BIOGRAPHICAL SKETCH

NAME: Gabriele Casirati

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Università degli Studi di Milano, Italy	Doctor of medicine (MD), cum laude	07/2014	Medicine and Surgery
Università degli Studi di Milano, Italy	MD licensing exam	02/2015	Medicine and Surgery
Università Vita-Salute San Raffaele, Milan, Italy	Hematology specialty, cum laude	11/2019	Hematology and Bone Marrow Transplantation
Università Milano-Bicocca, Milan, Italy	Doctor of Philosophy (PhD)	04/2023	Translational and Molecular Medicine

A. Personal Statement

As a medical doctor and hematologist, my research interest has always been focused on hematological cancers and in particular acute myeloid leukemia (AML) which, despite our growing understanding of leukemogenesis and disease recurrence, continues to leave both adult and pediatric patients in tremendous need for novel and effective treatment options. Beyond my 5-year clinical experience in the treatment of hematological malignancies and hematopoietic stem cell transplantation, I dedicated part of my hematology residency to translational research in Dr. Bernhard Gentner's lab at San Raffaele Telethon Institute for Gene Therapy (SR-Tiget, Milan). In particular, I studied the role of miR-126 in AML leukemia stem cells (LSCs) and explored intra-tumoral heterogeneity at the single cell level by scRNAseq of primary AML samples (Naldini MM, Casirati G et al, *Nature Comm*). In light of our findings supporting the persistence of quiescent LSCs after chemotherapy and the remarkable advancements made possible by adoptive cancer immunotherapies over the past decade, extending their application to AML may provide significant benefits for high-risk patients that would otherwise fare poorly with conventional treatment options. On these grounds, upon graduation from the hematology specialty, I joined Dr. Pietro Genovese's lab at Dana Farber Cancer Institute - Boston Children's Hospital to tackle one of the major obstacles to the application of targeted immunotherapies for AML: the lack of actionable leukemia-specific antigens and the ensuing on-target/off-tumor toxicities. To this end, I developed the epitope-editing approach which, by exploiting precise base editing of donor HSPCs, can endow healthy hematopoietic cells with selective resistance to mAb or CAR-T cells, while still allowing eradication of AML cells (*Nature*).

<u>Ongoing projects include</u>:

(1) Epitope-edited HSPCs to enable immune-based in vivo selection and non-genotoxic conditioning for hematopoietic stem cell transplantation and gene therapy.
(2) Multi-specific CAR immunotherapies for AML, including novel, previously unexplored targets.
(3) Explore the mechanisms of AML escape under selective immunotherapeutic pressure.
(4) Development of novel genome engineering tools characterized by enhanced precision and minimal off-targets, including Base Editors with single-base selectivity.
(5) Improvement of T-cell on-target efficacy by endowing them with new killing mechanisms.

B. Positions and Honors

2023 New Investigator Award, American Society for Transplantation and Cellular Therapy
2023 New Investigator Award, The Pediatric Transplantation and Cellular Therapy Consortium
2020 – Research fellow – Dana-Farber Cancer Institute/Boston Children's Hospital, Boston MA

Advanced Genome Engineering Unit (Prof. Pietro Genovese)
2019 – 2020 Hematology consultant - IRCCS San Raffaele Milano – Milan
2015 – 2019 Hematology residency - Università Vita Salute San Raffaele, Milan
Dissertation: Deconvolution of AML cellular heterogeneity through single cell RNA sequencing
2015 Basic Life Support instructor
2008 – 2014 MD student - Università degli Studi di Milano – Milan
Dissertation: Skeletal and Bone Marrow Involvement in type 1 Gaucher disease

Other Experience and Professional Memberships
2023 – Frontiers in Genome Editing – Guest Editor
2023 – American Society of Hematology (ASH), Associate Member
2023 – Society for Immunotherapy of Cancer (SITC), Associate Member
2023 – International Society for Stem Cell Research (ISCCR), Associate Member
2022 – American Society of Gene and Cell Therapy (ASGCT), Poster abstract reviewer
2020 – American Society of Gene and Cell Therapy (ASGCT), Associate Member
2020 – American Association for Cancer Research (AACR), Associate Member
2018 – European Hematology Association (EHA), Member
2015 – 2019 Supervisor for medical students: clinical supervision in the hematology and bone marrow transplantation unit of Vita-Salute San Raffaele University students and interns.
Clinical research experience in clinical trials, both pharmaceutical company sponsored and investigator initiated trials

Fellowships
2021 – 2022 American-Italian Cancer Foundation Research Fellowship $40,000
Project: “Increase safety and efficacy of cancer adoptive immunotherapies”
2020 – 2021 American-Italian Cancer Foundation Research Fellowship $40,000
Project: “Increase safety and efficacy of cancer adoptive immunotherapies”

Funded Grants as Principal Investigator
07/01/23 – 07/01/25 New Investigator Award (PI: Casirati)
American Society for Transplantation and Cellular Therapy, $100,000
Project: “Epitope engineered hematopoiesis to enable safer non-genotoxic conditioning”
07/01/23 – 07/01/25 New Investigator Award (PI: Casirati)
The PTCTC Jeff Gordon Children’s Foundation, $100,000
Project: “Epitope-edited HSPCs to enable CD244 immunotherapies for acute leukemias”
09/01/23 – 08/31/25 P4P Research Grant, $50,000
Project: “CD244 targeted CAR-T cells for high-risk pediatric leukemias”

Pending Grants as Principal Investigator
03/01/2023 – 02/28/2025 Lauri Strauss Leukemia Foundation Research Grant, $50,000
11/01/2023 – 12/01/2024 ASGCT Career Development Award, $100,000
11/01/2023 – 12/01/2025 ASH Scholar Award, $100,000
04/01/2024 – 03/30/2026 Harrington Discovery Institute Scholar-Innovator Award, $100,000

Funded Grants as Key Research personnel
01/15/21 – 01/14/22 Emerging Scientist Award (PI: Genovese)
Children’s Cancer Research Fund $100,000
Project: “Empowering specificity of AML immunotherapies by HSC engineering”
04/01/21 – 03/31/22 Pilot Research Grant (PI: Genovese)
Research Executive council $50,000
Project: “Generation of “stealth” tyrosine kinase receptors for an immunotherapy resistant hematopoiesis”
07/01/21 – 06/30/22 Research Grant (Genovese)
National Blood Foundation (NBF) $75,000

Project: “Engineering immunotherapy resistant hematopoiesis to treat high-risk acute myeloid leukemia”

07/01/21 – 06/30/22 New Investigator Blood Cancer Research Grant (Genovese)
Leukemia Research Foundation $100,000
Project: “Generation of “stealth” tyrosine kinase receptors for an immunotherapy resistant hematopoiesis”

C. Contributions to Science

Research experience summary

2020 – current Boston Children’s Hospital / Dana Farber Cancer Institute, Boston

- **Epitope-engineering approach to enable adoptive immunotherapies targeting Acute Myeloid Leukemia** (AML). This strategy allows targeting genes essential for leukemia survival regardless of shared expression on HSPCs or mature cells, reducing on-target/off-tumor toxicity and the risk of tumor immune escape, through base editing of the target epitope in healthy HSPCs (*Nature*).

  - **Nature 2023** Epitope Editing Enables Targeted Immunotherapy of Acute Myeloid Leukemia **Casirati G** et al.
  - **CICON 2023** (presentation) **Casirati G** et al.
  - **ISSCR 2023** (poster presentation) **Casirati G** et al.
  - **Folkman Children's Hospital Research Day** (oral presentation) – Boston Children's Hospital
  - **ASGCT 2023** (oral presentation) **Casirati G** et al.
  - **Keystone X2** (oral presentation) **Casirati G** et al.
  - **ASH 2022** (oral presentation): **Casirati G** et al.
  - **PQG Conference 2022**, (oral presentation) **Casirati G** et al.
  - **Keystone RQ5** (oral presentation) Precision Genome Engineering (oral presentation): **Casirati G** et al.

- **KIT Epitope-editing for in vivo-selection of genome modified HSPCs and non-genotoxic conditioning.** KIT mAb are currently being investigated as an alternative to chemo/radiotherapy for transplant conditioning, but their use may result in on-target killing of transplanted HSCs. Epitope editing endows HSPCs with protection against mAb toxicity and allows progressive selection of transplanted cells in vivo. *This project has received the 2023 ASTCT New Investigator Award*.

  - **ISCCR 2023** (oral presentation) **Casirati G** et al.
  - **ASGCT 2023** (oral presentation) **Casirati G** et al.

- **Epitope-editing to explore CD244-targeted CAR-T cells for acute leukemias.** CD244 is a potential transformative target for AML, thanks to its expression in >95% of patients even on leukemia stem cells. Differently from other surface antigens, CD244 has also been reported to show over-expression at relapse. This project will generate proof-of-concept data of the feasibility of CD244 epitope-engineering and the functionality of CD244-targeted CAR-T cells. *This project has received the 2023 PTCTC New Investigator Award*. *The development of potent anti-CD244 CAR-T cells have received the 2023 P4P Research award.*

2018 – 2020 San Raffaele Telethon Institute for Gene Therapy, Milan

- **Explored the role of miR-126 in AML leukemia stem cells** (LSC) by means of lentivirus-encoded miRNA reporters and patient-derived AML xeno-grafts (PDX). Performed in vivo tracking of miR-126-associated intra-tumoral heterogeneity and explored LSC dynamics in response to chemotherapy and derived LSC-associated and LSC-depleted gene signatures. Performed single-cell RNA sequencing of primary AML samples of >30 bone marrow patient samples and investigated intra-patient leukemia heterogeneity, with relevant findings for outcome prediction and highlighted the role of residual quiescent LSCs early after chemotherapy (*Naldini M, Casirati G, Nature Communications*)

  - **Nature Communications 2023** Longitudinal single-cell profiling of chemotherapy response in acute myeloid leukemia, Naldini MM, **Casirati G** et al.
  - **ASH 2019** (oral presentation) Naldini MM, **Casirati G** et al.
  - **ASH 2018** and **Single Cell Biology Keystone Symposium L1 2019** (poster presentation) Naldini MM, **Casirati G** et al.
  - **EHA 2018** (poster presentation) Naldini MM, […] **Casirati G** et al.

2015 – 2019 Hematology and Bone Marrow Transplantation - Prof. Fabio Ciceri (San Raffaele Hospital, Milan)

Trained as hematology resident and gained clinical experience in the treatment of hematological malignancies (acute leukemias, myelodysplastic syndromes, lymphomas, etc.), non-malignant diseases (including bone marrow failure syndromes, acquired and genetic anemias, PNH, immunodeficiencies, etc.) and hematopoietic stem cell transplantation (autologous and allogeneic). Contributed to clinical research studies on the treatment of bacterial infectious diseases in immunocompromised patients, the long-term follow-up of HSCT patients and their complications, chemotherapy intensification strategies in AML patients, conditioning regimens for SCT in AML patients and B-ALL patients clinical outcome after HSCT.

- **Clinical Hematology International**, **2019** Jun; vol 1, issue 2:120 – 123. Nanosphere's Verigene® Blood Culture Assay to Detect Multidrug-Resistant Gram-Negative Bacterial Outbreak: A Prospective Study on 79 Hematological Patients in a Country with High Prevalence of Antimicrobial Resistance R Greco, [...] **Casirati G** et al.
- **ASH 2018** (poster presentation) Endocrinopathies Following Allogeneic Stem Cell Transplantation: 10 Years Follow-up in 402 Patients Lupo-Stanghellini MT, [...] **Casirati G** et al.
- **EHA 2018** (poster presentation) Feasibility and safety of an early intensification approach with FLAG-Ida in newly diagnosed Acute Myeloid Leukemia (AML) patients with morphological residual leukemia at day 14 bone marrow after 3+7 Pavesi F, [...], **Casirati G**, et al.
- **EBMT 2018** (poster presentation) – Allogeneic HSCT in Acute Lymphoblastic Leukemia: long-term outcome of 100 adult patients in 15 years; Pierini S, [...] **Casirati G** et al.
  EBMT 2018 (poster presentation) – Treosulfan, Fludarabine AND Cytarabine (FLAT) myeloablative conditioning for autologous stem cell transplantation in elderly patients with Acute Myeloid Leukemia in first complete remission Bernardi M, [...] **Casirati G** et al.
- **EBMT 2018** (poster presentation) – Immune status in long term survivors after allogeneic stem cell transplant is influenced by GvHD occurrence, donor type and timing: a cross-sectional evaluation Lupo-Stanghellini MT, [...] **Casirati G**, Ciceri F.

2013 – 2014 Rare Disease Centre, - Prof. Cappellini Maria Domenica (IRCCS Policlinico Ca' Granda, Milan)
I extensively **studied a cohort of 15 type 1 Gaucher disease patients**, their clinical history and explored the spectrum of skeletal and bone marrow involvement in adult patients, before and after enzyme replacement therapy (ERT). I evaluated the correlation between hematopoietic, hepatic, skeletal complications and ERT dose by considering imaging studies, biochemical markers of bone metabolism, disease activity markers and selected cytokines (GDF15, IL6). I also studied the consequences of ERT discontinuation due to the 2009-10 worldwide Imiglucerase shortage.

- **Blood Cells Mol Dis.** 2018 Feb; 68:148-152. doi: 10.1016/j.bcmd.2017.06.003. Skeletal involvement in type 1 Gaucher disease: Not just bone mineral density Baldini M, **Casirati G**, Ulivieri FM, Cassinerio E, Khouri Chalouhi K, Poggiali E, Borin L, Burghignoli V, Cesana BM, Cappellini MD.
- **SIMI National Congress 2015** (oral presentation) – Skeletal involvement in Type 1 Gaucher Disease: a comprehensive study of bone quality, quantity and turnover – **Casirati G**, Poggiali E, Baldini M, Ulivieri FM, Khouri Chalouhi K, Duca L, Nava I, Cassinerio E, Cappellini MD.

D. Awards

06/2023 – **ISSCR 2023** Travel Award, **ISSCR 2023** Abstract Merit Award
05/2023 – **Takara** SMART-Seq Pro Biomarker Discovery Contest
05/2023 – **ASGCT 2023** Excellence in Research Award and Meritorious Abstract Travel Award
03/2023 – **Keystone X2 Precision Genome Engineering 2023** Symposia Scholarship
12/2022 – **ASH 2022 Abstract Achievement Award** - ASH 2022
11/2022 – **Stellar Abstract Award** - Harvard PGQ conference

E. Additional Information: Research Support and/or Scholastic Performance

2015 Italian National Medical Residency Examination
Score: Hematology 125.2 (rank 1); Immunology 127.3 (rank 1); Clinical genetics 126.5 (rank 1).

F. Selected list of publications

1. Casirati, G. *et al.* Epitope Editing Enables Targeted Immunotherapy of Acute Myeloid Leukemia . *Nature* (2023).
2. Zeng, J., Casirati, G., Nguyen, M. A., Genovese, P. & Bauer, D. E. Base Editing of Human Hematopoietic Stem Cells. *Methods Mol Biol* **2606**, 43–62 (2023).
3. Naldini MM, Casirati G, Barcella M, Rancoita P, Cosentino A, Caserta C, Pavesi F, Zonari E, Desantis G, Gilioli D, Carrabba MG, Vago L, Bernardi M, Di Micco R, Di Serio C, Merelli I, Volpin, M, Montini E, C. F. & and Gentner B. Longitudinal single-cell profiling of chemotherapy response in acute myeloid leukemia. *Nat Commun* (2023).
4. Baldini, M., Casirati G. *et al.* Skeletal involvement in type 1 Gaucher disease: Not just bone mineral density. *Blood Cells Mol Dis* **68**, 148–152 (2018).