The undersigned

SURNAME Vago

FIRST NAME Luca Aldo Edoardo

born in Milano, Prov. Milano, on 19 / 09/ 1980

Unit: *Immunogenetics, Leukemia Genomics and Immunobiology*

Residency/Postgraduate: *Hematology and Bone Marrow Transplantation Unit/ Vita-Salute San Raffaele University*

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Role:

X Vita-Salute San Raffaele University Professor/Lecturer

☐ Vita-Salute San Raffaele University Researcher/Lecturer

X Group Leader of the hospital site Unit of Immunogenetics Genomics and Leukemia Immunobiology

☐ Project Leader of the hospital site _____

☐ Other_____

I hereby declare that, within the framework of the PhD Course for which I wish to submit the project described below:

X I am already a Supervisor;
☐ I am applying for the first time as a Supervisor (CV attached);
☐ I am applying as a Supervisor as three years have elapsed since my last Application as Supervisor and the submission of a research project (CV attached).

I further declare that (select the applicable option(s)):

☐ although I am less than four years away from retirement as a university professor/researcher, I will hold a documented institutional role at the hospital _____, for at least one year beyond the official duration of the course.

X I serve as Supervisor for no. 1 PhD candidates enrolled at other universities and I comply with the University requirement regarding the maximum number of five PhD candidates that may be supervised.

I would like to present a project:

X With a duration of three years

☐ **With a duration of two years within the Physician Scientist (PhS) programme**

as part of the PhD course in:

X Molecular Medicine

PhD Curriculum: ☐ Basic and Applied Immunology and Oncology

☐ Cell and Molecular Biology

☐ Clinical and Experimental Medicine

☐ Neurosciences and Experimental Neurology

X Gene and Cell Therapy

☐ Cognitive and Behavioural Sciences

The project consists in:

1.	Basic Research	☐
2.	Translational Research	**X**
3.	Basic/ Translational research using animal models	☐
4.	Clinical research	☐
5.	Clinical research involving interaction with patients	☐

If items 2 and/or 3 is/are selected, I declare that

☒ I HAVE OBTAINED the approval of the responsible Institutional Animal Care and Use Committee-IACUC number: 1316 (until May) and 1317

☐ I HAVE NOT YET OBTAINED the approval of the responsible Institutional Animal Care and Use Committee-IACUC

If items 4 and/or 5 is/are selected, I declare that the project:

☐ **HAS NOT YET OBTAINED** approval from the Ethics Committee (EC)

☒ **HAS OBTAINED**, or is part of a broader study that has obtained, approval from the Ethics Committee (EC); study code and date: research study code ALLO-RELAPSE last amendment 24/09/2025. Approved on 22/10/2025.

If items 4 and/or 5 is/are selected, I declare that the project:

☐ **HAS NOT OBTAINED** the resolution of the Institution

☐ **HAS OBTAINED** the resolution of the Institution on _____

I further declare (select the applicable option(s)):

☐ that I have the availability of the funds necessary to finance a scholarship for the proposed project and I confirm that I have contacted the Doctoral Office regarding the management of the administrative procedures related to the funding;

☒ that I have the availability of funds to support the research (i.e. funds for materials, reagents, and instruments required for research activities);

☐ that, in the case of a clinical research project, it will include a basic or translational research component to be carried out in a laboratory to be specified in the research plan, whose head will act as co-supervisor.

☒ that I have adequate workspace and a permanent workstation available for the PhD candidate who will be selected to carry out the project;

☒ that the proposed project can be reasonably completed within the three-year legal duration of the programme;

☒ that the PhD student, within the activities of the relevant PhD program, will carry out only their specific doctoral project;

☒ that the PhD student will be the first author/author of the main publication resulting from his/her project and of all publications (also after graduation) that are mainly based on his/her experimental work;

☒ that, in the event that the PhD student is not the recipient of a UniSR grant (i.e. has won a position without a grant), I am willing to cover the cost of their scholarship with funds at my disposal. I am aware that the grant must not amount to less than the minimum required by the Ministerial Decree of 23 February 2022, amounting to € 16,243 gross per year, for three years;

☐ that the study is co-funded by an industrial partner or that a commercial exploitation of the findings resulting from the project's research activity is conceivable, with a potential delay in the publication of the results. I therefore commit to promptly inform potential candidates of such circumstances.

Signature of the Supervisor 

Date 03/31/2026

When applicable:

Group Leader Prof. /Dr. Luca Aldo Edoardo Vago

Signature 

Date 03/31/2026

Please note that the information provided on the following pages (unless otherwise indicated) will be made public on the University website. Therefore, it is important not to include confidential information, in compliance with any confidentiality obligations towards third parties and to protect the potential patenting of such information. For any questions, please consult the PhD Office.

PROJECT

Supervisor:	Luca Aldo Edoardo Vago
Title:	Deciphering the epigenetic mechanisms by which lactic acid drives leukemia immune escape and relapse after allogeneic hematopoietic cell transplantation
Curriculum:	Gene and Cell Therapy
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/immunogenetics-leukemia-genomics-and-immunobiology.html

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

Background/gap of knowledge

Recent studies have highlighted the role of metabolic and epigenetic reprogramming in driving immune evasion in acute myeloid leukemia (AML) relapse following allogeneic hematopoietic cell transplantation (allo-HCT) (1–3). Among these processes, metabolic rewiring is emerging as a critical mechanism, with lactic acid (LA) identified as a key mediator of immune escape through both the impairment of T cell effector functions (2) and the modulation of oncogenic transcriptional programs via the histone modification "lactylation" (4). Growing evidence further indicates that lactylation of histone and non-histone proteins play a central role in regulating genes involved in resistance to cancer immunotherapy (4–7). However, the upstream regulators of lactylation, the gene targets and pathways it modulates in AML, and their potential role in mediating immune escape remain largely undefined.

Rationale and hypothesis

Recently, LA has emerged as a key immunosuppressive oncometabolite and epigenetic regulator, yet it remains unclear whether LA-driven signals intersect with epigenetic regulators to sustain post-transplant AML immune evasion. In this project, we aim to identify the actionable nodes within this metabolic–epigenetic axis to ultimately reinstate antitumor immunity. We will analyze paired AML samples collected at diagnosis and relapse after allo-HCT to quantify global (pan-Kla) and residue-specific histone lactylation and determine whether increased lactylation associates with distinct relapse patterns. We will characterize lactylation-driven chromatin remodeling by integrating ATAC-seq and CUT&Tag with transcriptional profiles to derive relapse-specific regulatory elements and transcription-factor (TFs) networks. In parallel, we will define lactylation-dependent proteomic changes using pan-Kla immunoprecipitation followed by LC-MS to identify differentially lactylated proteins, associated pathways, and top candidates

by targeted IP-MS to uncover associated regulators. We will functionally validate the role of priority TFs and lactylated proteins in immune evasion using CRISPR/Cas9 gene knockout, testing also whether combining genetic perturbation with epigenetic/metabolic agents reinstates antitumor immunity. This work will leverage collaborations with Dr. Davide Gabellini and Dr. Simone Cardaci (San Raffaele), and Dr. David Sumpton (CRUK Scotland Institute).

Objectives and specific aims

1)Decipher the LA–driven epigenetic alterations and the upstream regulators underlying post-transplant leukemia relapse

2)Characterize the LA-enriched proteome and identify the principal mediators of lactylation dynamics sustaining AML immune escape

3)Define the functional contribution of the most promising candidate regulators by ad hoc in vitro assays

Expected outcomes

Improved mechanistic understanding of lactylation-driven modifications in shaping immune-evasion programs after allo-HCT will identify novel actionable regulatory hubs that can be targeted to restore antitumor immunity, thereby supporting the rationale of new complementary therapeutic strategies.

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

The project features an equal balance of experimental and computational work. The student will gain proficiency in wet-lab techniques using primary patient samples, including libraries for sequencing. In parallel, a significant part of the project will require ability to program and develop bioinformatic pipelines for chromatin profiling and transcription-factor network inference. Through this integrative approach, the student will acquire a robust interdisciplinary skill set spanning molecular epigenetics, data science, and systems-level analysis of gene-regulatory mechanisms.

References (max. 15)

1)Toffalori, C et al. Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation. Nat. Med. 25, 603–611 (2019).

2)Uhl, F. M. *et al.* Metabolic reprogramming of donor T cells enhances graft-versus-leukemia effects in mice and humans. *Sci. Transl. Med.* **12**, eabb8969 (2020).

3)Gambacorta, V. *et al.* Integrated Multiomic Profiling Identifies the Epigenetic Regulator PRC2 as a Therapeutic Target to Counteract Leukemia Immune Escape and Relapse. *Cancer Discov.* **12**, 1449–1461 (2022).

4)Huang, Z.-W. *et al.* STAT5 promotes PD-L1 expression by facilitating histone lactylation to drive immunosuppression in acute myeloid leukemia. *Signal Transduct. Target. Ther.* **8**, 391 (2023). 5)Chen, H. *et al.* NBS1 lactylation is required for efficient DNA repair and chemotherapy resistance. *Nature* **631**, 663–669 (2024).

6)Wang, R. *et al.* H3K9 lactylation in malignant cells facilitates CD8+ T cell dysfunction and poor immunotherapy response. *Cell Rep.* **43**, 114686 (2024).

7)Jin, J. *et al.* Targeting lactylation reinforces NK cell cytotoxicity within the tumor microenvironment. *Nat. Immunol.* **26**, 1099–1112 (2025).

The information below will not be displayed on the University website in the description of the projects offered for the academic year, and will be used for internal project assessment only.

Experimental plan (Between 2,000 and 3,000 characters including spaces):

We will start our investigation by selecting n= 15 diagnosis/relapse pairs representative, for which extensive immunological, genetic, and epigenetic characterization is already available in the laboratory.

Under aim 1, we will define how histone lactylation shapes chromatin architecture and transcriptional regulation during relapse after allo-HCT by quantifying global (pan-Kla) and residue-specific lactylation in paired diagnosis–relapse samples, and by mapping chromatin regions and genes associated with increased chromatin accessibility and lactylated histones using ATAC-seq and CUT&Tag (targeting residues H3K18, H3K9, H4K5, H4K8, and H4K12). We will then integrate these datasets with established epigenetic marks (e.g., H3K4me3) and transcriptional profiles already available in the laboratory to identify lactylation-dependent regulatory elements and reconstruct transcription-factor networks that drive relapse-specific gene-expression programs.

In parallel, under Aim2, we will analyze the differences in the lactylation-enriched proteome of AML diagnosis and post transplantation relapses by performing immunoprecipitation (IP) with an antibody against lactylated lysines (pan-Kla), followed by liquid-chromatography mass spectrometry (LC-MS) of the eluted fractions. By this analysis we will derive proteins that are lactylated in each of the conditions analyzed, and the pathways they belong to. Priority candidates—selected based on the magnitude and significance of differential lactylation—will then undergo targeted validation through specific immunoprecipitation coupled with LC–MS/MS to identify potential lysine lactyltransferases responsible for regulating lactylation dynamics.

Under Aim3, we will validate priority transcription factors and lactylated proteins altered at relapse (identified in Aims 1–2) using orthogonal assays—including qPCR, western blot, immunophenotyping—in additional relapse samples from the EmaBank or external collaborators. Their functional role in immune evasion will be assessed by co-culturing CRISPR–Cas9- or shRNA-edited primary AML blasts with T cells enriched for leukemia-specificities, followed by flow-cytometry–based analyses of T-cell degranulation (CD107a), antigen-specific activation (4-1BB), target-cell apoptosis (Annexin V/Caspase-3), and proliferation (CFSE/CellTrace).

We will subsequently implement refined co-culture assays in which gene knockout is combined with exposure to epigenetic and metabolic agents—including HDAC inhibitors, hypomethylating agents already used in the post-transplant setting, and modulators of lactate metabolism such as LDHA or MCT1/4 inhibitors—to closely mimic pharmacological treatment conditions. These experiments will determine whether the combined perturbation modulates T-cell phenotype and effector function, in terms of enhancing GvL activity or increasing the susceptibility of leukemic cells to these therapeutic agents.

Available methods and experimental models (max. 600 characters including spaces):

ATAC-seq, CUT&Tag-seq, and MS sample-preparation workflows are regularly performed in our lab. The project will leverage the unit's expertise in hematology, immunology, molecular biology, and bioinformatics, together with the advanced infrastructures of our Institution, strong internal and external collaborations, and—thanks to a long-standing partnership with the Clinical Hematology Department—access to the well-annotated leukemia biobank (EmaBank), which collects longitudinal samples from AML patients.

Role of the PhD student (max. 600 characters including spaces):

The PhD student will conduct key experimental work on primary AML samples, including immunofluorescence, western blotting, immunoprecipitation, and preparation of NGS libraries. In parallel, he/she will process ATAC-seq, CUT&Tag-seq, and mass-spectrometry data, develop analytical pipelines, and validate candidate regulators, working closely with internal and external collaborators.

Impact of the expected results in the field of research (max. 600 characters including spaces):

These results will substantially advance the understanding of how lactic acid, through lactylation, shapes immune-evasion programs in post-transplant AML. By defining and targeting key epigenetic hubs, we aim to restore antitumor immunity, providing the rationale for new combined therapeutic strategies. This line of research could lead in a reasonable time to the validation of new epigenetic drugs for the treatment for post-transplantation relapses, and with potential extension to other tumors in which heightened lactate production and immunological rewiring have been documented.

In the case of clinical research, include the timeline for the project approval process up to the authorizing resolution of the Institution.

This project will not involve prospective clinical research or new sample collection. All analyses will be performed on pre-existing cryopreserved samples obtained from the San Raffaele Biobank (EMA-Bank). Sample use is authorized under the ALLO-RELAPSE retrospective protocol, last amended on 24/09/2025 and approved on 22/10/2025, which permits access to previously collected biological material and associated clinical data.

Period of attendance at a foreign institution

No period of activity at a foreign institution is currently planned.

For the use by the PhD Office

FOR OPINION – *(ONLY for Programs divided into Curricula)*

Signature of the Curriculum Supervisor _____ *Date* _____

FOR APPROVAL

Signature of the PhD Course Coordinator

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