The undersigned

SURNAME Chimienti

FIRST NAME Raniero

born in Taranto Prov. TA on 18 / 08 / 1988

Unit: Beta Cell Biology

Residency/Postgraduate School: Ph.D in Experimental Medicine and Medical Biotechnologies

Email address: chimienti.raniero@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 _____
- ☐ 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. 0 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:

- ☒ **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 1506, 1579 _____

☐ 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* 31/03/2026

When applicable:

Group Leader Prof. /Dr. Lorenzo Piemonti

Signature _____ *Date* 31/03/2026

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: Raniero Chimienti

Title: Balancing immune evasion and antiviral defense in hypoimmunogenic stem cell-derived islet therapy

Curriculum: Molecular Medicine - Gene and Cell Therapy

Link to the personal page of the University or relevant hospital site website:

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

<u>Background/gap of knowledge</u>

Hypoimmunogenic stem cell-derived islets (SC-islets) are emerging as a transformative strategy for β-cell replacement in type 1 diabetes (T1D), with the goal of restoring endogenous insulin production without chronic immunosuppression[1-3]. Gene editing approaches targeting immune recognition pathways (e.g., MHC class I ablation, deletion of activating ligands such as CD155 and B7-H3[4-5], or overexpression of inhibitory molecules such as HLA-E[6] and CD47[7-8]) have shown promising results in preventing alloimmune rejection. However, these immune-evasive strategies are primarily validated under steady-state conditions and may compromise immune surveillance mechanisms under stress. In particular, viral infections represent a major unresolved challenge, as pancreatic islets are highly susceptible to clinically relevant viruses (e.g., Coxsackievirus B, HCMV, influenza viruses, SARS-CoV-2), which can directly damage β cells and reshape the local immune microenvironment[9-12]. Whether immune-evasive SC-islets retain the ability to be recognized and cleared upon infection remains unknown, raising concerns about potential viral persistence or graft dysfunction.

<u>Rationale and hypothesis</u>

We hypothesize that viral infections induce stress pathways and inflammatory signals in engineered SC-islets that can partially restore immune visibility, enabling innate immune cells

to recognize and eliminate infected cells. However, the extent of this reactivation may vary depending on the immune-evasion strategy employed. A critical balance must be achieved between maintaining graft protection from alloimmunity and preserving sufficient immune competence to prevent viral persistence and uncontrolled infection.

Objectives and specific aims

Aim 1: Define the susceptibility of engineered SC-islets to pancreatic-tropic viruses and characterize infection-induced molecular and functional changes, including stress ligand expression and cytokine release.

Aim 2: Investigate innate immune responses and immune cell cross-talk (NK cells, γδ T cells, iNKT cells, macrophages, neutrophils) against the infected SC-islets under different engineering strategies.

Aim 3: Validate *in vivo* whether viral infection can selectively trigger immune-mediated clearance of infected cells while preserving overall graft function and metabolic efficacy.

Expected outcomes

This project will provide a comprehensive understanding of how hypoimmunogenic SC-islets behave under infectious stress. It will identify vulnerabilities and strengths of different immune-evasion strategies, define mechanisms of infection-induced immune reactivation, and establish key principles for designing safe and durable stem cell-based therapies that balance immune tolerance with effective immunosurveillance.

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

The student will develop advanced skills in stem cell biology and differentiation into SC-islets, CRISPR/Cas9-mediated genome editing, virology (*in vitro* and *in vivo* infection models, virus titration), and immunology (NK cell biology and innate immune assays). Additional training will include multiparametric flow cytometry, library preparation for single-cell transcriptomics, *in vivo* humanized mouse models. The student will also acquire competencies in experimental design and statistical analysis, as well as data interpretation, presentation of results and their integration into manuscript preparation.

References (max. 15)

[1] Balboa, D., et al. Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. Nat Biotechnol 40, 1042–1055 (2022).

[2] Reichman, T.W. et al. Stem cell-derived, fully differentiated islets for type 1 diabetes. N Engl J Med 393, 858–868 (2025)

[3] Upadhye, K.S. et al. Advances in stem cell-derived beta-cell therapy: a new frontier in type 1 diabetes treatment. Cell Transplant 35, 9636897261417623 (2026).

[4] Chimienti, R. et al. Engineering of immune checkpoints B7-H3 and CD155 enhances immune compatibility of MHC-I-/- iPSCs for β cell replacement. Cell Reports 40, (2022).

[5] Chimienti, R. et al. Knock-out of the NK activating ligands in MHC-I-null human stem cell-derived β cells avoids chronic allogeneic rejection and reverts hyperglycemia in diabetic humanized mice. Cytotherapy 26, S15 (2024).

[6] Gornalusse, G. G. et al. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. Nat Biotechnol 35, 765–772 (2017).

[7] Deuse, T. et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nat Biotechnol 37, 252–258 (2019).

[8] Hu, X. et al. Hypoimmune induced pluripotent stem cells survive long term in fully immunocompetent, allogeneic rhesus macaques. Nat Biotechnol https://doi.org/10.1038/s41587-023-01784-x (2023) doi:10.1038/s41587-023-01784-x.

[9] Vecchio, F. et al. Coxsackievirus infection induces direct pancreatic β cell killing but poor antiviral CD8+ T cell responses. Sci Adv 10, eadl1122 (2024).

[10] Smelt, M. J. et al. Susceptibility of human pancreatic β cells for cytomegalovirus infection and the effects on cellular immunogenicity. Pancreas 41, 39–49 (2012).

[11] Capua, I. et al. Influenza A viruses grow in human pancreatic cells and cause pancreatitis and diabetes in an animal model. J Virol 87, 597–610 (2013).

[12] Deng, W. et al. Infection with SARS-CoV-2 can cause pancreatic impairment. Signal Transduct Target Ther 9, 98 (2024).

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 is a translational experimental study integrating *in vitro* and *in vivo* approaches to investigate immunosurveillance of engineered SC-islets under viral stress.

A panel of human iPSC lines will be used, including wild-type, $B2M^{-/-}$, T-KO ($B2M^{-/-}/CD155^{-/-}/B7\text{-}H3^{-/-}$), $B2M^{-/-}/HLA\text{-}E^{+}$, and $B2M^{-/-}/CIITA^{-/-}/CD47^{+}$ lines. They will be differentiated into functional SC-islets and exposed to a panel of clinically relevant viruses with pancreatic tropism or immunomodulatory potential, including Coxsackievirus B, influenza A, HCMV, and EBV.

Aim 1: Viral susceptibility will be assessed by infecting SC-islets at different multiplicities of infection. Viral replication and tropism will be evaluated by flow cytometry and immunostaining of viral antigens, while cell viability will be assessed using Annexin V/PI assays. Functional impairment will be quantified through glucose-stimulated insulin secretion (GSIS). Infection-induced cytokine and chemokine production will be measured using multiplex assays. Molecular responses will be characterized by single-cell RNA sequencing and spatial transcriptomics (RNAscope), enabling identification of cell-type-specific susceptibility and immune remodelling.

Aim 2: Innate immune responses will be investigated using co-culture systems with primary human NK cells, γδ T cells, iNKT cells, macrophages, and neutrophils isolated from healthy donors. Both direct infection models and indirect priming approaches will be employed to recapitulate physiological immune activation. Functional assays will include cytotoxicity, degranulation (CD107a), cytokine secretion, and migration assays using transwell systems. Donor variability will be addressed through stratification based on immune phenotype and statistical mixed-effects models.

Aim 3: *In vivo* validation will be performed using humanized hIL-15 NOG mice transplanted with luciferase-expressing SC-islets. Mice will be infected with selected viruses to assess graft survival, immune infiltration, and metabolic function (C-peptide levels, imaging). This will allow evaluation of the balance between immune-mediated clearance of infected cells and preservation of graft function.

This is a non-pharmacological experimental study that does not involve investigational drugs. All experiments will be conducted using established research models and GLP-compliant laboratory equipment at the Diabetes Research Institute (San Raffaele Scientific Institute) and in collaboration with institutional core facilities.

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).*

Human iPSC-derived SC-islets, Cas9-based editing protocols using in-house electroporation systems, *in vitro* viral infection models, and *in vivo* transplantation/humanization models based on immunodeficient hIL-15 NOG, EXL-NOG and MHC-I/II DKO NSG mice are available. The project includes well-established methods for SC-islet differentiation, viral challenge, multiparametric flow cytometry, GSIS, imaging, and transcriptomic analyses. Innate immune cells (NK, γδ T, iNKT, macrophages) will be isolated and expanded using robust protocols already established and published by our group.

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

The PhD student will take an active role in both the design and execution of the project, with progressive scientific independence. He/She will generate and characterize engineered SC-islets, perform viral infection assays, and investigate innate immune responses using co-culture systems. The student will analyze and integrate functional and transcriptomic data, contribute to *in vivo* studies in humanized mice models, and be directly involved in data interpretation, manuscript writing, and dissemination of results.

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

This project addresses a critical and largely unexplored safety gap in cell therapies for T1D: the behaviour of hypoimmunogenic grafts under viral challenge. By moving beyond the conventional "rejection vs no rejection" paradigm, it will define whether engineered SC-islets can support selective clearance of infected cells while preserving graft function. The comparative analysis of gene editing strategies and the integration of viral responses will generate design rules for next-generation cell therapy that is both immune-evasive and infection-resilient, accelerating safe clinical translation.

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 _____