# Abstract: P1410

# Title: LONG-TERM EX VIVO HAEMATOPOIETIC STEM CELL (HSC) EXPANSION USING BONE-DERIVED REINVIGORATING MESENCHYMAL STEM CELLS (RMSCS).

**Abstract Type: Poster Presentation**

**Topic: Hematopoiesis, stem cells and microenvironment**

**Background:**

Hematopoietic stem cells (HSCs) are crucial for life-long hematopoiesis and are widely used in HSC transplantation (HSCT) as a key treatment for diverse blood disorders. *Ex vivo* expansion of HSCs offers a potential solution to the limited availability of functional HSCs, but maintaining their stemness during this process is challenging. HSCs reside within specific niches in the bone marrow (BM) and are in close contact with cells from the stroma. Mesenchymal stem/stromal cells (MSCs) are key components of the stromal compartment within the different BM niches that regulate and maintain HSCs and hematopoiesis.

**Aims:**

Based on the hypothesis that MSCs isolated from distinct areas of the bone could specifically enhance HSC expansion while preserving their progenitor potential, we aimed to develop a potent *ex vivo* expansion system to address limitations not only in HSCT, but also to facilitate and advance fundamental efforts in human HSC research.

**Methods:**

Our group developed (1) a robust pipeline for isolating bone derived reinvigorating MSCs (rMSCs) from mouse and human samples, based on serial enzymatic digestion of bone tissue, followed by fluorescence-activated sorting (FACS) selection of specific MSC markers; and (2) a long-term *ex vivo* expansion system for murine and human HSCs based in co-cultures with rMSCs.

**Results:**

Co-culturing of murine rMSCs and HSCs resulted in superior capacity for HSC expansion and maintenance in both bulk and single-HSC long-term expansion compared to traditional methods, as demonstrated by enhanced HSC engraftment potential in transplantation experiments. Significant efforts were directed towards adapting the murine expansion system for human applications, resulting in a similarly effective expansion increase. Moreover, long-term expanded human HSCs showed functional bone marrow reconstitution capacity. Multiomics approaches are conducted, with the potential to illuminate the mechanisms behind enhanced HSC expansion in rMSC co-cultures, the heterogeneity of the expanded HSC population, and the further identification of the rMSC subpopulation.

**Summary/Conclusion**

We have stablished a robust protocol to isolate and expand the distinct rMSC population, demonstrating their potential to support long-term *ex vivo* expansion of functional murine and human HSCs. This work provides promising insights for both research and therapeutic applications. Future efforts will concentrate on elucidating the mechanisms supporting this expansion potential and refining the human co-culture system.

**Keywords:** Hematopoietic stem cell, Mesenchymal stem cell, Ex vivo expansion, Bone marrow microenvironment