Tecnologie Convergenti per i Sistemi Biomolecolari (TeCSBi) Converging Technologies for Biomolecular Systems (TeCSBi)	
Progetto di ricerca **Research project**	*"Tracking the fate of genetically engineered HSCs by single cell multiome and barcoding technologies"*
Tipo/Type	Borsa finanziata da **Fondazione Telethon ETS** Scholarship funded by **Fondazione Telethon ETS**
Borse/Scholarships	1
Abstract	BACKGROUND: Hematopoietic stem cells (HSC) are an elusive cell type, whose presence can only be inferred retrospectively, from the outcome of time-consuming transplantation experiments. Since current state-of-the-art does not allow prospective HSC identification, today's cell and gene therapy technology has been mostly optimized on surrogate progenitor cells, which differ biologically from HSC. Nowadays, the HSC assessment predictive of in vivo function relies basically on mouse xenotransplantation experiments, that are costly, time-consuming, and affected by reproducibility issues. At SR-TIGET we are currently developing and optimizing an efficient expansion protocol with a specialized culture media that supports the proliferation, self-renewal, and differentiation of HSCs. This protocol allows to reach a critical mass that is needed to perform identification of this rare HSCs population. Reliable identification of most primitive HSC in culture is crucial to successfully develop in vitro characterization methods for candidate ATMPs. AIMS: The major goal of this project is to capture HSCs in the ex vivo culture, using a combination of innovative expansion conditions, iterative cell sorting and multi-omics single cell profiling. The first aim is to define the HSCs state and quantify primitive HSCs in culture by single cell multi-omics analyses leveraging both transcriptomic and epigenomic layers of information. Second goal of the project is to evaluate the self-renewal and multilineage differentiation capacity of engineered HSCs by employing a cutting-edge LV-barcoding technology adapted to single-cell multiome sequencing. This technique allows to track the fate of HSCs with their progeny and possibly discriminate between symmetric and asymmetric HSCs division. Moreover, tracking of LV-barcodes between in vitro and ex-vivo specimens may allow to link the in vitro HSCs footprint to in-vivo function. This is crucial for a potential future substitution of in vivo experiments with in-vitro readouts. Accurately predicting the in vivo potency of ATMPs and quantifying the HSC reserve of individual patients will be clinically important goals. WORK PLAN: The research plan is to primarily develop a data analysis workflow that allows the analysis of multiome data on multiple samples and identify the HSCs footprint in terms of transcriptomic and epigenomic profile. Computational methods to integrate scRNAseq and scATAC data, as well as methods to handle batch effect, will be tested, developed, and implemented. At the same time, the work will be strongly focused on the detailed annotation of the most primitive compartment characterized by HSC/MPP cells. The fine annotation of these cells will be performed by leveraging both the double layer of information provided by the multiome data as well as by gene signatures / gene modules obtained from in-house single cell datasets comprising highly purified HSCs. The second part of the project will be focused on the quantification of self-renewal and multilineage differentiation activity of engineered HSCs using the barcoding technology. To pursue this aim, the plan is to develop a data analysis framework that will allow to identify clones according to LV-

	barcoding sharing as well as classify them according to cell members identities obtained in the first part of the project. This information will unravel the fate of HSCs identified and at the same time provide the quantity as well as the omics profiles of HSCs undergoing either self-renewal or lineage-commitment/differentiation. The project also encompasses accurate statistical planning of the wet experiments, namely starting cell number as a function of the complexity of LV barcode library, depth and timepoint of sampling. Additional bioinformatics approaches, as well as trajectories-inference methods, ligand-receptor analyses and TF regulatory networks analyses will be applied to further define the state of HSCs sub-populations. IMPACT: Rapid, quantitative, and qualitative in vitro HSC assessment predictive of in vivo function may become a sustainable alternative to mouse xenotransplantation experiments. The in vitro HSC readout obtained from expansion protocols sets new standards in terms of throughput and turnaround time, allowing to test a multitude of HSC engineering conditions during process development, and predict in vivo function and potency of ATMPs. This new precision-based approach to ex vivo HSC gene therapy will be applied to samples from gene therapy trials and patients and correlated to clinical outcomes.
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Mesi previsti all'estero/Expected months abroad	Da definire/To be defined
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