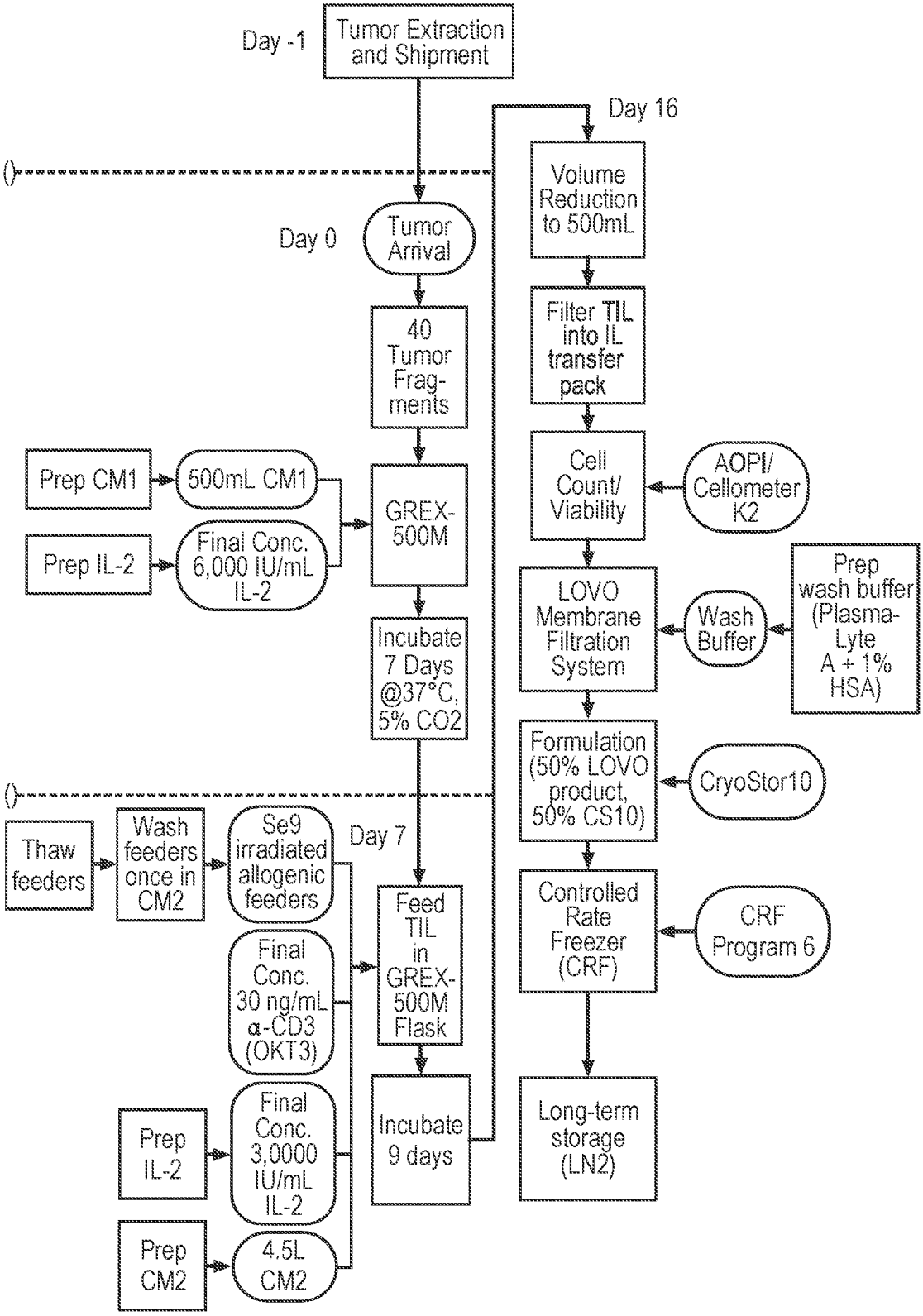
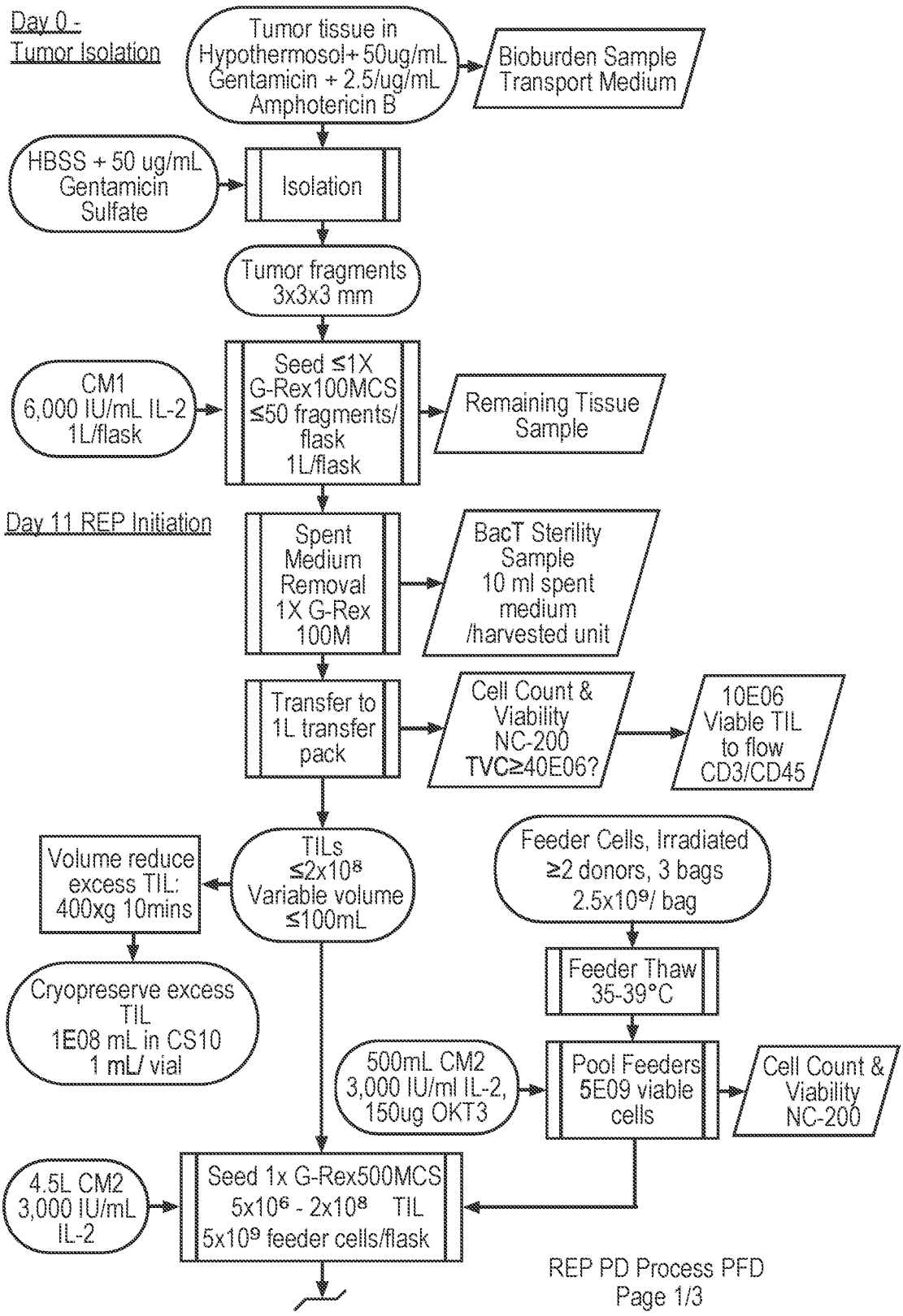

Day -1
Tumor Extraction and Shipment
Day 0
Tumor Arrival
40 Tumor Frag-ments
Prep CM1
500mL CM1
Prep IL-2
Final Conc. 6,000 IU/mL IL-2
GREX-500M
Incubate 7 Days @37°C, 5% CO2
Day 7
Thaw feeders
Wash feeders once in CM2
Se9 irradiated allogenic feeders
Final Conc. 30 ng/mL α-CD3 (OKT3)
Feed TIL in GREX-500M Flask
Prep IL-2
Final Conc. 3,0000 IU/mL IL-2
Prep CM2
4.5L CM2
Incubate 9 days
Day 16
Volume Reduction to 500mL
Filter TIL into IL transfer pack
Cell Count/ Viability
AOPI/ Cellometer K2
LOVO Membrane Filtration System
Wash Buffer
Prep wash buffer (Plasma-Lyte A + 1% HSA)
Formulation (50% LOVO product, 50% CS10)
CryoStor10
Controlled Rate Freezer (CRF)
CRF Program 6
Long-term storage (LN2)

Figure 6



REP PD Process PFD
Page 1/3

Figure 7A

Figure 7B

Figure 7A

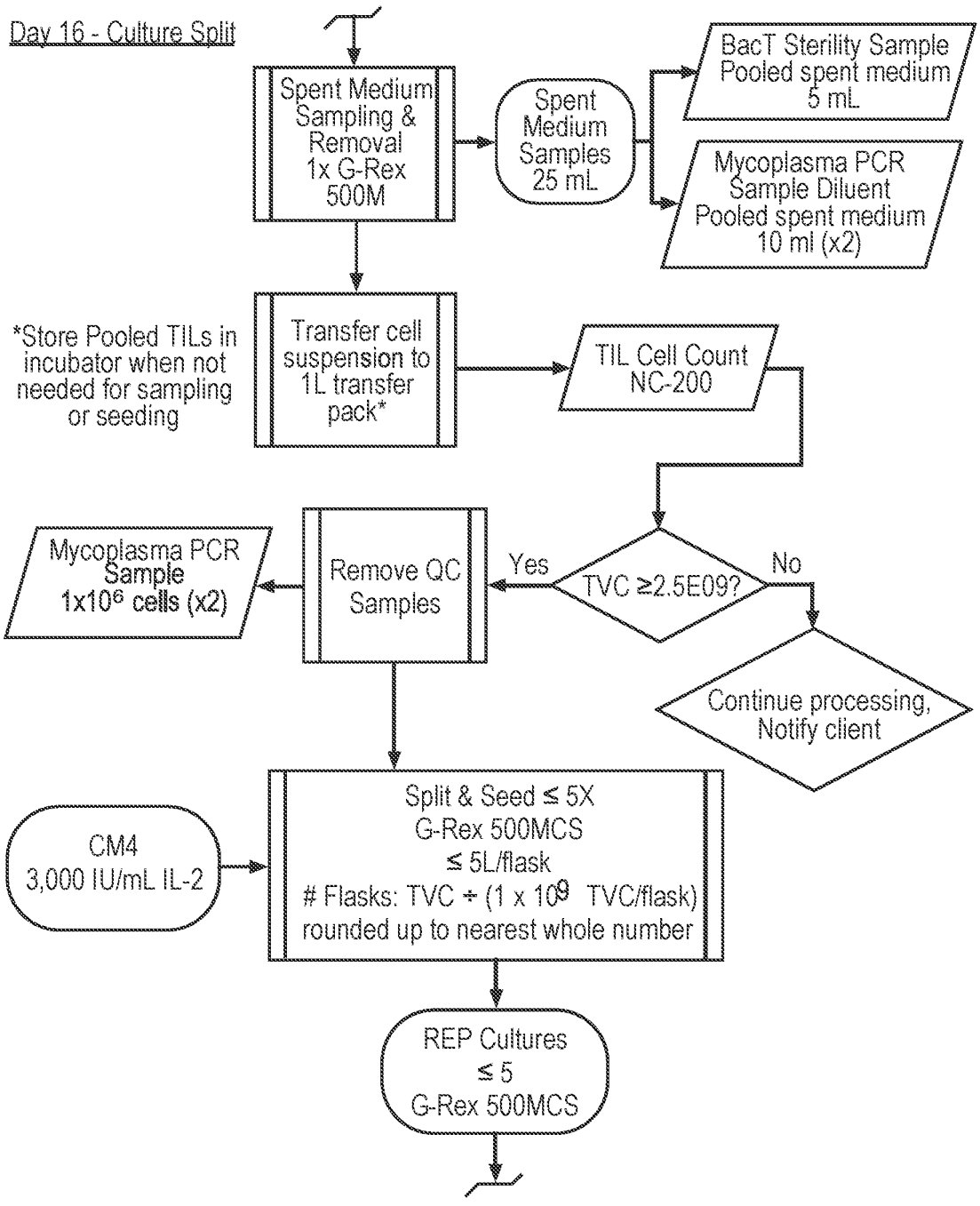


Figure 7C

REP PD Process PFD
Page 2/3

Figure 7B

Figure 7B

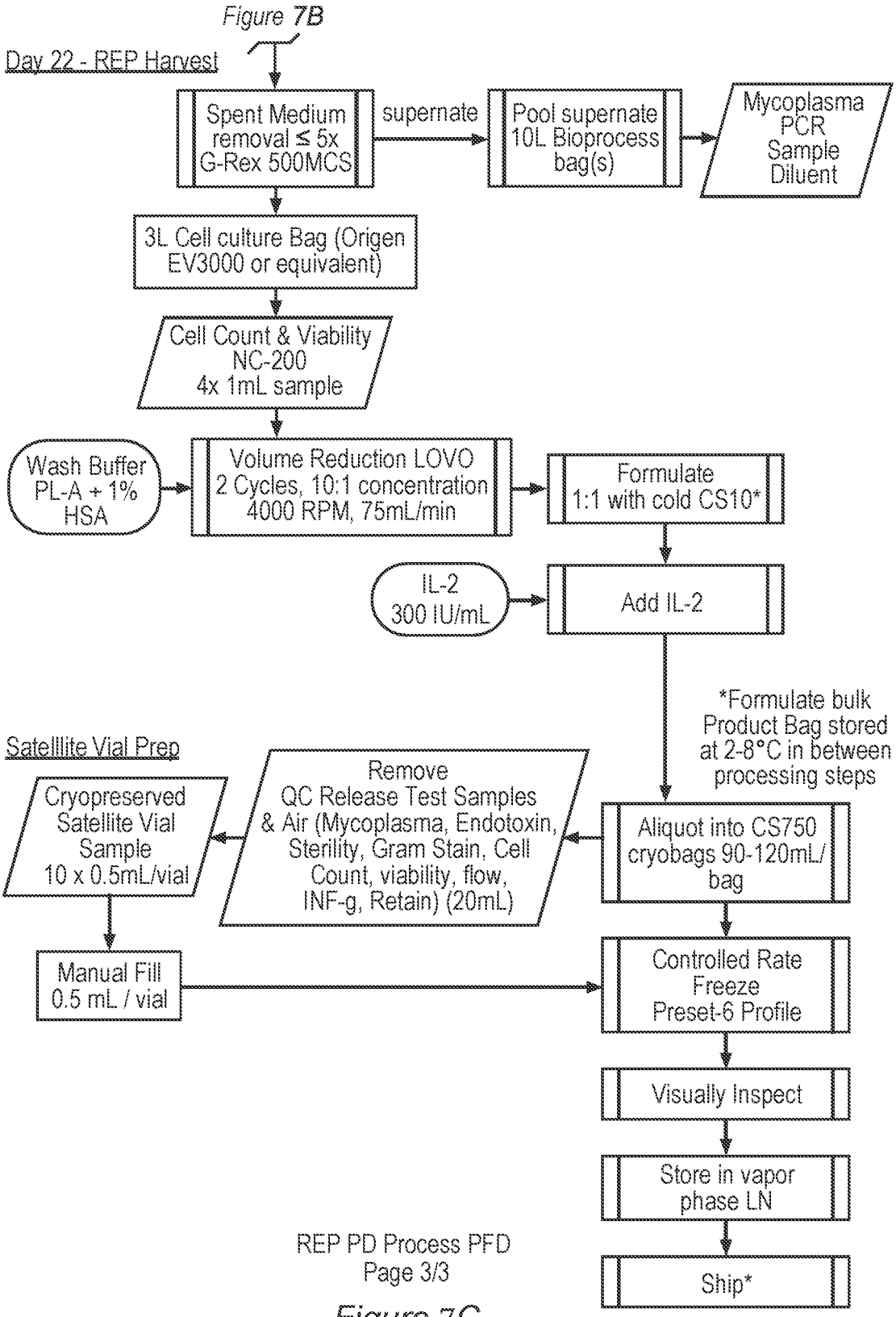


Figure 7C

Figure 8

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)

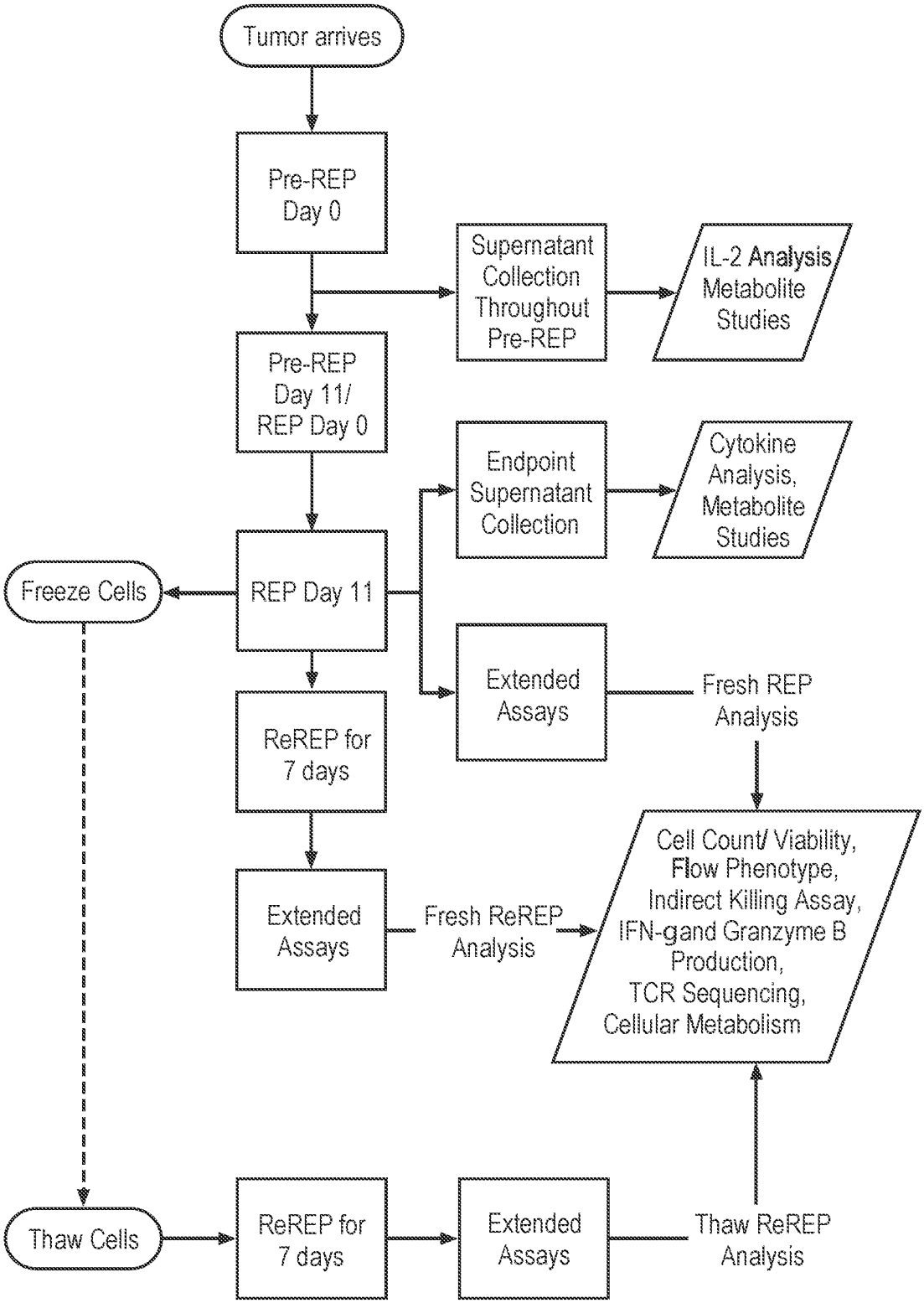

Tumor arrives
Pre-REP Day 0
Supernatant Collection Throughout Pre-REP
IL-2 Analysis Metabolite Studies
Pre-REP Day 11/ REP Day 0
Endpoint Supernatant Collection
Cytokine Analysis, Metabolite Studies
Freeze Cells
REP Day 11
Extended Assays
Fresh REP Analysis
ReREP for 7 days
Extended Assays
Fresh ReREP Analysis
Cell Count/ Viability, Flow Phenotype, Indirect Killing Assay, IFN-gand Granzyme B Production, TCR Sequencing, Cellular Metabolism
Thaw Cells
ReREP for 7 days
Extended Assays
Thaw ReREP Analysis

Figure 9

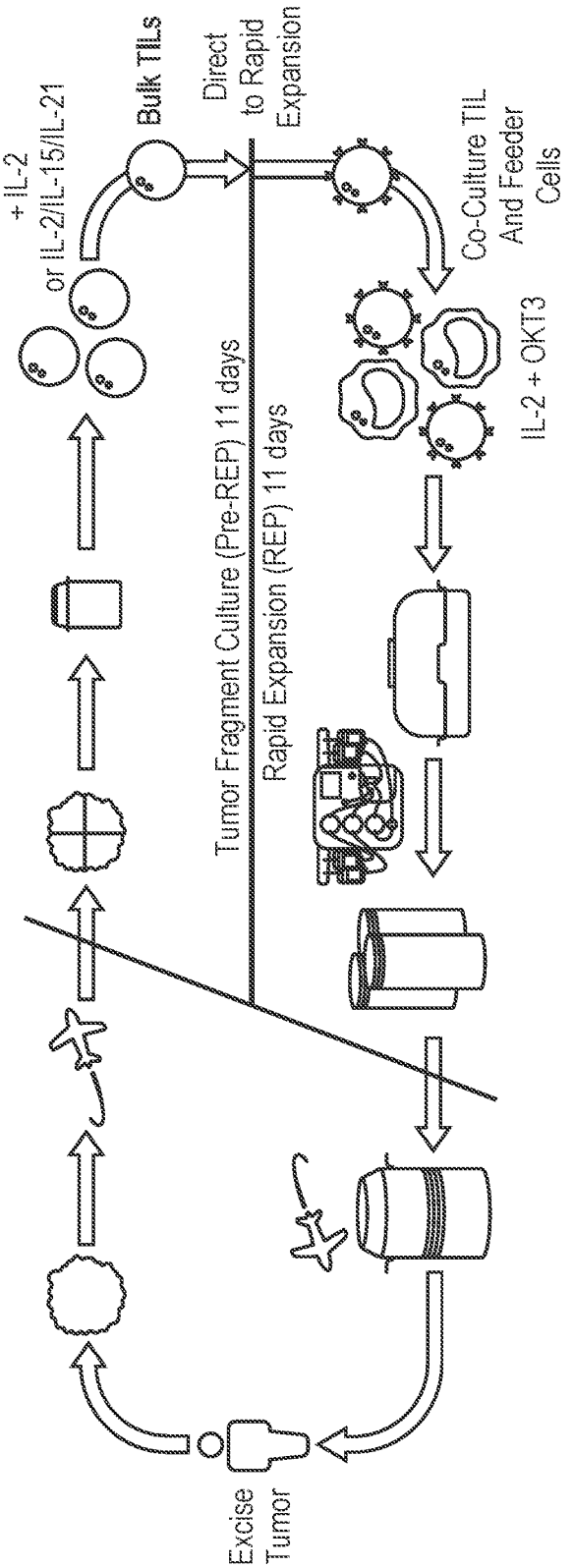

+ IL-2
or IL-2/IL-15/IL-21
Bulk TILs
Direct
to Rapid
Expansion
Co-Culture TIL
And Feeder
Cells
IL-2 + OKT3
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
Excise
Tumor

Figure 10

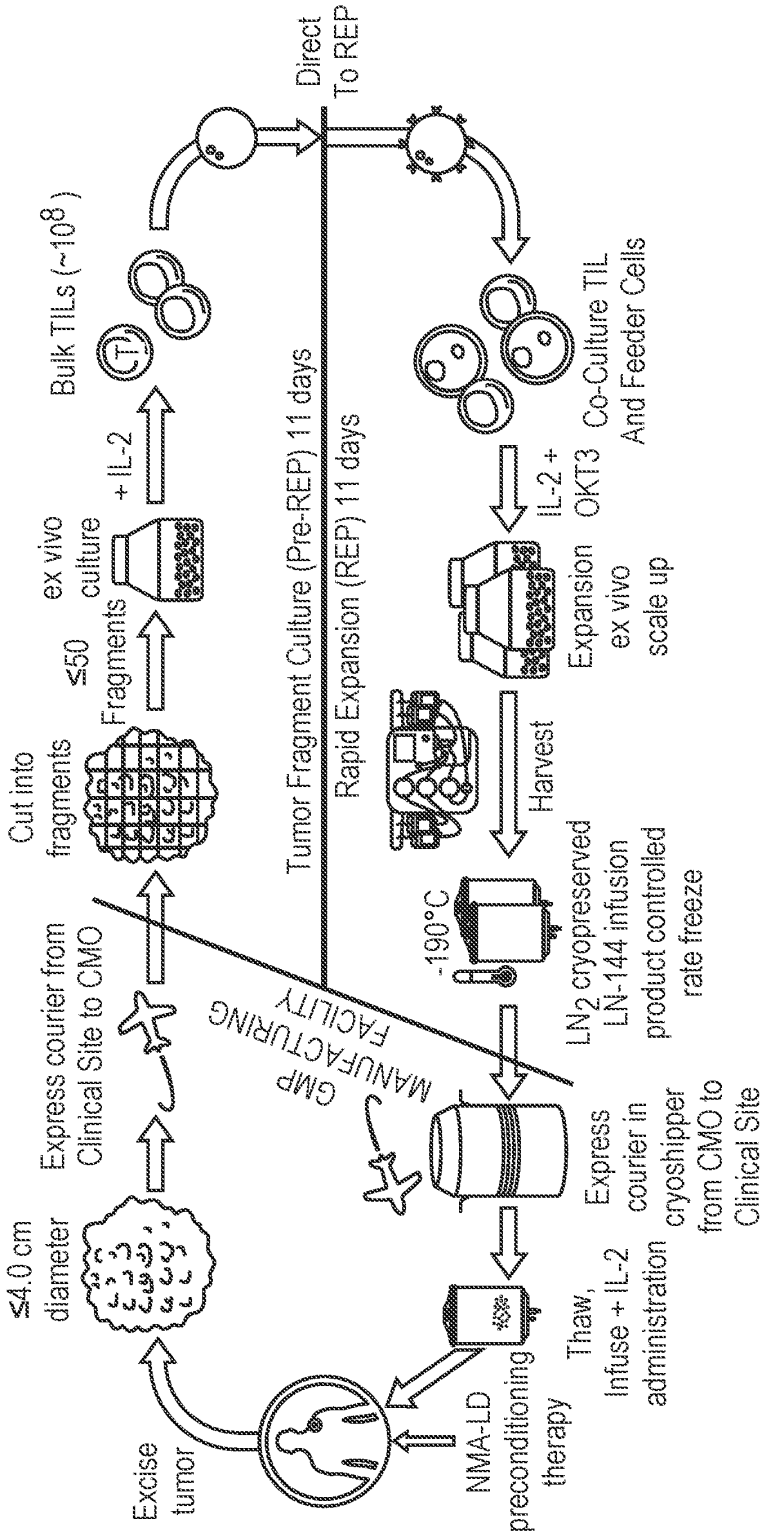

Excise tumor
≤4.0 cm diameter
Express courier from Clinical Site to CMO
Cut into fragments
≤50 Fragments
ex vivo culture
+ IL-2
Bulk TILs (~10^8)
CTL
Direct To REP
NMA-LD preconditioning therapy
Thaw, Infuse + IL-2 administration
GMP MANUFACTURING FACILITY
Express courier in cryoshipper from CMO to Clinical Site
-190°C
LN2 cryopreserved LN-144 infusion product controlled rate freeze
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
Harvest
Expansion ex vivo scale up
IL-2 + OKT3
Co-Culture TIL And Feeder Cells

Figure 11

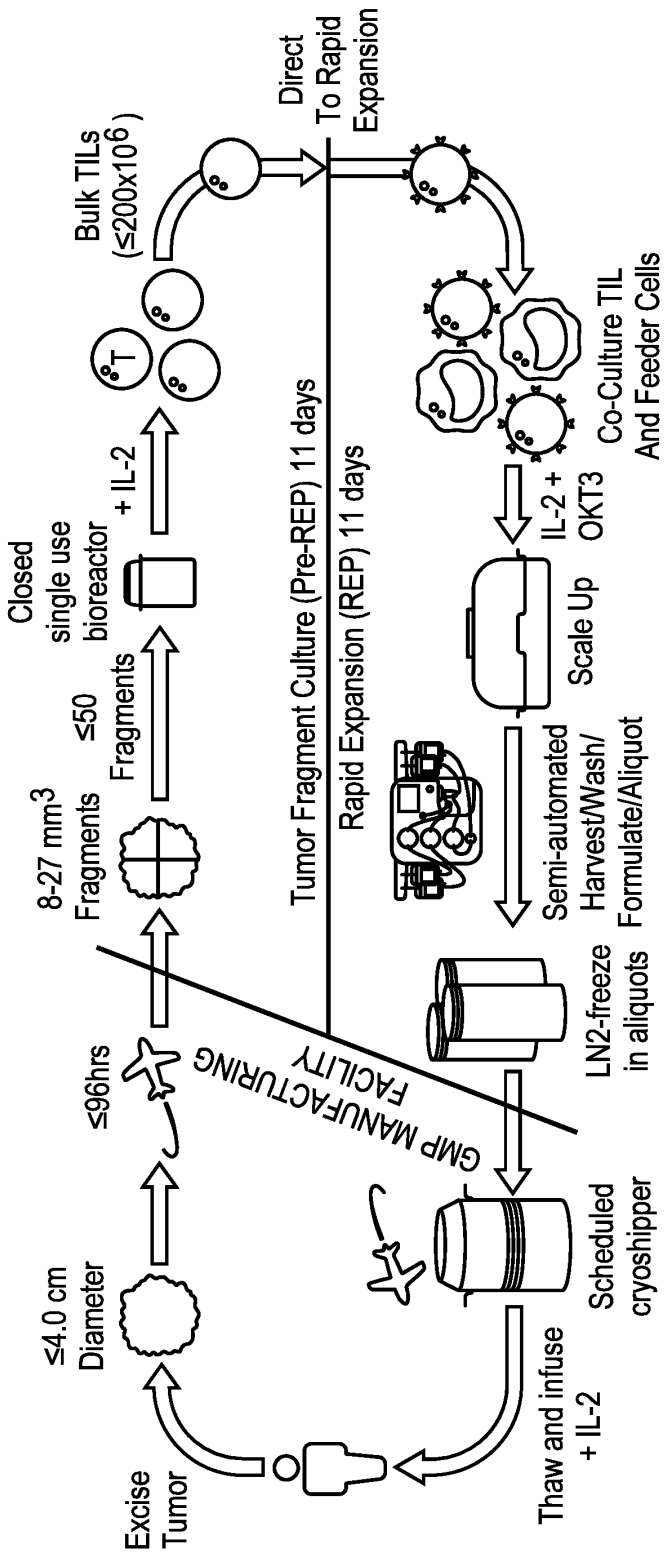

Excise Tumor
≤4.0 cm Diameter
≤96hrs
8-27 mm3 Fragments
≤50 Fragments
Closed single use bioreactor
+ IL-2
Bulk TILs (≤200x10^6)
Thaw and infuse + IL-2
Scheduled cryoshipper
GMP MANUFACTURING FACILITY
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
LN2-freeze in aliquots
Semi-automated Harvest/Wash/ Formulate/Aliquot
Scale Up
IL-2 + OKT3
Co-Culture TIL And Feeder Cells
Direct To Rapid Expansion

Figure 12

Figure 13

Process 1C: 43-55 Days for Steps A - E	Process 2A: about 22 days from Steps A - E
1. **STEP A** Obtain Patient Tumor Sample	1. **STEP A** Obtain Patient Tumor Sample
2. **STEP B** Fragmentation and First Expansion 11 days to 21 days	2. **STEP B** Fragmentation and First Expansion 3 days to 14 days
3. **STEP C** First Expansion to Second Expansion Transition Optional Storage until Selection	3. **STEP C** First Expansion to Second Expansion Transition No Storage and Closed System
4. **STEP D** Second Expansion IL-2, OKT-3, antigen-presenting feeder cells Optionally repeat one or more times	4. **STEP D** Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells Closed System
5. **STEP E** Harvest TILS from Step D	5. **STEP E** Harvest TILS from Step D Closed System
6. **STEP F** Final Formulation and/or Transfer to Infusion Bag	6. **STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 14

Process Step	Process 1C Embodiment	Process 2A Embodiment	Advantages
Pre-REP	• 4 fragments per 10 GREX-10 flasks • 11-21 day duration	• 40 fragments per 1 GREX-100M flask • 11 day duration	• Increased tumor fragments per flask • Shortened culture time • Reduced number of steps • Amenable to closed system
Pre-REP to REP Transition	• Pre-REP TIL are frozen until phenotyped for selection then thawed to proceed to the REP (~day 30) • REP requires >40×10^6 TIL	• Pre-REP TIL directly move to REP on day 11 • REP requires 25-200×10^6 TIL	• Shortened pre-REP-to-REP process • Reduced number of steps • Eliminated phenotyping selection • Amenable to closed system
REP	• 6 GREX-100M flasks on REP day 0 • 5×10^6 TIL and 5×10^8 PBMC feeders per flask on REP day 0 • Split to 18-36 flasks on REP day 7 • 14 day duration	• 1 GREX-500M flask on day 11 • 25-200×10^6 TIL and 5x10^9 PBMC feeders on day 11 • Split to ≤ 6 GREX-500M flasks on day 16 • 11 day duration	• Reduced number of steps • Shorter REP duration • Closed system transfer of TIL between flasks • Closed system media exchanges
Harvest	• TIL harvested via centrifugation	• TIL harvested via LOVO automated cell washing system'	• Reduced number of steps • Automated cell washing • Closed system • Reduced loss of product during wash
Final Formulation	• Fresh product in Hypothermosol • Single infusion bag • Limited shipping stability	• Cyropreserved product in PlasmaLyte-A + 1% HSA and CS10 stored in LN_2 • Multiple aliquots • Longer shipping stability	• Shipping flexibility • Flexible patient scheduling • More timely release testing
Overall Estimated Process Time	• 43-55 days	• 22 days	• Faster turnaround to patient

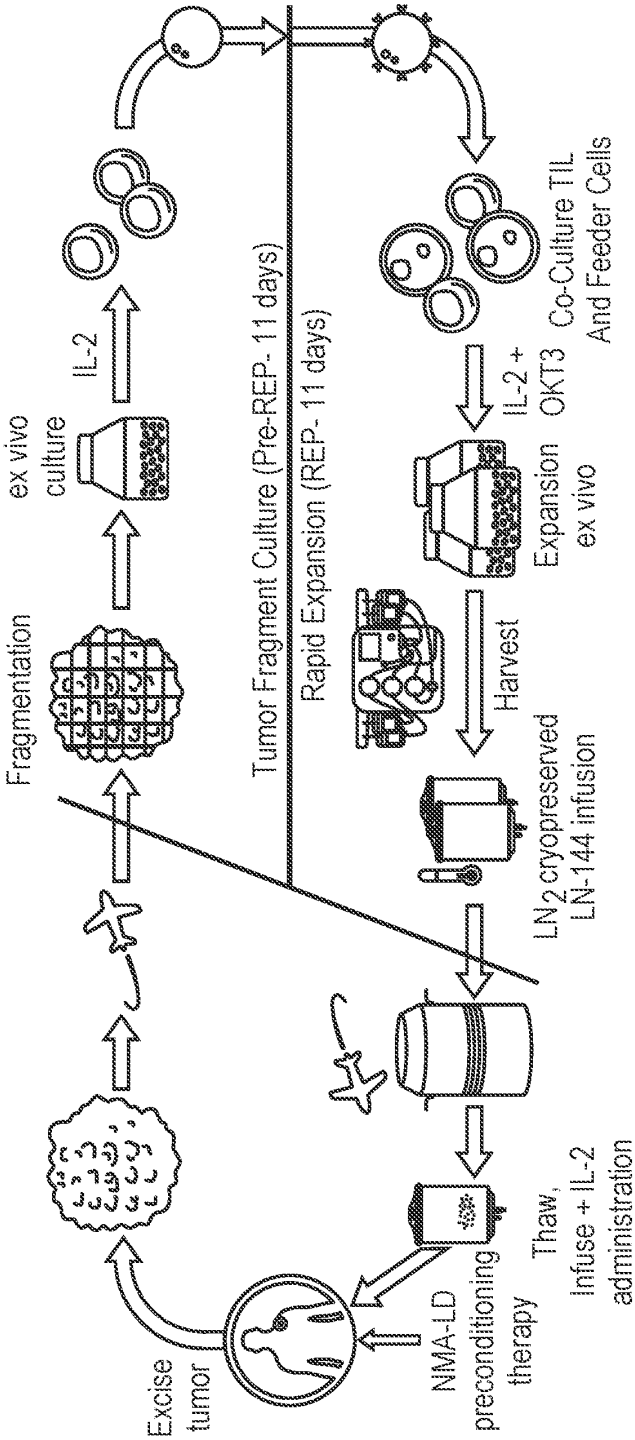

Excise tumor
Fragmentation
ex vivo culture
IL-2
Tumor Fragment Culture (Pre-REP- 11 days)
Rapid Expansion (REP- 11 days)
NMA-LD preconditioning therapy
Thaw, Infuse + IL-2 administration
LN2 cryopreserved LN-144 infusion
Harvest
Expansion ex vivo
IL-2 + OKT3
Co-Culture TIL And Feeder Cells

Figure 15

Figure 16

PROCESS STEP	GEN 1	GEN 2	IMPACT
Fragment Culture	≤21 days, multiple bioreactors, multiple operator interventions	≤11 days, single closed bioreactor, no intervention	Shortens culture, reduces interventions
TIL selection	IL-2 expanded TIL cryopreserved, tested, selection based on phenotype, thaw, rest, co-culture	Bulk TIL direct to co-culture	Reduces steps, eliminates testing, increases clonal diversity
Harvest/ Wash	Manual volume reduction and harvest. Manual wash and concentration	Closed semi-automated volume reduction and harvest. Automated wash and concentration	Reduces operator interventions, reduces processing time, maintains functionally closed system
Formulation	Fresh hypothermic product (2- 8^{o}C)	Cryopreserved product (≤ -150^{o}C)	Allows for global trials through increased flexibility in shipping and patient scheduling
Manufacturing Time	38 day process time	22 day process time	Turnaround to patient, clean room throughput, lower cost of goods

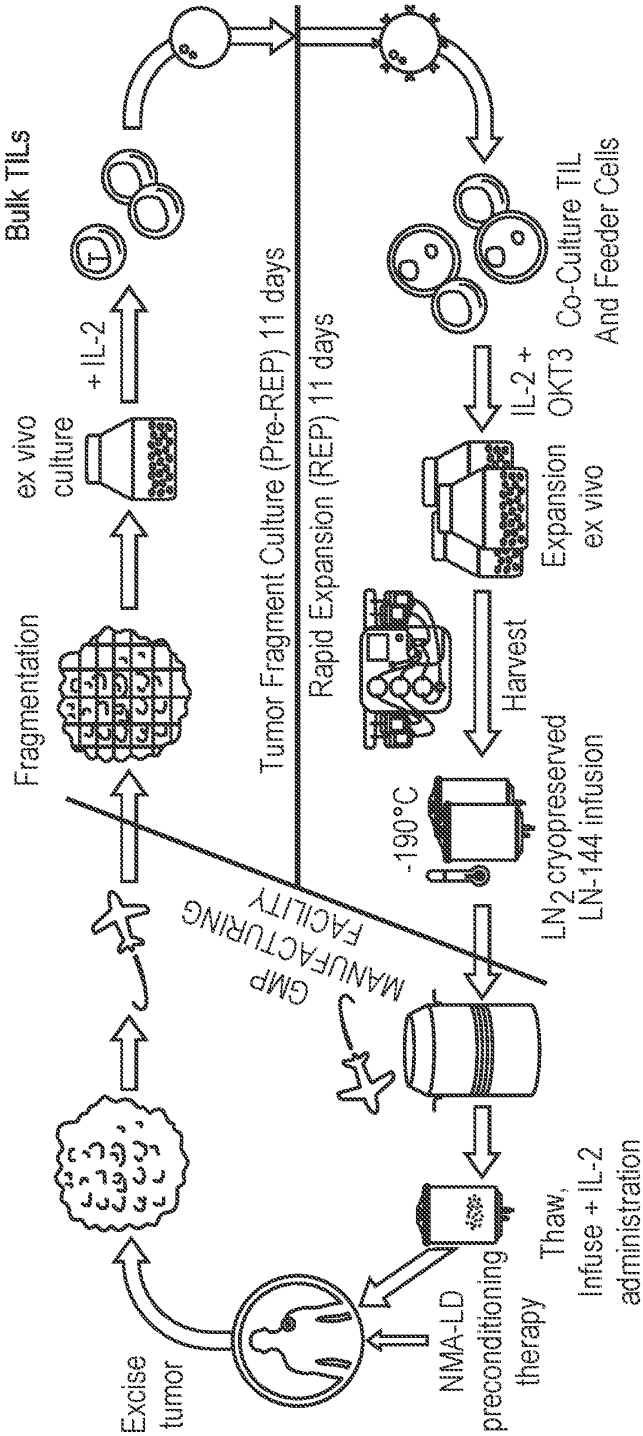

Bulk TILs
ex vivo culture
+IL-2
Fragmentation
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
Co-Culture TIL And Feeder Cells
IL-2 + OKT3
Expansion ex vivo
Harvest
-190°C
LN2 cryopreserved LN-144 infusion
GMP MANUFACTURING FACILITY
Excise tumor
NMA-LD preconditioning therapy
Thaw, Infuse + IL-2 administration

Figure 17

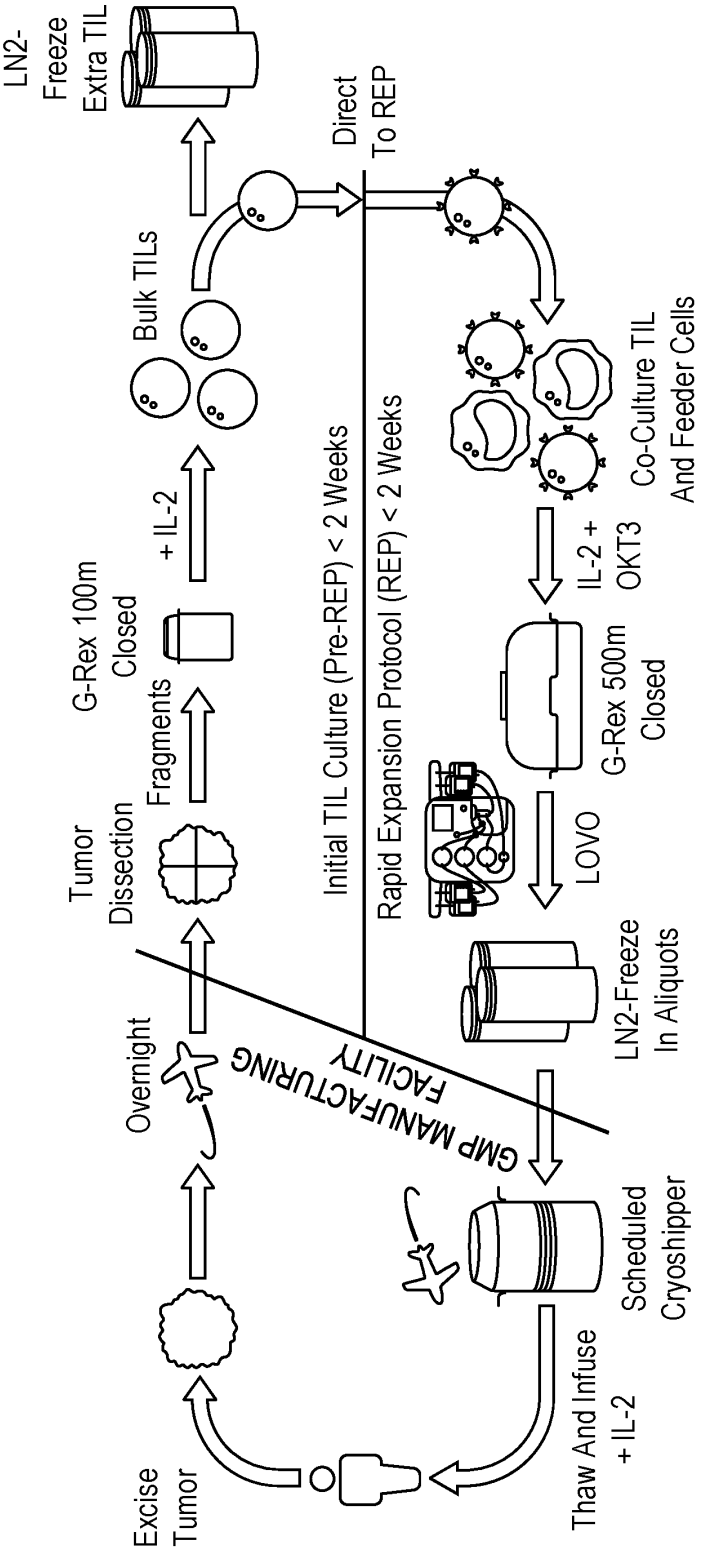

LN2-
Freeze
Extra TIL
Direct
To REP
Bulk TILs
+ IL-2
G-Rex 100m
Closed
Tumor
Dissection
Fragments
Initial TIL Culture (Pre-REP) < 2 Weeks
Rapid Expansion Protocol (REP) < 2 Weeks
Co-Culture TIL
And Feeder Cells
IL-2 +
OKT3
G-Rex 500m
Closed
LOVO
LN2-Freeze
In Aliquots
Overnight
GMP MANUFACTURING
FACILITY
Scheduled
Cryoshipper
Thaw And Infuse
+ IL-2
Excise
Tumor

Figure 18

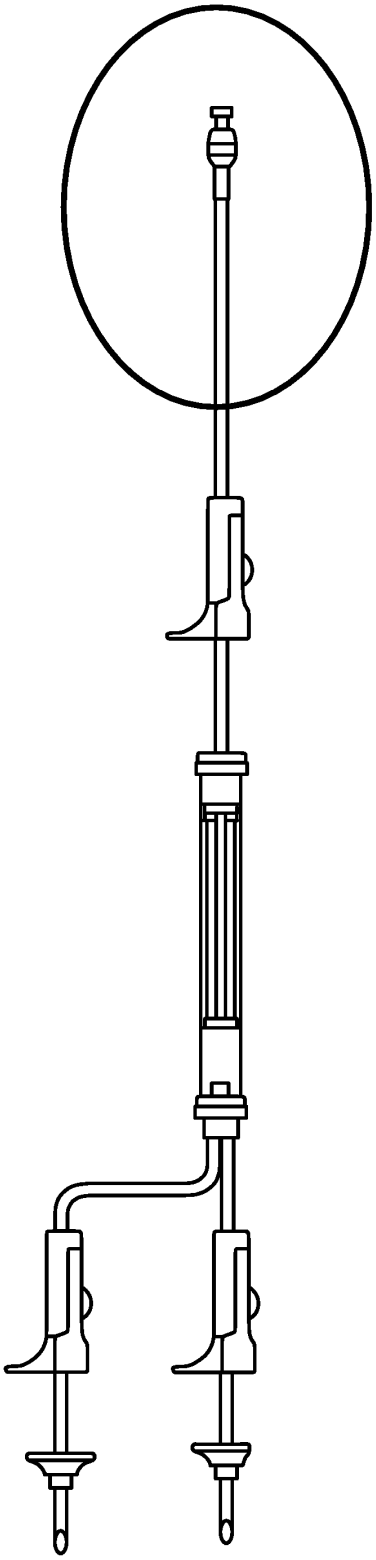


Figure 19

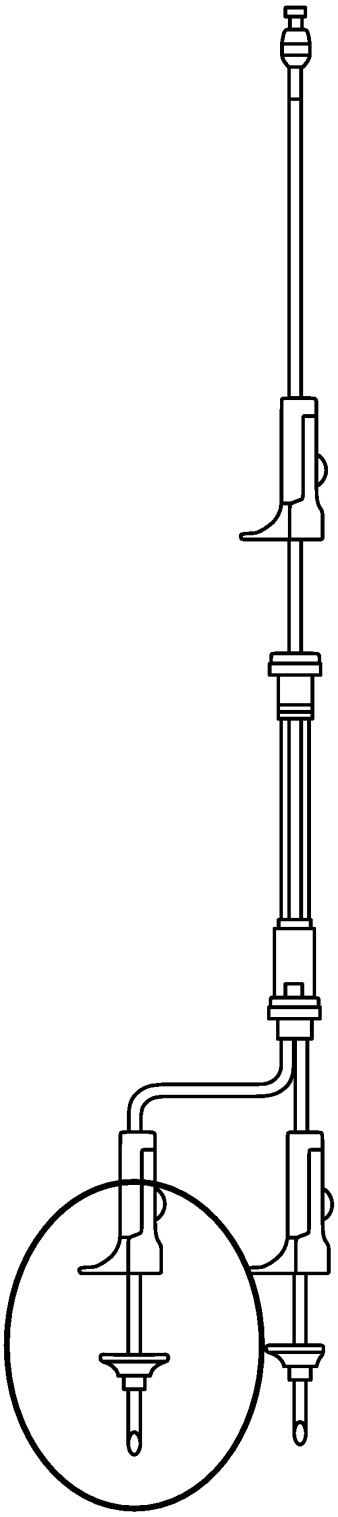


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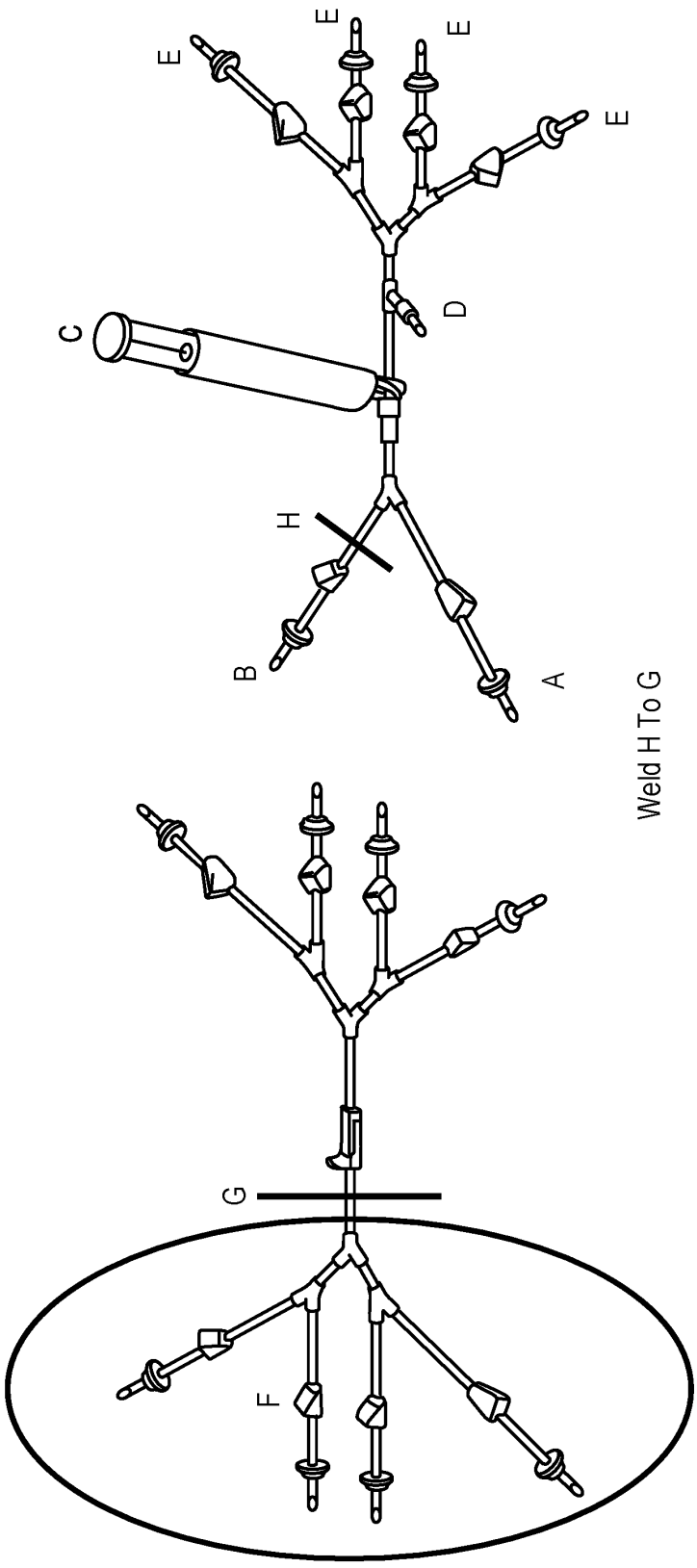


Figure 21

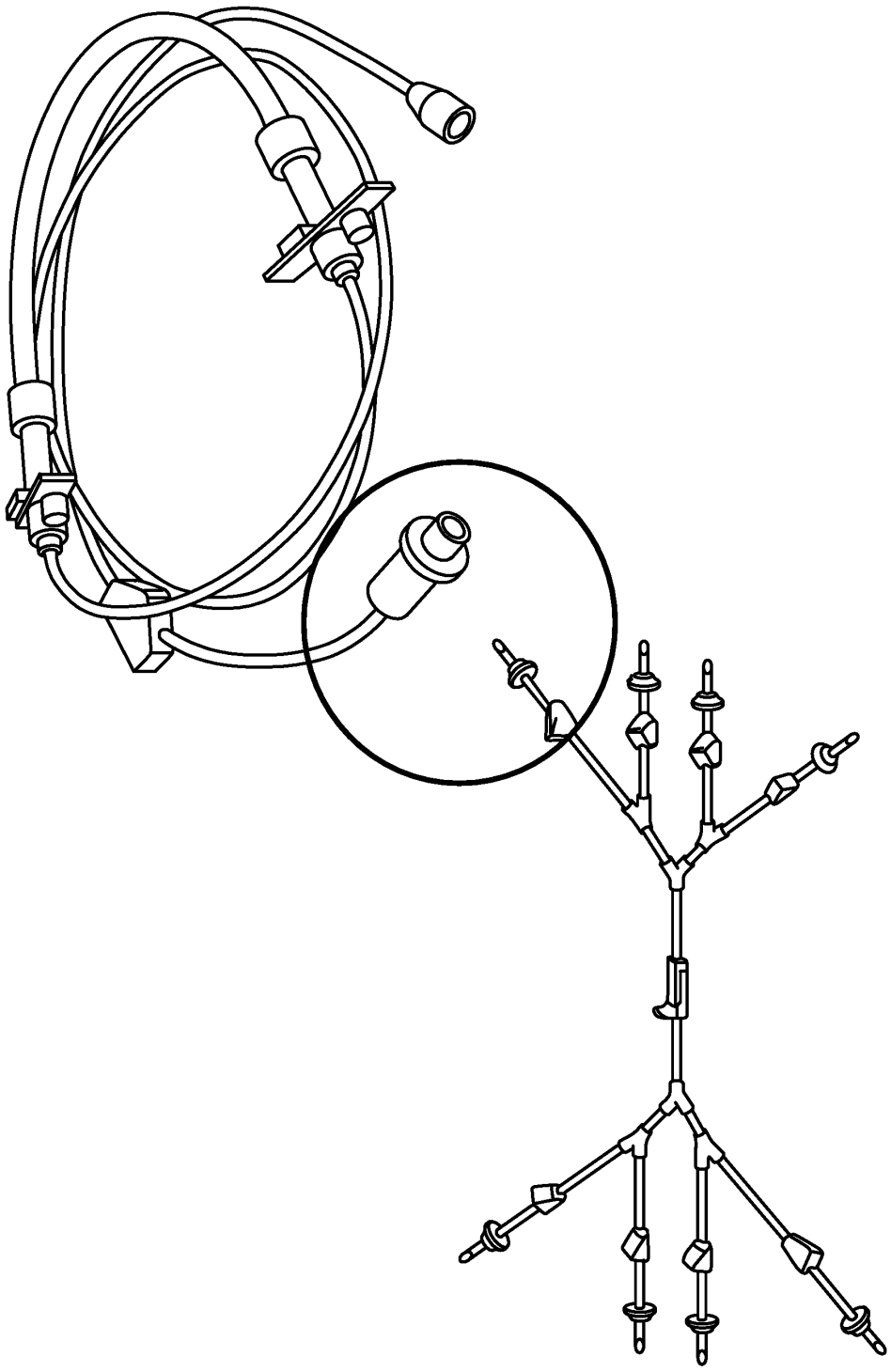


Figure 22

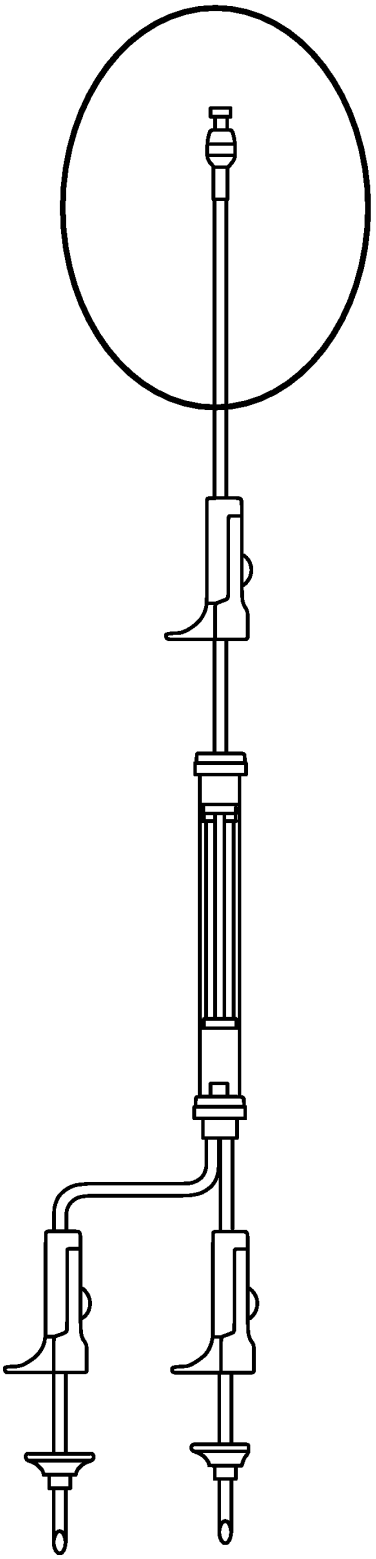


Figure 23

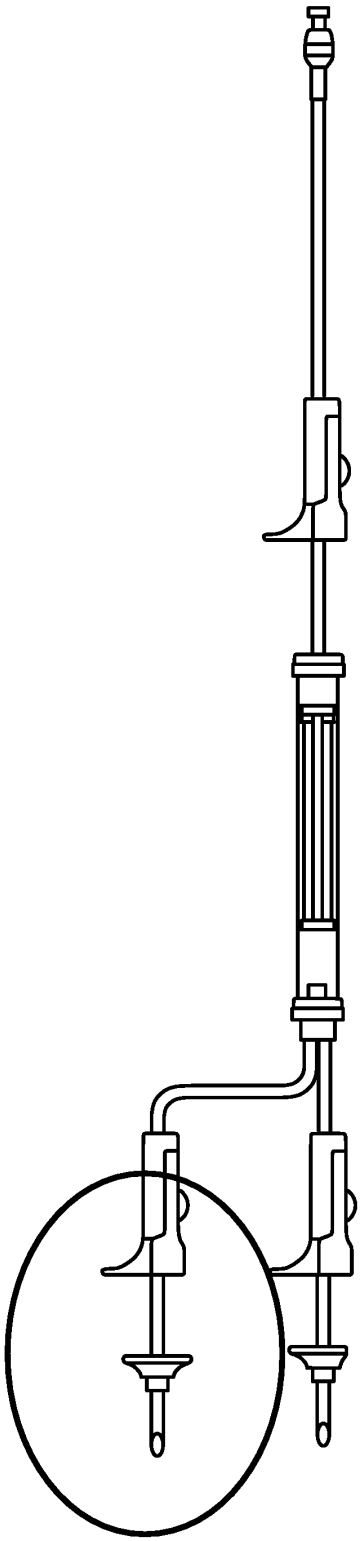


Figure 24

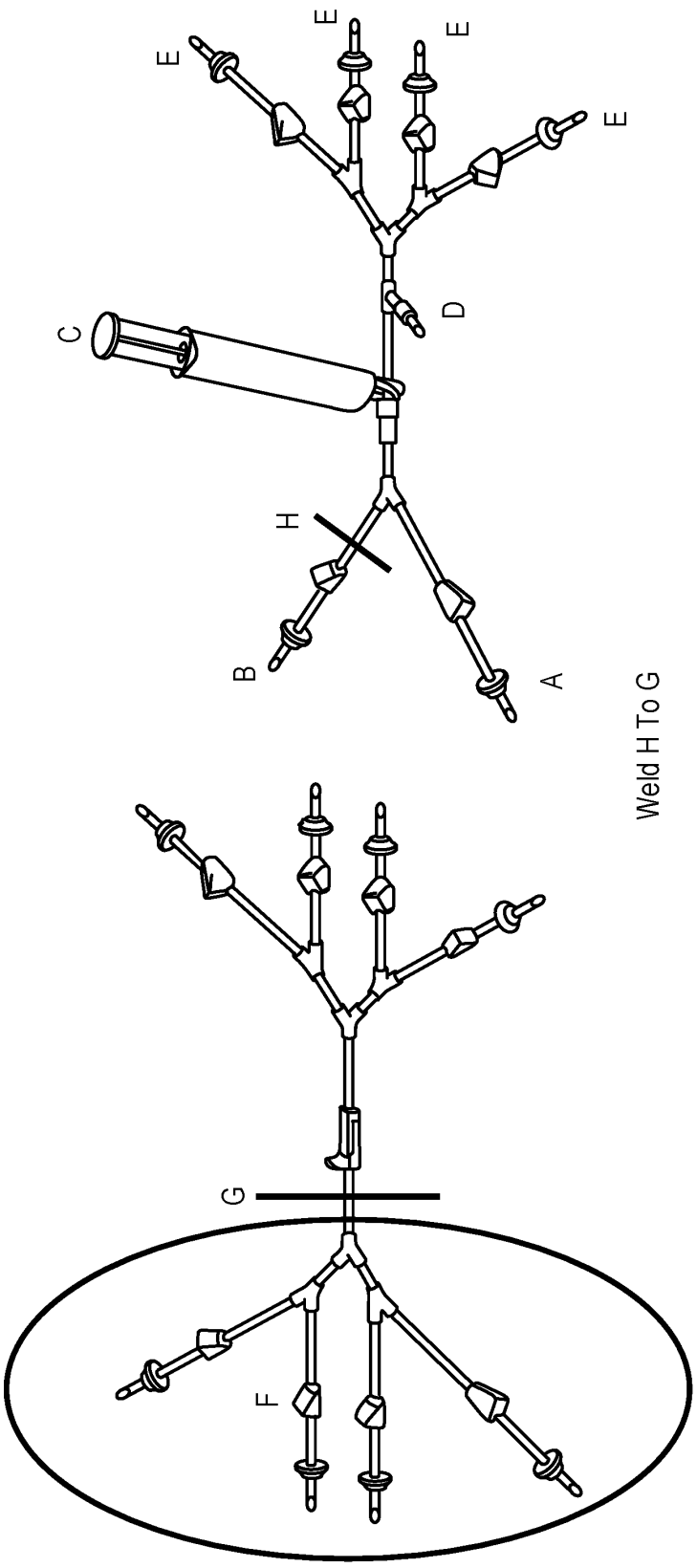


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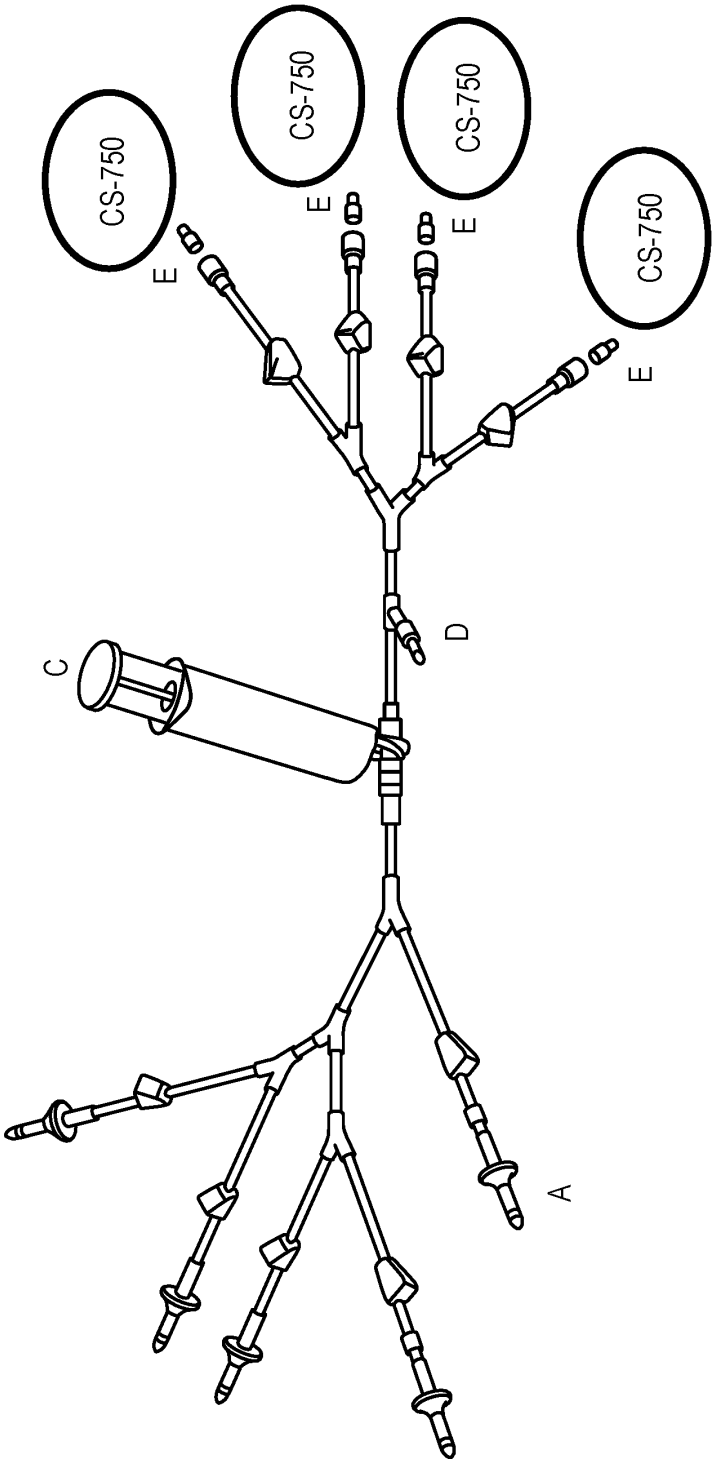


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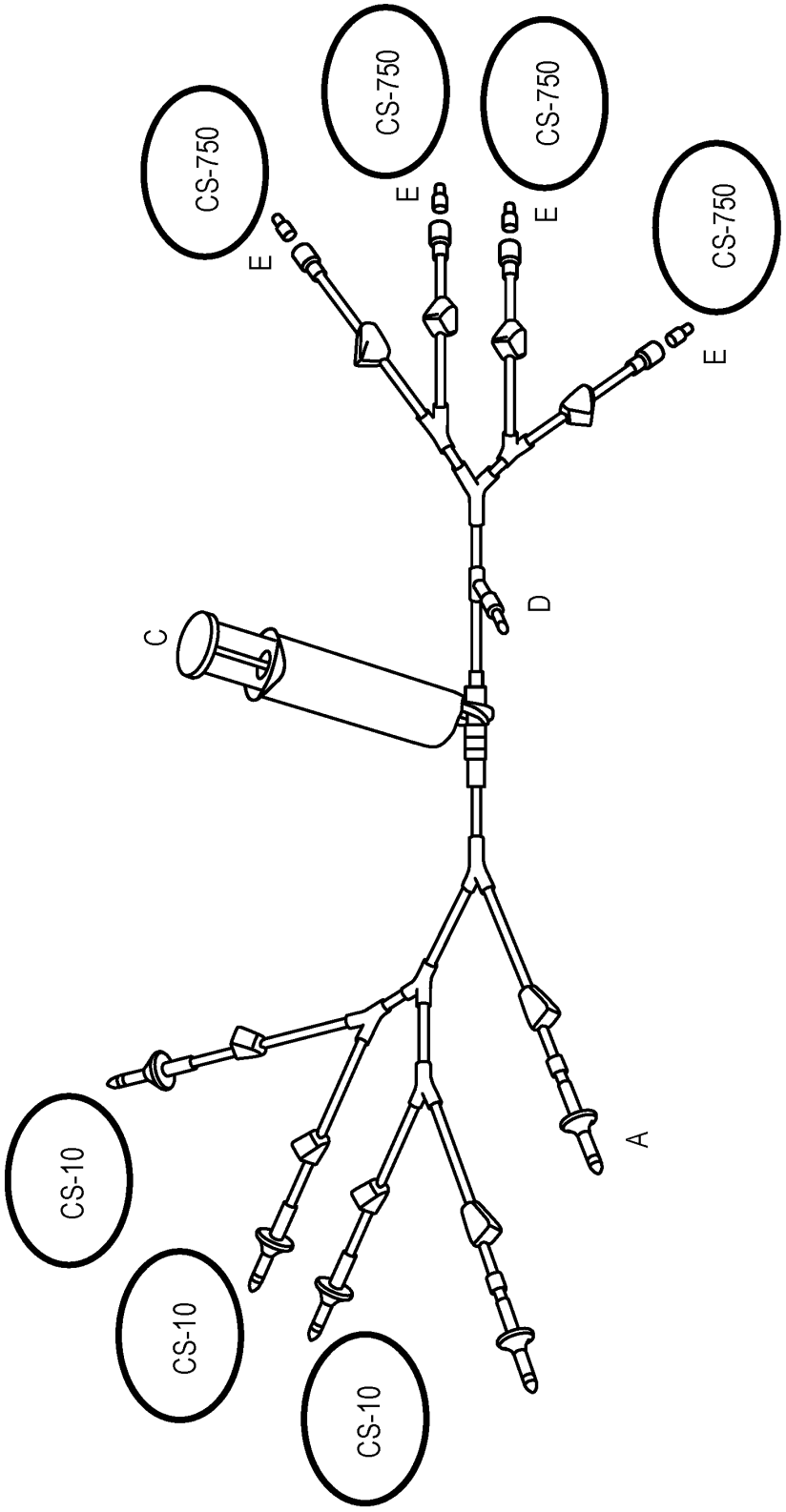


Figure 27

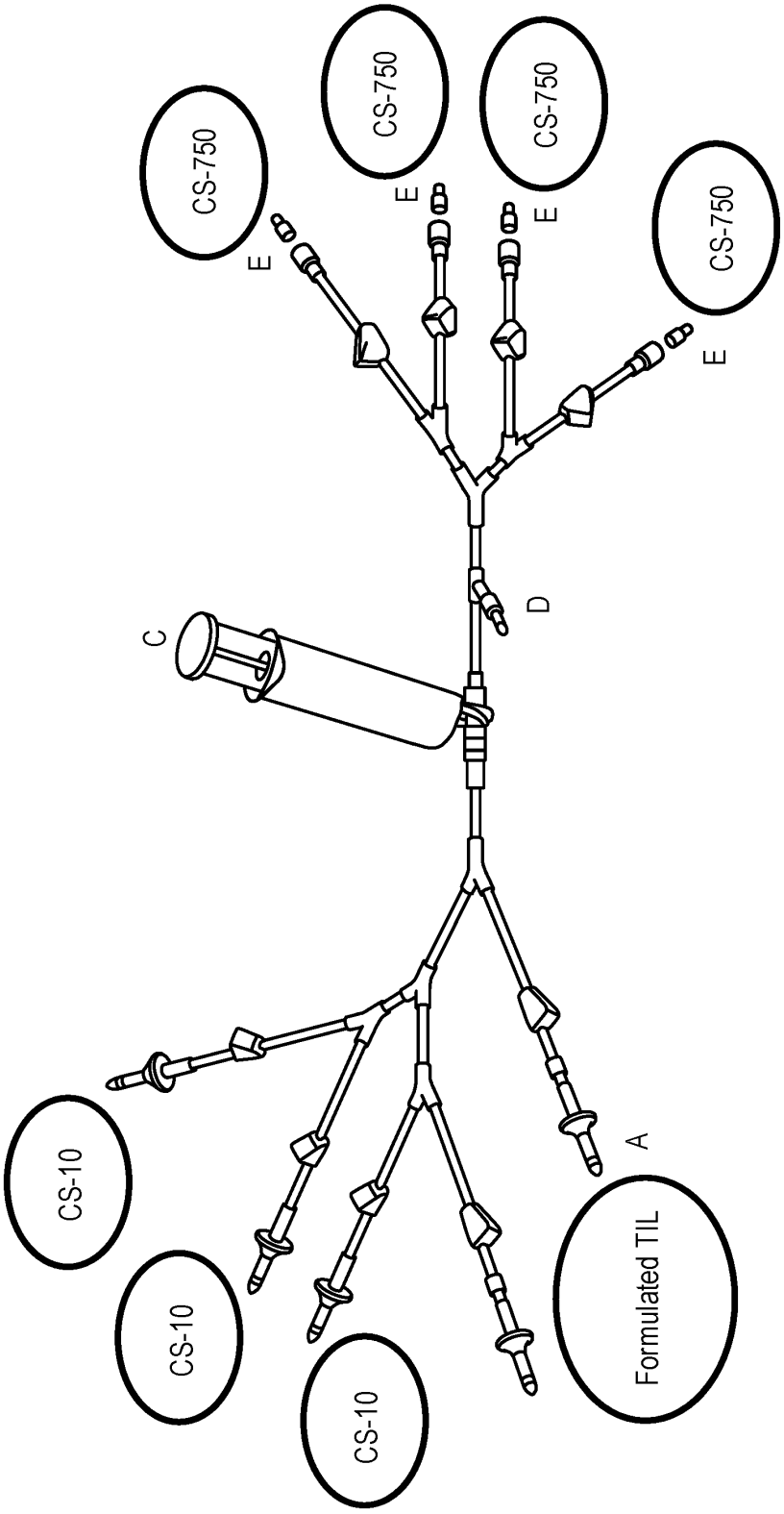

CS-750
CS-750
CS-750
CS-750
E
E
E
E
C
D
A
CS-10
CS-10
CS-10
Formulated TIL

Figure 28

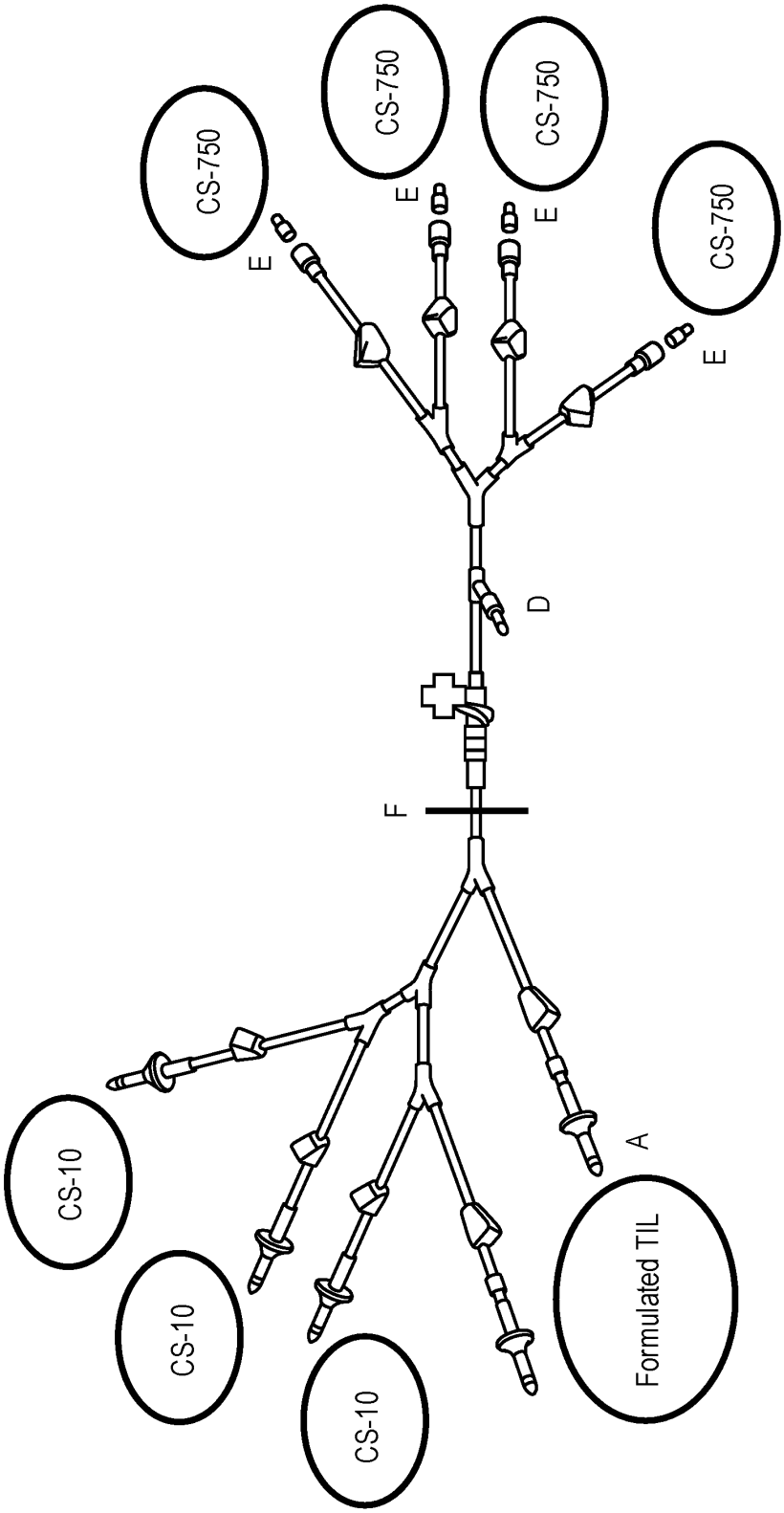

CS-750
CS-750
CS-750
CS-750
E
E
E
E
D
F
CS-10
CS-10
CS-10
A
Formulated TIL

Figure 29

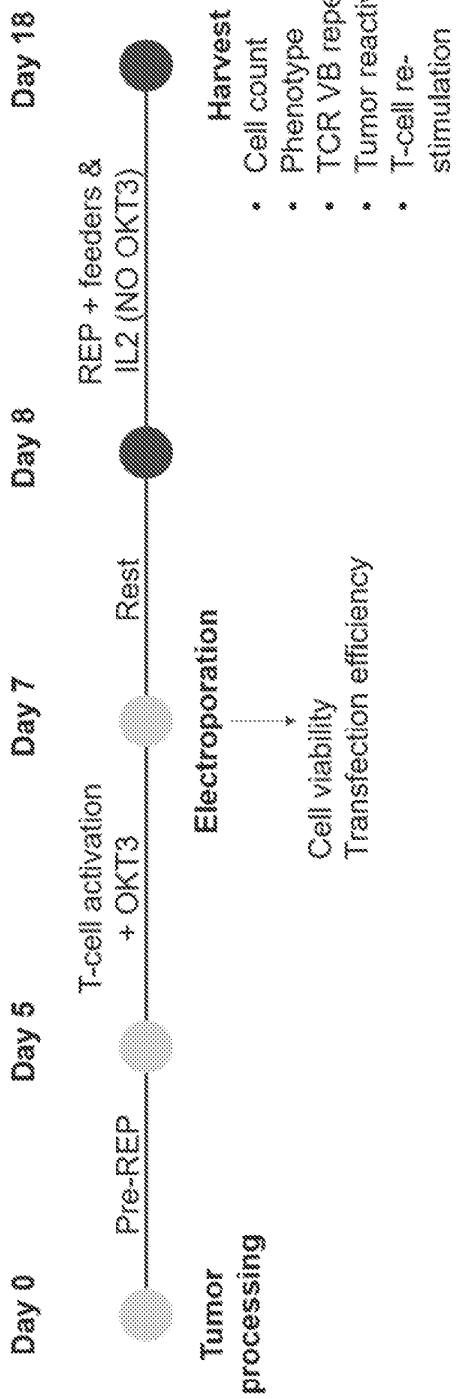

Day 0
Day 5
Day 7
Day 8
Day 18
Tumor processing
Pre-REP
T-cell activation + OKT3
Electroporation
Rest
REP + feeders & IL2 (NO OKT3)
Harvest
Cell count
Phenotype
TCR VB repertoire
Tumor reactivity
T-cell re-stimulation
Cell viability
Transfection efficiency

Figure 30

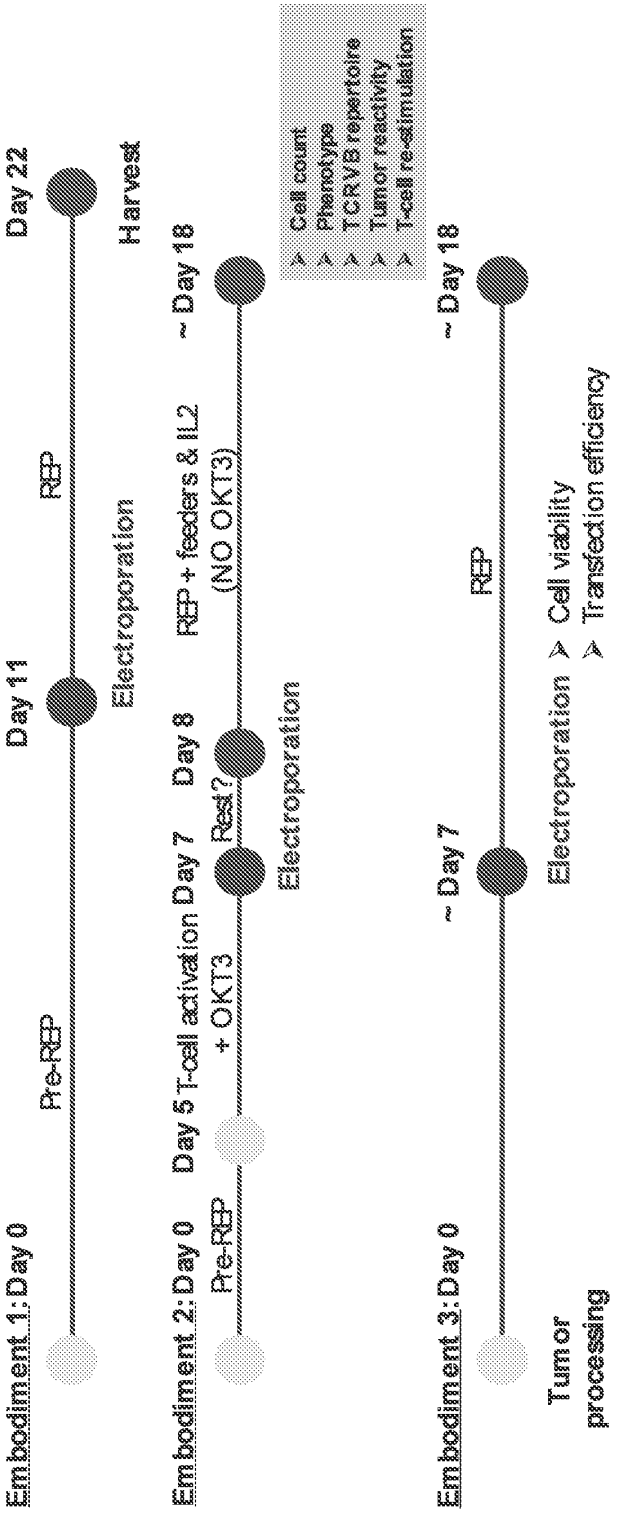

Embodiment 1: Day 0
Pre-REP
Day 11
Electroporation
REP
Day 22
Harvest
Embodiment 2: Day 0
Pre-REP
Day 5 T-cell activation
+ OKT3
Day 7
Rest?
Electroporation
Day 8
REP + feeders & IL2
(NO OKT3)
~ Day 18
Embodiment 3: Day 0
Tumor processing
~ Day 7
Electroporation
REP
~ Day 18
Cell viability
Transfection efficiency
Cell count
Phenotype
TCRVB repertoire
Tumor reactivity
T-cell re-stimulation

Figure 31

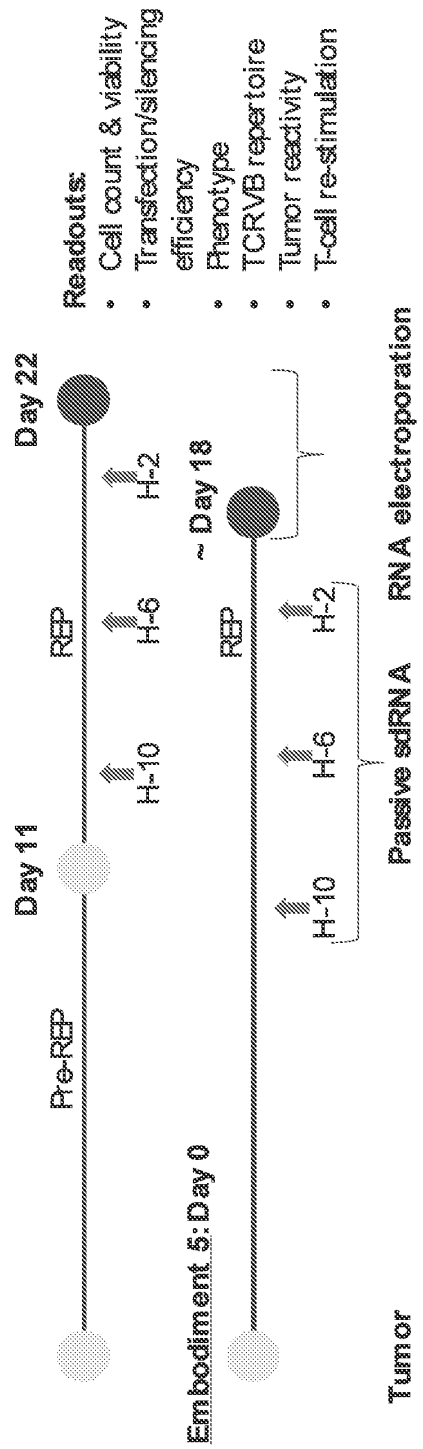
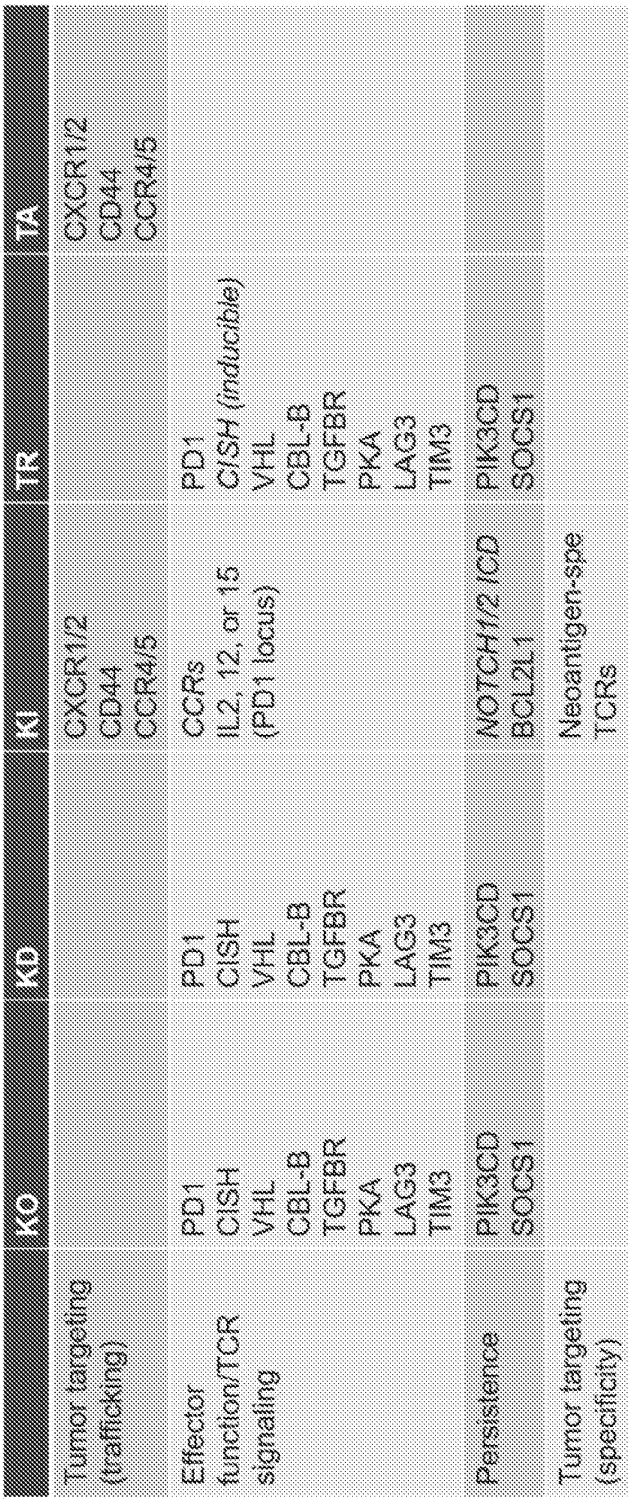
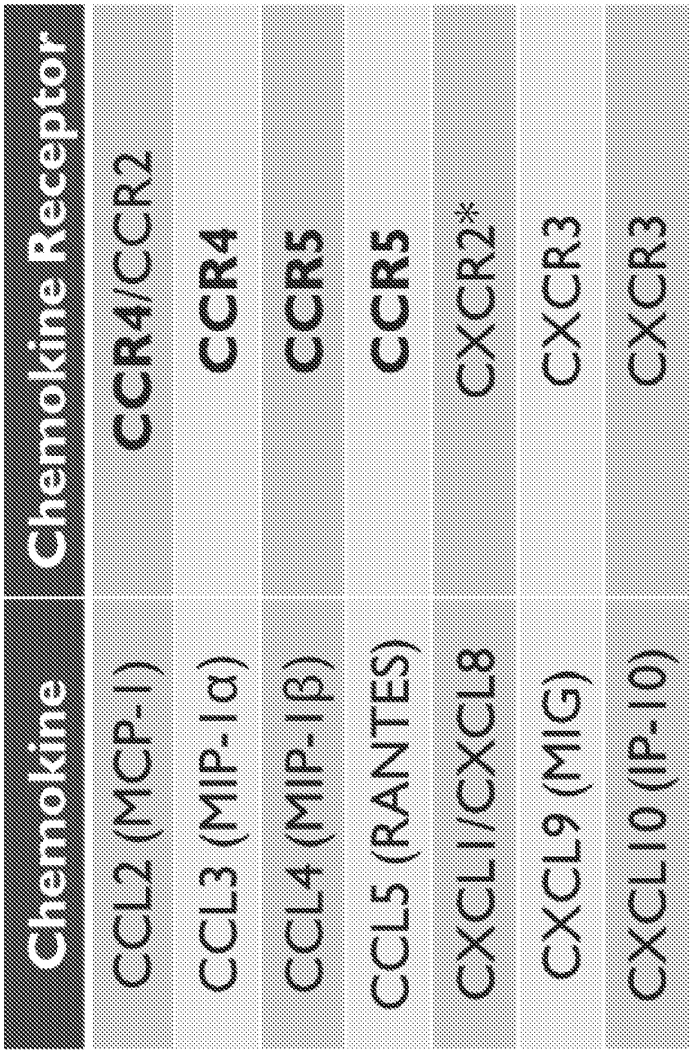

Embodiment 5: Day 0
Pre-REP
Day 11
REP
~ Day 18
REP
Day 22
Tumor processing
H-10
H-6
H-2
Passive sdRNA delivery
RNA electroporation
Readouts:
• Cell count & viability
• Transfection/silencing efficiency
• Phenotype
• TCRVB repertoire
• Tumor reactivity
• T-cell re-stimulation

Figure 32



	KO	KD	KI	TR	TA
Tumor targeting (trafficking)			CXCR1/2 CD44 CCR4/5		CXCR1/2 CD44 CCR4/5
Effector function/TCR signaling	PD1 CISH VHL CBL-B TGFBR PKA LAG3 TIM3	PD1 CISH VHL CBL-B TGFBR PKA LAG3 TIM3	*CCRs* IL2, 12, or 15 (PD1 locus)	PD1 *CISH (inducible)* VHL CBL-B TGFBR PKA LAG3 TIM3	
Persistence	PIK3CD SOCS1	PIK3CD SOCS1	*NOTCH1/2 ICD* BCL2L1	PIK3CD SOCS1	
Tumor targeting (specificity)			Neoantigen-spe TCRs		

Figure 33



Chemokine	Chemokine Receptor
CCL2 (MCP-1)	CCR4/CCR2
CCL3 (MIP-1α)	CCR4
CCL4 (MIP-1β)	CCR5
CCL5 (RANTES)	CCR5
CXCL1/CXCL8	CXCR2*
CXCL9 (MIG)	CXCR3
CXCL10 (IP-10)	CXCR3

Figure 34

Chemokines overexpressed	Chemokine receptors overexpressed
CCL2 (MCP-1) CCL3 (MIP-1α) CCL4 (MIP1-β) CCL5 (RANTES) CXCL1/CXCL8	• Healthy donor T cells were activated to overexpresss CCR1, CCR2, CCR5, CCR7, CXCR3, CXCR4. • CXCR3+, CCR5+ T cells showed highest recruitment in transwell assays
CCL22	CCR4 (Cytotoxic T cells modified to express CCR4 showed increased tumor engraftment and tumor killing)
CCL17	CCR4 (CD30+ CAR T cells modified to express CCR4 showed improved migration and enhanced anti-tumor activity)
CXCL1/CXCL8	CXCR2 (PBMC derived T cells modified to express CXCR2 showed improved migration and increase in IFN-γ production)

Figure 35

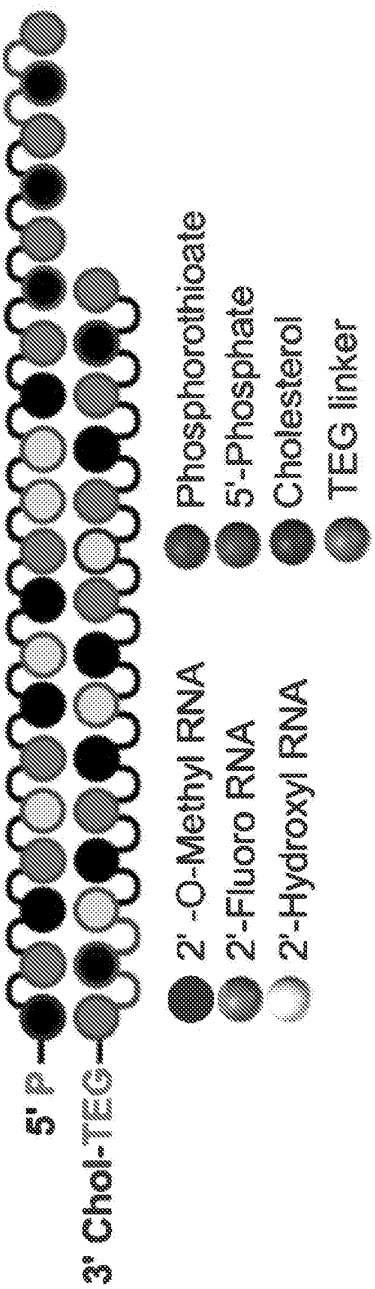

5' P
3' Chol-TEG
2' -O-Methyl RNA
2'-Fluoro RNA
2'-Hydroxyl RNA
Phosphorothioate
5'-Phosphate
Cholesterol
TEG linker

Figure 36

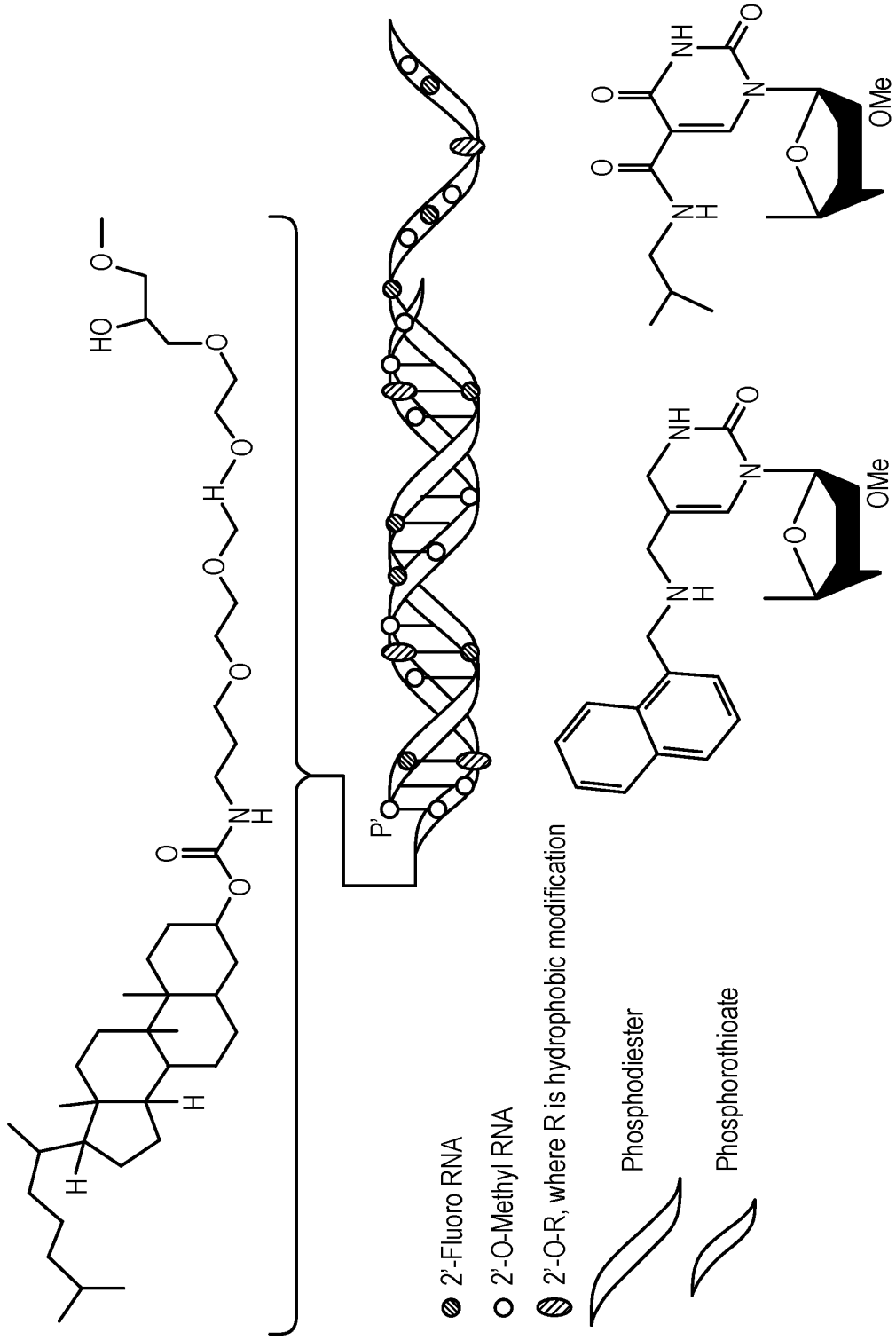

2'-Fluoro RNA
2'-O-Methyl RNA
2'-O-R, where R is hydrophobic modification
Phosphodiester
Phosphorothioate
P'
OMe
OMe

Figure 37

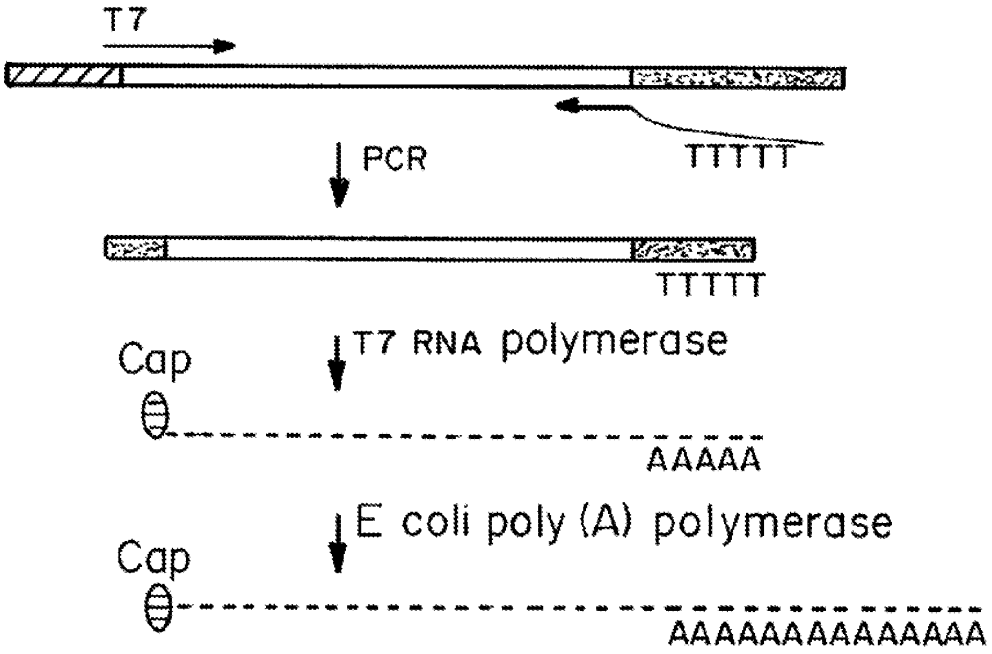

T7
PCR
TTTTT
TTTTT
T7 RNA polymerase
Cap
AAAAA
E coli poly (A) polymerase
Cap
AAAAAAAAAAAAAA

Figure 38

Target gene	sd-rxRNA sequence	RXI reagent	KD detection method
PDCD1	Not provided	Provided	Flow cytometry
TIM3 (HAVCR1)	Not provided	Provided	Flow cytometry
CBLB	Not provided	Provided	qPCR, Flow cytometry, Western blot
LAG3	Not provided	Provided	Flow cytometry
CISH	Not provided	Provided	qPCR, Flow cytometry, Western blot

Figure 39

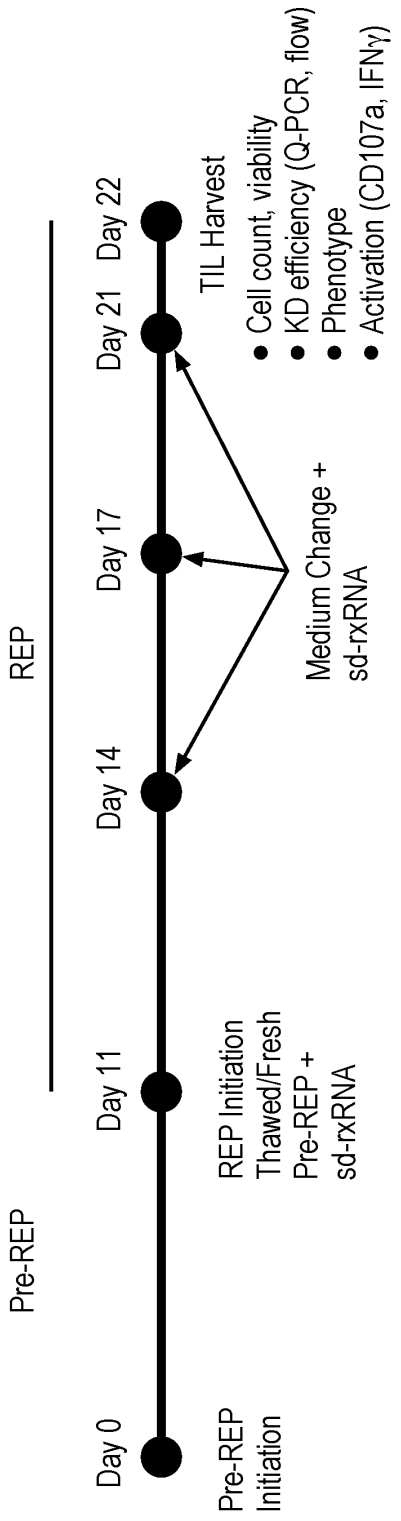

Pre-REP
REP
Day 0
Day 11
Day 14
Day 17
Day 21
Day 22
Pre-REP Initiation
REP Initiation Thawed/Fresh Pre-REP + sd-rxRNA
Medium Change + sd-rxRNA
TIL Harvest
Cell count, viability
KD efficiency (Q-PCR, flow)
Phenotype
Activation (CD107a, IFNγ)

Figure 40

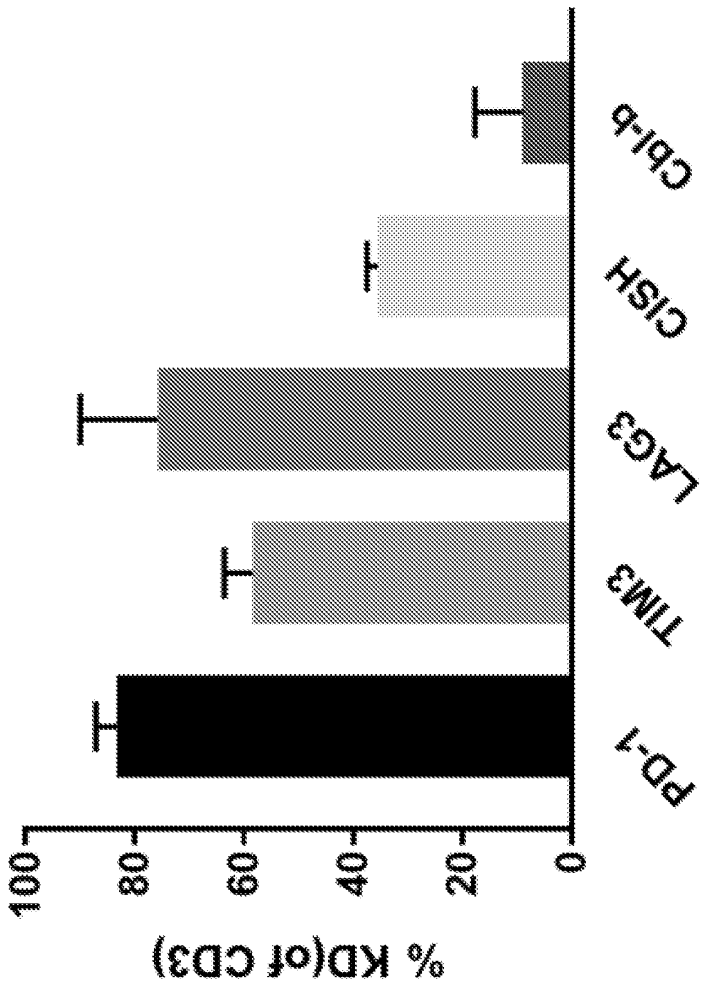


Figure 41

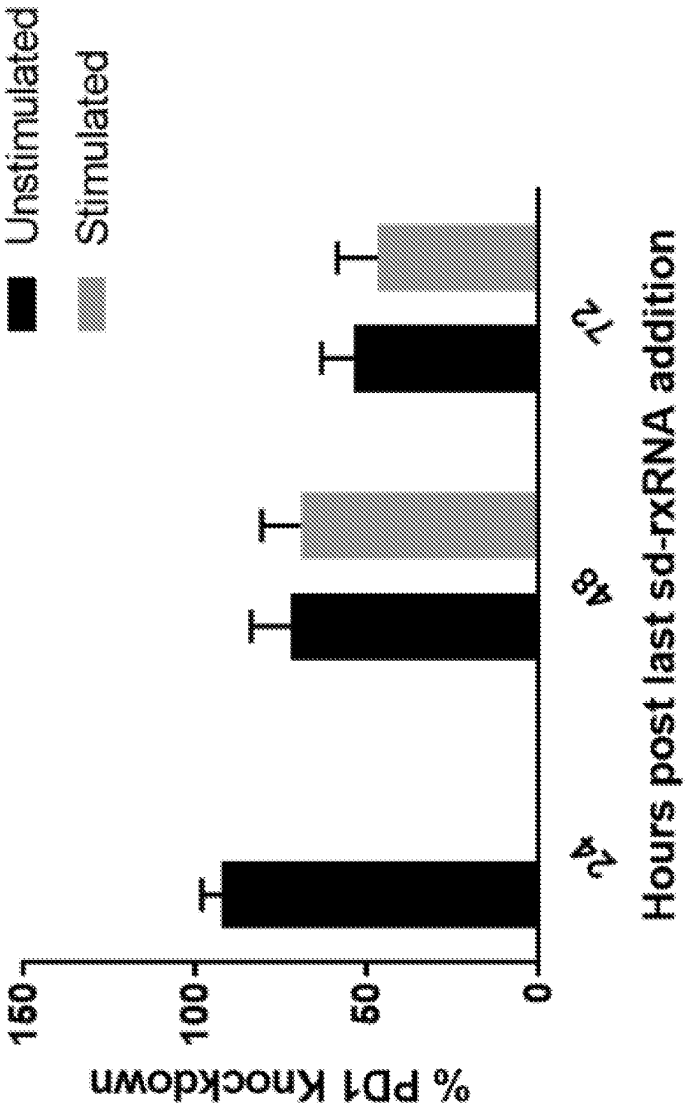

Unstimulated
Stimulated
% PD1 Knockdown
150
100
50
0
24
48
72
Hours post last sd-rxRNA addition

Figure 42

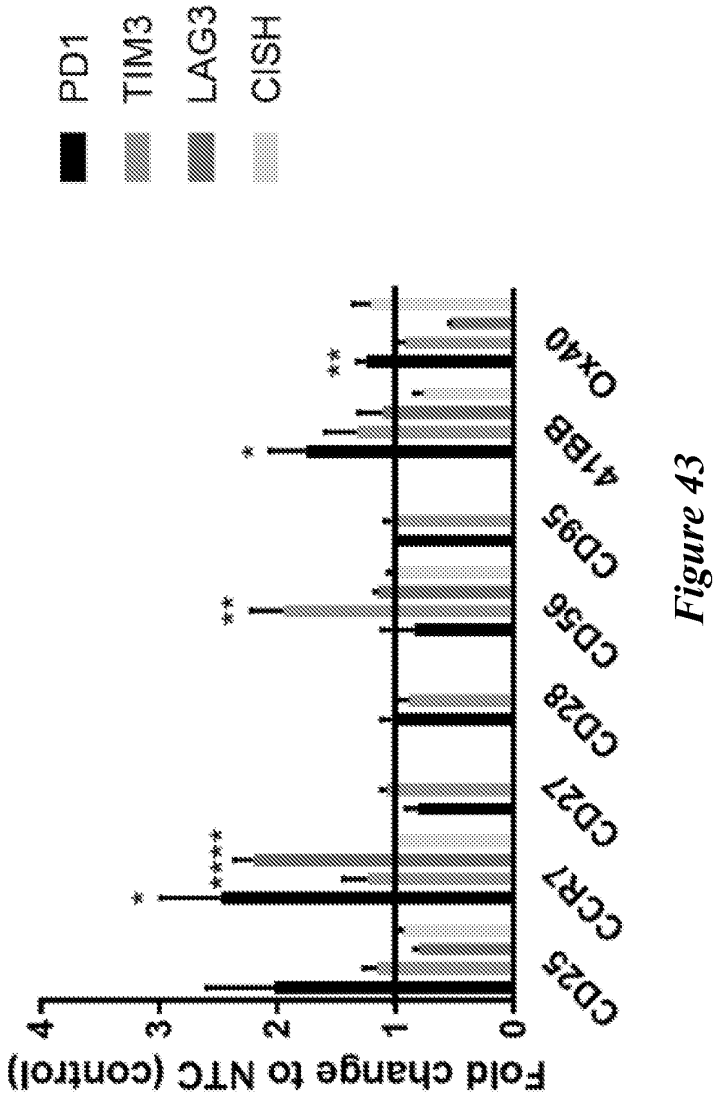
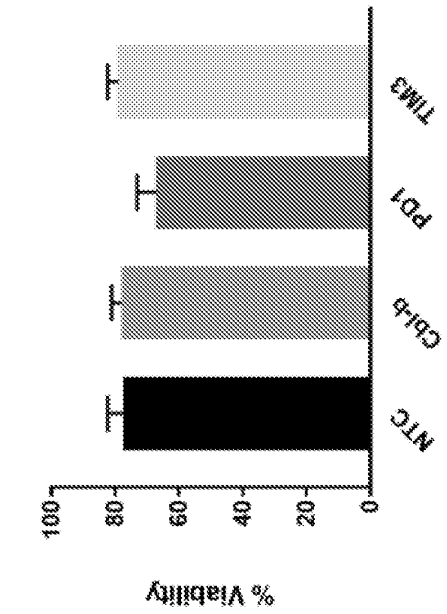

PD1
TIM3
LAG3
CISH
Fold change to NTC (control)
0
1
2
3
4
CD25
CCR7
CD27
CD28
CD56
CD95
4/BB
Ox40
*
****
**
*
**
*
**
*

Figure 43


% Viability
100
80
60
40
20
0
NTC
Cbl-b
PD1
TIM3

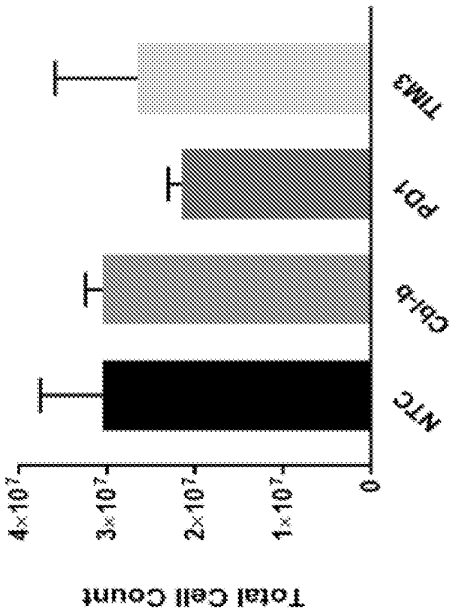

Total Cell Count
4×10^7
3×10^7
2×10^7
1×10^7
0
NTC
Cbl-b
PD1
TIM3

Figure 44

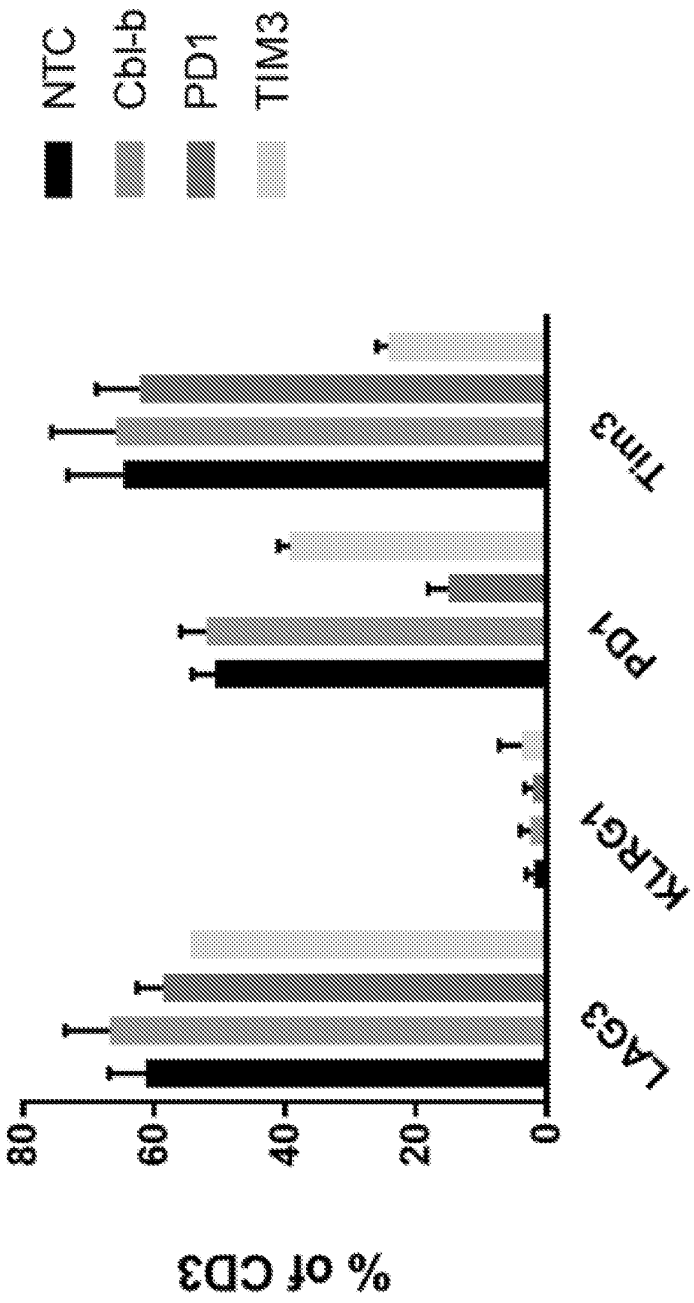


Figure 45A

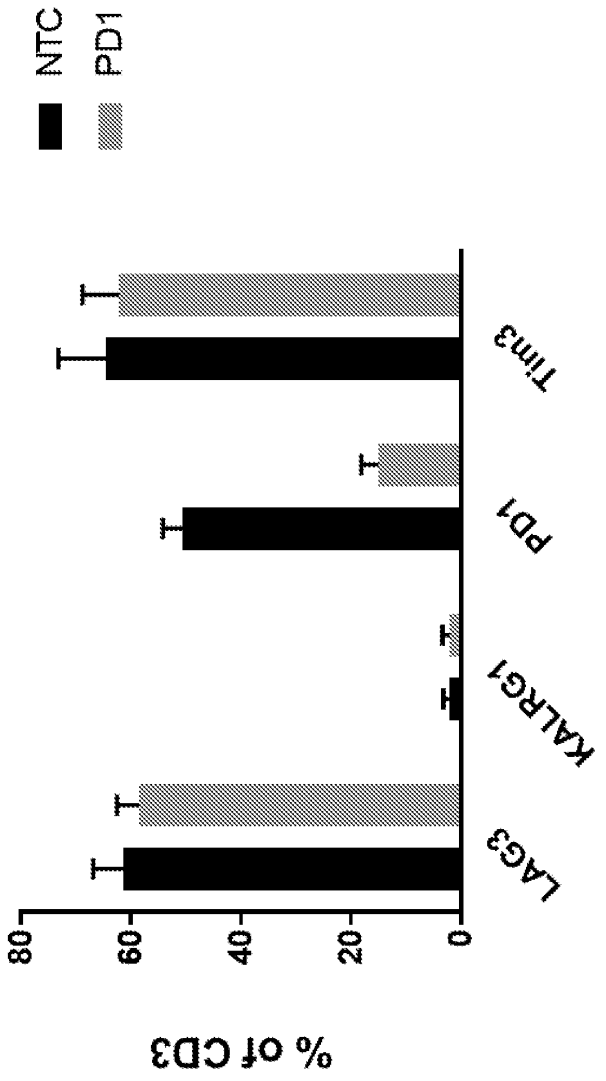


Figure 45B

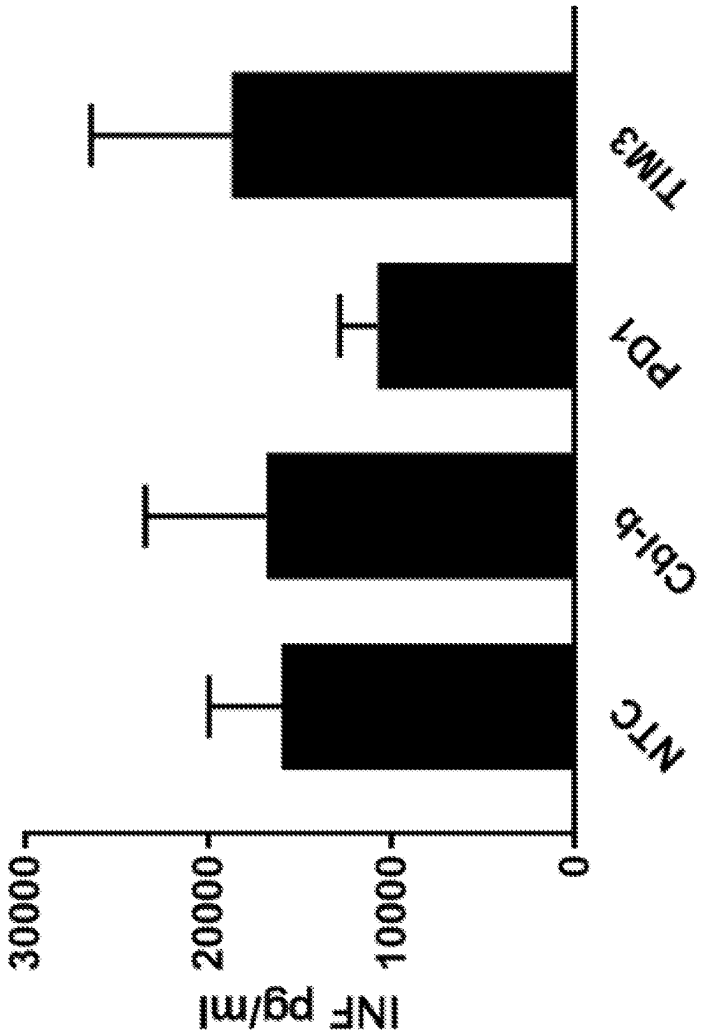


Figure 46

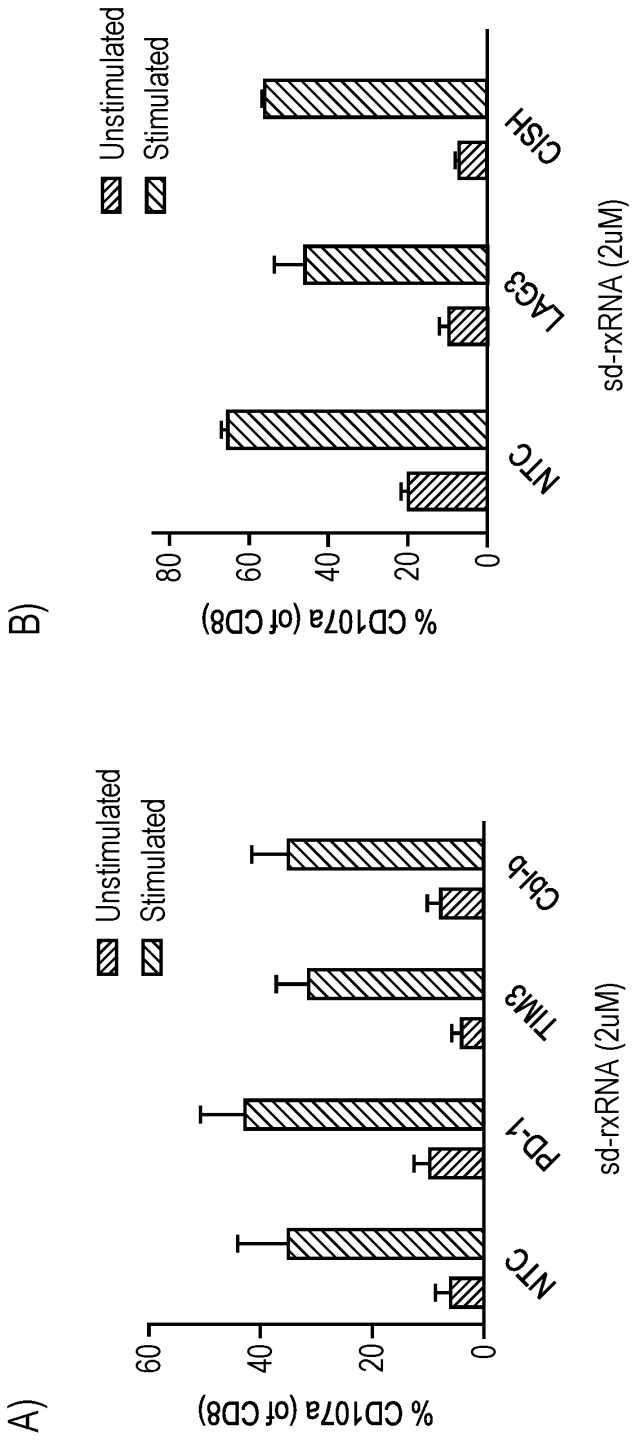
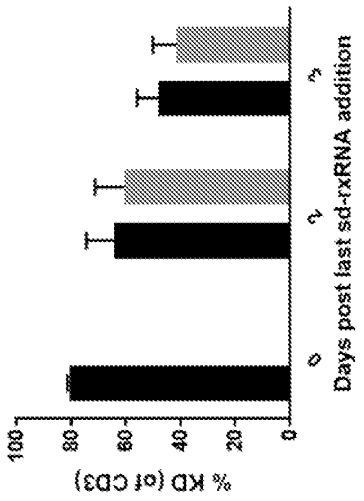

A)
% CD107a (of CD8)
0
20
40
60
Unstimulated
Stimulated
NTC
PD-1
TIM3
Cbl-b
sd-rxRNA (2uM)
B)
% CD107a (of CD8)
0
20
40
60
80
Unstimulated
Stimulated
NTC
LAG3
CISH
sd-rxRNA (2uM)

Figure 47A-B

C)



D)

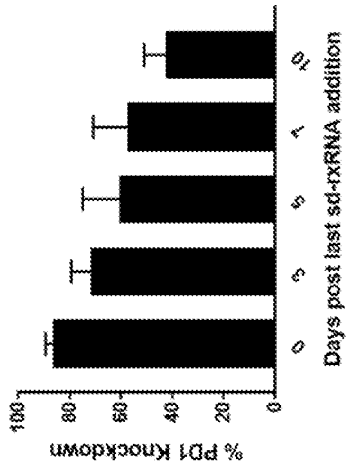


Figure 47C-D

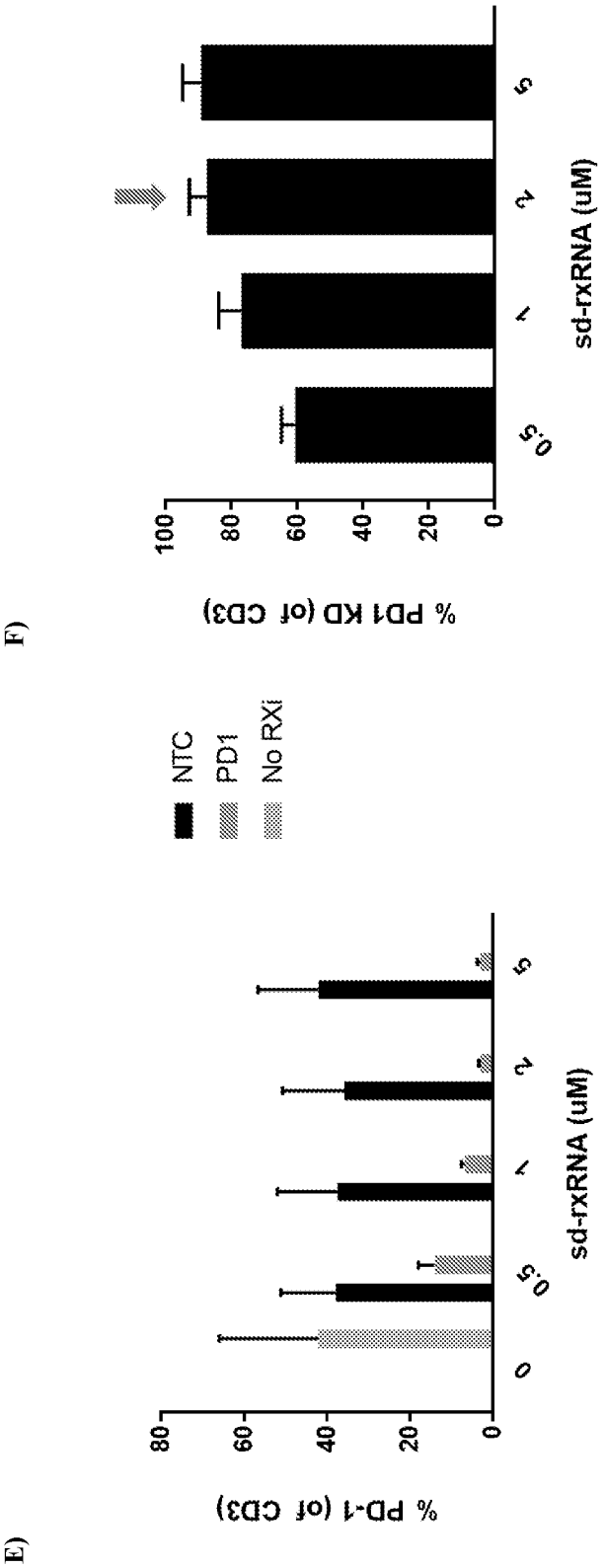

E)
% PD-1 (of CD3)
0
20
40
60
80
0
0.5
1
2
5
sd-rxRNA (uM)
NTC
PD1
No RXi
F)
% PD1 KD (of CD3)
0
20
40
60
80
100
0.5
1
2
5
sd-rxRNA (uM)

Figure 47E-F

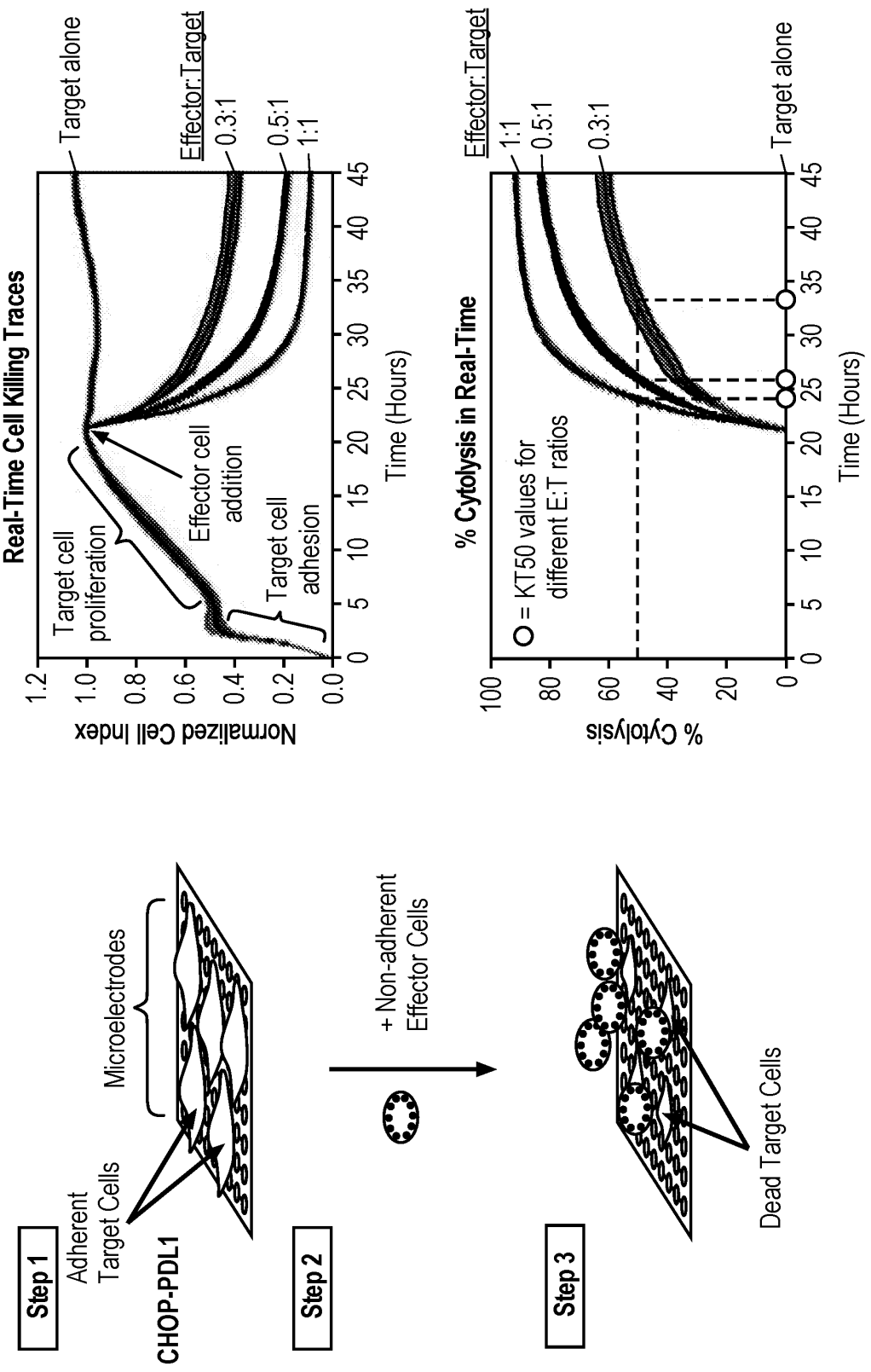

Real-Time Cell Killing Traces
Normalized Cell Index
Time (Hours)
Target alone
Effector:Target
0.3:1
0.5:1
1:1
Target cell proliferation
Effector cell addition
Target cell adhesion
% Cytolysis in Real-Time
% Cytolysis
O = KT50 values for different E:T ratios
Step 1
Adherent Target Cells
CHOP-PDL1
Microelectrodes
Step 2
+ Non-adherent Effector Cells
Step 3
Dead Target Cells

Figure 48

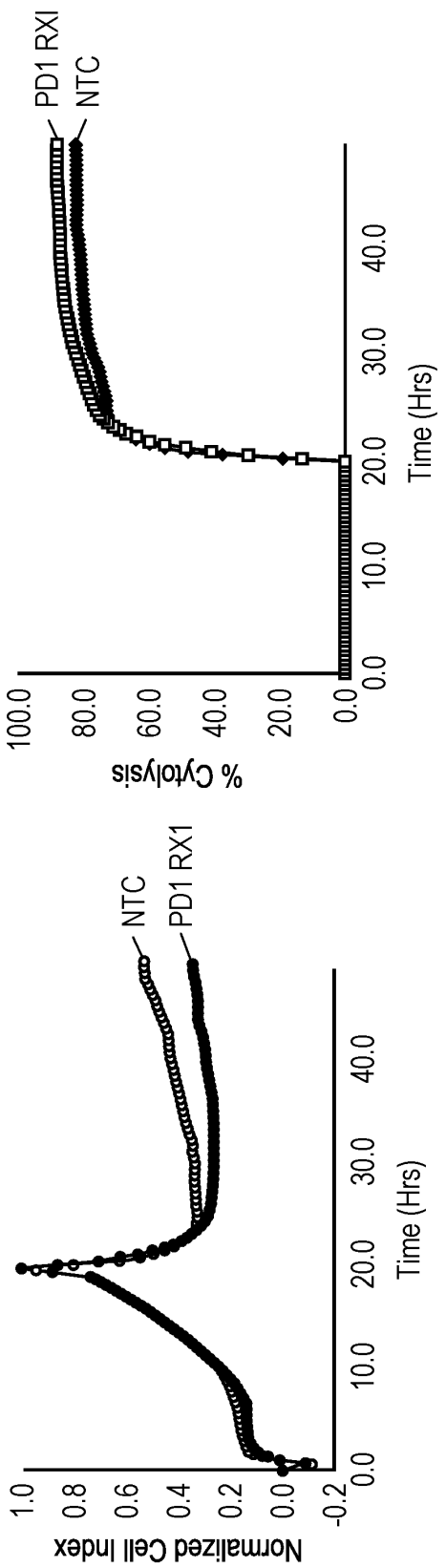

PD1 RXI
NTC
% Cytolysis
100.0
80.0
60.0
40.0
20.0
0.0
0.0
10.0
20.0
30.0
40.0
Time (Hrs)
NTC
PD1 RX1
Normalized Cell Index
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0.0
10.0
20.0
30.0
40.0
Time (Hrs)

Figure 49A

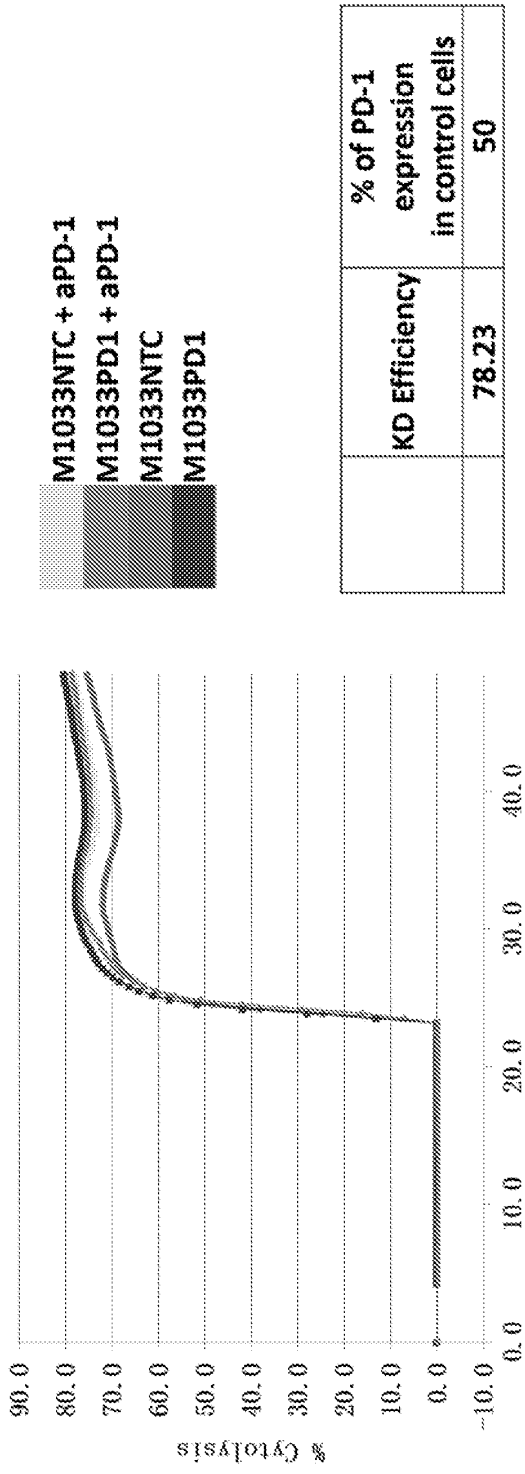

M1033NTC + aPD-1
M1033PD1 + aPD-1
M1033NTC
M1033PD1
% of PD-1 expression in control cells
KD Efficiency
78.23
50
% Cytolysis
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
0.0
10.0
20.0
30.0
40.0
Time (hrs)

Figure 49B

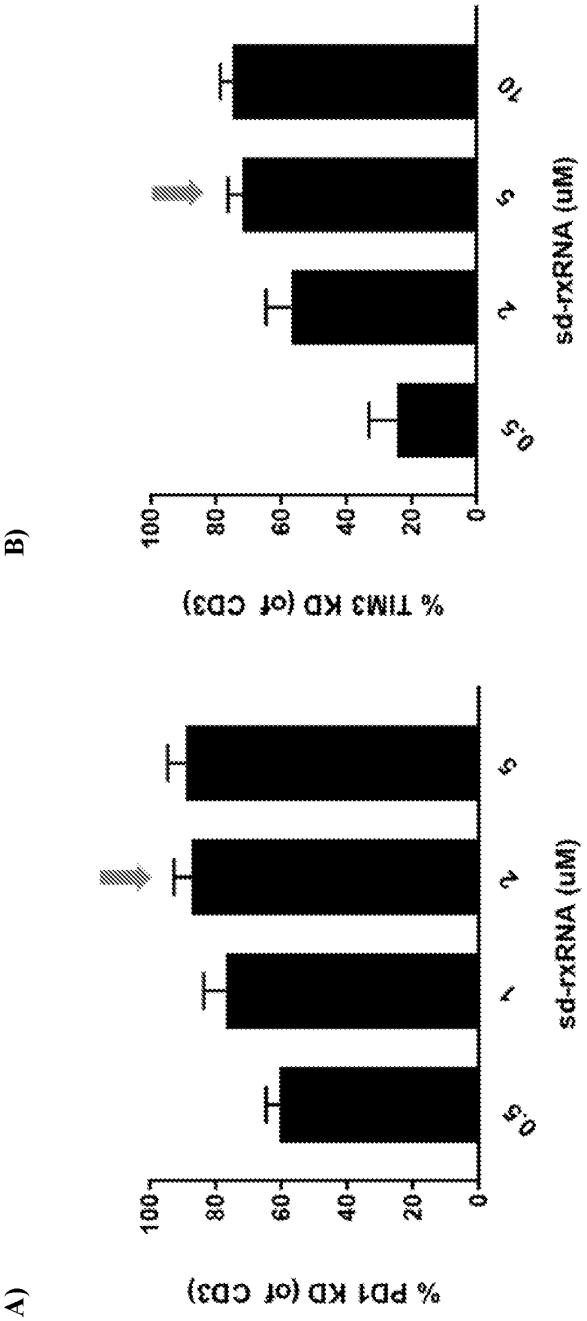

A)
% PD1 KD (of CD3)
100
80
60
40
20
0
0.5
1
5
sd-rxRNA (uM)
B)
% TIM3 KD (of CD3)
100
80
60
40
20
0
0.5
1
5
10
sd-rxRNA (uM)

Figure 50

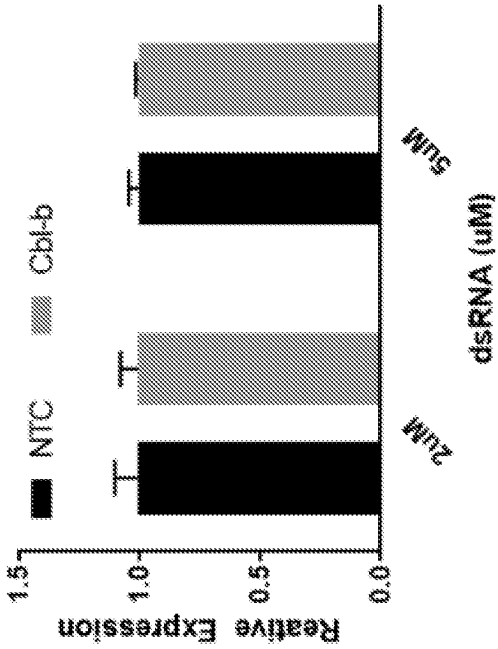


Figure 51A

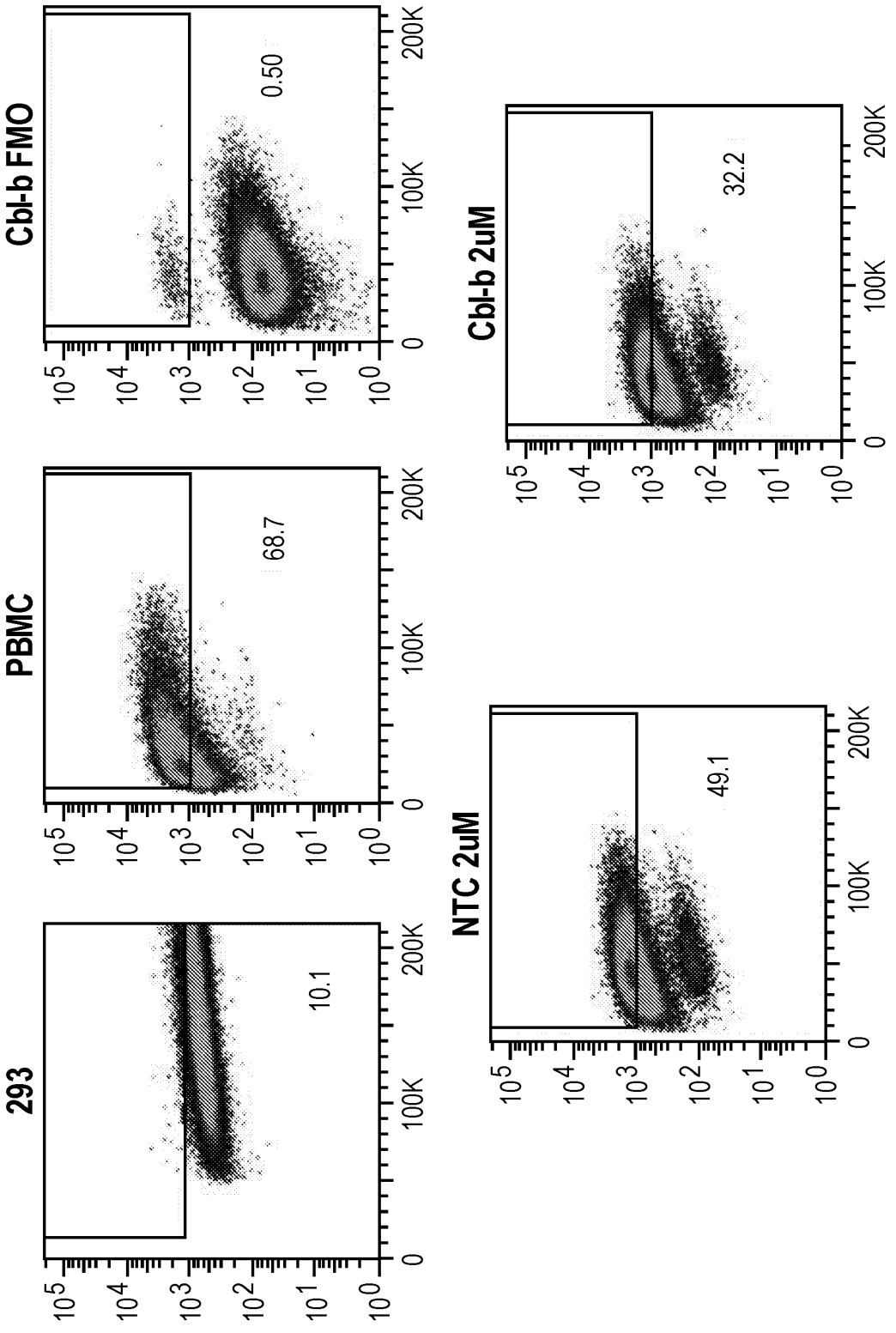

293
PBMC
Cbl-b FMO
NTC 2uM
Cbl-b 2uM
10.1
68.7
0.50
49.1
32.2
0
100K
200K
10^0
10^1
10^2
10^3
10^4
10^5

Figure 51B

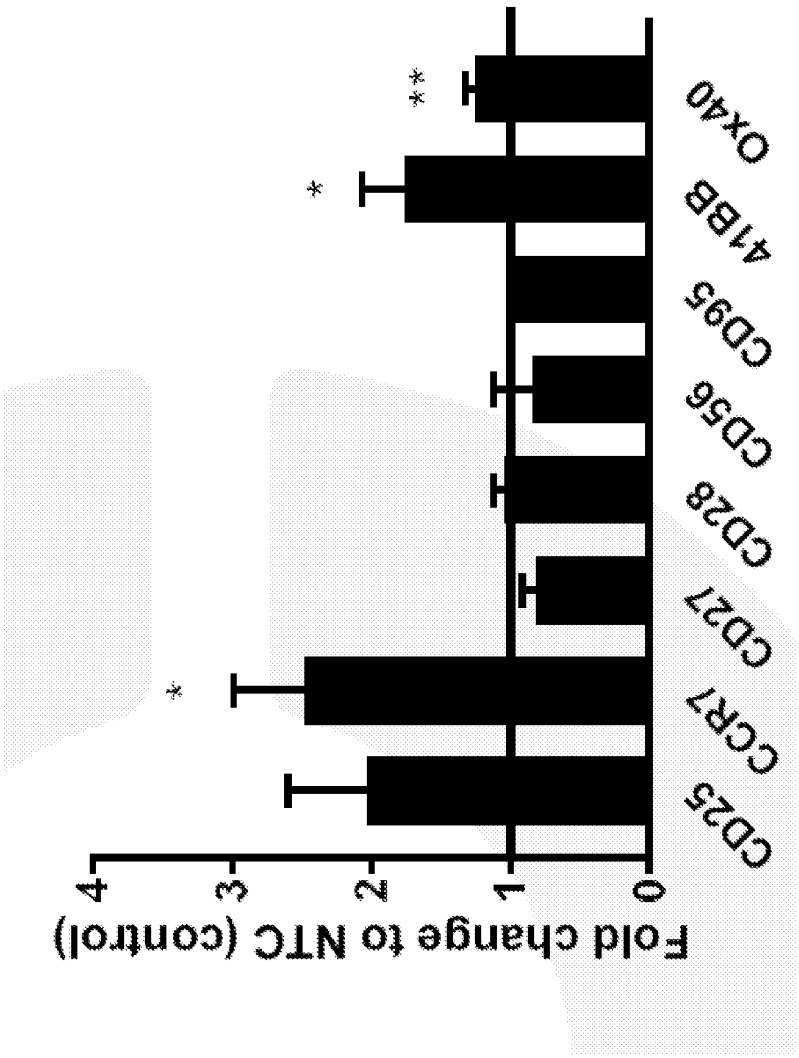

Fold change to NTC (control)
0
1
2
3
4
CD25
CCR7
CD27
CD28
CD56
CD95
4/BB
Ox40
*
*
**

Figure 52A

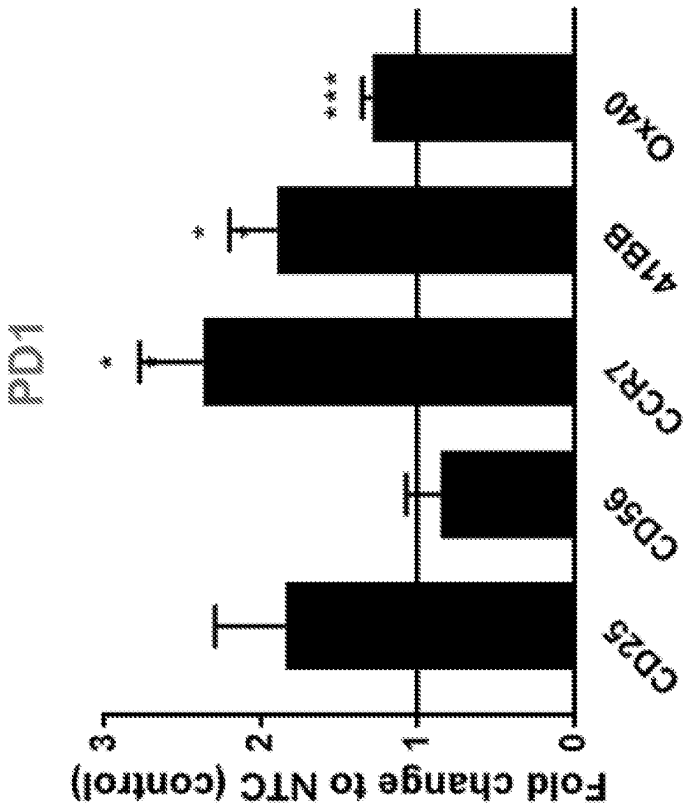


Figure 52B

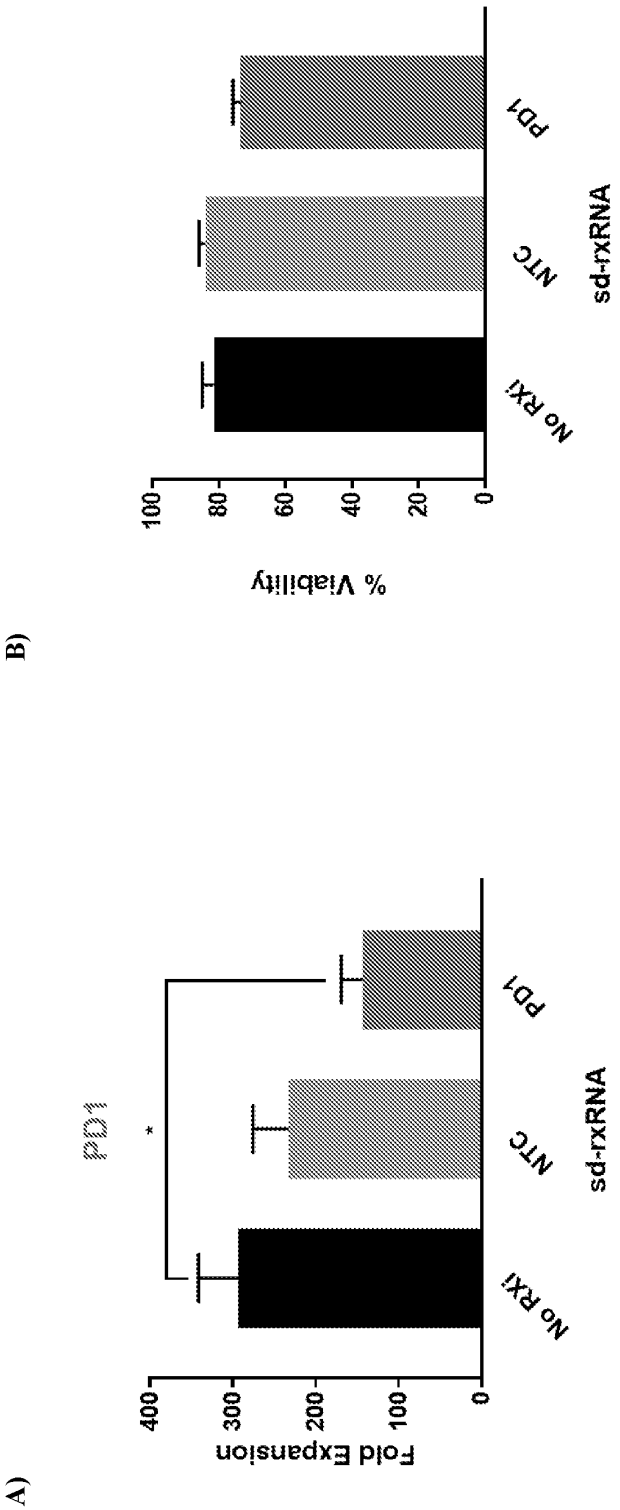

A)
PD1
*
Fold Expansion
400
300
200
100
0
No RXI
NTC
PD1
sd-rxRNA
B)
% Viability
100
80
60
40
20
0
No RXI
NTC
PD1
sd-rxRNA

Figure 53

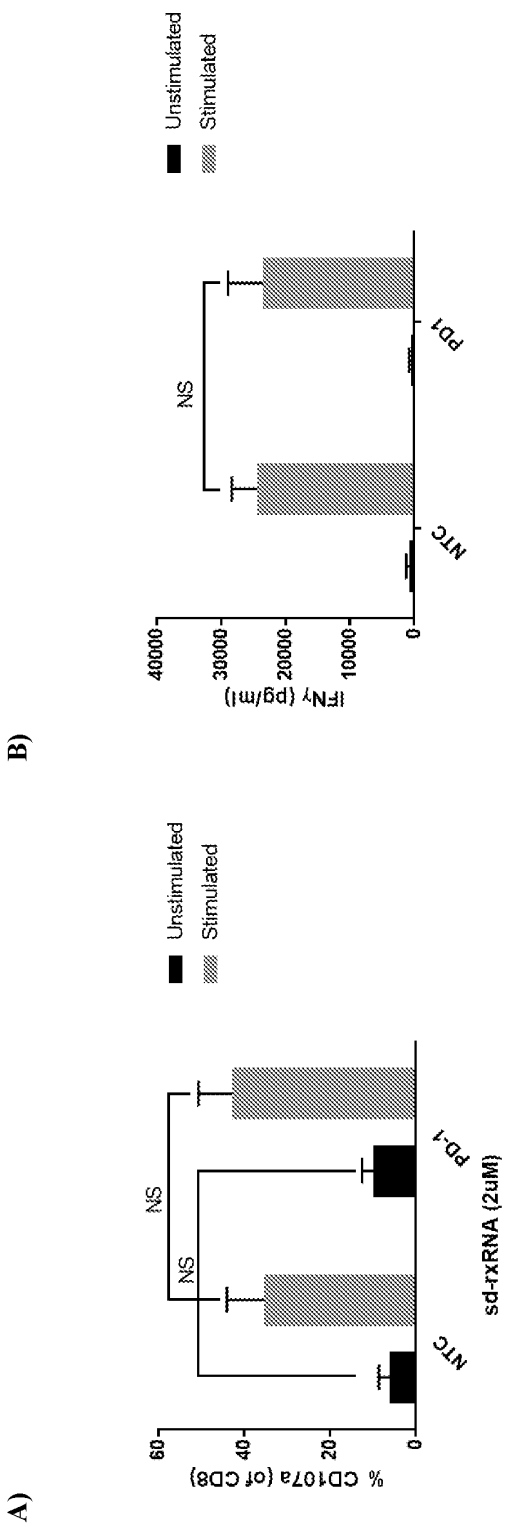

A)
Unstimulated
Stimulated
% CD107a (of CD8)
60
40
20
0
NS
NS
NTC
PD-1
sd-rxRNA (2uM)
B)
Unstimulated
Stimulated
IFNγ (pg/ml)
40000
30000
20000
10000
0
NS
NTC
PD1

Figure 54