BIOGRAPHICAL SKETCH

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NAME: Pietro Genovese, Ph.D.

eRA COMMONS USER NAME (agency login): PGENOVESE

POSITION TITLE: Assistant Professor of Pediatrics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Modena and Reggio Emilia, Italy	B.Sc. (Summa cum Laude)	09/2005	Hematopoiesis
"Vita Salute San Raffaele" University, Italy	M.Sc. (Summa cum Laude)	03/2008	Site-Specific Genome Editing Technology
"Vita Salute San Raffaele" University	Ph.D.	05/2013	Cancer Immunotherapy
San Raffaele Telethon Institute for Gene Therapy, Italy	Postdoctoral Fellow	06/2016	Gene correction in hematopoietic stem cells

A. Personal Statement

My long-term scientific interest is to develop innovative and translational genome engineering strategies to cure currently untreatable diseases. Throughout my training as a medical biotechnologist with Luigi Naldini's group at the San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), I helped pioneer this field since when zinc finger nucleases (ZFNs) were first shown to enhance gene targeting and be useful for therapeutic purposes. In 2007, I contributed to a breakthrough study that demonstrated, for the first time, the ability of artificial nucleases to direct the integration of exogenous DNA sequences into a predetermined genomic locus in several human cell types (Lombardo, Genovese, et al., Nat Biotech 2008). During my Ph.D. studies, I developed the T-cell receptor gene editing strategy to enhance the safety and efficacy of cancer-adoptive immunotherapies (Provasi*&Genovese* et al., Nat Med. 2012). This approach is now widely used in the immunotherapy field for generating allo-compatible T cells or expressing chimeric antigen receptors (CARs) under the endogenous TCR promoter. As postdoctoral associate, I developed the first protocol that allows targeted transgene integration and *in situ* gene correction in human hematopoietic stem cells (HSCs) capable of long-term multilineage repopulation (Genovese et al., Nature 2014). By assuming a senior role, I coordinated the pre-clinical development of these novel medical treatments for selected candidate diseases, chosen as a paradigm for testing their therapeutic potential. My first project as principal investigator, which focused on the gene correction of T cells and HSCs for HIGM1 (Vavassori et al., EMBO Mol Med, 2021), is now in the advanced phases of manufacturing development for a first-of-its-kind clinical testing. Since establishing my independent laboratory at Harvard Medical School and the Dana-Farber/Boston Children's Cancer and Blood Disorder Center, I have led multiple efforts to overcome key translational bottlenecks in the field. My group developed a single-cell barcoding system (BAR-Seq) to track the fate of edited HSCs and demonstrated that precise editing preserves clonal diversity and long-term engraftment (Ferrari et al., Nat Biotechnol 2020). We also uncovered a transient p53-mediated checkpoint that governs HSC tolerance to genome editing (Schiroli et al., Cell Stem Cell 2019), work that continues to inform clinical editing protocols. Most recently, we introduced the concept of epitope editing to render healthy donor HSCs resistant to immunotherapy toxicity by modifying surface antigens while preserving receptor function (Casirati et al., Nature 2023). My impression is that now that gene editing technology is already in clinical testing for relatively straightforward problems like monogenic blood diseases, we must raise the bar to reach new solutions for more complex biomedical problems, such as oncologic diseases and, more broadly, to reduce the toxicities of hematopoietic stem cell transplantation.

Ongoing and recently completed projects that I would like to highlight include:

R01 CA286036 (PI: Genovese) 08/01/24 – 07/31/29
NIH - NCI [score:13, percentile: 1]
Multiplex Epitope Editing to Enable Novel Immunotherapies for Acute Myeloid Leukemia

DoD Bone Marrow Failure Award (PI: Genovese; MPI Bauer:) 07/01/24 – 06/30/27
Department of Defense (DoD) - USAMRAA
Base Editing to Improve Conditioning and Transplant of Bone Marrow Failure Patients

R01 HL170629 (PI: Bauer; MPI: Genovese) 08/01/23 – 07/31/27
NIH - NHLBI [score:12, percentile: 1]
Chemotherapy-free cure of hemoglobin disorders through base editing

Translational Research Program (PI: Genovese) 07/01/23 – 06/30/26
Leukemia Lymphoma Society (LLS)
Towards clinical testing of epitope editing to enable novel adoptive immunotherapies.

Discovery Boost Grant (PI: Genovese) 07/01/23 – 06/30/26
American Cancer Society (ACS)
Immunotherapy resistant hematopoiesis to treat acute myeloid leukemia

Sponsored Research Agreement (PI: Genovese) 09/01/20 – 08/31/23
Takeda Oncology
Development of a new adoptive immunotherapy for breast cancer

"Giovani Ricercatori" - GR-2013-02358956 (PI: Genovese) 07/01/15– 06/30/19
Italian Ministry of Health.
Targeted genome editing of the CD40LG gene for the treatment of X-linked HIGM1

B. Positions and Honors

Research Training

2009 - 2013 PhD intern, Gene Editing for Adoptive Immunotherapy (Prof. Luigi Naldini), SR-Tiget
2008 - 2009 Research Fellow, Molecular and Cell Biology of Gene Transfer (Prof. Luigi Naldini), SR-Tiget
2005 - 2008 Intern, Molecular and Cell Biology of Gene Transfer (Prof. Luigi Naldini), San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)
2005 Intern, Molecular Biology and Gene Therapy (Prof. Fulvio Mavilio), University of Modena and Reggio Emilia, Dept. of Biomedical Sciences, Sect. of Biological Chemistry
2004 Intern, Molecular Oncology (Prof. Bruno Calabretta), University of Modena and Reggio Emilia, Dept. of Biomedical Sciences, Sect. of General Pathology

Appointments at Hospitals/Affiliated Institutions

2021 - Associate Member, Broad Institute of MIT and Harvard
2020 - Affiliate Faculty, Harvard Stem Cell Institute
2019 - Assistant Professor in Pediatrics, Harvard Medical School, Department of Pediatrics of Boston Children's Hospital and Harvard Medical School
2019 - Faculty member, Gene Therapy Program, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Dana-Farber Cancer Institute/Boston Children's Hospital
2016 - 2019 Project Leader, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), San Raffaele Scientific Institute, (Member of the SR-Tiget staff, voting)
2013 - 2016 Postdoctoral Fellow, Gene Editing of Hematopoietic Stem/Progenitor cells (Prof. Luigi Naldini), San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), San Raffaele Scientific Institute.

Other Professional Positions

2024 - Member of the Scientific Advisory Board, Ensoma, Inc.
2024 - Member of the Scientific Advisory Board, Vor Biopharma
2022 Scientific consultant, Guidepoint Global Advisors
2022 Scientific consultant, Patient Square Capital, L.P.
2020 - 2021 Scientific consultant, Dorian Therapeutics (NCI SBIR: 1R43CA265636-01)
2020 Scientific co-founder, GeneSpire Srl.
2020 Expert consultant, ALGM on behalf of Cellectis
2019 - 2020 Scientific consultant, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)
2018 - 2019 Scientific consultant, Janssen R&D (pharma R&D organization of Johnson & Johnson)

Professional Societies

2024 - Member, Society for Immunotherapy of Cancer (SITC)
2024 - Member, Società Italiana di Terapia Genica e Cellulare (SITGEC)
2022 - Member, American Society for Transplantation and Cellular Therapy (ASTCT)
2020 - Member, International Society for Stem Cell Research (ISSCR)
2019 - Active Member, American Society of Hematology (ASH)
2010 - Member, American Association for Cancer Research (AACR)
2009 - Member, The American Society of Gene and Cell Therapy (ASGCT)
2009 - Member, European Society of Gene and Cell Therapy (ESGCT)

Grant Review Activities

2024 - Reviewer for the Great Ormond Street Hospital Children's (GOSH) Charity – London, UK.
2024 Reviewer for the Young Investigator Grant of the Alex Lemonade Stand Foundation (ALSF)
2024 Member of the Immunology and Blood Cell Development (IBCD) peer review committee for the American Cancer Society (ACS)
2022, 2024 Reviewer for the Multi-round Research Grant of the Telethon Foundation - Italy
2021, 2023 Reviewer for Advance Grants of the European Research Council (ERC)
2021 Reviewer for the Children's Cancer Research Fund (CCRF)
2020 - Reviewer of proposals for the Astellas award, Boston Children's Hospital – Astellas Pharma
2009 - Reviewer for the French National Research Agency, Agence Nationale de la Recherche (ANR)
2009 Reviewer for the Dystrophic Epidermolysis Bullosa Research Association (DEBRA) International

Editorial Activities

2020 Co-coordination of a Research Topic (eBook) from *Frontiers in Genome Editing* journal: "Mutation Specific Gene Editing for Blood Disorders".
2019 - Associate Editor of the *Frontiers in Genome Editing* journal (Genome Editing in Blood Disorders).
2012 - Ad-hoc Reviewer for Nature Immunology Reviews, Blood, Blood Reviews, Nature Methods, Human Gene Therapy, Nature Communications, Molecular Therapy

Honors and Awards

2024 **Outstanding New Investigator Award** – American Society of Gene and Cell Therapy (ASGCT), Recognition for valuable contribution to the field of cell and gene therapy.
2023 Scholar Award of the Association for the Advancement of Blood & Biotherapies (aabb) Foundation
2023 Translation to CURE Award (T2C) - CURE Childhood Cancer, support for the project "Empowering pediatric immunotherapies by HSC engineering"
2021 Emerging Scientist Award - Children's Cancer Research Fund (CCRF), support for the project "*Empowering specificity of AML immunotherapies by HSC engineering*"
2021 Pilot Research Award - Research Executive Council, Boston Children's Hospital, support for the project "S*tealth tyrosine kinase receptors for an immunotherapy resistant hematopoiesis"*
2020 Merit Abstract Award - International Society for Stem Cell Research (ISSCR), Best Abstracts of the ISSCR meeting (Mentor of the winning Awardee)
2019 - 2023 Excellence in Research Awards - American Society of Gene and Cell Therapy (ASGCT), (Mentor of the winning Awardees in: 2019, 2020, 2021, 2022, and 2023)
2016 **Young Investigator Award** - European Society of Gene and Cell Therapy (ESGCT), Recognition for valuable contribution to the field of cell and gene therapy
2015 - Invited speaker at more than 10 national and 25 international scientific meetings, including the Annual Meetings of the American and the European Societies of Gene and Cell Therapy (ASGCT and ESGCT), the European Medicines Agency (EMA) and the European Society for Blood and Marrow Transplantation (EBMT) and the American Society of Hematology (ASH).
2014 Nicolò Copernico Award for Biomedical Science from the Promoting Committee of the "Giulio Natta and Nicolò Copernico Awards" for the Scientific Research and Technology Innovation
2014 Cecilia Cioffrese Award - Fondazione Carlo Erba, the best research followed by Italian young graduates in the field of cancer
2012 Van Bekkum Award - European Society for Blood and Marrow Transplantation (EBMT)
2012 Jon Van Rood Award - European Federation for Immunogenetics (EFI), Best Abstract submitted to the EFI annual congress
2010 - 2014 Meritorious Travel Grant Awards - Top Abstracts submitted to: the ESGCT meeting (Awardee in the years: 2011, 2013 and 2014); the ASGCT meeting (years: 2010, 2012, 2013 and 2014)
2010 Leslie Fairbairn Runner Up Award - Persisting Transgenesis (PERSIST) European Research Consortium, Best presentation from a young scientist at the Second PERSIST Meeting

C. Contributions to Science

(1) Since early in my studies, I contributed to several works that pioneered the use of targeted genome editing technology by developing innovative tools and protocols, which are today perceived as state-of-the-art technologies in the gene editing field. In these studies, we provided: *i.* the first proof-of-concept of targeted gene editing by engineered nucleases in therapeutically relevant cell types, including human embryonic stem cells and hematopoietic progenitors; *ii.* characterized the human *AAVS1* locus as a genomic safe harbor for the integration of therapeutic transgenes; and *iii*. developed pioneering approaches to assess the specificity of artificial nucleases by unbiased genome-wide studies.

a. Lombardo A, **GENOVESE P**, et al.. Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. Nature biotechnology. 2007. PMID: 17965707.
b. Lombardo A, Cesana D*, **GENOVESE P***, et al.. Site-specific integration and tailoring of cassette design for sustainable gene transfer. Nature Methods. 2011. PMID: 21857672. *** Equal contribution**.
c. Gabriel R*, Lombardo A*, Arens A, Miller JC, **GENOVESE P**, et al.. An unbiased genome-wide analysis of zinc-finger nuclease specificity. Nature Biotechnology. 2011. PMID: 21822255.
d. Montepeloso A., …, **GENOVESE P.**, Biffi A. Haploinsufficiency at the CX3CR1 locus of hematopoietic stem cells favors the appearance of microglia-like cells in the central nervous system of transplant recipients. Nature Communications. 2024. PMID: 39587072

(2) Starting from my Ph.D. training, I extended the application of these emerging technologies to the development of a new cancer-adoptive immunotherapy strategy. Here, I exploited the use of engineered nucleases to abrogate the expression of the endogenous T cell receptor (TCR) genes in primary human T lymphocytes and re-direct them against a tumor-associated antigen. By avoiding competition for surface expression between exogenous and endogenous TCR chains, and by abrogating the risk of inappropriate TCR pairing, the TCR editing approach permanently overcomes some of the major limitations of TCR gene transfer immunotherapy. This work was the first proof that gene editing can be used to genetically re-write the endogenous antigen specificity of cytotoxic T cells and enable the feasibility of a safe allogeneic T cell transplantation, thus providing the basis for several other studies in the rapidly expanding cancer immunotherapy field, some of which already entered clinical testing. My expertise in genome engineering and T-cell manipulation was then essential for expanding the potential of cancer-adoptive cellular therapies by developing: i) highly efficient bi-directional lentiviral vectors for co-expression of anti-AML CAR and a suicide gene, ii) multiplex gene inactivation of immune checkpoint inhibitors for potentiating the efficacy and persistence of anti-leukemic T cells, and iii) dissecting the molecular mechanism determining an improved anti-cancer activity of NKT cells engineered to express IL-12.

a. Provasi E*, **GENOVESE P***, et al.. Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nature medicine. 2012 PMCID: PMC5019824. *** Co-First Authorship**
b. Mastaglio S, **GENOVESE P**, et al.. NY-ESO-1 TCR single edited stem and central memory T cells to treat multiple myeloma without graft-versus-host disease. Blood. 2017. PMID: 28637663.
c. Casucci M, …, **GENOVESE P**, et al. CD44v6-targeted T cells mediate potent antitumor effects against acute myeloid leukemia and multiple myeloma. Blood. 2013. PMID: 24016461.
d. Landoni E, …, **GENOVESE P**, et al. IL-12 reprograms CAR-expressing natural killer T cells to long-lived Th1-polarized cells with potent antitumor activity. Nature Communications. 2024. PMID: 38167707

(3) As Postdoctoral Associate, I provided a major contribution to the gene therapy field by developing an effective gene editing strategy for targeted gene addition or *in situ* correction of inherited mutations in human hematopoietic stem cells (HSC). By tailoring culture conditions and gene delivery vehicles, I overcome the biologic barriers that specifically constrain gene targeting in the most primitive subset of hematopoietic progenitors and developed a protocol that allows targeted integration of a transgene expression cassette into a "safe harbor" site or direct correction of the *IL2RG* gene of HSCs from healthy donors and X-linked severe combined immunodeficiency (SCID-X1) patients. This work was the first proof that targeted gene modifications can be efficiently obtained in HSC that preserve their repopulation potential and formed the basis for several other studies in the field, some of which recently entered clinical testing. More recently, we assessed the impact of this editing procedure on the treated HSC by performing an unbiased single-cell transcriptomic analysis. These studies uncovered cumulative activation of P53-dependent DNA Damage Response (DDR), which receives multiple converging inputs during the editing procedure, including sensing of AAV6 used for DNA template delivery. However, we found that this functional impairment could be overcome by short transitory dampening of DDR during the editing procedure achieved through the delivery of mRNA encoding for a p53 inhibitor peptide (GSE56). Since AAV6 transduction was the principal driver of DDR activation, we transiently co-delivered during electroporation different factors that counteract this response. By this screening, we identified an adenoviral protein (E4orf6/7) that forced cell cycle progression, thus boosting homology-mediated editing and increasing

the yield of edited HSPC in xeno-transplanted NSG mice. To assess the clonality of the edited HSC, we developed a barcoding-based strategy that allows the monitoring of clonal behavior and hematopoietic graft composition after HDR-mediated editing (BAR-seq). By this strategy, we proved polyclonal reconstitution and preserved self-renewal and multi-potency of individual HSC edited with our optimized protocols. These findings provide molecular evidence of the feasibility of seamless targeted gene editing in HSPCs that can produce polyclonal, long-term engraftment, thus giving confidence to its prospective translation.

a. **GENOVESE P**, Schiroli G, et al.. Targeted genome editing in human repopulating hematopoietic stem cells. Nature. 2014 PMCID: PMC4082311.
b. Schiroli G, Conti A, … **GENOVESE P*,** Naldini L*, Di Micco R*. Precise Gene Editing Preserves Hematopoietic Stem Cell Function Following Transient p53-Mediated DNA Damage Response. Cell Stem Cell. 2019. PMID: 30905619. ***Co-senior authorship; co-corresponding author.**
c. Ferrari S, …, **GENOVESE P*,** Naldini L*. Efficient gene editing of human long-term hematopoietic stem cells validated by clonal tracking. Nature Biotechnology. 2020. PMID: 32601433 ***Co-senior authorship**.
d. Ferrari S, Beretta S, …, **GENOVESE P.** BAR-Seq clonal tracking of gene edited cells. Nature Protocols. 2021. **Senior authorship**.

(4) Building on these achievements, and thanks to my continuous interest in developing and applying improved molecular strategies for regenerative medicine, my research then focuses on the exploitation of tailored preclinical models to establish the conditions for safe and effective correction of two inherited immunologic diseases, the SCID-X1 and the HIGM1 syndrome. With these studies, we were able to model the impact of the edited cell product and its administration strategy on the timing and extent of immune reconstitution, establishing the rationale for clinical translation of these precise but challenging genetic engineering strategies.

a. Schiroli G, Ferrari S, … **GENOVESE P***, Naldini L*. Preclinical modeling highlights the therapeutic potential of hematopoietic stem cell gene editing for correction of SCID-X1. Science Translational Medicine. 2017 PMID: 29021165. ***Co-senior authorship; co-corresponding author.**
b. Cesana D, …, **GENOVESE P**, …, Montini E. Liquid-Biopsy-Integration-Site-Sequencing (LiBIS-Seq) for the retrieval of vector integrations from cell-free DNA. Nature Medicine. 2021
c. Vavassori V, Mercuri E, … Naldini L*, **GENOVESE P***. Preclinical Modelling Positions T-cell Ahead of Hematopoietic Stem Cell Gene Editing for the Treatment of X-linked Hyper IgM Syndrome. EMBO Molecular Medicine. 2021. ***Co-senior authorship; co-corresponding author.**
d. Castiello MC, … **GENOVESE P**, Naldini L*, Villa A*. Exonic knockout and knockin gene editing in hematopoietic stem and progenitor cells rescues RAG1 immunodeficiency. Science Trans. Med. 2024. PMID: 38324638

(5) Recently, I decided to exploit the power of gene editing tools 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, we developed the epitope-engineering approach that, by exploiting precise base editing of donor HSPCs, can endow healthy hematopoietic cells with selective resistance to monoclonal antibodies or CAR-T cells while still allowing the eradication of AML cells.

a. Casirati G, Cosentino A, Mucci A, Mahmoud MS, Ugarte-Zabala I, Zeng J, Ficarro SB, Klatt D, Brendel C, Rambaldi A, Ritz J, Marto JA, Pellin D, Bauer DE, Armstrong SA, **GENOVESE P**. Epitope Editing Enables Targeted Immunotherapy of Acute Myeloid Leukemia. *Nature*. 2023. PMID: 37648862 **Senior corresponding author**.
b. Zeng J, Casirati G, **GENOVESE P**, Bauer D. Base Editing of Human Hematopoietic Stem Cells. Methods Mol Biol. 2023. PMID: 36592307
c. Levesque S, Cosentino A, Verma A, **GENOVESE P**, Bauer DE. Nucleotide metabolism constrains prime editing in hematopoietic stem and progenitor cells. *Nature Biotechnology* 2024. PMID: 38806736

Complete List of Published Works: https://www.ncbi.nlm.nih.gov/myncbi/1peIE6ibSDpQ7/bibliography/public/