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DEFINING THE CLONAL COMPLEXITY AND BIOLOGY OF GENE-MODIFIED HEMATOPOIESIS IN GENE THERAPY

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Hematopoietic stem/progenitor cells (HSPC) have been successfully used in ex vivo HSPC-based gene therapy (HSPC-GT) to treat genetic or acquired disorders, showing a favorable benefit-risk profile in clinical trials, especially when lentiviral vectors (LV) are used[1-3].

In this context, longitudinal vector integration site (IS) retrieval and analyses, from peripheral blood (PB) and bone marrow (BM) samples of HSPC-GT patients, have been instrumental to monitor both safety and efficacy of the GT treatment as well as to study in vivo HSPC clonal dynamics in humans[4]. Over the years, we have applied our combinatorial pipeline, that includes both phenotypic and functional characterization along with IS clonal tracking, to measure clonal complexity of the engineered graft and to unveil the fate of distinct HSPC subpopulations after GT. Our work showed sustained long-term HSPC marking and multi-lineage potential of LV-transduced HSPC, measured the number of engineered HSPC that participate in hematopoietic reconstitution, proved that long-term hematopoietic stem cells (HSC), despite activation in vitro, remained capable of homing and resilience upon re-infusion[5-6]. In our recent study, we correlated the amount of primitive HSC undergoing ex vivo gene-correction with the long-term BM transduced cell chimerism and the clonality of engrafted stem cells. Of note, our results showed that, once engrafted, HSC display similar functional properties independently from their source (BM or mobilized peripheral blood, MPB)[7]. Finally, we have described that HSC clonogenic activity, lineage output, long-term lineage commitment and rates of somatic mutations as key variables influenced by the underlying disease, the extent of genetic defect correction and the hematopoietic stress imposed by the inherited disease[8]. With LV-GT patients reaching now >10 years of observations, we are currently studying the characteristics, functional properties, and clonality of long-term engrafted engineered HSPC exploiting innovative single cell tracing methods. Preliminary data show that the engrafted pool of engineered HSPC retain self-renewal properties preserving their number and clonality up to 12 years post-GT. Overall, our work will provide fundamental information on the biology and the clonal complexity of engineered HSPC, establishing gene therapy as a definitive, safe, and curative option for a broader range of diseases.

References

1. **Ferrari G, Thrasher AJ, Aiuti A.** — Nat Rev Genet. 2021. https://doi.org/10.1038/s41576-020-00298-5
2. **Ferrari S et al.** — Cell Stem Cell. 2023. https://doi.org/10.1016/j.stem.2023.04.014
3. **Tucci F et al.** — Nat Commun. 2022;13:1–16. https://doi.org/10.1038/s41467-022-28762-2
4. **Scala S, Aiuti A.** — Blood Advances. 2019. https://doi.org/10.1182/bloodadvances.2019000039
5. **Biasco L et al.** — Cell Stem Cell. 2016;19(1):107-19. https://doi.org/10.1016/j.stem.2016.04.016
6. **Scala S et al.** — Nature Medicine. 2018. https://doi.org/10.1038/s41591-018-0195-3
7. **Scala S et al.** — Nature Communications. 2023;14(1):3068. https://doi.org/10.1038/s41467-023-38448-y
8. **Calabria A et al.** — Nature. 2024;636(8041):162-171. https://doi.org/10.1038/s41586-024-08250-x