Figure 1

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)

Figure 2A

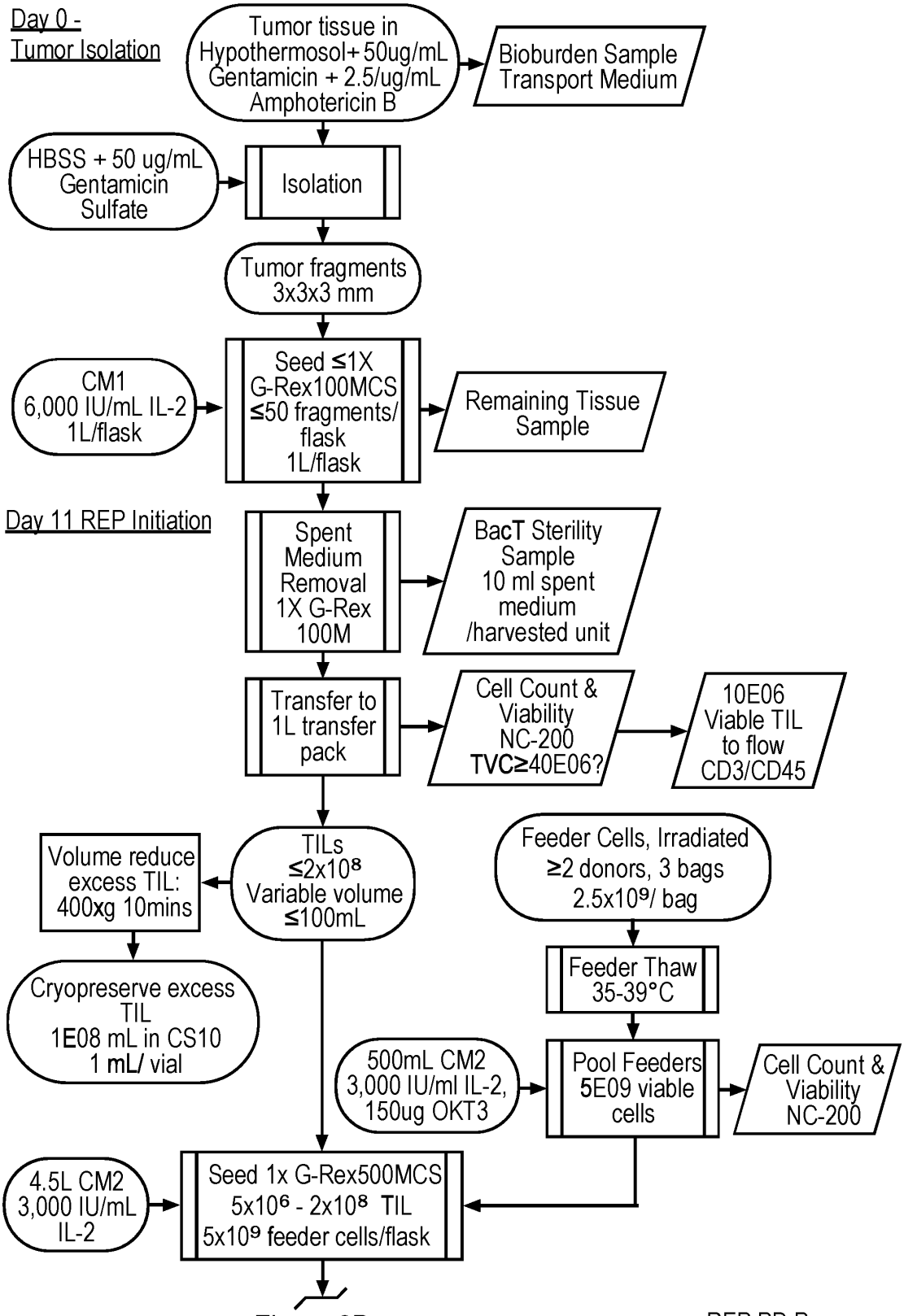


Figure 2B

REP PD Process
Page 1/3

Figure 2A

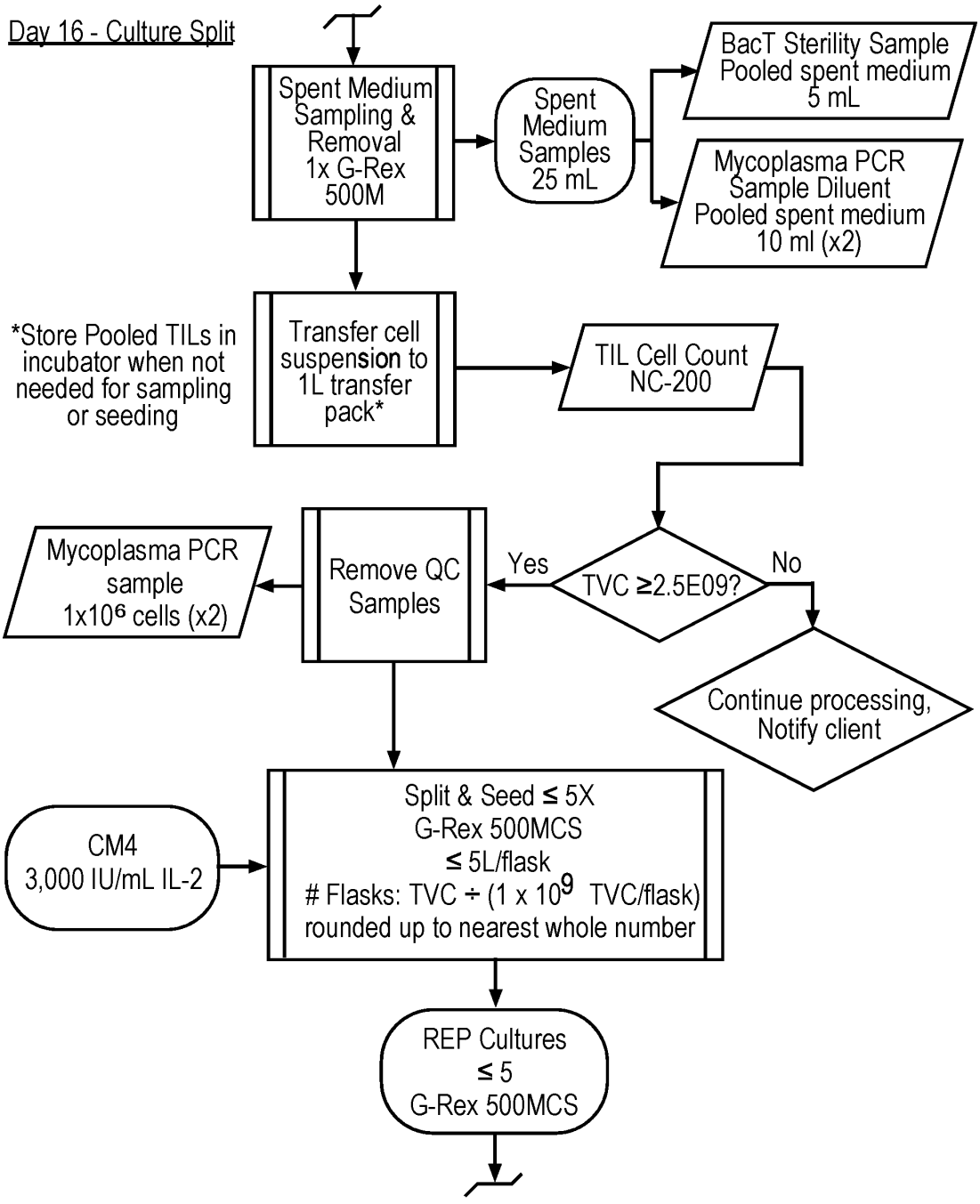
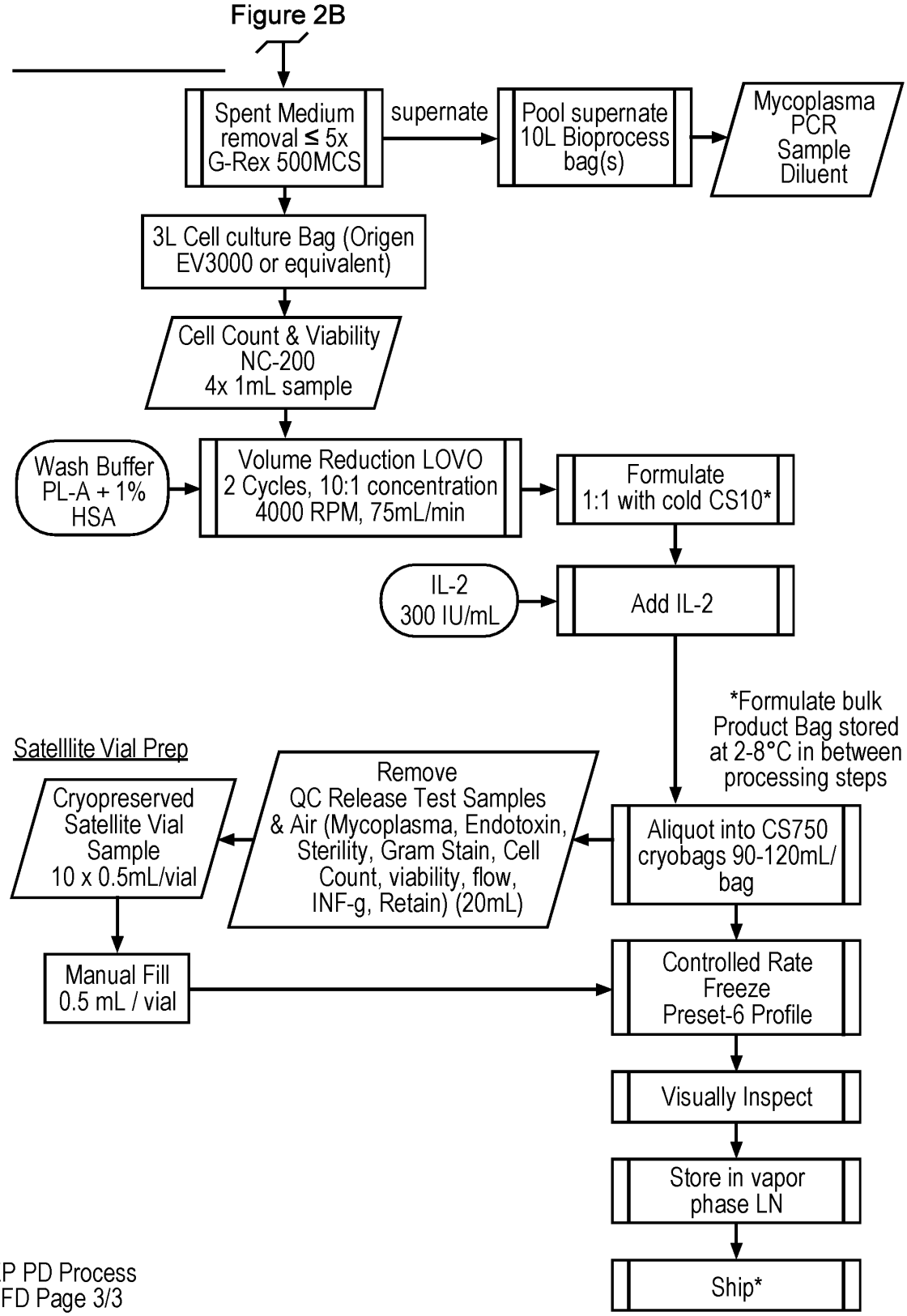


Figure 2C

Figure 2C

Figure 2B



REP PD Process
PFD Page 3/3

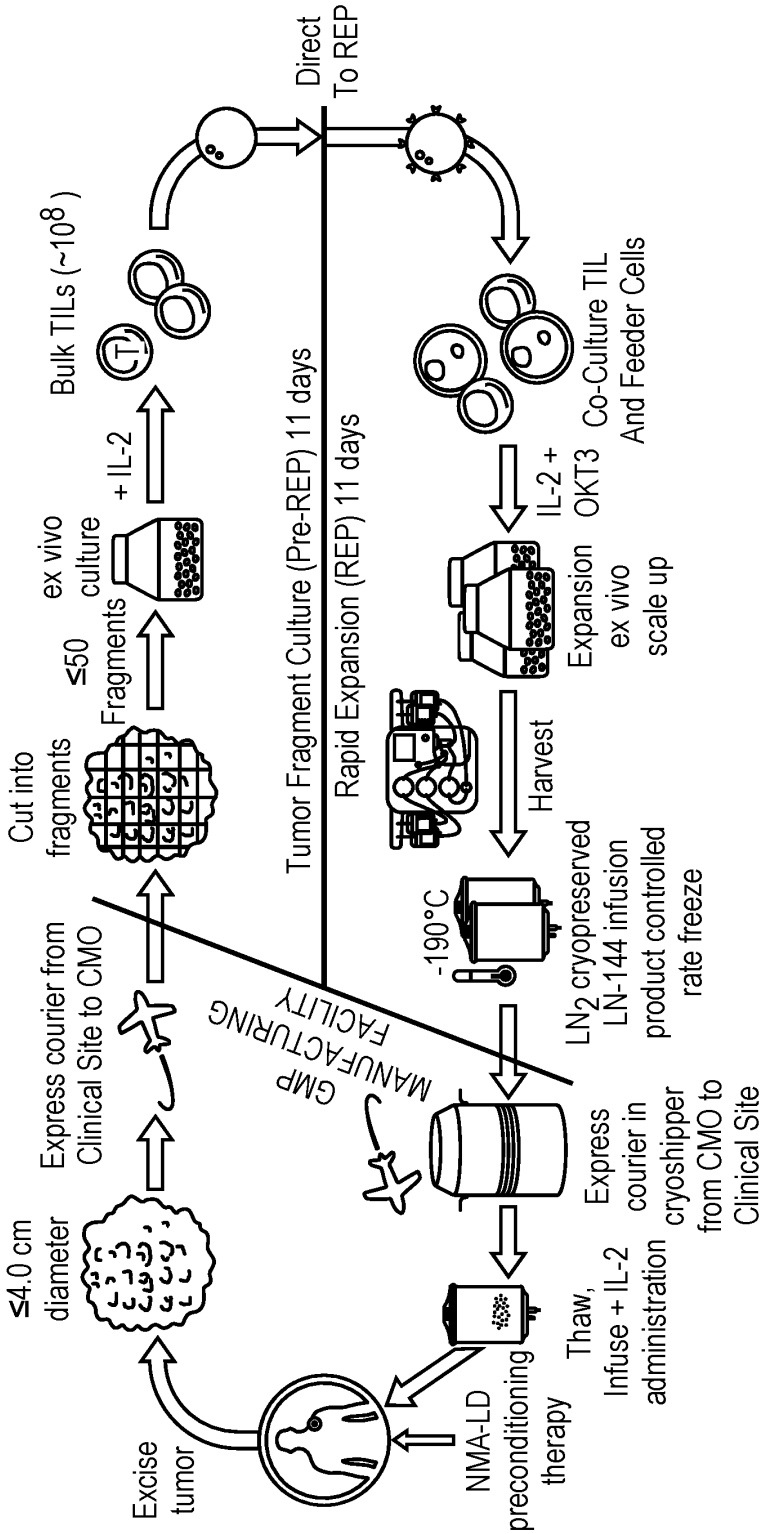

≤4.0 cm diameter
Excise tumor
Express courier from Clinical Site to CMO
Cut into fragments
≤50 Fragments
ex vivo culture
+ IL-2
Bulk TILs (~10^8)
GMP MANUFACTURING FACILITY
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
Direct To REP
NMA-LD preconditioning therapy
Thaw, Infuse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

Figure 3

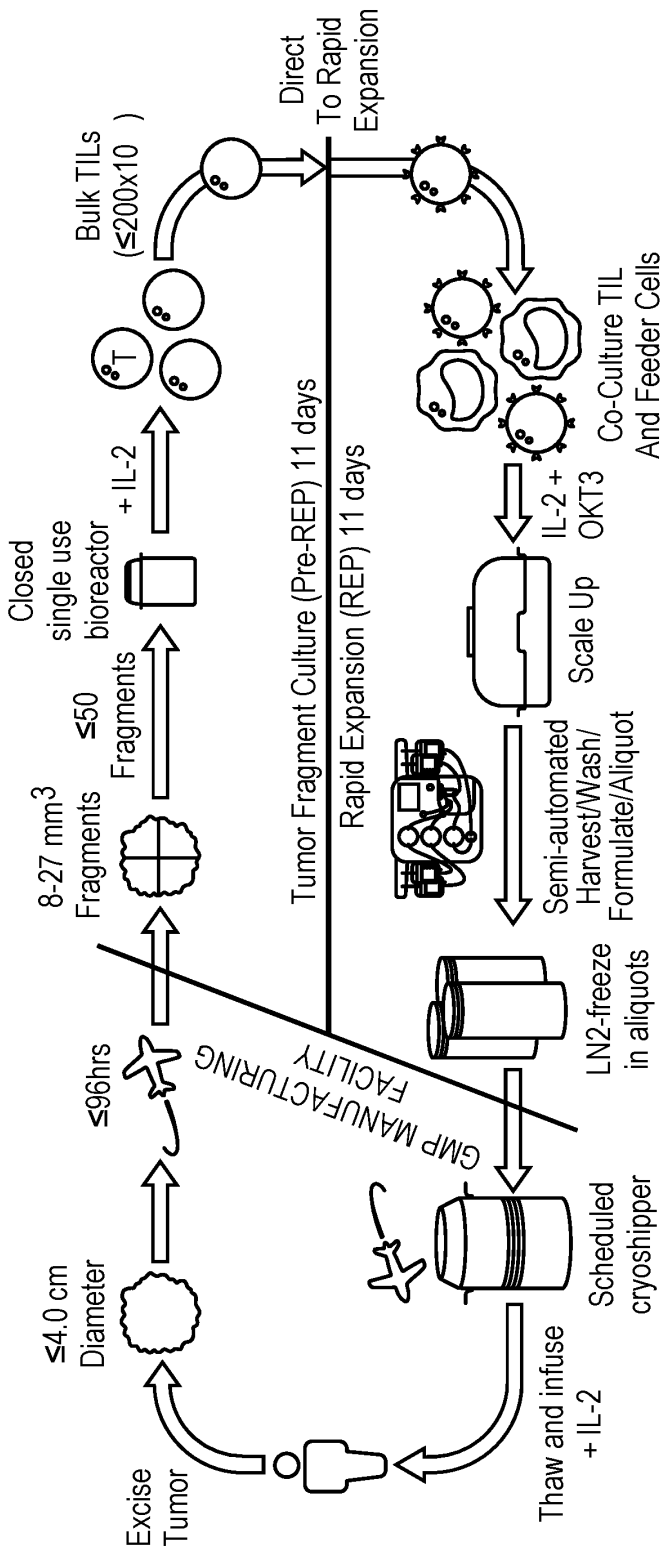

Excise Tumor
≤4.0 cm Diameter
≤96hrs
8-27 mm3 Fragments
≤50 Fragments
Closed single use bioreactor
+ IL-2
Bulk TILs (≤200x10)
Direct To Rapid Expansion
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
GMP MANUFACTURING FACILITY
Co-Culture TIL And Feeder Cells
IL-2 + OKT3
Scale Up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

Figure 4

Figure 5

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 6

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

Figure 7

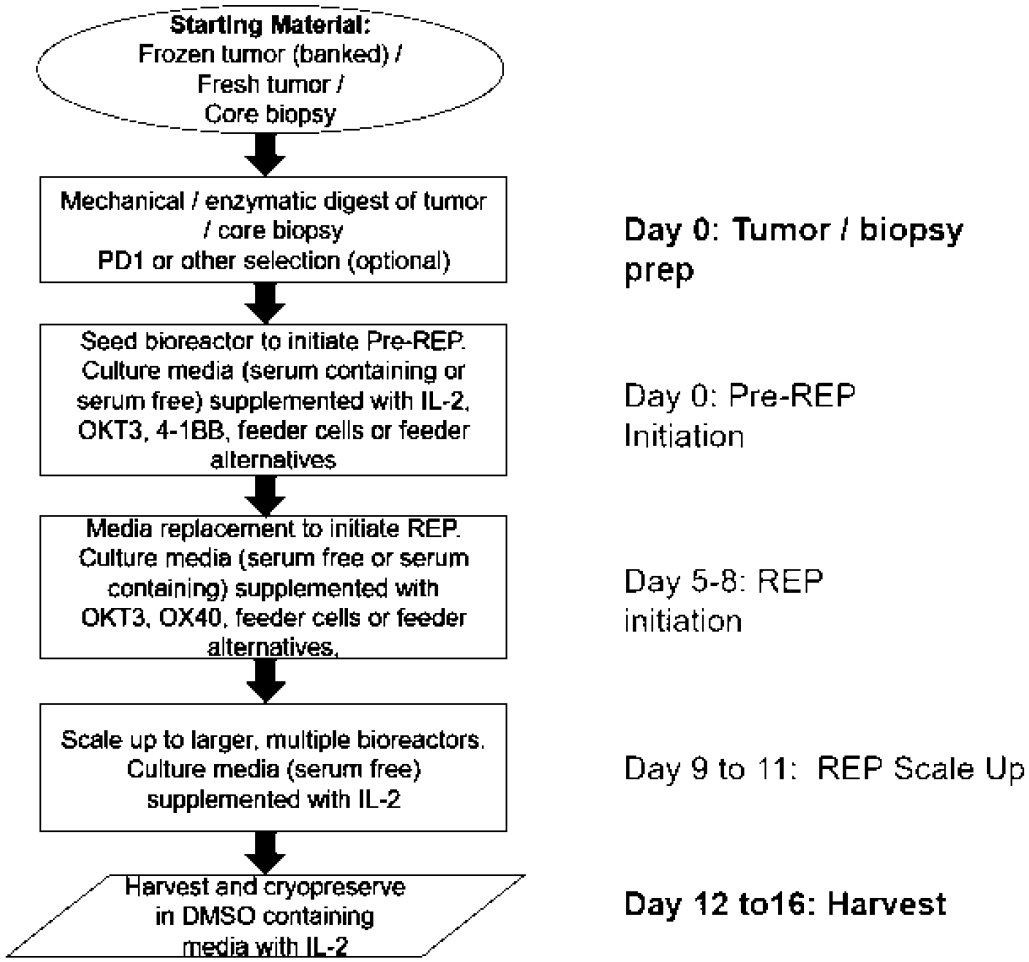

Starting Material:
Frozen tumor (banked) /
Fresh tumor /
Core biopsy
Mechanical / enzymatic digest of tumor / core biopsy
PD1 or other selection (optional)
Seed bioreactor to initiate Pre-REP. Culture media (serum containing or serum free) supplemented with IL-2, OKT3, 4-1BB, feeder cells or feeder alternatives
Media replacement to initiate REP. Culture media (serum free or serum containing) supplemented with OKT3, OX40, feeder cells or feeder alternatives,
Scale up to larger, multiple bioreactors. Culture media (serum free) supplemented with IL-2
Harvest and cryopreserve in DMSO containing media with IL-2
Day 0: Tumor / biopsy prep
Day 0: Pre-REP Initiation
Day 5-8: REP initiation
Day 9 to 11: REP Scale Up
Day 12 to16: Harvest

Figure 8A

Process 2A: about 22 days from Steps A - E	Process GEN 3: about 14-18 days from Steps A - E
STEP A Obtain Patient Tumor Sample (optionally can be frozen before Step B)	**STEP A** Obtain Patient Tumor Sample (optionally can be frozen before Step B)
STEP B First Expansion (physical fragmentation to at least 40 fragments per container grown for about 3 days to 14 days with media comprising IL-2)	**STEP B** Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7 days with media comprising IL-2, OKT-3, and antigen-presenting feeder cells)
STEP C First Expansion to Second Expansion Transition (Step B TILs directly move to Step D, optionally on Step B day 11)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7)
STEP D Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and antigen-presenting feeder cells in a closed container)	**STEP D** Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)
STEP E Harvest TILS from Step D (TILs harvested via closed system)	**STEP E** Harvest TILS from Step D
STEP F Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	**STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 8B

Process GEN 3: about 14-18 days from Steps A - E

STEP A
Obtain Patient Tumor Sample
(optionally can be frozen before Step B)

STEP B
Priming First Expansion
(physical fragmentation of up to 60 fragments per container grown for about 1 days to 7 days with media comprising IL-2, OKT-3, and antigen-presenting feeder cells)

STEP C
Priming First Expansion to Rapid Second Expansion Transition
(Step B TILs directly move to Step D on day 7)

STEP D
Rapid Second Expansion
(TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)

STEP E
Harvest TILS from Step D

STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 8C

Embodiment GEN 3.0: about 14-18 days from Steps A - E	Embodiment GEN 3.1 control: about 14-18 days from Steps A - E	Embodiment GEN 3.1 Test/F: about 14-18 days from Steps A - E
STEP A Obtain Patient Tumor Sample (optionally can be frozen before Step B)	**STEP A** Obtain Patient Tumor Sample (optionally can be frozen before Step B)	**STEP A** Obtain Patient Tumor Sample (optionally can be frozen before Step B)
STEP B Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 days with media comprising IL-2)	**STEP B** Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 days with media comprising IL-2, and OKT-3)	**STEP B** Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 days with media comprising IL-2, OKT-3, and antigen-presenting feeder cells)
STEP C Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)
STEP D Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)	**STEP D** Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)	**STEP D** Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)
STEP E Harvest TILS from Step D	**STEP E** Harvest TILS from Step D	**STEP E** Harvest TILS from Step D
STEP F Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	**STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	**STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 9

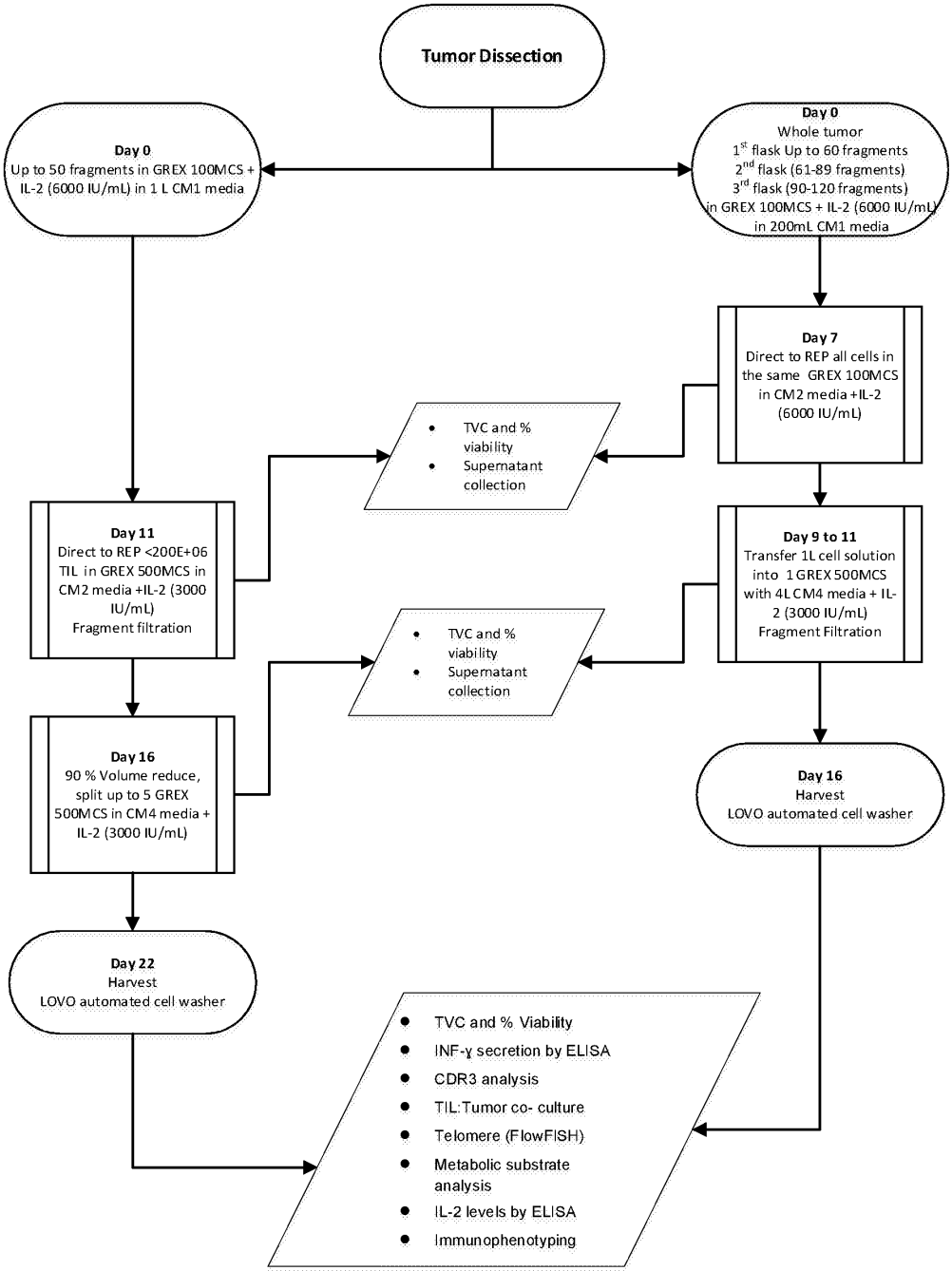
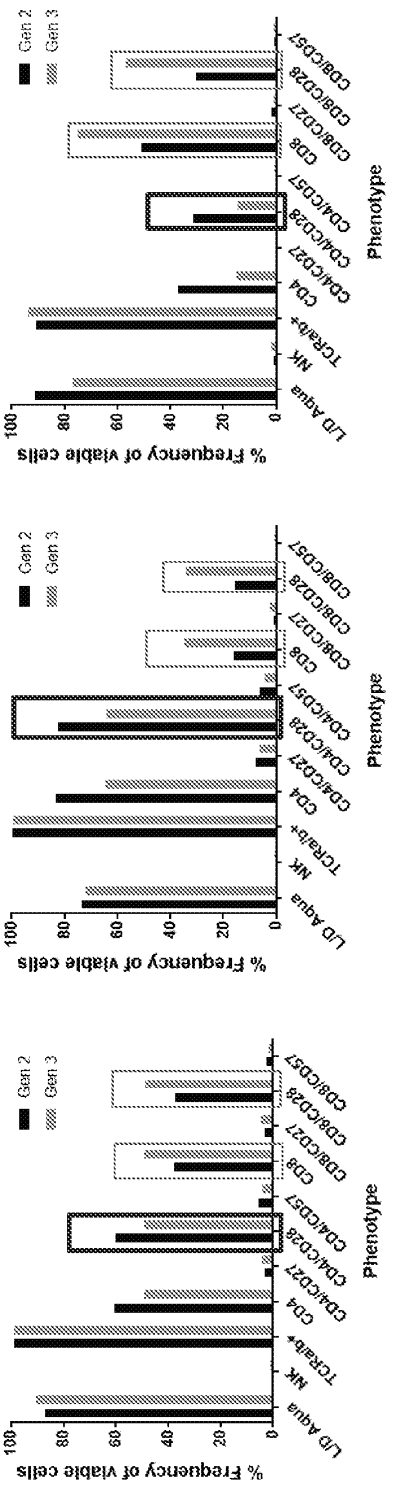

Tumor Dissection
Day 0
Up to 50 fragments in GREX 100MCS + IL-2 (6000 IU/mL) in 1 L CM1 media
Day 0
Whole tumor
1st flask Up to 60 fragments
2nd flask (61-89 fragments)
3rd flask (90-120 fragments)
in GREX 100MCS + IL-2 (6000 IU/mL) in 200mL CM1 media
Day 7
Direct to REP all cells in the same GREX 100MCS in CM2 media +IL-2 (6000 IU/mL)
TVC and % viability
Supernatant collection
Day 11
Direct to REP <200E+06 TIL in GREX 500MCS in CM2 media +IL-2 (3000 IU/mL)
Fragment filtration
Day 9 to 11
Transfer 1L cell solution into 1 GREX 500MCS with 4L CM4 media + IL-2 (3000 IU/mL)
Fragment Filtration
TVC and % viability
Supernatant collection
Day 16
90 % Volume reduce, split up to 5 GREX 500MCS in CM4 media + IL-2 (3000 IU/mL)
Day 16
Harvest
LOVO automated cell washer
Day 22
Harvest
LOVO automated cell washer
TVC and % Viability
INF-γ secretion by ELISA
CDR3 analysis
TIL:Tumor co- culture
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

Figure 10



Tumor ID	L4054		L4055		M1085T	
Process	Gen 2	Gen 3	Gen 2	Gen 3	Gen 2	Gen 3
# Fragments	21	21	24	24	16	16

Figure 11

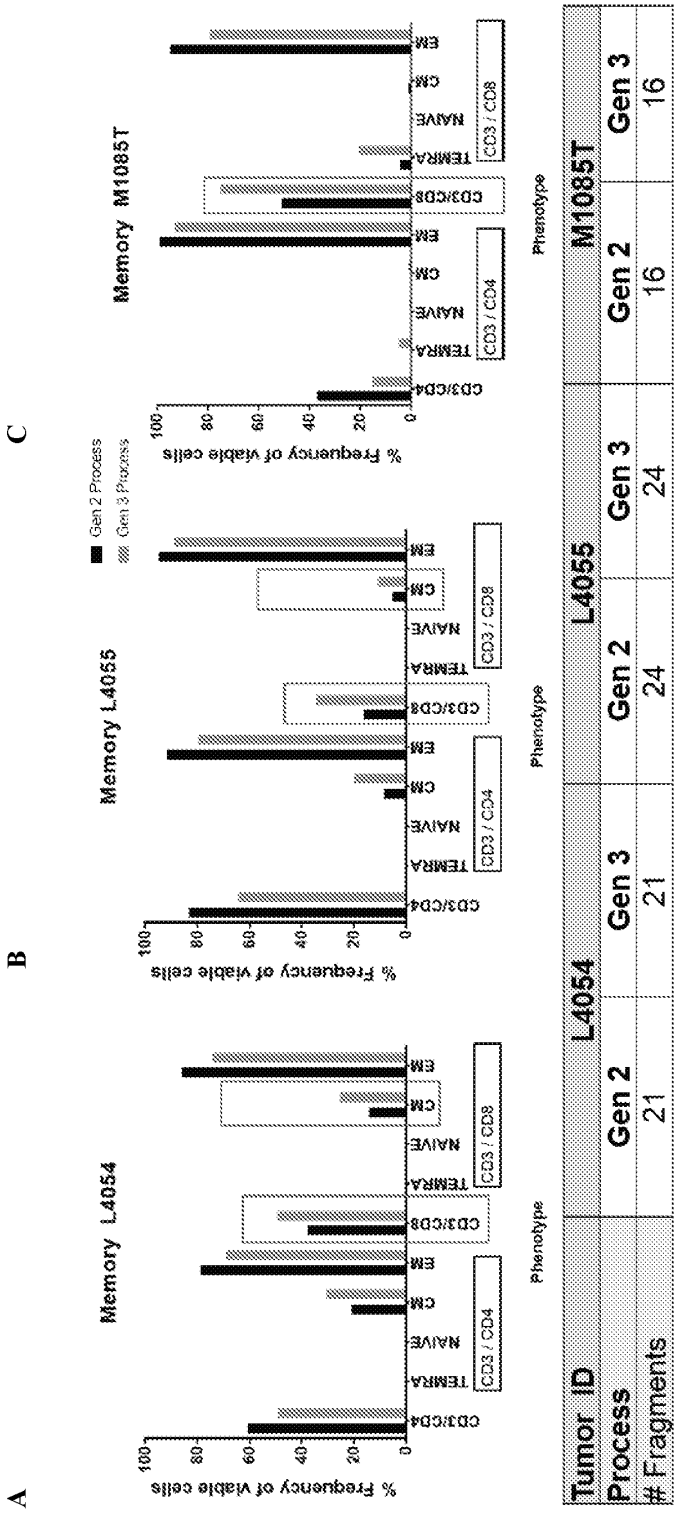

A
B
C
Memory L4054
Memory L4055
Memory M1085T
Gen 2 Process
Gen 3 Process
% Frequency of viable cells
Phenotype
CD3/CD4
TEMRA
NAIVE
CM
EM
CD3/CD8
CD3 / CD4
CD3 / CD8
Tumor ID
L4054
L4055
M1085T
Process
Gen 2
Gen 3
Fragments
21
21
24
24
16
16

Figure 12

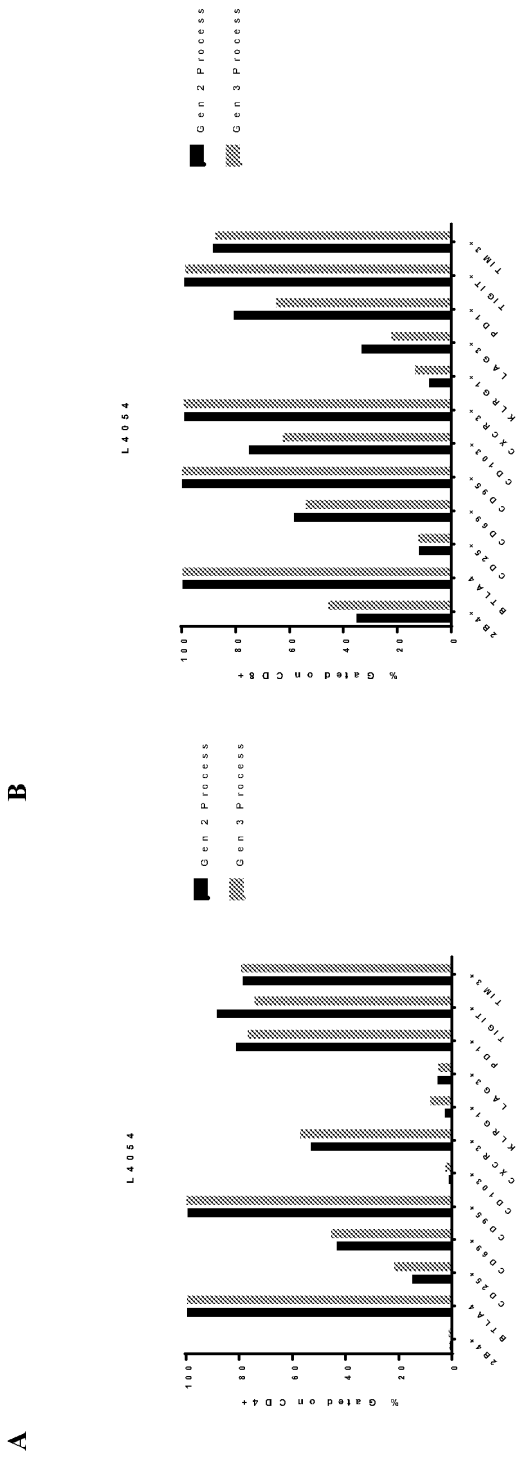

A
B
Gen 2 Process
Gen 3 Process
L40554
% Gated on CD4+
% Gated on CD8+
100
80
60
40
20
0
2B4+
BTLA4
CD25+
CD69+
CD95+
CD103+
CXCR3+
KLRG1+
LAG3+
PD1+
TIGIT+
TIM3+

Figure 13

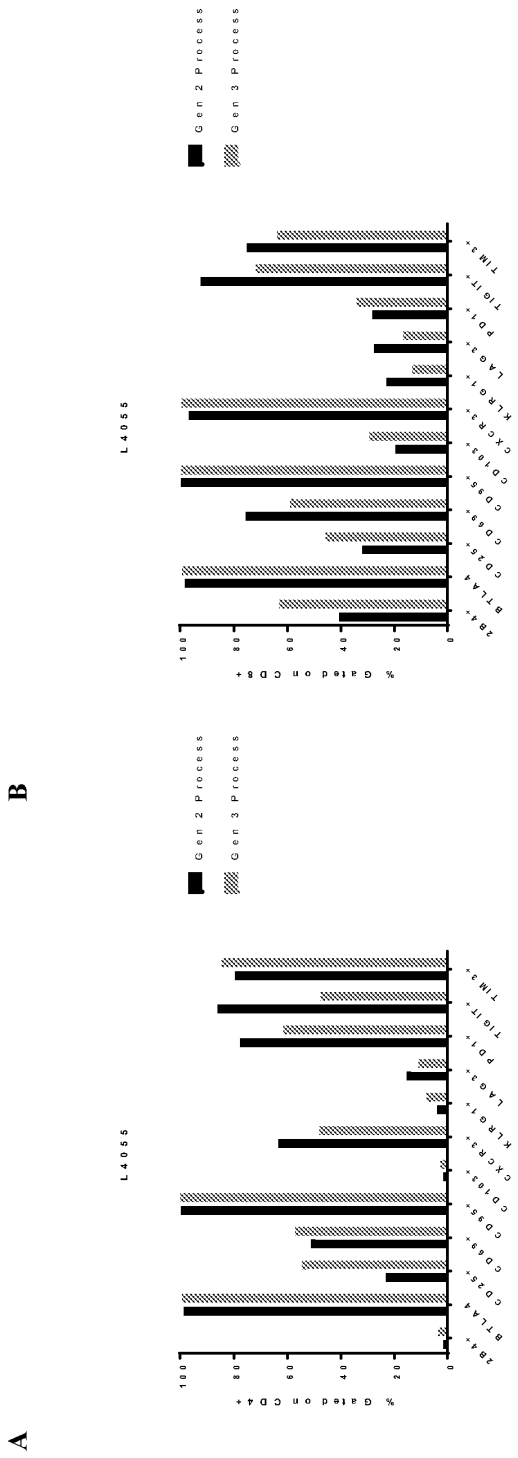

A
B
Gen 2 Process
Gen 3 Process
L4055
% Gated on CD4+
% Gated on CD8+
100
80
60
40
20
0
2B4+
BTLA4
CD25+
CD69+
CD95+
CD103+
CXCR3+
KLRG1+
LAG3+
PD1+
Tigit+
TIM3+

Figure 14

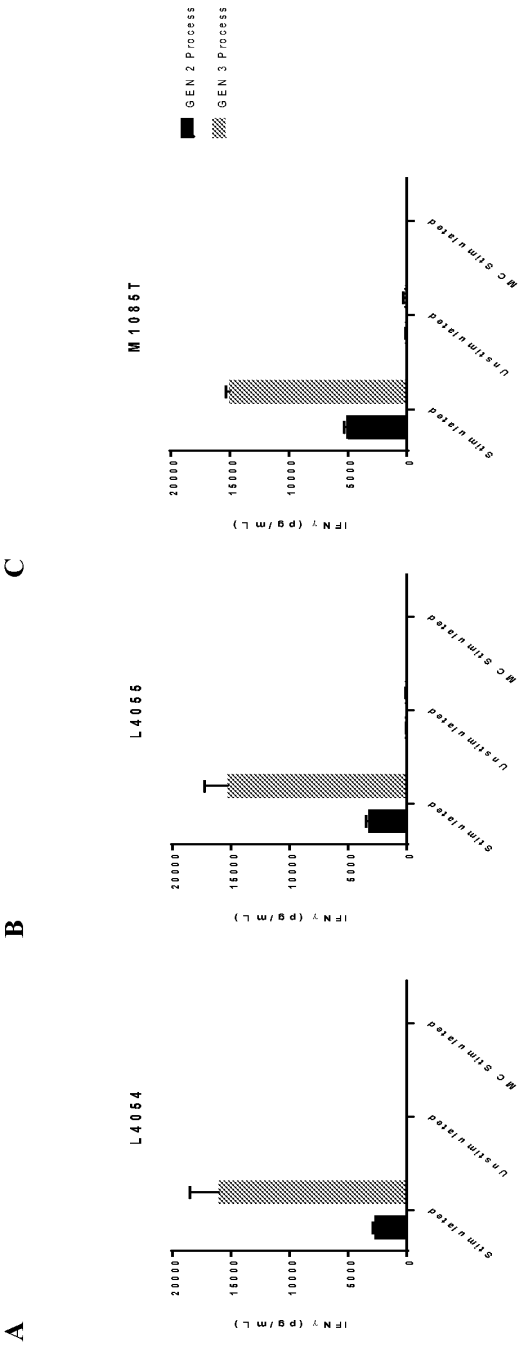

A
L4064
B
L4065
C
M1086T
GEN 2 Process
GEN 3 Process
IFNγ (pg/mL)
20000
15000
10000
5000
0
Stim u/tested
Unstim u/tested
MC Stim u/tested

Figure 15

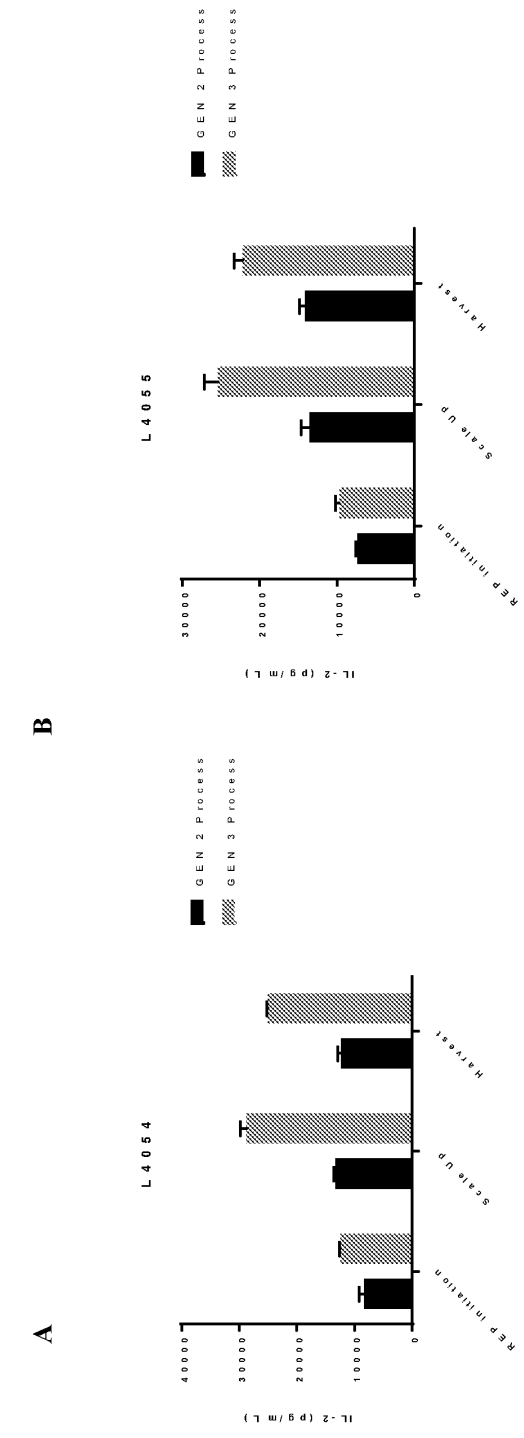

A
L4054
GEN 2 Process
GEN 3 Process
IL-2 (pg/mL)
40000
30000
20000
10000
0
Harvest
Scale Up
Rep Initiation
B
L4055
GEN 2 Process
GEN 3 Process
IL-2 (pg/mL)
30000
20000
10000
0
Harvest
Scale Up
Rep Initiation

Figure 16

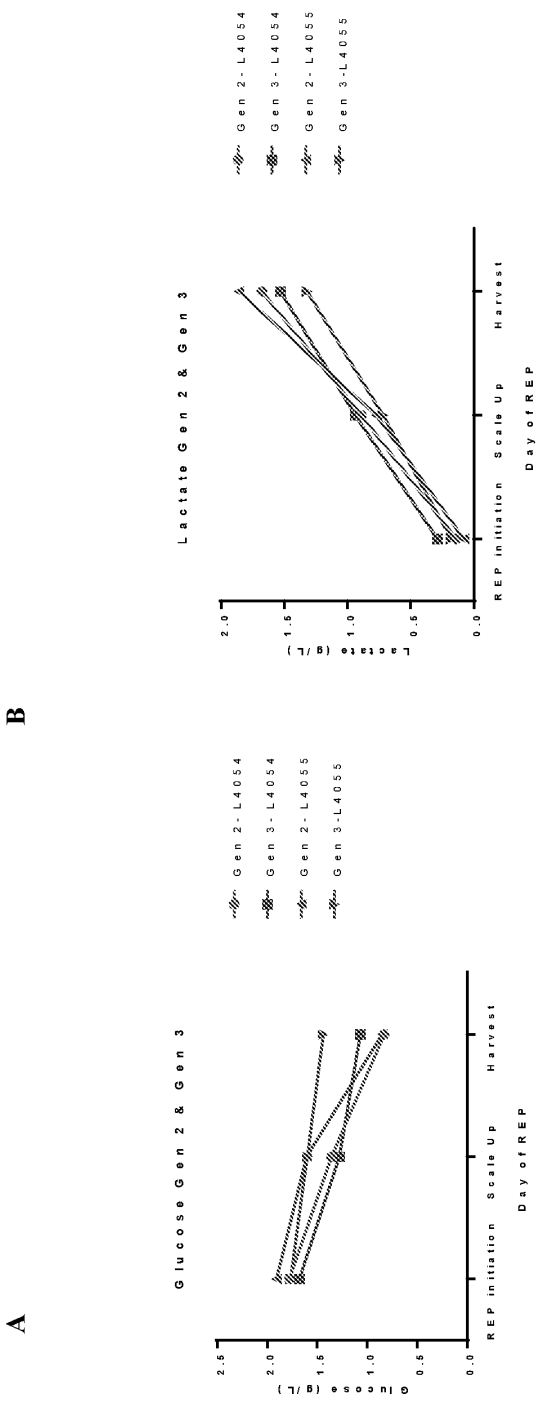
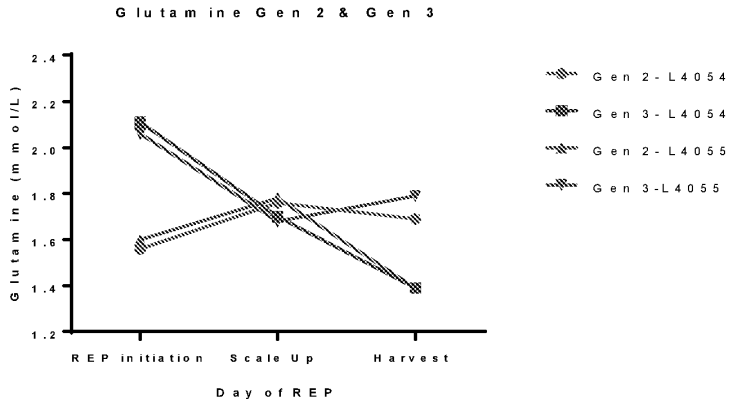

A
Glucose Gen 2 & Gen 3
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055
Glucose (g/L)
2.5
2.0
1.5
1.0
0.5
0.0
REP initiation
Scale Up
Harvest
Day of REP
B
Lactate Gen 2 & Gen 3
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055
Lactate (g/L)
2.0
1.5
1.0
0.5
0.0
REP initiation
Scale Up
Harvest
Day of REP

Figure 17

A


Glutamine Gen 2 & Gen 3
Glutamine (mmol/L)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
REP initiation
Scale Up
Harvest
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055

B

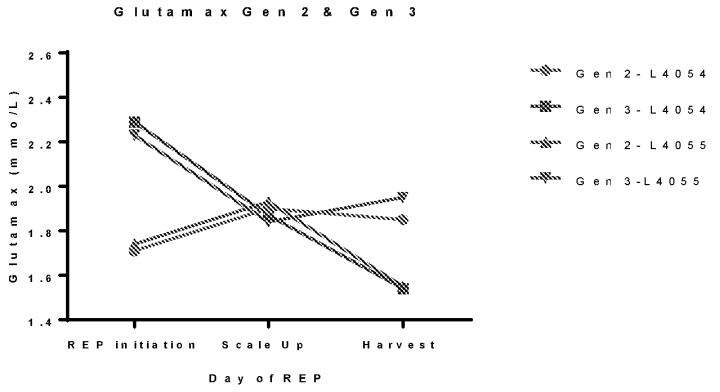

Glutamax Gen 2 & Gen 3
Glutamax (mmol/L)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
REP initiation
Scale Up
Harvest
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055

Figure 17

C

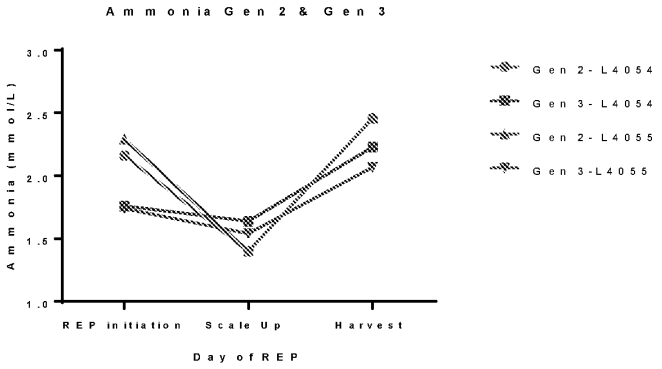

Ammonia Gen 2 & Gen 3
Ammonia (mmol/L)
3.0
2.5
2.0
1.5
1.0
REP initiation
Scale Up
Harvest
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055

Figure 18

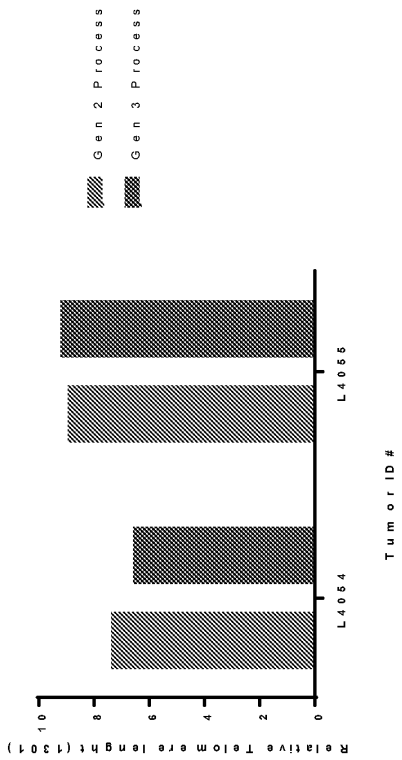

Gen 2 Process
Gen 3 Process
Relative Telomere length (1301)
0
2
4
6
8
10
L4054
L4055
Tumor ID#

Figure 19

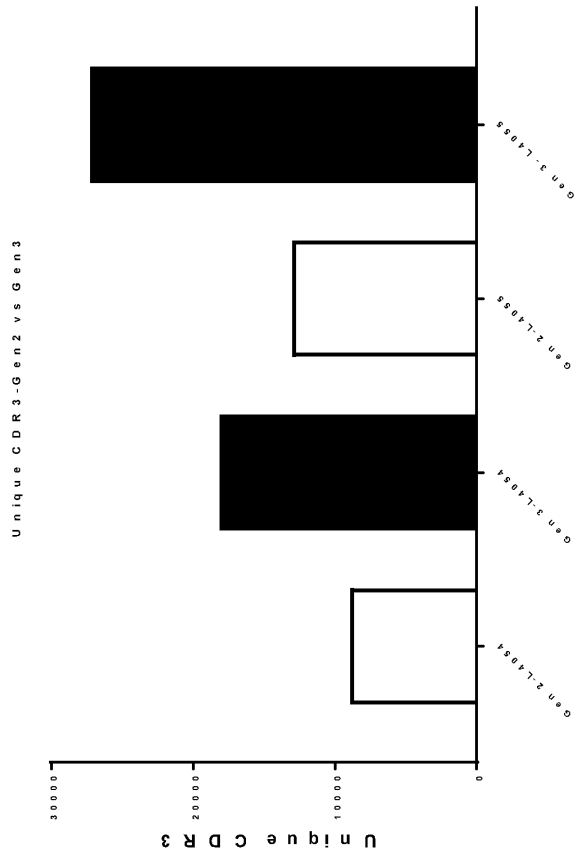

Unique CDR3-Gen2 vs Gen3
Unique CDR3
30000
20000
10000
0
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055

Figure 20

Frequency of uCDR3

# uCDR3 (% Overlap)	All uCDR3's		Top 80% uCDR3's	
	Gen 2	Gen 3	Gen 2	Gen 3
Gen 2-L4054	8915	4355 (48.85%)	205	199 (97.07%)
Gen 3-L4054	-	18130	-	223

- 199 sequences are shared between Gen 3 and Gen 2 final product, corresponding to 97.07% of top 80% of unique CDR3 sequences from Gen 2 shared with Gen 3 final product.

Figure 21

# uCDR3 (% Overlap)	All uCDR3's		Top 80% uCDR3's	
	Gen 2	Gen 3	Gen 2	Gen 3
Gen 2-14055	12996	6599 (50.77%)	1843	1833 (99.45%)
Gen 3-14055	-	27246	-	2616

- 1833 sequences are shared between Gen 3 and Gen 2 final product, corresponding to 99.45% of top 80% of unique CDR3 sequences from Gen 2 shared with Gen 3 final product.

Figure 22

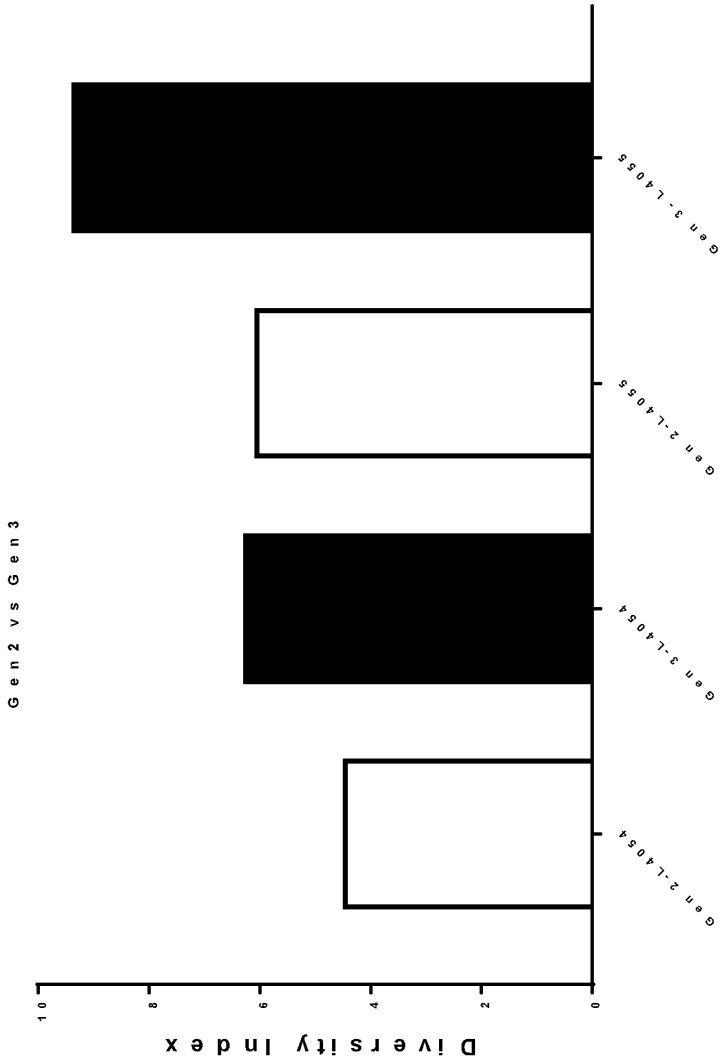

Gen2 vs Gen3
Diversity Index
0
2
4
6
8
10
Gen 2-L405a
Gen 3-L405a
Gen 2-L405s
Gen 3-L405s

Figure 23

Cell counts Day 7-Gen 3 REP initiation

Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
188	Total	2.46E+05	2.28E+05	2.31E+05	2.35E+05	4.42E+07
	Live	2.37E+05	2.27E+05	2.26E+05	2.30E+05	4.32E+07
	Dead	9.26E+03	7.03E+02	4.63E+03	4.86E+03	9.14E+05
	% Viability	96.20%	99.70%	98.00%	97.97%	
188	Total	8.26E+04	8.00E+04	7.66E+04	7.97E+04	1.50E+07
	Live	7.60E+04	7.54E+04	6.89E+04	7.34E+04	1.38E+07
	Dead	6.61E+03	4.63E+03	7.73E+03	6.32E+03	1.19E+06
	% Viability	92.00%	94.20%	89.90%	92.03%	
200	Total	2.44E+04	4.20E+04	1.05E+04	2.56E+04	5.13E+06
	Live	1.74E+04	2.80E+04	7.03E+03	1.75E+04	3.50E+06
	Dead	7.03E+03	1.40E+04	3.48E+03	8.17E+03	1.63E+06
	% Viability	71.20%	66.70%	66.90%	68.27%	

Figure 24

[0001] *Cell counts Day 11-Gen 2 REP initiation and Gen 3 Scale Up*

ID	Volume (mL)		Count 1 (cells/mL)	Count 3 (cells/mL)	Count 2 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3 #L4054 First Round	1000	Total	9.11E+05	1.01E+06	1.08E+06	1.00E+06	1.00E+09
		Live	8.60E+05	9.34E+05	1.01E+06	9.35E+05	9.35E+08
		Dead	5.09E+04	7.49E+04	6.74E+04	6.44E+04	6.44E+07
		% Viability	94.40%	92.60%	93.70%	93.57%	
Gen 3 #L4055 Second Round	1000	Total	8.56E+05	9.04E+05	8.81E+05	8.80E+05	8.80E+08
		Live	8.24E+05	8.67E+05	8.42E+05	8.44E+05	8.44E+08
		Dead	3.17E+04	3.70E+04	3.87E+04	3.58E+04	3.58E+07
		% Viability	96.30%	95.90%	95.60%	95.93%	
Gen 3 #M1085T	200	Total	2.32E+06	2.25E+06		2.29E+06	4.57E+08
		Live	1.68E+06	1.57E+06		1.63E+06	3.25E+08
		Dead	6.41E+05	6.79E+05		6.60E+05	1.32E+08
		% Viability	72.40%	69.90%		71.15%	
Gen 2 #L4054 First Round	142	Total	1.08E+06	9.84E+05	1.00E+06	1.02E+06	1.45E+08
		Live	1.06E+06	9.71E+05	9.79E+05	1.00E+06	1.42E+08
		Dead	2.05E+04	1.26E+04	2.12E+04	1.81E+04	2.57E+06
		% Viability	98.10%	98.70%	97.90%	98.23%	
Gen 2 #L4055 Second Round	96	Total	2.93E+05	3.05E+05	2.64E+05	2.87E+05	2.76E+07
		Live	2.88E+05	2.96E+05	2.55E+05	2.80E+05	2.68E+07
		Dead	4.72E+03	9.14E+03	8.26E+03	7.37E+03	7.08E+05
		% Viability	98.40%	97.00%	96.90%	97.43%	
Gen 2 #M1085T	200	Total	9.10E+04	5.60E+04		7.35E+04	1.47E+07
		Live	8.05E+04	4.21E+04		6.13E+04	1.23E+07
		Dead	1.04E+04	1.39E+04		1.22E+04	2.43E+06
		% Viability	88.50%	75.20%		81.85%	

Figure 25

[0002] *Cell counts Day 16-Gen 2 Scale Up and Gen 3 Harvest*

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3 #L4054 First Round -pre LOVO	1243.1	Total	1.40E+07	1.46E+07		1.43E+07	1.78E+10
		Live	1.32E+07	1.35E+07		1.34E+07	1.66E+10
		Dead	8.47E+05	1.12E+06		9.84E+05	1.22E+09
		% Viability	94.00%	92.40%		93.20%	
Gen 3 #L4054 First Round -post LOVO	330	Total	6.35E+07	6.07E+07		6.21E+07	2.05E+10
		Live	5.72E+07	5.44E+07		5.58E+07	1.84E+10
		Dead	6.35E+06	6.27E+06		6.31E+06	2.08E+09
		% Viability	90.00%	89.70%		89.85%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3 #L4055 Second Round -pre LOVO	1032.6	Total	9.49E+06	1.05E+07		1.00E+07	1.03E+10
		Live	8.90E+06	9.79E+06		9.35E+06	9.65E+09
		Dead	5.85E+05	6.75E+05		6.30E+05	6.51E+08
		% Viability	93.80%	93.60%		93.70%	
Gen 3 #L4055 Second Round -post LOVO	330	Total	3.31E+07	2.82E+07	3.18E+07	3.07E+07	1.01E+10
		Live	2.85E+07	2.46E+07	2.77E+07	2.66E+07	8.76E+09
		Dead	4.56E+06	3.61E+06	4.12E+06	4.09E+06	1.35E+09
		% Viability	86.20%	87.20%	87.10%	86.70%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3 #M1085T pre-LOVO	5000	Total	2.26E+06	2.64E+06		2.45E+06	1.23E+10
		Live	1.97E+06	2.31E+06		2.14E+06	1.07E+10
		Dead	2.85E+05	3.30E+05		3.08E+05	1.54E+09
		% Viability	87.40%	87.50%		87.45%	
Gen 3 #M1085T - post LOVO pre CS10 addition	150	Total	6.27E+07	5.44E+07		5.86E+07	8.78E+09
		Live	5.50E+07	4.74E+07		5.12E+07	7.68E+09
		Dead	7.70E+06	6.96E+06		7.33E+06	1.10E+09
		% Viability	87.70%	87.20%		87.45%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 2 #L4054 First Round	913	Total	3.53E+06	4.30E+06		3.92E+06	3.57E+09
		Live	3.35E+06	4.00E+06		3.68E+06	3.36E+09
		Dead	1.75E+05	3.03E+05		2.39E+05	2.18E+08
		% Viability	95.00%	93.00%		94.00%	
Gen 2 #L4055 Second Round	292	Total	1.29E+07	1.36E+07		1.33E+07	3.87E+09
		Live	1.16E+07	1.23E+07		1.20E+07	3.49E+09
		Dead	1.27E+06	1.23E+06		1.25E+06	3.65E+08
		% Viability	90.10%	90.90%		90.50%	
Gen 2 #M1085T	369	Total	6.75E+06	6.98E+06		6.87E+06	2.53E+09
		Live	5.22E+06	5.58E+06		5.40E+06	1.99E+09
		Dead	1.54E+06	1.40E+06		1.47E+06	5.42E+08
		% Viability	77.20%	79.90%		78.55%	

Figure 26

[0003] *Cell counts Day 22-Gen 2 Harvest*

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 2 #L4054 First Round -pre LOVO	1385.7	Total	3.55E+07	4.12E+07	4.03E+07	3.90E+07	5.40E+10
		Live	3.26E+07	3.73E+07	3.67E+07	3.55E+07	4.92E+10
		Dead	2.93E+06	3.87E+06	3.59E+06	3.46E+06	4.80E+09
		% Viability	91.70%	90.60%	91.10%	91.13%	
Gen 2 #L4054 First Round -post LOVO	330	Total	1.70E+08	1.79E+08	1.68E+08	1.72E+08	5.69E+10
		Live	1.49E+08	1.58E+08	1.48E+08	1.52E+08	5.01E+10
		Dead	2.16E+07	2.04E+07	2.00E+07	2.07E+07	6.82E+09
		% Viability	87.30%	88.60%	88.10%	87.95%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 2 #L4055 First Round -pre LOVO	1968.2	Total	3.15E+07	2.51E+07	2.97E+07	2.88E+07	5.66E+10
		Live	2.89E+07	2.25E+07	2.72E+07	2.62E+07	5.16E+10
		Dead	2.57E+06	2.61E+06	2.52E+06	2.57E+06	5.05E+09
		% Viability	91.80%	89.60%	91.50%	90.97%	
Gen 2 #L4055 First Round -post LOVO	330	Total	2.33E+08	1.89E+08	1.53E+08	1.92E+08	6.33E+10
		Live	2.03E+08	1.66E+08	1.33E+08	1.67E+08	5.52E+10
		Dead	3.00E+07	2.24E+07	1.94E+07	2.39E+07	7.90E+09
		% Viability	87.10%	88.10%	87.30%	87.50%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 2 #M1085T-pre LOVO	N/A	Total				#DIV/0!	#DIV/0!
		Live				#DIV/0!	#DIV/0!
		Dead				#DIV/0!	#DIV/0!
		% Viability				#DIV/0!	
Gen 2 #M1085T - post LOVO pre CS10 addition	150	Total	8.51E+07	9.05E+07		8.78E+07	1.32E+10
		Live	7.33E+07	7.79E+07		7.56E+07	1.13E+10
		Dead	1.18E+07	1.26E+07		1.22E+07	1.83E+09
		% Viability	86.10%	86.10%		86.10%	

For L4054 Gen 2, post LOVO count was extrapolated to 4 flasks, because was the total number of the study. 1 flask was contaminated, and the extrapolation was done for total = 6.67E+10

Figure 27

	S1/S2/All/Live | Freq. of Parent (%)	S1/S2/All/Live/CD14+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/CD3-/NK- | Freq. of null (%)	S1/S2/All/Live/CD14-/CD19- | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab+ | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD27+ | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD28+ | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD56- | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD57- | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q1: CCR7-PE-A-, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q2: CCR7-PE-A+, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q3: CCR7-PE-A+, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q4: CCR7-PE-A-, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q5: CD62L-BV421-A-, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q6: CD62L-BV421-A+, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q7: CD62L-BV421-A+, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q8: CD62L-BV421-A-, CD45RA-AF700-A- | Freq. of Parent (%)
											TEMRA	NAÏVE	CM	EM	TEMRA	NAÏVE	CM	EM
A1 gen2 1st.fcs	87.2	1.77E-03	0.66	0	98.9	60.6	3.35	60.1	0.06	5.34	0.25	0.073	3.37	96.3	0.056	0.032	21.1	78.8
A2 gen2 2nd.fcs	73.5	0	0.12	0	99.7	83.1	7.94	82.6	0.14	6.18	0.16	0.047	2.39	97.4	0.036	0.014	8.3	91.6
A3 gen3 1st.fcs	90.4	0	0.97	1.72E-03	98.8	49.1	4.5	48.9	0.18	4.02	0.2	0.059	2.29	97.5	0.059	0.049	30.7	69.2
A4 gen3 2nd.fcs	72.1	2.56E-03	0.44	0.018	99.3	64.4	6.27	64	0.26	4.3	0.25	0.18	7.12	92.5	0.12	0.072	20.1	79.7
A5 PBMC.fcs	93.7	6.07	11.6	5.12	70.9	41.1	35.6	40.4	0.041	0.31	0.91	36.1	16.2	46.9	11.4	23.1	16.4	49.1
area was include to make the graph																		
TCRab+ are = CD3+																		

S1/S2/All/Live/CD14-/TCRab/CD8 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/CD27- | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/CD28+ | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/CD56- | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/CD57- | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q1: CCR7-PE-A-, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q2: CCR7-PE-A+, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q3: CCR7-PE-A+, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q4: CCR7-PE-A-, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q5: CD62L-BV421-A-, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q6: CD62L-BV421-A+, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q7: CD62L-BV421-A+, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q8: CD62L-BV421-A-, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRgd | Freq. of null (%)
					TEMRA	NAÏVE	CM	EM	TEMRA	NAÏVE	CM	EM	
37.9	5.25	37.3	0.41	2.31	0.028	0	4.24	95.7	0.033	9.37E-03	14.1	85.9	0
16.1	1.08	15.8	0.67	0.17	0.042	0.014	4.76	95.2	0.071	0.014	5.24	94.7	0
49.2	4.83	48.5	0.71	1.53	0.056	6.97E-03	3.11	96.8	0.077	0.017	25.5	74.4	0
34.6	2.46	34	1.57	0.5	0.18	0.13	10.2	89.5	0.29	0.044	10.6	89.1	0
29.6	25.8	27.4	0.75	2.61	8.05	40.9	3.02	48	22.3	27.1	6.23	44.3	0

Figure 28

CD3/CD4								
	CD38	HLA-DR	CCR7- CD45RA+	CCR7+CD45RA+	CCR7+ CD45RA-	CCR7-CD45RA-		
GEN 3-Day 16-Harvest	42.4	95.2	4.96	0.7	1.22	93.1		
GEN 2-D22-Harvest	50.4	98	0.61	0.13	0.3	99		

CD3/CD8								
	CD38	HLA-DR	CCR7-CD45RA+	CCR7+CD45RA+	CCR7+CD45RA-	CCR7-CD45RA-		
CD3	77.6	83.2	90.3	20.5	0.013	0.06	79.5	
CD8	54.3	88	83.2	4.24	0.026	1.05	94.7	

Figure 29

			CD4												
	CD3+	CD4+	2B4+	BTLA+	CD103+	CD25+	CD69+	CD95+	CXCR3+	KLRG1+	LAG3+	PD1+	TIGIT+	TIM3+	
Gen 2-L4054			0.61	99.50	1.05	14.80	43.20	99.40	52.90	2.65	5.36	81.20	88.40	78.60	
Gen 3-L4054			1.11	99.50	2.28	21.70	45.40	99.70	56.90	8.06	5.07	76.70	74.10	79.30	
Gen 2-L4055			1.61	98.60	1.56	23.10	51.10	99.80	63.30	3.86	15.30	77.60	86.00	79.50	
Gen 3- L4055			3.57	99.30	2.61	54.40	57.00	99.90	48.00	8.02	10.80	61.40	47.50	84.60	

			CD8											
Gen 2-L4054		CD8+	2B4+	BTLA4+	CD103+	CD25+	CD95+	CD69+	CXCR3+	KLRG1+	LAG3+	PD1+	TIGIT+	TIM3+
Gen 3-L4054			35.20	99.50	74.90	11.80	99.80	58.30	99.10	8.03	33.10	80.60	99.10	88.30
Gen 2-L4055			45.50	99.60	62.40	12.10	99.90	54.00	99.30	13.40	22.10	64.90	98.50	87.50
Gen 3- L4055			40.60	98.20	19.50	32.00	99.70	75.50	96.80	22.80	27.40	28.10	92.30	75.10
			62.80	99.20	29.20	45.50	99.70	58.80	99.60	13.10	16.60	34.10	71.70	63.60

Figure 30

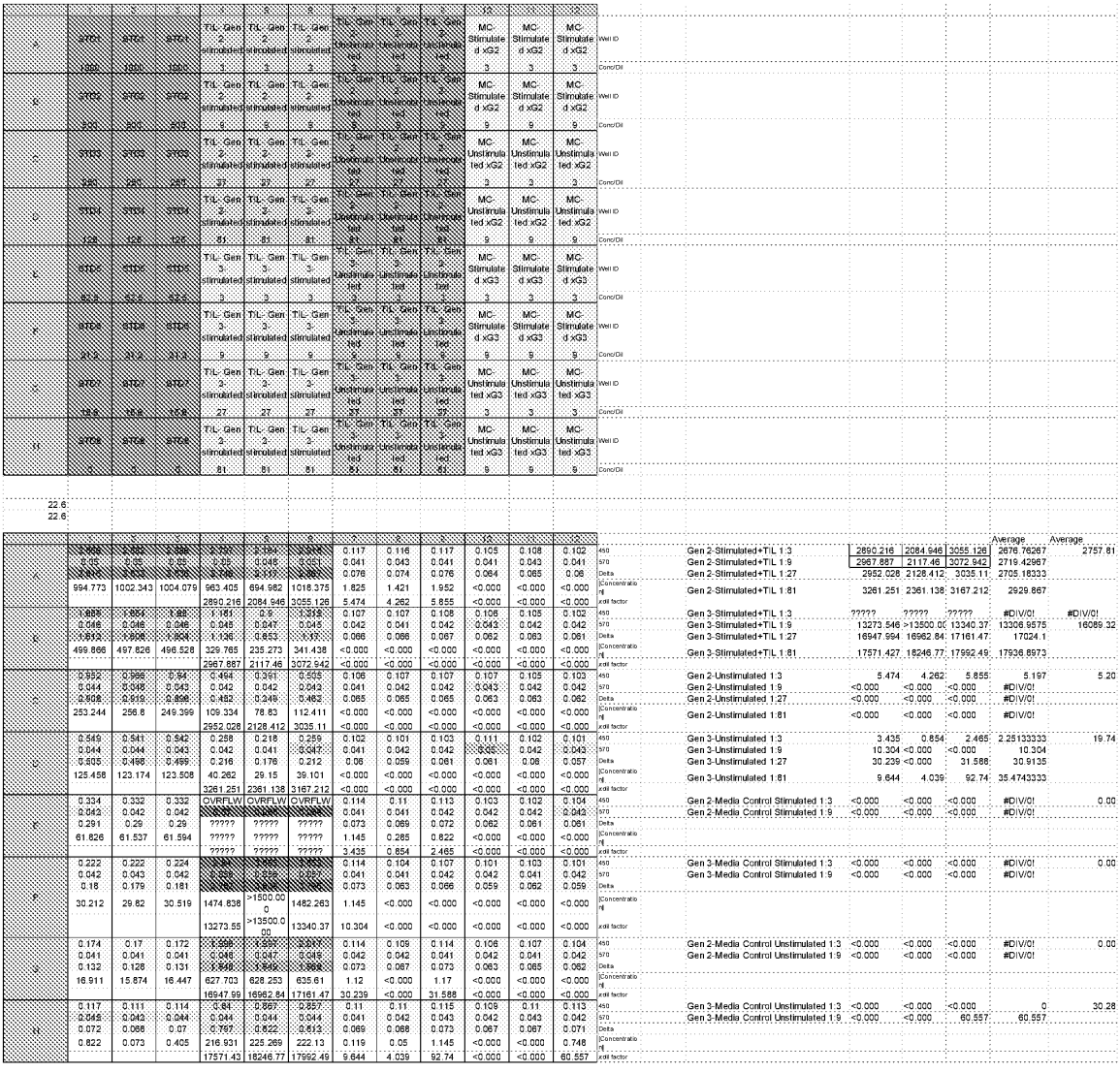


Figure 31

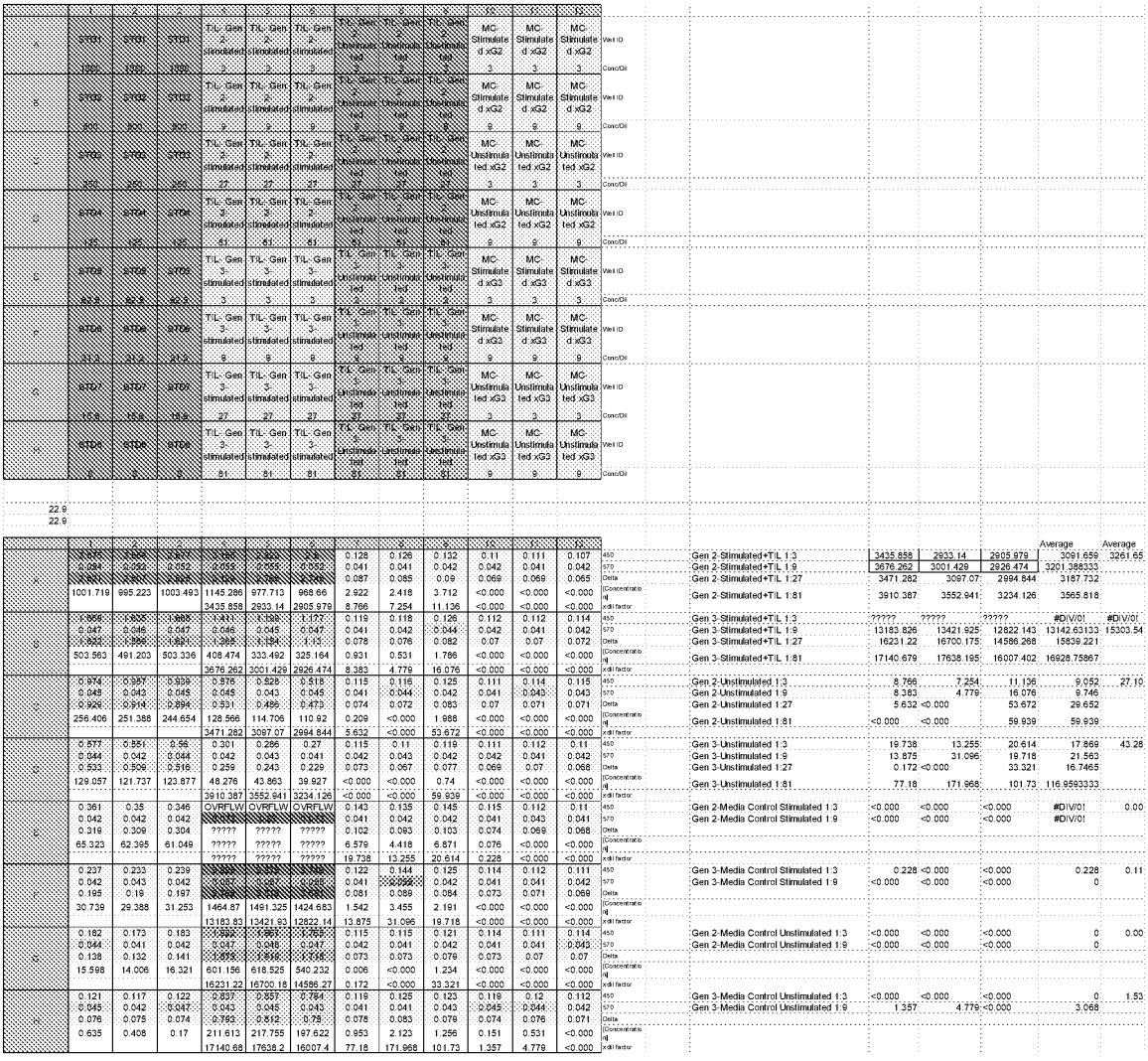


Figure 32

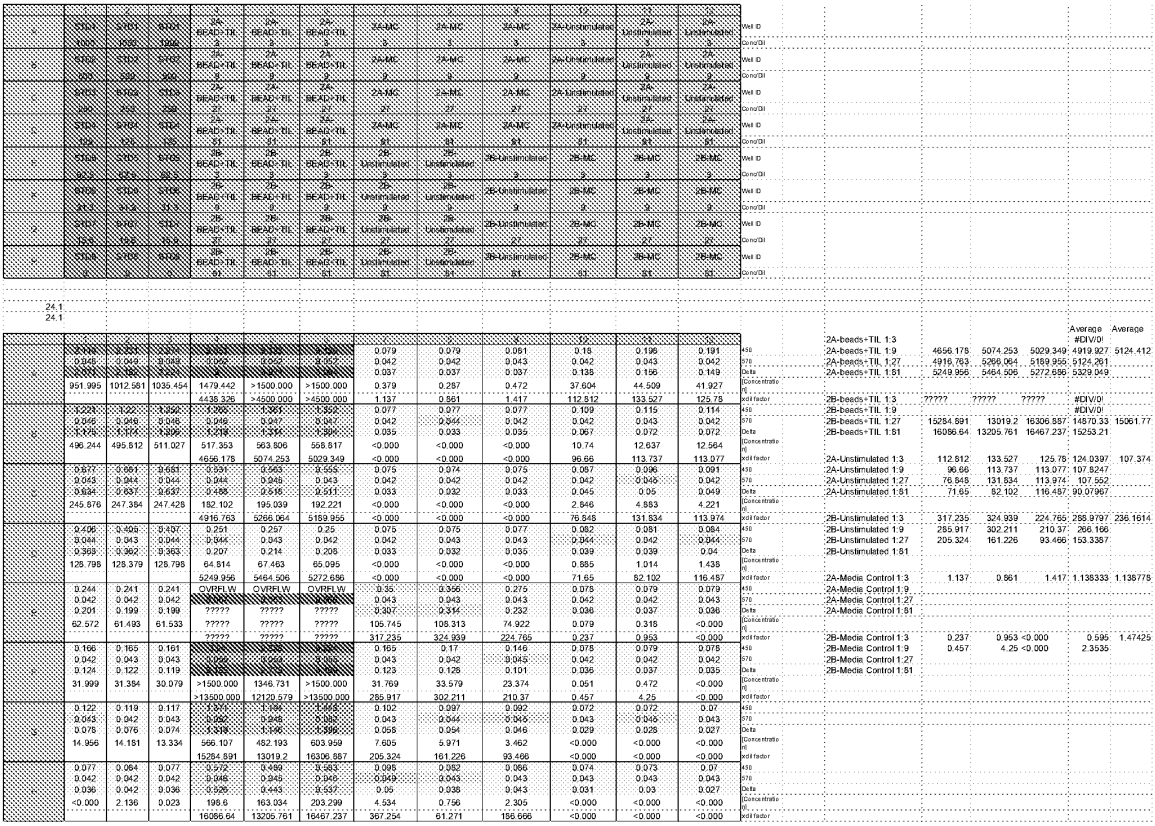
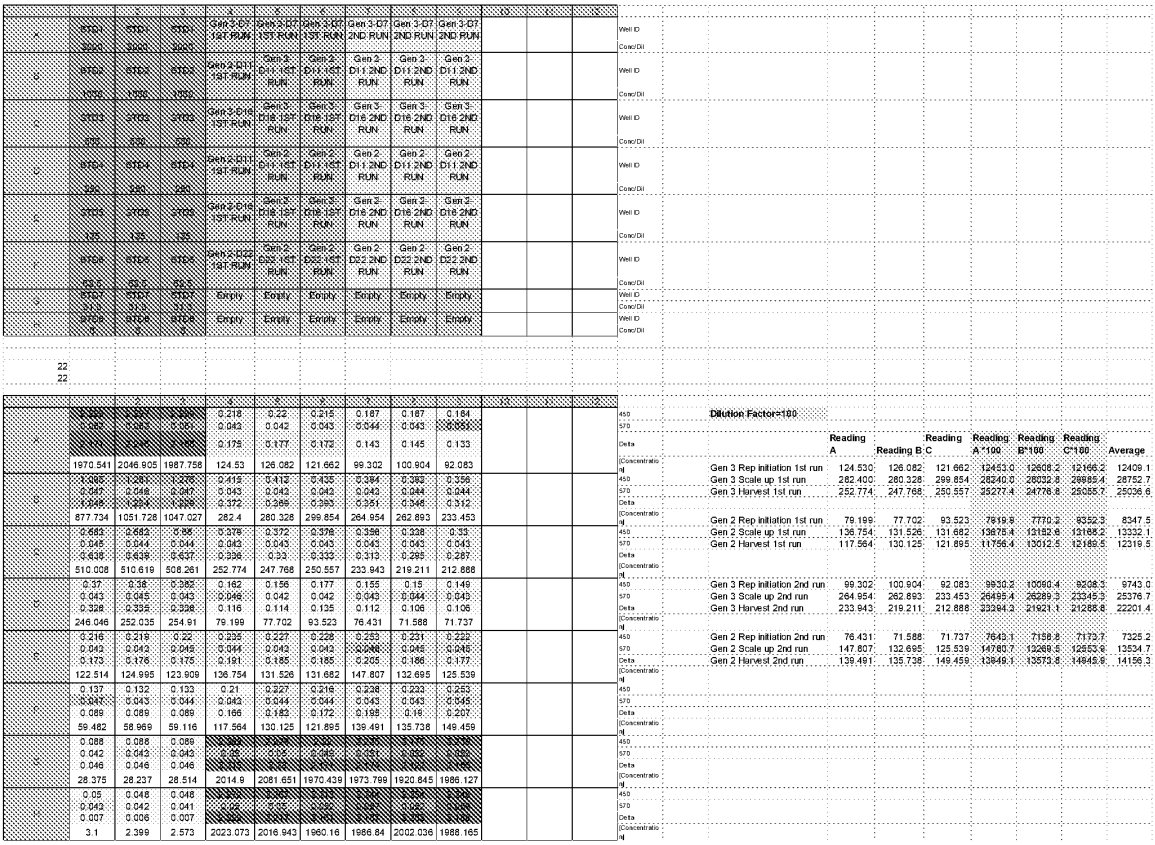


Figure 33



A	STD1	STD1	STD1	Gen 3-D7 1ST RUN	Gen 3-D7 1ST RUN	Gen 3-D7 1ST RUN	Gen 3-D7 2ND RUN	Gen 3-D7 2ND RUN	Gen 3-D7 2ND RUN			
B	STD2	STD2	STD2	Gen 2-D11 1ST RUN	Gen 3-D11 1ST RUN	Gen 2-D11 1ST RUN	Gen 3-D11 2ND RUN	Gen 3-D11 2ND RUN	Gen 3-D11 2ND RUN			
C	STD3	STD3	STD3	Gen 3-D16 1ST RUN	Gen 3-D16 1ST RUN	Gen 3-D16 1ST RUN	Gen 3-D16 2ND RUN	Gen 3-D16 2ND RUN	Gen 3-D16 2ND RUN			
D	STD4	STD4	STD4	Gen 2-D11 1ST RUN	Gen 2-D11 1ST RUN	Gen 2-D11 1ST RUN	Gen 2-D11 2ND RUN	Gen 2-D11 2ND RUN	Gen 2-D11 2ND RUN			
E	STD5	STD5	STD5	Gen 2-D16 1ST RUN	Gen 2-D16 1ST RUN	Gen 2-D16 1ST RUN	Gen 2-D16 2ND RUN	Gen 2-D16 2ND RUN	Gen 2-D16 2ND RUN			
F	STD6	STD6	STD6	Gen 2-D22 1ST RUN	Gen 2-D22 1ST RUN	Gen 2-D22 1ST RUN	Gen 2-D22 2ND RUN	Gen 2-D22 2ND RUN	Gen 2-D22 2ND RUN			
G	STD7	STD7	STD7	Empty	Empty	Empty	Empty	Empty	Empty			
H	STD8	STD8	STD8	Empty	Empty	Empty	Empty	Empty	Empty			

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A	0.365	0.361	0.359	0.218	0.22	0.215	0.187	0.187	0.184			
	0.043	0.043	0.043	0.043	0.042	0.043	0.044	0.043	0.043			
	0.322	0.318	0.316	0.175	0.177	0.172	0.143	0.145	0.133			
	1970.541	2046.905	1987.758	124.53	126.082	121.662	99.302	100.904	92.083			
B	0.395	0.391	0.388	0.415	0.412	0.435	0.384	0.393	0.356			
	0.047	0.048	0.047	0.043	0.043	0.043	0.043	0.044	0.044			
	0.348	0.343	0.341	0.372	0.369	0.393	0.341	0.349	0.312			
	877.734	1051.728	1047.027	282.4	280.328	299.854	264.954	262.893	233.453			
C	0.683	0.683	0.68	0.378	0.372	0.376	0.356	0.338	0.33			
	0.045	0.044	0.044	0.043	0.043	0.043	0.043	0.043	0.043			
	0.638	0.639	0.637	0.335	0.33	0.333	0.313	0.295	0.287			
	510.008	510.619	508.261	252.774	247.768	250.557	233.943	219.211	212.888			
D	0.37	0.38	0.382	0.162	0.156	0.177	0.155	0.15	0.149			
	0.043	0.045	0.043	0.046	0.042	0.042	0.043	0.044	0.043			
	0.328	0.335	0.338	0.116	0.114	0.135	0.112	0.106	0.106			
	246.046	252.035	254.91	79.199	77.702	93.523	76.431	71.588	71.737			
E	0.216	0.219	0.22	0.235	0.227	0.228	0.253	0.231	0.222			
	0.043	0.043	0.045	0.044	0.043	0.043	0.046	0.045	0.045			
	0.173	0.176	0.175	0.191	0.185	0.185	0.205	0.186	0.177			
	122.514	124.995	123.909	136.754	131.526	131.682	147.807	132.695	125.539			
F	0.137	0.132	0.133	0.21	0.227	0.216	0.228	0.233	0.253			
	0.042	0.043	0.044	0.043	0.044	0.044	0.043	0.043	0.045			
	0.089	0.089	0.089	0.166	0.182	0.172	0.198	0.18	0.207			
	59.462	58.969	59.116	117.564	130.125	121.895	139.491	135.738	149.459			
G	0.088	0.088	0.089	0.388	0.378	0.387	0.353	0.352	0.339			
	0.042	0.043	0.043	0.05	0.05	0.049	0.051	0.052	0.052			
	0.046	0.046	0.046	0.338	0.328	0.338	0.302	0.3	0.287			
	28.375	28.237	28.514	2014.9	2081.651	1970.439	1973.799	1920.845	1986.127			
H	0.05	0.048	0.048	0.385	0.385	0.391	0.349	0.339	0.343			
	0.042	0.042	0.041	0.05	0.05	0.051	0.051	0.052	0.052			
	0.007	0.006	0.007	0.335	0.335	0.34	0.298	0.287	0.291			
	3.1	2.399	2.573	2023.073	2016.943	1960.16	1986.84	2002.036	1988.165			

Dilution Factor=100

	Reading A	Reading B	Reading C	Reading A*100	Reading B*100	Reading C*100	Average
Gen 3 Rep initiation 1st run	124.530	126.082	121.662	12453.0	12608.2	12166.2	12409.1
Gen 3 Scale up 1st run	282.400	280.328	299.854	28240.0	28032.8	29985.4	28752.7
Gen 3 Harvest 1st run	252.774	247.768	250.557	25277.4	24776.8	25055.7	25036.6
Gen 2 Rep initiation 1st run	79.199	77.702	93.523	7919.9	7770.2	9352.3	8347.5
Gen 2 Scale up 1st run	136.754	131.526	131.682	13675.4	13152.6	13168.2	13332.1
Gen 2 Harvest 1st run	117.564	130.125	121.895	11756.4	13012.5	12189.5	12319.5
Gen 3 Rep initiation 2nd run	99.302	100.904	92.083	9930.2	10090.4	9208.3	9743.0
Gen 3 Scale up 2nd run	264.954	262.893	233.453	26495.4	26289.3	23345.3	25376.7
Gen 3 Harvest 2nd run	233.943	219.211	212.888	23394.3	21921.1	21288.8	22201.4
Gen 2 Rep initiation 2nd run	76.431	71.588	71.737	7643.1	7158.8	7173.7	7325.2
Gen 2 Scale up 2nd run	147.807	132.695	125.539	14780.7	13269.5	12553.9	13534.7
Gen 2 Harvest 2nd run	139.491	135.738	149.459	13949.1	13573.8	14945.9	14156.3

Figure 34

Sample number #	Description	Glucose (g/L)	Lactate (g/L)	Ammonia (mmol/L)	Glutamine (mmol/L)	Glutamax (mmol/L)	Glutamax-Glutamine (mmol/L)
1	Gen 2-Rep initiation First Round #L4054	1.78	0.14	2.16	1.56	1.71	0.16
2	Gen 2-Scale Up First Round #L4054	1.36	0.89	1.40	1.76	1.90	0.15
3	Gen 2-Harvest First Round #L4054	0.83	1.68	2.46	1.69	1.85	0.17
4	Gen 3-REP initiation First Round #L4054	1.68	0.29	1.76	2.11	2.29	0.18
5	Gen 3-Scale Up First Round #L4054	1.28	0.94	1.64	1.70	1.87	0.16
6	Gen 3-Harvest First Round #L4054	1.07	1.53	2.23	1.39	1.54	0.14
7	Gen 2-Rep initiation Second Round #L4055	1.91	0.08	2.29	1.60	1.74	0.14
8	Gen 2-Scale up Second Round #L4055	1.61	0.77	1.40	1.78	1.93	0.15
9	Gen 2-Harvest Second Round #L4055	0.85	1.86	2.46	1.39	1.55	0.16
10	Gen 3-REP initiation Second Round #L4055	1.77	0.18	1.74	2.06	2.23	0.17
11	Gen 3-Scale up Second Round #L4055	1.60	0.72	1.54	1.68	1.84	0.15
12	Gen 3-Harvest Second Round #L4055	1.44	1.32	2.07	1.79	1.95	0.16

Figure 35

	$FIL	S/L/1301 | Geometr	S/L/Pt | Geometric Mean (FL03-A)
A1 gen2L4054 ne	A1 gen2L4054 neg.fcs	34.5	3.24
A2 gen2L4054 ne	A2 gen2L4054 neg.fcs	34	3.19
A3 gen2L4054 po	A3 gen2L4054 pos.fcs	1834	70.6
A4 gen2L4054 po	A4 gen2L4054 pos.fcs	1872	70.3
A5 gen2L4055 ne	A5 gen2L4055 neg.fcs	31.9	4.23
A6 gen2L4055 ne	A6 gen2L4055 neg.fcs	32.4	4.87
A7 gen2L4055 po	A7 gen2L4055 pos.fcs	1941	88.7
A8 gen2L4055 po	A8 gen2L4055 pos.fcs	1933	91.1
B1 gen3L4054 ne	B1 gen3L4054 neg.fcs	31.2	3.45
B2 gen3L4054 ne	B2 gen3L4054 neg.fcs	31.9	3.39
B3 gen3L4054 po	B3 gen3L4054 pos.fcs	2016	61.3
B4 gen3L4054 po	B4 gen3L4054 pos.fcs	2014	76.2
B5 gen3L4055 ne	B5 gen3L4055 neg.fcs	33.7	6.76
B6 gen3L4055 ne	B6 gen3L4055 neg.fcs	35.4	6.7
B7 gen3L4055 po	B7 gen3L4055 pos.fcs	2158	99.4
B8 gen3L4055 po	B8 gen3L4055 pos.fcs	1938	99.5
Mean	-	998	43.3
SD	-	999	41.3

Figure 36

Study	Sample	Sample id	Species	Chain	Reads	CDR3	Unique CDR3	D50
20180511_Study1_FCA	Gen2-L4054	59990	TRB	h	1181732	1181732	8915	0
20180511_Study1_FCA	Gen3-L4054	59991	TRB	h	1145697	1145697	18130	0
20180511_Study1_FCA	Gen2-L4055	59987	TRB	h	1166465	1166465	12996	0.1
20180511_Study1_FCA	Gen3-L4055	59982	TRB	h	1059985	1059985	27246	0.9

Figure 37

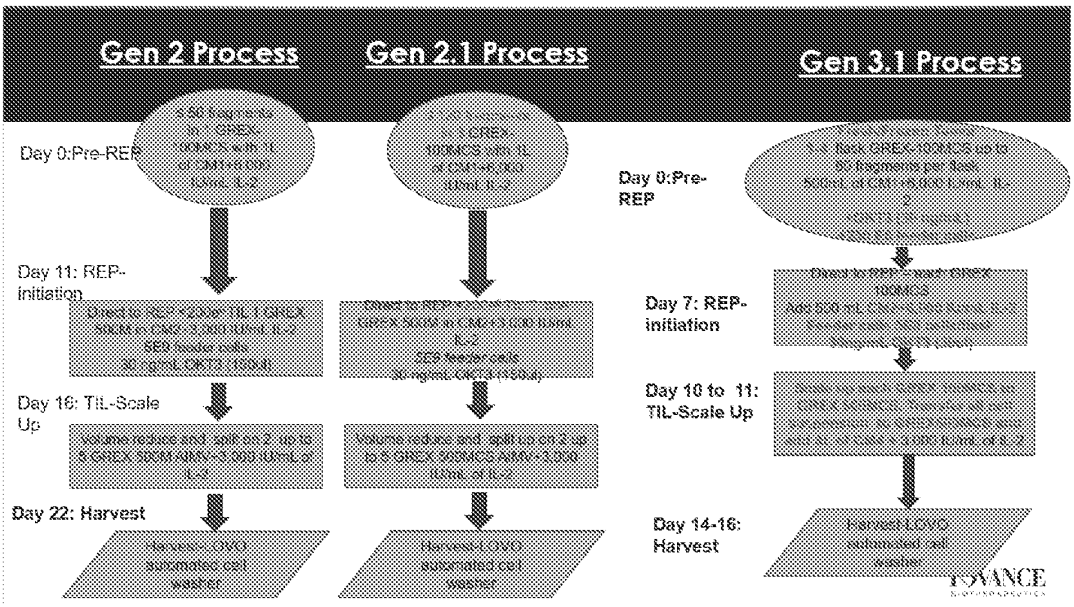

Gen 2 Process
Gen 2.1 Process
Gen 3.1 Process
Day 0:Pre-REP
Day 11: REP-initiation
Day 16: TIL-Scale Up
Day 22: Harvest
Day 0:Pre-REP
Day 7: REP-initiation
Day 10 to 11: TIL-Scale Up
Day 14-16: Harvest

Figure 38

STEP	Gen 2	Gen 2.1	Gen 3.0 Optimized
Pre REP- day 0	≤ 50 fragments/ 1 G-Rex 100MCS - **11 days**	≤ 180 fragments/ 3 G-Rex Pre-formulated CM1 warmed media 100MCS - **11 days**	***Fresh or Frozen Tumor*** Whole tumor with ≤ 30 fragments up to 50 fragments per 1 G-Rex. 100MCS (up to 4 G-Rex) preformulated warmed media - **7 days. Pre REP. Feeders 2.5 E8 cells + OKT-3 (30ng/mL)**
REP Initiation	Direct to REP- Day 11- **<200 E6 TIL** 1 G-Rex 500MCS	Direct to REP- Day 11- **<200 E6 TIL** Pre-formulated CM2 warmed media in one G-Rex 500MCS	Direct to REP- Day **7-all cells TIL- same G-Rex 100MCS (100MCS up to 4 GREX), Standard media or Defined Media (Serum free). Addition Feeders 5 E8 cells +OKT-3 (30ng/mL)**
TIL propagation or Scale up	1 to 5 G-REX 500MCS **Split day 16**	2 to 5 G-REX 500MCS Pre-formulated CM4 warmed media **Split day 16**	From G-REX 100MCS transfer TIL suspension to G-REX 500MCS - up to 4 GREX 500 MCS- Standard media or Defined Media (Serum Free) **Scale up on day 10 or 11**
Harvest	**Harvest day 22.** LOVO-automated cell washer	**Harvest day 22.** LOVO-automated cell washer (5 wash cycle)	**Harvest day 14 or 16** LOVO- automated cell washer (5 wash cycle)
Final formulation	Cryopreserved Product 300IU/ml IL-2- CS10 in LN_2 multiple aliquots	Cryopreserved Product 300IU/ml IL-2- CS10 in LN_2 multiple aliquots	Cryopreserved product 300IU/ml IL-2-CS10 in LN_2 multiple aliquots
Process time	**22 days**	**22 days**	**16 days**

Figure 39

Process Day	Conditions	Gen 3.1
Day 0- pre REP initiation	Media CM1	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (30ng/mL)	+
	Feeders (250 E+06)	+

Process Day	Conditions	Gen 3.1
Day 7- REP initiation	Media CM2	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (30ng/mL) added on Day 7	+
	Feeders Added on Day 7	500 E06
	Total Feeders at Day	750 E+06

Process Day	Conditions	Gen 3.1
Day 9-11 –Scale Up	From G-REX 100MCS transfer TIL suspension to 1 G-REX 500MCS (up to 3 GREX 500MCS)	Yes
Day 16- Harvest	LOVO- automated cell washer	Yes

Figure 40

Process Comparison	Key Process Changes	Benefit
Gen 2 : Gen 2.1	• Initiate process with two flasks instead of one flask • Divide REP initiation feeder layer between 2 G-Rex500MCS Flasks • Pre-formulate media and warm prior to use	• Potential doubling of final cell count (dose) with increased TIL repertoire. • Process redundancy throughout process
Gen 2.1 : Gen 3.1	• Fresh or Frozen tumor • 14-16 day process (from 22 day) • Reduce total feeder layer on process • Feeder layer and OKT3 present at Day 0 • REP initiated with fragments • 100MCS scales to 500MCS • Scales to multiple pre-REP flasks • Standard Media and Defined Media (Serum Free)	• Increased potency • Improved phenotype • Decreased process time • Reduced reagent testing • Decreased process variability • Defined reagents • Increased repertoire • Reduce impurities (feeder) • Comparable or Higher Dose.

Figure 41

Process Comparison	Key Process Changes	Desired Improvement	Criteria for Success	Outcome
Gen 2 : Gen 3.0	• 14-16 days • Initiate REP with fragments up to 4 flask. • 100MCS scales to 500MCS	• Increased potency • Improved phenotype • Decreased process time	• Increase potency as measured by INF-g ✓ • Comparable phenotype ✓ • Comparable Dose ✓ • Comparable purity ✓ (feeder cell) • Maintain clonal diversity ✓	• Potency increased over Gen2 • Improved expression of CD28 on CD8 cells • Maximum capacity of flask reached by day 16 on Gen 3.1 • Reduced feeder cell usage • Increased diversity

Figure 42

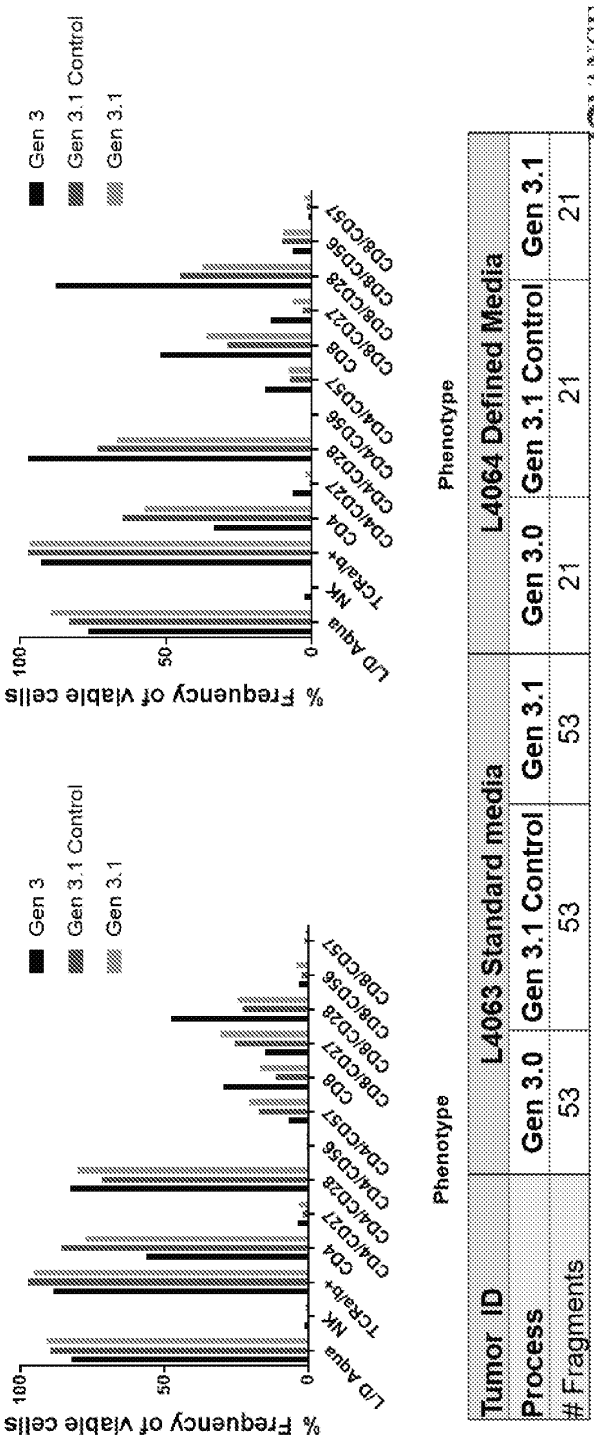
Process	Gen 2	Gen 3
L4054	Standard Media	Standard Media
L4055	Standard Media	Standard Media
M1085T	Standard Media	Standard Media

Process	Gen 3	Gen 3.1 control	Gen 3.1
L4063	Standard Media	Standard Media	Standard Media
L4064	Defined Media	Defined Media	Defined Media

Standard Media:
Pre REP: CM1
REP initiation : CM2
Split or Scale up : CM4

Defined Media:
CTS Optimizer (Serum Free Media) in each day of the process

Figure 43



Tumor ID	L4063 Standard media			L4064 Defined Media		
Process	Gen 3.0	Gen 3.1 Control	Gen 3.1	Gen 3.0	Gen 3.1 Control	Gen 3.1
# Fragments	53	53	53	21	21	21

Figure 44

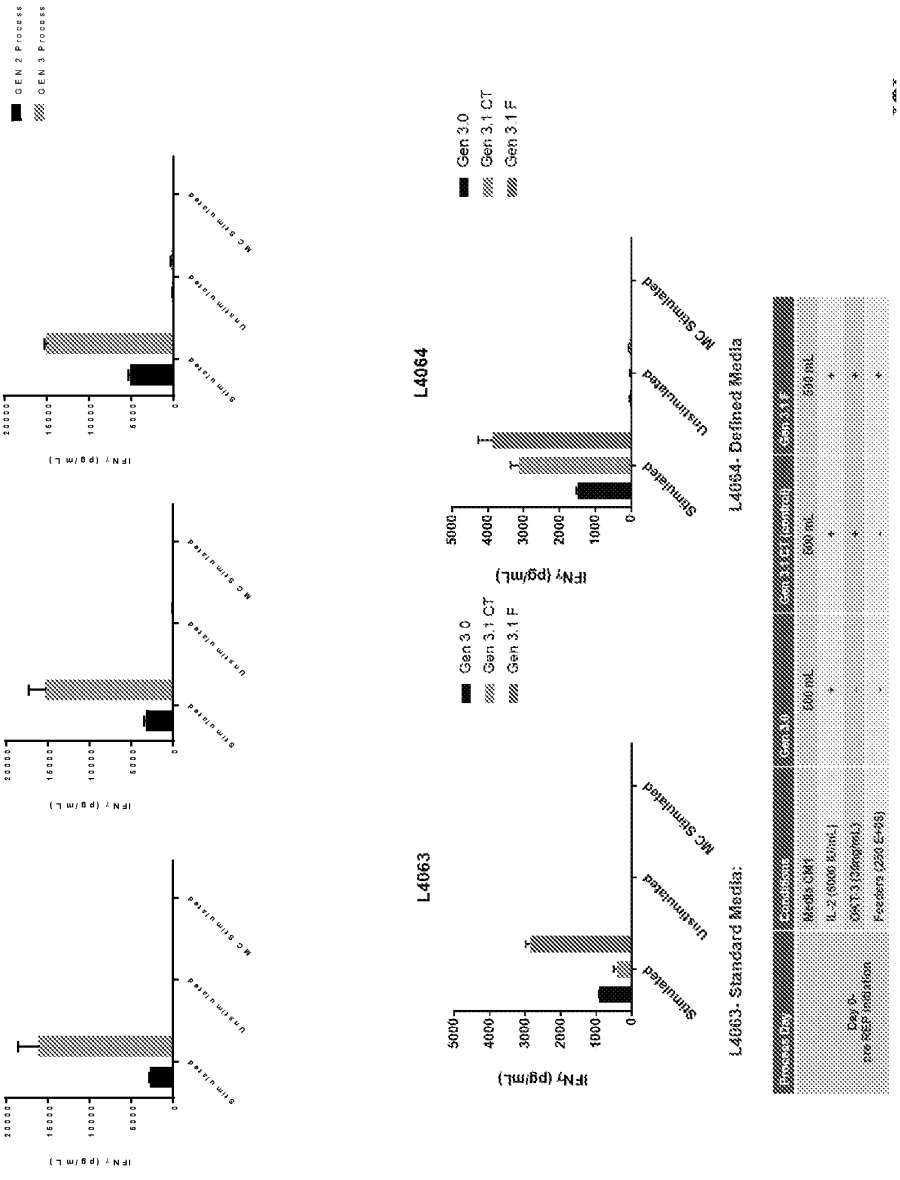

GEN 2 Process
GEN 3 Process
M10857
L4056
L4054
IFN γ (pg/mL)
Stimulated
Unstimulated
MC Stimulated
L4063
L4064
Gen 3.0
Gen 3.1 CT
Gen 3.1 F
IFNγ (pg/mL)
L4063- Standard Media:
L4064- Defined Media
Gen 3.0
Gen 3.1
600 mL
Media (MP)
IL-2 (6000 IU/mL)
Day 0 pre-REP initiation
Feeders (2E8 E+18)

Figure 45

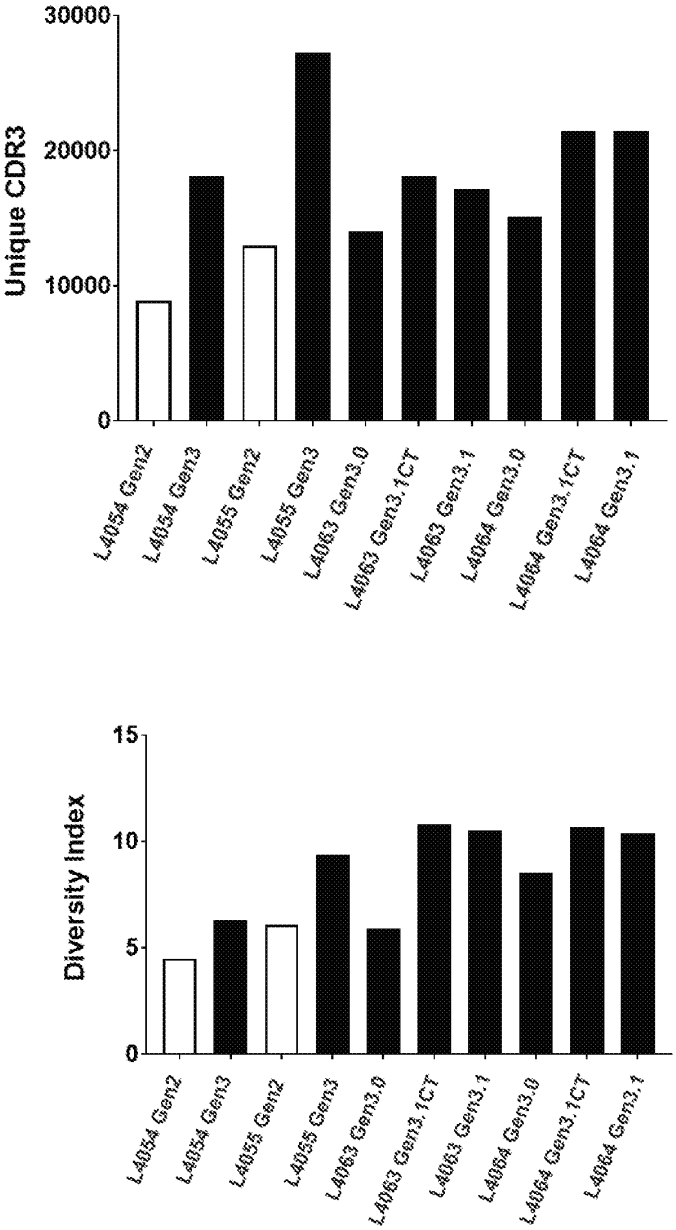

Unique CDR3
30000
20000
10000
0
L4054 Gen2
L4054 Gen3
L4055 Gen2
L4055 Gen3
L4063 Gen3.0
L4063 Gen3.1CT
L4063 Gen3.1
L4064 Gen3.0
L4064 Gen3.1CT
L4064 Gen3.1
Diversity Index
15
10
5
0
L4054 Gen2
L4054 Gen3
L4055 Gen2
L4055 Gen3
L4063 Gen3.0
L4063 Gen3.1CT
L4063 Gen3.1
L4064 Gen3.0
L4064 Gen3.1CT
L4064 Gen3.1

Figure 46

Conditions on Day 0	Gen 3.0	Gen 3.1 (control)	Gen 3.1
Media	500 mL	500 mL	500 mL
IL-2 (6E+06)	+	+	+
OKT-3 (30ng/mL)	-	+	+
Feeders (250 E+06)	-	-	+

	All uCDR3's			Top 80% uCDR3's		
Number of uCDR3 (% Overlap)	Gen 3.0	Gen 3.1 Control	Gen 3.1	Gen 3.1	Gen 3.1 control	Gen 3.1
Gen 3.0	14005	3240 (23)	2959 (21)	1105	1000 (90)	975 (88)
Gen 3.1 control	-	18149	-	-	2438	-
Gen 3.1	-	-	17181	-	-	2332

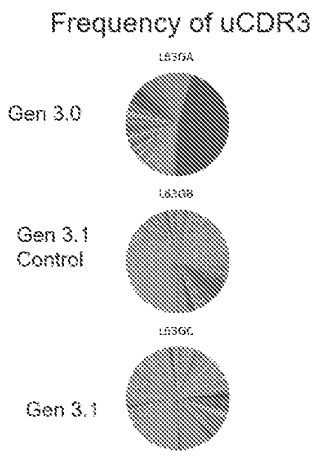


Figure 47

Conditions on Day 0	Gen 3.0	Gen 3.1 (control)	Gen 3.1
Media	500 mL	500 mL	500 mL
IL-2 (6E+06)	+	+	+
OKT-3 (30ng/mL)	-	+	+
Feeders (250 E+06)	-	-	+

Number of uCDR3 (% Overlap)	All uCDR3's			Top 80% uCDR3's		
	Gen 3.0	Gen 3.1 Control	Gen 3.1	Gen 3.0	Gen 3.1 Control	Gen 3.1
Gen 3.0	15107	5486 (36)	5541 (37)	2479	2186 (88)	2163 (87)
Gen 3.1 Control	-	21460	-	-	3247	-
Gen 3.1	-	-	21503	-	-	3264

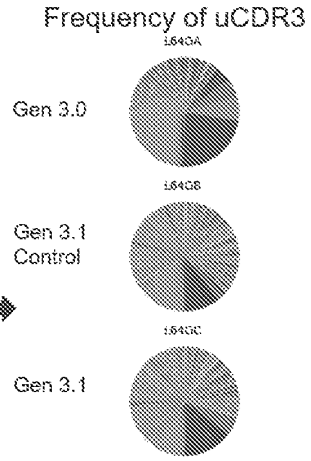


Figure 48

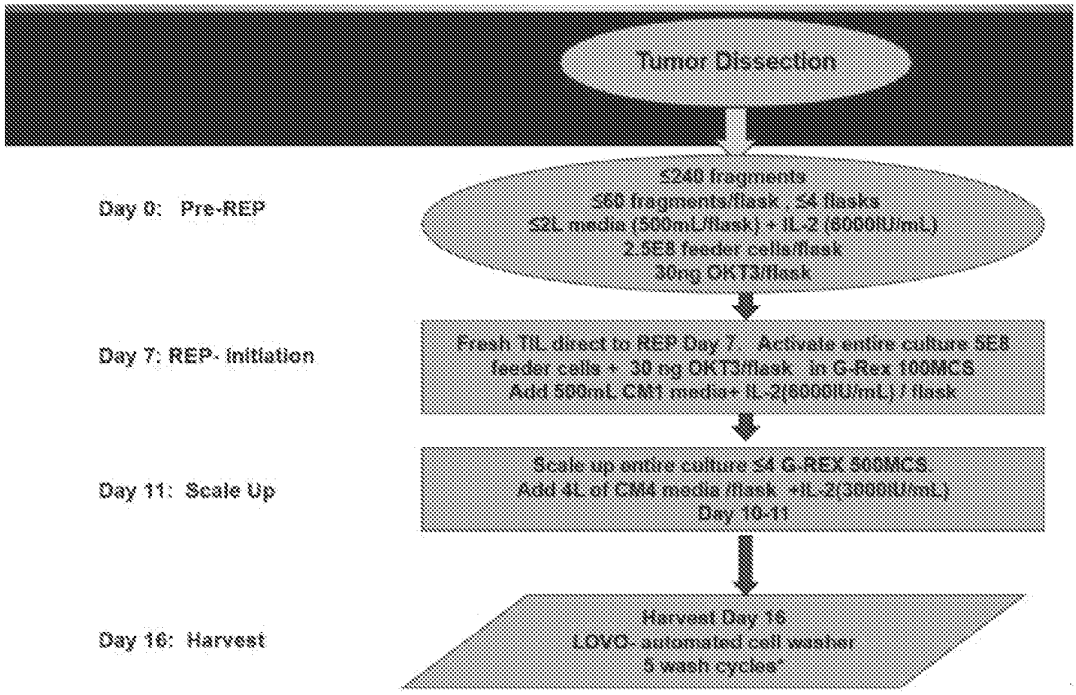

Tumor Dissection
Day 0: Pre-REP
≤240 fragments
≤60 fragments/flask , ≤4 flasks
≤2L media (500mL/flask) + IL-2 (6000IU/mL)
2.5E8 feeder cells/flask
30ng OKT3/flask
Day 7: REP- initiation
Fresh TIL direct to REP Day 7 Activate entire culture 5E8 feeder cells + 30 ng OKT3/flask in G-Rex 100MCS
Add 500mL CM1 media+ IL-2(6000IU/mL) / flask
Day 11: Scale Up
Scale up entire culture ≤4 G-REX 500MCS
Add 4L of CM4 media /flask +IL-2(3000IU/mL)
Day 10-11
Day 16: Harvest
Harvest Day 16
LOVO- automated cell washer
5 wash cycles*

Figure 49

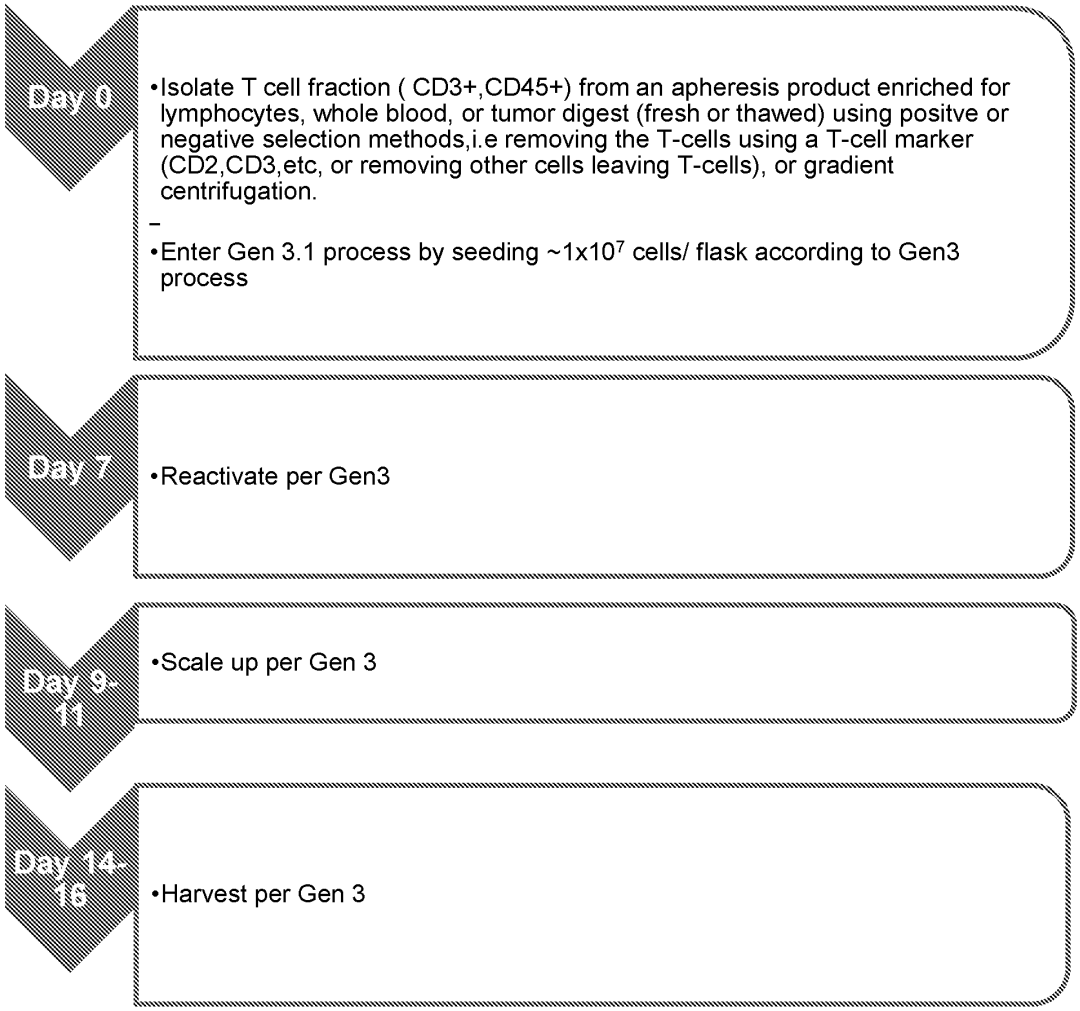

Day 0
•Isolate T cell fraction (CD3+,CD45+) from an apheresis product enriched for lymphocytes, whole blood, or tumor digest (fresh or thawed) using positve or negative selection methods,i.e removing the T-cells using a T-cell marker (CD2,CD3,etc, or removing other cells leaving T-cells), or gradient centrifugation.
–
•Enter Gen 3.1 process by seeding ~1x10^7 cells/ flask according to Gen3 process
Day 7
•Reactivate per Gen3
Day 9-11
•Scale up per Gen 3
Day 14-16
•Harvest per Gen 3

Figure 50

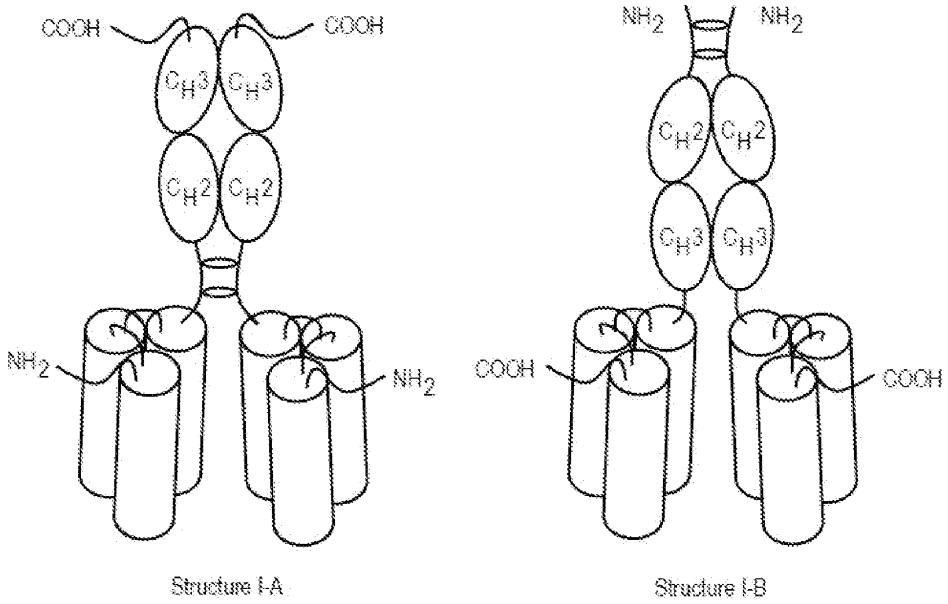

COOH
COOH
C_H3
C_H3
C_H2
C_H2
NH_2
NH_2
Structure I-A
NH_2
NH_2
C_H2
C_H2
C_H3
C_H3
COOH
COOH
Structure I-B

Figure 51

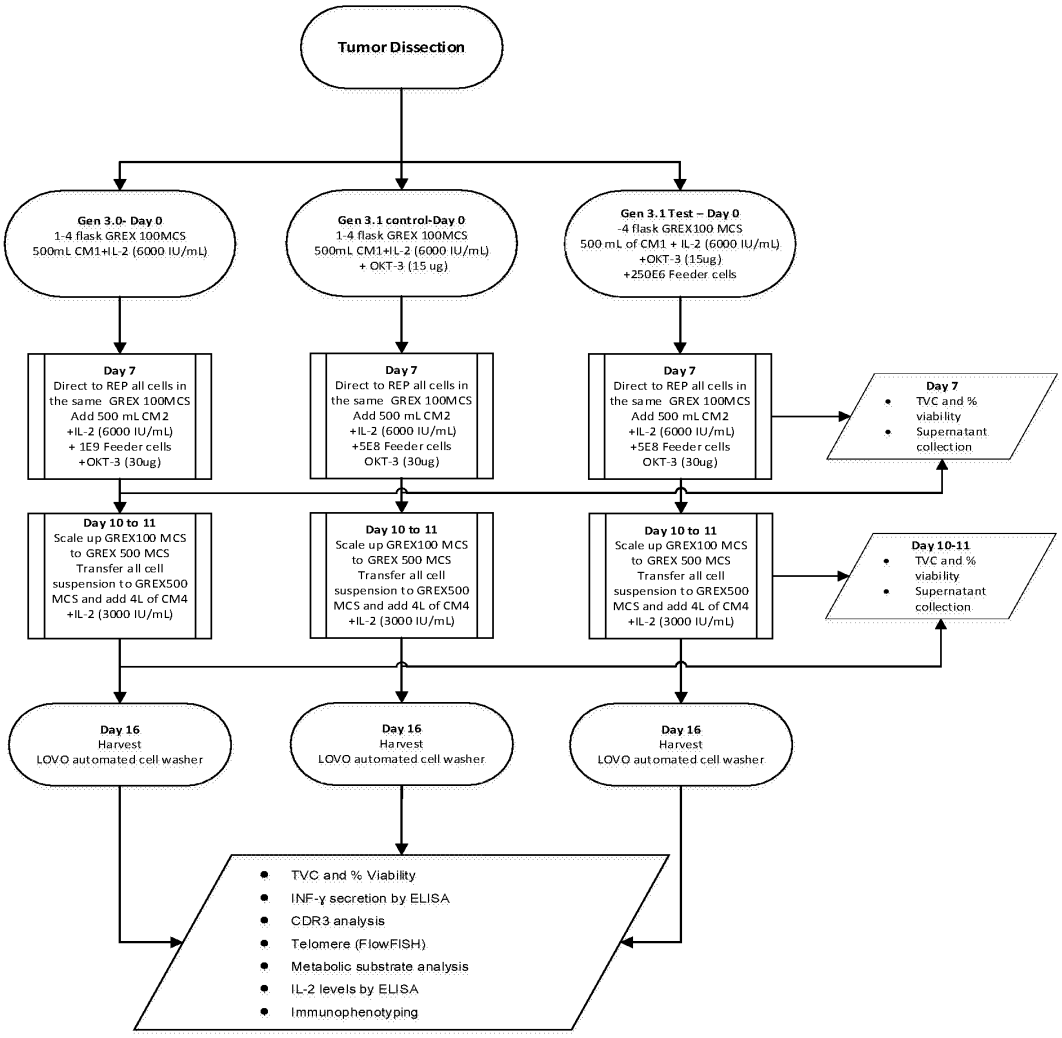
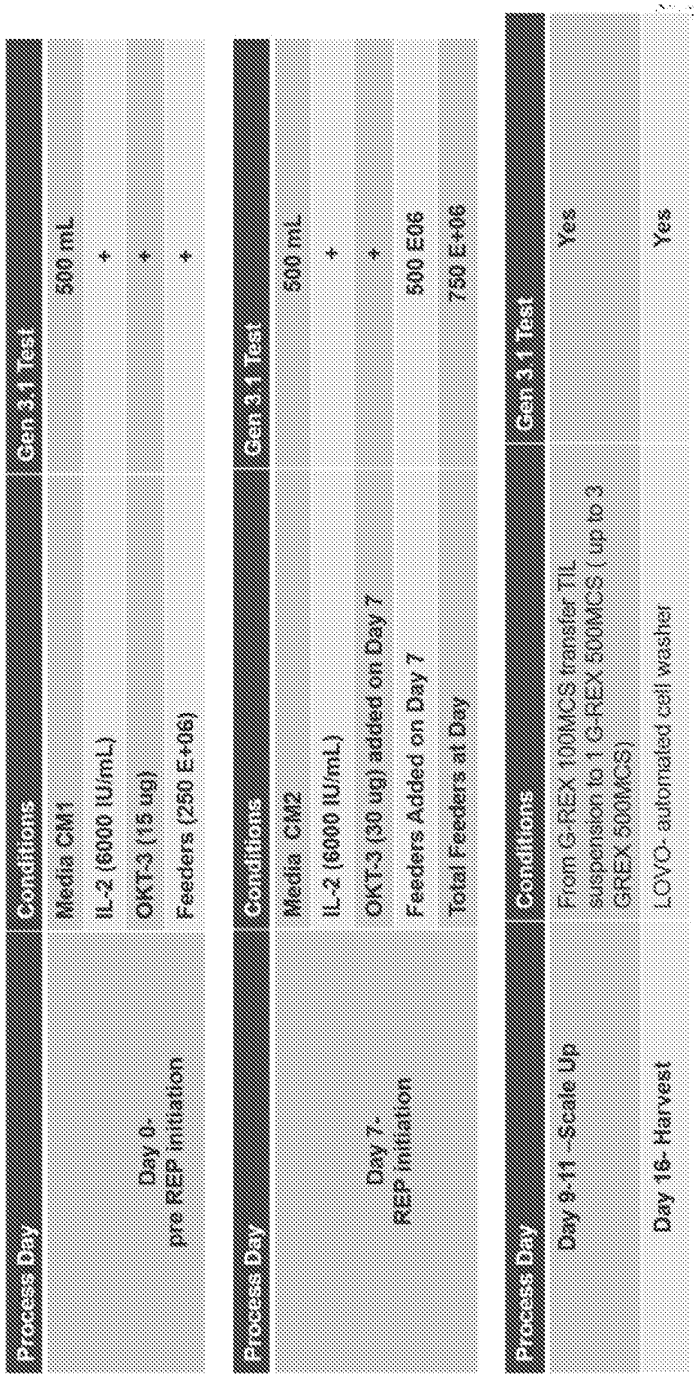

Tumor Dissection
Gen 3.0- Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
Gen 3.1 control-Day 0
1-4 flask GREX 100MCS
500mL CM1+IL-2 (6000 IU/mL)
+ OKT-3 (15 ug)
Gen 3.1 Test – Day 0
-4 flask GREX100 MCS
500 mL of CM1 + IL-2 (6000 IU/mL)
+OKT-3 (15ug)
+250E6 Feeder cells
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+ 1E9 Feeder cells
+OKT-3 (30ug)
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+5E8 Feeder cells
OKT-3 (30ug)
Day 7
Direct to REP all cells in the same GREX 100MCS
Add 500 mL CM2
+IL-2 (6000 IU/mL)
+5E8 Feeder cells
OKT-3 (30ug)
Day 7
TVC and % viability
Supernatant collection
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10 to 11
Scale up GREX100 MCS to GREX 500 MCS
Transfer all cell suspension to GREX500 MCS and add 4L of CM4
+IL-2 (3000 IU/mL)
Day 10-11
TVC and % viability
Supernatant collection
Day 16
Harvest
LOVO automated cell washer
Day 16
Harvest
LOVO automated cell washer
Day 16
Harvest
LOVO automated cell washer
TVC and % Viability
INF-γ secretion by ELISA
CDR3 analysis
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

Figure 52



Process Day	Conditions	Gen 3.1 Test
Day 0- pre REP initiation	Media CM1	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (15 ug)	+
	Feeders (250 E+06)	+

Process Day	Conditions	Gen 3.1 Test
Day 7- REP initiation	Media CM2	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (30 ug) added on Day 7	+
	Feeders Added on Day 7	500 E06
	Total Feeders at Day	750 E+06

Process Day	Conditions	Gen 3.1 Test
Day 9-11 –Scale Up	From G-REX 100MCS transfer TIL suspension to 1 G-REX 500MCS (up to 3 GREX 500MCS)	Yes
Day 16- Harvest	LOVO- automated cell washer	Yes

Figure 53

Tumor ID	L4063 in Standard Media			L4064 in CTS Optimizer Media		
Process	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Number of fragments	53	53	53	21	21	21
Average TVC per fragment	1.37E+08	3.19E+08	3.53E+08	5.90E+08	7.57E+08	9.29E+08
% viability at Harvest	79.53%	89.43%	94.80%	88.80%	81.90%	84.90%
TVC Harvest	**7.26E+09**	**1.69E+10**	**1.87E+10 ***	**1.24E+10**	**1.59E+10**	**1.95E+10 ***

Figure 54

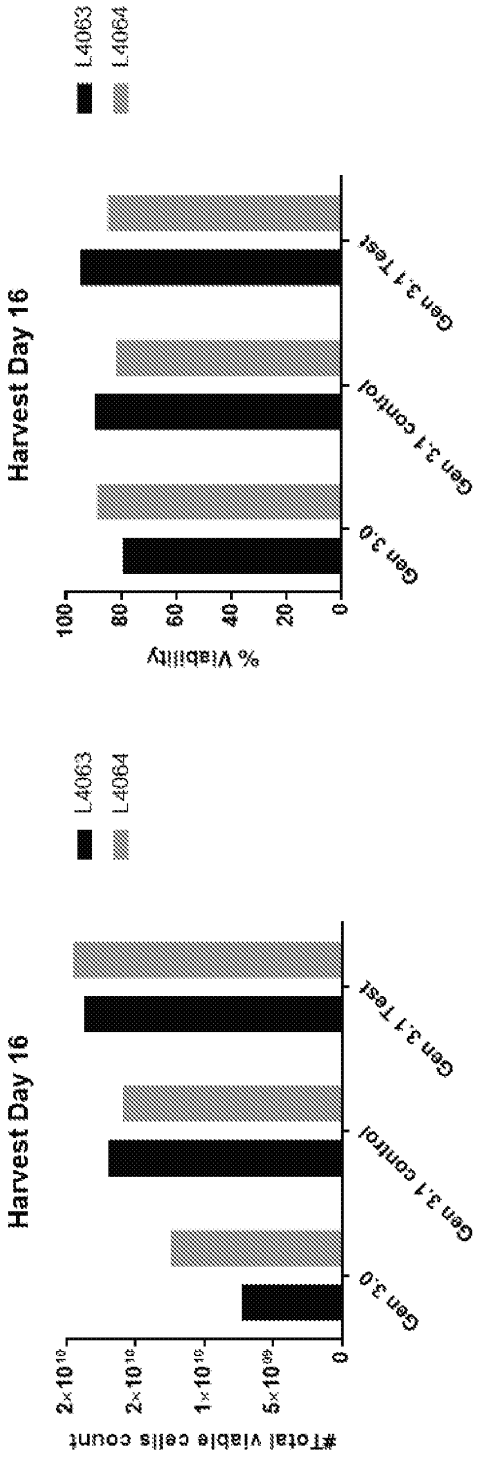
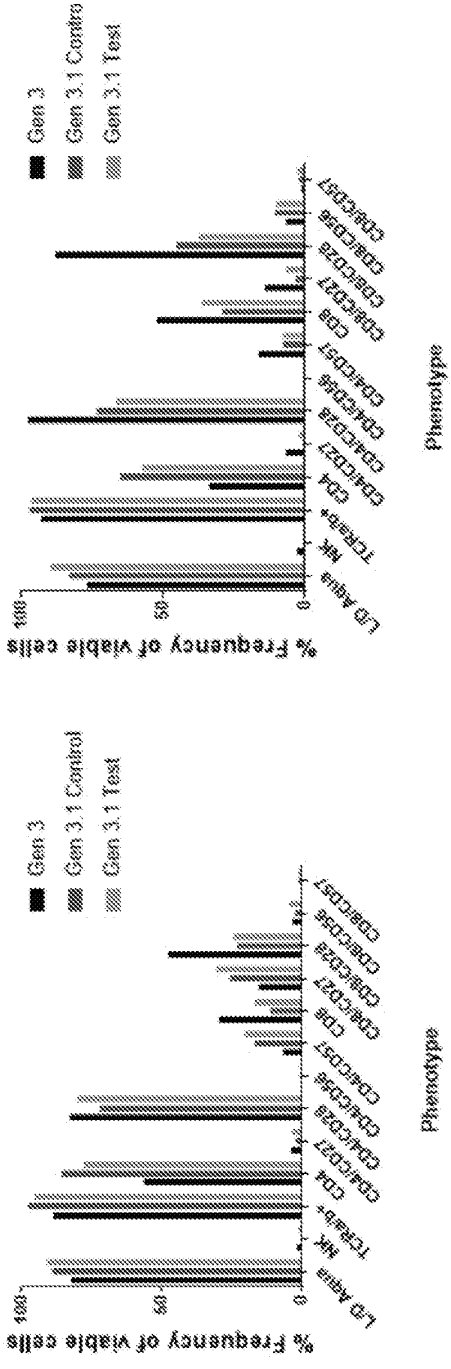

L4063
L4064
Harvest Day 16
% Viability
100
80
60
40
20
0
Gen 3.0
Gen 3.1 control
Gen 3.1 Test
L4063
L4064
Harvest Day 16
#Total viable cells count
2×10^10
2×10^10
1×10^10
5×10^09
0
Gen 3.0
Gen 3.1 control
Gen 3.1 Test

Figure 55



Tumor ID	L4063 Standard media			L4064 Defined Media		
Process	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
# Fragments	53	53	53	21	21	21

Figure 56

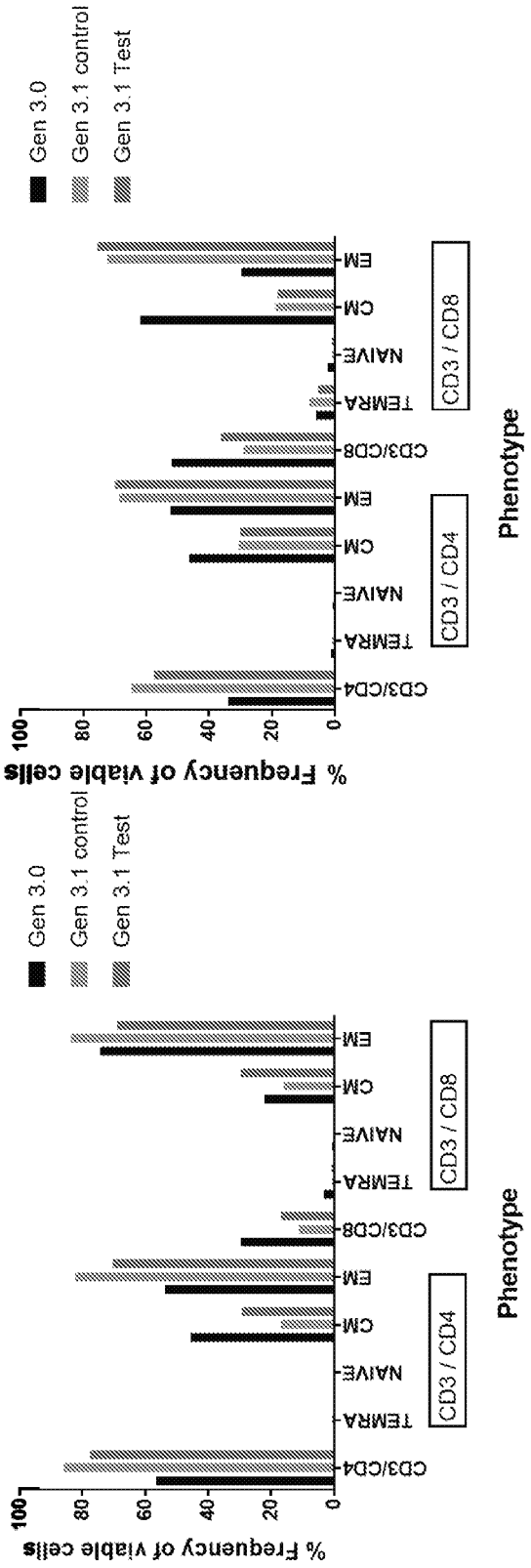
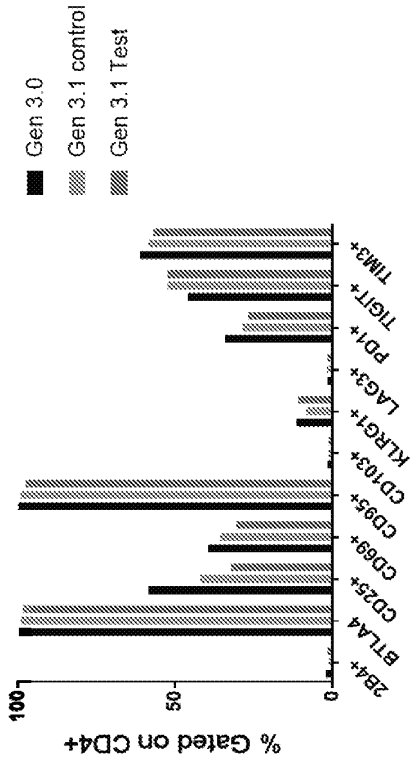

Gen 3.0
Gen 3.1 control
Gen 3.1 Test
L4064
% Frequency of viable cells
100
80
60
40
20
0
CD3/CD4
TEMRA
NAIVE
CM
EM
CD3/CD8
TEMRA
NAIVE
CM
EM
CD3 / CD4
CD3 / CD8
Phenotype
Gen 3.0
Gen 3.1 control
Gen 3.1 Test
L4063
% Frequency of viable cells
100
80
60
40
20
0
CD3/CD4
TEMRA
NAIVE
CM
EM
CD3/CD8
TEMRA
NAIVE
CM
EM
CD3 / CD4
CD3 / CD8
Phenotype

Figure 57


L4064
Gen 3.0
Gen 3.1 control
Gen 3.1 Test
% Gated on CD4+
100
50
0
2B4+
BTLA4
CD25+
CD69+
CD95+
CD103+
KLRG1+
LAG3+
PD1+
TIGIT+
TIM3+

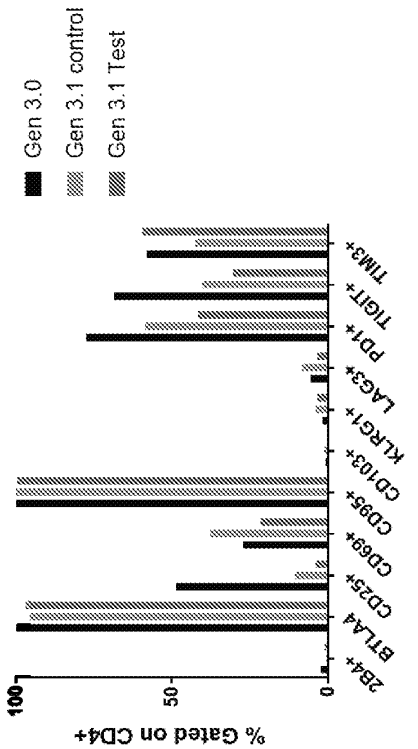

L4063
Gen 3.0
Gen 3.1 control
Gen 3.1 Test
% Gated on CD4+
100
50
0
2B4+
BTLA4
CD25+
CD69+
CD95+
CD103+
KLRG1+
LAG3+
PD1+
TIGIT+
TIM3+

Figure 58

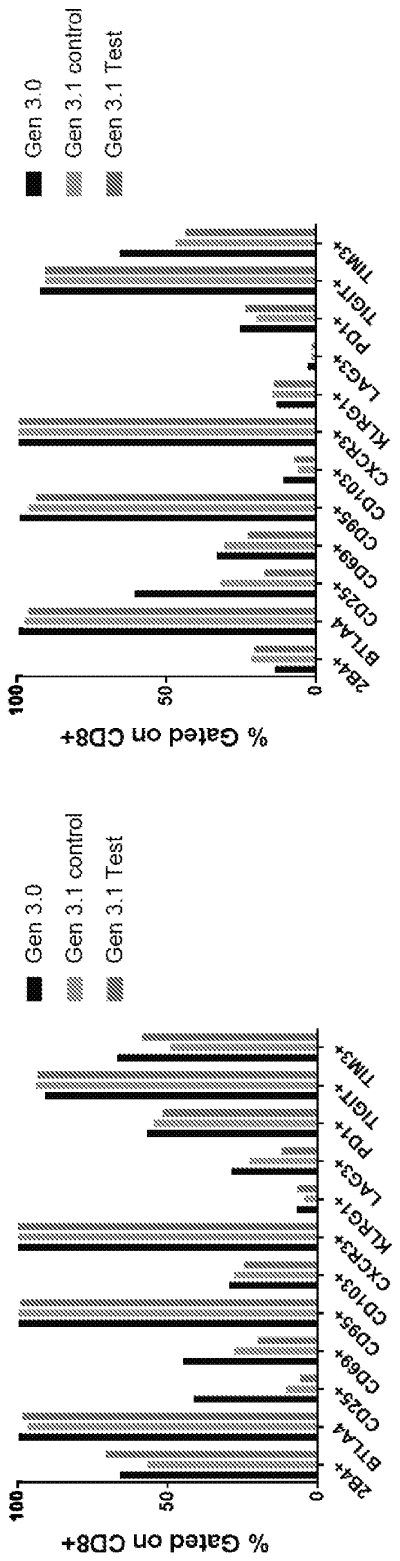
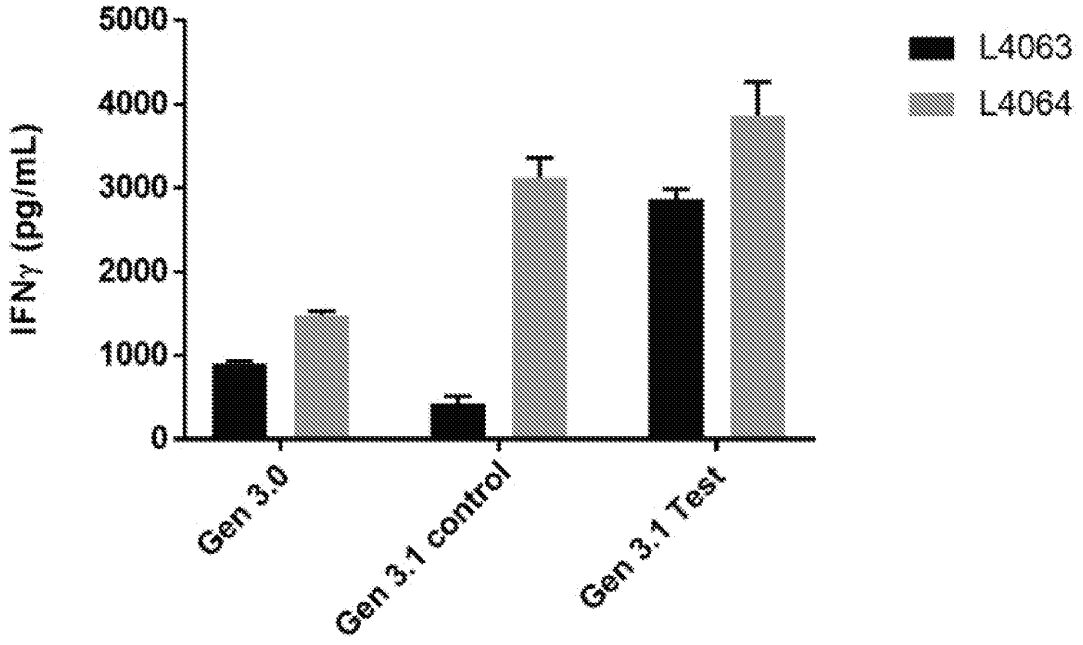

L4063
L4064
Gen 3.0
Gen 3.1 control
Gen 3.1 Test
% Gated on CD8+
100
50
0
2B4+
BTLA4
CD25+
CD69+
CD95+
CD103+
CXCR3+
KLRG1+
LAG3+
PD1+
TIGIT+
TIM3+

Figure 59



Process Day	Conditions	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Day 0- pre REP initiation	Media CM1	500 mL	500 mL	500 mL
	IL-2 (6000 IU/mL)	+	+	+
	OKT-3 (30ng/mL)	-	+	+
	Feeders (250 E+06)	-	-	+

Figure 60

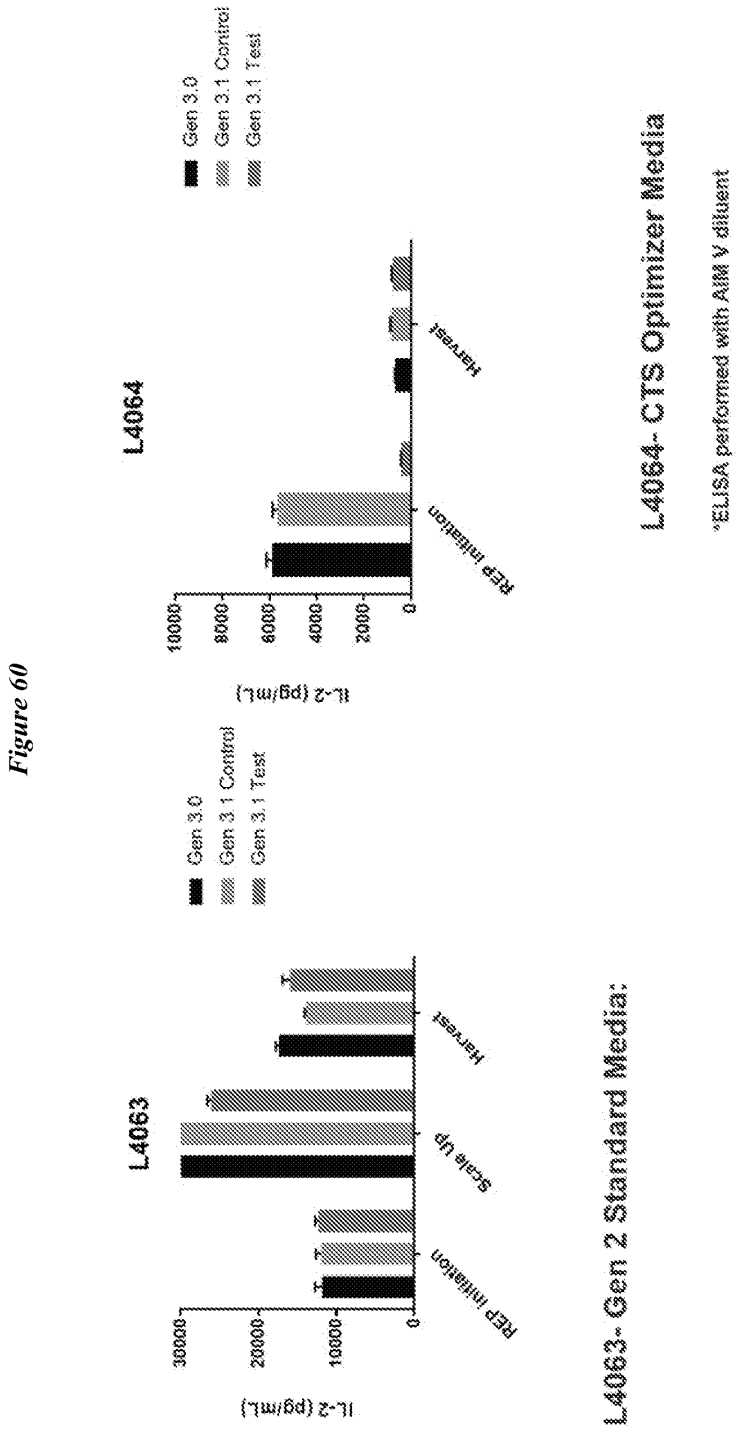

L4063
Gen 3.0
Gen 3.1 Control
Gen 3.1 Test
IL-2 (pg/mL)
30000
20000
10000
0
REP Initiation
Scale up
Harvest
L4063- Gen 2 Standard Media:
L4064
Gen 3.0
Gen 3.1 Control
Gen 3.1 Test
IL-2 (pg/mL)
10000
8000
6000
4000
2000
0
REP Initiation
Harvest
L4064- CTS Optimizer Media
*ELISA performed with AIM V diluent

Figure 61

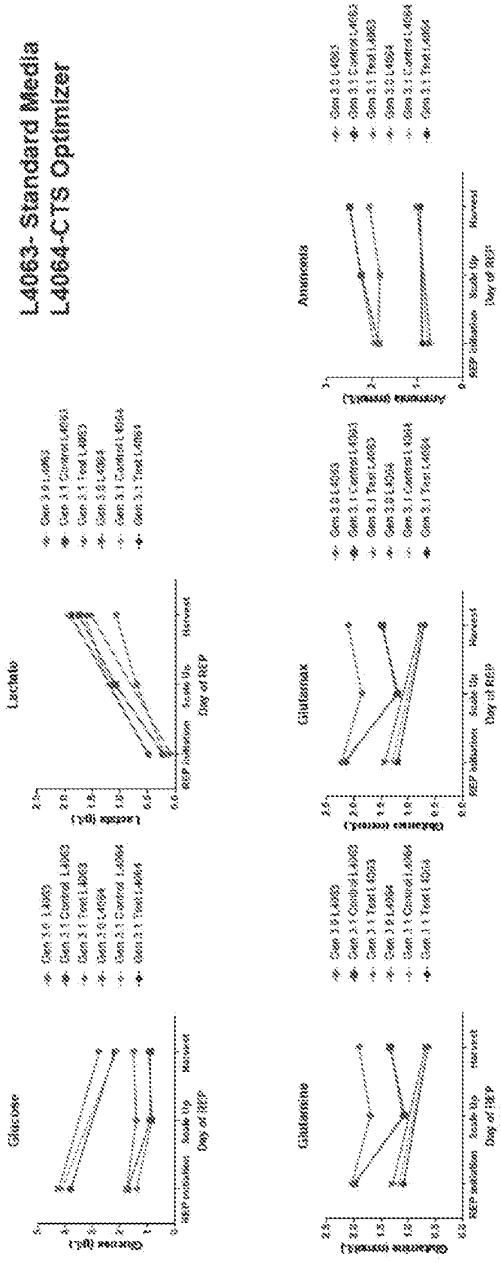

L4063- Standard Media
L4064-CTS Optimizer
Glucose
Lactate
Ammonia
Glutamine
Glutamax
Day of REP
REP Initiation
Scale Up
Harvest

Figure 62

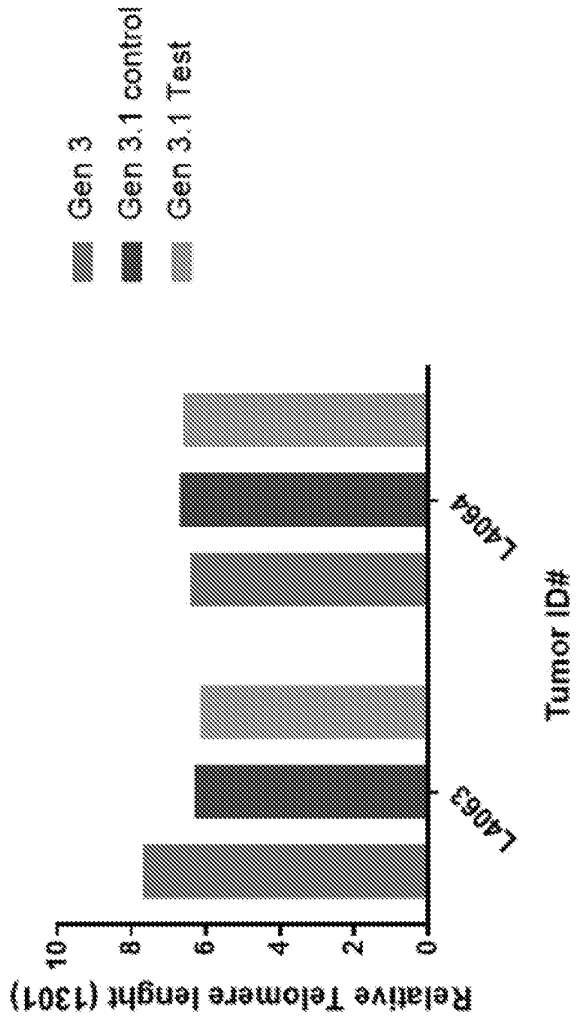

Gen 3
Gen 3.1 control
Gen 3.1 Test
Relative Telomere length (1301)
0
2
4
6
8
10
LA063
LA064
Tumor ID#

Figure 63

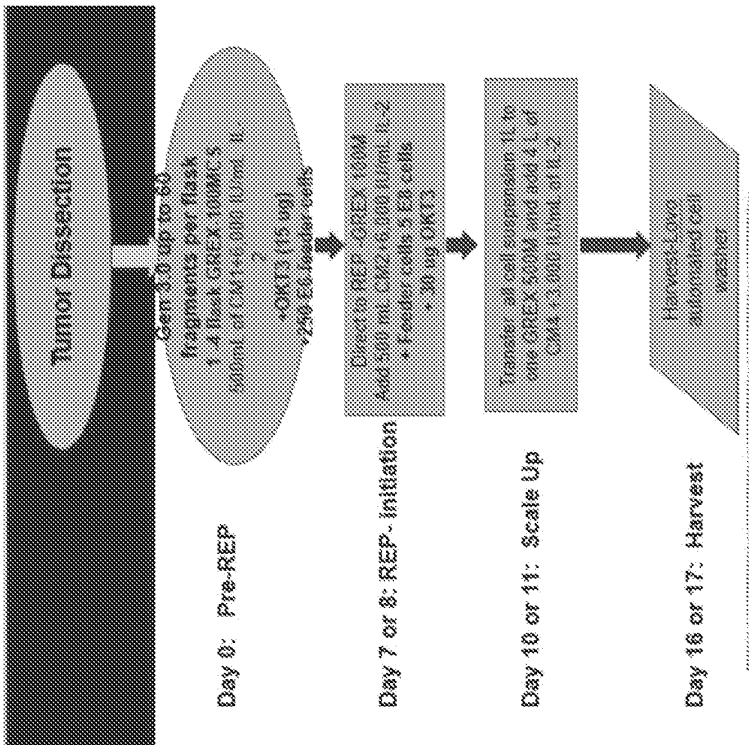

Tumor Dissection
Day 0: Pre-REP
Day 7 or 8: REP- initiation
Day 10 or 11: Scale Up
Day 16 or 17: Harvest

Figure 64

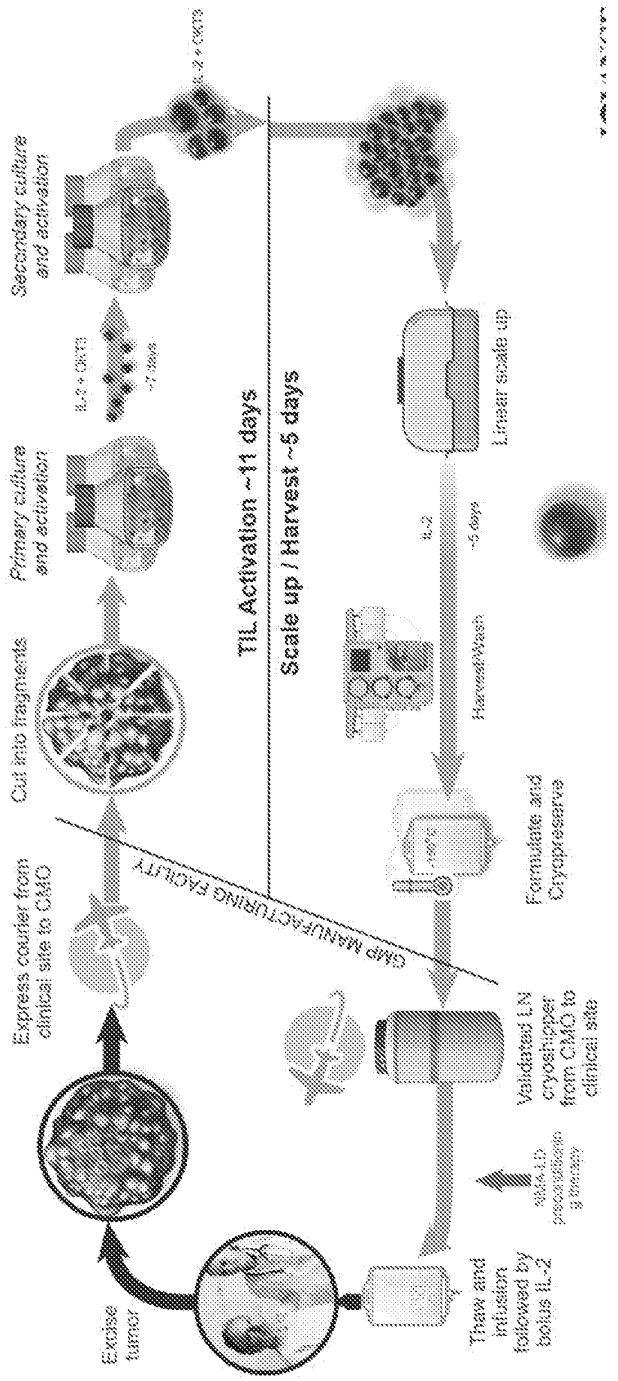

Excise tumor
Express courier from clinical site to CMO
Cut into fragments
Primary culture and activation
Secondary culture and activation
TIL Activation ~11 days
Scale up / Harvest ~5 days
GMP MANUFACTURING FACILITY
Harvest/Wash
Linear scale up
IL-2
~5 days
Formulate and Cryopreserve
Validated LN cryoshipper from CMO to clinical site
Thaw and infusion followed by bolus IL-2

Figure 65A

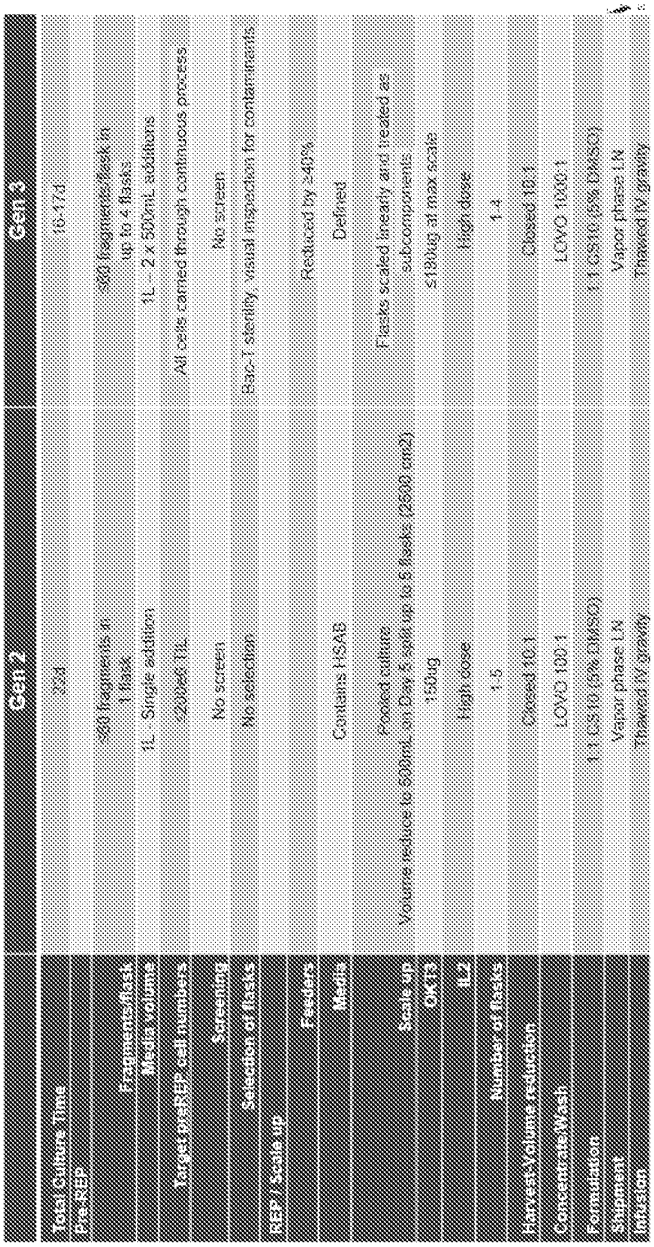

Gen 2
Gen 3
Total Culture Time
33d
10-17d
Pre-REP
Fragments/flask
~60 fragments in 1 flask
~60 fragments/flask in up to 4 flasks
Media volume
1L - Single addition
1L – 2 x 500mL additions
Target postREP cell numbers
~20e9 TIL
All cells carried through continuous process
Sampling
No screen
No screen
Selection of flasks
No selection
Bac-T sterility visual inspection for contaminants
REP / Scale up
Features
Reduced by ≥40%
Media
Contains HSAB
Defined
Scale up
Pooled culture
Volume reduce to 500mL on Day 5, split up to 5 flasks (2500 cm2)
Flasks scaled linearly and treated as subcomponents
OKT3
150ug
≤18ug at max scale
IL2
High dose
High dose
Number of flasks
1-5
1-4
Harvest Volume reduction
Closed 10:1
Closed 10:1
Concentrate/Wash
LOVO 100:1
LOVO 100:1
Formulation
1:1 CS10 10% DMSO
1:1 CS10 (5% DMSO)
Shipment
Vapor phase LN
Vapor phase LN
Infusion
Thawed IV gravity
Thawed IV gravity

Figure 65B


Gen 2
Gen 3
Total Culture Time
22d
16d
Fragment Culture
Fragments/flask
~66 fragments in 1 flask
~66 fragments/flask in up to 4 flasks
Media Volume
1L - Single addition
1L – 2 x 500mL additions
Target packed cell numbers
≤200e6 TIL
All cells carried through continuous process
Screening
No screen
No screen
Selection of flasks
No selection
Bac-T sterility, visual inspection for contaminants
REP / Scale up
Factors
Reduced by ≥40%
Media
Contains HSAB
Defined
Scale up
Rocked culture
Volume reduce to 500mL on Day 5 split up to 5 flasks (2500 cm2)
Flasks scaled linearly and treated as subcomponents
OKT3
150ug
≤180ug at max scale
IL2
High dose
High dose
Number of flasks
1-5
1-4
Harvest/Volume reduction
Closed 10:1
Closed 10:1
Concentrate/Wash
LOVO 100:1
LOVO 100:1
Formulation
1:1 CS10 (5% DMSO)
1:1 CS10 (5% DMSO)
Shipment
Vapor phase LN
Vapor phase LN
Infusion
Thawed IV gravity
Thawed IV gravity

Figure 66

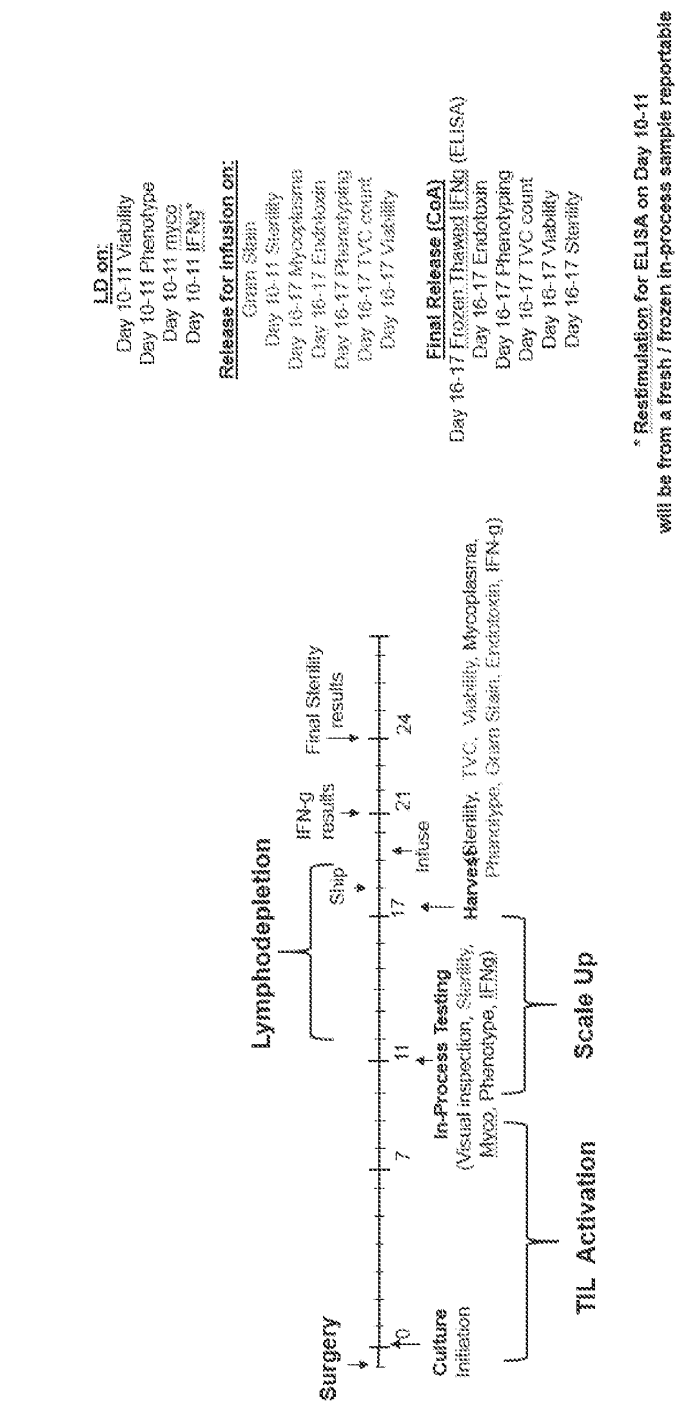

LD on:
Day 10-11 Viability
Day 10-11 Phenotype
Day 10-11 mycp
Day 10-11 IFNg*
Release for infusion on:
Gram Stain
Day 10-11 Sterility
Day 10-17 Mycoplasma
Day 10-17 Endotoxin
Day 10-17 Phenotyping
Day 10-17 TVC count
Day 10-17 Viability
Final Release (CoA)
Day 10-17 Frozen Thawed IFNg (ELISA)
Day 10-17 Endotoxin
Day 10-17 Phenotyping
Day 10-17 TVC count
Day 10-17 Viability
Day 10-17 Sterility
* Restimulation for ELISA on Day 10-11
will be from a fresh / frozen in-process sample reportable
On Day 14
Surgery
Culture Initiation
0
7
11
17
21
24
In-Process Testing
(Visual inspection, Sterility, Myco, Phenotype, IFNg)
Ship
IFN-g results
Infuse
Final Sterility results
Harvest
Sterility, TVC, Viability, Mycoplasma, Phenotype, Gram Stain, Endotoxin, IFN-g)
Lymphodepletion
TIL Activation
Scale Up

Figure 67

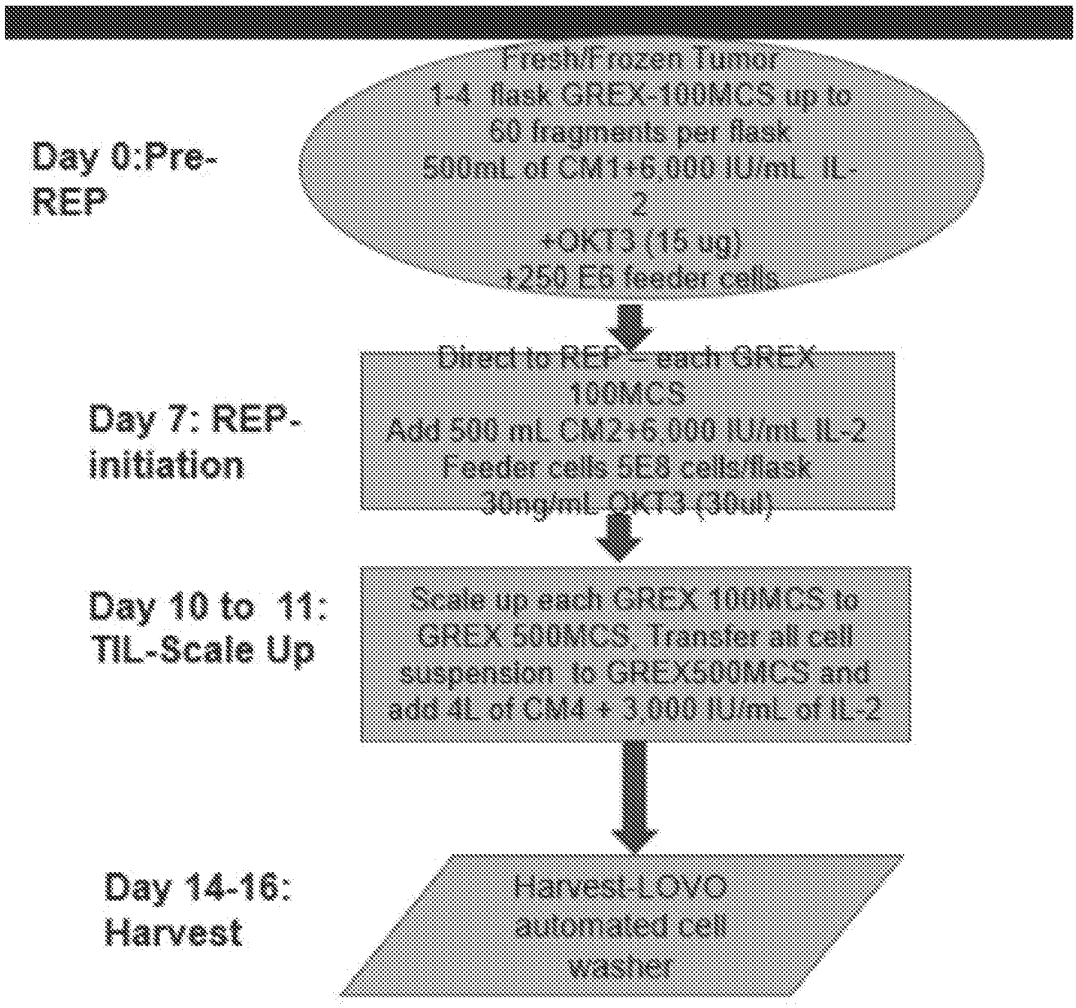
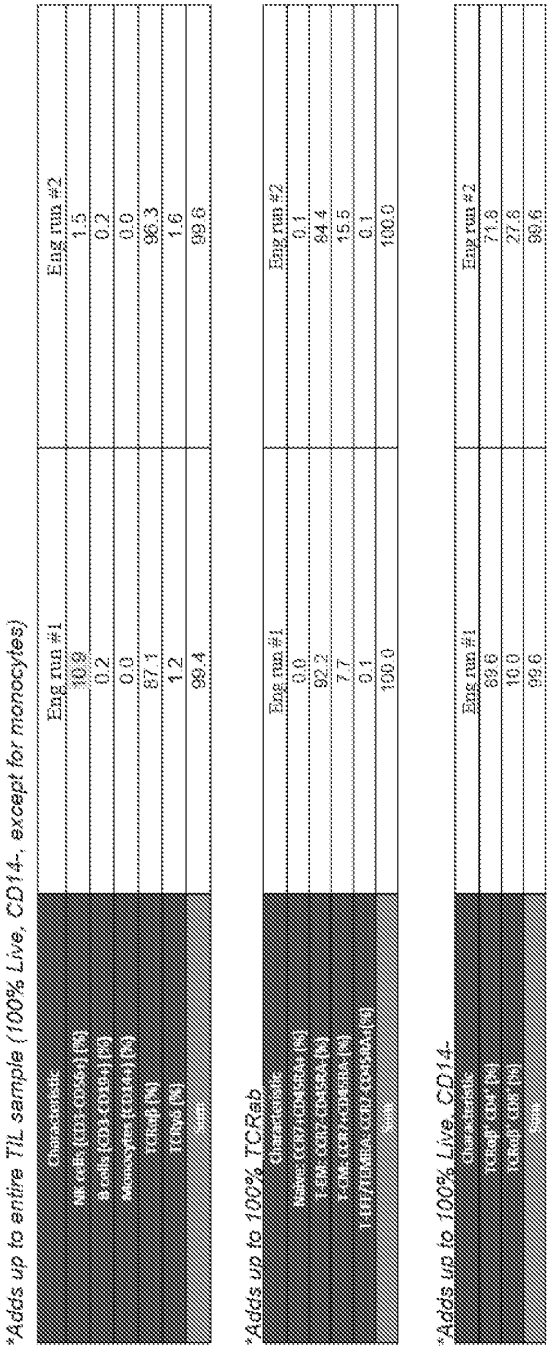

Day 0:Pre-REP
Fresh/Frozen Tumor
1-4 flask GREX-100MCS up to 60 fragments per flask
500mL of CM1+6,000 IU/mL IL-2
+OKT3 (15 ug)
+250 E6 feeder cells
Day 7: REP-initiation
Direct to REP = each GREX 100MCS
Add 500 mL CM2+6,000 IU/mL IL-2
Feeder cells 5E6 cells/flask
30ng/mL OKT3 (30ul)
Day 10 to 11: TIL-Scale Up
Scale up each GREX 100MCS to GREX 500MCS. Transfer all cell suspension to GREX500MCS and add 4L of CM4 + 3,000 IU/mL of IL-2
Day 14-16: Harvest
Harvest-LOVO automated cell washer

Figure 68

Data Summary of testing Day 16/17

Testing Parameter	1st Eng. Run Day 17 10 March 2019	2nd Eng. Run Day 17 11 March 2019	3rd Eng. Run Day 17 25 March 2019	Gen 2 (n=47)		
				Mean	Median	Stdev
Pre-LOVO TVC	17.3E+09	46.1E+09				
Pre-LOVO % Viability	90.5 %	94.3%				
Post-LOVO TVC	17.9E+09	59.4E+09	48.2E+09			
Post-LOVO % Viability	85.3%	90.7%	89%			
TVC Post LOVO % Recovery	103.5%	128.9%				
%CD45+/CD3+	88.0%	98.3%				
IFNγ (pg/mL)	11,618	19,235		10,005	9,637	5,128
GrzB (pg/mL)	11,326	27,441		19,929	16,040	
Sterility	Pending (*)	Pending (*)				
Endotoxin (total EU units)	154.2 EU	196.9 EU				
Mycoplasma	Negative	Negative				
Gram Staining	No organisms detected	No organisms detected				

Figure 69



*Adds up to entire TIL sample (100% Live, CD14-, except for monocytes)

Characteristic	Exp run #1	Exp run #2
NK cells (CD3-CD56+) [%]	10.8	1.5
B cells (CD3-CD19+) [%]	0.2	0.2
Monocytes (CD14+) [%]	0.0	0.0
TCRαβ [%]	87.1	96.3
TCRγδ [%]	1.2	1.6
Total	99.4	99.6

*Adds up to 100% TCRαb

Characteristic	Exp run #1	Exp run #2
Naive CD4+ cells [%]	0.0	0.1
TEM CD4+ cells [%]	92.2	84.4
TCM CD4+ cells [%]	7.7	15.5
CD4+ cells [%]	0.1	0.1
Total	100.0	100.0

*Adds up to 100% Live, CD14-

Characteristic	Exp run #1	Exp run #2
CD4+ [%]	83.6	71.8
CD8+ [%]	16.0	27.8
Total	99.6	99.6

Figure 70

**Adds up to entire TIL sample (100% Live, CD14-, except for monocytes)*

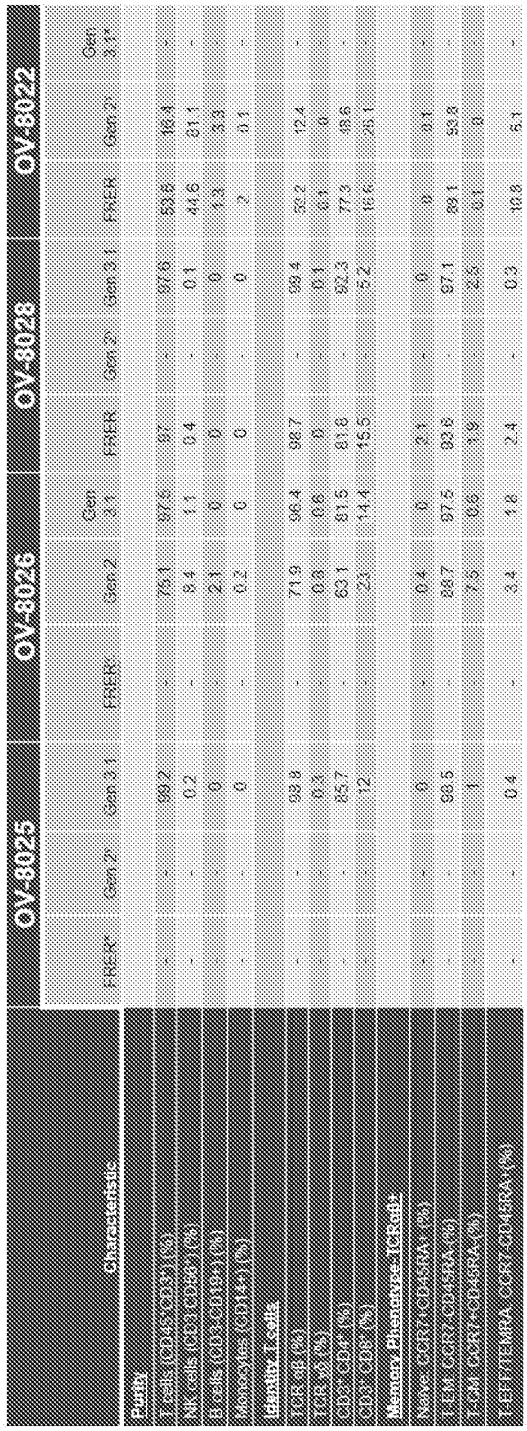
Characteristic	PD run #1 L4063	PD run #2 L4064
NK cells (CD3-CD56+) [%]	1.04	0.52
B cells (CD3-CD19+) [%]	0.0076	0.013
Monocytes (CD14+) [%]	0.032	0.043
TCRαβ [%]	95.5	96.7
TCRγδ [%]	2	1.86
Sum	98.6	99.14

**Adds up to 100% TCRab*

Characteristic	PD run #1 L4063	PD run #2 L4064
Naive: CCR7+CD45RA+ [%]	0.35	0.07
T-EM: CCR7-CD45RA- [%]	97.4	96.7
T-CM: CCR7+CD45RA- [%]	1.39	2.78
T-EFF/TEMRA: CCR7-CD45RA+ [%]	0.88	0.44
Sum	100.0	100.0

Characteristic	PD run #1 L4063	PD run #2 L4064
TCRαβ+ CD4+ [%]	77.5	57.4
TCRαβ+ CD8+ [%]	16.8	36.1
Sum	94.3	93.5

Figure 71



Characteristic	OV-3025			OV-3026			OV-3028			OV-3022		
	FRER*	Gen 2^	Gen 3.1	FRER*	Gen 2	Gen 3.1	FRER*	Gen 2^	Gen 3.1	FRER*	Gen 2^	Gen 3.1^
Purity												
T cells (CD1+CD3+) (%)	-	-	99.2	-	79.1	97.6	97	-	97.6	53.8	16.4	-
NK cells (CD1+CD56+) (%)	-	-	0.2	-	8.4	1.1	0.4	-	0.1	44.6	81.1	-
B cells (CD1+CD20+) (%)	-	-	0	-	2.1	0	0	-	0	1.3	3.3	-
Monocytes (CD1+CD14+) (%)	-	-	0	-	0.2	0	0	-	0	2	0.1	-
Identity - T cells												
TCR αβ (%)	-	-	93.8	-	71.9	96.4	98.7	-	99.4	32.2	12.4	-
TCR γδ (%)	-	-	0.3	-	0.8	0.6	0	-	0.1	0.1	0	-
CD4+ CD8- (%)	-	-	25.7	-	83.1	81.5	81.8	-	82.3	77.3	49.6	-
CD4- CD8+ (%)	-	-	72	-	23	14.4	15.5	-	5.2	16.6	26.1	-
Memory Phenotype - TCR αβ												
Naive CD1+CD45RA+ (%)	-	-	0	-	0.4	0	2.1	-	0	0	0.1	-
TEM CD1+CD45RA- (%)	-	-	98.5	-	88.7	97.5	93.8	-	97.1	93.1	53.8	-
TCM CD1+CD45RA- (%)	-	-	1	-	7.5	0.6	3.9	-	2.5	3.1	8	-
TEMRA CD1+CD45RA+ (%)	-	-	0.4	-	3.4	1.8	2.4	-	0.3	16.8	6.1	-

FR ER, Frozen tumor, Early REP
*, Condition not tested
^, Sampling issue, low TVC count or non-viable cells on thawing

Figure 72

Characteristic	Hierarchy	Exp run #1	Exp run #2
NK cells (CD3-CD56+) (%)	S1/S2/All/Live/CD14-/CD3-/NK | Freq. of null (%)	10.9	1.6
B cells (CD3-CD19+) (%)	S1/S2/All/Live/CD14-/CD19 | Freq. of null (%)	0.2	0.2
Monocytes (CD14+) (%)	S1/S2/All/Live/CD14+ | Freq. of Parent (%)	0.0	0.0
T cells (%)	S1/S2/All/Live/CD14-/TCRab | Freq. of null (%)	87.1	96.3
T cells (%)	S1/S2/All/Live/CD14-/TCRgd | Freq. of null (%)	1.2	1.6
T cells, CD4+ (%)	S1/S2/All/Live/CD14-/TCRab/CD4 | Freq. of Parent (%)	89.6	74.8
T cells, CD8+ (%)	S1/S2/All/Live/CD14-/TCRab/CD8 | Freq. of Parent (%)	10.0	27.8
Naive CD4+ (CD45RA+) (%)	S1/S2/All/Live/CD14-/TCRab/Q2: CCR7+, CD45RA+ | Freq. of Parent (%)	0.0	0.1
TEM CD4+ (CD45RA-) (%)	S1/S2/All/Live/CD14-/TCRab/Q4: CCR7-, CD45RA- | Freq. of Parent (%)	92.2	84.4
TCM CD4+ (CD45RA-) (%)	S1/S2/All/Live/CD14-/TCRab/Q3: CCR7+, CD45RA- | Freq. of Parent (%)	7.7	15.5
TEFF/ITEMRA CD4+ (CD45RA+) (%)	S1/S2/All/Live/CD14-/TCRab/Q1: CCR7-, CD45RA+ | Freq. of Parent (%)	0.1	0.1
Naive CD8+ (CD45RA+) (%)	S1/S2/All/Live/CD14-/TCRab/Q6: CD62L+, CD45RA+ | Freq. of Parent (%)	0.2	0.1
TEM CD8+ (CD45RA-) (%)	S1/S2/All/Live/CD14-/TCRab/Q8: CD62L-, CD45RA- | Freq. of Parent (%)	47.7	49.5
TCM CD8+ (CD45RA-) (%)	S1/S2/All/Live/CD14-/TCRab/Q7: CD62L+, CD45RA- | Freq. of Parent (%)	52.1	50.4
TEFF/ITEMRA CD8+ (CD45RA+) (%)	S1/S2/All/Live/CD14-/TCRab/Q5: CD62L-, CD45RA+ | Freq. of Parent (%)	0.1	0.0

**Adds up to CD3+CD45+ %*

Characteristic	Exp run #1	Exp run #2
T cells (%)	87.1	96.3
T cells (%)	1.2	1.6
Total (%)	88.3	97.9

Figure 73

Characteristic	Hierarchy	CD4+		CD8+	
		Eng run #1	Eng run #2	Eng run #1	Eng run #2
CD22+ (%)	S1/S2/All/Live/CD14-/TCRab/CD4 or CD8/CD45+/8+CD27+ | Freq. of Parent (%)	0.7	2.0	2.1	12.7
CD28+ (%)	S1/S2/All/Live/CD14-/TCRab/CD4 or CD8/CD45+/8+CD28+ | Freq. of Parent (%)	41.2	50.1	3.6	13.9
CD57+ (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD45+/8+CD57+ | Freq. of Parent (%)	1.0	6.9	0.1	3.7
2B4+ (%)	S/S/Live/CD3/CD4 or CD8/2B4 | Freq. of Parent (%)	4.5	5.5	69.1	55.7
BTLA4+ (%)	S/S/Live/CD3/CD4 or CD8/2b4 | Freq. of Parent (%)	67.5	86.1	79.6	89.1
CCR4+ (%)	S/S/Live/CD3/CD4 or CD8/CCR4+ | Freq. of Parent (%)	94.1	93.6	84.2	96.6
CD25+ (%)	S/S/Live/CD3/CD4 or CD8/CD25 | Freq. of Parent (%)	63.1	61.0	47.5	51.8
CD69+ (%)	S/S/Live/CD3/CD4 or CD8/CD69 | Freq. of Parent (%)	47.0	22.8	45.4	25.8
CD95+ (%)	S/S/Live/CD3/CD4 or CD8/CD95 | Freq. of Parent (%)	99.2	98.1	99.1	97.6
CD103+ (%)	S/S/Live/CD3/CD4 or CD8/CD103 | Freq. of Parent (%)	4.2	10.7	25.0	36.9
CXCR3+ (%)	S/S/Live/CD3/CD4 or CD8/CXCR3 | Freq. of Parent (%)	58.1	76.7	89.3	88.3
KLRG1+ (%)	S/S/Live/CD3/CD4 or CD8/KLRG1 | Freq. of Parent (%)	0.3	8.9	3.1	35.8
LAG3+ (%)	S/S/Live/CD3/CD4 or CD8/Lag3 | Freq. of Parent (%)	5.0	3.3	19.4	21.2
PD1+ (%)	S/S/Live/CD3/CD4 or CD8/PD1 | Freq. of Parent (%)	29.3	22.1	11.2	7.2
TIGIT+ (%)	S/S/Live/CD3/CD4 or CD8/Tigit | Freq. of Parent (%)	68.1	53.2	74.9	65.8
TIM3+ (%)	S/S/Live/CD3/CD4 or CD8/TIM3 | Freq. of Parent (%)	93.6	81.8	91.2	84.4

Figure 74

	1:100 Stim/1:60 Unstim			1:200 Stim/1:100 Unstim			1:400 Stim/1:200 Unstim						
Sample	1	2	3	4	5	6	7	8	9	Avg	Stim-Unstim	Fold Induction	
Eng 1 Stimulated	13021.49	16033.00	14083.24	11068.85	15254.59	12534.24	9985.83	14237.23	11689.82	13089.81	11325.97	7.42	
Eng 1 Unstimulated	2155.31	2445.64	2108.37	1727.43	1982.96	1566.52	1119.087	1611.132	1158.179	1763.84			
Eng 1 Stimulated Ctrl	26491.92	31766.20	31601.13	26385.05	30852.42	29752.34	25175.43	33462.43	29728.52	29579.45	13486.52	1.84	
Eng 1 Unstimulated Ctrl	16209.90	16478.69	16936.51	15659.69	16385.60	17083.36	15419.07	15683.49	14960.40	16092.97			

	1:100 Stim/1:60 Unstim			1:200 Stim/1:100 Unstim			1:400 Stim/1:200 Unstim					
Sample	1	2	3	4	5	6	7	8	9	Avg	Stim-Unstim	Fold Induction
Eng 2 Stimulated	31509.54	30435.33	33822.81	27296.28	29091.87	29432.13	29027.45	30693.94	31138.70	30271.45	27441.29	10.70
Eng 2 Unstimulated	3236.56	3442.58	3630.97	2754.12	2839.66	2892.96	2047.795	2291.994	2324.838	2830.16		
Eng 2 Stimulated Ctrl	21515.93	20346.31	19425.54	16295.25	17398.51	18131.38	18835.27	18412.15	16278.94	18559.30	6189.32	1.48
Eng 2 Unstimulated Ctrl	13716.23	13445.60	13563.89	12782.28	12678.37	13122.55	11898.75	11573.48	12046.29	12769.97		

Figure 75A

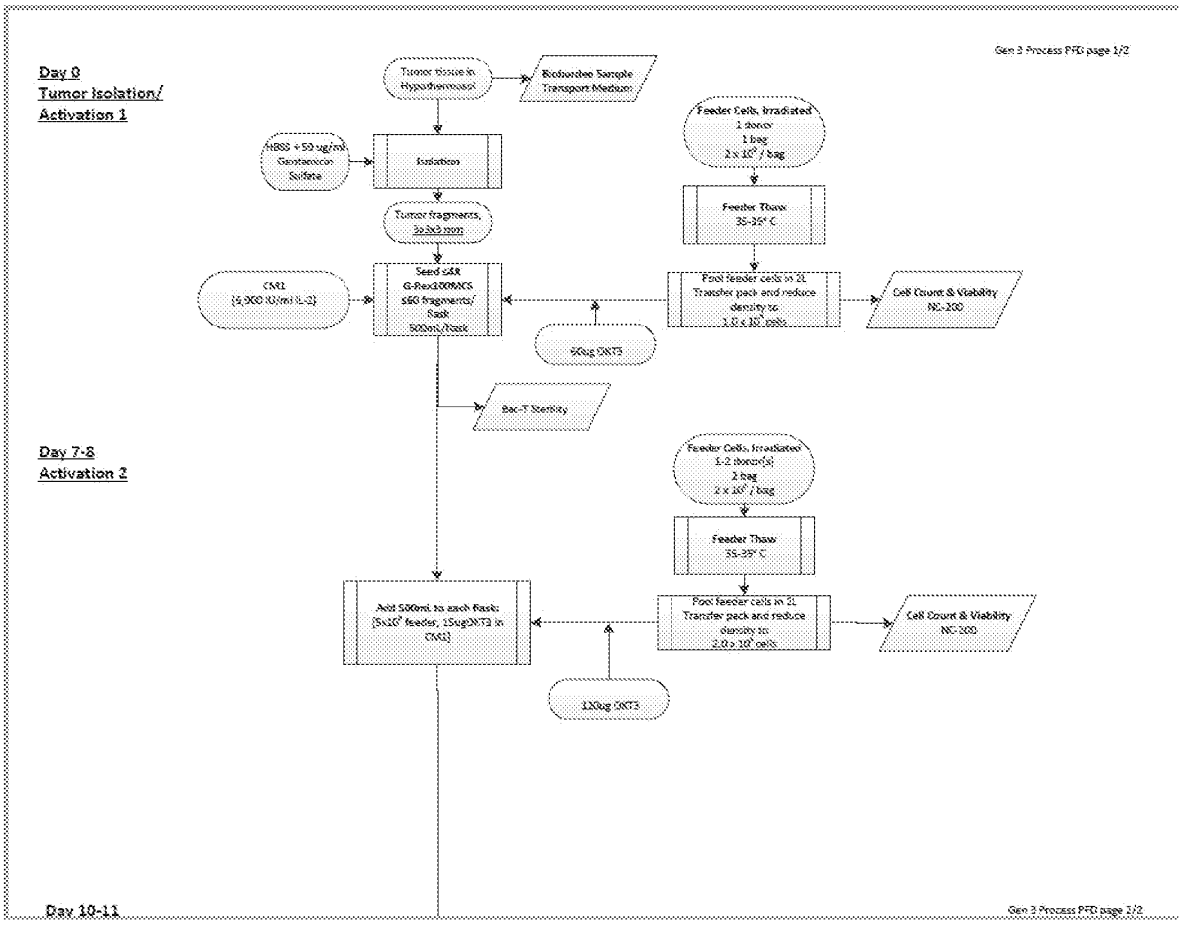

Gen 3 Process PFD page 1/2
Day 0
Tumor Isolation/
Activation 1
Tumor tissue in Hypothermosol
Biobanker Sample Transport Medium
HBSS +50 ug/ml Gentamycin Sulfate
Isolation
Tumor fragments, 30-50 mm
Feeder Cells, Irradiated
1 donor
1 bag
2 x 10^9 / bag
Feeder Thaw
35-37° C
CM1
(6,000 IU/ml IL-2)
Seed 1AR
G-Rex100MCS
≤60 fragments/
flask
500ml/flask
Pool feeder cells in 2L
Transfer pack and reduce
density to
1.0 x 10^9 cells
Cell Count & Viability
NC-200
90ug OKT3
Bac-T Sterility
Day 7-8
Activation 2
Feeder Cells, Irradiated
1-2 donor(s)
1 bag
2 x 10^9 / bag
Feeder Thaw
35-37° C
Add 500ml to each flask:
[5x10^8 feeder, 15ug OKT3 in
CM1]
Pool feeder cells in 2L
Transfer pack and reduce
density to:
2.0 x 10^9 cells
Cell Count & Viability
NC-200
120ug OKT3
Day 10-11
Gen 3 Process PFD page 2/2

Figure 75B

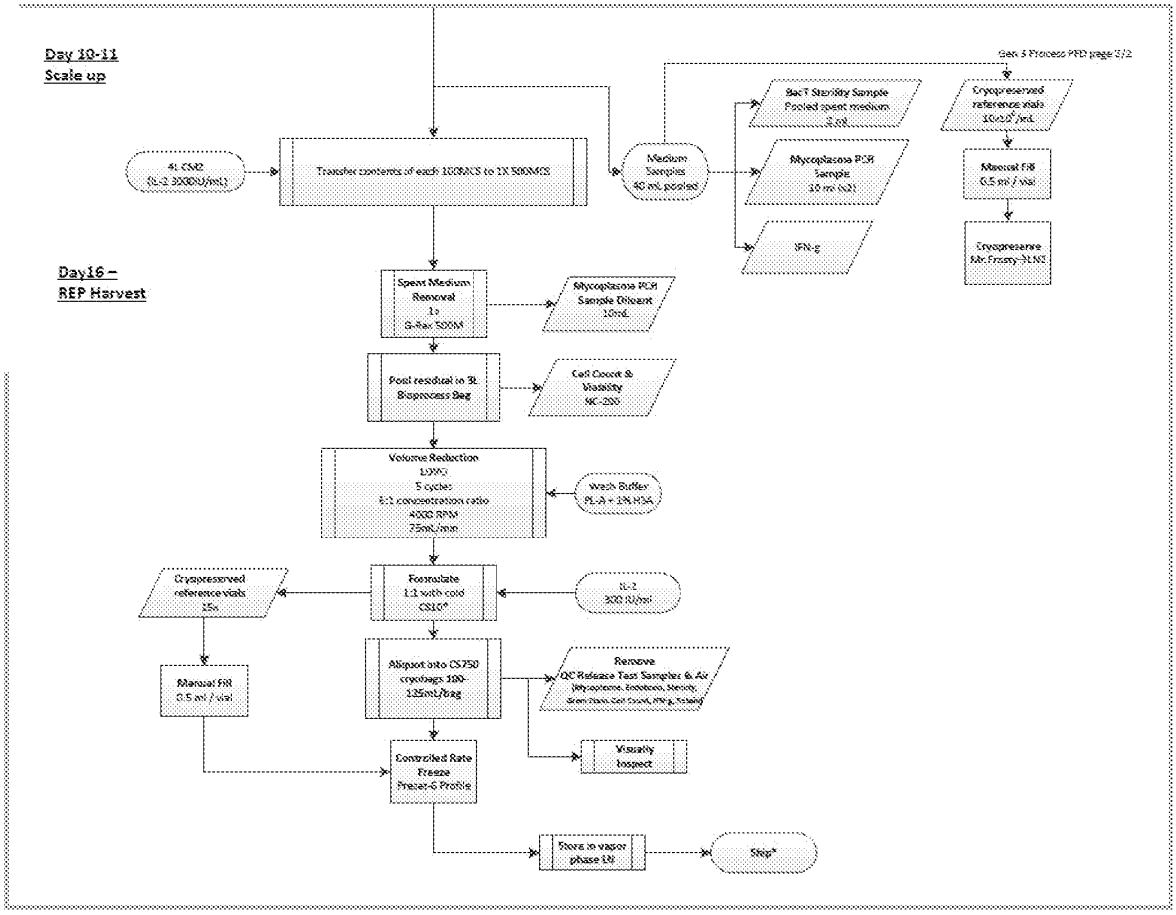

Day 10-11
Scale up
Gen 3 Process PFD page 1/2
4L CM2
(IL-2 3000IU/mL)
Transfer contents of each 1000MCS to 1X 5000MCS
Medium Samples
40 mL pooled
BacT Sterility Sample
Pooled spent medium
3.0 ml
Mycoplasma PCR Sample
10 ml (x2)
IFN-g
Cryopreserved reference vials
10x10^6 /mL
Mascot Fill
0.5 ml / vial
Cryopreserve
Mr. Frosty -LN2
Day16 –
REP Harvest
Spent Medium Removal
1x
0.8um 5000M
Mycoplasma PCR Sample Diluent
10mL
Post residual in 3L Bioprocess Bag
Cell Count & Viability
NC-200
Volume Reduction
LOVO
5 cycles
5:1 concentration ratio
4000 RPM
75mL/min
Wash Buffer
PL-A + 1% HSA
Cryopreserved reference vials
25x
Formulate
1:1 with cold CS10*
IL-2
3000 IU/ml
Manual Fill
0.5 ml / vial
Aliquot into CS750 cryobags 100-125ml/bag
Remove
QC Release Test Samples & Ali
Visually Inspect
Controlled Rate Freeze
Freezer-6 Profile
Store in vapor phase LN
Ship*

Figure 76

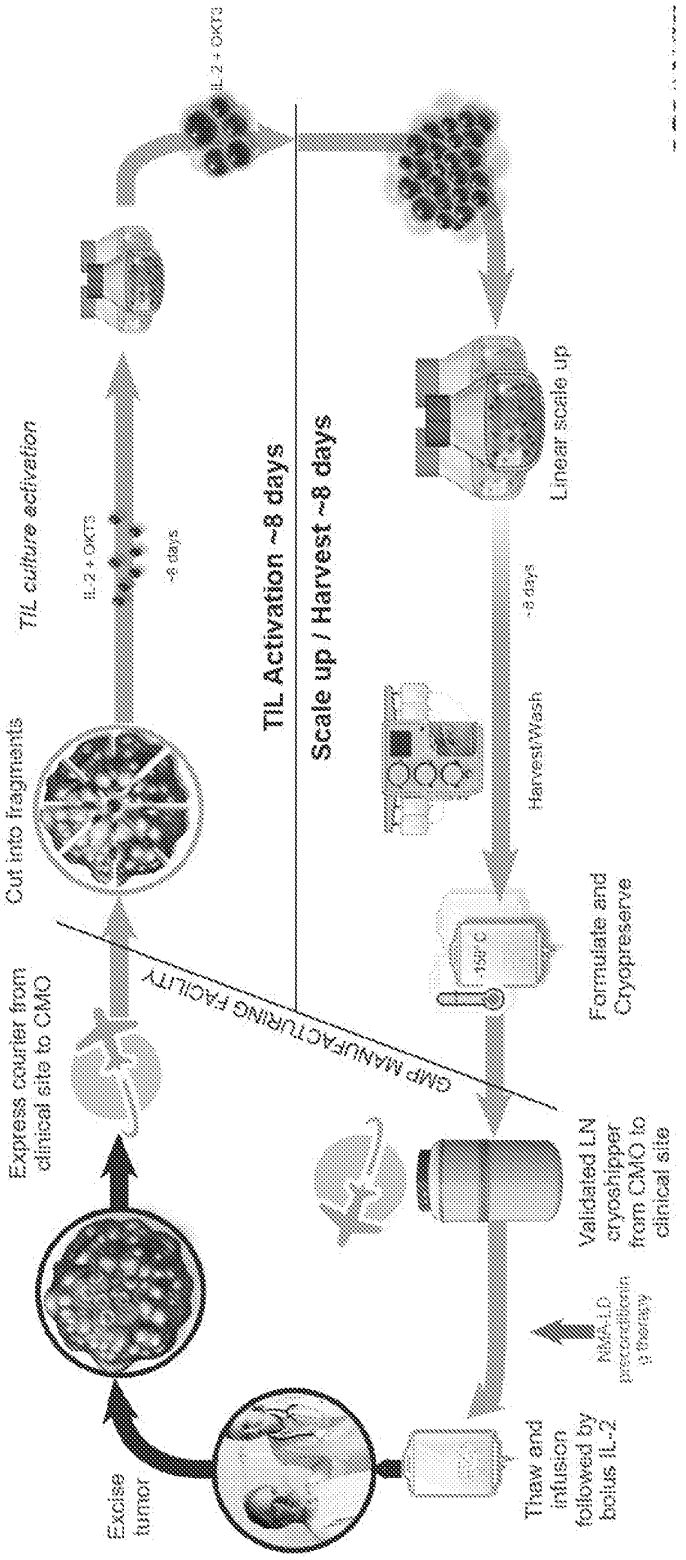

Express courier from clinical site to CMO
Cut into fragments
TIL culture activation
IL-2 + OKT3
~8 days
IL-2 + OKT3
TIL Activation ~8 days
Scale up / Harvest ~8 days
GMP MANUFACTURING FACILITY
Linear scale up
~8 days
Harvest/Wash
Formulate and Cryopreserve
Validated LN cryoshipper from CMO to clinical site
Thaw and infusion followed by bolus IL-2
Excise tumor

Figure 77

STEP	Gen 2	Gen 2.1	Gen 3.0
Pre REP- day 0	≤ 50 fragments/ 1 G-Rex 100MCS - **11 days**	≤ 180 fragments/ 3 G-Rex, Pre-formulated CM1 warmed media 100MCS - **11 days**	*Fresh or Frozen Tumor* Whole tumor with ≤ 30 fragments up to 60 fragments per 1 G-Rex 100MCS (up to 4 G-Rex), preformulated, warmed media - **7 days. Pre REP; Feeders 250e6 cells + OKT-3 (15ug)**
REP Initiation	Direct to REP- Day 11- **<200e^6 TIL** 1 G-Rex 500MCS	Direct to REP- Day 11- **<200 e^6 TIL** Pre-formulated CM2 warmed media in one G-Rex 500MCS	Direct to REP- Day **7-all cells TIL- same G-Rex 100MCS (100MCS up to 4 GREX), Standard media or Defined Media (Serum free). Addition Feeders 500e6 cells +OKT-3 (30ug)**
TIL propagation or Scale up	1 to 5 G-REX 500MCS **Split day 16**	2 to 5 G-REX 500MCS Pre-formulated CM4 warmed media **Split day 16**	From G-REX 100MCS transfer TIL suspension to G-REX 500MCS . up to 4 GREX 500 MCS- Standard media or Defined Media (Serum Free) **Scale up on day 10 or 11**
Harvest	**Harvest day 22,** LOVO-automated cell washer	**Harvest day 22,** LOVO-automated cell washer (5 wash cycle)	**Harvest day 14 or 16** LOVO- automated cell washer (5 wash cycle)
Final formulation	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2, multiple aliquots	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2, multiple aliquots	Cryopreserved product 300IU/ml IL-2-CS10 in LN_2, multiple aliquots
Process time	**22 days**	**22 days**	**16 days**

Figure 78

Process Comparison	Process Changes	Differences
Gen 2 : Gen 2.1	• Initiate process with two flasks instead of one flask • Divide REP initiation feeder layer between 2 G-Rex500MCS Flasks • Pre-formulate media and warm prior to use	• Potential doubling of final cell count (dose) with increased TIL repertoire. • Process redundancy throughout process
Gen 2.1 : Gen 3.1	• Fresh or Frozen tumor • 14-16 day process (from 22 day) • Reduce total feeder layer on process • Feeder layer and OKT3 present at Day 0 • REP initiated with fragments • 100MCS scales to 500MCS • Scales to multiple pre-REP flasks • Standard Media and Defined Media (Serum Free)	• Increased potency • Improved phenotype • Decreased process time • Reduced reagent testing • Decreased process variability • Defined reagents • Increased repertoire • Reduce impurities (feeder) • Comparable or Higher Dose.

Figure 79

Table 2: Description of Gen 3 optimization conditions.

Process Day	Conditions	Gen 3.0	Gen 3.1 control	Gen 3.1 Test
Day 0 : Tumor Fragment Isolation and Activation	Media (*)	500 mL	500 mL	500 mL
	IL-2	6000 IU/mL	6000 IU/mL	6000 IU/mL
	OKT-3	-	15 ug	15 ug
	Feeders	-	-	2.5E+06
Process Day	**Conditions**	**Gen 3.0**	**Gen 3.1 control**	**Gen 3.1 Test**
Day 7- 8 : TIL Culture Reactivation	Media (*)	500 mL	500 mL	500 mL
	IL-2	6000 IU/mL	6000 IU/mL	6000 IU/mL
	OKT-3	30 ug	30 ug	30 ug
	Feeders	1 E+09	500 E+06	500E+06
	Total Feeders added through Day 7	1 E+09	500 E+06	750E+06
Process Day	**Conditions**	**Gen 3.0**	**Gen 3.1 control**	**Gen 3.1 Test**
Day 10-11 : Culture Scale Up	From GREX 100 transfer whole TIL suspension to 1 GREX 500 containing 4L media with IL-2 (3000 IU/mL)			
Process Day	**Conditions**	**Gen 3.0**	**Gen 3.1 control**	**Gen 3.1 Test**
Day 16 –17: Harvest/Wash/Formulate	LOVO automated cell washer and cryopreservation with CS10.			

(*) Media can be standard media or CTS serum free media.

Figure 80

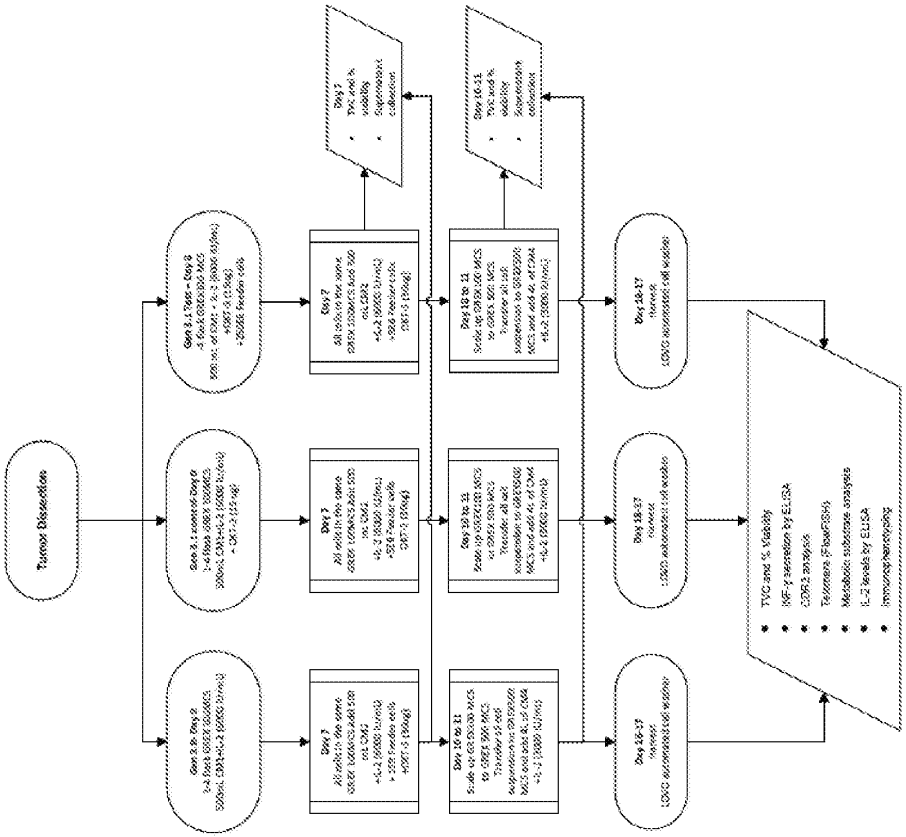

Tumor Dissection
TVC and % Viability
INF-γ secretion by ELISA
CD69 analysis
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

Figure 81

Process Day	L4063 in Standard Media			L4064 in Serum Free Media		
	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Number of fragments	53	53	53	21	21	21
Average TVC at harvest per fragment	1.37E+08	3.19E+08	3.53E+08	5.90E+08	7.57E+08	9.29E+08
TVC day 7 (*)	2.59E+07	6.43E+07	1.45E+08	7.35E+06	9.28E+07	1.39E+08
TVC day 10/11 (*)	6.07E+08	1.66E+09	1.83E+09	7.48E+08	1.28E+09	1.72E+09
TVC harvest day 16/17	7.26E+09	1.69E+10	1.87E+10	1.24E+10	1.59E+10	1.95E+10
Fold Expansion (day 10 or 11 /day 7)	23.4	25.8	12.6	101.8	13.8	12.4
Fold Expansion (Harvest day 16 / day 7)	280.3	262.8	129.0	1687.1	171.3	140.3

(*) Cell counts for Day 7 and Day 10/11 were taken FIO. Based on the design of the process, samples were pulled directly from each flask after swirling its contents without volume reduction. As such, analysis and conclusions of cell count data from these samples is limited by the nature of the sampling methodology.

Figure 82

	L4063 in Standard media			L4064 in CTS Serum Free media		
Process Day	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Reactivation	74.6%	92.1%	88.7%	88.0%	92.8%	95.0%
Scale up	92.1%	95.4%	94.0%	90.4%	94.3%	95.1%
Harvest	79.5%	89.4%	94.8%	88.8%	81.9%	84.9%

Figure 83

Tumor ID	L4063 in Standard media			L4064 in CTS Serum Free media		
Markers	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
L/D Aqua	82.2	89.4	91.0	76.6	83.1	89.5
NK	1.3	0.4	1.0	2.5	0.2	0.5
TCRa/b+	88.6	97.5	95.5	93.0	97.3	96.7
CD4	56.4	85.8	77.5	33.8	64.8	57.4
CD4/CD27	3.8	2.1	3.4	6.7	0.7	2.2
CD4/CD28	82.7	71.8	80.2	97.5	73.5	66.8
CD4/CD56	0.4	0.5	0.7	0.5	0.5	0.3
CD4/CD57	6.7	17.0	20.5	16.0	7.6	8.0
CD8	29.6	11.2	16.8	51.9	28.9	36.1
CD8/CD27	15.0	25.6	30.5	14.1	3.3	6.7
CD8/CD28	47.5	23.0	24.5	88.0	45.1	37.3
CD8/CD56	3.3	2.3	4.2	6.6	10.3	10.0
CD8/CD57	0.3	1.2	1.1	1.3	2.0	2.5

Figure 84

Tumor ID	L4063 in Standard media			L4064 in CTS Serum Free media		
Markers	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
CD3/CD4	56.4	85.8	77.5	33.8	64.8	57.4
TEMRA of CD4	0.4	0.7	0.4	1.1	0.7	0.2
NAIVE of CD4	0.3	0.2	0.2	0.5	0.1	0.0
CM of CD4	45.6	16.8	29.3	46.3	30.6	29.9
EM of CD4	53.7	82.3	70.2	52.1	68.6	69.9
CD3/CD8	29.6	11.2	16.8	51.9	28.9	36.1
TEMRA of CD8	3.1	0.5	0.9	6.1	8.0	5.4
NAIVE of CD8	0.7	0.1	0.4	2.2	0.7	1.0
CM of CD8	22.0	15.8	29.7	61.9	18.9	18.2
EM of CD8	74.3	83.5	69.0	29.8	72.4	75.5

Figure 85

Tumor ID	L4063 in Standard media			L4064 in CTS Serum Free media		
Markers	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
2B4+	2.1	0.6	1.0	2.0	1.2	1.3
BTLA4	99.7	95.4	96.9	99.5	98.8	98.1
CD25+	48.6	10.5	3.9	58.2	41.7	32.1
CD69+	27.1	37.7	21.5	39.4	35.6	30.5
CD95+	99.9	99.9	99.5	99.6	99.0	97.3
CD103+	0.7	1.2	0.4	1.4	1.3	1.2
KLRG1+	1.6	3.7	3.3	11.4	8.3	10.7
LAG3+	5.6	8.2	3.3	1.5	1.6	1.4
PD1+	77.4	58.4	41.6	34.1	28.4	26.7
TIGIT+	68.5	40.3	30.3	45.8	52.1	52.2
TIM3+	58.0	42.4	59.4	61.0	58.4	56.8

Figure 86

Tumor ID	L4063 in Standard media			L4064 in CTS Serum Free media		
Markers	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
2B4+	65.8	56.6	70.5	13.6	21.5	20.7
BTLA4	99.5	96.6	98.2	99.3	97.4	96.1
CD25+	41.4	10.5	5.9	60.6	32.0	17.1
CD69+	44.9	28.0	20.0	33.2	30.6	22.7
CD95+	99.6	99.6	98.9	98.9	96.1	93.5
CD103+	29.4	27.9	24.5	10.9	6.0	7.3
CXCR3+	99.9	99.9	99.8	99.3	99.2	99.2
KLRG1+	7.1	4.6	6.7	13.2	14.5	14.1
LAG3+	28.6	22.5	11.9	2.8	1.6	1.4
PD1+	56.9	54.5	51.7	25.4	20.0	23.6
TIGIT+	90.8	93.7	93.3	92.1	90.4	90.5
TIM3+	66.7	49.1	58.6	65.6	46.8	43.6

Figure 87

TIL Characterization	L4063 in Standard media			L4064 in CTS Serum Free media		
	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
IFNγ (pg/mL)	907	428	2863	1485	3130	3863

Results are reported as mean of triplicate samples from one representative experiment

Figure 88

Process Day	L4063 in Standard media			L4064 in CTS Serum Free media		
	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Reactivation	12319.6	11928.7	11786.7	5891.6	5671.8	389.4[(1)]
Scale Up	32095.1	30368.6	26145.0	*	*	*
Harvest	17309.3	13828.4	16019.2	656.0	826.2	768.9

Results are reported as mean of triplicate samples from one representative experiment

(*) Spent media from day 10 /11 on L4064 was not collected during the execution of the runs.

(1) The data for Gen 3.1 Test L4064 on reactivation day was significantly below the corresponding values for Gen 3.1 control and Gen 3.0 conditions. Due to the low amount of sample taken, it was not possible to repeat the assay to verify the accuracy of this number.

Figure 89

Glucose (g/L)						
	L4063 in Standard media			L4064 in CTS Serum Free media		
Process Day	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Reactivation	1.8	1.7	1.4	4.3	4.1	3.8
Scale Up	1.4	0.9	0.8	*	*	*
Harvest	1.5	0.9	1.0	2.8	2.1	2.2

(*) Spent media from day 10 /11 on L4064 was not collected during the execution of the runs.

Figure 90

Lactate (g/L)						
	L4063 in Standard media			L4064 in CTS Serum Free media		
Process Day	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Reactivation	0.2	0.2	0.5	0.1	0.2	0.5
Scale Up	0.7	1.1	1.2	*	*	*
Harvest	1.1	1.7	1.6	1.5	2.0	1.9

(*) Spent media from day 10 /11 on L4064 was not collected during the execution of the runs.

Figure 91

Glutamine (mmol/L)						
	L4063 in Standard media			L4064 in CTS Serum Free media		
Process Day	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Reactivation	2.0	2.0	2.0	1.3	1.2	1.1
Scale Up	1.7	1.1	1.1	*	*	*
Harvest	1.9	1.3	1.3	0.7	0.6	0.6

(*) Spent media from day 10 /11 on L4064 was not collected during the execution of the runs.

Figure 92

Glutamax (mmol/L)						
	L4063 in Standard media			L4064 in CTS Serum Free media		
Process Day	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Reactivation	2.2	2.2	2.2	1.4	1.3	1.2
Scale Up	1.9	1.2	1.2	*	*	*
Harvest	2.1	1.5	1.5	0.8	0.7	0.7

(*) Spent media from day 10 /11 on L4064 was not collected during the execution of the runs.

Figure 93

Ammonia (mmol/L)						
	L4063 in Standard media			L4064 in CTS Serum Free media		
Process Day	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Reactivation	1.9	1.9	2.0	0.7	0.8	0.9
Scale Up	1.8	2.2	2.3	*	*	*
Harvest	2.1	2.5	2.5	1.0	1.0	0.9

(*) Spent media from day 10 /11 on L4064 was not collected during the execution of the runs.

Figure 94

	L4063 in Standard media				L4064 in CTS Serum Free media			
TIL Characterization	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test		Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	
Relative Telomere Length	7.7	6.3	6.1		6.4	6.7	6.6	

Figure 95

TIL Characterization	L4063 in Standard media			L4064 in CTS Serum Free media		
	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
uCD3	14005.0	18149.0	17181.0	15107.0	21460.0	21503.0
Shanon Entropy Index	5.9	10.8	10.5	8.5	10.7	10.4

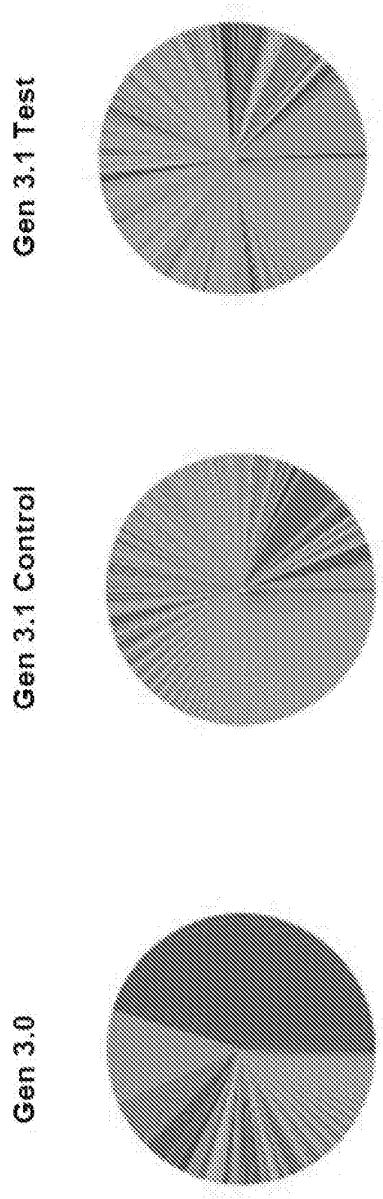


Figure 96

Figure 97

Number of u CDR3 (%overlap) L4063	All uCDR3's			Top 80% uCDR3's		
	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Gen 3.0	14005	3240 (23%)	2959 (21%)	1104	1000 (90 %)	975 (88%)
Gen 3.1 Control	-	18149	-	-	2437	-
Gen 3.1 Test	-	-	17181	-	-	2331

Figure 98

Number of u CDR3 (%overlap) L4064	All uCDR3's			Top 80% uCDR3's		
	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Gen 3.0	15107	5486 (36%)	5541 (36%)	2478	2186 (88%)	2163 (87%)
Gen 3.1 Control	-	21460	-	-	3246	-
Gen 3.1 Test	-	-	21503	-	-	3263

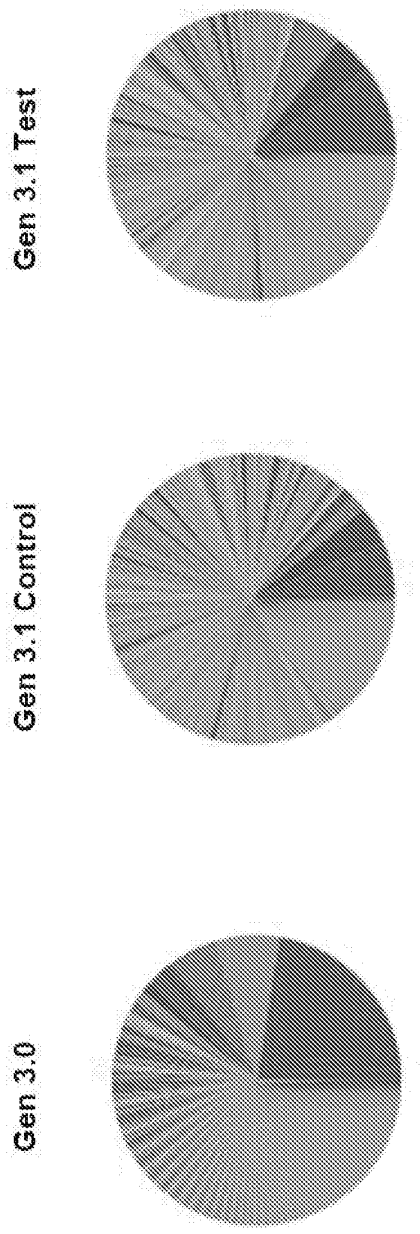


Figure 99

Figure 100

Step	Process Gen 3-Optimized
Day 0 Tumor isolation and Activation	≤240 fragments ≤60 fragments/flask ≤4 flasks ≤2L media (500mL/flask) IL-2 (6000IU/mL) $2.5x10^8$ feeder cells/flask 15ug OKT3/flask
Day 7 - 8 Reactivation	Fresh TIL direct to REP Activate entire culture $5x10^8$ feeder cells 30 ug OKT3/flask G-Rex 100MCS Add 500mL media+ IL-2(6000IU/mL)
Day 10 – 11 Scale up or TIL Sub-culture	≤4 G-REX 500MCS Scale up entire culture transferring 1L from GREX 100MCS into GREX 500MCS and add 4L of media +IL-2 (3000 IU/mL) /flask
Day 16 -17 Harvest	Harvest LOVO- automated cell washer Cryopreservation on Plasmalyte 1% HSA: CS10

Figure 101

Test	Acceptance Criteria	Gen 3.1Test vs Gen 3.0 Process
Cell Count (TVC)	Gen 3.1 > 30% to Process Gen 3.0	Met
% Viability	≥70% Viability	Met
Immunophenotyping (%CD3+/ %CD45+)	≤5% difference between Gen 3.1 and Gen 3.0 process	Met
IFNγ secretion	Gen 3.1 ≥ to Process Gen 3.0	Met

Figure 102

Cell counts reactivation Day

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3.0 (A) L4063	500	Total	7.10E+04	6.81E+04		6.96E+04	3.48E+07
		Live	5.27E+04	5.09E+04		5.18E+04	2.59E+07
		Dead	1.83E+04	1.72E+04		1.78E+04	8.88E+06
		% Viability	74.30%	74.80%		74.55%	
Gen 3.1 Control L4063	500	Total	1.41E+05	1.38E+05		1.40E+05	6.98E+07
		Live	1.31E+05	1.26E+05		1.29E+05	6.43E+07
		Dead	9.84E+03	1.21E+04		1.10E+04	5.49E+06
		% Viability	93.00%	91.20%		92.10%	
Gen 3.1 Test L4063	500	Total	3.26E+05	3.28E+05		3.27E+05	1.64E+08
		Live	2.83E+05	2.96E+05		2.90E+05	1.45E+08
		Dead	4.25E+04	3.16E+04		3.71E+04	1.85E+07
		% Viability	87.00%	90.30%		88.65%	
Gen 3.0 (A) L4064	500	Total	1.59E+04	1.75E+04		1.67E+04	8.35E+06
		Live	1.39E+04	1.55E+04		1.47E+04	7.35E+06
		Dead	1.98E+03	2.02E+03		2.00E+03	1.00E+06
		% Viability	87.50%	88.50%		88.00%	
Gen 3.1 Control L4064	500	Total	2.02E+05	1.98E+05		2.00E+05	1.00E+08
		Live	1.88E+05	1.83E+05		1.86E+05	9.28E+07
		Dead	1.38E+04	1.51E+04		1.45E+04	7.23E+06
		% Viability	93.20%	92.40%		92.80%	
Gen 3.1 Test L4064	500	Total	2.70E+05	3.15E+05		2.93E+05	1.46E+08
		Live	2.55E+05	3.01E+05		2.78E+05	1.39E+08
		Dead	1.51E+04	1.38E+04		1.45E+04	7.23E+06
		% Viability	94.40%	95.60%		95.00%	

Figure 103

Cell counts Scale Up Day

ID	Volume (mL)		Count 1 (cells/mL)	Count 3 (cells/mL)	Count 2 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3.0 (A) L4063	1000	Total	6.57E+05	6.61E+05		6.59E+05	6.59E+08
		Live	6.00E+05	6.14E+05		6.07E+05	6.07E+08
		Dead	5.71E+04	4.68E+04		5.20E+04	5.20E+07
		% Viability	91.30%	92.90%		92.10%	
Gen 3.1 Control L4063	1000	Total	1.66E+06	1.82E+06		1.74E+06	1.74E+09
		Live	1.59E+06	1.72E+06		1.66E+06	1.66E+09
		Dead	7.17E+04	9.07E+04		8.12E+04	8.12E+07
		% Viability	95.70%	95.00%		95.35%	
Gen 3.1 Test L4063	1000	Total	1.88E+06	2.00E+06		1.94E+06	1.94E+09
		Live	1.76E+06	1.89E+06		1.83E+06	1.83E+09
		Dead	1.20E+05	1.15E+05		1.18E+05	1.18E+08
		% Viability	93.60%	94.30%		93.95%	
Gen 3.0 (A) L4064	1000	Total	7.95E+05	8.58E+05		8.27E+05	8.27E+08
		Live	7.17E+05	7.78E+05		7.48E+05	7.48E+08
		Dead	7.85E+04	8.05E+04		7.95E+04	7.95E+07
		% Viability	90.10%	90.60%		90.35%	
Gen 3.1 Control L4064	1000	Total	1.38E+06	1.32E+06		1.35E+06	1.35E+09
		Live	1.31E+06	1.24E+06		1.28E+06	1.28E+09
		Dead	7.20E+04	8.13E+04		7.67E+04	7.67E+07
		% Viability	94.80%	93.80%		94.30%	
Gen 3.1 Test L4064	1000	Total	1.85E+06	1.76E+06		1.81E+06	1.81E+09
		Live	1.76E+06	1.67E+06		1.72E+06	1.72E+09
		Dead	8.62E+04	8.95E+04		8.79E+04	8.79E+07
		% Viability	95.30%	94.90%		95.10%	

Figure 104

Cell counts Harvest L4063

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3.0 (A) L4063 pre LOVO	602.4	Total	1.68E+07	1.70E+07		1.69E+07	1.02E+10
		Live	1.52E+07	1.54E+07		1.53E+07	9.22E+09
		Dead	1.60E+06	1.65E+06		1.63E+06	9.79E+08
		% Viability	90.50%	90.30%		90.40%	
Gen 3.1 Control L4063 pre LOVO	497.1	Total	3.39E+07	3.90E+07		3.65E+07	1.81E+10
		Live	3.25E+07	3.73E+07		3.49E+07	1.73E+10
		Dead	1.41E+06	1.63E+06		1.52E+06	7.56E+08
		% Viability	95.80%	95.80%		95.80%	
Gen 3.1 Test L4063 pre LOVO	552.3	Total	3.71E+07	3.62E+07		3.67E+07	2.02E+10
		Live	3.56E+07	3.51E+07		3.54E+07	1.95E+10
		Dead	1.50E+06	1.09E+06		1.30E+06	7.15E+08
		% Viability	96.00%	97.00%		96.50%	
Gen 3.0 (A) L4063 post LOVO	165	Total	5.37E+07	5.73E+07	5.50E+07	5.53E+07	9.13E+09
		Live	4.32E+07	4.52E+07	4.36E+07	4.40E+07	7.26E+09
		Dead	1.04E+07	1.21E+07	1.14E+07	1.13E+07	1.86E+09
		% Viability	80.50%	78.80%	79.30%	79.53%	
Gen 3.1 Control L4063 post LOVO	165	Total	1.21E+08	1.01E+08	1.22E+08	1.15E+08	1.89E+10
		Live	1.10E+08	8.85E+07	1.09E+08	1.03E+08	1.69E+10
		Dead	1.11E+07	1.23E+07	1.27E+07	1.20E+07	1.99E+09
		% Viability	90.90%	87.80%	89.60%	89.43%	
Gen 3.1 Test L4063 post LOVO	175	Total	1.05E+08	1.20E+08		1.13E+08	1.97E+10
		Live	9.93E+07	1.14E+08		1.07E+08	1.87E+10
		Dead	5.51E+06	6.14E+06		5.83E+06	1.02E+09
		% Viability	94.70%	94.90%		94.80%	

Figure 105

Cell counts Harvest L4064

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3.0 (A) L4064 pre LOVO	641.3	Total	1.99E+07	1.94E+07		1.97E+07	1.26E+10
		Live	1.83E+07	1.94E+07		1.89E+07	1.21E+10
		Dead	1.60E+06	1.68E+06		1.64E+06	1.05E+09
		% Viability	92.00%	92.00%		92.00%	
Gen 3.1 Control L4064 pre LOVO	493.4	Total	3.98E+07	3.87E+07		3.93E+07	1.94E+10
		Live	3.65E+07	3.55E+07		3.60E+07	1.78E+10
		Dead	3.31E+06	3.22E+06		3.27E+06	1.61E+09
		% Viability	91.70%	91.70%		91.70%	
Gen 3.1 Test L4064 pre LOVO	593.4	Total	3.92E+07	3.79E+07		3.86E+07	2.29E+10
		Live	3.67E+07	3.54E+07		3.61E+07	2.14E+10
		Dead	2.55E+06	2.50E+06		2.53E+06	1.50E+09
		% Viability	93.50%	93.40%		93.45%	
Gen 3.0 (A) L4064 post LOVO	165	Total	8.58E+07	8.34E+07		8.46E+07	1.40E+10
		Live	7.56E+07	7.46E+07		7.51E+07	1.24E+10
		Dead	1.02E+07	8.79E+06		9.50E+06	1.57E+09
		% Viability	88.10%	89.50%		88.80%	
Gen 3.1 Control L4064 post LOVO	165	Total	1.13E+08	1.22E+08	1.15E+08	1.18E+08	1.94E+10
		Live	9.21E+07	1.01E+08	9.45E+07	9.66E+07	1.59E+10
		Dead	2.14E+07	2.13E+07	2.07E+07	2.14E+07	3.52E+09
		% Viability	81.20%	82.60%	82.10%	81.90%	
Gen 3.1 Test L4064 post LOVO	165	Total	1.33E+08	1.45E+08		1.39E+08	2.29E+10
		Live	1.14E+08	1.22E+08		1.18E+08	1.95E+10
		Dead	1.94E+07	2.26E+07		2.10E+07	3.47E+09
		% Viability	85.40%	84.40%		84.90%	

Figure 106

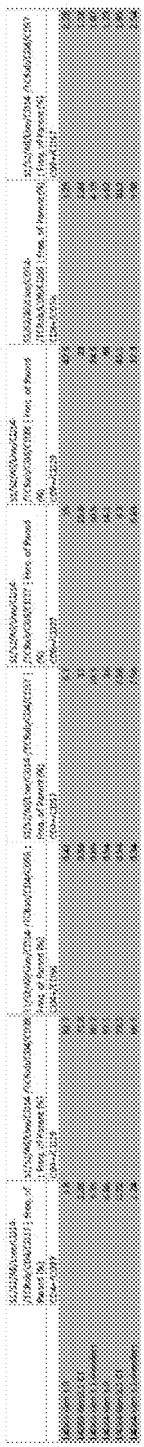


Figure 107

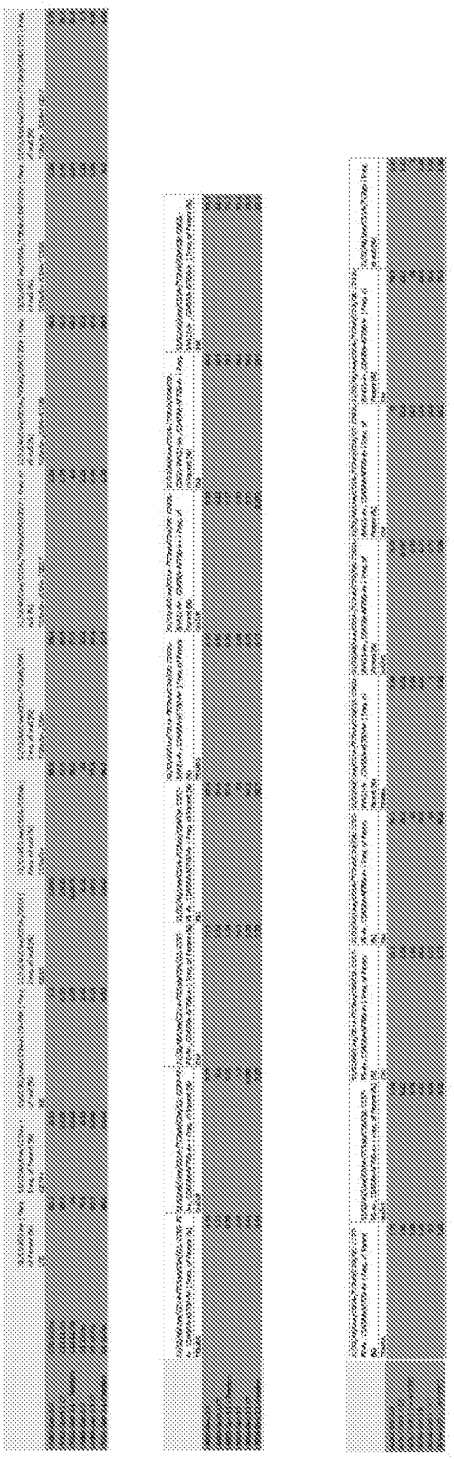


Figure 1108



Figure 109



Figure 110

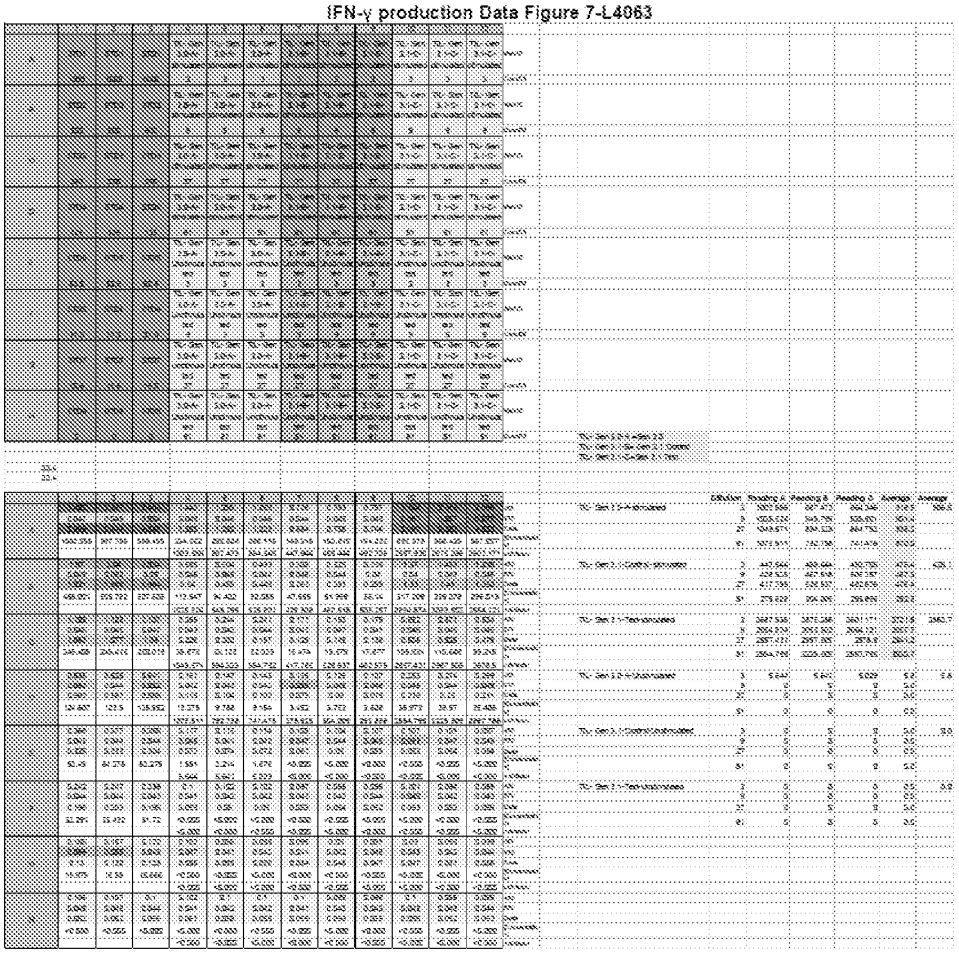


Figure 111

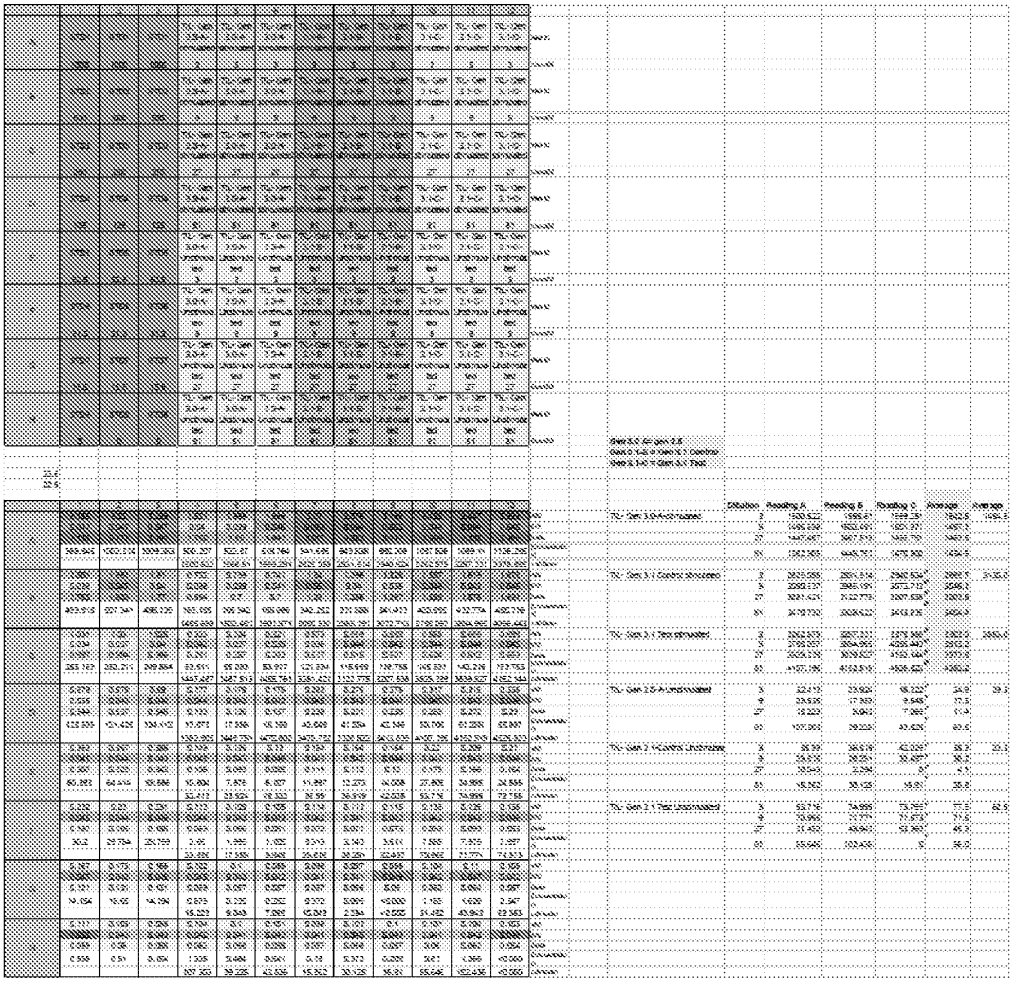


Figure 112

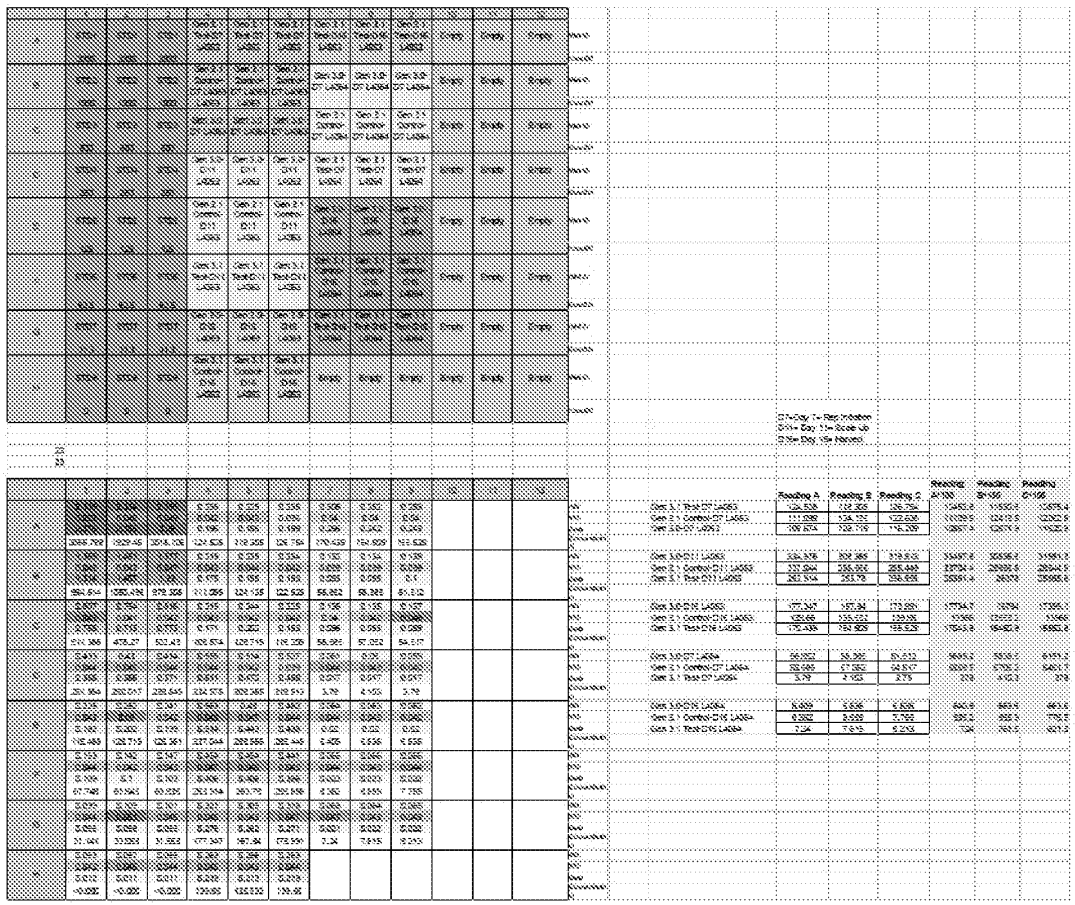


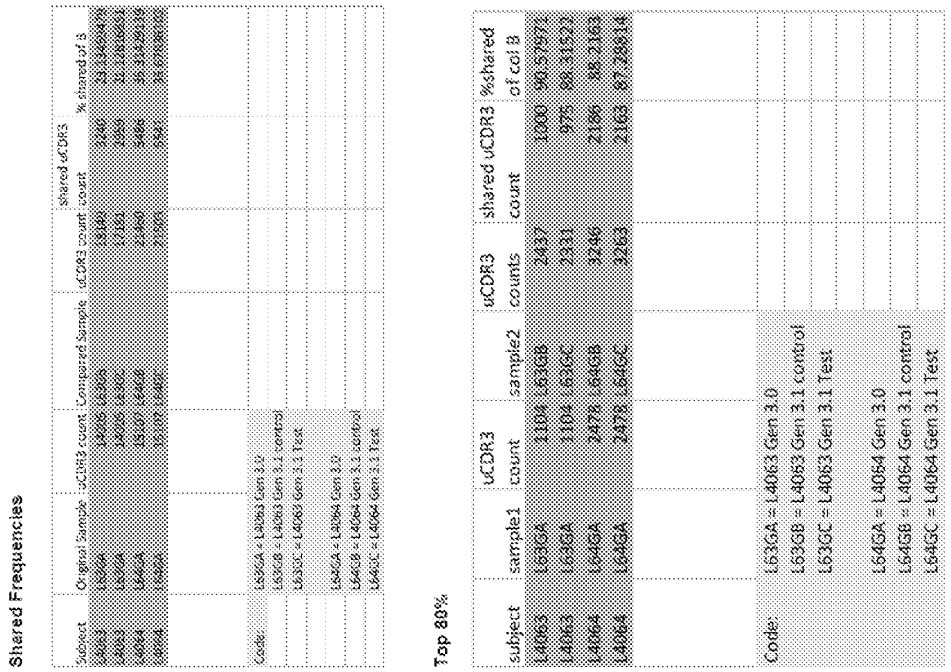
Figure 113

Sample number #	Description Gen 3.1 Optimization	Glucose (g/L)	Lactate (g/L)	Ammonia (mmol/L)	Glutamine (mmol/L)	Glutamax (mmol/L)	Glutamax-Glutamine (mmol/L)
13	Gen 3.0-Day 16 First Round #L4063	1.52	1.07	2.06	1.91	2.11	0.20
14	Gen 3.1 Control-Day 16 First Round #L4063	0.90	1.74	2.50	1.34	1.49	0.15
15	Gen 3.1 Test-Day 16 First Round #L4063	0.96	1.64	2.49	1.33	1.47	0.14
16	Gen 3.0-Day 11 First Round #L4063	1.42	0.70	1.83	1.70	1.86	0.15
17	Gen 3.1 Control-Day 11 First Round #L4063	0.90	1.08	2.24	1.07	1.19	0.11
18	Gen 3.1 Test-Day 11 First Round #L4063	0.84	1.21	2.25	1.11	1.23	0.12
19	Gen 3.0-Day 7 First Round #L4063	1.76	0.20	1.88	2.04	2.23	0.19
20	Gen 3.1 Control-Day 7 First Round #L4063	1.73	0.22	1.86	2.00	2.19	0.19
21	Gen 3.1 Test-Day 7 First Round #L4063	1.41	0.50	1.95	1.98	2.15	0.17
22	Gen 3.0-Day 16 Second Round #L4064	2.76	1.52	1.00	0.68	0.77	0.09
23	Gen 3.1 Control-Day 16 Second Round #L4064	2.14	1.95	0.99	0.64	0.73	0.08
24	Gen 3.1 Test-Day 16 Second Round #L4064	2.20	1.89	0.94	0.64	0.71	0.07
25	Gen 3.0-Day 7 Second Round #L4064	4.26	0.08	0.71	1.29	1.43	0.14
26	Gen 3.1 Control-Day 7 Second Round #L4064	4.14	0.22	0.78	1.17	1.29	0.12
27	Gen 3.1 Test-Day 7 Second Round #L4064	3.82	0.49	0.89	1.09	1.20	0.11

Figure 114

Order	Name		Geometric Mean Probe-fitc-A	Geometric Mean Probe-fitc-A	Average 1301	Average T	Length relative to 1301
A1	Gen 3.0-L4063	A1 10.1.fcs	86	42.5			
A2		A2 10.2.fcs	85.4	42.3	85.7	42.4	
A3		A3 10.3.fcs	4585	207			
A4		A4 10.4.fcs	4389	216	4487	211.5	7.684093
A5	Gen 3.1 Control-L4063	A5 11.1.fcs	82.7	33.3			
A6		A6 11.2.fcs	86	32.9	84.35	33.1	
A7		A7 11.3.fcs	4696	180			
A8		A8 11.4.fcs	4641	175	4668.5	177.5	6.299968
B1	Gen 3.1 Test-L4063	B1 12.1.fcs	95.1	28.8			
B2		B2 12.2.fcs	84.7	26.7	89.9	27.75	
B3		B3 12.3.fcs	4764	169			
B4		B4 12.4.fcs	4800	174	4782	171.5	6.12732
B5	Gen 3.0 -L4064	B5 13.1.fcs	101	40.3			
B6		B6 13.2.fcs	89.6	34.1	95.3	37.2	
B7		B7 13.3.fcs	4300	174			
B8		B8 13.4.fcs	4367	172	4333.5	173	6.408381
C1	Gen 3.1 Control-L4064	C1 14.1.fcs	92.1	31.1			
C2		C2 14.2.fcs	88.6	29.6	90.35	30.35	
C3		C3 14.3.fcs	4111	169			
C4		C4 14.4.fcs	4443	172	4277	170.5	6.69509
C5	Gen 3.1 Test-L4064	C5 15.1.fcs	89.7	25.7			
C6		C6 15.2.fcs	85.7	24.7	87.7	25.2	
C7		C7 15.3.fcs	4342	172			
C8		C8 15.4.fcs	4560	166	4451	169	6.591341

Figure 115



Shared Frequencies

Subject	Original Sample	uCDR3 count	Compared Sample	uCDR3 count	shared uCDR3 count	% shared of B
L63C	L63GA	13008	L63GA	3330	3240	33.1349436
L63C	L63GB	14206	L63GB	11281	2353	31.1188653
L64C	L64GA	15139	L64GA	2468	3484	35.1352548
L64C	L64GB	15139	L64GB	2468	3541	35.6760618

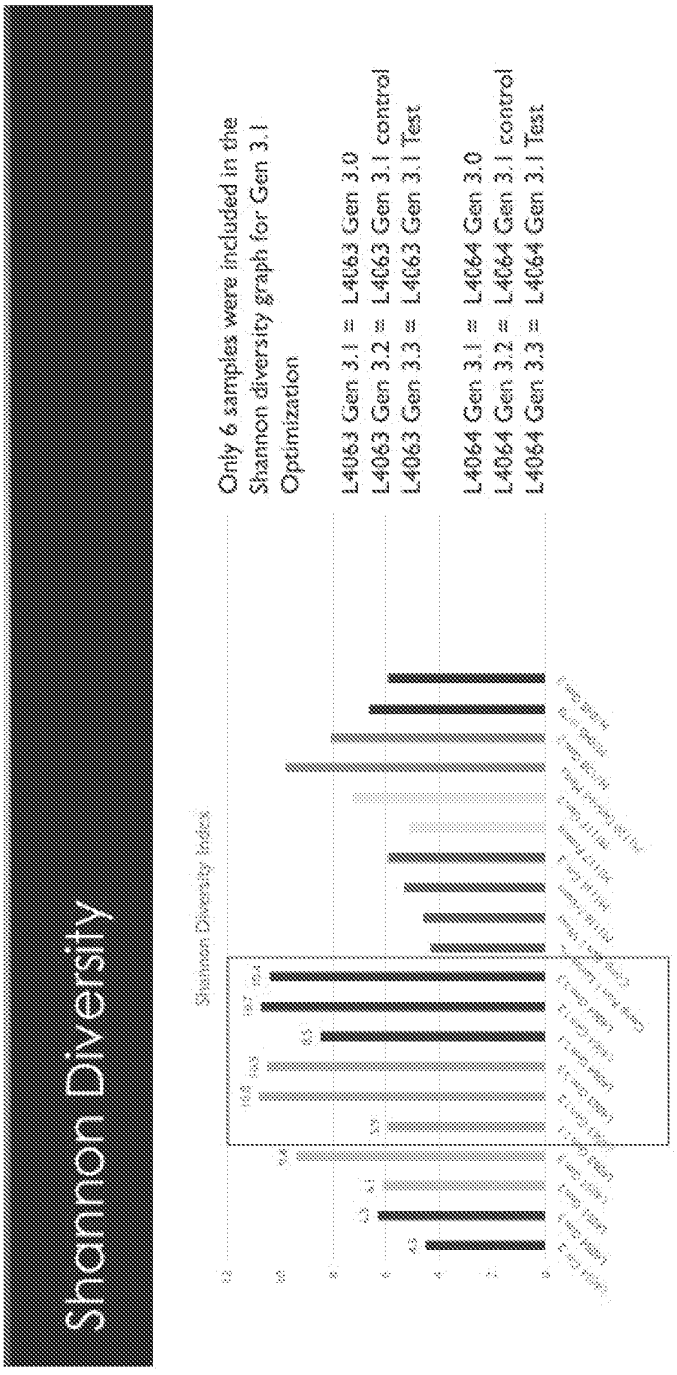
Code:	
	L63GA = L4063 Gen 3.0
	L63GB = L4063 Gen 3.1 control
	L63GC = L4063 Gen 3.1 Test
	L64GA = L4064 Gen 3.0
	L64GB = L4064 Gen 3.1 control
	L64GC = L4064 Gen 3.1 Test

Top 80%

subject	sample1	uCDR3 count	sample2	uCDR3 counts	shared uCDR3 count	%shared of col B
L4063	L63GA	1104	L63GB	2437	1000	90.57971
L4063	L63GA	1104	L63GC	2331	975	88.31522
L4064	L64GA	2478	L64GB	3246	2186	88.2183
L4064	L64GA	2478	L64GC	3263	2163	87.28814

Code:	
	L63GA = L4063 Gen 3.0
	L63GB = L4063 Gen 3.1 control
	L63GC = L4063 Gen 3.1 Test
	L64GA = L4064 Gen 3.0
	L64GB = L4064 Gen 3.1 control
	L64GC = L4064 Gen 3.1 Test

Figure 116


Shannon Diversity
Only 6 samples were included in the Shannon diversity graph for Gen 3.1 Optimization
L4063 Gen 3.1 = L4063 Gen 3.0
L4063 Gen 3.2 = L4063 Gen 3.1 control
L4063 Gen 3.3 = L4063 Gen 3.1 Test
L4064 Gen 3.1 = L4064 Gen 3.0
L4064 Gen 3.2 = L4064 Gen 3.1 control
L4064 Gen 3.3 = L4064 Gen 3.1 Test
Shannon Diversity Index