

 <p>UniSR Università Vita-Salute San Raffaele</p>	<p>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</p>	<p>MO 20-5 ed. 01 del 21/02/2025 PO 20 Page 4 of 11</p>
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PROJECT

Supervisor:

Samuele Ferrari

Title:

Unravelling the mechanism of inflammatory-driven evolution in
Clonal Hematopoiesis by targeted genome editing and single-cell
multi-omics

Curriculum:

PhS - Gene and Cell therapy

Link to the personal page of the
University or relevant hospital site
website:

<https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/novel-gene-therapy-strategies/samuele-ferrari.html>

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Clonal Hematopoiesis (CH) is an age-related somatic mosaicism, characterized by the presence of acquired mutations in the Hematopoietic Stem/Progenitors Cells (HSPCs), which carried an increased risk of progression into hematologic malignancies¹. This process is also related to heightened cardiovascular risk, probably due to a state of chronic systemic inflammation². CH of indeterminate potential (CHIP) often result from mutations in key hematopoietic genes involved in epigenetic regulation or DNA methylation (such as DNMT3A, TET2, ASXL1) or other master cellular functions, which have been commonly associated with clonal dominance in elderly individuals. However, the mechanisms driving the initiation, maintenance, and progression of CHIP are not entirely understood³.

VEXAS (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) syndrome is a recently discovered hematoinflammatory disease that leads to multiple organ involvement⁴. This disease is caused by an acquired mutation in *UBA1* gene which occur in hematopoietic stem cells, resulting in an inflammatory disorder associated with hematological abnormalities, often including also other CHIP mutations (i.e., DNMT3A, TET2)⁵.

The similarities between CHIP and VEXAS syndrome are still elusive. Moreover, CHIP and VEXAS syndrome are bonded through a bidirectional mechanism driven by systemic inflammation but the influence of *UBA1* and CHIP mutations on one another when co-occurring in a VEXAS patient or in the same stem cell remains insufficiently characterized^{5,6}.



Rationale and hypothesis

By developing VEXAS xenograft models generated through base editing and characterizing them through immunophenotypic and single-cell analyses⁷ we recently found that UBA1-mutated HSPCs are more resistant to the exhaustion and overwhelming states induced by the inflammatory milieu in VEXAS syndrome. Since this disease represent a peculiar type of CH, the main hypothesis of this project is that we can leverage more broadly on humanized murine models precisely recapitulating the genetics of CHIP and VEXAS syndrome to elucidate the interplay between clonal expansion and inflammation during aging. Moreover, using multiplex editing may uniquely enable the study of the impact of co-occurring CH mutations in VEXAS patients in terms of prognosis and clonal expansion dynamics.

To this goal, the candidate will elucidate the different mechanisms of clonal evolution of different mutations associated with CHIP and VEXAS syndrome by combining precision genome editing to develop humanized xenograft models and multiomic technologies with single-cell resolution⁸⁻¹⁰.

Objectives and specific aims

The main goal of this project is to shed light on the mechanisms and clonal evolution dynamics of the hematopoietic system in the context of CH through:

1. Developing humanized xenograft models of CHIP through base and prime editing to assess the mechanisms of clonal dominance.
2. Evaluating the impact of CHIP mutations in VEXAS syndrome, focusing on how the inflammation substrate change the severity and/or dynamic of progression.

Expected outcomes

We expect that this approach could lead to the identification of biological pathways and mechanisms that can explain CH progression, its interrelation with systemic inflammation, and the impact of CHIP mutations in the prognosis of VEXAS patients. Also, we expect that the development of preclinical models that recapitulate the key feature of pathophysiology could serve for the testing of new therapeutic or preventive approaches.

Skills that the student should acquire (max. 600 characters including spaces):

The candidate will leverage their clinical expertise to conduct experiments and enrich the projects with more practical and accessible insights, bridging the gap between basic research and clinical application. In particular, the candidate will transfer his/her clinical expertise to



design and implement multi-omic studies to explore complex biological questions. The candidate will also gain hands-on experience with gene editing technologies. Additionally, the candidate will acquire expertise in mouse models, investigating disease mechanisms and evaluating potential prognosis factor. The candidate will regularly present and discuss the results of the project with supervisor and peers.

References (max. 15)

1. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med.* 2014 Dec;20(12):1472-8. doi: 10.1038/nm.3733.
2. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N Engl J Med.* 2017 Jul 13;377(2):111-121. doi: 10.1056/NEJMoa1701719.
3. Fabre MA, Vassiliou GS. The lifelong natural history of clonal hematopoiesis and its links to myeloid neoplasia. *Blood.* 2024 Feb 15;143(7):573-581. doi: 10.1182/blood.2023019964.
4. Beck DB, Ferrada MA, Sikora KA, et al. Somatic Mutations in UBA1 and Severe Adult-Onset Autoinflammatory Disease. *N Engl J Med.* 2020 Dec 31;383(27):2628-2638. doi: 10.1056/NEJMoa2026834.
5. Gutierrez-Rodriguez F, Kusne Y, Fernandez J, et al. Spectrum of clonal hematopoiesis in VEXAS syndrome. *Blood.* 2023 Jul 20;142(3):244-259. doi: 10.1182/blood.2022018774.
6. Challen GA, Goodell MA. Clonal hematopoiesis: mechanisms driving dominance of stem cell clones. *Blood.* 2020 Oct 1;136(14):1590-1598. doi: 10.1182/blood.2020006510.
7. Molteni R, Fiumara M, Campochiaro C et. al. Mechanisms of hematopoietic clonal dominance in VEXAS syndrome. *Nat Med* 2025. doi:10.1038/s41591-025-03623-9
8. Rodriguez-Meira A, Norfo R, Wen S, et al. Single-cell multi-omics identifies chronic inflammation as a driver of TP53-mutant leukemic evolution. *Nat Genet.* 2023 Sep;55(9):1531-1541. doi: 10.1038/s41588-023-01480-1. Epub 2023 Sep 4. PMID: 37666991; PMCID: PMC10484789.
9. Ferrari S, Valeri E, Conti A, et al. Genetic engineering meets hematopoietic stem cell biology for next-generation gene therapy. *Cell Stem Cell.* 2023 May 4;30(5):549-570. doi: 10.1016/j.stem.2023.04.014.



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10. Fiumara M, Ferrari S, Omer-Javed A, et al. Genotoxic effects of base and prime editing in human hematopoietic stem cells. *Nat Biotechnol.* 2024 Jun;42(6):877-891. doi: 10.1038/s41587-023-01915-4. Epub 2023 Sep 7.