PROJECT

Supervisor:	Giulia Casorati
Title:	**Optimizing CD1c-Redirected Adoptive Cell Therapy for Acute Leukemia via Cooperative TCR/CAR and CCR Targeting**
Curriculum:	Basic and Applied Immunology and Oncology
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/immunology-transplantation-and-infectious-diseases/experimental-immunology/giulia-casorati.html

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

Background/gap of knowledge

Adoptive T cell immunotherapy, particularly through genetically engineered T cells expressing chimeric antigen receptors (CARs) or T cell receptors (TCRs), represents a transformative approach for the treatment of hematological malignancies, including acute leukemias and non-Hodgkin lymphomas (NHL). While these therapies have demonstrated remarkable clinical efficacy, their broader application is constrained by the limited availability of tumor-specific antigens that are absent from healthy tissues and independent of MHC restriction. These factors are critical for the development of universal, allogeneic “off-the-shelf” therapies, which could greatly expand patient access.

Alternative immunotherapeutic strategies that exploit non-polymorphic antigen-presenting molecules offer a promising solution. The monomorphic CD1 molecules, which present lipid antigens, allows MHC-independent recognition. Group 1 CD1 molecules, particularly CD1c, are selectively expressed on antigen-presenting cells and frequently in hematological malignancies, including AML, B- and T-ALL, and multiple NHL subtypes. We have identified CD1c-restricted, self-reactive T cell clones that recognize leukemia-associated lipid antigens enriched in malignant cells but minimally present in healthy tissues. Building on this, a lead TCR candidate, DN4.99-TCR, has demonstrated potent anti-leukemic activity in vitro and in xenograft models while sparing normal CD1c-expressing cells.

However, in vivo studies have highlighted mechanisms of tumor escape, including downregulation of CD1c and functional exhaustion of engineered T cells. These limitations underscore the need for strategies to improve T cell persistence and enhance anti-tumor activity. Integration of chimeric costimulatory receptors (CCRs), which provide sustained activation signals, and the development of CD1c-targeting CAR constructs are promising

approaches to overcome these challenges. Additionally, we have generated a transgenic mouse model expressing human CD1c to evaluate efficacy and on-target/off-tumor effects in an immunocompetent system.

Rationale and hypothesis

CD1c-restricted T cells enable donor-unrestricted, MHC-independent tumor targeting. Nevertheless, tumor immune evasion and limited T cell persistence reduce therapeutic efficacy. We hypothesize that:

1. CCR co-expression will enhance the functional capacity, persistence, and durability of DN4.99-TCR/CD1c-CAR engineered T cells.
2. Comparative evaluation of DN4.99-TCR and CD1c-CAR T cells will identify the most effective strategy under varying tumor burden conditions.
3. Assessment in an immunocompetent, human CD1c-expressing model will establish safety and efficacy, guiding clinical translation.

Objectives and specific aims

1. **Optimize DN4.99-TCR functionality:** Incorporate CCRs into DN4.99-TCR and CD1c-CAR T cells and evaluate impact on activation, persistence, and resistance to exhaustion.
2. **Compare CD1c-targeting strategies:** Perform head-to-head in vitro and in vivo comparisons with DN4.99-TCR and CD1c-CAR T cells under different tumor burden conditions.
3. **Evaluate safety and efficacy in vivo:** Utilize a human CD1c transgenic mouse model to assess therapeutic activity, persistence, and on-target/off-tumor effects in an immunocompetent system.

Expected outcomes

This project is expected to demonstrate that CCR co-expression enhances the durability and anti-tumor activity of CD1c-restricted TCR or anti CD1c-CAR-engineered T cells.

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

The student will acquire different skills including molecular biology protocols to generate new constructs and lentiviral vectors, production of lentivirus and transduction of primary lymphocytes. Purification, transduction and expansion of primary human lymphocytes. Tissue culture, immunological cellular assays, animal handling and modelling, and high dimensional flow cytometry.

References (max. 15)

1. Lepore M, de Lalla C, Gundimeda SR, Gsellinger H, Consonni M, Garavaglia C*, et al.* A novel self-lipid antigen targets human T cells against CD1c(+) leukemias. J Exp Med 2014;211:1363-77. doi: 10.1084/jem.20140410

2. Consonni M, Garavaglia C, Grilli A, de Lalla C, Mancino A, Mori L*, et al.* Human T cells engineered with a leukemia lipid-specific TCR enables donor-unrestricted recognition of CD1c-expressing leukemia. Nat Commun 2021;12:4844. doi: 10.1038/s41467-021-25223-0