BIOGRAPHICAL SKETCH

NAME: Pietro Genovese, Ph.D.

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POSITION TITLE: Assistant Professor of Pediatrics – Harvard Medical School

EDUCATION/TRAINING

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. Positions and Honors

Research Training

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

Appointments at Hospitals/Affiliated Institutions

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.
2016 - 2019 Project Leader, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), San Raffaele Scientific Institute, (Member of the SR-Tiget staff)
2019 - Faculty member, Gene Therapy Program, Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Dana-Farber Cancer Institute/Boston Children's Hospital
2019 - Assistant Professor in Pediatrics, Harvard Medical School, Department of Pediatrics of Boston Children's Hospital and Harvard Medical School
2020 - Affiliate Faculty, Harvard Stem Cell Institute

Other Professional Positions

2018 - 2019 Scientific consultant, Janssen R&D (pharma R&D organization of Johnson & Johnson)
2019 – 2020 Scientific consultant, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget)
2020 Expert consultant, AMGL for Cellectis
2020 Scientific Founder, GeneSpire Therapeutics

Professional Societies

2009 - Member, European Society of Gene and Cell Therapy
2009 - Member, The American Society of Gene and Cell Therapy
2010 Associate Member, American Association for Cancer Research (AACR)
2019 - Associate Member, America Society of Hematology (ASH)
2020 - Member, International Society for Stem Cell Research (ISSCR)

Editorial Activities

2019- Associate Editor of the *Frontiers in Genome Editing* journal (section: Genome Editing in Blood Disorders).
2012 - Ad-hoc Reviewer for Blood, Blood Reviews, Nature Methods
2012 - 2019 Support of mentor in ad-hoc Revision for Nature, Nat. Med., Nat. Biotech., Cell, Cell Stem Cell, Molecular Therapy, Blood

Honors and Prizes

2010 Leslie Fairbairn Runner Up Award - Persisting Transgenesis (PERSIST) European Research Consortium, Best presentation from young scientist at the Second PERSIST Meeting
2010 - 2014 Meritorious Travel Grant Award - American Society of Gene and Cell Therapy (ASGCT), Best Abstract submitted to the ASGCT meeting (Awardee in the years: 2010, 2012, 2013 and 2014)
2011 - 2014 Meritorious Travel Grant Award - European Society of Gene and Cell Therapy (ESGCT), Best Abstract submitted to the ESGCT meeting (Awardee in the years: 2011, 2013 and 2014)
2012 Jon Van Rood Award - European Federation for Immunogenetics (EFI), Best Abstract submitted to the EFI annual congress
2012 Van Bekkum Award - European Society for Blood and Marrow Transplantation (EBMT) the best abstract submitted to the physician's program at the EBMT annual congress
2014 Cecilia Cioffrese Award - Fondazione Carlo Erba, the best research followed by Italian young graduates on the field of cancer
2014 Nicolò Copernico Award for Biomedical Science - the Promoting Committee of the Awards «Giulio Natta and Nicolò Copernico for the Scientific Research and Technology Innovation of Ferrara, Best publication from an Italian young researcher in a Scientific Journal (link2)
2016 Young Investigator Award - European Society of Gene and Cell Therapy, Recognition for the valuable contribution in the field of cell and gene therapy (link)
2015 – 2019 Invited speaker at 6 national and 18 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).

B. Contributions to Science

Selected Publications:

In my early studies, I contribute to several works that pioneered the use of targeted genome editing technology by developing innovative tools and protocols, which are today perceived as the state-of-the-art technologies in 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 integration of therapeutic transgenes; and *iii*. developed pioneering approaches to assess the specificity of artificial nucleases.

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.

During 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 lymphocyte 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 overcome some of the major limitations of TCR gene transfer immunotherapy. This work was the first poof 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.

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. * Equal contribution
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. PubMed 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 PubMed PMID: 24016461.
d. Cianciotti B, …, **GENOVESE P**, et al. LAG-3, but not Tim-3, disruption in TCR gene edited human memory stem T cells enhance the anti-tumor activity against multiple myeloma. Manuscript in preparation.

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 poof 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 will shortly enter clinical testing.

a. **GENOVESE P**, Schiroli G, et al.. Targeted genome editing in human repopulating hematopoietic stem cells. Nature. 2014 PMCID: PMC4082311.

More recently, we assess 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 thought 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 clonality of the edited HSC, we developed a barcoding-based strategy that allows monitoring 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 HSPC able to polyclonal, long-term engraftment, thus giving confidence to its prospective translation in humans.

a. 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.
b. Ferrari S, Jacob A, …, **GENOVESE P*,** Naldini L*. Clonal Tracking Uncovers Barriers and Validates New Strategies to Enhance Gene Editing in Human Hematopoietic Stem Cells. Nature Biotechnology. 2020. *Co-senior authorship; co-corresponding author.

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 timing and extent of immune reconstitution, establishing the rationale for clinical translation of this 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. Manuscript under revision.
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. Manuscript under revision. *Co-senior authorship; co-corresponding author

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/pubmed/?term=Pietro+genovese

Dr. Genovese is inventor of more than 8 patent applications on gene editing in hematopoietic stem cells, T cell engineering and cell selection owned and managed by the San Raffaele Scientific Institute and the Telethon Foundation.