

## PhD project No. 1, Dr. Brouard/Dr. Léon

<b>Scientific Area</b>	Haematopoiesis and immune cell differentiation
<b>Two project titles</b>	A) Stiffness sensing of bone marrow endothelial cell B) Matrix stiffness sensing and proplatelet formation
<b>Host country</b>	France
<b>Supervisor, institution</b>	Dr. Catherine Léon, University of Strasbourg, France Dr. Nathalie Brouard, University of Strasbourg, France
<b>Co-Supervisor, institution</b>	Dr. Rémi Peyronnet, University of Freiburg, Germany
<b>Mentor, institution</b>	“to be determined later”.
<b>Secondment institution</b>	A) and B) University of Strasbourg, France and INSERM S1255
<b>Short description of the supervisor’s lab with introduction to the topic</b>	
<p>Our group is interested in the mechanisms of platelet formation in physiopathology and focused on the role of the mechanical microenvironment. Platelets are produced in the bone marrow by megakaryocytes (MKs). The last steps of MK differentiation and platelet release occur when MKs are in contact with endothelial cells (EC) constituting the sinusoid vessels. We recently observed that human ECs from marrow vessels are capable to promote the formation of platelets more efficiently than ECs from other vascular territories. Modifications of mechanical properties of the marrow occur in myeloproliferative neoplasms that usually leads to myelofibrosis, pathological conditions associated with excessive matrix protein deposition around sinusoid vessels and abnormal platelet production.</p>	
<b>Topic description, including techniques to be used</b>	
<p><b>Project A)</b> This project will aim at evaluating the matrix-stiffness-sensing properties of marrow ECs compared to ECs from other vascular territories using i) <i>in vitro</i> approaches through human marrow endothelial cell adhesion onto matrices with various stiffness and analysis of their behavior including cell stiffness and ii) <i>in vivo</i> approaches by comparing single cell RNAseq transcriptome of ECs isolated from normal vs. myelofibrotic mouse bone marrow.</p> <p><u>Techniques:</u> Primary EC culture, flow cytometry cell sorting and analysis, immunofluorescence, confocal microscopy, FLIM microscopy, nanoindentation, transcriptomic, RT-qPCR, western blots, mouse models.</p> <p><b>Project B)</b> This project will aim at i) identifying matrix proteins specifically deposited at the interface between MKs and sinusoid ECs by transcriptomic analysis (MKs and ECs from control vs. myelofibrotic mice will be used); ii) evaluating <i>in vitro</i> the impact of substrate stiffness on platelet formation by MKs following interaction with matrix proteins overexpressed under myelofibrosis.</p> <p><u>Techniques:</u> Hematopoietic stem cell culture, primary EC culture, flow cytometry cell sorting and analysis, immunofluorescence, confocal microscopy, FLIM microscopy, transcriptomic, RT-qPCR, mouse models.</p>	
<b>Recommended applicant’s training (technical expertise and knowledge)</b>	
<p>Techniques: cell culture, RT-PCR, confocal microscopy, ability to work with mice Knowledge: cell biology, mechanobiology, signalling, some knowledge in transcriptomic analysis would be a plus.</p>	
<b>Maximum two relevant publications</b>	
<p>Aguilar A, Pertuy F, Eckly A, Strassel C, Collin D, Gachet C, Lanza F, Léon C, 2016, Blood: Importance of environmental stiffness for megakaryocyte differentiation and proplatelet formation Bornert A, Boscher J, Pertuy F, Eckly A, Stegner D, Strassel C, Gachet C, Lanza F, Léon C, 2020, Haematologica: Cytoskeletal-based mechanisms differently regulate in vivo and in vitro proplatelet formation.</p>	

## Ethics description

<b>1. Humans</b>	
This research involves human participants.	YES <input type="checkbox"/> / NO <input checked="" type="checkbox"/>
This research involves physical interventions on the study participants.	YES <input type="checkbox"/> / NO <input checked="" type="checkbox"/>
<b>2. Human Cells /Tissues</b>	
This research involves human cells or tissues, such as blood.	YES <input checked="" type="checkbox"/> / NO <input type="checkbox"/>
<b>3. Personal Data</b>	
This research involves personal data collection and/or processing.	YES <input type="checkbox"/> / NO <input checked="" type="checkbox"/>
This research involves further processing of previously collected personal data (secondary use).	YES <input type="checkbox"/> / NO <input checked="" type="checkbox"/>
<b>4. Animals</b>	
This research involves animals, such as mice.	YES <input checked="" type="checkbox"/> / NO <input type="checkbox"/>