The undersigned

SURNAME Ditadi

FIRST NAME Andrea

born in *Dolo (VE)* on 15 / 03 / 1979

Unit: *Human hematopoietic development and disease modelling unit*

Residency/Postgraduate School[1]: *NA*

Email address: *ditadi.andrea@hsr.it*

Role:

☐ Vita-Salute San Raffaele University Professor/Lecturer

☐ Vita-Salute San Raffaele University Researcher/Lecturer

X Group Leader of the hospital site Ospedale San Raffaele – via Olgettina

☐ 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.

☐ 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 1337 (to be renewed)

☐ 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;

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: March 24th 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:	Dr. Andrea Ditadi
Title:	Dissecting ribosome-regulated protein expression dynamics during human erythroid development
Curriculum:	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/human-hematopoietic-development-and-disease-modeling.html

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

Background/gap of knowledge

Disrupted ribosomal biogenesis is a hallmark of both congenital and acquired bone marrow failure (BMF) (1). In particular, mutations in ribosomal protein genes (RPGs) underlie a class of diseases, termed ribosomopathies, that at their onset affect specific hematopoietic lineages (1). Of these, Diamond-Blackfan Anemia (DBA) is especially intriguing since it holds the rare distinction of being an intrinsic genetic disorder of erythroid progenitor cells. The mechanism by which impaired ribosome biogenesis specifically affects adult erythropoiesis remains poorly understood. Red blood cells (RBCs), are produced in three distinct waves (2, 3); one "primitive" wave (herein defined as PrimE) followed by a "transient" (TransE) and a subsequent "definitive" (DefE) (4, 5). DefE is sustained by hematopoietic stem cells (HSCs) capable of long-term reconstitution, starts in the fetal liver (FL) and post-natal bone marrow (BM) and generates RBCs needed throughout postnatal life. However, in the embryo, RBC progenitors are first produced in the yolk sac (YS), beginning with PrimE that sustains RBC production until placentation and is then succeeded by TransE fetal erythropoiesis that originates from YS-derived precursor that mature in the FL. Interestingly, although RBCs are needed throughout the entire life, including during embryonic development, where mutations affecting RBCs numbers are lethal, RPGs mutations leading to DBA strongly affect RBCs only postnatally, specifically the HSC-derived DefE wave. In fact, DBA onset is usually observed concomitantly with the switch to definitive erythropoiesis (1, 6, 7). Why only some developmental progenitors display sensitivity to DBA mutations is currently unknown.

Rationale and hypothesis

We therefore hypothesize there are fundamental biological differences associated with ribosome biogenesis between the waves of erythropoiesis that explain why only definitive erythropoiesis is impaired by ribosomal protein (RP) deficiency. Our aim is to use DBA as a "natural" model to understand how translation is controlled between pre- and post-natal hematopoietic cells, in particular in RBCs, and how defects in ribosome biogenesis, a universal and essential process for all cells, can lead to divergent outcomes during development.

Objectives and specific aims

1) To build a developmental model of RBC development using wt and DBA human iPSCs

2) To dissect gene expression dynamics of developing RBCs throughout human development with wt and DBA human iPSCs

3) To identify specific developmental vulnerabilities and validate DBA therapeutic targets in vitro and in vivo

Expected outcomes

The successful completion of this project will establish a robust and reproducible human model of developmental erythropoiesis. This will enable the mechanistic interrogation of gene expression control during hematopoietic development as well as the modeling genetic forms of anemia, leading to the identification of new therapeutic targets of these diseases.

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

Analysis of stemness, pluripotency and developmental stages; analysis of signaling pathways; cell and tissue cultures with human pluripotent stem cells; hematopoietic assays; erythroid cell characterization; flow cytometry and cell sorting; molecular biology; data analysis; system biology literacy; scientific communication.

References (max. 15)

1. Narla A, Ebert BL. Ribosomopathies: human disorders of ribosome dysfunction. Blood. 2010 Apr 22;115(16):3196-205. PubMed PMID: 20194897

2. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016 Aug;14(8):e1002533. PubMed PMID: 27541692

3. Palis J. Primitive and definitive erythropoiesis in mammals. Front Physiol. 2014;5:3. PubMed PMID: 24478716

4. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. Cell. 2008 Feb 22;132(4):631-44. PubMed PMID: 18295580

5. Palis J. Ontogeny of erythropoiesis. Current opinion in hematology. 2008 May;15(3):155-61. PubMed PMID: 18391778

6. Schechter AN. Hemoglobin research and the origins of molecular medicine. Blood. 2008 Nov 15;112(10):3927-38. PubMed PMID: 18988877

7. Da Costa L, Narla A, Mohandas N. An update on the pathogenesis and diagnosis of Diamond-Blackfan anemia. F1000Res. 2018;7. PubMed PMID: 30228860

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.*

Three types of PSC lines will be used throughout the project: a control PSC line for which hematopoietic differentiation has been successfully reported; iPSCs derived from DBA patients carrying RPS19 mutations; isogenic gene-corrected control lines. All PSC lines will be differentiated into hematopoietic progenitors of the different developmental waves.

The multipotency of PSC-derived hematopoietic progenitors will be assessed using multipotency assay (MPA) and lineage-specific differentiation protocols. These assays will quantify the ability of each cell line to generate myeloid, lymphoid, and erythroid lineages, with particular emphasis on identifying defects in erythroid differentiation that recapitulate the DBA phenotype.

Second, the self-renewal capacity will be interrogated through serial colony-forming unit (CFU) replating assays, comparing patient-derived and control cells.

Third, the in vivo engraftment potential of PSC-derived hematopoietic progenitors will be evaluated by transplanting them into NBSGW mice. After five months, engraftment will be assessed in peripheral blood, bone marrow, and spleen to determine long-term repopulation and multilineage contribution.

To provide proof of principle that this model is suitable for therapeutic screening, the student will test small molecules previously shown to rescue erythroid defects in DBA in vitro. Restoration of erythroid maturation in treated DBA-derived cells will validate the model's predictive value.

Finally, the student will perform molecular analysis such as RNA-seq, proteomic analyses and ribosome profiling and footprint on erythroid progenitors and mature erythroid cells derived from DBA and control lines to identify dysregulated pathways and novel druggable targets.

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).*

All the hPSC differentiation protocols and read-outs have been already developed and characterized in our lab. The hPSC lines at the basis of the proposal have been already established. A preliminary pipeline for the analysis of the bioinformatic results has been generated and will be refined once raw data will be acquired.

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

The PhD student will be the main actor of the project. Once deemed mature and once solid data have been collected, a master thesis student might be placed under her/his supervision to develop management and leadership skills.

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

The establishment of this in vitro iPSCs based developmental model of DBA will provide the scientific community with a novel and robust model of DBA. It is important to note that this model has additional applications in the therapeutic field beside the ones outlined in this project. As an example, it could serve as a platform for high-throughput screening of new molecules to treat the disease, given the highly scalable nature of this iPSCs based model.

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

_____