The undersigned

SURNAME OMER

FIRST NAME Attya

born in Paris Prov. Ile-De-France on 14/10/1989

Unit: Novel Gene Therapy Strategies unit

Residency/Postgraduate School[1]: *N/A*

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Role:

☐ Vita-Salute San Raffaele University Professor/Lecturer

☐ Vita-Salute San Raffaele University Researcher/Lecturer

☐ Group Leader of the hospital site _____

X Project Leader of the hospital site SR-TIGET, Dibit 1

☐ Other _____

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

☐ I am already a Supervisor;
X 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.

☐ I serve as Supervisor for no. __ 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.

[1] To be indicated only for research projects associated with the Physician Scientist programme

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	☐
3.	Basic/ Translational research using animal models	X
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 _____
- X I <u>HAVE NOT YET OBTAINED the approval</u> 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 _____

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)):

X 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;

X 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.

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

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

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

X 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 27/03/2026*

When applicable:

Group Leader Prof. Luigi Naldini

Signature _____  *Date 27/03/2026*

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:	OMER Attya
Title:	Mobilization and Antibody-Mediated Conditioning: A Dual Approach to Maximize Chemotherapy-free Hematopoietic Stem Cell Therapy
Curriculum:	Medicina Molecolare/*Molecular Medicine* > Terapia Genica e Cellulare/*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.html

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

Background/gap of knowledge

Current hematopoietic stem and progenitor cell gene therapy (HSPC-GT) requires the mobilization, ex vivo genetic correction, and infusion of hematopoietic stem cells (HSPCs) following myeloablative conditioning. This process traditionally involves chemo/radiotherapy, which can lead to significant acute and long-term side effects. Although alternatives like antibody-drug conjugates (ADCs) promise reduced toxicity, their clinical application is complicated by off-target effects and prolonged antibody half-lives, which delay subsequent therapeutic interventions and potentially compromise patient outcomes [1-3].

Rationale and hypothesis

To overcome these challenges, we propose a non-genotoxic conditioning strategy that synergistically combines HSPC mobilization with targeted, antibody-mediated conditioning [4]. This strategy leverages the natural mobilization process to enhance the accessibility of HSPCs to conditionally active antibodies, potentially reducing the required antibody dosage and associated toxicity. We hypothesize that this approach will enable more effective and safer engraftment of gene-edited HSPCs, achieving clinically relevant levels of chimerism without the adverse effects associated with traditional conditioning methods.

Objectives and specific aims

The primary aim of this study is to develop and validate a novel, synergistic conditioning protocol, termed Mobilization/Antibody/RNA-based Strategy (MARS), to enhance the engraftment of gene-edited HSPCs without relying on irradiation or chemotherapy. Specifically, our project aims to:

1. Validate the efficacy of engineered antibody variants targeting CD117 with reduced half-lives through comprehensive in vitro and in vivo assessments [5-6].

2. Implement mRNA-based transient overexpression of a drug-resistant CD117 and the homing factor CXCR4 in HSPCs to optimize engraftment efficiency [7-8].

3. Explore the synergy between mobilization and antibody-based conditioning to enhance the survival and persistence of transplanted HSPCs, minimizing the risk of adverse events.

Expected outcomes

By integrating cutting-edge gene editing technologies, innovative antibody engineering, and advanced delivery systems, this project expects to:

- Establish a novel, non-genotoxic conditioning regimen that significantly reduces the toxicity associated with hematopoietic stem cell transplantation.
- Demonstrate improved engraftment efficiency and survival of gene-edited HSPCs in humanized mouse models, setting the stage for clinical trials.
- Expand the therapeutic applications of HSPC-GT by providing a safer, more effective alternative to current conditioning protocols, thereby improving outcomes for patients with hematologic disorders and genetic diseases amenable to gene editing.

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

The PhD candidate will strongly implement his/her competences thanks to the training-through-research activities: in the field of HSPCs biology (*in vitro* HSPCs culture, clonality, and transcriptional landscape); in the field of editing technology (Cas9 nuclease and base/prime editor applications); in the field of gene therapy (AAV/LV transduction, conditioning regimens and *in vivo* mouse modeling approaches). Importantly, the rich environment for scientific discussion in Naldini's lab will strengthen the candidate analytic and communication skills essential for research dissemination.

References (max. 15)

[1] Russkamp NF, Myburgh R, Kiefer JD, Neri D, Manz MG. Anti-CD117 immunotherapy to eliminate hematopoietic and leukemia stem cells. Exp Hematol. 2021 Mar;95:31-45. doi: 10.1016/j.exphem.2021.01.003. Epub 2021 Jan 20. PMID: 33484750.

[2] Marone R, Landmann E, Devaux A, et al. Epitope-engineered human hematopoietic stem cells are shielded from CD123-targeted immunotherapy. J Exp Med. 2023;220(12):e20231235. doi:10.1084/jem.20231235

[3] Wellhausen N et al. Epitope base editing CD45 in hematopoietic cells enables universal blood cancer immune therapy. Sci Transl Med. 2023;15(714):eadi1145. doi:10.1126/scitranslmed.adi1145

[4] A. Omer Javed, G. Pedrazzani, L. Albano, S. Ghaus, C. Latroche, M. Manzi, S. Ferrari, M. Fiumara, A. Jacob, V. Vavassori, A. Nonis, D. Canarutto, and L. Naldini. *Mobilization-based chemotherapy-free engraftment of gene-edited human hematopoietic stem cells.* Cell (2022)

[5] Stapleton, N.M., Brinkhaus, M., Armour, K.L. et al. Reduced FcRn-mediated transcytosis of IgG2 due to a missing Glycine in its lower hinge. Sci Rep 9, 7363 (2019). https://doi.org/10.1038/s41598-019-40731-2

[6] Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, Korman AJ. Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. Cancer Immunol Res. 2013 Jul;1(1):32-42. doi: 10.1158/2326-6066.CIR-13-0013. Epub 2013 Apr 7. PMID: 24777248.

[7] Casirati G. et al. Epitope editing enables targeted immunotherapy of acute myeloid leukaemia. Nature. 2023;621(7978):404-414. doi:10.1038/s41586-023-06496-5

[8] Vavassori V, Ferrari S, Beretta S, Asperti C, Albano L, Annoni A, Gaddoni C, Varesi A, Soldi M, Cuomo A, Bonaldi T, Radrizzani M, Merelli I, Naldini L. Lipid nanoparticles allow efficient and harmless ex vivo gene editing of human hematopoietic cells. Blood. 2023 Aug 31;142(9):812-826. doi: 10.1182/blood.2022019333. PMID: 37294917; PMCID: PMC10644071.

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):

To be completed for all types of projects; however, for CLINICAL PROJECTS, please specify:

1. *If Observational prospective, cross-sectional, or retrospective) or retro/prospective, quality of life, pharmacological, pathophysiology, genetics, epidemiological, registry/data collection, biobank, diagnostic accuracy, in vitro diagnostic device (IVD), nutraceutical/supplement, appropriateness; OR interventional (pharmacological, surgical, procedure, or medical device, and if a drug will be used, indicate the phase – I, II, III, or IV);*
2. *If a drug will be used, specify whether it has a marketing authorisation (MA), whether it will be used according to the MA or whether it does not have a MA;*
3. *If the study does not regard a drug, specify what will be studied (e.g. medical device, surgical procedure, diagnostic procedure, food supplement, etc.). If the study will use a medical device, please specify: whether it is CE marked. If CE marked, please indicate whether it will be used according to the approved use or for a new use.*
4. *Indicate the laboratory on which you intend to rely for the basic or translational part.*

The proposed research encompasses a multifaceted approach, integrating cutting-edge technologies and methodologies to achieve our research aims, leveraging platforms such as base and prime editing, lipid nanoparticles, and protein engineering. The research plan includes the following key steps:

1. In vivo assessment of antibody half-life and efficacy: A major hurdle for MARS lies in the prolonged half-life of the antibody in vivo, estimated to be around 15-20 days, while mRNA-based transgene overexpression can last for up to 7 days. To address this, we have identified specific mutations that, when introduced in the Fc region of the antibody, can dramatically reduce its half-life [5-6]. Humanized mouse models will serve as a valuable tool for evaluating the in vivo performance of antibody variants. We will assess their ability to target HSCs within the BM niche, both with and without mobilization. This will provide crucial insights into the pharmacokinetics and depleting efficiency of the engineered antibodies in a physiological context, ultimately establishing the optimal combination of Ab dose and timing combined with HSC mobilization.

2. In vitro validation of cell shielding: Recent work by our collaborator Casirati et al. has unveiled a CD117 variant that evades recognition antibodies, allowing cell survival in the presence of the drug by editing the receptor [7]. Our approach entails the transient overexpression of this variant, with subsequent assessment of HSPC viability in presence of the drug.

3. Implementing MARS in mouse models: This comprehensive approach will involve transient overexpression of drug-resistant KIT and the homing factor CXCR4 in ex vivo cultured HSPCs, which will be infused in mice conditioned with the established combination of mobilization and Ab-based treatment. To track the fate and engraftment of ex vivo modified cells, we will employ lentivirus transduction or cutting-edge editing technologies, such as base or prime editing. Moreover, by introducing distinguishable markers into the modified cells, we aim to demonstrate the compatibility of our strategy with different gene therapy platforms and ensure accurate tracking of transplanted cells in vivo.

Through this research plan, we aim to advance our understanding of non-genotoxic conditioning strategies and their application in HSPC-GT. By combining innovative technologies and translational approaches, we seek to optimize the efficacy and safety of antibody-mediated conditioning while paving the way for improved outcomes in hematologic disorders and genetic diseases amenable to gene editing approaches.

Methods: we will use advanced methodologies already validated in the lab for mRNA *in vitro* transcription and delivery in primary HSPCs (e.g. electroporation or lipid nanoparticles) [8], and cutting-edge gene editing platforms such as base and prime editing. Moreover, we already established and validated a powerful mobilization protocol in humanized mouse model.

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

To be completed for all types of projects; however, for CLINICAL PROJECTS, please specify:

1. *whether participants (patients and/or healthy volunteers) will be recruited;*
2. *whether biological samples will be taken from participants (patients and/or healthy volunteers);*
3. *whether the biological samples will be stored in a Biobank (specify which Biobank);*
4. *whether biological samples are already stored and available in a Biobank (specify which Biobank);*

5. *whether biological samples or data will be collected in addition to those already included in the routine standard of care from routine practice (specify type of samples/data, quantity and timing);*
6. *whether procedures will be required in addition to those already included in the routine standard of care from routine practice (e.g. Consultations, laboratory tests, clinical/instrumental examinations). Specify the additional procedures, quantity and timing).*

This project will exploit the following methods and models:

- Gene editing by CRISPR/Cas of human hematopoietic cells from healthy subjects (in compliance with the TIGET-09 and TIGET-HPCT ethical protocol approved by the OSR Ethical Committee)
- Xenotransplantation studies in immunodeficient NBSGW and NSGW41 mice (in compliance with IACUC submitted to the Italian MoH)
- Production of non-pathogenic viral vectors (AAV and LV) to be used for ex-vivo transduction of human cells (in compliance with the MI/IC/Op2/13/010 protocol approved by the Italian MoH)

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

The PhD candidate will be mainly engaged in the aforementioned project. Under the supervision of the DoS and post-doctoral fellows, the PhD candidate will design the experimental plan, perform the experiment, analyze the subsequent data obtained and present/summarize the data, which will all contribute to strengthen his/her analytic and project management skills. The candidate will take part in the weekly lab meetings, annual progress presentation and international congresses, improving his/her scientific communication skills and professional network. Moreover, the participation to the SR-TIGET and OSR retreat will allow to facilitate the exchange of multi-disciplinary expertise and to foster intra-sectorial collaborations. These trainings will be instrumental to reach professional maturity and independency in science.

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

The successful implementation of MARS could revolutionize HSCT by reducing associated toxicities, eliminating the need for chemotherapy, and thus making treatments safer and accessible to a broader patient population. By optimizing mRNA expression for drug-resistant CD117 and CXCR4, this approach aims to improve the engraftment efficiency of gene-edited stem cells, potentially reducing treatment frequency and costs. Collaborative efforts will streamline the clinical translation of these findings, facilitating quicker regulatory approvals and improving patient outcomes.

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

Period of attendance at a foreign institution

Mandatory for the PhD course in Cognitive and Behavioral Sciences

The PhD course in Cognitive and Behavioral Sciences encourages attendance at foreign universities and research institutes, promoting the acquisition of advanced skills and methodologies in international contexts.

Please indicate whether a period of activity at a foreign institution is planned. If so, specify:

- *Host institution (name of the University/Institute and country)*
- *Duration of stay (not less than 3 months)*
- *Integration with the research project (describe how this experience will contribute to the objectives of the proposed project)*

The information provided is not binding and may be subject to modifications based on the project's development and available opportunities.

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 _____