# Abstract: S250

# Title: UNVEILING THE BIOLOGICAL ROLE OF PERIPHERAL BLOOD HUMAN CIRCULATING HEMATOPOIETIC STEM AND PROGENITOR CELLS

**Abstract Type: Oral Presentation**

**Session Title: Hematopoiesis, stem cells, and their niche**

**Background:**

Although most hematopoietic stem/progenitor cells (HSPC) reside in the bone marrow (BM), few circulating HSPC (cHSPC) can be also found in the peripheral blood (PB) at steady state.

**Aims:**

The aim of our project is to unveil the contribution of cHSPC to hematopoietic homeostasis and their relationship with BM-resident counterpart.

**Methods:**

We phenotypically characterized cHSPC composition by applying multi-parametric flow cytometry on 110 PB and, as control, 48 BM samples of healthy donors (HD) of different age groups. These analyses were combined with single cell RNA sequencing (scRNAseq) and *ad hoc* designed *in vitro* and *in vivo* assays to investigate the transcriptional and functional properties of steady-state cHSPC subpopulations. To study cHSPC vs BM-HSPC differentiation *in vivo* in humans, we exploited integration site (IS) clonal tracking of cHSPC, BM HSPC and mature PB lineages isolated from 8 HSPC-gene therapy (GT) treated patients once steady-state hematopoiesis is established.

**Results:**

cHSPC show a progressive reduction in number during aging and a different composition than BM counterpart, with Multi Lymphoid Progenitors (MLP) displaying the highest PB circulation propensity. Applying scRNAseq on PB- and BM-derived HSPC, we identified a unique transcriptional profile of both primitive and lineage-committed cHSPC subpopulations, characterized by lower replicative, metabolic and transcriptional activity, but increased differentiation-, adhesion- and immune response-priming than BM counterpart.

From a functional point of view, cHSPC were endowed with BM homing capability and multilineage differentiation potential both in vitro and in vivo. We also detected a reduced long-term human cell engraftment in cHSPC- than BM HSPC-transplanted mice. This latter finding might be explained by the low primitive Hematopoietic Stem Cell (HSC) content and the transcriptional pre-activated state observed in steady-state PB HSC.

Finally, in line with the enrichment of lymphoid transcriptional signature observed in trafficking HSPC, we found a higher IS sharing between PB mature lymphoid compartment and steady-state PB-derived HSPC. Moreover, a substantial fraction of trafficking HSPC displayed an enriched expression of the gene signature associated with thymus seeding progenitor type 1 (TSP1), a group of low-cycling immature lymphoid progenitors with thymus-emigrant properties. Although at lower level with respect to PB cells, we detected a TSP1 signature also in BM dataset, thus suggesting that few thymus-emigrant lymphoid progenitors originate in the BM and preferentially egress into the PB in order to seed lymphoid organs.

**Summary/Conclusion:**

Altogether, our findings indicate PB trafficking HSPC as a peculiar steady-state reservoir of low-cycling, pre-activated hematopoietic progenitors, which are endowed with BM homing and repopulation potential. Furthermore, our results of IS analyses combined with the higher expression of TSP1 signature in PB vs. BM HSPC indicate the key function of steady-state trafficking HSPC in the seeding of the thymus, with the aim of locally

differentiating into lymphoid progeny. Overall, our work represents one of the most comprehensive studies on cHSPC, unveiling fundamental insights on their biological role in humans.

**Keywords:** Stem cell mobilization, Stem cell gene therapy, Hematopoietic stem and progenitor cells