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;

X 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. Bonini *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:	Development of superpowered engineered T cells 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

Despite clinical success of chimeric antigen receptor (CAR)-T cells for selected hematological malignancies, several hurdles, such as tumor heterogeneity, impaired T cell function and trafficking, and the immunosuppressive signals from the tumor microenvironment limit the full exploitation of genetically engineered T cells to treat solid tumors.

Rationale and hypothesis

Here, we hypothesize that multiple genome editing into T cells could allow to overcome these hurdles and boost T cell functions against gastrointestinal (GI) tumors.

Objectives and specific aims

We shall redirect T cell specificity through our TCR gene editing protocol (Provasi, Genovese et al., Nat Med 2012; Ruggiero Science Transl. Med. 2022) and CAR gene transfer protocol (Casucci Blood 2013; Greco STM 2022). We will select antigens highly expressed in GI tumors and exploit TCR-gene editing coupled with lentiviral transduction of a TCR and a CAR construct. We shall investigate the metabolic reprogramming of CAR and TCR-T cells**.** Reprogramming of energetic metabolism is a critical feature of T cells responses in cancer. Glycolysis and mitochondrial functions are finely tuned during lymphocyte activation. Accordingly, growing evidence indicates that immunotherapy efficacy and, in turn, T-cell exhaustion, are highly influenced by the metabolic requirements of immune cells. We already reported that it is possible to knock-down CD39 in TCR edited (TCR_{ED}) T cells and that Oxygen Consumption Rate and ExtraCellular Acidification Rate are reduced in activated TCR_{ED} $CD39_{KO}$ redirected cells, and that these cells are more potent anti-tumor effectors than CD39 competent counterparts (Potenza, Balestrieri et

al., GUT 2023). Here, we will increase the fitness of engineered CAR and TCR T cell products, by CD39/CD73/A2AR gene manipulation. Furthermore, we will investigate the metabolic mechanism enhancing T cell activity under constant antigen stimulation, nutrients deprivation and suppressive conditions in the TME, and perform single-cell RNA sequencing, to reveal novel modulators to harness for boosting Adoptive Cellular Therapy (ACT). Besides metabolic reprogramming, in the TME of GI tumors both stromal and tumor cells overexpress multiple immune checkpoint ligands, which bind to their cognate receptor on T cell surface, activating a suppressive cascade. We will select the most relevant inhibitory axis in GI tumors and generate ATMPs with tailored modifications, with the aim to improve their efficacy in the TME. Moreover, to enhance the persistence of these super-powered ATMPs, we will genetically engineer T cells to harbor not only the anti-tumor receptor, but also the membrane-bound IL15, which mimics physiological trans-presentation of the cytokine and enhances persistence. Engineered cells will be tested *in vitro* and *in vivo* against cell lines and patients' derived organoids, already available in the lab.

Expected outcomes

Results of this study will lead to the implementation of innovative therapeutic cellular products for the treatment of patients affected by aggressive cancer.

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, AAV6 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. Cianciotti BC, et al., TIM-3, LAG-3, or 2B4 gene disruptions increase the anti-tumor response of engineered T cells. Front Immunol. 2024 Feb 29;15:1315283. doi: 10.3389/fimmu.2024.1315283.

3. 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.

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 enhance the efficacy of adoptive cellular therapy (ACT) for gastrointestinal (GI) tumors by combining T cell receptor (TCR) gene editing and chimeric antigen receptor (CAR) gene transfer with targeted metabolic reprogramming. The first objective is to generate engineered T cell products with improved anti-tumor activity. Primary human T cells will be isolated and subjected to TCR editing to disrupt endogenous TCR expression, followed by transduction with selected TCR and CAR constructs. In parallel, we will introduce genetic modifications targeting key mediators of the adenosinergic pathway, including CD39, CD73, and A2AR, to enhance T cell fitness. The second objective focuses on metabolic reprogramming. Engineered T cells will be analyzed for metabolic activity using measurements of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) under basal and activated conditions. Functional assays will be performed under conditions mimicking the tumor microenvironment (TME), including chronic antigen stimulation, nutrient deprivation, and immunosuppressive signals. These experiments will allow us to identify metabolic adaptations associated with improved T cell persistence and cytotoxicity. To further dissect the molecular mechanisms underlying enhanced functionality, we will perform single-cell RNA sequencing on *ex-vivo* retrieved engineered T cells exposed to tumor organoids in mice. This analysis will enable the identification of transcriptional programs and novel metabolic regulators that can be targeted to optimize ACT. In addition to metabolic interventions, we will address immune suppression mediated by checkpoint pathways. The most relevant inhibitory receptor-ligand axes in GI tumors will be selected, and T cells will be genetically modified to resist these signals. Furthermore, to improve *in vivo* persistence, engineered T cells will be modified to express membrane-bound IL-15.

The third objective of the project will be to test the anti-tumor efficacy of these next-generation engineered T cells *in vitro* and *in vivo*. Functional assays will include cytotoxicity, proliferation, and cytokines production against tumor cell lines and patient-derived organoids. In vivo studies will assess tumor control, persistence, and safety. Overall, this experimental approach combines antigen targeting, metabolic engineering, and resistance to immunosuppression to develop more potent and durable T cell therapies for GI cancers.

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; metabolic profiling; multidimensional flow cytometry; functional assays (cytotoxicity, proliferation, cytokines); single-cell RNA sequencing; 3D cultures (patient-derived organoids); *in vivo* orthotopic 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 is expected to advance the development of more effective and durable T cell therapies for GI tumors by integrating antigen targeting, metabolic reprogramming, resistance

to immunosuppression and improved persistence. This work may identify novel strategies to empower T cells against GI tumors, contributing to the generation of innovative medicinal product and supporting the translation of next-generation adoptive cellular therapies into clinical practice.

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

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