**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the PhD project:** Fetal liver hematopoiesis: characterization of the hematopoietic stromal compartment that sustains stem cell expansion.

**Keywords:** fetal liver; stroma; hematopoiesis

**Department:** Immunology

**Name of the lab:** Lymphopoiesis

**Head of the lab:** Ana Cumano

**PhD advisor:** Ana Cumano

**Email address:** ana.cumano@pasteur.fr

**Web site address of the lab:** https://research.pasteur.fr/en/team/lymphopoiesis/

***Doctoral school affiliation and University*:** BioSPC, Paris 7, Université Paris Diderot

Presentation of the laboratory and its research topics:

The Unit Lymphopoiesis, at the Pasteur Institute, has as main research interests understanding the molecular cues that determine the development of the hematopoietic and immune systems. The group of Rachel Golub studies the development of innate lymphoid cells, the group of Paulo Vieira studies the role of cytokines in the development of the immune system and the group of Ana Cumano focus in the molecular basis of lymphoid lineage commitment.

Description of the project:

Fetal liver hematopoiesis: characterization of the hematopoietic stromal compartment that sustains stem cell expansion.

In mammals, blood cells are constantly produced by the differentiation of progenitors that originate from hematopoietic stem cells (HSC). The niche hypothesis was proposed in 1978 by Schofield1 and postulated that HSC are associated with other non-hematopoietic cell types, generally designated as stromal cells that provide signals to self-renewal HSCs. The characterization of the stromal components, in the bone marrow (BM), that contribute to hematopoiesis indicates that endothelial and mesenchymal cells, belonging to the osteogenic lineage, are the major cell types of the hematopoietic stroma2–4. During embryonic life, before the bone cavity is formed, hematopoiesis occurs in the fetal liver (FL) that, like the BM, provides signals that promote differentiation of hematopoietic progenitors but also the expansion of HSC. In the BM, HSCs are in the G0 cell cycle stage5, in contrast, in FL their numbers increase by more than 30 fold between embryonic day (E) 12 and E166. Moreover, our laboratory7,8 reported that the FL stroma plays an important role not only in the expansion of the HSC compartment but also in lineage commitment of the hematopoietic progenitors.

The projects we propose aim at the characterization of the FL stromal cells and at the identification of the environmental cues that induce expansion and lineage commitment of hematopoietic progenitors. Preliminary evidence from our laboratory and consistent with previous reports9 indicates that a population of hepatoblasts produces mRNA encoding the major hematopoietic cytokines (interleukin 7, kit ligand, thrombopoietin). This observation indicates that, like in the BM, in FL resident tissue specific cells contribute the hematopoietic stromal compartment.

The project will be developed in sequential stages, some of which already started: **1.** Development of a multicolor panel of fluorescent antibodies (15-18 to be used in the BD-Symphony analyzer) to identify, by flow cytometry, non-hematopoietic FL subsets. **2.** RNA sequencing analysis of different FL populations identified in 1. compared to endothelial cells. **3.** Analysis of the RNA sequence data and identification of transcripts specifically expressed the different hepatic subsets. We will focus on the populations that express known hematopoietic cytokines (interleukin 7, kit ligand, Flt3 ligand) **4.** Single cell multiplex RT-PCR analysis of the transcripts identified in 3. to define co-expression and to determine heterogeneity of the stromal populations identified in 1. **5.** Engineering mouse models where cytokine producing FL stroma can be traced and specifically deleted. This stage involves the identification of genes that are exclusively expressed in the cytokine producing subsets of liver cells and to introduce a Cre recombinase cDNA under the control of the regulatory sequences of these genes, in the mouse germ-line. These alleles can be combined with to a Rosa floxed stop RFP to trace the cells and to an inducible diphtheria toxin receptor allele to allow elimination of cytokine producing cells. **6.** Characterization of hematopoiesis in mice where the different FL stromal subsets were eliminated. We will analyze the progenitor and the committed hematopoietic compartment. These experiments will allow distinguish the different stages of hematopoietic differentiation and the different roles of FL stroma in the processes leading to blood production **7.** The development of cultures of FL organoids. This step involves isolating extra-cellular matrix (ECM) elements, developing 3D culture conditions where the different stromal cells types at different concentrations can be associated with hematopoietic progenitors. These experiments will indicate the relative contribution of each stromal subset to blood cell production and will complement the experiments in 6.

References:

*1. Schofield, R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells* ***4****, 7-25 (1978).*

*2. Ding, L., Saunders, T. L., Enikolopov, G. & Morrison, S. J. Endothelial and perivascular cells maintain haematopoietic stem cells. Nature* ***481****, 457-462 (2012).*

*3. Lo Celso, C. & Scadden, D. T. The haematopoietic stem cell niche at a glance. J Cell Sci* ***124****, 3529-3535 (2011).*

*4. Cordeiro Gomes, A. et al. Hematopoietic Stem Cell Niches Produce Lineage-Instructive Signals to Control Multipotent Progenitor Differentiation. Immunity* ***45****, 1219-1231 (2016).*

*5. Passegue, E., Wagers, A. J., Giuriato, S., Anderson, W. C. & Weissman, I. L. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. J Exp Med* ***202****, 1599-1611 (2005).*

*6. Ema, H. & Nakauchi, H. Expansion of hematopoietic stem cells in the developing liver of a mouse embryo. Blood* ***95****, 2284-2288 (2000).*

*7. Ramond, C. et al. Two waves of distinct hematopoietic progenitor cells colonize the fetal thymus. Nat Immunol* ***15****, 27-35 (2014).*

*8. Berthault, C. et al. Asynchronous lineage priming determines commitment to T cell and B cell lineages in fetal liver. Nat Immunol AOP (2017).*

*9. Chou, S. & Lodish, H. F. Fetal liver hepatic progenitors are supportive stromal cells for hematopoietic stem cells. Proc Natl Acad Sci U S A* ***107****, 7799-7804 (2010).*

Expected profile of the candidate (optional):

We seek a hard-working, highly motivated candidate with some knowledge of Immunology and laboratory experience.

Contact:

ana.cumano@pasteur.fr