

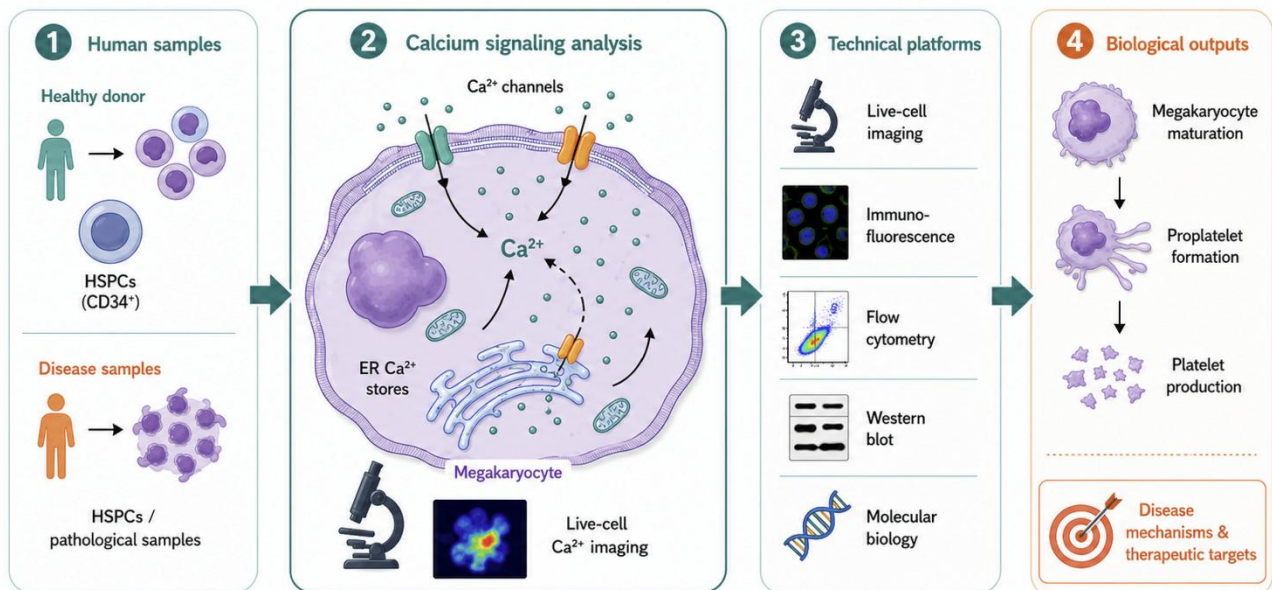
**Project Title:** Calcium signaling, bone marrow niche, and blood cell production in health and disease

**Supervisor:** Christian A. Di Buduo

**Laboratory:** Laboratory of studies on megakaryocyte function, Department of Molecular Medicine

**Research Theme:** Clinical Biochemistry, Hematopoiesis

## Abstract



Hematopoietic stem and progenitor cells give rise to all mature blood cell lineages within the bone marrow. This process depends on the coordinated interaction between intrinsic cellular programs and extrinsic cues provided by the bone marrow microenvironment, including cytokines, growth factors, extracellular matrix components, and specialized cellular niches.

Among the intracellular mechanisms that integrate these signals, calcium (Ca<sup>2+</sup>) signaling plays a central role in regulating cell fate decisions, lineage commitment, differentiation, and functional maturation.

Within this broader hematopoietic context, megakaryocytes represent a highly specialized lineage responsible for platelet production and deeply influenced by both Ca<sup>2+</sup>-dependent signaling pathways and microenvironmental cues.

The host laboratory has established strong expertise in the study of human megakaryopoiesis, platelet production, Ca<sup>2+</sup> signaling networks, and advanced *in vitro* systems modeling selected features of the bone marrow niche. Previous work from the group has shown that cytosolic Ca<sup>2+</sup> dynamics in megakaryocytes are finely regulated by plasma membrane channels, intracellular Ca<sup>2+</sup> stores, and organelle-dependent signaling mechanisms. Alterations of these pathways may profoundly affect megakaryocyte maturation, proplatelet formation, and platelet function, and may contribute to hematological diseases, including myeloproliferative neoplasms.

This Ph.D. project will investigate how  $\text{Ca}^{2+}$  signaling and organelle function contribute to the regulation of physiological and pathological hematopoiesis, with a specific focus on megakaryocyte differentiation and platelet production. The candidate will combine primary human cell cultures from healthy donors and disease-relevant samples with advanced cellular, molecular, and functional assays. Particular attention will be given to the analysis of  $\text{Ca}^{2+}$  fluxes, cation channel activity, intracellular organelle function, and the influence of microenvironmental signals on hematopoietic and megakaryocytic differentiation.

The project may also exploit complex *in vitro* culture systems designed to reproduce selected aspects of the bone marrow microenvironment. These models will allow the candidate to explore how niche-related cues regulate hematopoietic cell behavior, megakaryocyte maturation, organelle remodeling, extracellular matrix interactions, and platelet production. In this way, the project will provide a platform to study not only lineage-specific mechanisms but also broader principles of hematopoietic regulation in health and disease.

By the end of the Ph.D. program, the candidate is expected to generate new mechanistic insights into  $\text{Ca}^{2+}$ -dependent signaling networks and microenvironment-driven regulation of hematopoiesis. The project may help identify altered signaling pathways and cellular interactions that could represent novel therapeutic targets for hematological disorders characterized by defective hematopoiesis, megakaryocyte dysfunction, or impaired platelet production.

### **Main activities**

The candidate will be involved in the culture and differentiation of human hematopoietic progenitors, with particular focus on the megakaryocytic lineage and platelet production. Experimental activities will include the analysis of hematopoietic cell differentiation, megakaryocyte maturation, proplatelet formation,  $\text{Ca}^{2+}$  signaling dynamics, cation channel activity, and organelle-associated signaling pathways. The candidate will also be trained in the use of advanced *in vitro* culture systems that model selected features of the bone marrow microenvironment, with the aim of studying how niche-derived signals influence hematopoiesis under physiological and pathological conditions.

### **Techniques**

Primary human hematopoietic cell culture; megakaryocyte differentiation assays; live-cell  $\text{Ca}^{2+}$  imaging; immunofluorescence microscopy; confocal microscopy; flow cytometry; western blotting; molecular biology; advanced *in vitro* culture systems modeling selected features of the bone marrow microenvironment.

## Selected references

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