The undersigned

SURNAME RUGGIERO

FIRST NAME ELIANA born in BENEVENTO Prov. BENEVENTO on 29/01/1983

Unit: *EXPERIMENTAL HEMATOLOGY UNIT*

Residency/Postgraduate *School*[1]:

_____

Email address: *ruggiero.eliana@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 Ospedale San Raffaele

☐ 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

X I HAVE OBTAINED the approval of the responsible Institutional Animal Care and Use Committee-IACUC number 1478

☐ 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 Elena Ruggiero

Date March 30th, 2026

When applicable:

Group Leader Prof. Chiara Bonini

Signature 

Date March 30th, 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:	Eliana Ruggiero
Title:	Harnessing the immunosuppressive leukemic microenvironment to engineer T cells with enhanced anti-tumor functionality
Curriculum:	Gene and cell therapy
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/division-of-immunology-transplantation-and-infectious-diseases/experimental-hematology/eliana-ruggiero.html

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

Background/gap of knowledge

Despite major advances in understanding disease biology, the treatment of acute myeloid leukemia (AML) remains challenging. Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative option, highlighting the disease's sensitivity to immunotherapy; however, most patients eventually relapse. While chimeric antigen receptor (CAR) T cells have achieved remarkable success in treating B cell malignancies, their efficacy in AML has been limited. This is largely due to disease heterogeneity, the lack of optimal surface antigens, and insufficient T cell persistence[1]. A promising strategy to overcome these limitations is the engineering of T cells with novel T cell receptors (TCRs), enabling recognition of a broader range of tumor antigens and enhancing T cell persistence[2]. Nevertheless, the altered bone marrow microenvironment in AML adds further complexity and remains a significant barrier to effective immune-based therapies.

Rationale and hypothesis

Given the critical role of donor-derived T cells after HSCT, we aim to leverage the key pitfalls of this setting, where T cells can be effective yet relapse still occurs, to uncover mechanisms of immune evasion. We hypothesize that defining these failure points will guide the design of improved engineered T cell therapies, ultimately enhancing outcomes for patients with AML.

Objectives and specific aims

Our overarching goal is to develop novel living drugs in the form of engineered T lymphocytes capable of sensing immunosuppressive signals within the leukemic

microenvironment and responding with enhanced cytotoxic activity. To achieve this, we will integrate advanced methodologies, including TCR repertoire sequencing[3], single cell multiomics, 3D culture systems, high throughput phenotypic T cell analysis, genome engineering, synthetic biology, and murine AML models. Specifically, we aim to: 1) identify and characterize novel anti-tumor TCRs, with thorough evaluation of their safety and efficacy[4]; 2) apply genome engineering approaches to generate robust and functional anti-tumor T cell populations[5]; 3) elucidate key pathways and mechanisms underlying cancer–T cell co-evolution and immune evasion. Rather than solely attempting to prevent immune escape, our approach seeks to reprogram leukemic cells into functional allies that enhance T cell activity.

Expected outcomes

This project is expected to yield innovative "living drug" therapies that can be evaluated as standalone or combination treatments for AML. Importantly, the immunosuppressive mechanisms identified within the bone marrow are shared across multiple cancer types. As such, our findings may provide a foundation for the development of durable, broadly applicable immunotherapies for a wide range of malignancies.

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

The student will receive hands-on training in cutting-edge cellular and molecular biology techniques, including single-cell omics, T cell profiling, T cell engineering, immunological assays, flow cytometry and in vivo models.

Beyond technical expertise, the student will be encouraged to cultivate a critical and analytical approach to scientific literature, while developing strong skills in experimental design and data interpretation. Active participation in scientific meetings and seminars will strengthen communication abilities, broaden perspective, and foster professional growth.

References (max. 15)

1. Atilla E. and Benabdellah K. The Black Hole: CAR T Cell Therapy in AML Cancers (Basel). 2023 May 11;15(10):2713. doi: 10.3390/cancers15102713.

2. Harris DT, Kranz DM. Adoptive T Cell Therapies: A Comparison of T Cell Receptors and Chimeric Antigen Receptors. Trends Pharmacol Sci. 2016;37:220–30. doi: 10.1016/j.tips.2015.11.004

3. Ruggiero, E. et al. High-resolution analysis of the human T-cell receptor repertoire. Nat. Commun. (2015). doi: 10.1038/ncomms9081.

4. Ruggiero, E. et al. CRISPR-based gene disruption and integration of high-avidity, WT1-specific T cell receptors improve antitumor T cell function. Science Translational Medicine (2022). doi: 10.1126/scitranslmed.abg8027

5. Provasi, E. et al. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat. Med. (2012). doi: 10.1038/nm.2700

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.*

Anti-tumor T cell receptors will be isolated from healthy donor peripheral blood mononuclear cells (PBMCs) using an optimized version of our previously established pipeline[4]. The newly identified receptors will be reconstructed, cloned into lentiviral vectors (LVs), and used to redirect T cell specificity by CRISPR/Cas9-mediated disruption of the endogenous TCR repertoire. Functional characterization of the engineered T cells will include target cell killing (using cell lines and primary leukemic blasts), T cell activation, secretion of proinflammatory cytokines, expression of degranulation markers, and assessment of TCR T cell avidity using the z-Movi analyzer. Additionally, T cell synthetic circuits will be constructed, cloned into LVs and employed for T cell engineering. The safety of engineered T cells will be assessed by evaluating both on target/off tumor and off target cross-reactivities. To examine on target/off tumor effects, T cells will be co-cultured with a panel of cells expressing the target antigen. If the target is present on hematopoietic cells, studies will progress to in vivo testing using a humanized mouse model. Off target cross-reactivity will be investigated via peptide scanning to identify potential human proteins recognized by the engineered T cells.

In the first year the focus will be on identifying and functionally characterizing novel anti-tumor T cell receptors, as well as designing and validating the therapeutic circuits.

In the second year the PhD student will establish *in vitro* assays that mimic immunosuppression within the tumor microenvironment and evaluate the effects of the therapeutic circuits in modulating T cell functionality. This will involve both standard T cell co-cultures with target cells and more advanced 3D cultures.

The third year will be dedicated to *in vivo* validation of the engineered T cells, with an emphasis on the study of T cell/tumor co-evolution using single-cell multiomics approaches.

For bioinformatic data, the student will collaborate closely with a bioinformatician from Ruggiero's team, gaining insight into the analysis process and interpretation of results.

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).*

The following models and procedures are already established in the lab:

- TCR hunting pipeline

- TCR gene editing procedure using CRISPR/Cas9

- design and validation of synthetic circuits

- comprehensive immune and cancer cell profiling through high-dimensional flow cytometry and single-cell multiomics

- well-established mouse models of AML for in vivo evaluation of safety and efficacy of the engineered T cells

Importantly, the institutional biobank Biological Resource Center (CRB-OSR) houses a large collection of patient-derived leukemic blasts, accompanied by essential clinical data.

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

The PhD student will drive the implementation of the experimental plan, encompassing the generation and functional validation of genetically engineered T lymphocytes in both in vitro and in vivo settings. The student will establish and optimize assays to assess the anti-tumor activity and the safety of these newly engineered "smart" T cells. In addition, the PhD student will be responsible for rigorous data analysis and interpretation and will actively disseminate findings through presentations at internal meetings and international scientific conferences.

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

We expect that our findings will provide key hints for generating novel targeted and combined therapeutic strategies able to provide tumor immunosurveillance, patrol for tumor recurrence and overcome the immunosuppression in the bone marrow microenvironment. In addition, if successful, this approach could be applied to different tumor entities, thus enlarging the therapeutic window of T cell-based therapies in the clinical arena.

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 _____