The undersigned

SURNAME Bonini

FIRST NAME Maria Chiara born in *Padova* Prov. PD on 21 / 08 / 1969

Unit: *Experimental Hematoloy*

Residency/Postgraduate School[1]:

Email address: bonini.chiara@hsr.it

Role:

X 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:

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.

I would like to present a project:

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

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 *n° 594/2024-PR*

☐ 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 LIMET V7_Emendamento n.6 (del 14/01/2026)

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.

☒ 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 C. Bovini *Date 30/3/2026*

When applicable:

Group Leader Prof. /Dr. _____

Signature _____ *Date* _____

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:	Chiara Bonini
Title:	Dual CAR and TCR gene editing for cancer treatment
Curriculum:	Cell and gene 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/chiara-bonini.html

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

Background/gap of knowledge

By acting as living drugs, T cell immunotherapies are revolutionizing cancer treatment. Gene transfer technologies endow T cells with the ability to specifically recognize a selected tumor antigen through a chimeric antigen receptor (CAR) or a T cell receptor (TCR). While CAR T cells induce exceptional clinical results against selected hematological malignancies, their efficacy against solid tumors is still limited by several hurdles including their property to recognize only extracellular molecules and their poor in vivo persistence. On the other hand, TCRs can target with very high sensitivity a broad range of extracellular and intracellular antigens, and TCR triggering better promotes T cell persistence. However, HLA restriction limits the feasibility of TCR-based therapeutics. Finally, both CARs and TCR-T cell efficacies are limited by T-cell exhaustion signals, abundant in the tumor microenvironment

Rationale and hypothesis

CAR and TCR T cells have historically been developed as two independent lines of therapy; our hypothesis is that a combined approach could empower T-cell based immunotherapy against solid tumors.

Objectives and specific aims

We aim at comparing and combining the relative strengths of CARs and TCRs, while improving the resilience and persistence abilities of engineered T cells, to ultimately deliver innovative, safe and effective advanced medicinal products (ATMPs) for cancer treatment. We will focus on

pancreatic and ovarian cancers, two tumor entities sharing silent progression and late diagnosis and overexpressing a pool of common tumor-associated antigens, such as the Human Epidermal Growth Factor Receptor 2 (HER2; Potenza, Balestrieri et al. Gut 2023) and Mesothelin (MSLN; Spiga, Potenza et al. Nat Comm 2026). We plan to perform a comparative analysis of engineered CARs and TCRs-T cells through the study of their activation kinetics and anti-tumor responses and test their combination to maximize therapeutic efficacy. To further enhance the functionality of dual-engineered CAR/TCR T cells, we will genetically modify T lymphocytes to address two main limitations to T cell therapy efficacy: functional exhaustion and poor persistence. Functional assays will be performed using a set of tumor organoids and ascites cultures, already available in the hosting institution, as target cells. This availability of a large biobank of tumor organoids and ascites cultures well characterized and with variable levels and numbers of antigen expressed, will allow to assess the role of antigens expression and other immunological variables on CAR and TCR redirected T cells, thus permitting a fine tuning of the cellular products and of the most effective and safe combinations. Safety will be carefully monitored in vitro and in vivo by the assessment of TCR and CAR specificity, risk of cytokine release syndrome and ICANS.

Expected outcomes

The combination of CAR- and TCR-engineered T cells harbors the promise to produce safe, effective, profound and prolonged anti-tumor responses in diseases that still represent major unmet clinical needs.

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

Hard skills: Genome editing and gene transfer with Crispr-Cas9 technology, base editing, lentiviral vectors; functional testing *in vitro* and in complex xenograft mouse models. Advanced transcriptomics and proteomics analysis. Flow cytometry.

Soft skills: Critical analysis of scientific literature; Design and management of scientific projects; Data presentation and discussion at internal lab meetings; Scientific presentations at Institutional progress reports and at international conferences; Thesis and manuscript writing.

References (max. 15)

1. Potenza A, Balestrieri C, et al., Revealing and harnessing CD39 for the treatment of colorectal cancer and liver metastases by engineered T cells. Gut. 2023 Oct;72(10):1887-1903. doi: 10.1136/gutjnl-2022-328042

2. Spiga M, Potenza A, et al. TIGIT disruption rescues the antitumor activity of low avidity TCR-engineered T cells by increasing TCR signal strength. Nat Commun. 2026 Jan 8;17(1):568. doi: 10.1038/s41467-025-67263-w.

3. Kondo et al. Engineering TCR-controlled fuzzy logic into CAR T cells enhances therapeutic specificity. Cell. 2025 May 1;188(9):2372-2389.e35. doi: 10.1016/j.cell.2025.03.017.

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 aims to systematically compare and integrate the strengths of CAR- and TCR-engineered T cells, to develop advanced therapeutic strategies for pancreatic and ovarian cancers. These malignancies share late diagnosis and poor prognosis and commonly overexpress tumor-associated antigens such as HER2 and Mesothelin, which will be targeted in this study. In the first part of the project, primary human T cells will be engineered to express either CARs or TCRs specific for selected antigens. A parallel set of dual-engineered T cells co-expressing both CAR and TCR constructs will also be generated to assess potential synergistic effects. These cellular products will be characterized for TCR/CAR expression, viability, and functionality. Comparative analyses will be performed to evaluate activation kinetics, proliferation, cytokine production, and cytotoxic activity upon antigen recognition. This will allow the identification of functional differences and complementary properties between CAR- and TCR-T cells. Afterwards, we will investigate strategies to enhance T cell resilience and persistence. Engineered T cells will be further modified to reduce functional exhaustion and improve long-term activity. Their performance will be evaluated under conditions mimicking the tumor microenvironment, including chronic antigen exposure. This project includes the use of a large and well-characterized biobank of patient-derived tumor organoids and ascites cultures. These tumor models will enable functional testing of engineered T cells in clinically relevant settings. By correlating T cell responses with antigen density and tumor-specific features, we will define the parameters that influence efficacy, allowing the optimization of the most effective CAR, TCR, or combined approach. Finally, safety will be rigorously assessed through *in vitro* and *in vivo* studies, aimed at evaluating the specificity of engineered receptors, as well as potential adverse effects such as excessive cytokine release and neurotoxicity. These analyses will ensure that the most promising cellular products combine high anti-tumor activity with a favourable safety profile.

Overall, this experimental plan integrates comparative and combinatorial approaches with functional optimization and safety evaluation to guide the development of next-generation engineered T cell therapies for solid tumors.

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).*

Primary human T cell isolation and stimulation, gene editing, lentiviral transduction multidimensional flow cytometry; functional assays (cytotoxicity, proliferation, cytokines); single-cell RNA sequencing; 3D cultures (patient-derived organoids and ascites); *in vivo* orthotopic and humanized mice models.

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

The PhD student will design and perform experiments and set up novel methodologies, analyze and interpret data. Also, the PhD student will prepare scientific reports and present results at meetings and national and international conferences, gaining experience in scientific communication and project management and fostering collaborations.

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

This project will advance the field of cancer immunotherapy by defining specific features of CAR and TCR-engineered T cells and providing novel combination that will improve the efficacy of adoptive cell therapy for pancreatic and ovarian cancers. The use of clinically relevant models, such as patient-derived organoids and ascites cultures, will support translation into safer and more effective advanced therapy medicinal products.

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.

n.a.

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 _____