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*

Email address: omer.attya@hsr.it

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:

☐ **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 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 _________________

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:	Attya Omer
Title:	Identifying key regulators of human HSPCs engraftment in an *in vivo* hematochimeric model using a gain-of-function screen
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

Hematopoietic stem/progenitor cell gene therapy (HSPC-GT) is successfully used in the clinic to treat patients with hematopoietic malignancies and several inherited hematopoietic diseases[1]. This approach involves mobilization and harvesting of HSPCs, ex vivo genetic correction (via gene transfer or gene editing), and reinfusion following myeloablative conditioning to create space in the bone marrow (BM). Corrected HSPCs home to the BM, engraft, and regenerate a functional hematopoietic system.

Gene editing of human HSPCs offers the possibility of precise correction of disease-causing mutations. However, current ex vivo manipulation protocols—including cell culture, electroporation, and viral vector exposure—negatively impact HSPC fitness[2,3]. These stresses reduce long-term repopulating potential, induce transient cell cycle arrest and apoptosis, and lead to a contraction of the clonal repertoire.

Although recent advances have improved editing efficiency in long-term repopulating HSPCs, the proportion of edited cells that successfully engraft remains limiting. This represents a major bottleneck for achieving safe and effective therapeutic outcomes. Furthermore, while candidate-based approaches have identified some regulators of engraftment, there is a lack of unbiased, genome-wide strategies[4-6] to systematically uncover novel factors that enhance HSPC engraftment.

Rationale and hypothesis

Improving engraftment efficiency of edited HSPCs—without compromising their long-term repopulating capacity—is critical for advancing HSPC-GT. A transient enhancement of engraftment potential could provide edited cells with a competitive advantage in the BM niche while preserving safety.

We hypothesize that:

- Key regulators of HSPC engraftment can be identified through an unbiased gain-of-function screening approach.

- Transient overexpression of these factors will enhance homing, retention, and engraftment of HSPCs without long-term perturbation.

- Such transient modulation can be integrated with state-of-the-art gene editing platforms to improve therapeutic outcomes.

Objectives and specific aims

The overall objective is to identify and validate novel regulators of human HSPC engraftment and leverage them to enhance the efficacy of gene-edited HSPCs.

Specific Aim 1: Genome-wide identification of engraftment regulators To identify key regulators of HSPC engraftment, we will perform a gain-of-function screen using a lentiviral sgRNA library combined with a dCas9-based transcriptional activation system (dCas9-TA). Human HSPCs will be transduced with this system, and the most primitive HSC population will be isolated. These cells will then be transplanted via intrabone injection into immunodeficient NSGW41 mice. Samples will be collected at early (1 week) and late (12 weeks) time points post-transplantation, and next-generation sequencing (NGS) will be performed to identify genes that confer a competitive engraftment advantage.

Specific Aim 2: Validation of candidate regulators Top candidate genes will be prioritized based on their enrichment in the screen and their novelty. These selected hits will be validated through transient mRNA overexpression in human HSPCs. The engineered cells will then be transplanted into immunodeficient NSG mice. Engraftment efficiency, transcriptional profiles, and clonal composition will be assessed and compared to control cells electroporated with GFP mRNA.

Specific Aim 3: Integration with gene editing platforms Validated candidate regulators will be combined with gene-edited HSPCs to evaluate whether their transient overexpression confers a selective advantage to edited cells over unedited counterparts. Finally, long-term repopulation capacity and safety will be assessed to determine the translational potential of this approach.

Expected outcomes

This project is expected to lead to the identification of novel and previously unrecognized regulators of HSPC engraftment through an unbiased genome-wide approach. It will demonstrate that transient gain-of-function strategies can enhance engraftment efficiency without compromising the long-term repopulating capacity of hematopoietic stem cells. In addition, the study will validate a set of actionable targets that can be readily integrated into our existing gene editing workflows[7]. Altogether, these findings will establish a new conceptual and technological framework to improve HSPC gene therapy. Ultimately, this work is anticipated to contribute to the development of next-generation HSPC-based therapies by enabling safer, more efficient, and clinically robust engraftment of genetically corrected cells.

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

The PhD candidate will work in a stimulating and collaborative environment, under close daily supervision by an appointed senior lab member and progressively taking the lead on the project and learning how to design and perform experiments and critically interpret the results. Technical skills to be acquired include: design of gene editing tools and their application in primary cells; manipulation and functional characterization of HSPCs; -omics studies; design and production of vectors; experiments in mouse models. The candidate will regularly discuss the results of the project with the supervisors and peers at institutional and international meetings.

References (max. 15)

1 Naldini. Genetic engineering of hematopoiesis: current stage of clinical translation and future perspectives. EMBO Molecular Medicine (2019).

2 Schiroli et al. Precise gene editing preserves hematopoietic stem cell function following transient p53-Mediated DNA damage response. Cell Stem Cell (2019).

3 Ferrari et al. Efficient gene editing of human long-term hematopoietic stem cells validated by clonal tracking. Nature Biotechnology (2020).

4 Sanson et al. Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities. Nature Communication (2018).

5 Horlbeck et al. Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation. Elife (2016).

6 Joung et al. Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. Nature Protocols (2017).

7 Javed Omer, A. et al. Chemotherapy-free engraftment of gene edited human hematopoietic stem cells leveraged on mobilization and mRNA-based engineering. Cell (2022).

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.*

To identify key regulators of engraftment, the candidate will use a genome-wide activation strategy based on a deactivated Cas9 (dCas9), paired with a transcriptional activation (TA) domain. To this date multiple libraries and many transcriptional activations have been described in the literature, but none has been shown as effective in human HSPCs. To assess which library would allow a robust overexpression, the candidate will first compare *in vitro* the ability of three different libraries ability to overexpress 10 genes, which are involved in HSC retention, engraftment and quiescence. The library that will yield the highest number of gene overexpression will be selected for the subsequent *in vivo* experiments. Briefly, these HSPCs are collected from the peripheral blood of healthy consented donors injected with Granulocyte-Colony Stimulating Factor (G-CSF). After HSPCs transduction, cells will be sorted for the most primitive HSC compartment and transplanted via intrabone injection in the BM of immunodeficient NSGW41 mice. Cells will be collected at 1- and 12-weeks post-transplantation followed by next-generation sequencing (NGS) to identify the top hits conferring a competitive engraftment advantage. The OSR bioinformatics group will help with the post-acquisition data processing. Only the most resilient cells with a considerable engraftment advantage will be retained and proliferate despite the poorly permissive BM environment. The 5 top hits (prioritized by novelty) resulting from the NGS analysis will be validated by *in vivo* assays. The candidate will first stably express the 5 top hits, independently, in HSPCs using a lentiviral-platform. Transduced HSPCs will be transplanted in irradiated immunodeficient NSG mice (for all transplantation assays), followed by subsequent analysis of the transcriptional landscape, engraftment efficiency, and clonal composition to monitor an eventual increased engraftment, compared to

HSPCs infected with a control cell surface reporter (NGFR). The top 3 genes conferring the highest advantage will be used for the subsequent optimization step.

The cells of interest are patients' HSPCs, required to be gene corrected. The stable overexpression of a gene raises safety concerns. To overcome issues associated with stable integration, the candidate will exploit a transient overexpression platform, already established in the host lab. The candidate will first investigate whether a mRNA-based transient expression of the top 3 genes confer a competitive engraftment advantage. Briefly, HSPCs will be electroporated with the mRNA from the top 3 genes identified, kinetics of expression will be measured *in vitro*, paralleled to the transplantation in NSG mice. Subsequently, clonal composition and engraftment advantage will be monitored longitudinally, as compared to HSPCs electroporated with GFP mRNA as control. Finally, the candidate will use a similar approach on gene-edited HSPCs in order to investigate the engraftment benefit, induced by a transient expression, in engineered HSPCs. Briefly, HSPCs will be electroporated with a ribonucleoprotein complex, including CRISPR/Cas9 nuclease and specific gRNAs for the AAVS1 locus. The complex will be delivered to HSPCs by electroporation, followed by a transduction with an AAV6 vector carrying a donor template. The candidate will exploit the electroporation step to transiently co-deliver the top mRNA hit identified and identify its effect on long-term engraftment.

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-HPCT ethical protocol approved by the OSR Ethical Committee)
- Xenotransplantation studies in immunodeficient NSG 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):

If successful, the increased engraftment efficiency investigated in this proposal will provide a novel way to increase the robustness of *ex vivo* editing, expanding the patients' pool and conferring long-term therapeutic benefits with considerably less long-term toxicity to patients.

The project involves the optimization of a genome-wide activation library in human HSPCs. This methodology has never been described in human HSPCs due to the poor permissiveness of HSPCs to lentiviral infection. The host lab knowhow in terms of HSPCs transduction and genome-wide screenings will allow to develop and optimize the experimental conditions in a relatively short time. The proposed use of genome-wide activation will allow to investigate the cell

retention processes in an unprecedented *in vivo* model, to shed new light on the molecular mechanisms of engraftment and the bone marrow biology.

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 ____