The undersigned

SURNAME APRILE

FIRST NAME ANNAMARIA

Born in TORTONA Prov. AL on 11 / 11 / 1986

Unit: GENE TRANSFER INTO STEM CELLS (SR-Tiget)

Residency/Postgraduate School[1]: _____

Email address: : aprile.annamaria@hsr.it

Role:

☐ Vita-Salute San Raffaele University Professor/Lecturer

☐ Vita-Salute San Raffaele University Researcher/Lecturer

☐ Group Leader of the hospital site _____

☒ Project Leader of the hospital site IRCCS Ospedale San Raffaele (SR-Tiget)

☐ 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;
☐ 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.

I would like to present a project:

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

☒ **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:

☒ Molecular Medicine

PhD Curriculum:

☐ Basic and Applied Immunology and Oncology

☐ Cell and Molecular Biology

☐ Clinical and Experimental Medicine

☐ Neurosciences and Experimental Neurology

☒ 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 ☒
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 1401 and 1333

☐ 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)):

☐ 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;

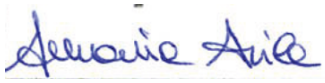
☒ that the PhD student, within the activities of the relevant PhD program, will carry out only their specific doctoral project;

☒ 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 25/03/2026

When applicable:

Group Leader Prof. GIULIANA FERRARI

Signature

Date 25/03/2026

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:	APRILE ANNAMARIA
Title:	Dissecting and targeting the hematopoietic stem cell niche to improve gene therapy for hemoglobinopathies
Curriculum:	GENE AND CELL THERAPY (GCT)
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/gene-transfer-into-stem-cells.html

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

Background/gap of knowledge

The hematopoietic stem cells (HSC) lie at the apex of the hierarchical process of hematopoiesis and reside in a specialized bone marrow (BM) microenvironment, termed niche[1]. The complex regulation of HSC and the BM niche remains underexplored in inherited disorders, as hemoglobinopathies. Hemoglobinopathies, β-thalassemia (BThal)[2] and sickle cell disease (SCD)[3], are globally widespread genetic disorders of hemoglobin. Despite differences in etiology, in both BThal and SCD, anemia causes chronic stress in different organs, including in the BM[4]. We provided the first demonstration of impaired HSC function caused by an altered BM stromal niche in BThal[5,6] and we have recently identified fibroblast growth factor-23 (FGF23)[7] as the molecular link connecting anemia, bone and the HSC niche[8]. FGF23 is high in BThal patients and *in vivo* inhibition of FGF23 signaling rescues bone defects and the BM niche, thus restoring HSC function. Alterations of HSC and BM niche have been described also in SCD and our preliminary data showed increased levels of FGF23 in SCD patients.

Rationale and hypothesis

Definitive cure for hemoglobinopathies is achieved by HSC transplantation from healthy donors or gene therapy transplantation of autologous HSC from patients[9]. The quality and the engraftment of HSC depend on the BM environment, thus studying the molecular basis and developing strategies targeting the HSC-niche crosstalk might be relevant for transplant outcome. How FGF23 is involved in BM niche regulation still need to be elucidated. Our hypothesis

is that FGF23 is the missing link at the crossroads of anemia and HSC niche defects in hemoglobinopathies through common and disease-specific mechanisms and that pharmacological inhibition of FGF23 might restore the HSC-BM niche crosstalk, thus contributing to a better clinical outcome of transplantation and gene therapy.

Objectives and specific aims

The main objectives of the project are to investigate the molecular mechanisms of the altered BM niche in BThal and SCD, focusing on the role of FGF23, and to target the identified defects to ameliorate the outcome of gene therapy transplantation. The specific aims are (1) to investigate the molecular mechanisms regulating the role of FGF23 in BThal and SCD HSC niche by single-cell transcriptomics, barcoding[10] and imaging approaches[11], (2) to test our FGF23 inhibition strategy[8] in the mouse model of SCD, (3) to perform preliminary studies on the combination of FGF23 inhibition with transplantation and gene therapy for hemoglobinopathies.

Expected outcomes

The expected outcomes of the project include the identification of the role of FGF23 in the BM niche by integrating single-cell RNA sequencing and *in vivo* barcoding strategies in the murine models of BThal and SCD, the evaluation of the safety and efficacy of FGF23 inhibition in SCD and the assessment of a protocol to combine FGF23 inhibition with gene therapy approaches.

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

These studies will be performed by using *Hbb*$^{th3/+}$ BThal and Townes SCD mice, as well as *in vivo* tracing and reporter strains, i.e. the CARLIN and α-catulin^{GFP} mice. The student will acquire both cellular and molecular techniques from immunophenotype analyses to *in vitro* and *in vivo* functional assays, histological, barcoding and single cell-RNA sequencing analyses. The student will be followed in acquiring specific skills for the analysis and critical interpretation of research data.

References (max. 15)

(1) Crane, G.M., et. al. *Adult haematopoietic stem cell niches* (2017) Nat Rev Immunol. doi: 10.1038/nri.2017.53

(2) Taher, A.T., et. al. *β-Thalassemias* (2021) N Engl J Med. doi:10.1056/NEJMra2021838

(3) Ware, R.E., et al. *Sickle cell disease* (2017) Lancet. doi: 10.1016/S0140-6736(17)30193-9

(4) Aprile A., et al. *Targeting the Hematopoietic Stem Cell Niche in beta-Thalassemia and Sickle Cell Disease* (2022) Pharmaceuticals (Basel). doi: 10.3390/ph15050592

(5) Aprile A., et al. *Hematopoietic stem cell function in beta-thalassemia is impaired and is rescued by targeting the bone marrow niche* (2020) Blood. doi: 10.1182/blood.2019002721

(6) Crippa S., et al. *Bone marrow stromal cells from beta-thalassemia patients have impaired hematopoietic supportive capacity* (2019) J Clin Invest. doi: 10.1172/JCI123191

(7) Edmonston D., et al. *FGF23 at the crossroads of phosphate, iron economy and erythropoiesis* (2020) Nat Rev Nephrol. doi: 10.1038/s41581-019-0189-5

(8) Aprile A., et al. *Inhibition of FGF23 is a therapeutic strategy to target hematopoietic stem cell niche defects in beta-thalassemia* (2023) Sci Transl Med. doi: 10.1126/scitranslmed.abq3679

(9) Marktel S. et al. *Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent β-thalassemia* (2019) Nat Med. doi: 10.1038/s41591-018-0301-6

(10) Bowling S., et al. *An engineered CRISPR-Cas9 mouse line for simultaneous readout of lineage histories and gene expression profiles in single cells* (2020) Cell. doi: 10.1016/j.cell.2020.06.018

(11) Acar M., et al. *Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal* (2015) Nature. doi: 10.1038/nature15250

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.*

This project will investigate HSC-BM niche interactions in hemoglobinopathies, focusing on the role of FGF23 as the missing link at the crossroads of anemia and the BM microenvironment. Pharmacological inhibition of FGF23 signaling might restore bone homeostasis and BM niche-HSC crosstalk in hemoglobinopathies, thus contributing to a better clinical outcome of transplantation and gene therapy, with a 'two birds with one stone strategy'. The proposed research will be performed in mouse models of BThal and SCD and will be organized following this general experimental plan.

1) To unravel the molecular mechanisms regulating FGF23 in BThal and SCD HSC niche the student will analyze the expression of FGF23 by bone, erythroid cells and other niche populations and the effect of pathophysiological triggers (e.g. erythropoietin, iron, inflammation, etc.) stimulating FGF23 accumulation in hemoglobinopathies. Moreover, transgenic barcoding and reporter strains crossed to the disease models will be exploited to dissect the contribution of FGF23 to HSC niche defects in BThal and SCD. In particular, the CARLIN mouse model will be used to simultaneously study single-cell transcriptomics and lineage tracing in hematopoietic and stromal cells[10]. To evaluate HSC interactions within local BM niches of BThal and SCD mice the α-catulin^{GFP} mouse strain[11] will provide genetic

marking of HSC to directly visualize their localization. Experiments will be performed in BThal and SCD untreated conditions and after FGF23 inhibition.

2) Our published FGF23 inhibition strategy by the cFGF23 peptide[8] will be tested in the mouse model of SCD to rescue *in vivo* the HSC-BM niche crosstalk. Treated animals will be analyzed to assess the safety (i.e. animal survival, clinical signs and off-target effects) and efficacy of the treatment (i.e. effects on the bone density, bone quality and deposition). Immunophenotypical, histological and *in vivo* functional assays will be performed to establish the rescue of the BM niche and HSC activity.

3) To develop *in vivo* FGF23 inhibition strategies in combination with transplantation and gene therapy for BThal and SCD, the long-term effect and the persistence of the efficacy after treatment discontinuation will be tested. The student will set HSC transplantation experiments in the disease mouse models to assess the efficacy of FGF23 inhibition on HSC function and rescue of the recipient BM niche and will collect preliminary evidence on combining the administration of cFGF23 with gene therapy strategies.

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).*

Hbb$^{th3/+}$ BThal, Townes SCD mice, CARLIN *in vivo* tracing and α-catulin^{GFP} reporter strains will be used. *In vivo* studies will involve breeding, drug administration and HSC transplantation. Cellular

biology methods will include isolation and genetic engineering of HSC, BM stromal cell isolation and culture, immunophenotype analysis by flow cytometry and immunofluorescence. Molecular biology techniques including ddPCR, scRNAseq library preparation and ELISA assays will be used. Bioinformatic analysis will be performed with the support of the SR-Tiget Bioinformatic Core.

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

The student will be followed in acquiring a rigourous scientific method to achieve the project objectives under the supervision of the Supervisor. She/he will gain *in vivo*, cellular and molecular techniques as well as the ability to critically interpret experimental data and to present results in written and oral presentations. The student is expected to develop strong organizational skills and to actively work in a team towards the acquisition of a progressive independence. The results of the project are expected to be included in a peer-reviewed publication with the student as first author.

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

The research on hemoglobinopathies has been mostly focused on erythropoiesis, leaving the study of the HSC niche underexplored. We have demonstrated that HSC function in BThal is negatively affected by an altered BM niche and others revealed defects in the BM niche also in SCD. This project might exploit a genetic disorder to shed light into general molecular mechanisms of stem cell biology. Moreover, the niche targeting strategy might lead to the development of novel therapeutic approaches aimed to preserve BM niche and HSC function in gene therapy transplantation for hemoglobinopathies.

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 _____