January 27th, 2023

HEU: Ex-vivo γδ CBMC panel #1 - 2023

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
Inf 182-1 a-2	HU S953		13µl		bloody est	3	3.69 ~~8.22~~	~~7.68~~ 5.06	15.18 HM	198µl 5.06 → ~~2.06~~ mls	2.06
Inf ~~182-7~~ a-1 135-9	HEU-lo S720		16µl		bloody	2	~~5.60~~ ~~8.11~~	~~8.08~~ 8.13	16.26	5.42 ~~8.13~~ →	3.42 ~~6.13~~ ml
Inf039-2 a-2	HEU-hi RUT3				limited	2	4.92 ~~5.76~~	7.85	15.70	~~5.23~~ ~~7.85~~ →	3.23 ~~5.85~~ ml
NY058	Adult		10E6	12-16-15		2	~~4.08~~ 5.24	7.65	15.3	5.10 → ~~7.65~~ →	3.10 ~~5.65~~ l

4:12pm Inf specimens pulled

5:22pm count done

6:28pm Culture in, Fc wells aliquoted

7 resuspensions for BCG w/ needle

L/D @ 6:58pm

Spinning down also

No RBC lysis for aurora unstained

sc's got RBC lysed ✓

T/O spin @ 7:16pm

Surface @ 7:34 → ~~7:54~~ pm

Sc's @ 7:44pm → 8:04 pm

Intracellulars done @ 7:52pm

★ 8:01pm Samples post RBC lysis ★

& spin @ 8:13pm

Strep @ 8:15 P2 → 8:30pm

→ P's + sc's resuspended in 150µl

Strep P2 wash @ 8:32pm

Fix Perm @ 8:44 → 54 → 04

2nd wash @ 9:18

Intrac @ 9:31

Done @ 10:23 pm

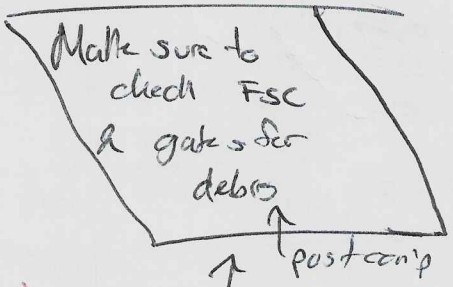
Larissa on @ 10:30 AM, bit sheathe fluid refill

Running contrad: (appears to be clean)

↑ SS R780
↑ 530 V450
V525 ↑ 580
V690 ↑ 550

what changed vs last year

Cross contaminants relatively low as long as 3 drops fall



avoid having additional issues going forward

SC's Acquired at 11:02 AM (add only 5000 events)

FSC for samples ↑ 700 3500 evts/sec 11:05 AM

Runtime / sample: 13 min to get 2400 events
1,973,397 events (58640 evets aborted)

Inf 135 @ running high speed → 6500 evets/sec (5 min) to hit 1000 events threshold

NR62A stripe (spin better?)

P2 panel strong Vd2 CD3 stripe (CD3 V525 scaled up to high?) pattern

Overwrote P1 for B2

PD1/CD16 stripe

Overall P1 clearest abs wise / comp (due to PeDazzle?) ~ treatment matched

Bleach high 5 min @ 12:19 pm
Contrad @ 12:26 pm

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Compensation (/ issues screening)...

P1 → V450 ✗ V670 ✓

V525 V670

R670 > ~~R780~~ R780

R610 > V610

R780 ✗ Y780

CD45RA R780

↓ lower your FSC for p1

V450 PD1 CD16 → two smears unidentified (AF?)

CD16 & PD1

& CD45RA

↑ some decompensation

So... CD25 B710 pattern

CD3 Y780 spread negative

P2 →

R780 L/D ✗ Y780 NKG2A B530

V02

V450 stripe NKG2D V670

CD3 V525 decomp

↑ raise FS

CD3 V525 > Perf B530 at VD2 messy border?

PD1 Y590 > Y780 NKG2A

some stripes

P4b

V450/V525 V670 Y590 neg split? Granz B

R670 VD2 a lot non-specific in CD3-

Y780 ✗ R780 ✓

V670 > V450

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Ex-vivo Gd-P4b

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	3	Intracellular 40min @RT	3
1	V450	BV421	PD1			2	6		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.8	5.4		
4	B530	Alexa 488	Perforin					3	9
5	B710	PerCPeF710	CD3			1.5	4.5		
6	Y590	PE	GzmB					1.5	4.5
	Y615	PE-Dazzle							
7	Y780	PE-Vio770	NKG2A			0.6	1.8		
8	R670	APC	Vδ2			1	3		
9	R780	APC-Fire750	CD16			1	3		
					Antibody Total	7.9	23.7	4.5	13.5
					PBS	12.6	37.8	16	48
					Pippette draw volume / sample	19.5		19.5	

Ex-vivo Gd-P2

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	3	Streptavidin	Intracellular 40min @RT	3
1	V450	BV421	Vδ2			1	3			
2	V525	BV510	CD3			1.5	4.5			
3	V670	BV650	NKG2D			2	6	1.5		
4	B530	Alexa 488	Perforin						3	9
5	B710	PerCPVio700	CD56			1	3			
6	Y590	PE	PD1			1.5	4.5			
	Y615	PE-Dazzle								
7	Y780	PE-Vio770	NKG2A			0.6	1.8			
8	R670	APC	Vδ1			1	3			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
					Antibody Total	8.6	25.8	4.5	3	9
					PBS	11.9	35.7	57	17.5	52.5
					Pippette draw volume / sample	19.5		19.5	19.5	

Ex-vivo Gd-P1

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	3
1	V450	BV421	PD1			2	6
2	V525	BV510	L/D Aqua		<1:500>		
3	V670	BV650	CD16			1.5	4.5
4	B530	FITC	Vδ2			1.2	3.6
5	B710	PerCPeF710	CD25			2	6
6	Y590	PE	CD28			2	6
7	Y615	PE-Dazzle	CD27			1.5	4.5
8	Y780	PE-Vio770	CD3			0.5	1.5
9	R670	APC	Vδ1			1	3
10	R780	APC-Fire750	CD45RA			1.8	5.4
					Antibody Total	13.5	40.5
					PBS	7	21
					Pippette draw volume / sample	19.5	

Ex-vivo gd-CK: PMA-ionomycin

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	6	Intracellular 40min @RT	6
1	V450	BV421	PD1			2	12		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD27			1.5	9		
4	B530	Alexa 488	Vδ2			1.2	7.2		
5	B710	PerCPeF710	CD3			1	6		
6	Y590	PE	CD56			0.5	3		
	Y615	PE-Dazzle							
7	Y780	PE-Vio770	IFNγ					0.6	3.6
8	R670	Alexa 647	TNFα					2	12
9	R780	APC-Fire750	CD45RO			2	12		
					Antibody Total	8.2	49.2	2.6	15.6
					PBS	12.3	73.8	17.9	107.4
					Pippette draw volume / sample	19.5		19.5	

Culture

Thaw cells - > into 9 ml cold R10 -> spin 1200 rpm 10 min
Remove supernatant, resuspend pellet, add 40-80uL of DNAse
Resuspend in 1-3 mL of R10 (pellet guestimate), stain for CD45 and count on Guava

Bring cell concentration to 3.0E+6 Lym+M cells/ml R10
Plate 1ml (3E6 cells) in a 12 well polystyrene plate

BCG::::
3 million cells in 1 ml.
Raise to 2 ml with 1 ml 3E6 CFU BCG
Add 20ul IL-2

Zol:::
3 million cells in 2 ml.
Add 20 uL Zol (10uL/ml)
Add 20ul IL-2 (10 uL/ml)

IL-2:::
3 million cells in 2 ml.
Add 20ul IL-2 (10 uL/ml)

D0, D3, D7, D10
D14

BCG: 7 tubes, waterbath, all into single falcon tube with 6 ML cold R10
Volume will approximate 12 ml-ish
Spin 4000g for 15 min
Discard supernatant, estimate remaining. Raise to 3.1mL of R10
18G 3 ML syringe up down 5 times.
Distribute 1 mL to every BCG well

Staining

Aliquot 1.5 E6 Lym in 500 ul R10 in 5mL polystyrene tube
Bring volume up to 1 ml R10, add 2 ul PMA / Ctrl respectively
Cap, shake and incubate at 37*C for 6 hours

Aliquot 1-1.5 E6 Lym in 5mL polystyrene tube.
Wash with 1-2 ml PBS, spin down 1300rpm 8min
800 ul of LiveDead mix (2:1000, 1:1000) @RT for 15min
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add SurfaceStain mix, incubate @ 4C for 20min
Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 2 ml 5% PBS-FBS 1300 rpm, 8min

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

First PermWash: 1 ml PermWash 1400 rpm 6 min
Second Perm Wash: 1 ml PermWash 1400 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
Final PermWash: 2 ml PermWash 1400 rpm 6 min

Resuspend in 250-300 ul 0.4% PFA-PBS

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