PROJECT 1

DoS: Alessandro Aiuti

Title: ***Elucidating the role of human circulating hematopoietic stem/progenitor cells in phsyiological and pathological conditions***

Curriculum: Cellular and Molecular Physiopathology

Link to OSR/UniSR personal page:

http://www.unisr.it/k-teacher/aiuti-alessandro/

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Hematopoietic stem/progenitor cells (HSPC) are rare bone marrow (BM) resident cells that continuously produce all blood lineages (*1*). Integration Sites (IS) analysis represents a powerful tool to track the activity of hematopoietic cells not only in animal models but also in Gene Therapy (GT) treated patients. Given the concept that each gene-corrected cell and its progeny become univocally marked by a distinct IS upon transduction, we are currently studying the hematopoietic reconstitution dynamics in HSPC-GT patients. Our experimental strategy combines multi-parametric phenotypic characterization, molecular analyses and mathematical modelling (*2*). Under physiological conditions, few circulating HSPC (cHSPC) are found in peripheral blood (PB) of untreated subjects and their amount is emerging as a marker of altered BM function (*3*). In the mouse, the re-circulation of HSPC between the BM and PB is important for the maintenance of hematopoietic homeostasis or for the local production of immune cells during infections. However, little information are available on the role of human cHSPC both at steady state and in stressed conditions, such as transplantation or sepsis. Moreover, the direct correlation between BM HSPC and cHSPC in terms of function and hematopoietic potential is still to be proven. Thus, the aim of this project is to study the properties of human cHSPC and their relationship with BM counterpart under homeostatic conditions, after transplantation or infections. In order to evaluate cHSPC biology in healthy human hematopoiesis, the PhD student involved in this project will characterize cHSPC frequency, subset composition, transcriptome profile, cell cycle status and differentiation potential in comparison with the BM counterpart in adult healthy donors. Moreover, the student will explore the role of cHSPC during physiological maturation of the hematopoietic system studying their functional properties and their composition at birth, in the first years of life (1-4 years), in child and adolescents (6-18 years), adults (20-60 years) and during senescence (>75 years). At the same time, the student will be involved in studying the function of cHSPC during hematopoietic reconstitution after transplant in GT patients. He/she will analyze through IS analysis their relationship with resident BM CD34+ cells and their hematopoietic output estimating the level of identical IS shared with BM HSPC and with distinct mature PB subsets. These studies will be performed in the first hours/days post-infusion of transduced CD34+ cells and after re-establishment of steady state, physiological hematopoiesis. These analyses will be complemented with phenotypic and functional characterization of single cHSPC subpopulations (from HSC to lineage committed) in comparison with the healthy-donor dataset. Finally, IS will allow to gain information about cHPSC functions in homeostatic vs. stressed hematopoiesis, such as in case of acute infections and/or sepsis that can occur in GT treated patients. The combinatorial nature of this study will generate fundamental information on the biology of the human hematopoiesis at steady state or

in response of acute infections, generating a novel frame of reference for the development of novel therapeutic approaches.

Skills to be acquired by the student:

The PhD student will learn to perform phenotypic, molecular and functional characterization of rare HSPC subpopulations in healthy donors and GT patients. She/he should be able to correctly and independently conduct his/her experiments, to critically discuss about the obtained results and to assess their biological significance. The student should be able to acquire the required background of knowledge in order to propose advancement of his/her own project. The student will provide update of his/her project during internal and institutional lab meeting. She/he should be able to present the results of the project in international meetings.

References (max. 3)

1. From haematopoietic stem cells to complex differentiation landscapes. Laurenti, E. & Göttgens B. *Nature* 2018.

2. In Vivo Tracking of Human Hematopoiesis Reveals Patterns of Clonal Dynamics during Early and Steady-State Reconstitution Phases. Biasco L. et al. *Cell Stem Cell* 2016

3. Circulating CD34+ progenitor cell frequency is associated with clinical and genetic factors. Cohen, K S et al. *Blood* 2013