European School of Molecular Medicine

RESEARCH ACTIVITY SHEET

2025 PhD selections

YOUR DETAILS

* Name & Surname: Alice Giustacchini

* Affiliation HT

PHD PROJECT DETAILS

* Title of the proposed project

Engineering Dual-Target CAR T-Cells for Pediatric AML

* Short description of the project (up to 300 words)

Our laboratory aims to tackle the challenge of therapeutic resistance in pediatric acute myeloid leukemia (pAML) by advancing next-generation immunotherapies. Our experimental focus is twofold:

1) **CAR T-cell development:**
   We plan to design and validate CAR T-cell strategies tailored for the molecular and phenotypic heterogeneity of pAML. Recognising that single-target CARs often fail due to subclonal antigen loss, we systematically identified combinations of surface antigens for dual or multi-targeting. Using state-of-the-art single-cell multi-omics, CITE-seq and long-read sequencing, we define the expression patterns of candidate targets across diverse AML subtypes, with special emphasis on leukemic stem cell populations that sustain relapse. These data will inform rational CAR construct design, including co-transduction and sequential targeting strategies. We also examine the fitness, activation/exhaustion states and persistence of engineered CAR T-cells, leveraging preclinical in vitro co-culture assays and cytotoxicity readouts.
2) **Modelling CAR-tumor-immune interactions in organoid systems:**
   To better predict therapeutic responses and resistance, we are developing iPSCs-derived bone marrow organoid models that recapitulate key features of the leukemia niche and that can be engrafted with primary pAML samples. These 3D co-culture systems incorporate primary leukemia cells, allogeneic or autologous immune cells, and supportive stromal elements, allowing us to study the spatial and functional dynamics of CAR T-cell function, synapse formation, killing efficiency, and the emergence of antigen-negative escape. Super-resolution imaging and molecular tension probes are applied to dissect the biophysical and molecular mechanisms of immune synapse formation under different CAR designs.

   Together, these experimental approaches aim to establish a robust translational pipeline from antigen discovery to functional validation in relevant ex vivo models, ultimately informing the development of more durable, personalized immunotherapies for high-risk pAML patients.

* Indicate the main research area for the project described above Cancer Biology

If needed indicate a second research area for the project described above Molecular Theraphy

* Provide up to 3 key words for project:

Leukemia, CAR T, organoids

YOUR LABORATORY ACTIVITIES DETAILS

* Main topic/s of the lab

Leukemia stem cells, CAR T, single cell multi-omics

* Short description of the lab activity (up to 500 words)

Immunotherapy development for pediatric AML informed by single cell-multiomics, with a focus on innovative multi-target CAR T-cell engineering, functional modelling in patient-derived organoid systems, and biological insights into leukemia stem cells, target identification, mechanisms of transformation, and molecular kinetics in CAR T-cell therapy.

* Recent bibliography (max 5 references)

1. Michelozzi IM, Gomez-Castaneda E, Pohle RVC, *et al.*, Giustacchini A. *Activation priming and cytokine polyfunctionality modulate the enhanced functionality of low-affinity CD19 CAR T cells.* **Blood Adv**. 2023 May; 7:1725.
2. Michelozzi IM, Sufi J, Adejumo TA, Amrolia PJ, Tape CJ, Giustacchini A. *High-dimensional functional phenotyping of preclinical human CAR T cells using mass cytometry.* **STAR Protoc**. 2022 Mar; 3:101174.
3. Michelozzi IM, Kirtsios E, Giustacchini A. *Driving CAR T Stem Cell Targeting in Acute Myeloid Leukemia: The Roads to Success.* **Cancers**. 2021 Jun; 13.
4. Giustacchini A, Thongjuea S, Barkas N, *et al. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia.* **Nat Med**. 2017 Jun; 23:692.

* Group composition: total members, and roles distribution (PhD, postdoc, technician, etc.)

The lab currently includes 2 PhD students, 1 senior technician, and 1 postdoctoral researcher, with 2 additional postdocs and 1 PhD student joining soon. The team operates as a hybrid research environment, balanced approximately 50% experimental and 50% computational.

Institutional page link

https://humantechnopole.it/en/research-groups/giustacchini-group/

Lab website link, if any

Social media links, if any

If you prepare a video to promote your lab/project, please include the link below