My InSPIREd Life

In Vitro Drug Sensitivity Assay on CLL cells

Fudan University
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09/09/2022

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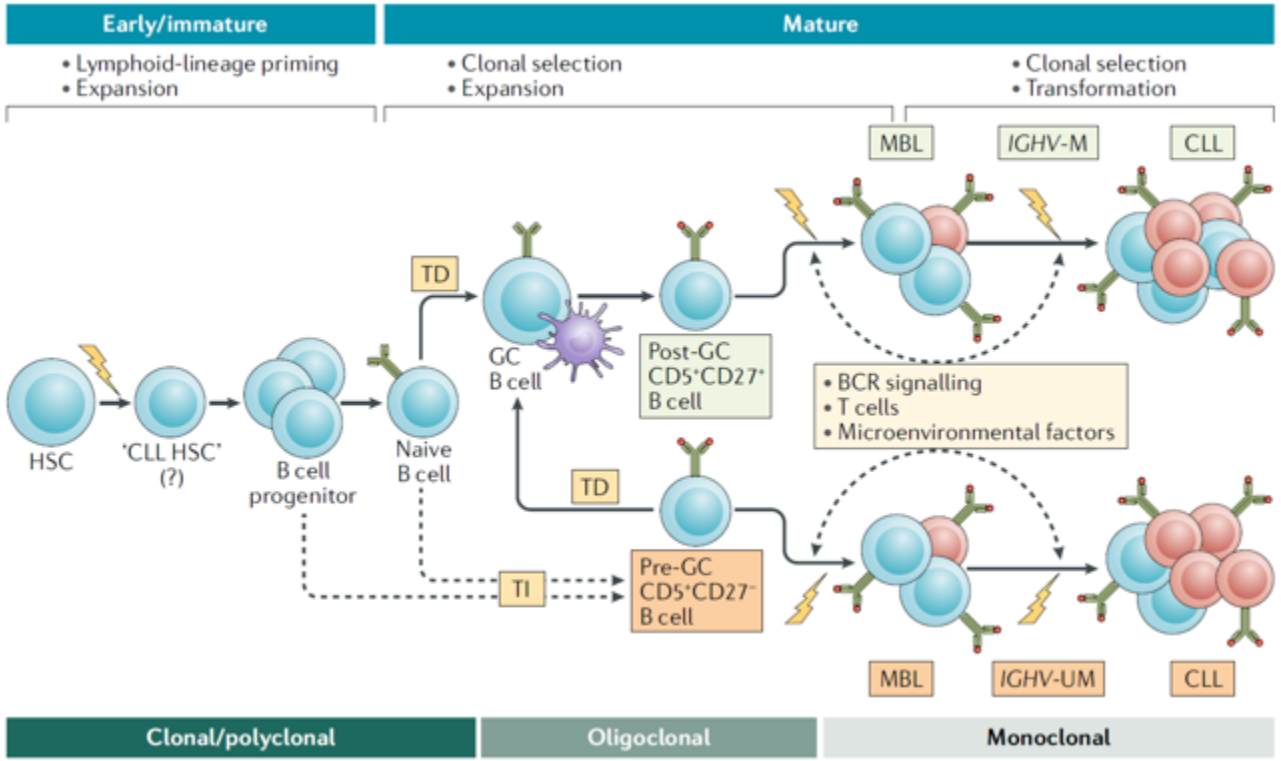
Project Background: Chronic lymphocytic leukaemia



Immunophenotype in CLL and other lymphoproliferative disorders.

Markers	CLL	MCL	SMZL	B-PLL
CD19	+	+	+	+
CD5	+	+	+/–	+/–
CD20	dim	+	+	+
CD23	+	–	+/–	–
FMC7	–	+	+/–	+
Light chain	dim	Bright	Bright	Bright
Heavy chain	IgM/IgD	IgM/IgD	IgM/IgD	IgM/IgD
CD200	+	–	+/–	+/–

CLL: chronic lymphocytic leukaemia; MCL: mantle cell lymphoma; SMZL: splenic marginal zone lymphoma; B-PLL: B-cell prolymphocytic leukaemia.

Chronic lymphocytic leukaemia (CLL) is a type of slow-growing leukaemia that affects developing B-lymphocytes with a high incidence.

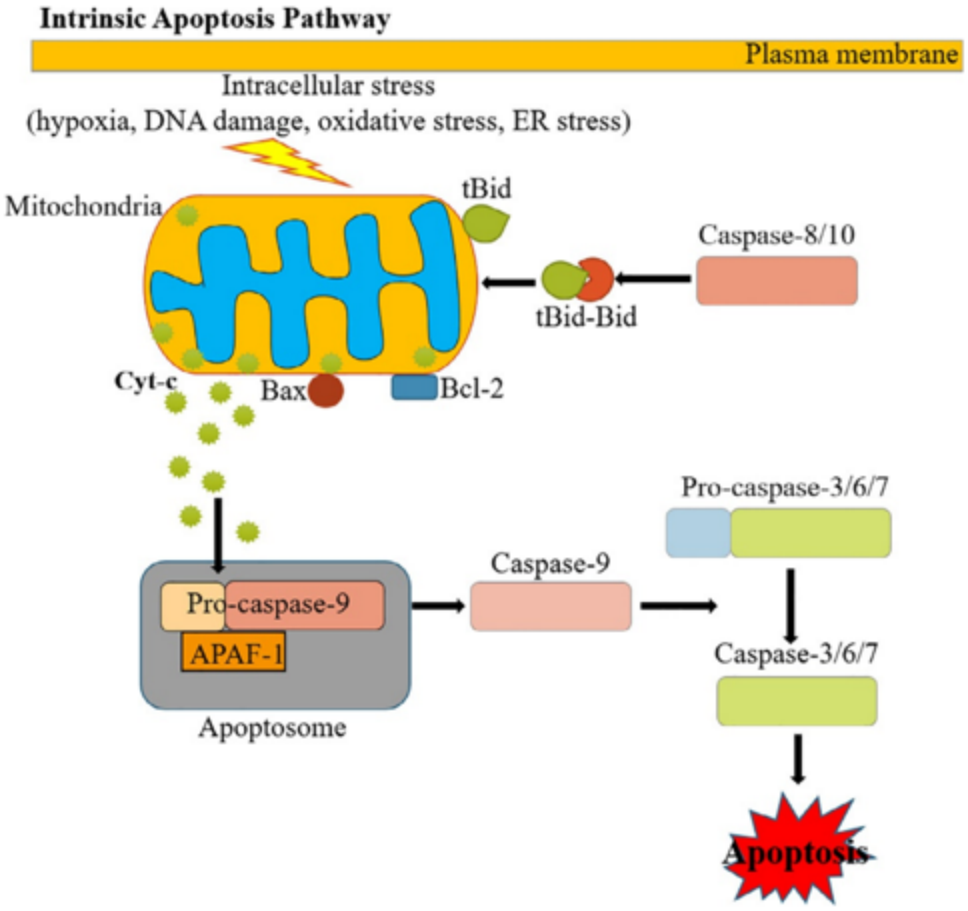
CLL cell surface marker:

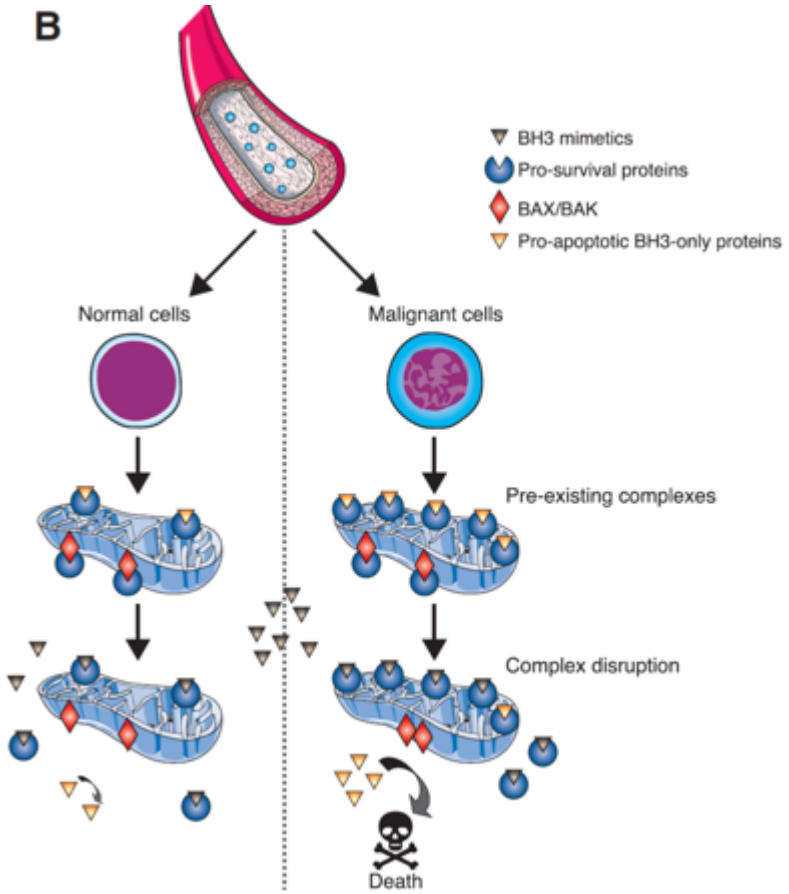
CD5 + CD19 +

doi: 10.1038/s41571-019-0239-8.
doi: 10.1016/j.critrevonc.2016.06.003.

Project Background: Treatments

BH3-mimetic Drugs: Venetoclax





Venetoclax is a Bcl-2 inhibitor, playing its role by attaching to Bcl-2 to block its function. This activates BAX and BAK, making cells begin to apoptosis.

Hypothesis:

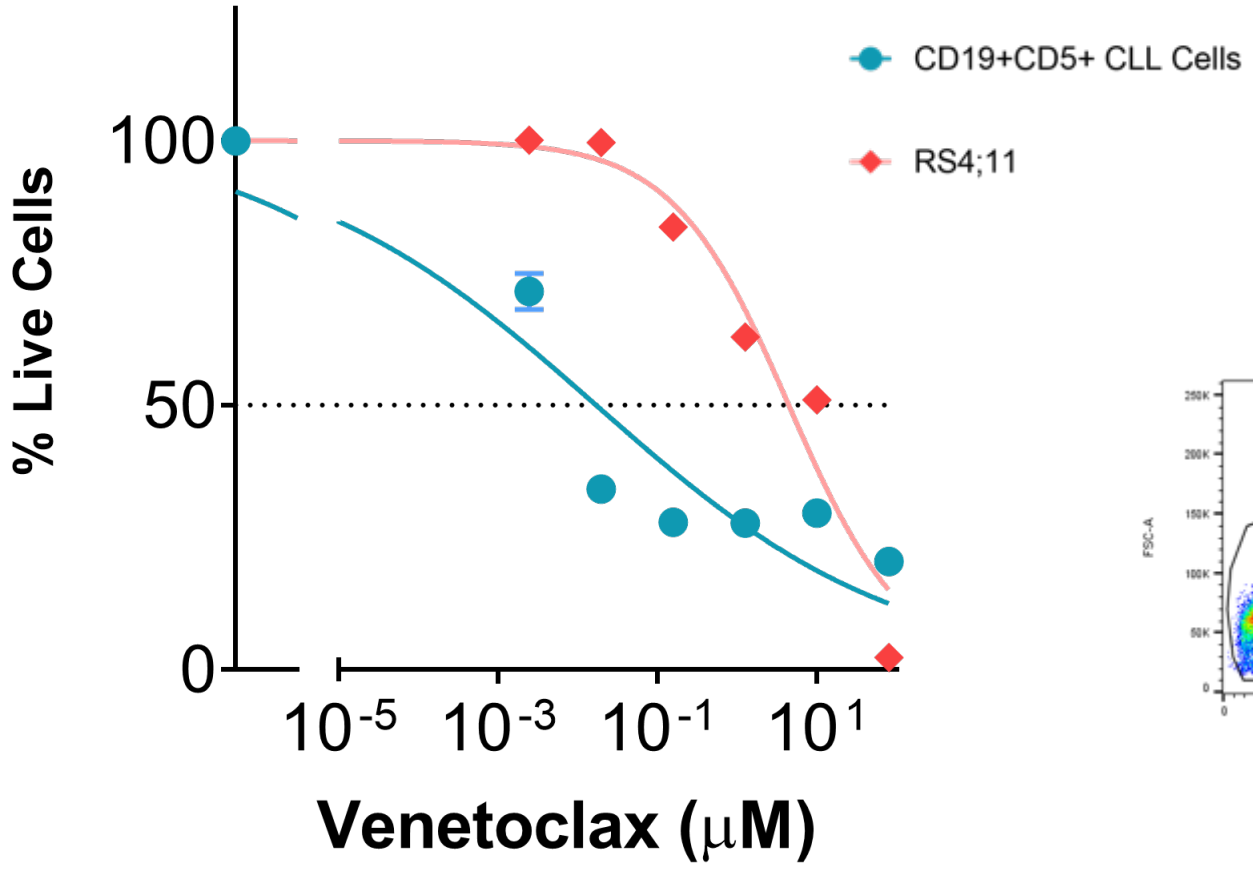
CLL cells can be killed by Venetoclax, and Emrisacan can strengthen the effect by activating necroptosis.

doi: 10.1016/j.ccell.2018.11.004.

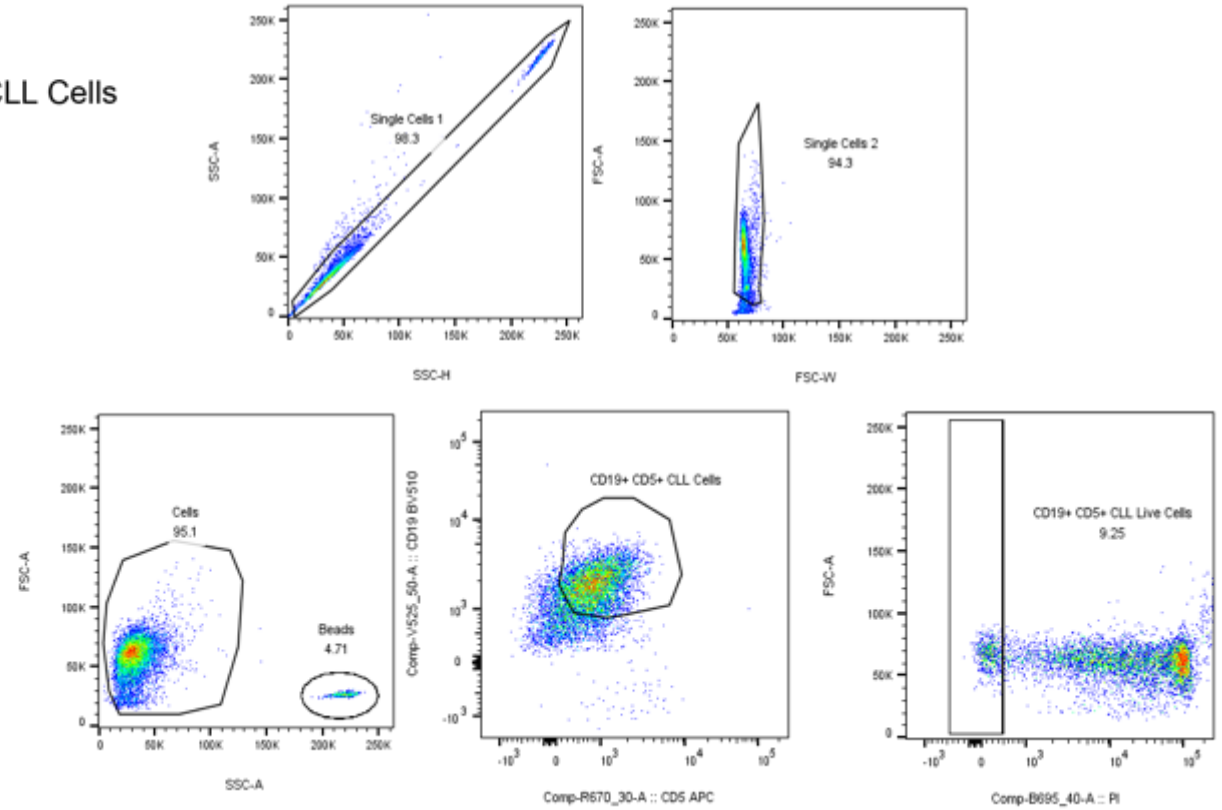
doi: 10.1016/bs.apcsb.2021.01.003.

Project: In Vitro Drug Sensitivity Assay on CLL Cells

Venetoclax Kill Curve



	CD19+CD5+ CLL Cells	RS4;11
HillSlope	-0.2300	-0.5980
IC50	0.01691	4.416

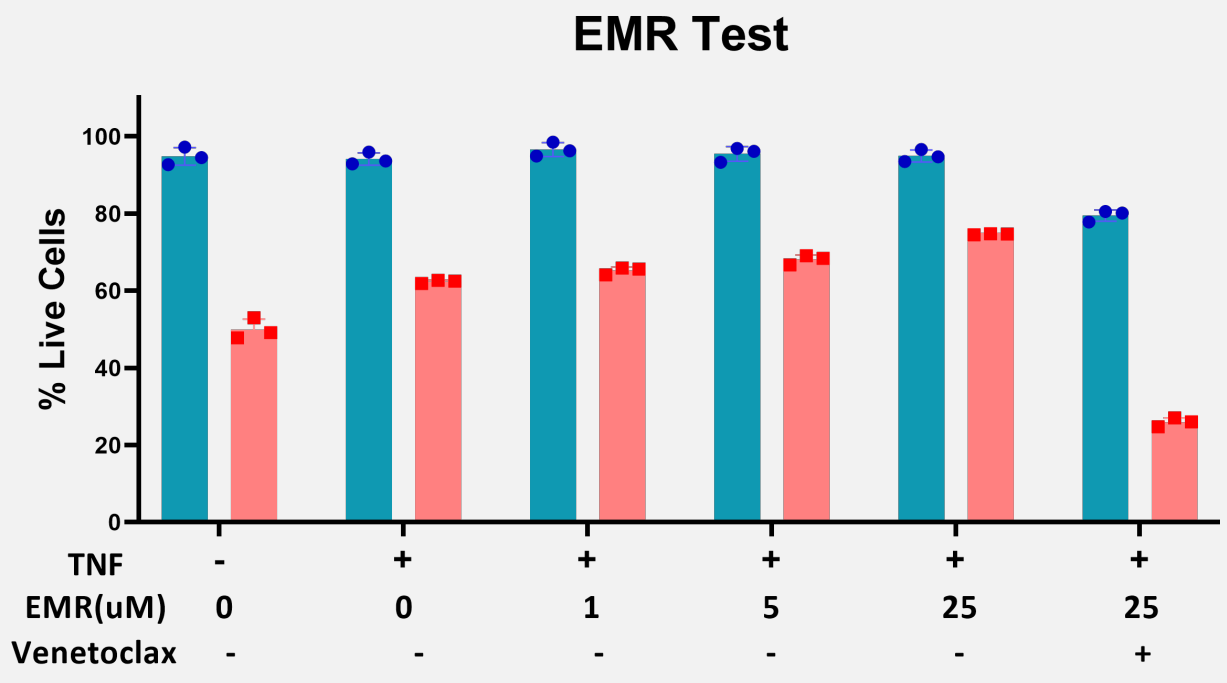


CLL cells are sensitive to killing by Venetoclax

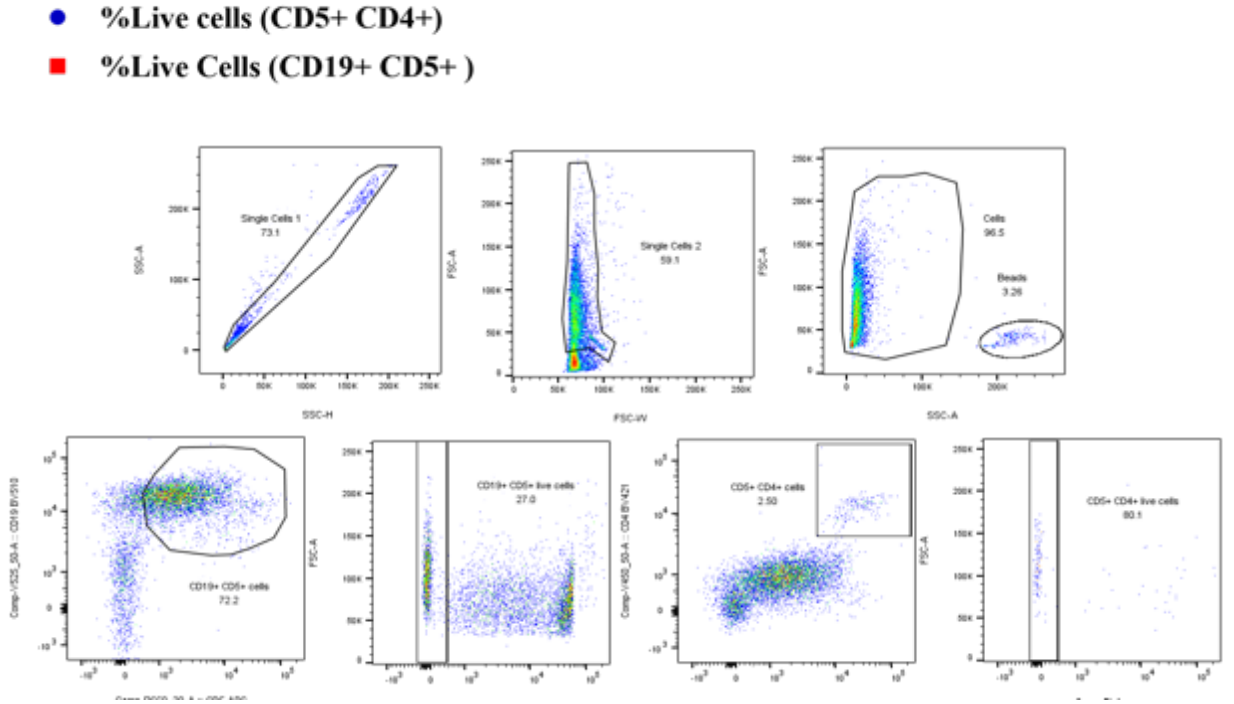
Project: In Vitro Drug Sensitivity Assay on CLL Cells

Drug Assay on CLL Cells

Emricasan & TNF



Live Cell Concentrations (cells/uL)						
TNF (10ng/mL)	-	+	+	+	+	+
EMR(uM)	0	0	1	5	25	25
Venetoclax (2.5uM)	-	-	-	-	-	+
CD19+ CD5+	92.29	210.92	211.49	229.09	426.04	64.18
CD5+ CD4+	6.32	8.19	8.71	8.73	14.33	6.61



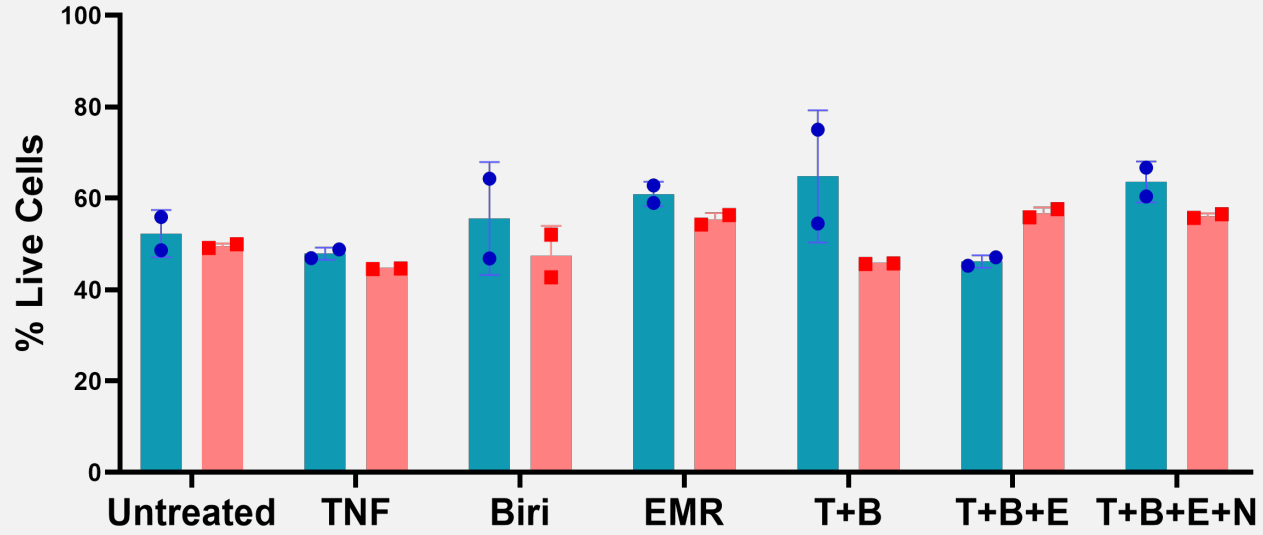
CLL cells are not sensitive to killing by Emricasan

Project: In Vitro Drug Sensitivity Assay on CLL Cells

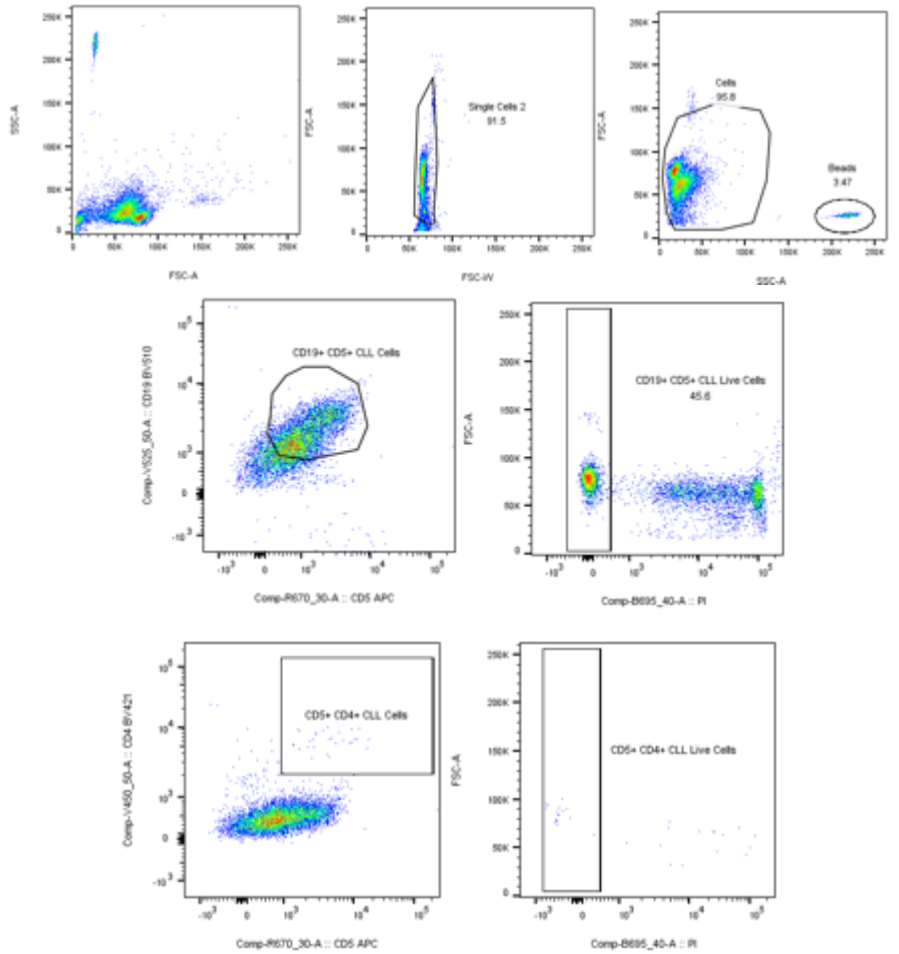
Drug Assay on CLL Cells

- %Live cells (CD5+ CD4+)
- %Live Cells (CD19+ CD5+)

TNF (T): 10ng/mL
Biri (B): 0.5uM
Emrisacan (E): 25uM
Necrostatin (N): 50uM



Live Cell Concentrations (cells/uL)							
	Untreated	TNF only	Smac mimetic only (Biri)	Emricasan	T+S	T+S+I	T+S+I+N
CD19+ CD5+	47.07	43.80	58.11	63.84	55.11	63.38	81.67
CD5+ CD4+	0.29	0.29	0.52	0.46	0.47	0.26	0.66



Project: In Vitro Drug Sensitivity Assay on CLL Cells

Summary

- **CLL cells are very sensitive to Venetoclax.**
- **CLL cells would show resistance to Venetoclax (20%).**
- **Emricasan is a potential way to solve the problem but it can not be used with TNF.**

Seminars and Workshops

WEHI
brighter together

Seminars

All welcome

Wednesday Seminars

1pm - Visitors Welcome
Revised 6 July 2022

13 July 2022
Associate Professor Aaron Jex
Laboratory Head, Population Health & Immunity division
Infection mechanisms, drug resistance and surveillance of zoonotic and neglected pathogens
WEHI L7C Davis Auditorium

10 August 2022
Dr Donia Moujalled
Research Fellow - Wei Laboratory, Blood Cells & Blood Cancer division
Title to be advised
WEHI L7C Davis Auditorium

20 July 2022
Dr Christoph Grohmann

eventbrite

Order no. 4016196119

Regulatory T cell Mini Symposium

In-person attendance ticket

G004 Auditorium, The Peter Doherty Institute for Infection and Immunity, 792 Elizabeth Street, Melbourne, VIC 3000, Australia

Wednesday, 13 July 2022 from 1:00 pm to 5:30 pm (AEST)

IMMUNOLOGY SEMINAR SERIES 2022

MONTH	DATE	SPEAKER(S)	CHAIR	VENUE
JULY	7	Lab Head Session: Charlotte Slade	Evan Thomas	DA/TEAMS
	14	Raymond Qin	Cindy Audiger	DA/TEAMS
	21	Sara Tomei (1hr)	Lucille Rankin	TEAMS ONLY
	28	Charis Teh Matthias Mulazzani	Esther Bandala Sanchez	DA/TEAMS
AUGUST	4	Lab Head Session: Misty Jenkins	Caleb Dawson	DA/TEAMS
	11	Lauren Howson Melinda Hardy	Charis Teh	DA/TEAMS
	18	Esmaeel Azadian Leesa Lertsumitkul	Julie Tellier	DA/TEAMS
	25	No Seminar: Andrew Lew Symposium		
SEPTEMBER	1	Lab Head Session: Vanessa Bryant	Matthias Mulazzani	DA/TEAMS
	8	Invited Speaker: Pirooz Zareie (Monash)	Lauren Howson	DA/TEAMS

MELBOUR
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Advancing immunotherapy through collabora

Reconnect Symposium 2022, Tue

Registration Opens : 8.30 AM – 9:00 AM, WE

9:00AM - 9:10AM Davis auditorium
Welcome to country
Introduction to MIN and the new MIN committee:
Prof. Nick Huntington (MONASH)

Welcome to MIN Reconnect 2022:
Dr Lorraine O'Reilly/Dr Cynthia Louis (WEHI)

Order #4064904619

ory Symposium

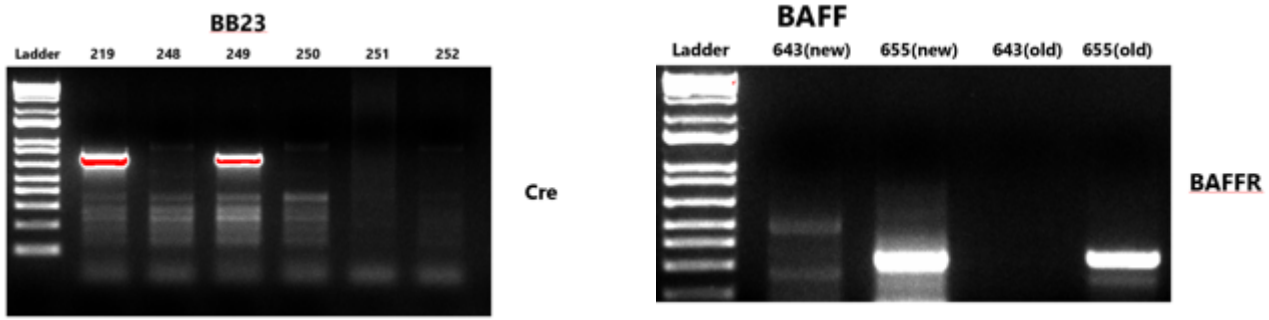
earch, 1G Royal Parade, Level 7, Parkville, VIC

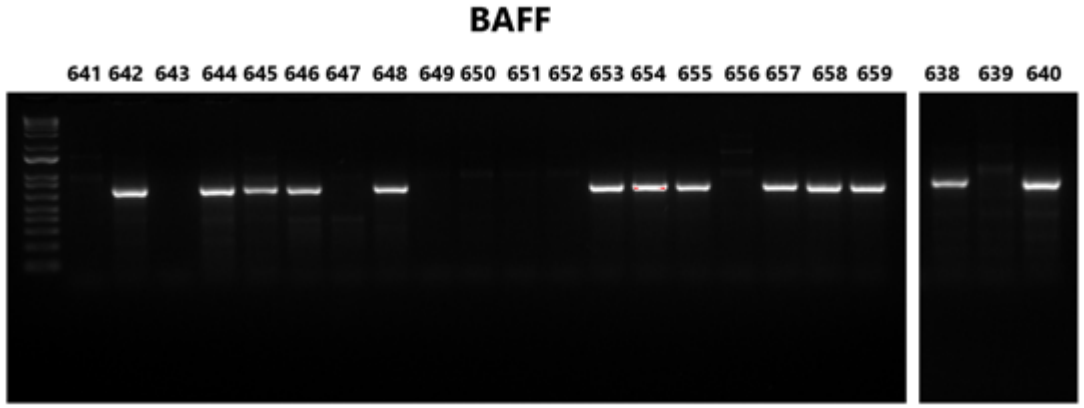
o 5:45 pm (AEST)

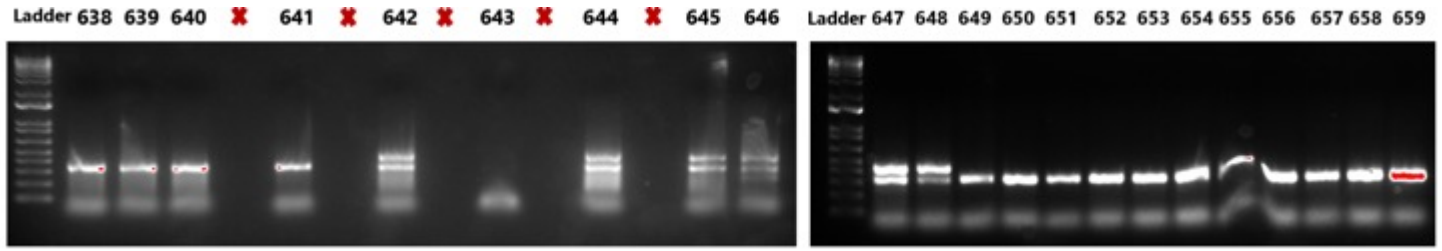
n 12 July 2022 4:49 pm

40649046196526623709001

Experiments: Genotyping

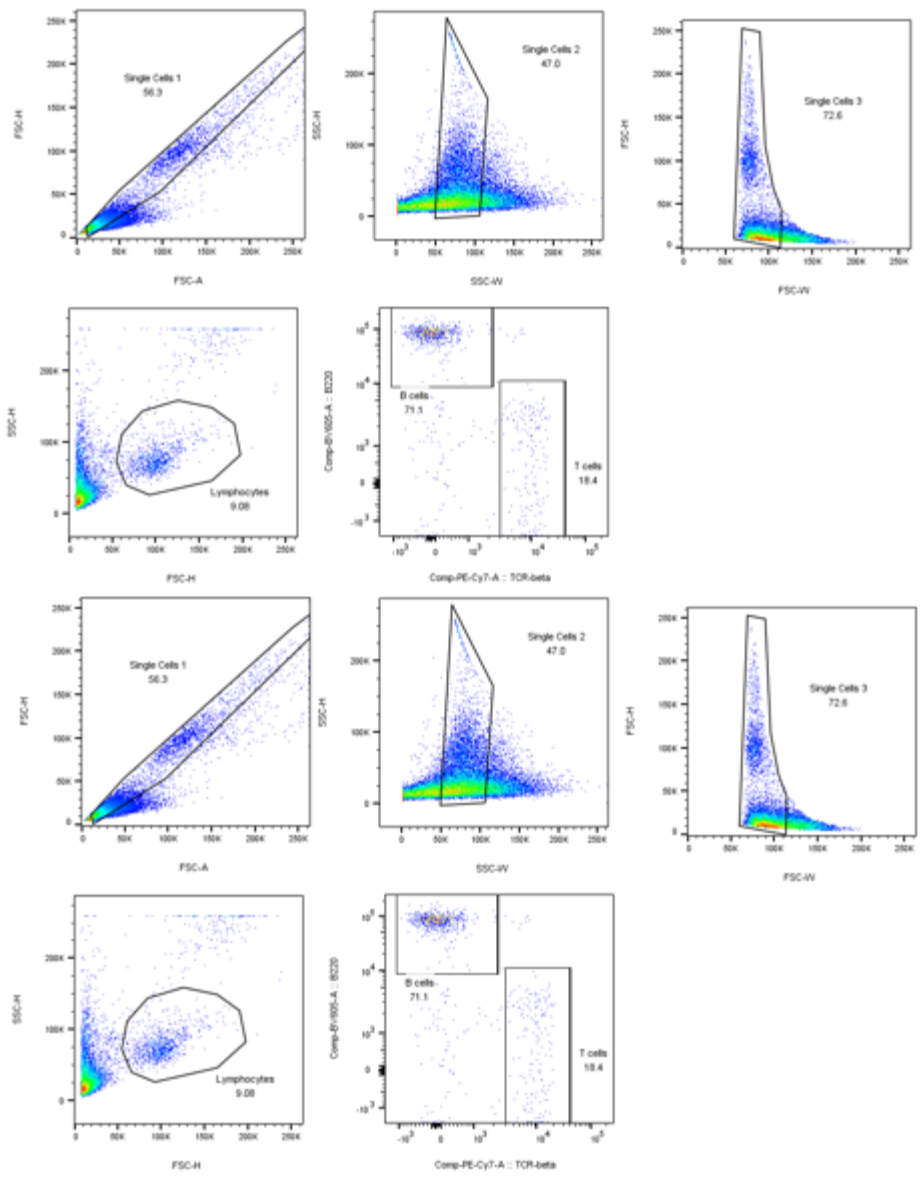


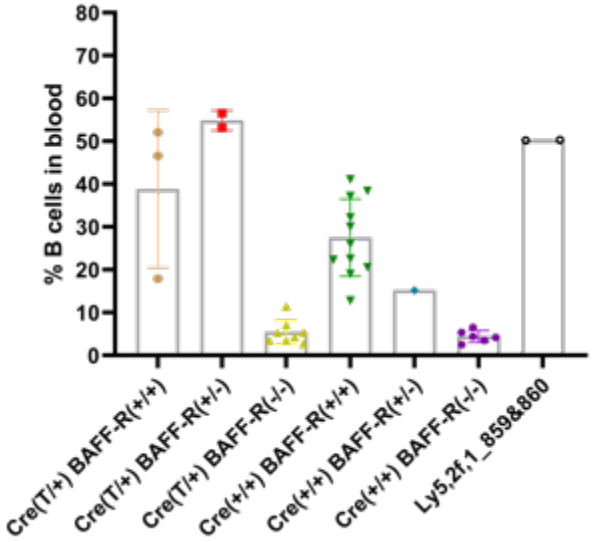


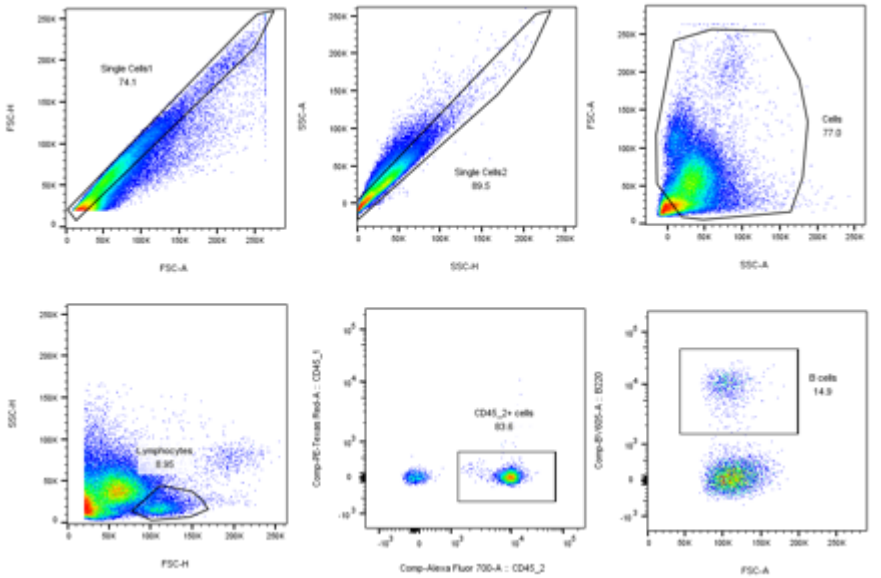


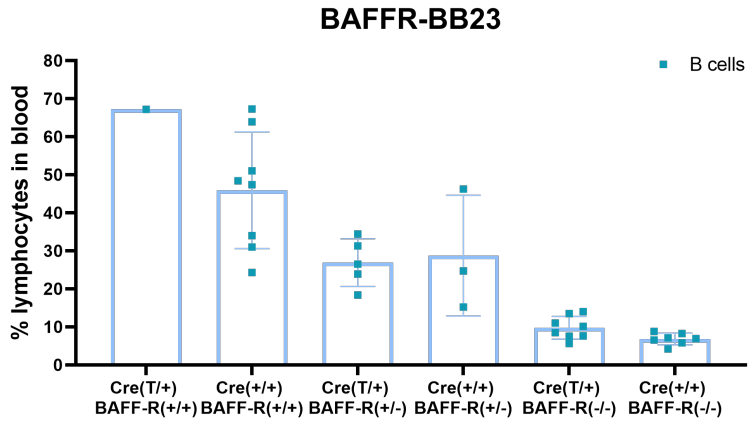
- Digesting the samples
- PCR
  - Preparing a PCR mix vial
  - Preparing a PCR test
  - Running a PCR program
- Running a Gel

Experiments: Mice Bleed (Cells sorting)



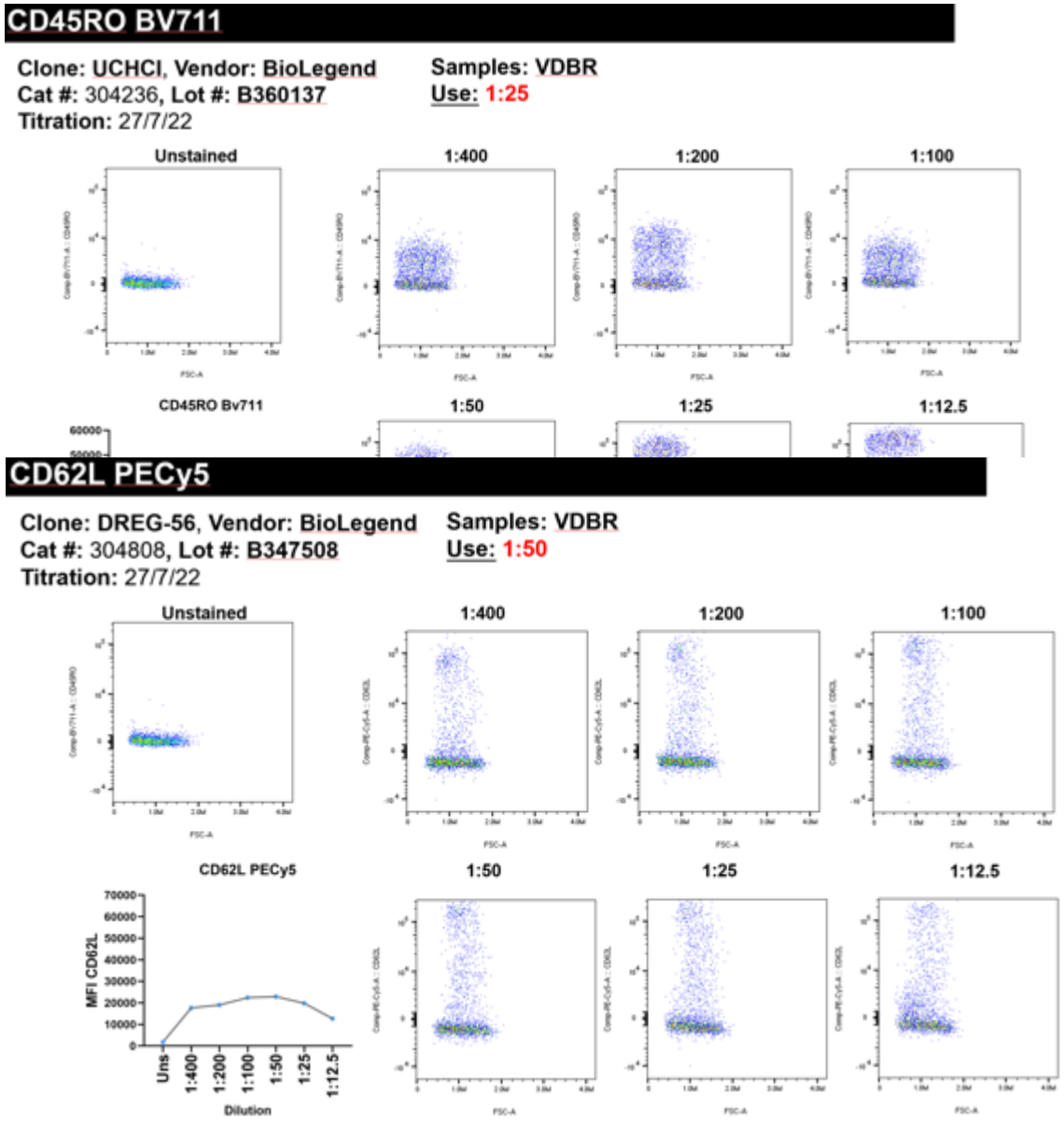


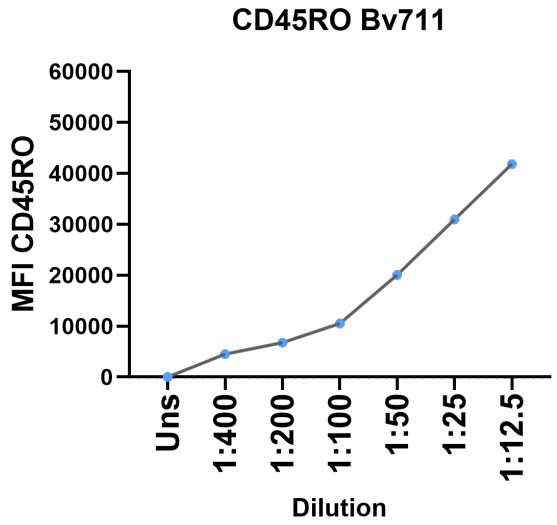


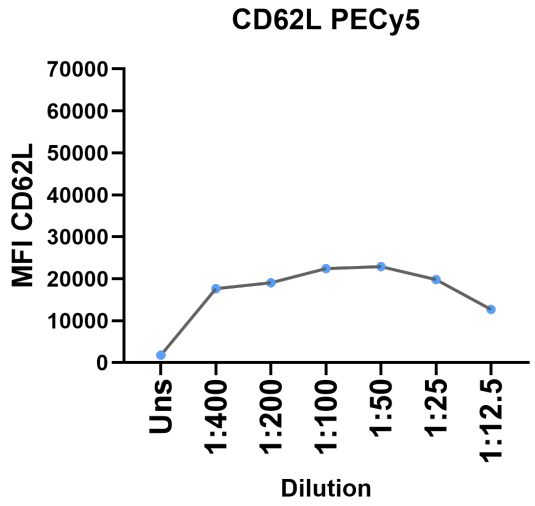


- **BAFF R KO mice have low B cells numbers in the bleed.**
- **A dose effect exists.**

Experiments: Antibody Titration







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Host Family

Family & Friends

Thank you

WEHI_research WEHIresearch WEHImovies WEHI_research Walter and Eliza Hall Institute