March 27th, 2023

<p/>

1:100

2023_ILT_07

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
Inf055-0 a-3	HU♂ RVQ6	(4)	<10.6>			1.5	5.98	9.75	8.97		
a-4	RVQ7	(7)	<9.0>			1.5	6.72	10.70	10.08		
Inf287-7 a-3	HEU-lo♀ SLYY	(3)	<17.0>			1.5	3.59	6.90	5.38		
a-4	SLYZ	(6)	<17.5>			1.5	3.32	6.67	4.98		
Inf052 a-2	HEU-hi♂ RVJB	(5)	<9.4>			3.0	5.56	8.27	16.7		
ND050	Adult Norm	(1)	15E6 <16>	1/13/23		1.5	6.46	9.17	9.69		
ND006	Adult SCs	(2)	15E6 <10.7>	1/20/23		1.5	9.59	12.2	14.39		

1680c/1/1 <60>

1610c/1/1 <30>

a30c/1/1 <20>

a00c/1/1 <80>

1023c/1/1 <30>

525c/1/1 <40>

700c/1/1 <40>

7:29 am thaw start
7:37am cord 1st spin
DNAse @ 7:55am adults
8:18 am final DNAse
8:35am count stain
9:00am cone done
9:20 cells done
9:40 am aliquots done now

All specimens/RBC's today (10.5) (12)

1,000,000 × = 25

		0.5	0.5	3M
Inf055	19.05 × 3			
Inf287	10.36 × 3			
Inf052	16.7 × 3			
ND050	9.69 × 1.5			
ND006	14.39 × 1.5			

Incubation @ 10:39 am → 4:39 pm

Sample spin @ 4:49 pm

L/D @ 5:06 pm

5:46pm Hot Samples → 6:16 pm

5:55pm sc's aliquoted & spun in 1ml PBS

sc hot @ 6:08pm → 48

6:44pm tetramers → 6:24-21

Va7 @ 6:57 →

sc's cold @ 7:08pm → 38pm

Samples FBS post tet spin @ 7:31pm

Samples cold @ 7:52pm → 8:22pm

FixPerm scs @ 8:00 pm → 8:10 → 8:20 pm

Last FBS wash + scs 1st permwash @ 8:33 →

RBC lysis @ 8:29

PMA-ionomycin

1 ctrl × 2	4 PMA × 2	
22µl	8µl	
4 ctrl ×33	4 pma ×5.33	
1.32µl	1.32µl	
1 ctrl	4 ctrl 1.64	1 pma 1.64
1µl	6.64µl	1.66µl

BD Golgistop Protein Transport Inhibitor (containing Monensin)

sold as (554724)

.7ml

2) Golgi Plug Brefeldin A

cat # ???

Samples FixPerm @8:54→9:04→9:14

9:14 sc's into →54µl

Samples 1st permwash @9:17pm

2nd permwash @9:29pm

Intra-c samples 9:44 → 10:24pm

scs 25µl

Set's done @ 10:10 pm ✓

Final span @ 10:31 pm

Done @ 10:43 pm

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Acquisition @ 9:35 am

~30 µl/min

Info55 cord ctrl

CCR4 staining ≠ great

CXCR3 disaster

resume @ 10:29

- CD16 distinct different in cord
- CD56 on cord also distinct
- CD16 way brighter on cord

NK620?

11:23 ← noise tech repair

how do you count systematically dying s?

~ experimental/technical variance

Unmixing 03-28-23 07:48 pm.

Moved main CXCR3 samples into SC

Added NDO50 untained to samples

Getting to unmix stats before separating the files for Aborus Tel.

AF extract + CD16/CD56/CD4 as cord SCs. + CD3 AF647

CD16 blatantly easy gate cord

CD56 less easy but noticable

CD16 (lips)

return to adult PBMCs...

... drumroll ladies & gents ...

Similarity Matrix 14.05

Sample	Total	Volume	Concentration	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF055	19.05	3	6.35	0.47	0.43	0.39	0.35	0.31	0.28	0.24	0.20	0.16	0.12	0.08
INF287	10.36	3	3.45	0.87	0.80	0.72	0.65	0.58	0.51	0.43	0.36	0.29	0.22	0.14
INF052	16.7	3	5.57	0.54	0.49	0.45	0.40	0.36	0.31	0.27	0.22	0.18	0.13	0.09
ND050	9.69	1.5	6.46	0.46	0.43	0.39	0.35	0.31	0.27	0.23	0.19	0.15	0.12	0.08
ND006	14.39	1.5	9.59	0.31	0.29	0.26	0.23	0.21	0.18	0.16	0.13	0.10	0.08	0.05

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
R10	INF055	0.53	0.483	0.439	0.40	0.351	0.307	0.26	0.219	0.176	0.13	0.088
	INF287	0.13	0.120	0.109	0.10	0.087	0.076	0.07	0.054	0.043	0.03	0.022
	INF052	0.46	0.422	0.384	0.35	0.307	0.269	0.23	0.191	0.153	0.12	0.077
	ND050	0.54	0.490	0.446	0.40	0.356	0.312	0.27	0.223	0.178	0.13	0.090
	ND006	0.69	0.629	0.572	0.52	0.458	0.401	0.34	0.286	0.229	0.17	0.115

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
PMA	INF055	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF287	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF052	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	ND050	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	ND006	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M
CD107a	INF055	6	5.5	5	4.5	4	3.5	3
	INF287	6	5.5	5	4.5	4	3.5	3
	INF052	6	5.5	5	4.5	4	3.5	3
	ND050	6	5.5	5	4.5	4	3.5	3
	ND006	6	5.5	5	4.5	4	3.5	3

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#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	15	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	15	ColdStain 30min @4C	15	RBC Lyse, then Fix/Perm	Spiked 30 min @RT	15
1	UV2				BUV395	CD62L	SK11								1.2	18.0			
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	RPA-T8								0.7	10.5			
4	UV10				BUV563	CD69	FN50								0.5	7.5		1	15.0
5	UV11				BUV615	CCR4	1G1						2.0	30					
6	U14				BUV661	Vδ2	B6								0.7	10.5		0.5	7.5
7	UV16				BUV737	CXCR3	1C6/CXCR3								1.3	19.5			
8	V1				BUV805	CD4	SK3						1.5	22.5				0.5	7.5
9	V3				BV421	CD127	A019D5						1.5	22.5					
	V3				Pacific Blue	CD14	M5E2								2.0	30.0			
10	V5				Pacific Blue	CD19	SJ25C1								2.0	30.0			
11	V7				BV480	CD161	HP-3G10						2	30					
					BV510	CD45RA	HI100								0.7	10.5			
12	V10				BV605	CD56	5.1H11												
13	V11				BV650	CCR7	G043H7						1.0	15					
14	V13				BV711	CD7	M-T701						1	15				1	15.0
15	V14				BV750	IFNγ	B27						1	15					
16	V15				BV786	CCR6	11A9											1.5	22.5
17	B2				FITC/AF488	Va24/hCD1d	6B11						1.5	22.5					
18	B3				Spark blue 550	CD3	SK7												
19	B4				PE	NKG2D	1D11								1.2	18.0		0.5	7.5
20	B6				PE-CF594	CD26	M-A261						1.5	22.5				0.5	7.5
21	B8				PE-Cy5	CD25	M-A251						1.2	18					
22	B10				PerCP-Cy5.5	TNFα	MAb11						1.2	18				0.5	7.5
23	B13				PE-vio770	PD1	PD1.3.1.3											2.5	37.5
24	R1				APC	CD16	3G8								1.5	22.5		0.5	7.5
25	R2				AlexaFluor647	Va7.2/hMR1	3C10								0.7	10.5			
26	R4				APC-R700	CD107a	H4A3		6.0	90									
27	R6				Zombie NIR	L/D	N/A				<1:2500>								
28	R7				APC/Fire 750	CD27	O323												
29	R8				APC/Fire 810	CD38	HIT2						2	30					
													1.6	24					
							Antibody Total		6.0	90	Antibody Total		19.0	285	12.5	187.5		9	135
And UNSTAINED CONTROLS !!!							R10 Media		14.5	217.5	Brilliant Stain		50	750	50.0	750		50	750
							Pippette draw volume /sample		19.5		Pippette draw volume /sample		66		59.5			56	

Simplified Protocol

Thaw cells, DNAse, count.
Collect, count, aliquot cells 2-3.0E+6 Cells R10 / 5ml polystyrene tube
Bring volume upto 1 ml R10, add 2 ul PMA/Ctrl and CD107a
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of LiveDead mix (1:2500) @RT for 15min
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add HotStain mix, incubate @37C for 30 min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add Tetramers, incubate @RT for 10 min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add ColdStain mix, incubate @ 4C for 30min
Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

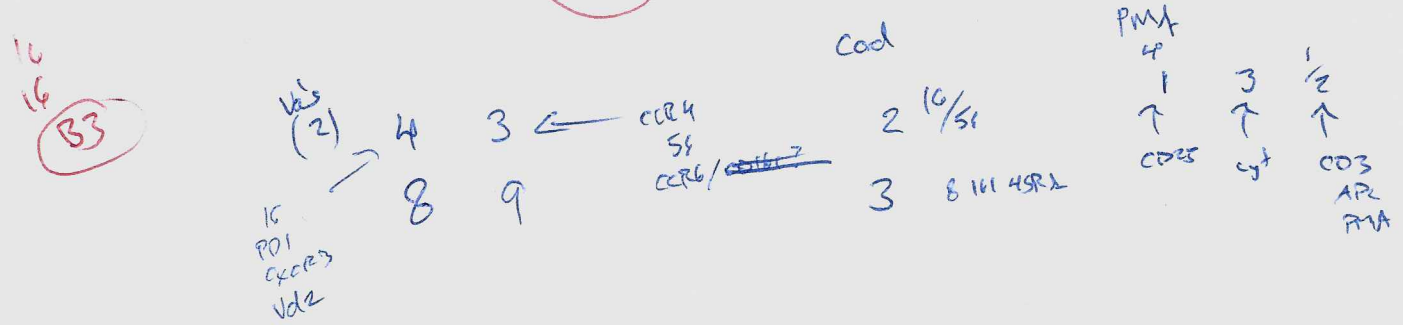
300 ul BD FixPerm, incubate @ 4C for 20min
(vortex every 10 minutes)

First PermWash: 1 ml PermWash 1500 rpm 6 min
Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 2 ml PermWash 1500 rpm 6 min

Resuspend in 70 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

1.5/1.2 antibody



ILT & NK SFC Panel

3/27/2023

Spectrum	UV				Violet				Blue				Red			
373	UV1															
388	UV2	BUV395	[2]	CD62L												
428	UV3				V1	BV421	[4]	CD127								
443	UV4				V2											
458	UV5				V3	PacBlue	[1]	CD14/19								
473	UV6	AF			V4											
508					V5	BV480	[3]	CD161	B1							
514	UV7	BUV496	[2]	CD8												
525					V6				B2	FITC/AF488	[1.5]	Va24/hCD1d				
542	UV8				V7	BV510	[1.5]	CD45RA	B3	SparkBlue 550	[1]	CD3				
582	UV9	BUV563	[3]	CD69	V8	BV570			B4	PE	[4]	NKG2D				
598					V9				B5							
613	UV10	BUV615	[3]	CCR4	V10	BV605	[3]	CD56	B6	PE-CF594	[4]	CD26				
664	UV11	BUV661	[3]	Vd2	V11	BV650	[3.5]	CCR7	B7				R1	APC	[3.5]	CD16
679									B8	PE-Cy5	[4.5]	CD25	R2	AF647	[3.5]	Va7.2/hMR1
697	UV12				V12				B9	PerCP-Cy5.5	[2]	TNFa	R3			
717	UV13				V13	BV711	[4]	CD7	B10				R4	APC-R700	[3]	CD107a
738									B11				R5			
750	UV14	BUV737	[3]	CXCR3	V14	BV750	[2.5]	IFNg								
760									B12				R6	Zombie NIR	[ ]	L/D
783	UV15				V15	BV786	[3]	CCR6	B13	Pe-Vio770	[3]	PD1	R7	APC-Fire 750	[2]	CD27
812	UV16	BUV805	[1]	CD4	V16				B14				R8	APC-Fire 810	[3]	CD38

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