PROJECT 1

DoS: Ditadi Andrea, PhD

Title: **Understanding the onset of the human immune system through hematopoietic development and disease modeling using hiPSC**

Curriculum: Cellular and Molecular Physiopathology

Link to OSR/UniSR personal page:
http://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/human-hematopoietic-development-and-disease-modeling.html

http://www.unisr.it/medicina-chirurgia/ddr-internazionale-in-medicina-molecolare/andrea-ditadi/

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):
The ability to generate transplantable hematopoietic cells from human pluripotent stem cells (hPSC; comprising human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC)) in culture would represent an important step forward, as it would provide an unlimited source of these cells for therapeutic applications as well as a model for studying human hematopoietic development and disease *in vitro*. The success in this endeavor will depend on the accurate recapitulation of embryonic hematopoiesis[1].

Using this developmental approach we have successfully generated the specialized cell population known as hemogenic endothelium (HE)[2-4], that represents the progenitor population that generates adult hematopoietic cells during human hematopoietic development. This HE has been already successfully used to model *in vitro* the onset of X-linked Severe Combined Immunodeficiency with hPSCs[5].

This project intends to understand the sequence of events that promotes the development of blood cells from hPSC. By using state-of-the-art tools, the PhD student will investigate the molecular mechanisms regulating human hematopoietic commitment using hPSC as well as mouse PSCs and embryos as model systems. Patient-specific hiPSC will be used to identify the genetic requirements of specific critical stages of the development of the immune system, representing the ultimate frontier of functional genetics. Both normal and diseased hematopoietic progenitors will be interrogated for signaling requirements, as well as transcriptional and epigenetic regulators. *In vivo* and *in vitro* assays will be used to evaluate developmental potential and functionality of the hPSC-derived populations.

Skills to be acquired by the student:

Analysis of stemness, pluripotency and developmental stages; analysis of signaling pathways; cell and tissue cultures with mouse and human pluripoternt stem cells, mouse embryos and primary samples; flow cytometry and cell sorting; molecular biology; animal models; embryo dissections; data analysis; scientific communication;

References (max. 3)

1 Murry, C. E. & Keller, G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell* **132**, 661-680 (2008).

2 Ditadi, A. *et al.* Human definitive haemogenic endothelium and arterial vascular endothelium represent distinct lineages. *Nature cell biology* **17**, 580-591 (2015).

3 Kennedy, M. *et al.* T lymphocyte potential marks the emergence of definitive hematopoietic progenitors in human pluripotent stem cell differentiation cultures. *Cell reports* **2**, 1722-1735 (2012).

4 Sturgeon, C. M., *et al.* Wnt signaling controls the specification of definitive and primitive hematopoiesis from human pluripotent stem cells. *Nature biotechnology* **32**, 554-561 (2014).

5 Li, L. B. *et al.* Silent IL2RG Gene Editing in Human Pluripotent Stem Cells. *Molecular therapy : the journal of the American Society of Gene Therapy*, doi:10.1038/mt.2015.190 (2015)