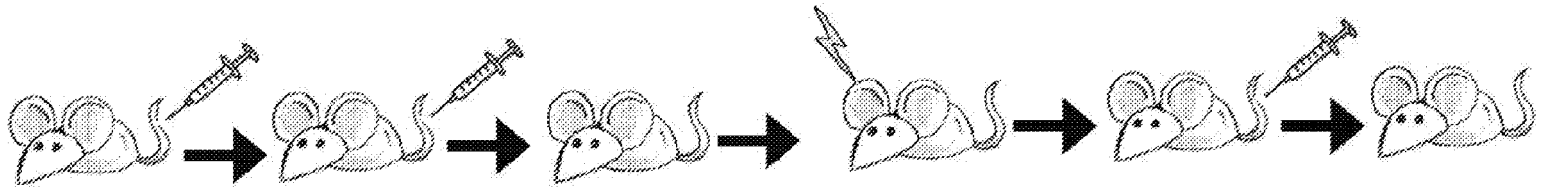
FIG. 1



Day -38	Day -24	Day -16	Day -1	Day 0	Days 30,60,90
Immunization 75ug OVA+CFA s.c. to OT-1RAGCD45.1 hosts	Immunization Boost 75ug OVA+IFA s.c. to OT-1RAGCD45.1 hosts	Initiation of anti-3rd party Co-culuture of immunized OT-1 spleens against irradiated OVA splenocytes enriched for CD8+CD44+ OR WT splenocytes vs. irradiated FVB	TBI 6/6.25/6.5Gy C57 host	20*10^{6} BALB/C NuBM ± 5*10^{6} CD8+CD44+ derived OT-1 Tcm OR C57BL/6 Tcm	Chimerism check in peripheral blood

FIG. 2A

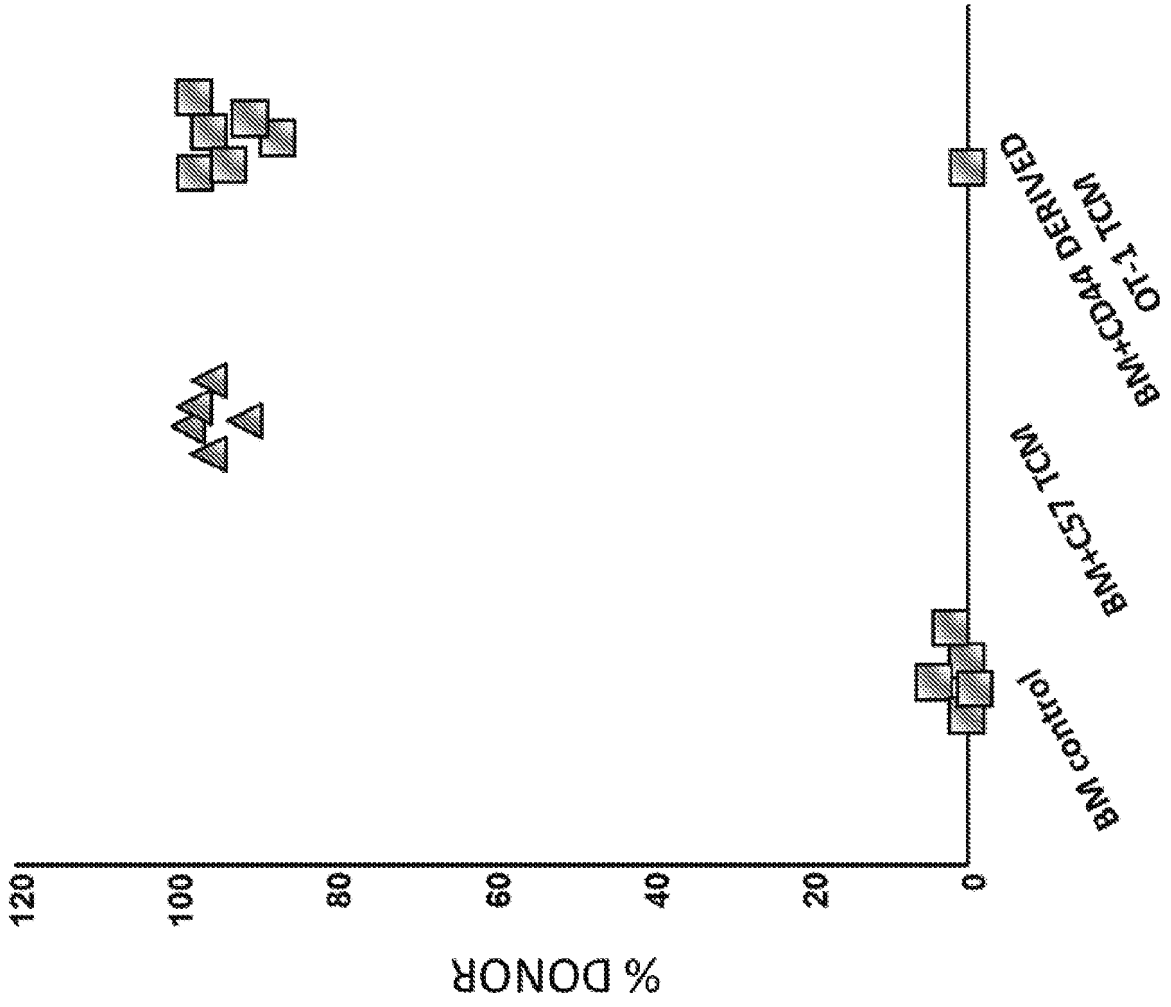

% DONOR
120
100
80
60
40
20
0
BM control
BM+C57 TCM
BM+CD4 DERIVED OT-1 TCM

FIG. 2B

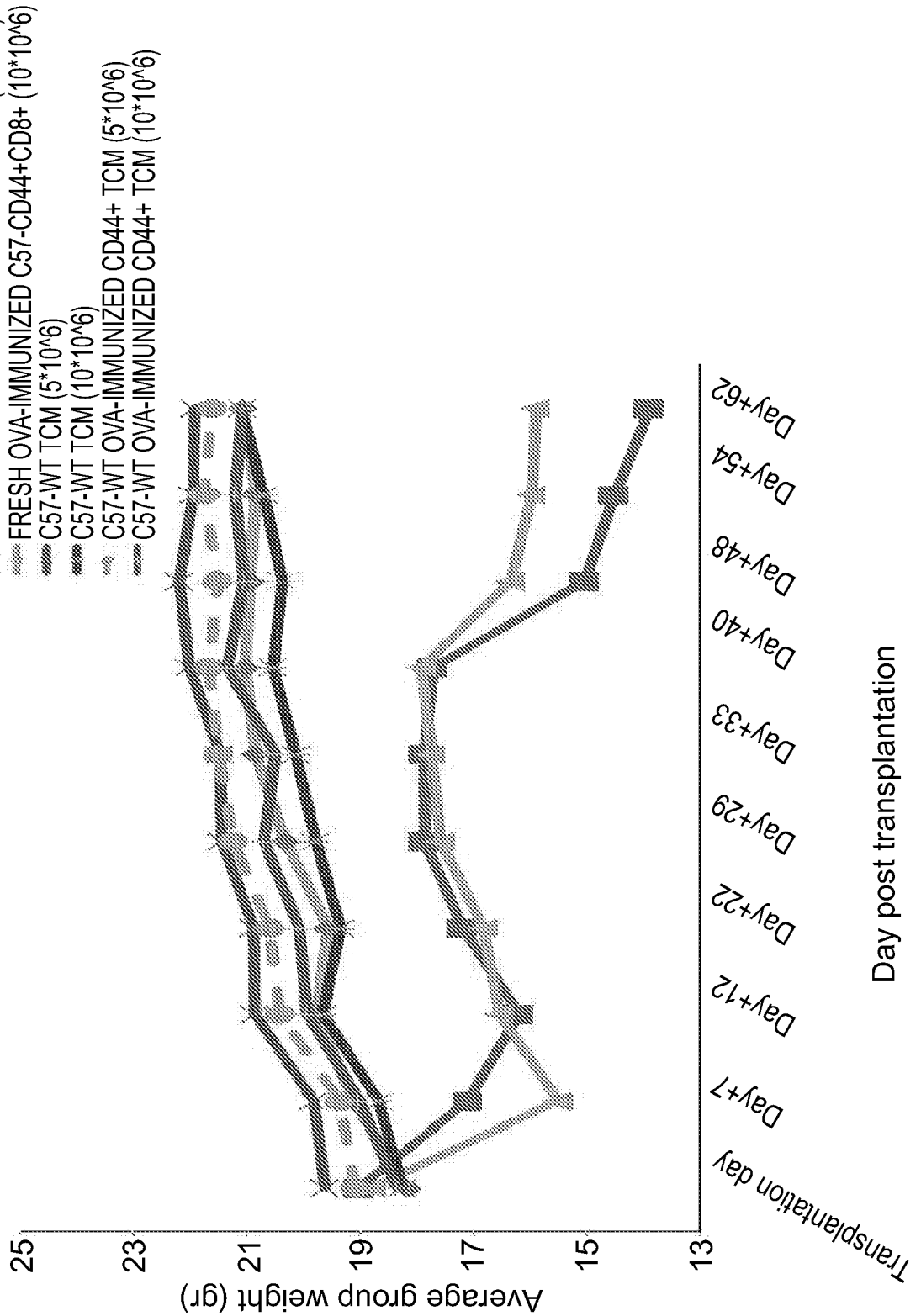

Irradiation CNTL (5Gy)
FRESH OVA-IMMUNIZED C57-CD44+CD8+ (5*10^6)
FRESH OVA-IMMUNIZED C57-CD44+CD8+ (10*10^6)
C57-WT TCM (5*10^6)
C57-WT TCM (10*10^6)
C57-WT OVA-IMMUNIZED CD44+ TCM (5*10^6)
C57-WT OVA-IMMUNIZED CD44+ TCM (10*10^6)
Average group weight (gr)
25
23
21
19
17
15
13
Transplantation day
Day+7
Day+12
Day+22
Day+29
Day+33
Day+40
Day+48
Day+54
Day+62
Day post transplantation

FIG. 2C

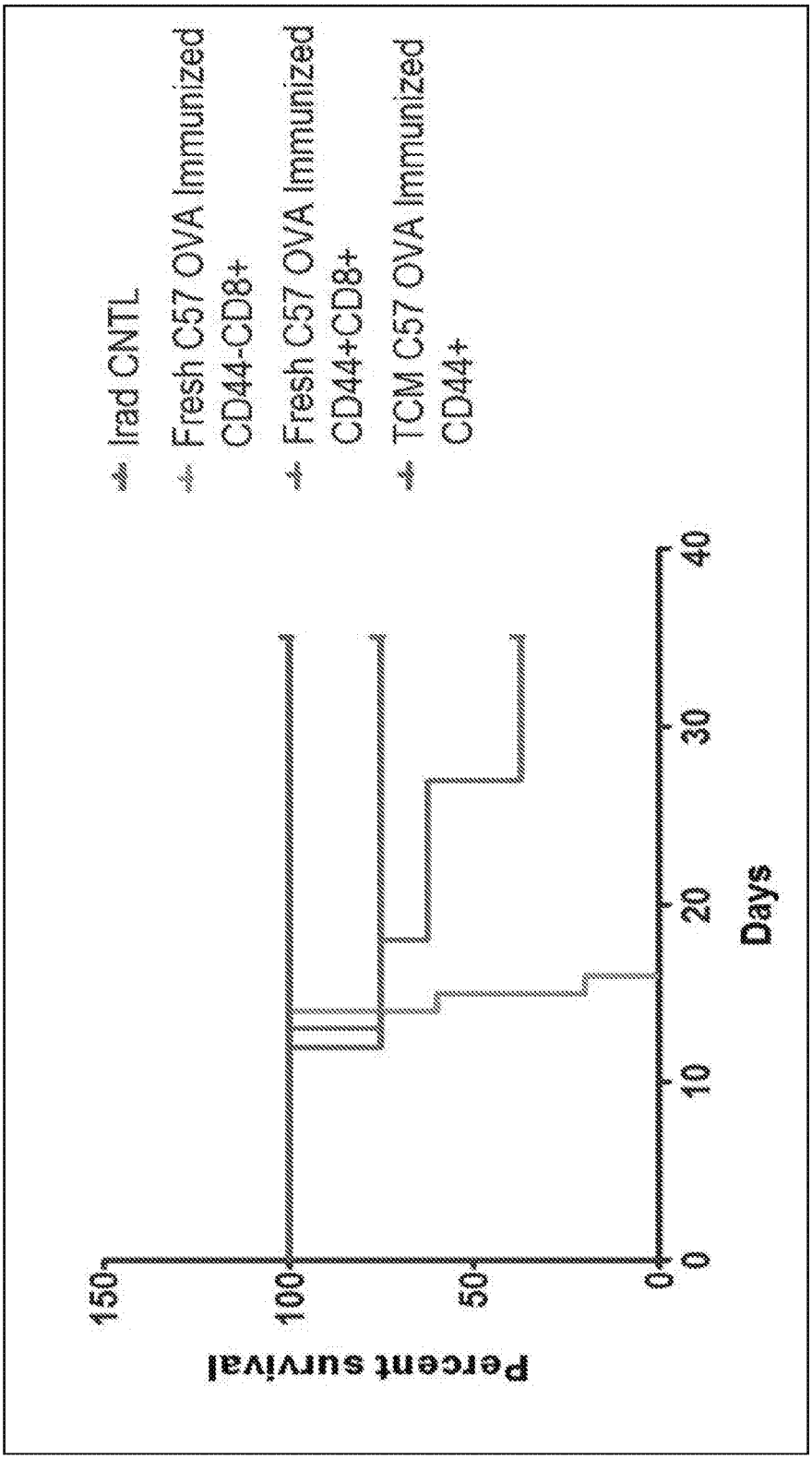
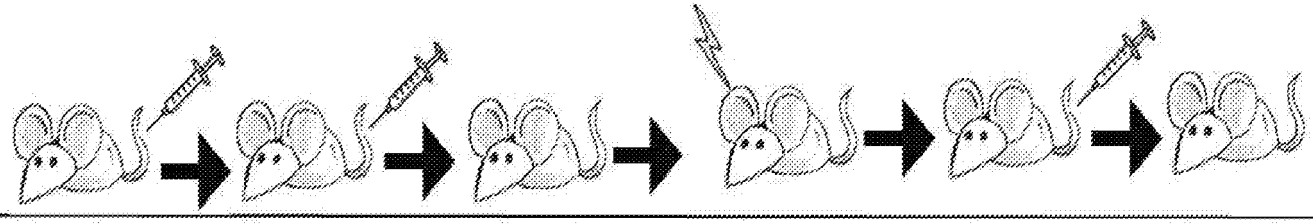

Irad CNTL
Fresh C57 OVA Immunized CD44-CD8+
Fresh C57 OVA Immunized CD44+CD8+
TCM C57 OVA Immunized CD44+
Percent survival
150
100
50
0
0
10
20
30
40
Days

FIG. 2D



Day -38	Day -24	Day -16	Day -1	Day 0	Days 30,60,90
Immunization 75ug OVA+CFA s.c. to OT-1RAGCD45.1 hosts	Immunization Boost 75ug OVA+IFA s.c. to OT-1RAGCD45.1 hosts	Initiation of anti-3rd party co-culuture of immunized spleens enriched for CD8+CD44+ against irradiated OVA splenocytes OR WT splenocytes vs. irradiated FVB	TBI 6/6.25/6.5Gy C57 host	$20*10^6$ BALB/C NuBM ± $5*10^6$ Tcm	Chimerism check in peripheral blood

FIG. 2E

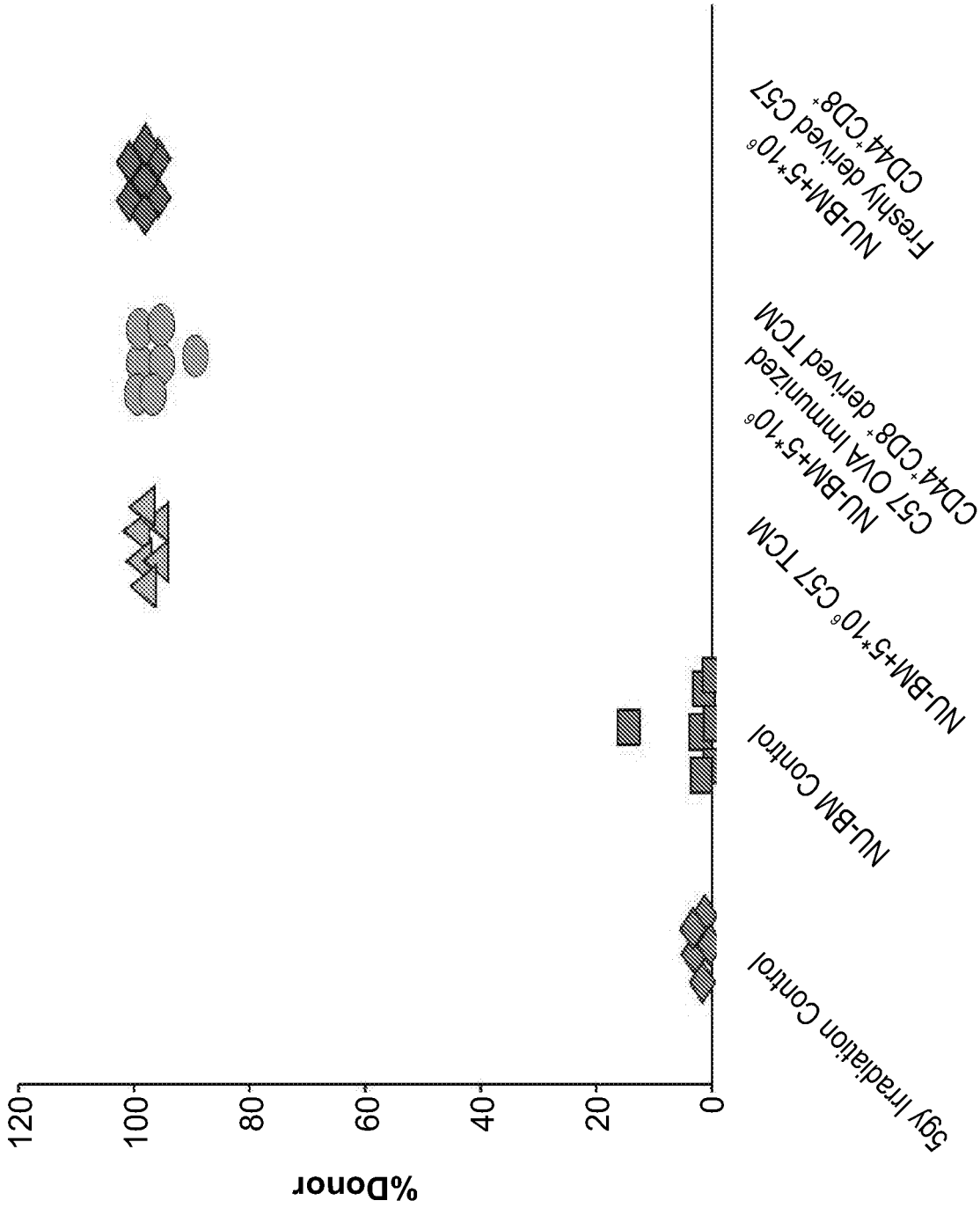

%Donor
120
100
80
60
40
20
0
5gy irradiation Control
NU-BM Control
NU-BM+5*10⁶ C57 TCM
NU-BM+5*10⁶ C57 OVA immunized CD44⁺CD8⁺ derived TCM
NU-BM+5*10⁶ Freshly derived C57 CD44⁺CD8⁺

FIG. 3A

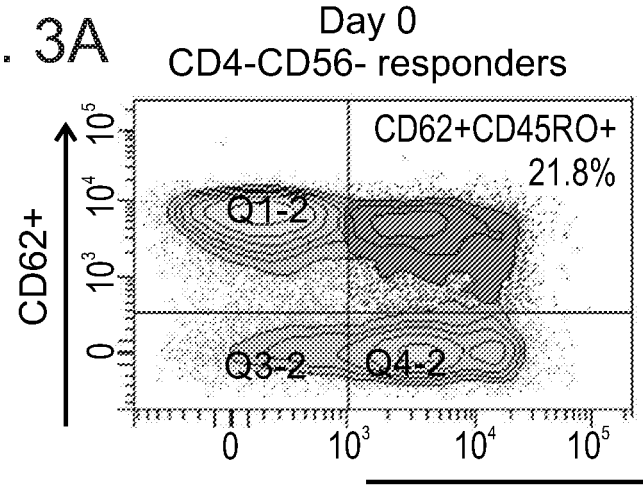


FIG. 3B

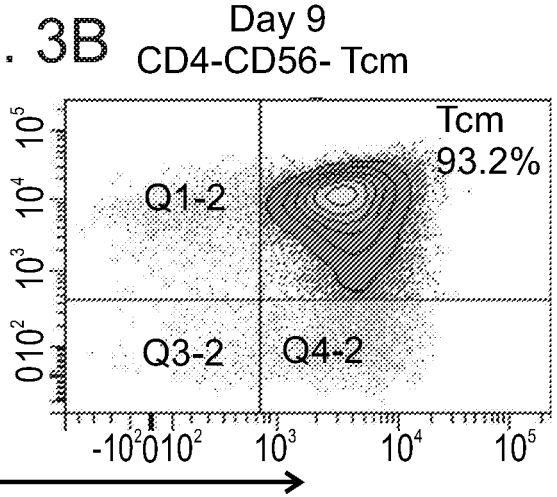
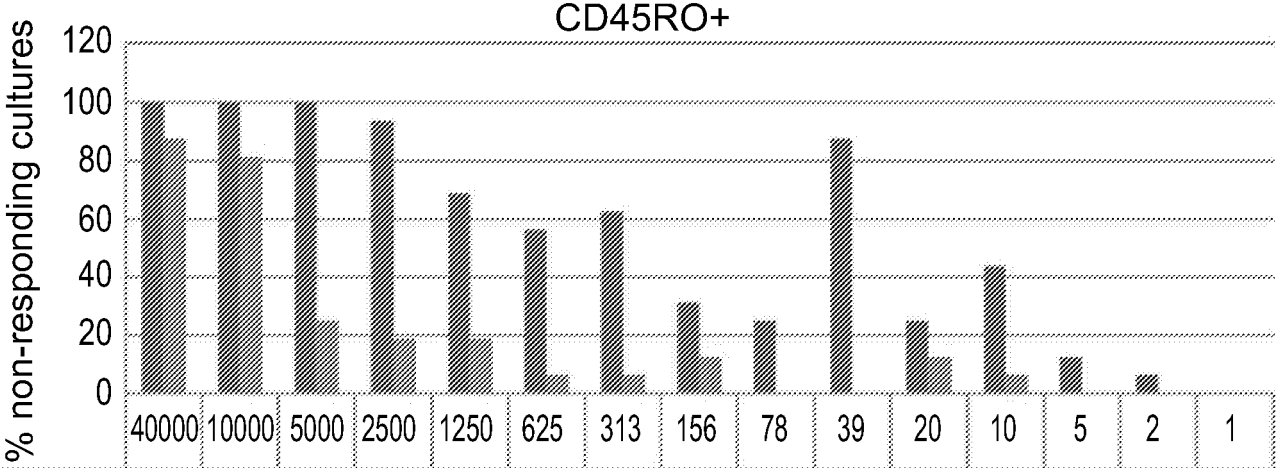


FIG. 3C



	40000	10000	5000	2500	1250	625	313	156	78	39	20	10	5	2	1
Fresh Cd4-CD56-CD19-	100.0	100.0	100.0	93.75	68.75	56.25	62.50	31.25	25.00	87.50	25.00	43.75	12.50	6.25	10.00
Tcm CD4-CD56-	87.5	81.25	2.5	18.75	18.75	6.25	6.25	12.5	0	0	12.5	6.25	0	0	0

FIG. 3D

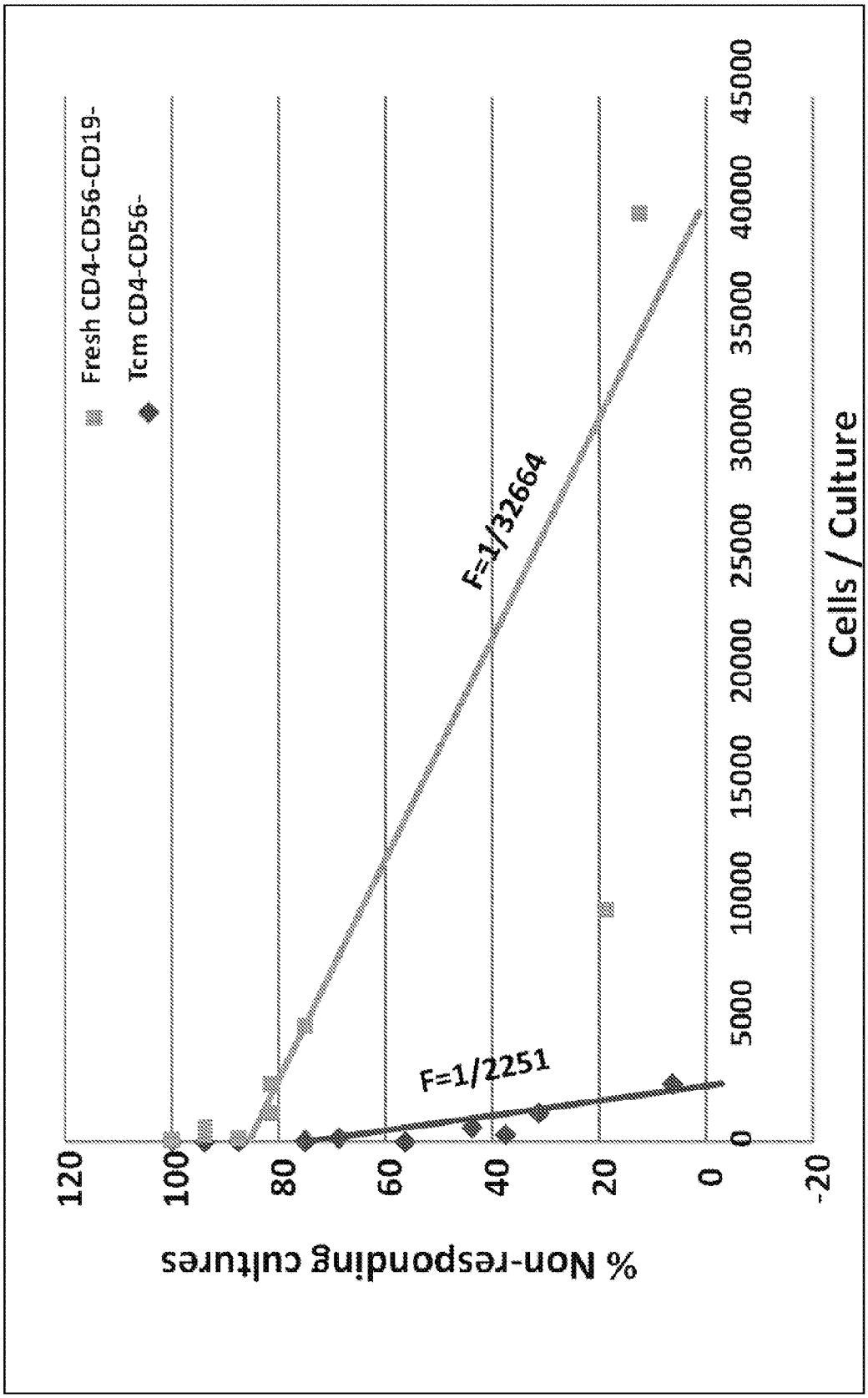

% Non-responding cultures
120
100
80
60
40
20
0
-20
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
Cells / Culture
Fresh CD4-CD56-CD19-
Tcm CD4-CD56-
F=1/32664
F=1/2251

FIG. 4A

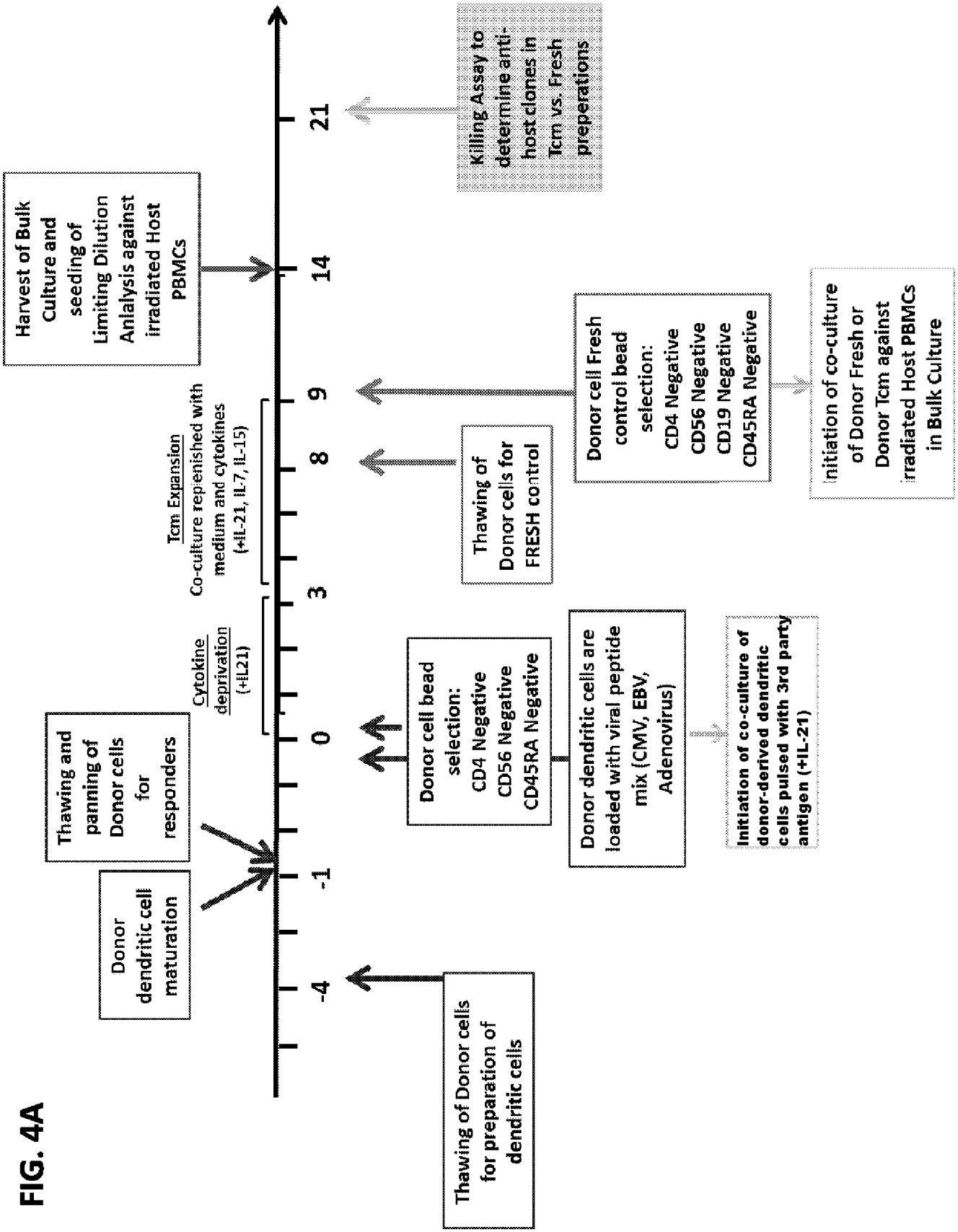
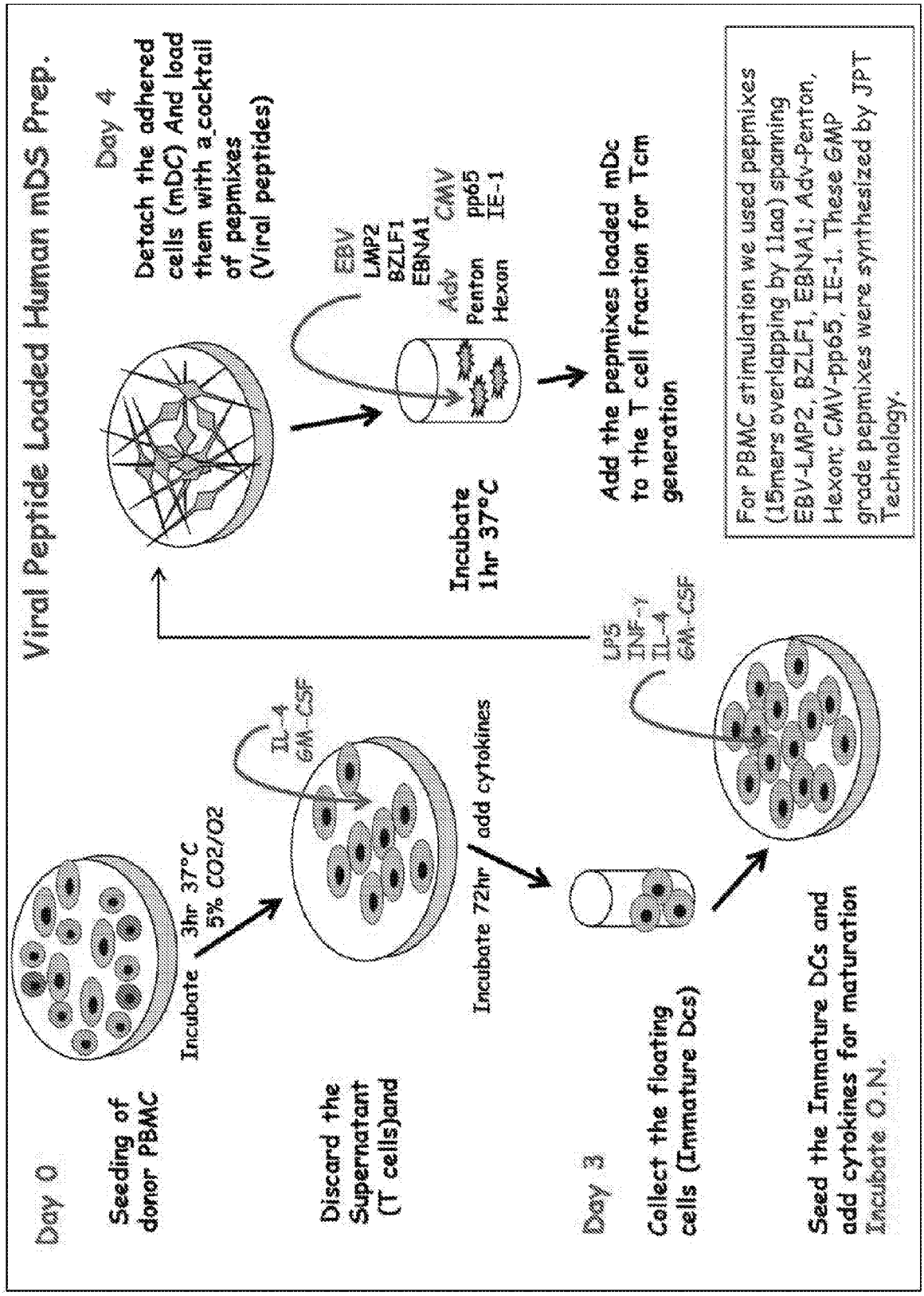

Harvest of Bulk Culture and seeding of Limiting Dilution Anlalysis against irradiated Host PBMCs
Killing Assay to determine anti-host clones in Tcm vs. Fresh preperations
Tcm Expansion
Co-culture replenished with medium and cytokines (+IL-21, IL-7, IL-15)
Cytokine deprivation (+IL21)
Thawing and panning of Donor cells for responders
Donor dendritic cell maturation
Thawing of Donor cells for preparation of dendritic cells
Donor cell bead selection: CD4 Negative CD56 Negative CD45RA Negative
Donor dendritic cells are loaded with viral peptide mix (CMV, EBV, Adenovirus)
Initiation of co-culture of donor-derived dendritic cells pulsed with 3rd party antigen (+IL-21)
Thawing of Donor cells for FRESH control
Donor cell Fresh control bead selection: CD4 Negative CD56 Negative CD19 Negative CD45RA Negative
Initiation of co-culture of Donor Fresh or Donor Tcm against irradiated Host PBMCs in Bulk Culture
-4
-1
0
3
8
9
14
21

FIG. 4B


Viral Peptide Loaded Human mDS Prep.
Day 0
Seeding of donor PBMC
Incubate 3hr 37°C 5% CO2/O2
Discard the Supernatant (T cells)and
IL-4 GM-CSF
Incubate 72hr add cytokines
Day 3
Collect the floating cells (Immature Dcs)
Seed the Immature DCs and add cytokines for maturation Incubate O.N.
LPS INF-γ IL-4 GM-CSF
Day 4
Detach the adhered cells (mDC) And load them with a_cocktail of pepmixes (Viral peptides)
EBV LMP2 BZLF1 EBNA1
Adv Penton Hexon
CMV pp65 IE-1
Incubate 1hr 37°C
Add the pepmixes loaded mDc to the T cell fraction for Tcm generation
For PBMC stimulation we used pepmixes (15mers overlapping by 11aa) spanning EBV-LMP2, BZLF1, EBNA1; Adv-Penton, Hexon; CMV-pp65, IE-1. These GMP grade pepmixes were synthesized by JPT Technology.

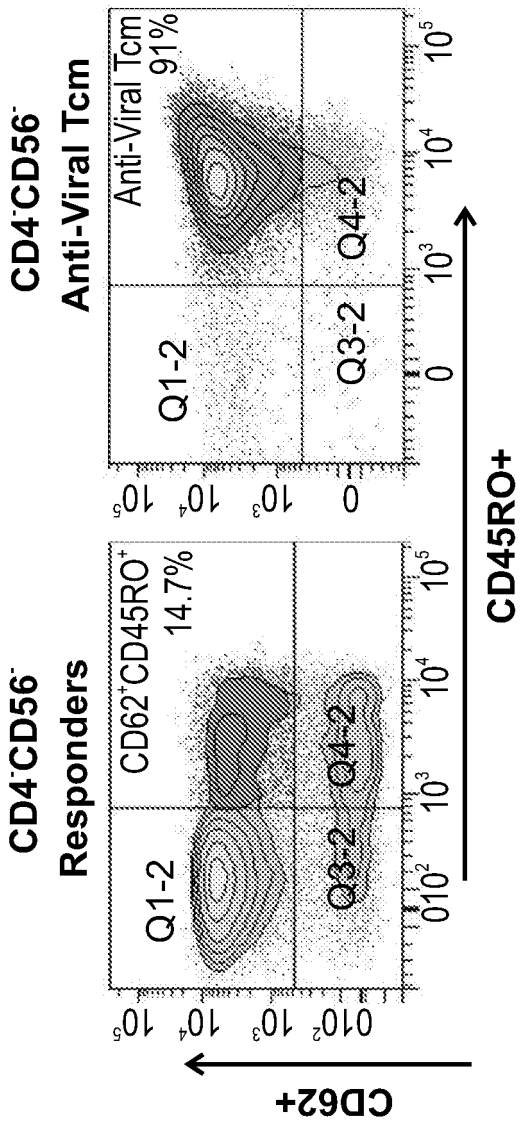

CD4-CD56-
Responders
CD62+CD45RO+
14.7%
Q1-2
Q3-2
Q4-2
CD4-CD56-
Anti-Viral Tcm
Anti-Viral Tcm
91%
Q1-2
Q3-2
Q4-2
CD62+
CD45RO+

FIG. 5A

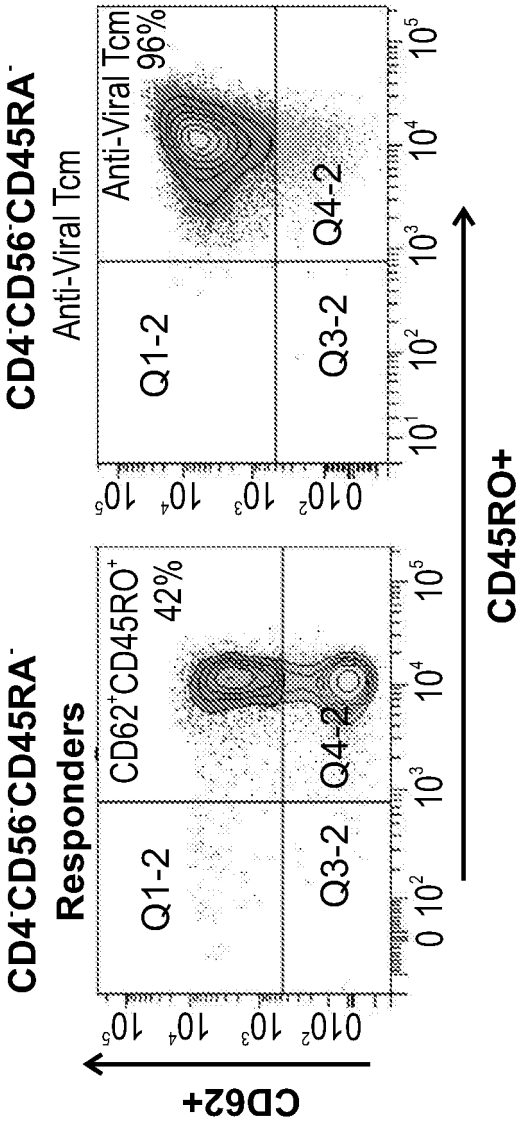

CD4-CD56-CD45RA-
Responders
CD62+CD45RO+
42%
Q1-2
Q3-2
Q4-2
CD4-CD56-CD45RA-
Anti-Viral Tcm
Anti-Viral Tcm
96%
Q1-2
Q3-2
Q4-2
CD62+
CD45RO+

FIG. 5B

FIG. 5C

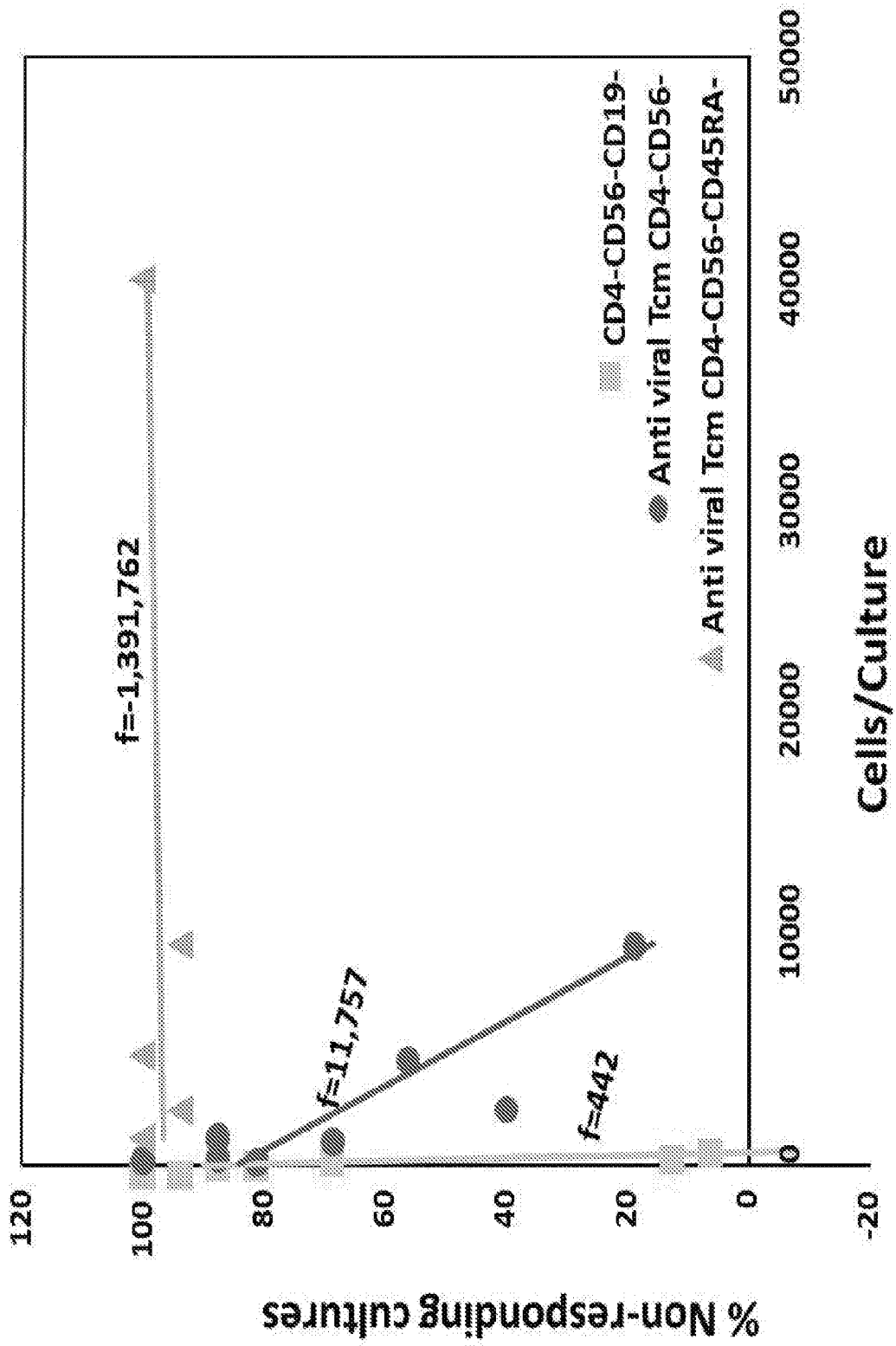
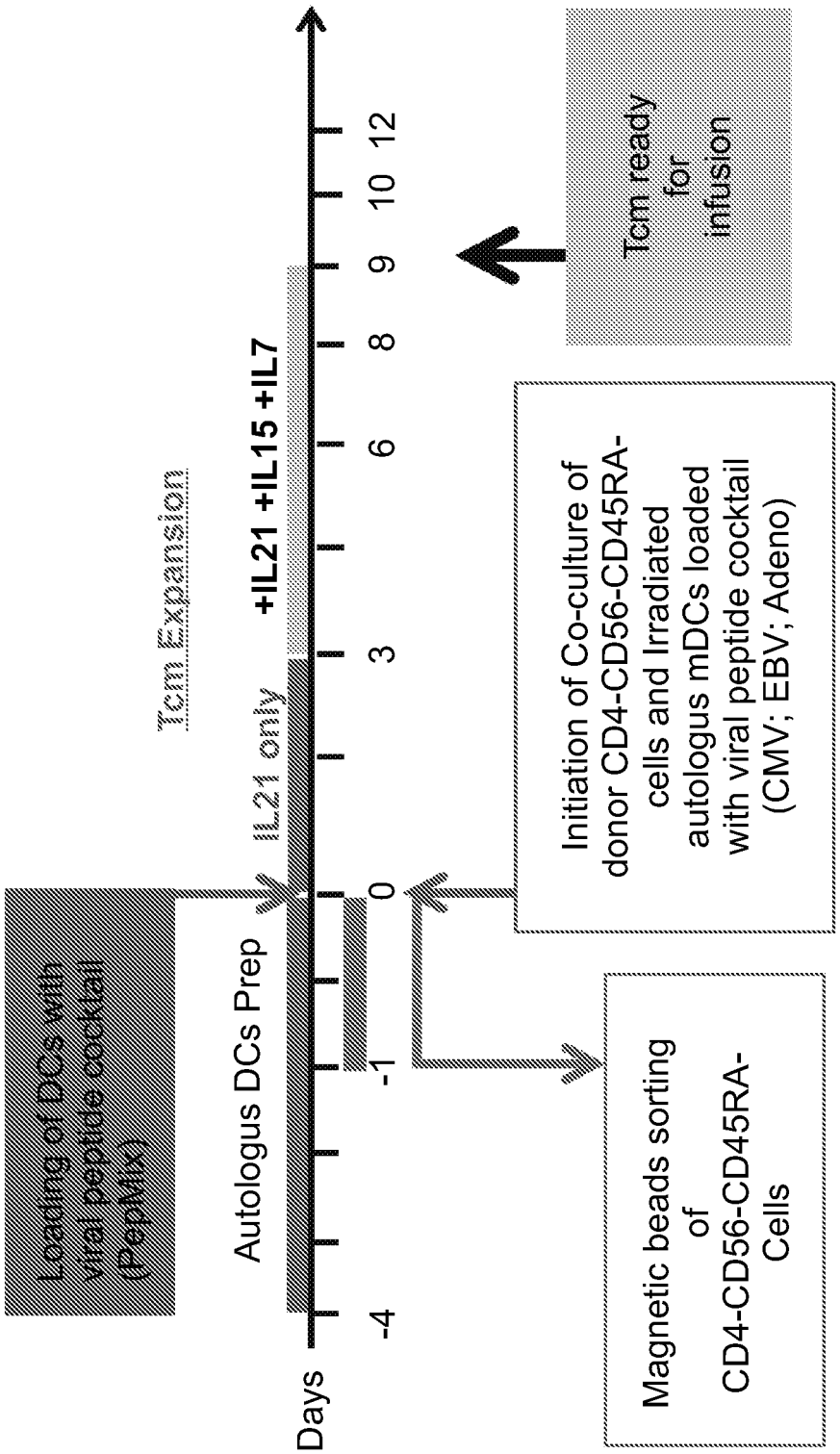

% Non-responding cultures
120
100
80
60
40
20
0
-20
0
10000
20000
30000
40000
50000
Cells/Culture
Anti viral Tcm CD4-CD56-CD45RA-
Anti viral Tcm CD4-CD56-
CD4-CD56-CD19-
f=-1,391,762
f=11,757
f=442

FIG. 6

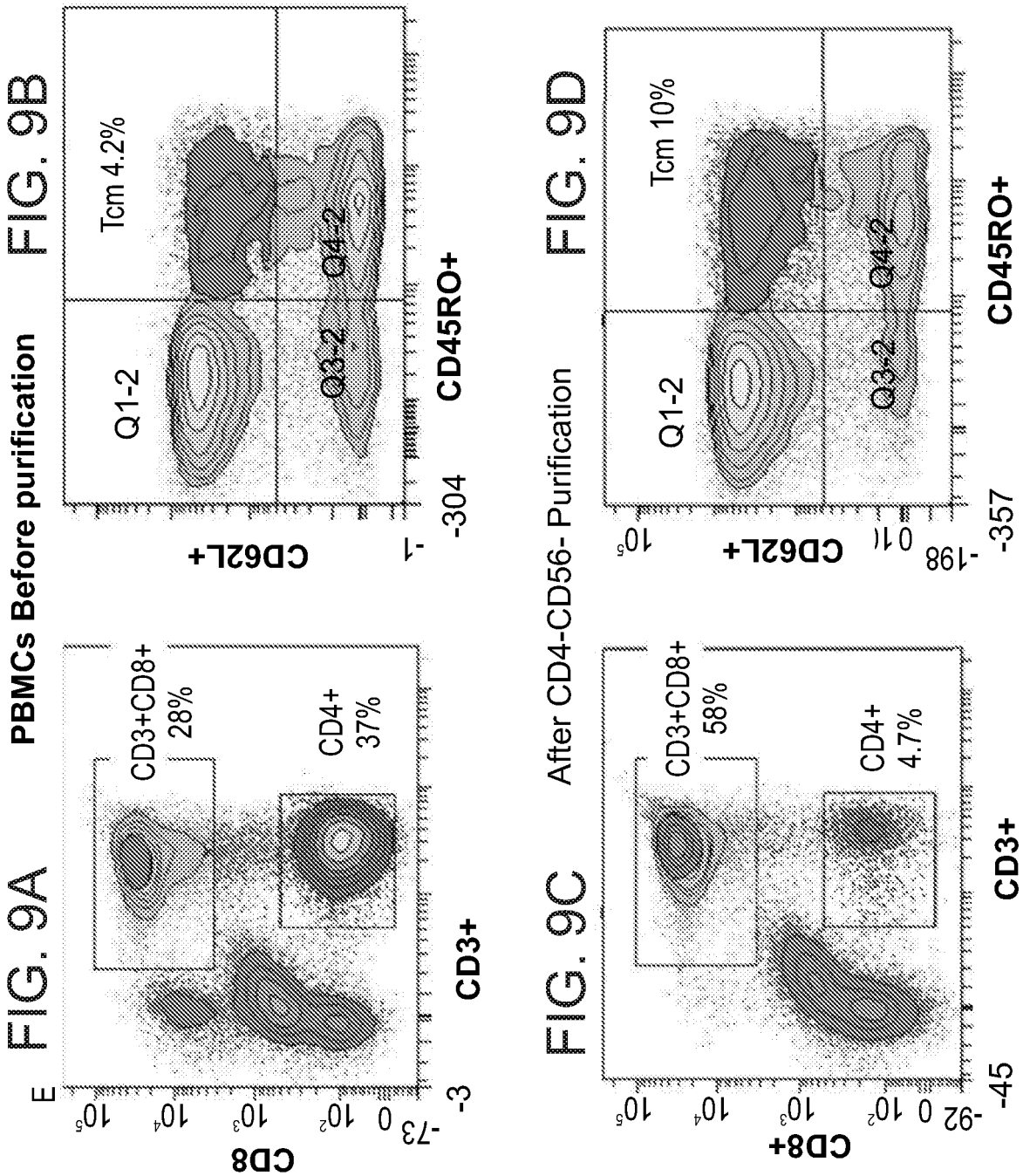
Cell Fraction	Cell number Seeded for bulk culture Vs host (Day 9)	Cell number Harvested after bulk culture (Day 14)	Anti-host CTL-p frequency based on LDA	Total anti-host CTL-p ($x10^6$) based on LDA (Normalized to $100x10^6$)	Depletion factor
Fresh CD4-56-19-	$50x10^6$	$30x10^6$	1/442	0.136	
Anti-viral Tcm CD4-56-	$200x10^6$	$30x10^6$	1/11,757	0.00128	106
Anti-viral Tcm CD4-56-RA-	$200x10^6$	$60x10^6$	1/-1,391,762	0.000022	6181

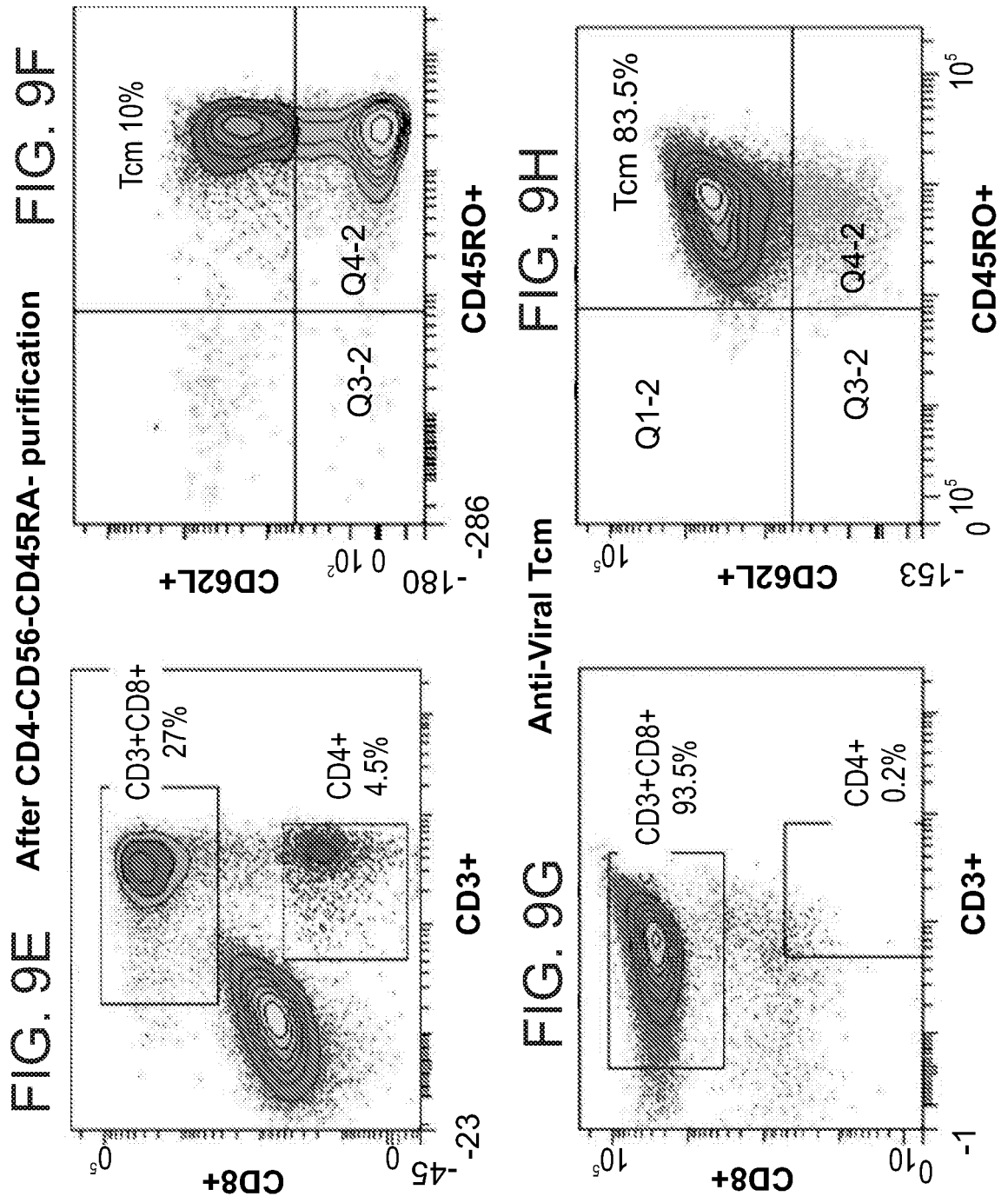
FIG. 7


Tcm Expansion
Loading of DCs with viral peptide cocktail (PepMix)
Autologus DCs Prep
IL21 only
+IL21 +IL15 +IL7
Days
-4
-1
0
3
6
8
9
10
12
Magnetic beads sorting of CD4-CD56-CD45RA- Cells
Initiation of Co-culture of donor CD4-CD56-CD45RA- cells and Irradiated autologus mDCs loaded with viral peptide cocktail (CMV; EBV; Adeno)
Tcm ready for infusion

		Day -1	Day -1	Day 0	Day 0	Day 0		Day 9		Day 9
	# Vials	Before thawing	After thawing	After Penning	After CD4-CD56	After CD4-CD56-RA-		Anti-viral Tcm		Fold Expansion
		($x10^6$)	($x10^6$)	($x10^6$)	($x10^6$)	Cell#($x10^6$)	%	Cell#($x10^6$)	%	
Donor A (AV of 3 Exp) Total Cell #	32	3167	2308	1083	462	26		1158		
Donor B (AV of 2 Exp) Total Cell #	22	2200	1450	1000	400	78		404		
Donor C (AV of 2 Exp) Total Cell #	30	3000	2025	1150	325	67		338		
Donor D (1 Exp) Total Cell #	30	3000	2120	1000	276	58		583		
Donor E (AV of 2 Exp) Total Cell #	30	2950	1943	1300	340	47		783		
Average	29	2863	1969	1107	361	55		653		11.8
SD	4	380	321	125	72	20		331		
Donor A (AV of 3 Exp) Cell # of CD3+CD8+ fraction						17	67	1088	94	
Donor B (AV of 2 Exp) Cell # of CD3+CD8+ fraction						19	24	349	86	
Donor C (AV of 2 Exp) Cell # of CD3+CD8+ fraction						16	25	318	92	
Donor D (1 Exp) Cell # of CD3+CD8+ fraction						31	53	550	94	
Donor E (AV of 2 Exp) Cell # of CD3+CD8+ fraction						14	30	731	93	
Average						19	40	607	92	31.5
SD						7	19	316	3	
Donor A (AV of 3 Exp) Cell # of CD62L+CD45RO+ fraction						7	27	897	77	
Donor B (AV of 2 Exp) Cell # of CD62L+CD45RO+ fraction						8	10	187	47	
Donor C (AV of 2 Exp) Cell # of CD62L+CD45RO+ fraction						8	12	228	65	
Donor D (1 Exp) Cell # of CD62L+CD45RO+ fraction						12	20	466	80	
Donor E (AV of 2 Exp) Cell # of CD62L+CD45RO+ fraction						5	12	699	89	
Average						8	16	495	72	63.8
SD						2	7	304	16	

FIG. 8


FIG. 9A
PBMCs Before purification
CD3+CD8+
28%
CD4+
37%
CD8
CD3+
FIG. 9B
Tcm 4.2%
Q1-2
Q3-2
Q4-2
CD62L+
CD45RO+
FIG. 9C
After CD4-CD56- Purification
CD3+CD8+
58%
CD4+
4.7%
CD8+
CD3+
FIG. 9D
Tcm 10%
Q1-2
Q3-2
Q4-2
CD62L+
CD45RO+


FIG. 9E
After CD4-CD56-CD45RA- purification
CD3+CD8+
27%
CD4+
4.5%
CD8+
CD3+
FIG. 9F
Tcm 10%
Q4-2
Q3-2
CD62L+
CD45RO+
FIG. 9G
Anti-Viral Tcm
CD3+CD8+
93.5%
CD4+
0.2%
CD8+
CD3+
FIG. 9H
Tcm 83.5%
Q1-2
Q4-2
Q3-2
CD62L+
CD45RO+