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

Andi Roy
University of Oxford
Is leukaemia the same disease at any age?

<table>
<thead>
<tr>
<th></th>
<th>INFANCY</th>
<th>CHILDHOOD</th>
<th>ADULTHOOD</th>
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<tbody>
<tr>
<td>Proportion of patients (%)</td>
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<tr>
<td>infant ALL</td>
<td>53.8%</td>
<td></td>
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<tr>
<td>paediatric ALL</td>
<td>91.5%</td>
<td></td>
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<tr>
<td>adult ALL</td>
<td>39%</td>
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Overall Survival

- paediatric ALL: 91.5%
- infant ALL: 53.8%
- adult ALL: 39%
• 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

CD34

HSC → MPP → LMPP → CLP → ProB → B cell

CD19-10+

CD19+10+

High frequency of PreProB progenitors in FBM

Sanz et al (CB), 2010
Roy et al (FL), 2012
Roy et al (fetal vs postnatal), 2017
Are fetal specific B progenitors site/ stage specific

HSC ➔ MPP ➔ LMPP ➔ ELP ➔ PreProB ➔ ProB ➔ B cell

% of Lin-2-CD34+

FL (n= 49) ➔ FBM (n= 27)

HSC MPP LMPP ELP PreProB ProB

Post conceptional weeks

% of Lin2-CD34+

FL ELP FBM ELP

Post conceptional weeks

% of Lin2-CD34+

FL PreProB FBM PrePro B

Post conceptional weeks

% of Lin2-CD34+

FL ProB FBM Pro B

Post conceptional weeks
Are upstream FBM progenitors ‘lymphoid primed’?

HSC → MPP → LMPP → ELP

PreProB → CD19+10-

ProB → CD19+10+

B cell

Lineage-CD34+

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

Chromium 10 X
HT single cell gene expression

30,000 cells
Are upstream FBM progenitors ‘lymphoid primed’?

<table>
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<tr>
<th>ERYTHROID</th>
<th>MYELOID</th>
<th>LYMPHOID</th>
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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

CD34

HSC → MPP → LMPP → ELP

- PreProB (CD19+10-)
- ProB (CD19+10+)

B cell

Immunophenotyping

Functional

Gene expression

Epigenetics

O’Byrne et al, manuscript under revision
Cellular hierarchy of human fetal B lymphopoiesis

FUNCTIONAL

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<thead>
<tr>
<th></th>
<th>HSC</th>
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**O’Byrne et al, manuscript under revision**
Cellular hierarchy by gene expression

HSC → MPP → LMPP → ELP → PreProB → ProB → B cell

- CD19+10-
- CD19+10+

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

HSC → MPP → LMPP → ELP → PreProB → ProB → B cell

CD19+10-  CD19+10+

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

O’Byrne et al, manuscript under revision
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)

PreProB
ProB

Lineage and leukaemia associated genes

PreProB
ProB

(log2 FC vs. 0)

RNA-SEQ

HOXA5
KIT
LIN28B
RUNX2
MPO
CSF1R
CD244
CD3D

(*)FDR<0.1
Pre ProB progenitors are distinct from ProB progenitors

Chromatin accessibility (ATAC sequencing)

PC1

PC2

PreProB

ProB

Lin+ MNC

PreProB

ProB

Lin+ MNC

MME/CD10

LIN28B

DNTT

PROM1

RAG1

MPO

CD19+10-
(2000 cells)

CD19+10+
(2000 cells)

CD34-Lin+
(5000 cells)

S O’Byrne
C Garnett
Cellular hierarchy by IgH status

HSC → MPP → LMPP → ELP → PreProB → ProB → B cell

RAG1 and DNTT

NO REARRANGEMENT

D-J?

D-J?

D-J?/VDJ

V-D-J

IgH rearrangements

VDJ

DJ

nil

S O’Byrne
S Rice
G Wright (GOSH)
Cellular hierarchy using *in vivo* models

HSC → MPP → LMPP → ELP

CMP → GMP → MEP

PreProB (CD19+10-)

ProB (CD19+10+)

B cell

Lineage-CD34+19-10-

Week 2-3 bone marrow

Check for engraftment and differentiation

S Rice
N Fordham
S O’Byrne
Cellular hierarchy using *in vivo* models

- HSC
- MPP
- LMPP
- ELP
- PreProB
- ProB
- B cell

Lineage-CD34+19-10-

- CMP
- GMP
- MEP

% hCD45+

- CD34+19-10-
- PreProB
- ProB

Graphs showing the distribution of hCD45+ cells in different stages.
There are 2 different B cell progenitors in fetal life based on CD10 expression:

- 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

- HSC
- MPP
- LMPP
- ELP
- PreProB
- ProB
- B cell
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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