

BIOGRAPHICAL SKETCH

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NAME: **Alessandro Bertero**

eRA COMMONS USER NAME (credential, e.g., agency login): **abertero**

POSITION TITLE: **Associate Professor**

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 Turin (Italy)	BSci	09/2009	Biotechnology
University of Turin (Italy)	MSci	07/2011	Medical Biotechnology
University of Cambridge (UK)	MRes	10/2012	Cardiovascular Disease
University of Cambridge (UK)	PhD	10/2016	Stem Cell & Developmental Biology
University of Washington (USA)	Postdoc	06/2019	Cardiac Development and Disease

A. Personal Statement

My group's long-term mission is to elucidate and reverse engineer the gene regulatory mechanisms that drive abnormal development. This commitment became deeply personal with the birth of **my second son, Riccardo, who suffers from Cat Eye Syndrome (CES)**. Together with my wife Dr. Elisa Balmas, who is also my co-lab head, I am uniquely positioned to advance the mechanistic understanding of this poorly characterized disorder. Through this proposal, we aim to establish a foundation for research that may ultimately enable improved diagnosis and therapeutic strategies for Riccardo and other individuals affected by CES.

Our funded programs investigate how three-dimensional chromatin organization contributes to congenital heart disease (CHD) and identify regulators of heart development through loss- and gain-of-function screens in human cells. We leverage differentiation of human pluripotent stem cells into cardiomyocytes and other cardiac lineages, CRISPR-based engineering, genomic and imaging assays of nuclear architecture, and 2D/3D models of morphogenesis and physiology. This proposal aligns with these strengths yet inaugurates a **new, currently unfunded research direction focused on CES**. To catalyze this effort, we are seeking support from the Human Technopole National Facility for Genome Editing and Disease Modeling (NF GEDM) and the Data Handling and Analysis (DHA) platform to derive induced pluripotent stem cells and perform trio whole-genome sequencing from our son (proband) and both parents, enabling mechanistic studies and variant interpretation. This support will be key for our goal of preparing a competitive Telethon Multiround application for 2026.

I have the expertise, resources, and mentorship skills required to serve as Principal Investigator for this project. During my PhD training with Ludovic Vallier at the University of Cambridge, I focused on the epigenetic and epitranscriptional regulation of TGF β signaling, while in parallel developing genetic engineering approaches for robust gain- and loss-of-function studies in hPSC-derived cells. As a postdoctoral fellow in Chuck Murry's laboratory at the University of Washington, I led NIH 4D Nucleome-funded studies defining the functional dynamics of chromatin topology in human cardiogenesis and disease. After joining the UW faculty in July 2019, I established an independent research program on CHD, supported by both intra- and extramural funding. In 2021, with the support of the prestigious Giovanni Armenise-Harvard Foundation Career Development Award, I relocated my group to the University of Turin, where it has since grown to include 3 senior scientists, 2 postdoctoral fellows, 5 PhD students, and 3 undergraduates. Over the last three years, our work has been further supported by the ERC, NIH, FEBS, and other funding bodies, and our first project results have already appeared in leading journals, including *Genome Research*, *EMBO Molecular Medicine*, and *Open Research Europe*.

Key publications relevant to the proposal

1. **Bertero A**[@], Pawlowski M, Ortmann D, Snijders K, Yiangou L, Cardoso de Brito MC, Brown S, Bernard WG, ... [11 authors], Vallier L[@] (2016). @co-corresponding. Optimized inducible shRNA and CRISPR/Cas9 platforms for in vitro studies of human development using hPSCs. *Development* 143, 4405–18
2. **Bertero A**, Fields PA, Ramani V, Bonora G, Yardimci GG, Reinecke H, Pabon L, Noble WS, Shendure J, Murry C.E. (2019). Dynamics of genome reorganization during human cardiogenesis reveal an RBM20-dependent splicing factory. *Nature Communications* 10, 1538
3. Balma E, Sozza F, Bottin S, Ratto ML, Savorè G, Becca S, Snijders KE, **Bertero** (2023). Manipulating and studying gene function in human pluripotent stem cell models. *FEBS Letters* 597, 2250-87
4. Hirstov BH, Noble WS, **Bertero A** (2024). Systematic identification of inter-chromosomal interaction networks supports the existence of RNA factories. *Genome Research*, 34, 1610-1623
5. Balmas E, Ratto ML, Snijders K, Calogero R, Mendjan S, **Bertero A** (2024). Single cell transcriptional perturbome in pluripotent stem cell models. *Sneak Peek* doi: 10.2139/ssrn.4854180

Ongoing extramural research support relevant to the proposal

Note: none of these awards supports research on CES described in this proposal

ERC Starting Grant 2022 – LS1 Principal Investigator European Research Council <i>TRANS-3. Beyond the chromosome: unravelling the interplay between inter-chromosomal genome architecture and mRNA biogenesis</i>	1 Sept 2023 – 31 Ago 2028
PRIN 2022 Co-Principal Investigator Ministry of University and Research <i>Decoding and leveraging the molecular determinants of myogenic fate through integrative genomics and cell engineering</i>	29 Sept 2023 – 28 Sept 2025
FEBS Excellence Award 2022 Principal Investigator Federation of European Biochemical Societies <i>Chromatin topology dysregulation in cardiac cohesinopathies</i>	1 Jan 2023 – 31 Dec 2025
AV Single Ventricle Research Fund 2021 Principal Investigator Additional Ventures, USA <i>Transcriptional and morphogenetic signatures of congenital heart disease pathways</i>	15 Feb 2022 – 14 Feb 2025
GAHF Career Development Award 2020 Principal Investigator Giovanni Armenise-Harvard Foundation, USA <i>Functional dynamics of chromatin topology in congenital heart disease</i>	01 Jul 2021 – 30 Jun 2026

Completed research support relevant to the proposal (last 5 years)

NIH R01 HL 149734 Co-Investigator National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health, USA <i>Mechanotransduction by Melusin in Cardiac Hypertrophy</i>	01 Jul 2020 – 30 Jun 2025
UW ISCRM Innovation Pilot Award Principal Investigator University of Washington Institute for Stem Cell and Regenerative medicine, USA <i>Functional Dynamics of Chromatin Topology in Congenital Heart Disease</i>	01 Jul 2019 – 30 Jun 2020

B. Positions, Scientific Appointments, and Honors

Professional Experience

06/2007 - 09/2011	Undergraduate Research Assistant, University of Turin, Turin, Italy
07/2009 - 08/2009	Research Intern, EPFL, Lausanne, Switzerland
10/2011 - 09/2012	Rotation Student, University of Cambridge, Cambridge, UK
10/2012 - 09/2016	Doctoral Candidate, University of Cambridge, Cambridge, UK
10/2016 - 06/2019	Senior Fellow/Senior Fellow Trainee, University of Washington, Seattle, WA, USA
07/2019 – 04/2021	Acting Assistant Professor, University of Washington, Seattle, WA, USA
07/2021 – present	Associate Professor, University of Turin, Turin, Italy

Scholarships and Fellowships

07/2009 - 08/2009	EPFL Summer Research Program, École Polytechnique Fédérale de Lausanne
10/2011 - 09/2015	BHF 4-year MRes-PhD Scholarship, British Heart Foundation
08/2017 - 07/2019	EMBO Long-Term Fellowship, European Molecular Biology Organization
07/2021 – present	GAHF Career Development Award, Giovanni Armenise-Harvard Foundation

Honors, Awards, and Named Lectures

2010	Optime Prize for Bachelor Dissertation, Unione Industriale Torino, Turin, Italy
2012	Optime Prize for Master Dissertation, Unione Industriale Torino, Turin, Italy
2018	National Academic Qualification - Associate Professor Level, MIUR, Italy
2019	Guido Tarone Memorial Lecture, XXII SIRC National Congress, Imola, Italy
2021	Next Generation of Leaders, International Society for Stem Cell Research
2022	FEBS Excellence Award, Federation of European Biochemical Societies
2024	Silver Prize, StarCup Piemonte Val D'Aosta, Italy
2025	Public Service Award, International Society for Stem Cell Research

Intra and Extramural Service

2018 –	<i>Ad Hoc</i> Reviewer for peer-reviewed journals (including <i>Nat. Genet.</i> , <i>Nat. Commun.</i> , <i>Stem Cell Rep.</i> , <i>Dev. Cell</i> , <i>JACC Transl. Med</i> , <i>Circ. Res</i> , <i>Cardiovasc. Res.</i> , and <i>J. Mol. Cell. Cardiol.</i>)
2020 – 22	Study Section, American Heart Association Career Development Award – Cardiac Sciences 2
2020	Junior Leadership Team, ISCRM, University of Washington
2020	Steering Committee, Center for Cardiovascular Biology, University of Washington
2022 – 25	Expert Reviewer, AAPG, Agence Nationale de la Recherche
2023 – 25	Expert Reviewer, Additional Ventures Single Ventricle Research Program
2023 –	Infrastructure Committee, Molecular Biotechnology Center “Guido Tarone”, University of Torino

Relevant Professional Membership

2015 –	International Society for Stem Cell Research (ISSCR) - Next Generation Leader (2021), Early Career Scientist Comm. (2022), Manufacturing, Clinical Translation & Regulatory Comm. (2023)
2019 –	Heart Failure Association (HFA) or the European Society of Cardiology (ESC)
2019 –	Società Italiana di Ricerche Cardiovascolari (SIRC)
2023 –	ERC in Italy
2025	Italian Association for Cell and Developmental Biology (A.B.C.D.)

C. Contributions to Science

1. The Melusin signaling pathway in cardioprotection.

During my undergraduate training, I spent four years investigating the cardioprotective role of the muscle-specific protein Melusin, whose function at the time was poorly understood. Through biochemical, pharmacological, and mouse genetic approaches, I contributed to two key discoveries: (i) Melusin responds to mechanical stimuli by activating ERK1/2 through direct interactions with FAK and the scaffold protein IQGAP1; and (ii) IQGAP1 is essential for the cardiac hypertrophic response. Building on this early work.

As faculty, I secured R01 funding to revisit the pathway using human iPSC-based models. These studies revealed that Melusin also modulates lipid metabolism, thereby limiting oxidative damage and enhancing cardiac resilience. Together, these findings have illuminated a novel cardioprotective signaling axis with strong potential for translational applications in heart disease.

1. Sbroggiò M, **Bertero A**, ... Tarone G (2011). ERK1/2 activation in heart is controlled by melusin, focal adhesion kinase and the scaffold protein IQGAP1. *J. Cell Sci.* 124, 3515–24.
2. Sbroggiò M, Carnevale D, **Bertero A**, ... Tarone G (2011). IQGAP1 regulates ERK1/2 and AKT signalling in the heart and sustains functional remodelling upon pressure overload. *Cardiov. Res.* 91, 461–70.
3. Sorge M, ... [28 authors], **Bertero A**[#], Brancaccio M[#] (2024). ^{#co-senior}. An intrinsic mechanism of metabolic tuning promotes cardiac resilience to stress. *EMBO Molecular Medicine*, 16, 2450-85

2. Epigenetic and epitranscriptional control by TGFβ signalling.

The focus of my doctoral research was to clarify the molecular mechanisms by which the Activin/Nodal/TGFβ–SMAD2/3 pathway controls early cell fate decisions in hPSCs. Although SMAD2/3 was canonically regarded as a transcription factor, how it regulated complex gene expression networks in hPSCs was at the time poorly understood. In a first study, I showed that TGFβ maintains pluripotency both in vitro and in vivo by shaping the H3K4me3 epigenetic landscape through SMAD2/3 in cooperation with NANOG and the MLL/SET1A complexes. In a second study, through large-scale proteomic screening, I revealed that SMAD2/3 associates not only with transcriptional and epigenetic cofactors, but also with multiple complexes involved in DNA repair, mRNA biogenesis, and RNA modification. I followed up this unexpected result and demonstrated that TGFβ regulates the stability of key pluripotency transcripts by promoting deposition of the N6-methyladenosine (m6A) mark through SMAD2/3 interaction with the m6A methyltransferase complex. This was the first mechanistic demonstration linking extracellular signaling to epitranscriptomic regulation. More broadly, my work revealed the multifaceted functions of TGFβ signaling, providing a conceptual advance with broad implications across biology and disease.

1. **Bertero A**, ... Vallier L (2015). Activin/Nodal signaling and NANOG orchestrate human embryonic stem cell fate decisions by controlling the H3K4me3 chromatin mark. *Genes & Dev.* 29, 702–17. 126 citations
2. **Bertero, A**, ... Vallier, L. (2018). The SMAD2/3 interactome reveals that TGFβ controls m6A mRNA methylation in pluripotency. *Nature* 555, 256–59. 261 citations

3. Gene editing methods for robust loss- or gain-of-function studies.

Another major aspect of my doctoral research was the development of improved methods to manipulate gene expression in hPSCs and their derivatives. I combined an optimized tetracycline-inducible shRNA/sgRNA system with gene targeting into safe genomic harbors and CRISPR/Cas9 technology to create the OPTimized inducible KnockDown and KnockOut (OPTiKD and OPTiKO) platforms. In parallel, I co-developed an OPTimized inducible Overexpression (OPTiOX) platform based on safe-harbor targeting of an optimized doxycycline-inducible cDNA cassette. The potential of these technologies was demonstrated by forced expression of developmental transcription factors in hPSCs, enabling efficient derivation of multiple mature cell types including skeletal muscle, neurons, and oligodendrocytes. The OPTiKD/KO/OX technologies were patented and licensed to bit.bio, a cell engineering and manufacturing company in Cambridge, with sub-licenses to Meatable, a Dutch startup on cultivated meat.

As faculty, my lab has further advanced these approaches. We modified OPTiKD to enable pooled loss-of-function screens with single-cell RNA-seq (iPS2-seq), which we applied to dissect the function of candidate CHD genes in cardiac organoid models. We extended OPTiOX to support growth factor-independent pluripotency, enabling cost-effective PSC expansion for efficient differentiation, a technology with strong potential for cultivated meat. More recently, we resolved a major bottleneck in inducible systems by developing strategies to prevent silencing of doxycycline-inducible promoters during differentiation, including into hiPSC-derived cardiomyocytes. Finally, we created a complementary platform based on combinatorial CRISPR activation and interference, enabling scRNA-seq-based gain- and loss-of-function screens in hiPSCs. These latter technologies have been submitted as patent applications.

1. **Bertero A**[@], ... [17 authors], Vallier L[@] (2016). ^{@corresp}. Optimized inducible shRNA and CRISPR/Cas9 platforms for in vitro studies of human development using hPSCs. *Development* 143, 4405–18.
2. Pawlowski M*, Ortmann D*, **Bertero A***, ... [3 authors] Kotter MRN (2017). ^{*equal contribution}. Inducible and Deterministic Forward Programming of Human Pluripotent Stem Cells into Neurons, Skeletal Myocytes, and Oligodendrocytes. *Stem Cell Reports* 8, 803–812.
3. Balmas E, Ratto ML, Snijders K, Calogero R, Mendjan S, **Bertero A** (2024). Single cell transcriptional perturbome in pluripotent stem cell models. *Sneak Peek* doi: 10.2139/ssrn.4854180
4. Guichardaz M Bottini S, Balmas E, **Bertero A** (2024). Overcoming the silencing of doxycycline-inducible promoters in hiPSC-derived cardiomyocytes. *Open Res. Europe* doi: 10.12688/openreseurope.19024.1

4. Chromatin topology dynamics in cardiac development and disease.

Since my postdoctoral work, I have used hPSC-derived cardiomyocytes (hPSC-CMs) to investigate how chromatin organization influences cardiac development and disease. In the developmental context, I discovered that during differentiation heterochromatin compacts and large cardiac genes transition from repressive (B) to active (A) compartments. I identified a network of such loci that increase their inter-chromosomal associations and are targets of the splicing factor RBM20. Genome editing studies demonstrated that *TTN* pre-mRNA nucleates RBM20 foci, which recruit other RBM20 targets into proximity and facilitate their alternative splicing. These findings uncovered a cardiac-specific Trans-Interacting chromatin Domain (TID) functioning as a “splicing factory,” suggesting that RNA-driven hubs may represent a new principle of chromatin organization. In the pathological context, I modeled cardiac laminopathy using hiPSC-CMs from patients carrying *LMNA* mutations. I tested the “chromatin hypothesis,” which posits that laminopathy arises from aberrant chromatin compartmentalization. My studies showed that lamin A/C haploinsufficiency disrupts electrophysiology, contractility, gene expression, and chromosomal topology. Surprisingly, however, chromatin compartment changes were limited, with most dysregulated genes lying outside these hotspots. One exception was the neuronal calcium channel gene *CACNA1A*, whose misexpression contributes to the electrophysiological abnormalities observed in hiPSC-CMs.

As faculty, my lab has extended this line of research by exploring the concept that RNA factories regulate nucleic acid biogenesis through inter-chromosomal contact hubs. We developed a bioinformatic tool to detect such hubs from genomics data, trans-C, providing support for this model. Most recently, we co-developed *o-MAP*, a nascent transcript-based approach that revealed the molecular architecture of a single-locus nuclear compartment organized by RBM20 and *TTN* RNA, offering direct experimental support for RNA-driven genome organization.

1. **Bertero A**, ... [8 authors], Murry CE (2019). Dynamics of genome reorganization during human cardiogenesis reveal an RBM20-dependent splicing factory. *Nature Communications* 10, 1538
2. **Bertero A**, ... [10 authors], Murry CE (2019). Chromatin compartment dynamics in a haploinsufficient model of cardiac laminopathy. *Journal of Cell Biology* 218, 2919-44
3. Hirstov BH, Noble WS, **Bertero A** (2024). Systematic identification of inter-chromosomal interaction networks supports the existence of RNA factories. *Genome Research*, 34, 1610-1623
4. Kania E, ... [3 authors], Bianchi S, Hristov B, ... [7 authors], **Bertero A**[@], Murry CE[@], Shechner D[@] (2024). ^{@corresponding}. Nascent transcript O-MAP reveals the molecular architecture of a single-locus subnuclear compartment built by RBM20 and the *TTN* RNA. *bioRxiv* doi: 10.1101/2024.11.05.622011

5. Cardiac regeneration with hPSC-derived cardiomyocytes.

Since my postdoctoral training, I have focused on applying gene editing to improve the safety and efficacy of cardiac regeneration therapies based on hPSC-derived cardiomyocytes (hPSC-CMs). As part of the UW Heart Regeneration Program (HRP), I led a team of three scientists responsible for generating gene-edited hPSC lines with the primary goal of reducing hPSC-CM arrhythmogenicity. We generated RNA-seq datasets of hPSC-CM maturation in vivo and selected several candidate genes for editing. After testing more than a dozen hPSC lines, we identified a combination of three knockouts (*HCN4*, *CACNA1H*, *NCX*) and one overexpression (*KCNJ2*) that yielded quiescent yet excitable hPSC-CMs, which elicited no engraftment arrhythmia. In parallel, I contributed to complementary studies showing that hPSC-CM engraftment and maturation can be enhanced by co-transplantation with epicardial supporting cells, and that a pharmacological combination of Amiodarone and Ivabradine mitigates engraftment arrhythmia.

As faculty, my group has extended this translational focus by developing refined in-house media that enable cost-effective, weekend-free hPSC culture. This advance supports scalable production of hPSC-CMs for both research and therapeutic applications, while significantly lowering cost and labor barriers.

1. **Bertero A**, and Murry C.E. (2018). Hallmarks of cardiac regeneration. *Nature Reviews in Cardiology* 15, 579–80
2. Marchiano S, ... [26 authors], **Bertero A**[#], Murry CE[#] (2023). ^{#co-senior}. Gene editing to prevent ventricular arrhythmias associated with cardiomyocyte cell therapy. *Cell Stem Cell* 30, 396-414
3. Truszkowski L, Bottini S, [15 authors] ... Balmas E, Elton C, **Bertero A** (2024). Refined and benchmarked homemade media for cost-effective, weekend-free human pluripotent stem cell culture. *Open Res. Europe* doi: 10.12688/openreseurope.18245.2

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