# Transcriptome analysis of EPLM subpopulations with various developmental potentials

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## Objectives

The aim of this study is the molecular characterization of the EPLM subpopulations in order to determine:

1) Is the EPLM heterogeneity reflected in different sets of potentials in the subpopulations?

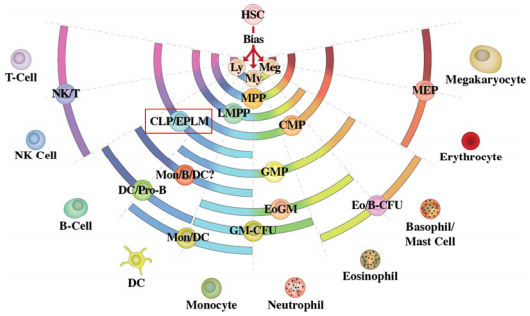
2) Is there a precursor product relationship between the EPLM subpopulations?

## RNA-seq

An RNA sequencing experiment was performed in order to obtain the transcriptome profile of the Ly6D$^+$, TN and TP EPLM subpopulations. The preB CD19$^+$ cells were used as a control of B cell commitment. Cells were sorted from a Flt3L transgenic mice, a source of large numbers of hematopoietic progenitor cells (2).

## Background

### New cyclic model of hematopoiesis

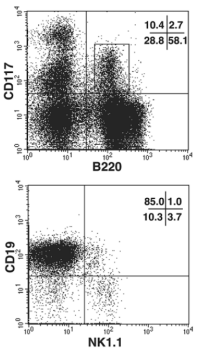



The classical models of hematopoiesis consist of a clear myeloid-lymphoid lineage separation and describe a single and irrevocable route to each end-cell type. However, our own and other research findings over the past 15 years point towards a more plastic hematopoietic development than previously anticipated. Consequently, hematopoiesis is now better illustrated in a cyclic model that not assume lineage branching patterns nor prescribe a single preferred route to a particular end-cell fate. In this model, the various combinations of lineage potentials that exist within each hematopoietic progenitor are represented as a continuum *via* arcs (1).

### EPLM (Early Progenitor with Lymphoid and Myeloid potential)

EPLM is one of the progenitor cell that contradicts the classical lymphoid/myeloid dichotomy because has the capacity to differentiate into lymphoid and myeloid cells. This cell, identified as B220$^+$ cKit$^{low}$ CD19$^-$ and NK1.1$^-$, represent 0.1-0.2% of total nucleated bone marrow cells in adult C57BL/6 mice (A).

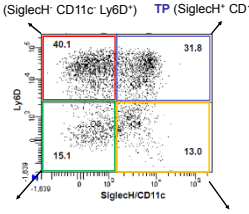
**(A) EPLM identification**




EPLM is an heterogeneous population that can be further divided into at least four subpopulations based on the cell surface markers SiglecH, Ly6D and CD11c (B). Red: single positives Ly6D; Blue: triple positives (TP); Green: triple negatives (TN); Yellow: single positives CD11c.

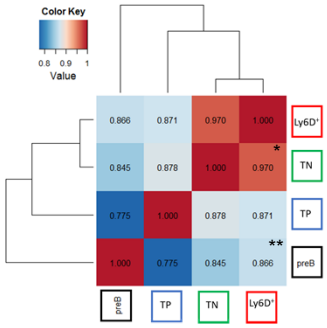
**(B) EPLM subpopulations**

Ly6D$^+$ (SiglecH$^-$ CD11c$^-$ Ly6D$^+$) TP (SiglecH$^+$ CD11c$^+$ Ly6D$^+$)




TN (SiglecH$^-$ CD11c$^-$ Ly6D$^-$) CD11c$^+$ (SiglecH$^-$ CD11c$^+$ Ly6D$^-$)

## 1. Transcriptome comparison



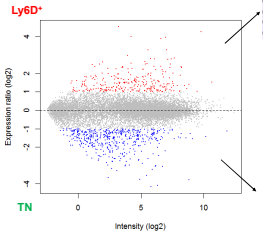

**Heatmap showing the correlation levels when comparing the whole transcriptome between the different EPLM subpopulations**. Ly6D$^+$ and TN are the closest populations (*). The EPLM subpopulation that is more proximal to B cell commitment (preB cells) is the Ly6D$^+$ (**).

## 2. Ly6D$^+$ cells are biased to the B cell lineage and the TN population retain myeloid potential

**(A) Differentially expressed genes**

Ly6D$^+$ vs TN

| | n° | % |
|---|---|---|
| Total expressed genes | 16567 | 100 |
| Differentially expressed | 757 | 4,57 |
| Overexpressed | 274 | 36,20 |
| Downregulated | 483 | 63,80 |



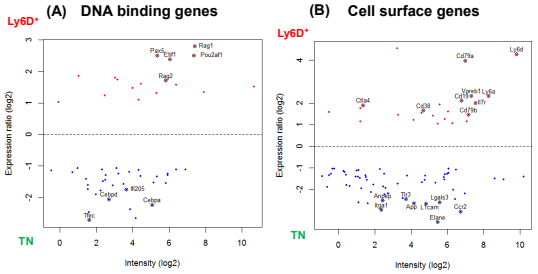

**(B) Enriched Biological Processes (BP)**

| Term | Genes Count | Fold Enrichment |
|---|---|---|
| GO:0033151~V(D)J recombination | 4 | 35,95 |
| GO:0042100~B cell proliferation | 5 | 33,70 |
| GO:0050853~B cell receptor signalling pathway | 4 | 23,11 |
| GO:0002360~T cell lineage commitment | 2 | 23,11 |
| GO:0050852~T cell receptor signalling pathway | 3 | 11,03 |
| GO:0030217~T cell differentiation | 9 | 9,58 |
| GO:0042102~positive regulation of T cell proliferation | 4 | 9,24 |
| GO:0042113~B cell activation | 8 | 8,30 |
| GO:0002377~immunoglobulin production | 3 | 8,09 |
| GO:0042110~T cell activation | 10 | 6,97 |
| GO:0042129~regulation of T cell proliferation | 4 | 5,48 |

| Term | Genes Count | Fold Enrichment |
|---|---|---|
| GO:0042116~macrophage activation | 4 | 15,10 |
| GO:0032680~regulation of TNF production | 7 | 9,79 |
| GO:0030593~neutrophil chemotaxis | 4 | 8,88 |
| GO:0002274~myeloid leukocyte activation | 8 | 8,63 |
| GO:0006909~phagocytosis | 11 | 8,47 |
| GO:0030100~regulation of endocytosis | 8 | 5,81 |
| GO:0019882~antigen processing and presentation | 12 | 5,21 |
| GO:0006954~inflammatory response | 29 | 4,86 |
| GO:0031349~positive regulation of defence response | 7 | 4,64 |
| GO:0045087~innate immune response | 13 | 4,59 |
| GO:0009611~response to wounding | 40 | 4,35 |
| GO:0050817~coagulation | 8 | 4,31 |

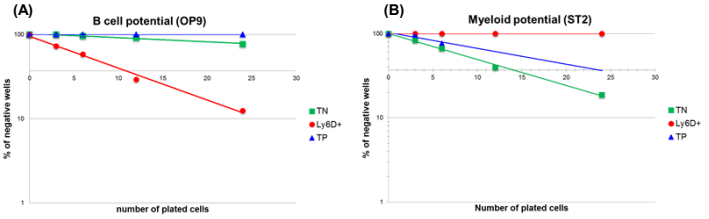
Table and graphical representation of the differentially expressed genes when comparing Ly6D$^+$ and TN EPLM subpopulations. Each dot represents a gene (red: Ly6D$^+$ overexpressed genes, blue: Ly6D$^+$ downregulated genes) (A). The genes upregulated in the Ly6D$^+$ cells are enriched in lymphoid BP (B upper table) while the upregulated genes in the TN cells are myeloid related (B lower table).

**(A) DNA binding genes** **(B) Cell surface genes**




**Differentially expressed DNA binding (GO:0003677) (A) and cell surface (GO:0009986) genes (B)** when comparing Ly6D$^+$ and TN EPLM subpopulations. Ly6D$^+$ cells upregulate DNA binding genes and cell surface markers associated to the lymphoid lineage (Rag1, Rag2 and Il7R) and mostly related to the B cell lineage such as CD19, a marker for B cell commitment (red dots). This confirms a B cell like genetic signature in the Ly6D$^+$ population. However, TN cells upregulate myeloid related genes (blue dots) anticipating that these cells might retain myeloid potential.

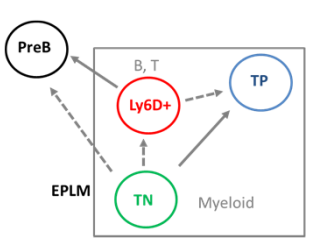
## *In vitro* developmental potentials




**Limiting dilution assay showing the developmental potential of the EPLM subpopulations to give rise to B cells (A) or myeloid cells (B)**. Cultures (48 replicates) containing graded numbers of sorted cells were plated in 96 well plates on OP9 stromal cells in the presence of IL-7 (A) or on ST2 stromal cells without cytokines (B). After 7 days (A) and 14 days (B), the number of wells containing colonies was counted under an inverted microscope. Ly6D$^+$ can efficiently differentiate into B cells but not into myeloid cells. TN is very inefficient differentiating into B cells but retain myeloid potential.

**Thus, the biases seen at the molecular level are confirmed with functional analysis.**

## Conclusion




Localization, developmental potential and precursor product relationship between the TN, Ly6D$^+$ and TP EPLM subpopulations.

## Future Work

1. **Single cell RNA seq** to determine if Ly6D$^+$ and TN populations are heterogeneous and if there are better markers to classify the EPLM cells

2. Test some of the differentially expressed genes by **overexpression** and **silencing** in HSC followed by mouse BM transplantation

## References

1. Geoffrey Brown, Ciaran J Mooney, Llucia A Servera, Lilly von Münchow, Kai-Michael Toellner, Rhodri Ceredig and Antonius G Rolink. 2014. Versatility of stem and progenitor cells and the instructive actions of cytokines shape haematopoiesis. Critical Reviews in Clinical Laboratory Sciences (In press)

2. Balciunaite G, Ceredig R, Massa S, Rolink AG. A B220+ CD117+ CD19- hematopoietic progenitor with potent lymphoid and myeloid developmental potential. Eur J Immunol. 2005; 35(7):2019-2030.

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