Re-ordering human B lymphopoiesis: Discovery of a CD10-ve fetal B progenitor

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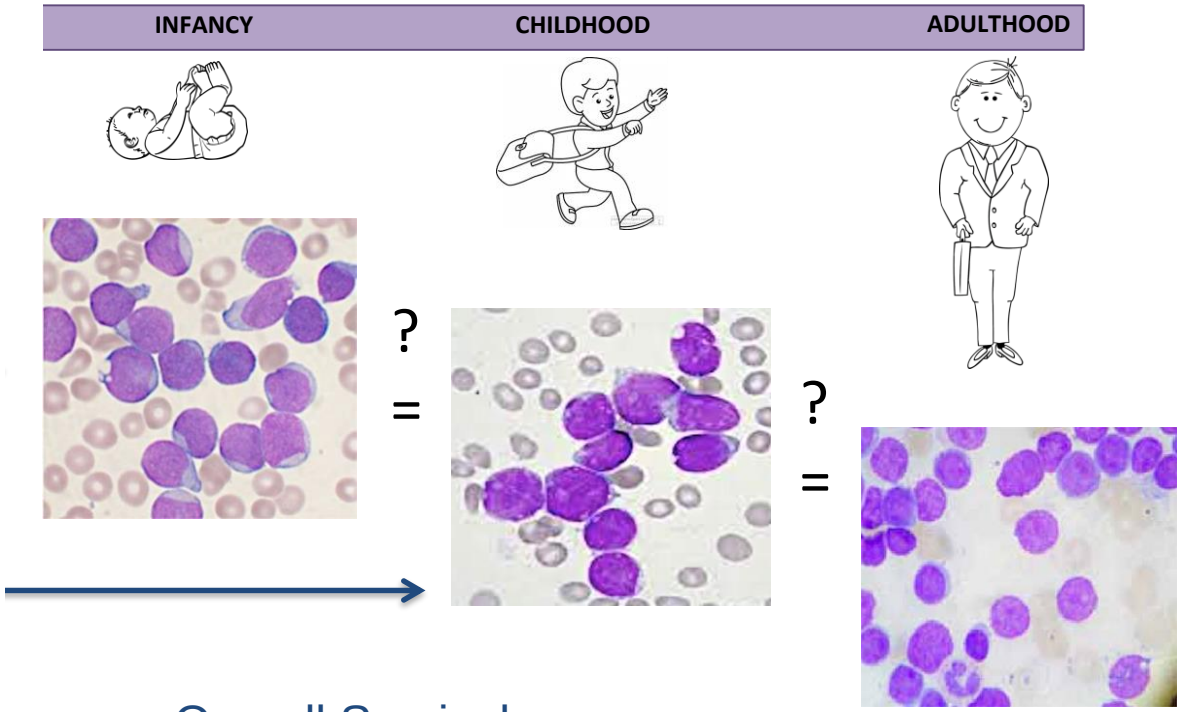
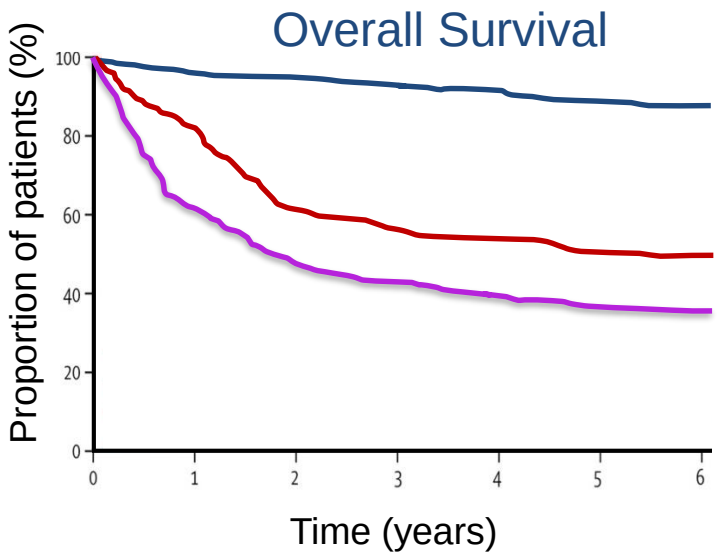
Is leukaemia the same disease at any age?



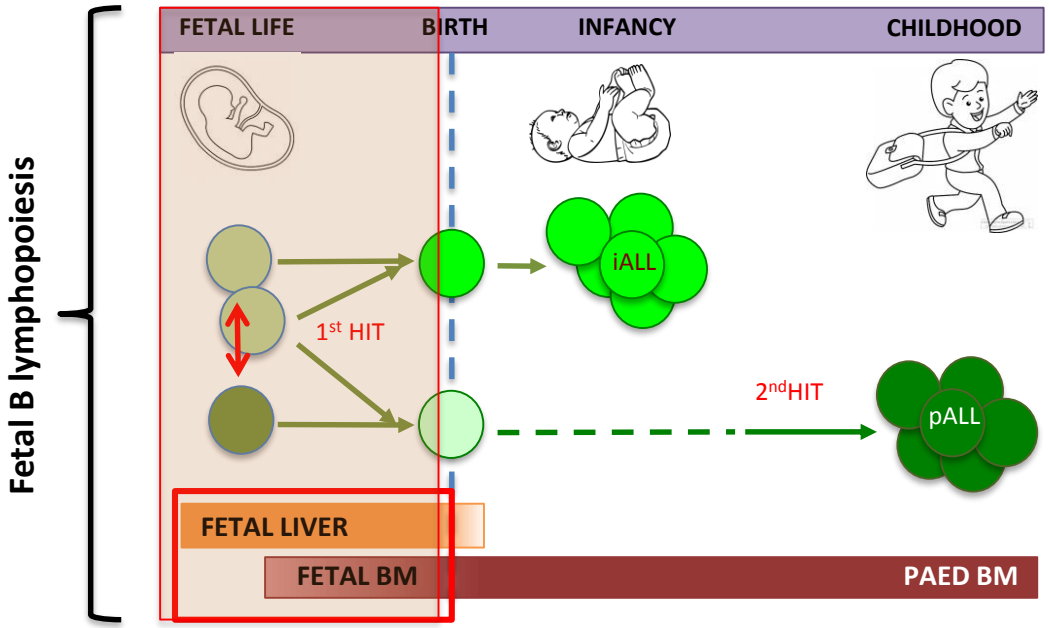


paediatric ALL: 91.5%

infant ALL: 53.8%

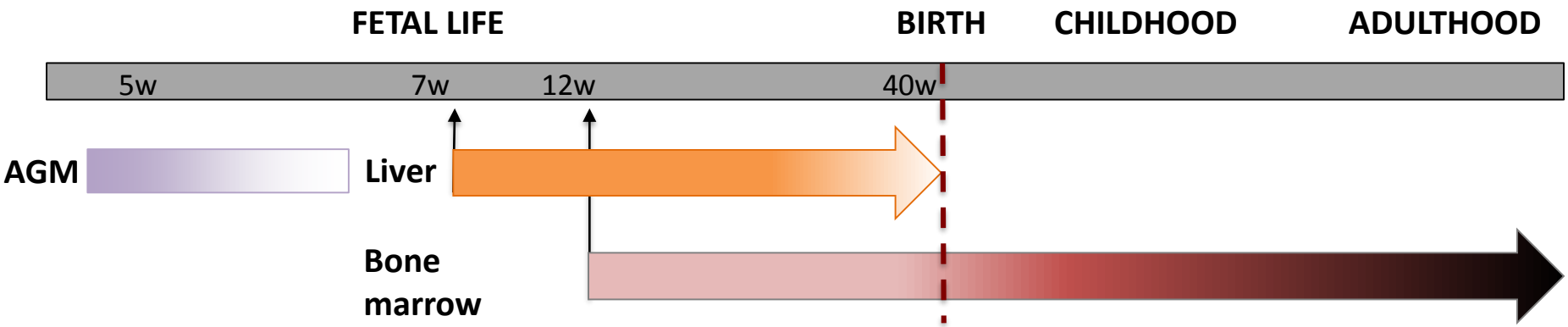
adult ALL: 39%

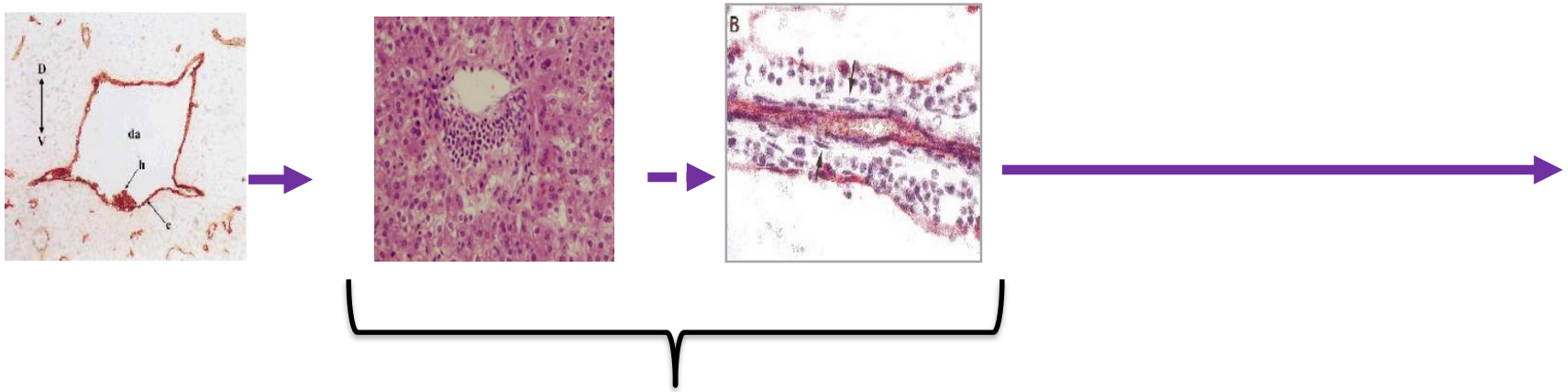
Fetal origins of infant ALL



- What is the target (fetal) cell for leukaemic transformation in infant/ childhood ALL?
- Where do these lie in the B cell developmental hierarchy?
- Are these progenitors restricted to a particular developmental stage or site?
- Do the fetal specific characteristics of these progenitors define the biology of the leukaemia?

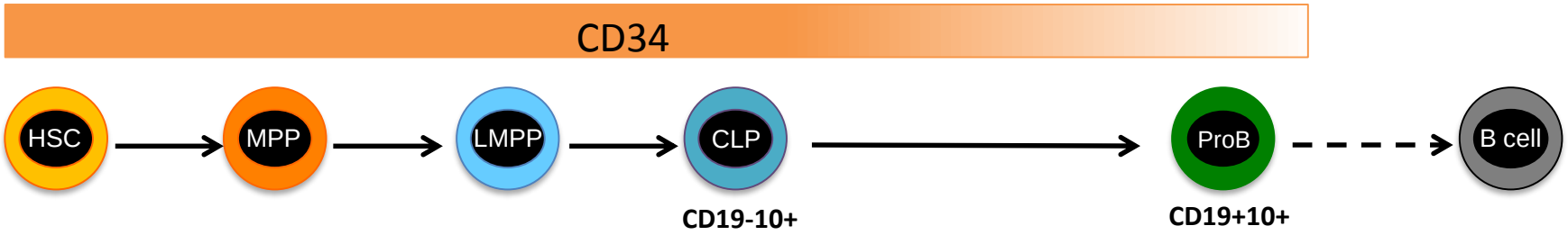
Sites of haematopoiesis through ontogeny





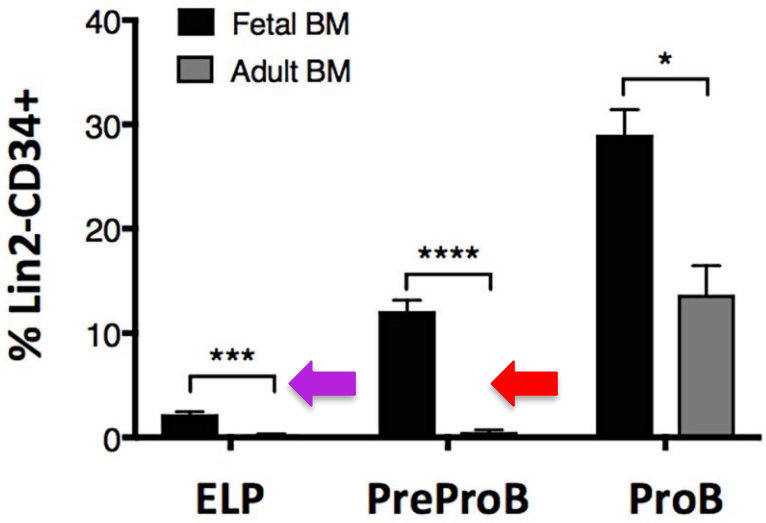
FL and FBM from 6 weeks- 21 post conceptional weeks

Fetal specific B progenitors



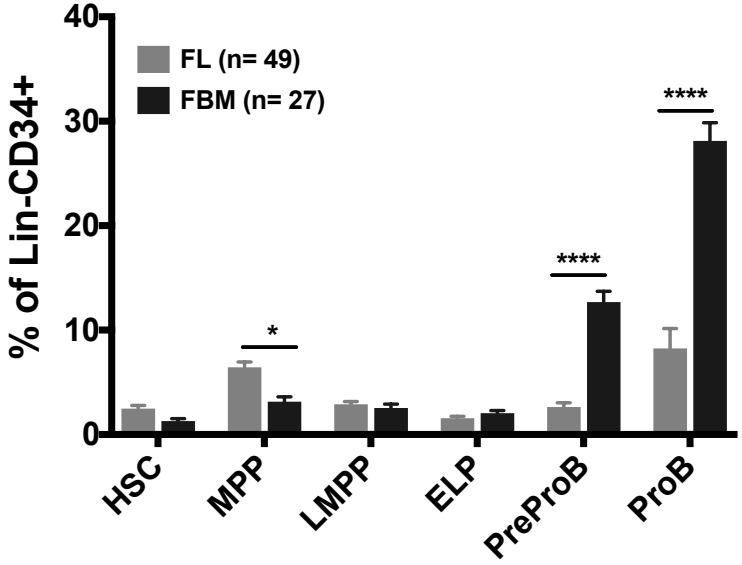
Sanz et al (CB), 2010
Roy et al (FL), 2012

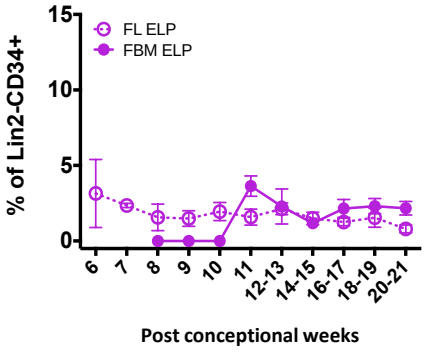
Roy et al (fetal vs postnatal), 2017

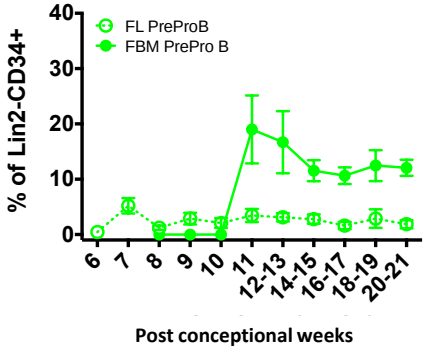


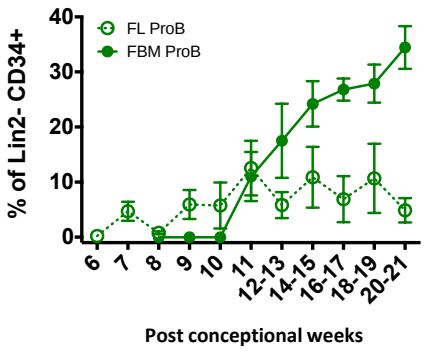
High frequency of PreProB progenitors in FBM

Are fetal specific B progenitors site/ stage specific

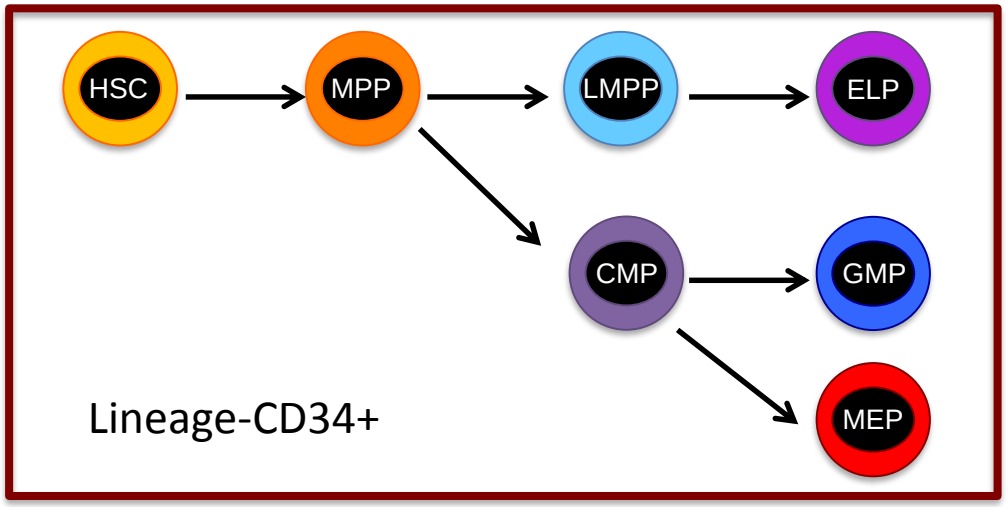


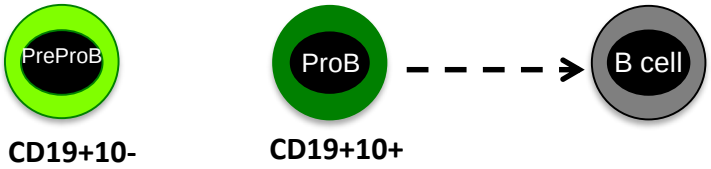


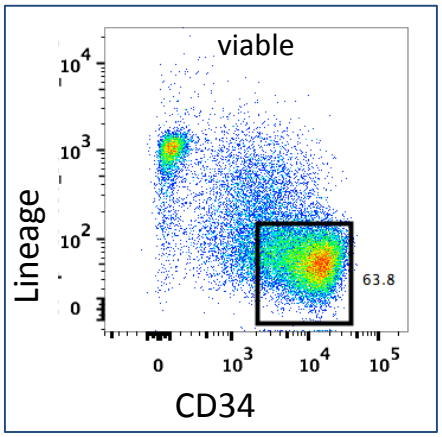




Are upstream FBM progenitors ‘lymphoid primed’?





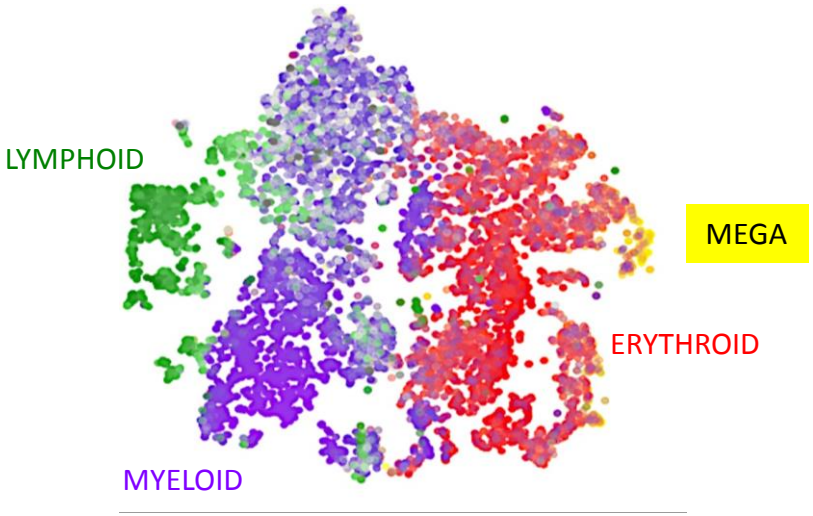


30,000 cells

SAMPLES
2 x Fetal liver (FL)
2 x Fetal BM (FBM)

Chromium 10 X
HT single cell gene expression

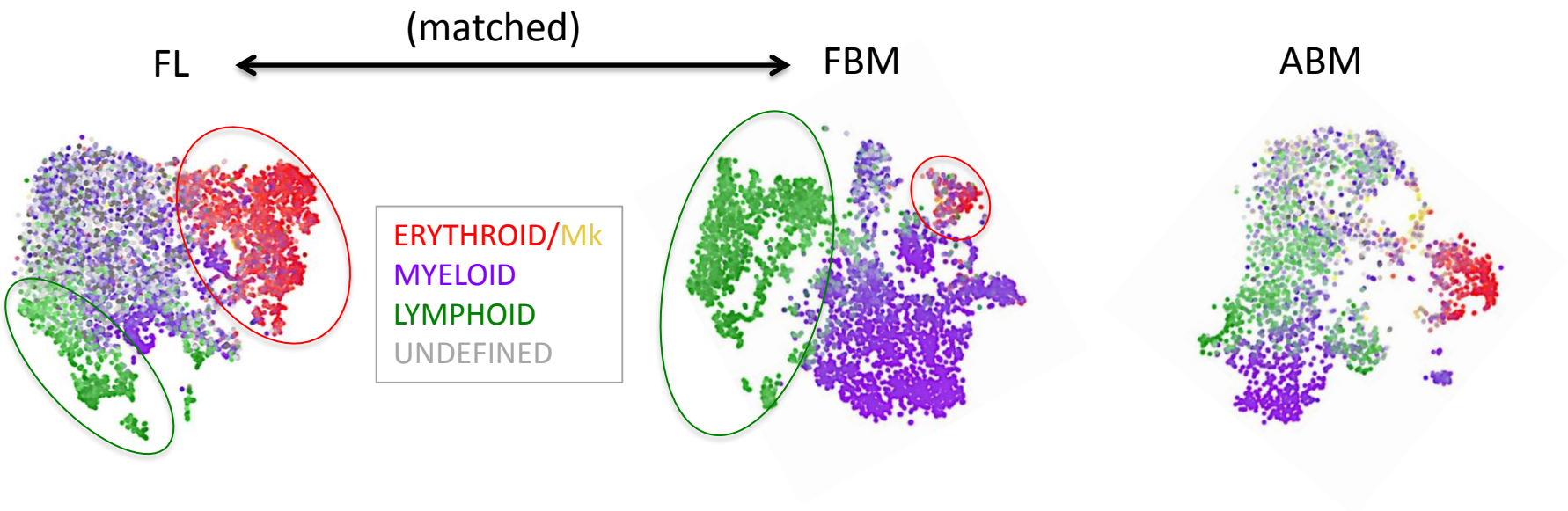
Are upstream FBM progenitors ‘lymphoid primed’?



ERYTHROID	MYELOID	LYMPHOID	MEGAKARYOCYTIC
EPOR	ELANE	CD79A	C6orf25
KLF1	AZU1	VPREB1	CD9
TFR2	PRTN3	VPREB3	CLU
TFR1	CFD	IL7R	PF4
CSF2RB	MPO	EBF1	GP9
APOE	CSF3R	IGHM	ITGA2B
APOC1	CSF1R	JCHAIN	ITGAB3
CNRIP1	LYZ	CD22	CLEC1B
	MS4A3		GP6
	CST7		PDGFA
	CTSG		FLI1
			THBS1
			SELP

N Ashley
B Psaila
S Thongjuea

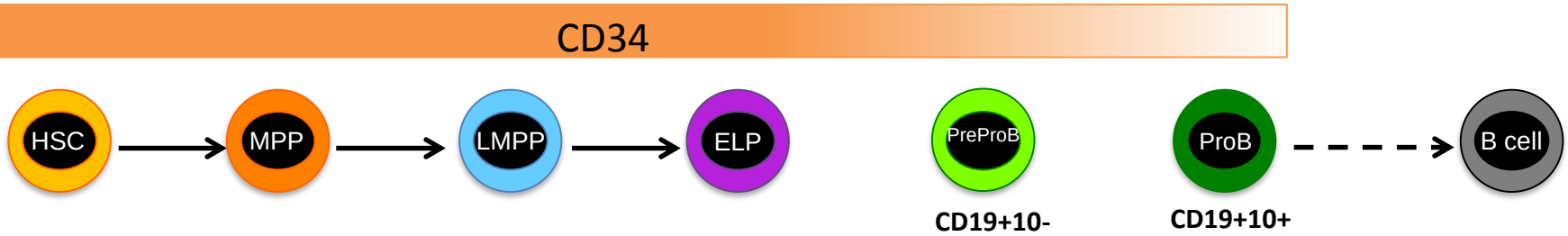
Are upstream FBM progenitors ‘lymphoid primed’?



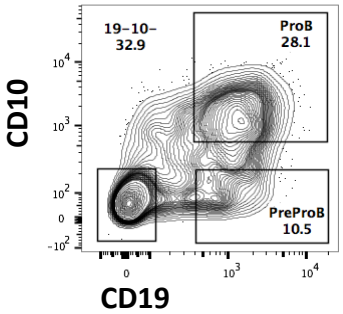
Erythroid affiliated HSPC compartment predominant in FL and lymphoid affiliated in FBM

The Lin-CD34+ HSPC compartment varies in its composition and lineage specification in a site- specific manner in the same fetus and is most likely, directed by cell intrinsic and/or specific microenvironmental factors

Characterising human FBM B lymphopoiesis



Immunophenotyping



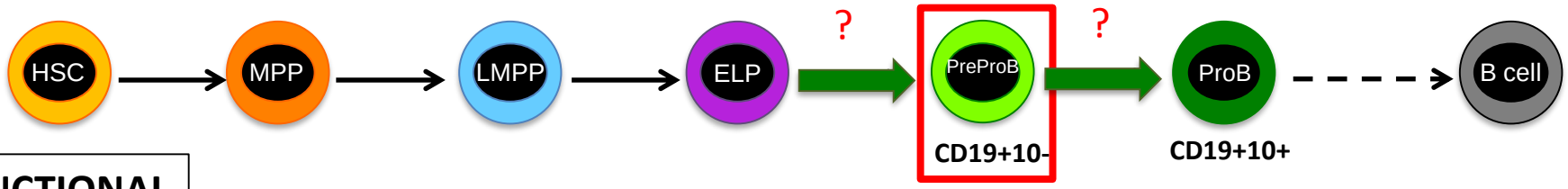
Functional

Gene expression

Epigenetics

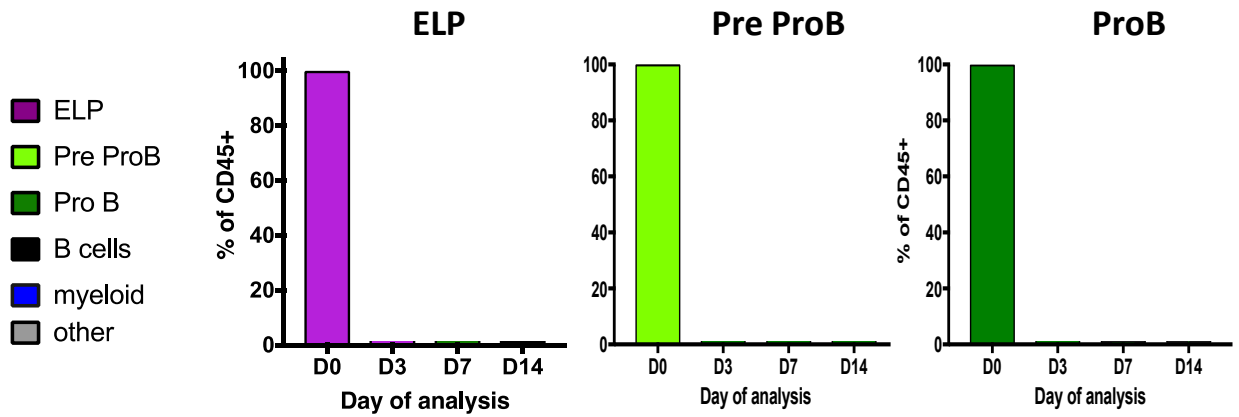
O'Byrne *et al*, manuscript under revision

Cellular hierarchy of human fetal B lymphopoiesis



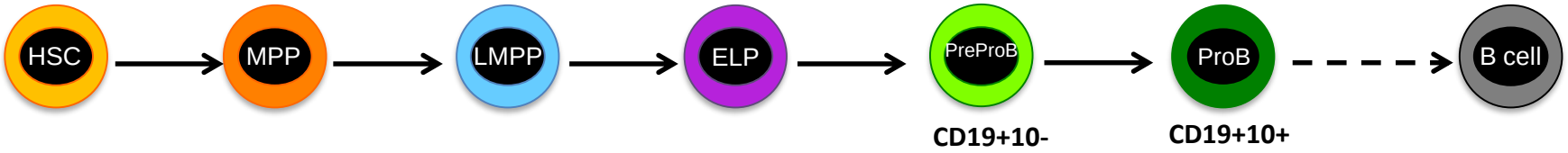
FUNCTIONAL

	HSC	MPP	LMPP	ELP	Pre ProB	ProB
erythroid	✓	✓				
myeloid	✓	✓	✓	✓		
T	✓	✓	✓	✓		
NK	✓	✓	✓	✓		
B	✓	✓	✓	✓	✓	✓

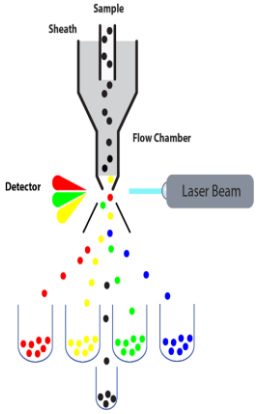


O'Byrne *et al*, manuscript under revision

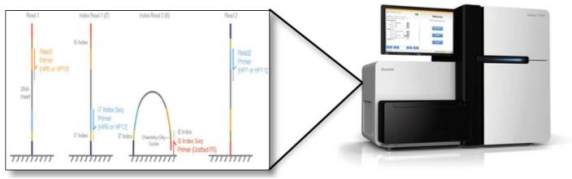
Cellular hierarchy by gene expression



BULK FACS SORTING
(100 cells/population)

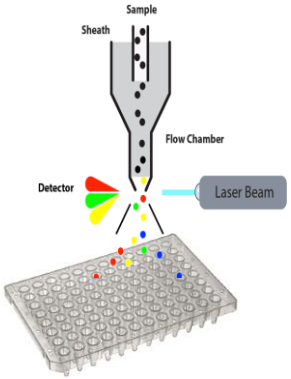


RNA SEQUENCING

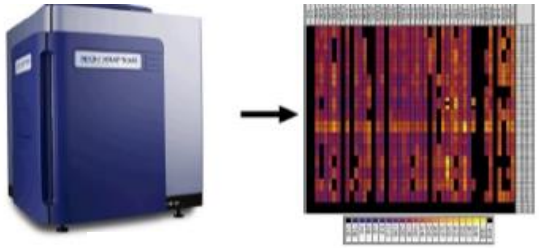


BIOINFORMATICS

SINGLE CELL INDEX SORTING



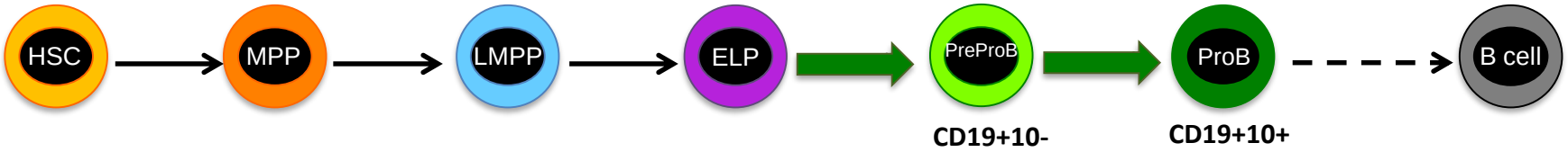
BIOMARK SINGLE CELL RQ-PCR



96 GENE PANEL
(lineage/lymphoid/ iALL associated genes)

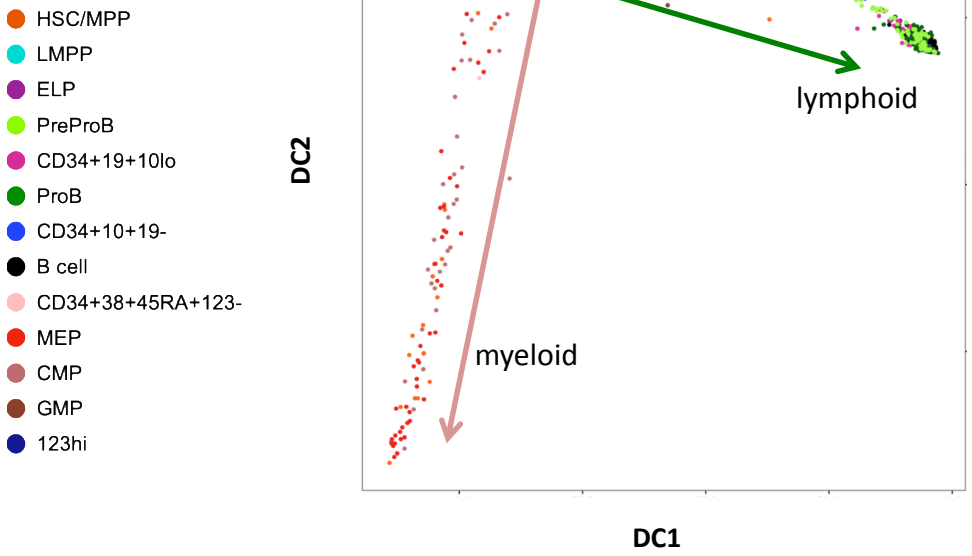
Natalina Elliott
Gemma Buck
S O'Byrne

Cellular hierarchy by gene expression

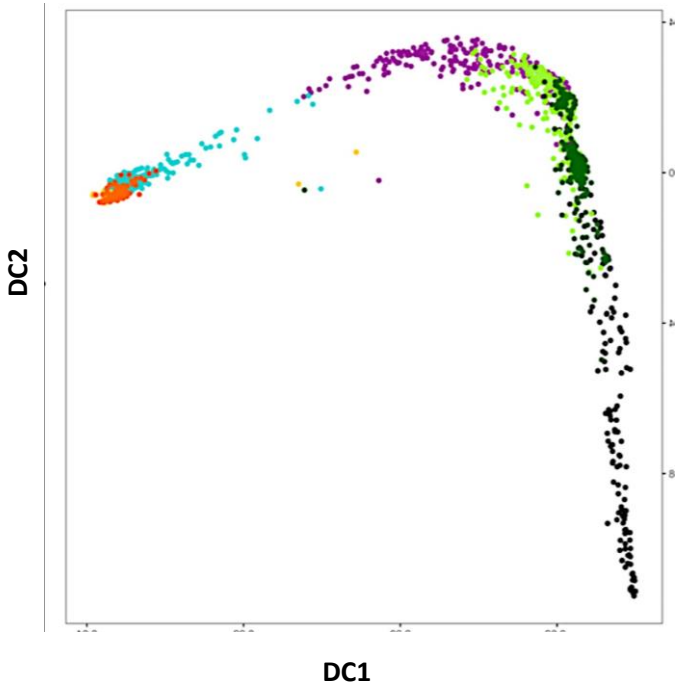


Single Cell analysis

Index sorted CD34+ cells



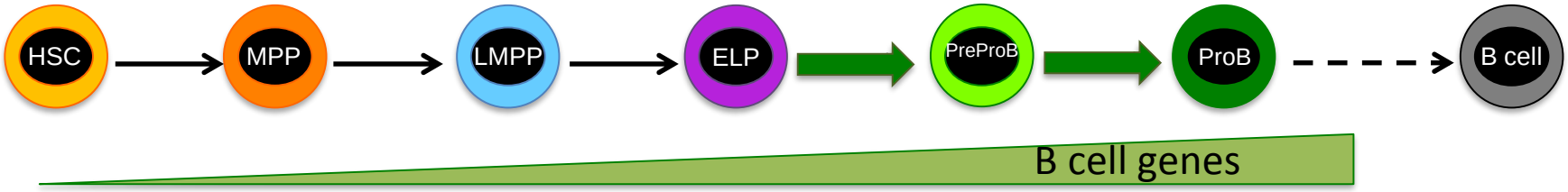
Index sorted lymphoid populations

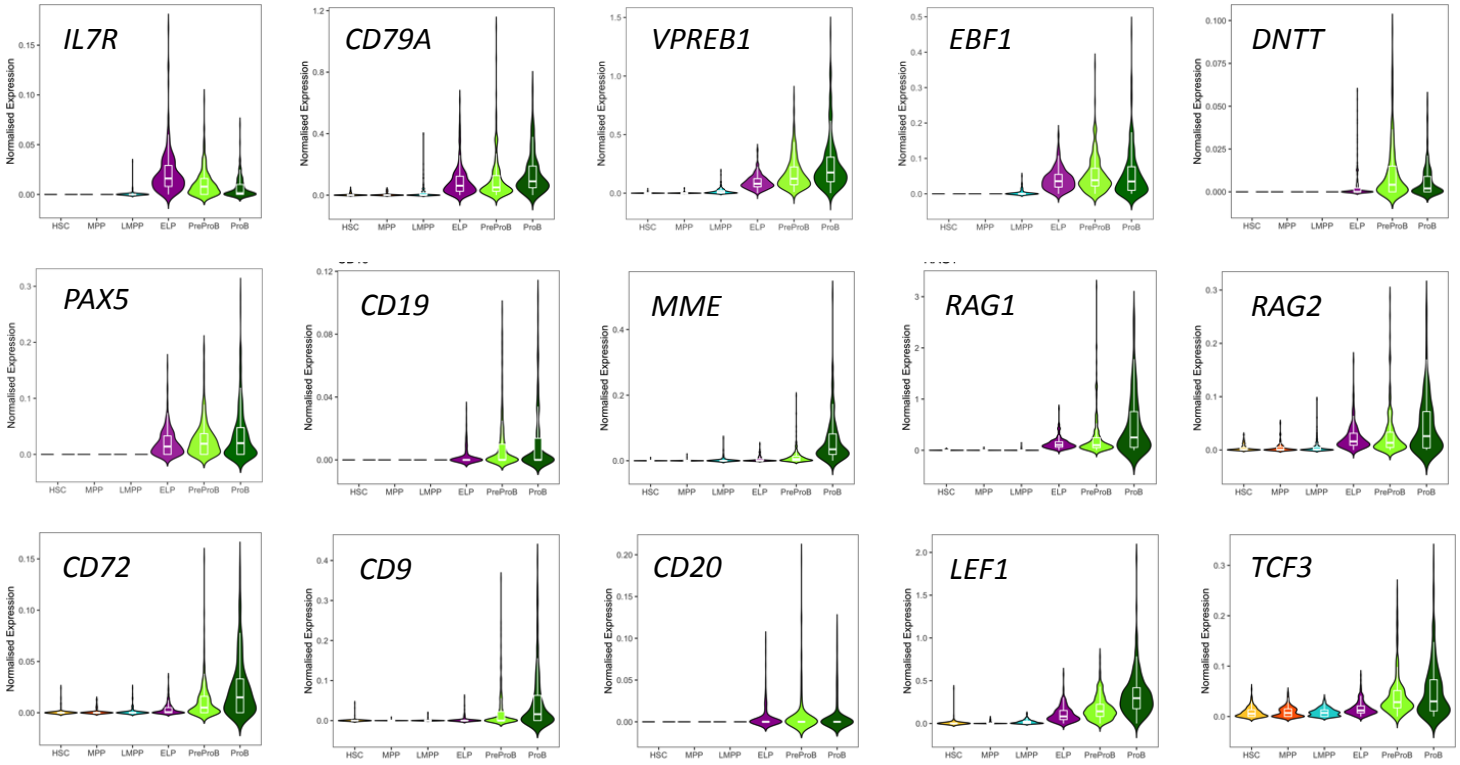


- B lymphoid differentiation trajectory : HSC->MPP->LMPP->ELP->PPB->PB->B

O'Byrne *et al*, manuscript under revision

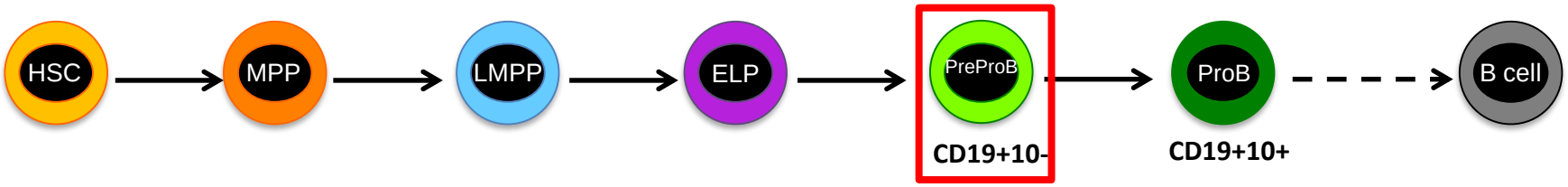
Cellular hierarchy by gene expression



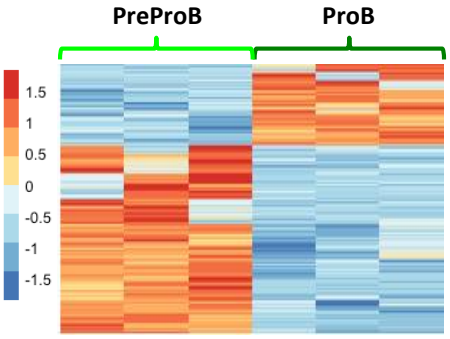


SC RQ-PCR analysis shows upregulation of B cell specific gene expression from ELP → PreProB → ProB

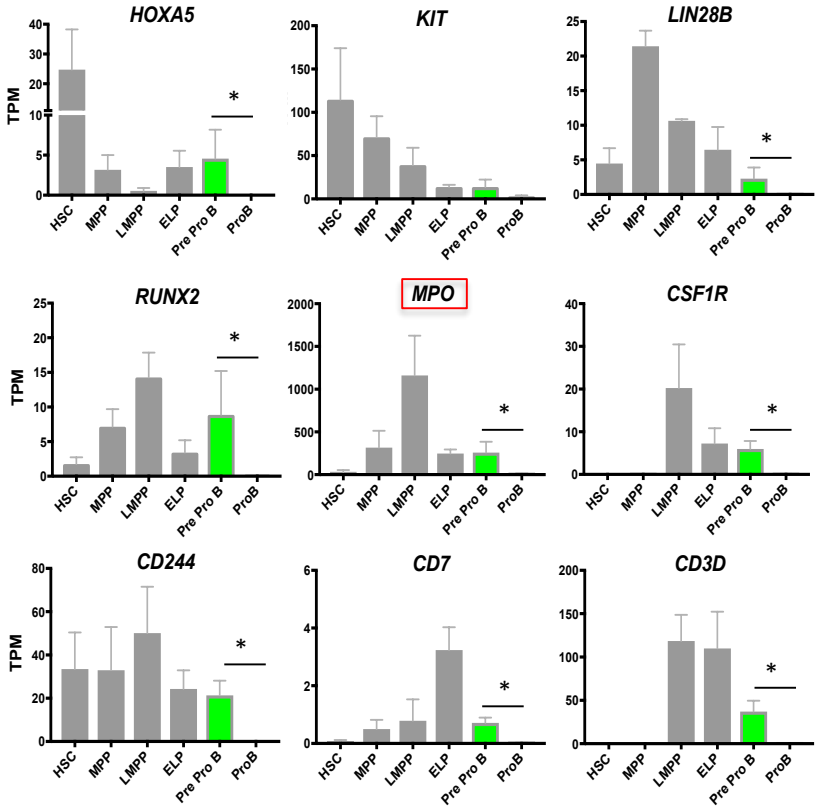
Pre ProB progenitors are distinct from ProB progenitors



RNA-SEQ

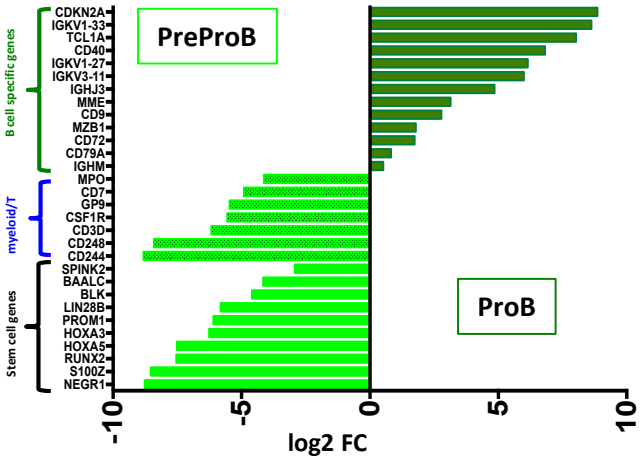


808 genes DE (FDR<0.1)



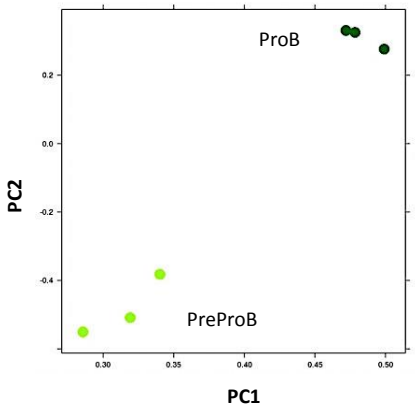
(*FDR<0.1)

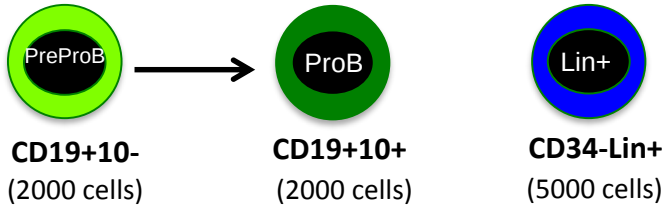
Lineage and leukaemia associated genes



Pre ProB progenitors are distinct from ProB progenitors

Chromatin accessibility (ATAC sequencing)

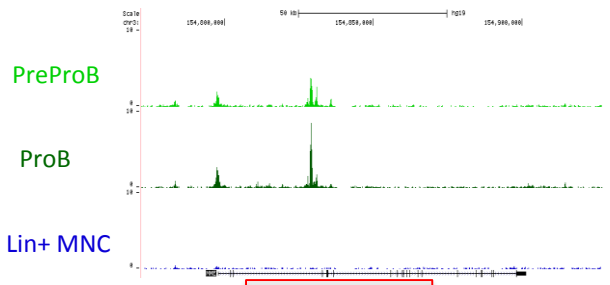




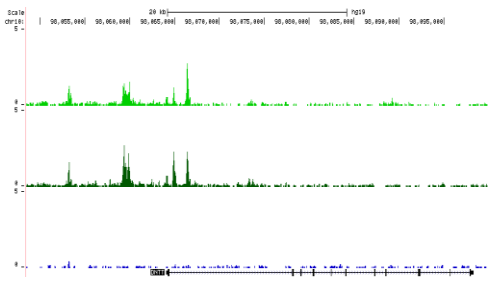
CD19+10- (2000 cells)

CD19+10+ (2000 cells)

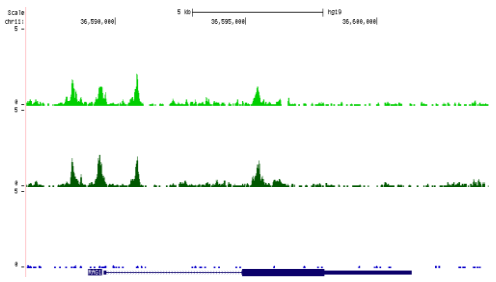
CD34-Lin+ (5000 cells)



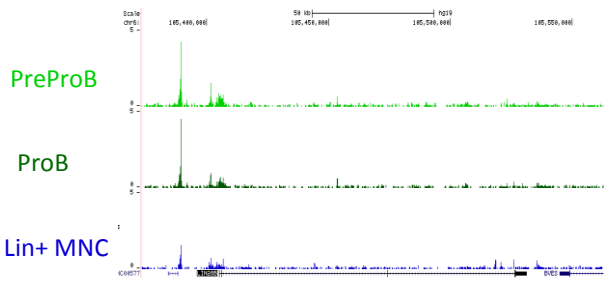
MME/CD10



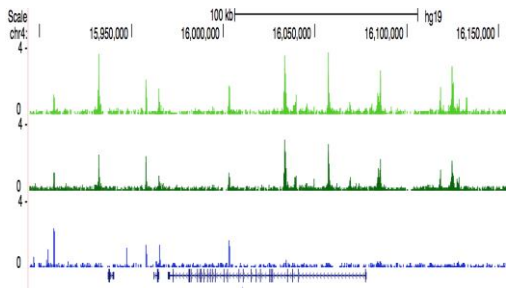
DNTT



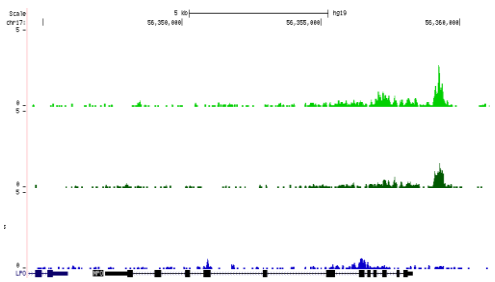
RAG1



LIN28B



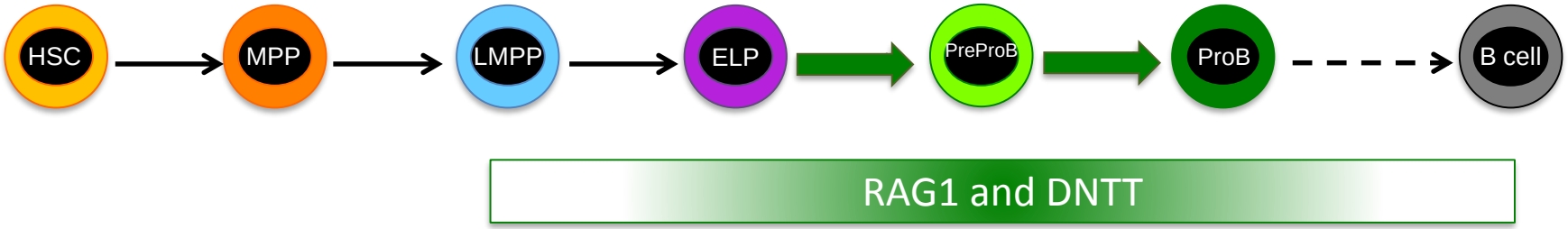
PROM1



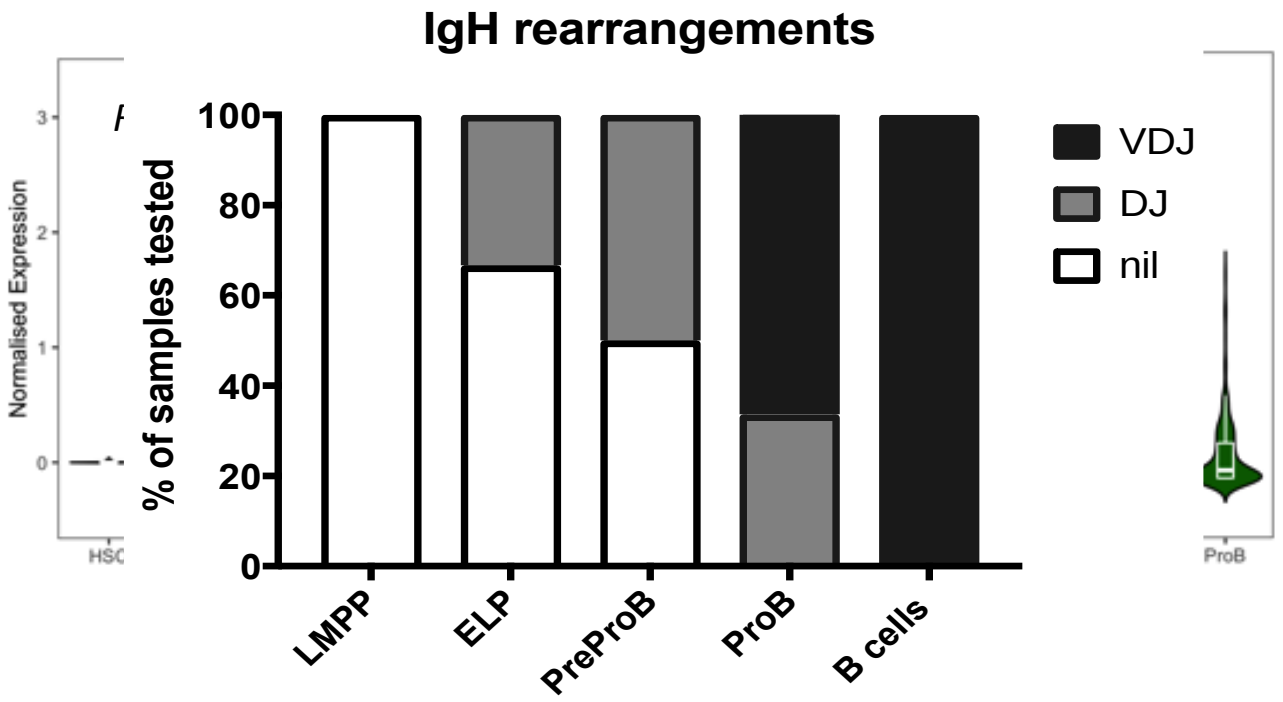
MPO

S O'Byrne
C Garnett

Cellular hierarchy by IgH status

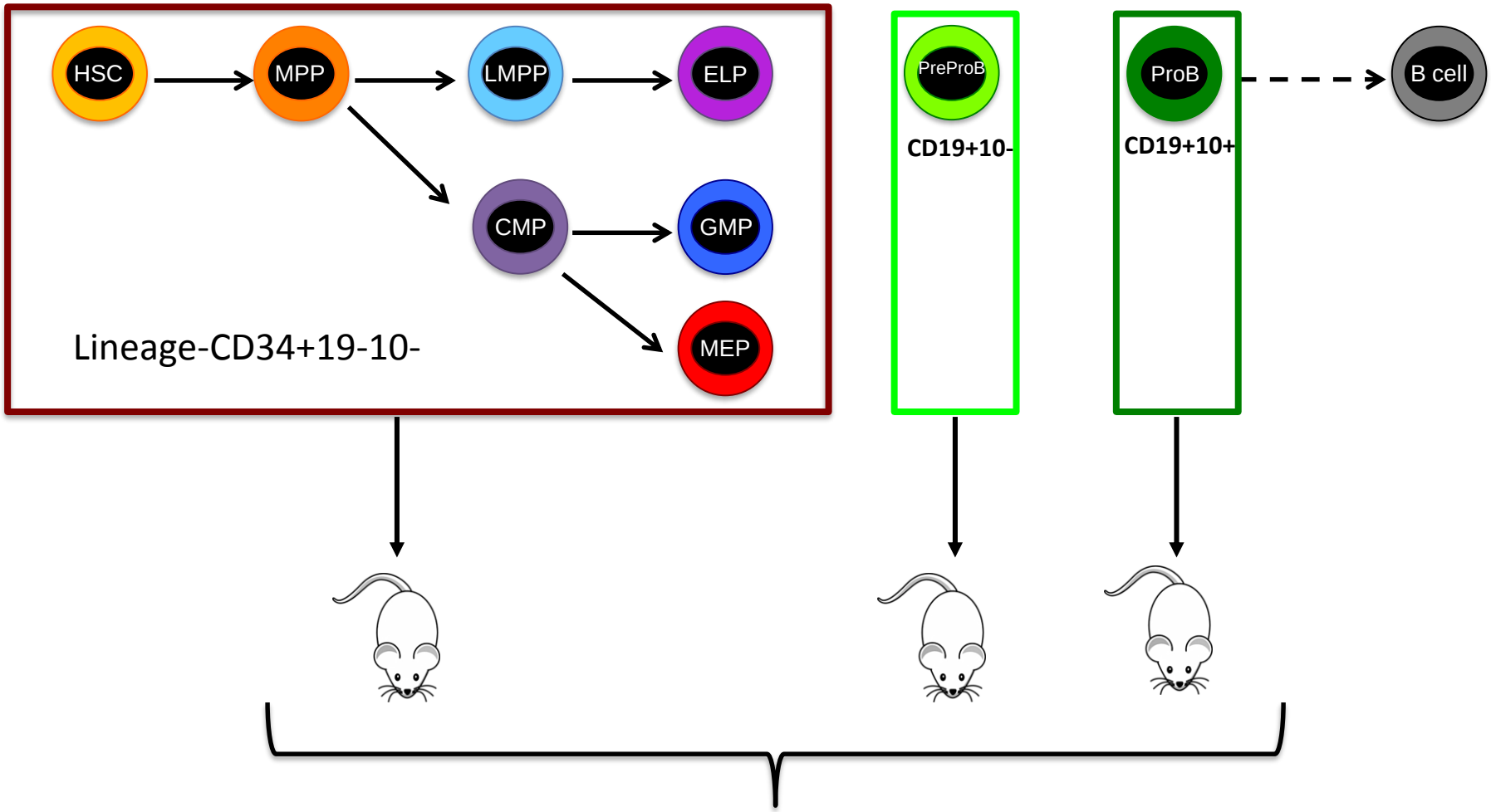


NO REARRANGEMENT D-J? D-J? D-J?/VDJ V-D-J



S O'Byrne
S Rice
G Wright (GOSH)

Cellular hierarchy using *in vivo* models

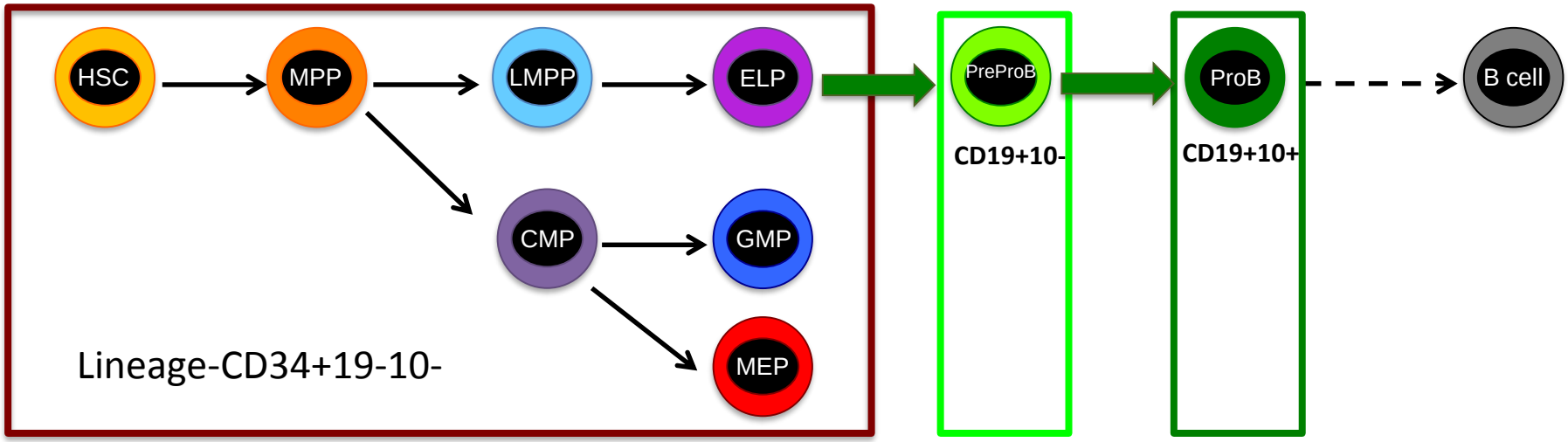


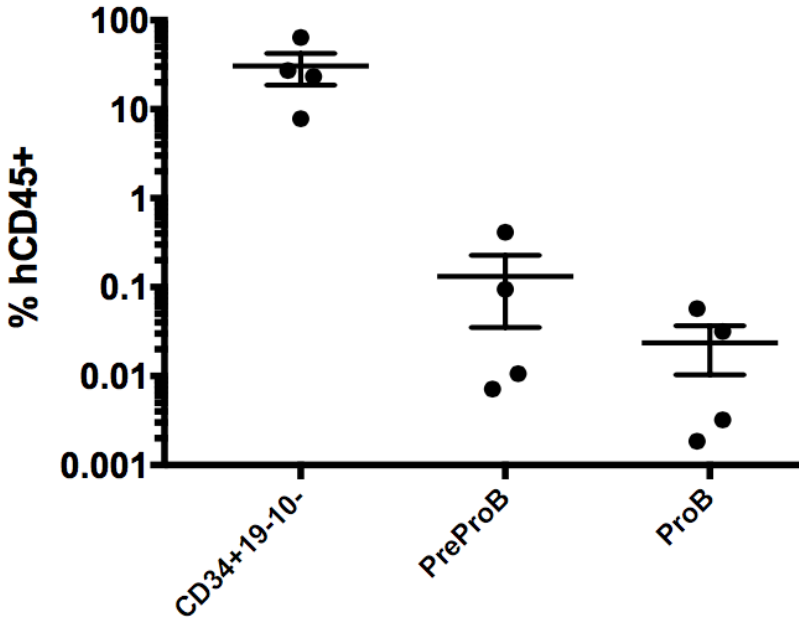
Week 2-3 bone marrow

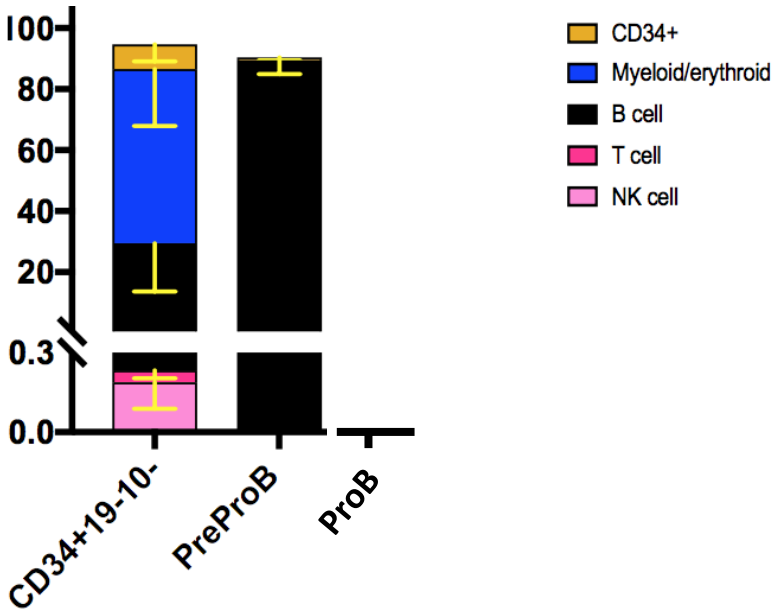
Check for engraftment and differentiation

S Rice
N Fordham
S O'Byrne

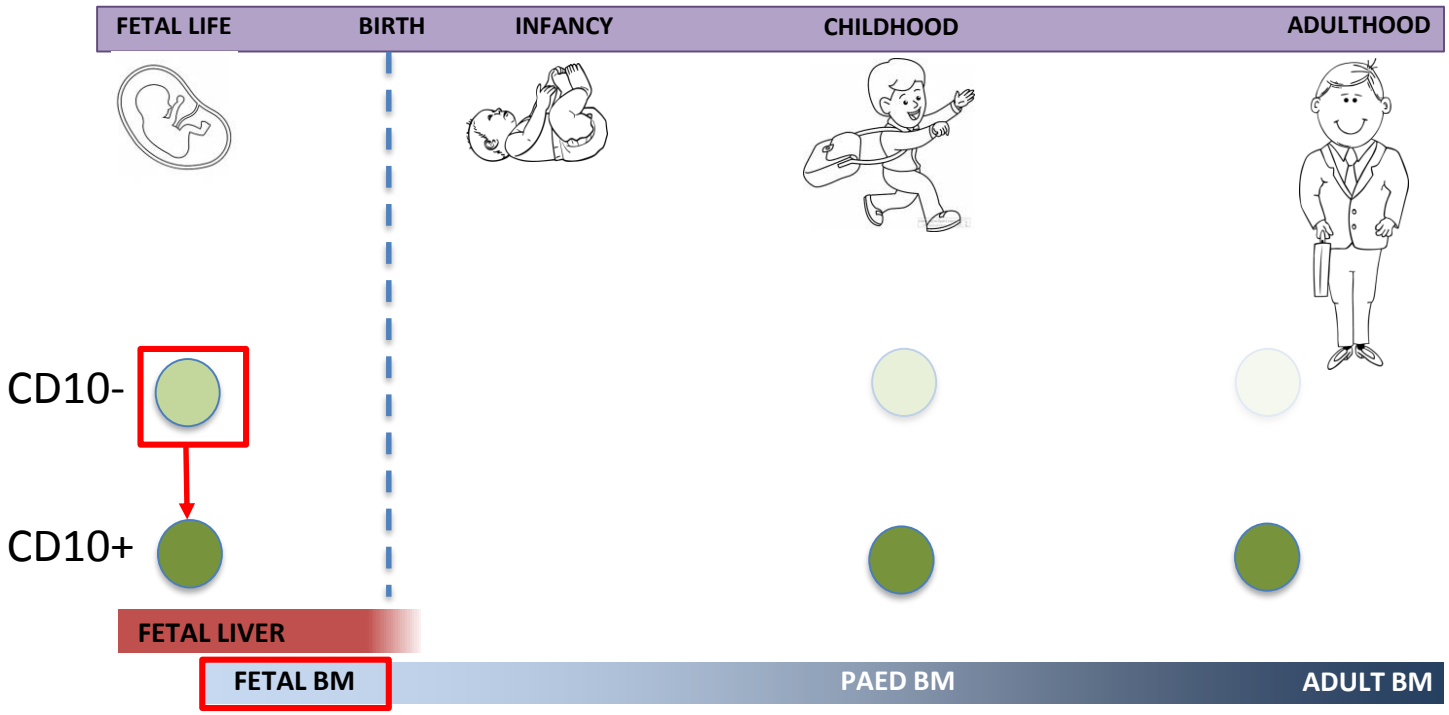
Cellular hierarchy using *in vivo* models





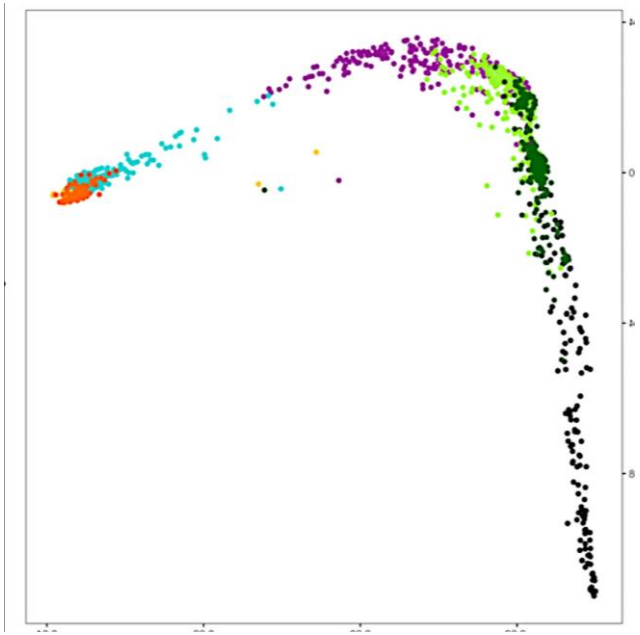


Summary

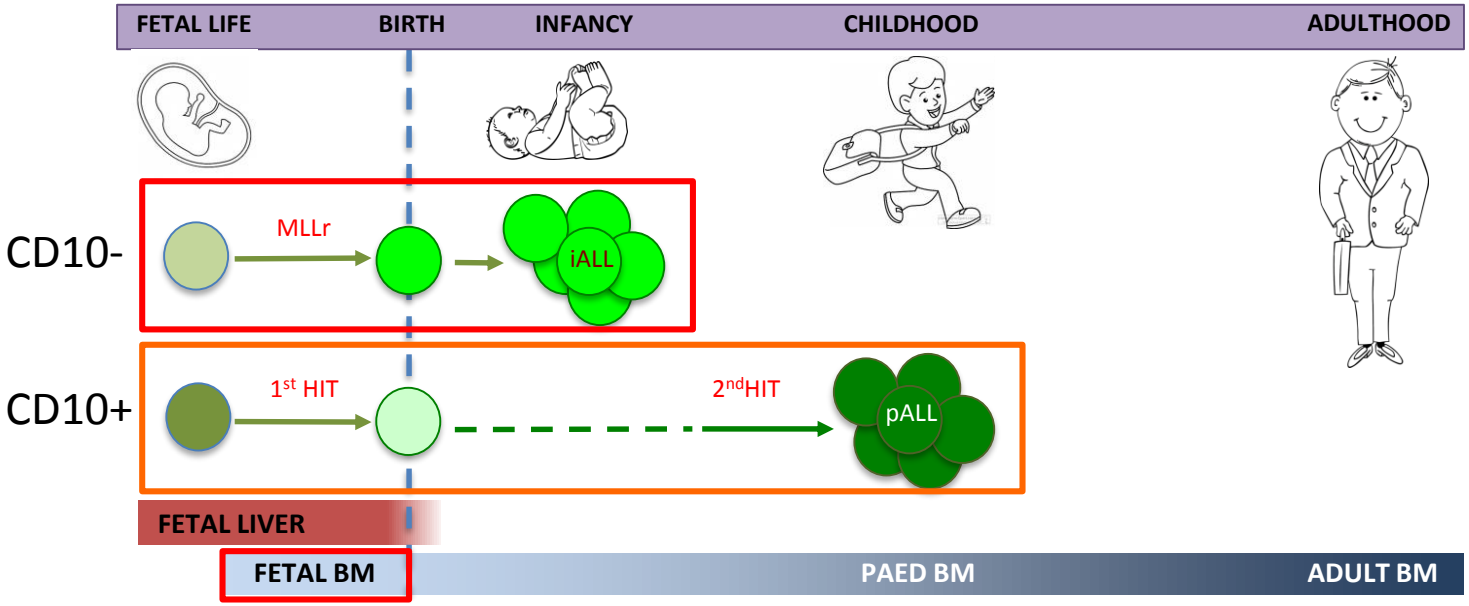


- There are 2 different B cell progenitors in fetal life based on CD10 expresssion
- The CD10- Pre ProB progenitor is more abundant in fetal BM compared to FL
- It is distinct from and lies upstream of the CD10+ ProB progenitor
- It is fetal specific and virtually absent in adult BM

Cellular hierarchy of fetal B lymphopoiesis



Why is this important?



- All infant and many paediatric ALL originate in fetal life
- The CD10- Pre ProB progenitor may be the target for infant ALL
- The CD10+ ProB progenitor may be the target for paediatric ALL

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