November 9-10th, 2021

HEU: Ex-vivo γδ CBMC panels #10 & ILT novo 3

Inf 171 n1 = 356 + 344

Inf 124 n1 = 500 + 200

Inf 171 n2 = 295 + 405

Inf 124 n2 = 472 + 228

NY062 434 + 246

10 PMA (20) a ctrl

x.3

3 ml 2.7 ml

+10% 3.3 31.5 2.84 ml

Used old protein transport inhibitor (2019) as not enough

7:21 pm → spindown

Tet f/D @ 7:36

everything worked @ 8:08 pm

Tetramers @ 8:33 pm

Adult PBMC libraries setup

NY062 → stimw/ PMA

→ ctrl w/o PMA.

Te

Mass single color spin @ 9:56

37°C out @ → 10:04 pm

10 µl Fix Perm

4°C @ 10:25 pm → 10:55

Mass Fixperm @ vortex @ 27: 37:47

Rbc lyse @ 11:02 pm

Tet Fix Perm @ 11:26 pm

Intra @ 00:13 am

4,100,000 / 30 → 1.3K cells/tube in ~100 µl volume → 1.3E6

Not confident anything is in these, but we shall see tomorrow if they are, or we just wasted time, effort & materials

scs intoac @ 23:50

black - PMA ?

blue - ctrl

November 9-10th, 2021 Current Stim Panel

#	Filter	Single color (ul)	Ref ctrl		Fluorochrome	Marker	During stimulation	Tetramer (RT) for 40 min	37oC (RT) for 30min	x6	Surface staining 4oC for 30min	6	Lysing, then CytoFix/Perm	RT for 40min Universal Intra mix (10x diluted)	6
1	UV2	1.25	Cells	C	BUV395	CD62L (DREG-56)					1.2	7.2			
2	UV7	~~1.25~~ 1.5	Cells	C	BUV496	CD8 (RPA-T8)					1.0	6.0		0.25	1.5
3	UV9	1.25	Cells	C	BUV563	CD69 (FN50)					1.0	6.0		0.25	1.5
4	UV10	1.25	Cells	C	BUV615	CCR4 (1G1)			2.0	12					
5	UV11	1.25	Cells	C	BUV661	Vd2 (B6)					1.0	6.0			
6	U14	1.25	Cells	C	BUV737	CXCR3 (1C6/CXCR3)			2.5	15					
7	UV16	1.25	Cells	C	BUV805	CD4 (SK3)					1.5	9.0		0.25	1.5
8	V1	1.25	Cells	C	BV421	CD127 (A019D5)	0.8				1.0	6.0			
	V3	1.25	Cells	C	Pacific Blue	CD14 (M5E2)					2.0	12.0			
9	V3	1.25	Cells	C	Pacific Blue	CD19 (HIB19)					2.0	12.0			
10	V5	1.25	Cells	C	BV480	CD161 (REA631)	0.8		1	6				0.5	3
11	V7	1.25	Cells	C	BV510 (dim)	CD45RA (HI100)					1.0	6.0			
	V10	-	Cells	C	BV605										
	V11		Cells	C	BV650										
	V13		Cells	C	BV711										
12	V14	1.25	Cells	C	BV750	IFNg (B27)								1.5	9
13	V15	1.25	Cells	C	BV786	CCR6 (11A9)			1.8	10.8					
14	B2	1.25	Cells	C	Alexa488	hCD1d F:tc VD2		<1:750>							
15	B3	1.25	Cells	C	Spark blue 550	CD3 (SK7)					1.2	7.2		0.25	1.5
	B4		Cells	C	PE										
16	B6	1.25	Cells	C	PE-CF594	CD26 (M-A261)			1.2	7.2					
17	B8	1.25	Cells	C	PE-Cy5	CD25 (M-A251)					1.2	7.2		0.25	1.5
18	B10 9	1.5 (<=10^4)	Cells	C	PerCP-Cy5.5	TNFa (MAB11)								2.5	15
	B13	-	Cells	C	PE-vio770										
19	R1	1.25	Cells	C	APC	IL-4								1.5	9
20	R2	1.25	Cells	C	Alexa Fluor 647	hMR1 5OP-RU		<1:500>							
21	R4	1.25	Cells	C	APC-R700	CD107a (H4A3)	6.0								
22	R6	1:2500	Cell	P	Zombie NIR	Viability									
23	R7	1.3	Cells	P	APC/Fire 750	CD27 (O323)			2	12					
24	R8	1.3	Cells	C	APC/Fire 810	CD38 (HIT2)			1.6	9.6					
						Total volume	7.6		12.1	72.6	14.1	84.6		7.25	44
		unstained Cells						Brilliant Stain:	50		50			50	
		24 color CBMC						Draw Volume:	57.1		59.1			52.3	

Protocol

Aliquot cells		
PMA-Ionomycin stimulation		
Golgi blockers		
PBS 2ml		1400rpm, 6min
Live/Dead @RT 15min		
PBS-FBS 2ml		1400rpm, 6min
Tetramers @RT 40min		
PBS-FBS 2ml		1400rpm, 6min
	CCR4	CD26
Surface	CXCR3	CD27
37oC 30min	CD161	CD38
	CCR6	
PBS-FBS 2ml		1400rpm, 6min
Surface mix @ 4oC 30min		
Lysing solution 300-500ul		
PBS-FBS 2ml		1400rpm, 6min
Cytofix/perm @ 4oC 20min		
wash twice PermWash 1 ml		1500rpm, 6min
Intra Staining @ RT for 40min		
wash once w/ 2ml		1500rpm, 6min
Resuspend 100 ul PFA-PBS		

-CXCR3 in iNKT
+new CCR4 in iNKT
+new CD4

¿Did I add them? (PD1, CD56, MR1 5OP?)

¿PMA + ctrl's got switched?
Forgot to record on 17Z held in
Good thing we have Vz1

↑ ≈3 is still safe → 60μl

20:50 Lots

Hot @ 8:56 → 26 (10 min late)
9:04 surface 1/3 2/3 @ 9:09
Cold @9:15 → 45
25 → 55
:20 hrs → 53
Tef ¿ Hot? :34 → 6:04 pm
unstained Rbclyse @ 9:49
mass spin @ 9:56

November 9-10th, 2021 Current Stim Panel

PBMC controls

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13	V15	1.25	Cells	C	BV786	CCR6 (11A9)			1.8	10.8					
14	B2	1.25	Cells	C	FITC	Va24Ja18					0.5	3.0			
15	B3	1.25	Cells	C	Spark blue 550	CD3 (SK7)					1.2	7.2		0.25	1.5
	B4		Cells	C	PE										
16	B6	1.25	Cells	C	PE-CF594	CD26 (M-A261)			1.2	7.2					
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						Brilliant Stain:			50		50			50	
						Draw Volume:			57.1		59.6			52.3	

unstained Cells

24 color CBMC

Protocol	
Aliquot cells	
PMA-Ionomycin stimulation	
Golgi blockers	
PBS 2ml	1400rpm, 6min
Live/Dead @RT 15min	
PBS-FBS 2ml	1400rpm, 6min
Tetramers @RT 40min	
PBS-FBS 2ml	1400rpm, 6min
Surface 37oC 30min	CCR4, CD26, CXCR3, CD27, CD161, CD38, CCR6
PBS-FBS 2ml	1400rpm, 6min
Surface mix @ 4oC 30min	
Lysing solution 300-500ul	
PBS-FBS 2ml	1400rpm, 6min
Cytofix/perm @ 4oC 20min	
wash twice PermWash 1 ml	1500rpm, 6min
Intra Staining @ RT for 40min	
wash once w/ 2ml	1500rpm, 6min
Resuspend 100 ul PFA-PBS	

stim+/- stim+/- stim+/-

Va ○○ ○○ ○○

hMR1 ○○ ○○ ○○

Running PMA+I I11 Va extra first

on the ref control, ... group + unstained control

strange

6x5 = 30

4-36 6FP
ditto for OP-RU

.2
x 6
7.2

1.9

No CD56, PD1 or NKG2D got added last night. CXCR3 ≠

my Va24ja18 stain at 0.5 not present.

hMR1 + IL-4 really ugly gate

hMR1 vs udz.

IFNg / TNFa not here?

¿ did I use TNFa?

Yeah ~ IL/4 ~ Va24ja18

DTR- PBMC- a/b ____ -2021 11/11/21

- Questions: Blue / Black — PMA / ctrl ? (did it even PMA?)

- are their any cells there?

syringe ≠ retract (no apparent PMA well issue, no stain)

Vortex every time!! w/dilution ~ 200 events

there are cells there ~ 220 events.

CD62L → ran out, included "dying" $10^{3½} \to 10^6$ ... not trustworthy

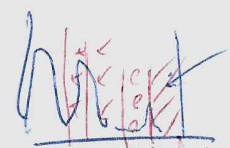
CD8 → looks PMA-ish CD69 looks activated ✓

the CD62L → suggest yup did work!

you can remove tubes!! ✓

Black → PMA

Quite a few odd events 10^6, gains not set?



¿ What does it walk?

- bcell CD19 looks odd — important pop markers do 50,000 (CD161)

↳ CCR6 also in this context!

- yup these stims worked... wtf happened other wells?

brightest CD3's (not the dominant stim ones)

no overwrite until total cell collected

NKG2D ran to 50k (not that bright).

TNFa ≠ there? (vs yes for IFNg?) → is there ✓

- We want the highest PD1 expressors, ecoll it to 50k -

Ref control next (moment of truth)

woah nailed it!

Start on 2nd set @ 9:55pm

DTR-PBMCb- -2021

↑ ctrl

May not have enough vD2's, ½ blue segments across spectrum

Some specimens, shift to the left you gain significance values (cleared overspread?)

Full cohort →

Alex Mei

Distinguishing AF junk from highest expressors when gating.

highest CD3's ≥ ah/yd's?

"CD25 just the tip"

It's the brightest that show ~~over~~compensation/unmixing issues, the average are generally fine.

... only vD2? ...

forgot to ~~gate~~ adjust I-7 or whatever panel

11:53pm