

BIOGRAPHICAL SKETCH

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NAME: Azzoni, Emanuele

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POSITION TITLE: Associate Professor of Cell and Experimental Biology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Parma, Italy	B. Sc.	07/2003	Biotechnology
University of Bologna, Italy	M.Sc.	10/2005	Medical Biotechnology
University of Milano-Bicocca, Italy	Ph. D.	03/2011	Molecular and Translational Medicine
University of Oxford, UK	Post-doctoral training	01/2019	Developmental hematopoiesis

A. Personal Statement

I am a cellular and molecular biology basic research scientist with a strong interest in experimental hematology, in both physiological and pathological contexts. After a Ph.D. in Translational and Molecular Medicine (University of Milano-Bicocca, Italy) and a post-doctoral fellowship (University of Oxford, UK) in 2019 I established my own research group at the School of Medicine and Surgery of University of Milano-Bicocca in Monza, Italy. I am currently leading a group of four post-docs, two Ph.D. students, one technician and one undergraduate master student. More information about our group can be found at: <https://hevaresearch.unimib.it/>

During my post-doc, my research focused on the developmental biology of hematopoietic stem and progenitor cells (HSPCs). I studied the role of micro-environmental factors such as cytokines and blood flow in HSPCs emergence in the mouse embryo (Azzoni et al., *EMBO Reports*, 2018; Azzoni et al., *Cell Reports*, 2021). As an independent investigator, I continued working on projects aimed at investigating the cellular origins of embryonic and fetal hematopoiesis, primarily using the mouse as a model system. We have recently discovered and characterized the emergence of fetal-restricted multipotent hematopoietic progenitors in the early mouse embryo, and highlighted this progenitor subset as the main contributor to fetal lympho-myelopoiesis (Barone et al., under revision; <https://www.biorxiv.org/content/10.1101/2024.07.11.603050v1>).

Moreover, I am strongly interested in pediatric myeloid malignancies with prenatal onset, including juvenile myelomonocytic leukemia (JMML) and childhood acute myeloid leukemia (cAML). We recently demonstrated that HSC-independent progenitors are susceptible to leukemic transformation upon acquisition of the MLL-Af9 translocation (Barone et al., *Cancers* 2023).

In our lab, we have established novel transgenic murine models that allowed the investigation of the biology underlying the prenatal origins of JMML (Quattrini et al., in preparation). Currently, we are using genetic and pharmacological approaches to study the role of genes involved in molecular pathways of cellular stress response and inflammation in JMML onset and progression. We recently expanded our array of models to humanized murine models of JMML, enabling a smoother translation of our findings to the clinic.

In summary, my expertise puts me and my research group in a great position to successfully carry out the proposed research project.

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

- Worldwide Cancer Research
2024-2027; Role: PI.
“Targeting inflammatory pathways in juvenile myelomonocytic leukemia”,
- Ministry of University and Research (PRIN 2022 PNRR)
2023-2025; Role: Unit PI (Project PI: A.Fantin)
“Novel roles for CXCR4 signaling in HEmatovascular development (CHEVron)”
- Ministry of University and Research (PRIN 2022)
2023-2025; Role: Unit PI (Project PI: T.Genova)
“Exploiting organ-on-a-chip technology to unravel the dynamic adaptation of microvasculature to biomechanical environmental cues (OC2AdMirE)”
- CARIPLO-Telethon Alliance
2023-2026; Role: PI.
“Novel targets modulating the inflammatory response in juvenile myelomonocytic leukemia”
- Leukemia Research Foundation (LRF) New Investigator Blood Cancer Research Grant
2021-2022; Role: PI.
“Generating a new model of juvenile myelomonocytic leukemia to study its cellular origins and to identify therapeutic vulnerabilities”
- CARIPLO Biomedical research conducted by young researchers
2010-2023; Role: PI.
“Childhood Acute Myeloid Leukemia: defining the cellular origins in the embryo and the pre-leukemic molecular signature”

B. Positions, Scientific Appointments, and Honors

Nov 2024 - present	Associate Professor in Cell and Experimental Biology, School of Medicine and Surgery, University of Milano-Bicocca, Monza (Italy)
Nov 2021 – Oct 2024	Tenure-track Assistant Professor in Cell and Experimental Biology, School of Medicine and Surgery, University of Milano-Bicocca, Monza (Italy)
Feb 2019 – Oct 2021	Junior Assistant Professor in Cell and Experimental Biology, School of Medicine and Surgery, University of Milano-Bicocca, Monza (Italy)
Dec 2012 – Jan 2019	Post-Doctoral Researcher, PI: Prof. Marella de Bruijn, MRC Molecular Hematology Unit, Weatherall Institute of Molecular Medicine (WIMM), University of Oxford (UK)
Apr 2012 – Nov 2012	Post-Doctoral Researcher, PI: Prof. Bruno Péault, Centre for Regenerative Medicine, University of Edinburgh, Edinburgh (UK)
Apr 2011 – Apr 2012	Post-Doctoral Researcher, PI: Prof. Silvia Brunelli, Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Scientific Institute, Milan (Italy)
Jan 2008 – Mar 2011	PhD student, PI: Prof. Silvia Brunelli, University of Milano-Bicocca and San Raffaele Scientific Institute, Milan (Italy)

Honors

2023	Worldwide Cancer Research Award
2021	Leukemia Research Foundation “Hollis Brownstein” New Investigator Blood Cancer Award
2019	CARIPLO Biomedical Research conducted by Young Researchers Award
2019	Eligibility for Associate Professorship (ASN) in Applied and Cell Biology
2016	Award for Excellence, Radcliffe Department of Medicine, University of Oxford

C. Contributions to Science

I have so far authored **26 publications** in peer-reviewed international journals (**H-index: 14**; Total number of citations: **2,778**). Several articles are currently under revision. Listed below are my top 5 contributions to science.

Contribution #1 – Hematopoietic stem/progenitor biology and their microenvironment

The complex array of cells in our blood is constantly replenished by a pool of specific stem cells. Haematopoietic stem cells (HSCs) have the capacity to form all types of blood cells. HSCs first appear in the embryo during development, and originate in the embryonic arteries from so-called haemogenic endothelium. The role of micro-environmental factors in HSC emergence in the mouse embryo is poorly understood. I have identified Kit Ligand (Kitl) as a common regulator of embryonic hematopoietic niches *in vivo* (Azzoni et al., *EMBO Reports*, 2018). Next, I have used a combination of 3D imaging, functional assays and single cell transcriptomics to show that the onset of circulation promotes the transition of haemogenic endothelium into HSC precursors, and also showed that this process is associated to previously unidentified key metabolic changes, required for normal HSC development (Azzoni et al., *Cell Reports*, 2021). Later, I have collaborated with the group of Christiana Ruhrberg (UCL) to demonstrate that despite being widely expressed in the embryonic endothelium, the Kitl receptor Kit is not involved in the vascularisation of embryonic organs, though its requirement in the hematopoietic system indirectly impacts vascular development (Tacconi et al *Angiogenesis* 2022). Collaborative work with the group of Ana Cumano (Institut Pasteur, Paris, France) mapped the fetal liver hematopoietic microenvironment at unprecedented resolution (Mesquita Peixoto et al., under revision). Our latest work characterized the emergence of fetal-restricted hematopoietic stem/progenitor cells (HSPCs) in the embryo. We found that those HSPCs contribute the majority of lympho-myelopoiesis up until the end of gestation and emerge from a specific subset of haemogenic endothelium (Barone et al., under revision).

1. **Azzoni E***, Frontera V, Anselmi G, Rode C, James C, Deltcheva EM, Demian AS, Brown J, Barone C, Patelli A, Harman J, Nicholls M, Conway SJ, Morrissey E, Jacobsen SE, Sparrow DB, Harris AL, Enver T, de Bruijn MFTR*, "The onset of circulation triggers a metabolic switch required for endothelial to hematopoietic transition", *Cell Reports*, 2021 Dec 14;37(11):110103. doi: 10.1016/j.celrep.2021.110103. *: corresponding author.
2. **Azzoni E***, Frontera V, McGrath KE, Harman J, Carrelha J, Nerlov C, Palis J, Jacobsen SEW, De Bruijn MFTR* "Kit ligand has a critical role in mouse yolk sac and aorta-gonad-mesonephros hematopoiesis", *EMBO Reports* 2018 Oct;19(10). pii: e45477. doi: 10.15252/embr.201745477. Epub 2018 Aug 30. *: corresponding author
3. Tacconi C, Plein A, Colletto C, Villa E, Denti L, Barone C, Javanmardi Y, Moeendarbary E, **Azzoni E**, Fantin A, Ruhrberg C, "KIT is dispensable for physiological organ vascularisation in the embryo", *Angiogenesis* 2022 Apr 13. doi: 10.1007/s10456-022-09837-6.
4. Mesquita Peixoto M, Soares-da-Silva F, Bonnet V, Zhou Y, Ronteix G, Santos RF, Mailhe MP, Nogueira G, Feng X, Pereira JP, **Azzoni E**, Anselmi G, de Bruijn M, Perkins A, Baroud CN, Pinto-do-Ó P, Cumano A "Spatiotemporal dynamics of fetal liver hematopoietic niches", under revision. (Preprint available at: <https://www.biorxiv.org/content/10.1101/2023.08.24.554612v1>)
5. Barone C, Orsenigo R, Cazzola C, ... Fantin A, Brunelli S, Piazza R and **Azzoni E***, "Hemogenic endothelium of the vitelline and umbilical arteries is the major contributor to mouse fetal lympho-myelopoiesis", under revision. (Preprint available at: <https://www.biorxiv.org/content/10.1101/2024.07.11.603050v1>)

Contribution #2 – Biology of erythro-myeloid progenitors (EMPs)

A significant part of my research in collaboration with other groups has contributed to elucidate the biology of HSC-independent hematopoietic progenitors. In collaboration with Frederic Geissmann (King's College, London, UK), we made the seminal discovery that yolk sac (YS) erythro-myeloid progenitors (EMPs) seed the fetal liver and are the source of the vast majority of adult tissue-resident macrophages persisting throughout adulthood in multiple tissues (Gomez-Perdiguero et al., *Nature* 2015). I had a key role in this project, as I was in charge of the *ex vivo* functional assays required to demonstrate the true EMP identity of the YS Csf1r+ progenitors contributing to tissue-resident macrophages. This ground-breaking work profoundly impacted the field and has been cited more than 1,650 times so far. In collaboration with the groups of Adam Mead (University of Oxford, UK) and Georges Lacaud (University of Manchester, UK), I have helped demonstrating that the epigenetic regulator Ezh2 is intrinsically dispensable for HSC function in the FL, though required in the microenvironment (Neo et al., *Blood*, 2018). We next demonstrated that Ezh2 is essential for EMP function through modulation of Wnt signaling (Neo et al., *Nature Communications*, 2021). Recently, we have continued our collaboration with the group of Georges Lacaud to characterize and profile YS hemogenic endothelium (Neo et al., under revision).

1. *Perdiguero EG, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, Garner H, Trouillet C, De Bruijn MF, Geissmann F, Rodewald HR "Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors" Nature 2015 Feb 26; 518 (7540):547-51.*
2. *Neo WH, Meng Y, Rodriguez-Meira A, Fadlullah M, Booth C, Azzoni E, Thongjuea S, de Bruijn MFTR, Jacobsen SE, Mead A, Lacaud G "Ezh2 is essential for the generation of functional yolk sac derived erythro-myeloid progenitors", Nature Communications, 2021 Dec 2;12(1):7019. doi: 10.1038/s41467-021-27140-8.*
3. *Neo WH, Booth CAG, Azzoni E, Chi L, Delgado-Olguin P, De Bruijn MFTR, Jacobsen SEW, Mead AJ "Cell-extrinsic hematopoietic impact of Ezh2 inactivation in foetal liver endothelial cells", Blood. 2018 Mar 19. pii: blood-2017-10-811455. doi: 10.1182/blood-2017-10-811455.*
4. *Neo WH, Fadlullah MZH, Bhatnagar H, Barone C, Quattrini G, Timóteo-Ferreira F, Carrelha J, Sala G, Weightman W, Breitwieser W, Moncaut G, Thambyrajah R, Jacobsen SE, Iqbal M, Murtuza Baker S, Azzoni E, Lie-A-Ling M, Lacaud G "Single cell profiling reveals three endothelial to hematopoietic transitions with divergent isoform expression landscapes", under revision.*

Contribution #3 – Biology of fetal lymphoid progenitors

In collaboration with the group of Sten Eirik W. Jacobsen (University of Oxford, UK and Karolinska Institutet, Sweden), I helped identifying and characterizing the first lymphoid-restricted progenitors emerging during development before HSCs, termed LMPPs (Böiers et al., *Cell Stem Cell* 2013). This was a seminal paper that deeply influenced the field, as it showed that the onset of lymphoid potential is uncoupled from HSCs; it has been cited more than 210 times so far. In this project, I had a key role as I performed the experiments that led to conclusively demonstrate a yolk sac origin for these progenitors. Later, in collaboration with the same group, I contributed to the demonstration that the first progenitors seeding the embryonic thymus are in fact lympho-myeloid immune-restricted HSC-independent progenitors (Luis et al., *Nature Immunology* 2016). For this project, I established a novel method for whole-mount imaging of the embryonic thymus. Ongoing collaboration with the Jacobsen and Böiers research teams continues to characterize B lymphopoiesis in the embryo in physiology and disease. Indeed, we recently identified the first emerging B cell-restricted progenitor in mouse development (Strålin et al., under revision).

1. *Böiers C, Carrelha J, Lutteropp M, Luc S, Green JC, Azzoni E, et al., "Lymphomyeloid contribution of an immune-restricted progenitor emerging prior to hematopoietic stem cells" Cell Stem Cell 2013 Nov 7;13(5):535-48*
2. *Luis TC, Luc S, Mizukami T, Boukarabila H, Thongjuea S, Woll PS, Azzoni E, et al., "Initial seeding of the embryonic thymus by immune-restricted lympho-myeloid progenitors", Nature Immunology, 2016 Dec;17(12):1424-1435. doi: 10.1038/ni.3576.*
3. *Strålin KB, Seki M, Green J, Loughran SJ, Högstrand K, Markljung E, Winroth A, Hillen A, Chari E, Böiers C, Azzoni E, Carrelha J, Yoshizato T, Woll P, Jacobsen SEW, "Identification of a B-cell restricted progenitor emerging early in mouse fetal development", under revision.*

Contribution #4 – Development and characterization of new models to study the biology of myeloid neoplasms

This is a line of research that I initiated upon starting my independent research group in Milan. We have developed and characterized new transgenic mouse models to investigate the biology of pediatric and adult myeloid neoplasms. In collaboration with the group of Rocco Piazza (University of Milano-Bicocca), we have contributed to characterize a new transgenic mouse model of SETBP1-mutated myelofibrosis (Crespiatico et al., *Blood* 2024). Work in our lab is centered around pediatric myeloid malignancies with prenatal origin. We recently demonstrated that HSC-independent progenitors are susceptible to leukemic transformation upon acquisition of the MLL-Af9 translocation (Barone et al., *Cancers* 2023). Our ongoing projects are focused on juvenile myelomonocytic leukemia (JMML). We have developed new preclinical models for this disease, and are taking advantage of those in order to investigate understudied aspects such as the nature of the cell of origin and the impact of inflammatory and cellular stress response pathways on leukemia onset and progression.

1. *Barone C, Orsenigo R, Cazzola A, D'Errico E, Patelli A, Quattrini G, Vergani B, Bombelli S, De Marco S, D'Orlando C, Bianchi C, Leone BE, Meneveri R, Biondi A, Cazzaniga G, Rabbitts TH, Brunelli S and Azzoni E*. Hematopoietic Stem Cell (HSC)-Independent Progenitors Are Susceptible to MLL-Af9-Induced Leukemic Transformation. Cancers. 2023; 15(14):3624. <https://doi.org/10.3390/cancers15143624> *: corresponding author.*
2. *Crespiatico I, Zaghi M, Mastini C, D'Aliberti D, Mauri M, ..., Azzoni E, Sessa A, Gambacorti-Passerini C, Elli EM, Mologni L, Piazza R. "First-hit SETBP1 mutations cause a myeloproliferative disorder with bone marrow fibrosis", Blood 2024 Apr 4;143(14):1399-1413. doi: 10.1182/blood.2023021349.*

Contribution #5 – Biology of embryonic and adult mesoangioblasts

As a Ph.D. student, I investigated the developmental origin of embryonic mesoangioblasts, vessel-associated progenitors recently discovered in the lab of Giulio Cossu (San Raffaele Scientific Institute). To this aim, I used a

genetic lineage tracing strategy based on the Cdh5-CreER^{T2} transgenic mouse in order to permanently label and follow the fate of a population of VE-Cad⁺ endothelial cells within the yolk sac and in the placenta. We found that these VE-cad⁺ derived cells expressed hematopoietic but not myeloid/macrophage markers, and displayed mesoangioblast features both *in vitro* and *in vivo* (Azzoni et al., *Development* 2014). Specifically, this novel progenitor population could contribute to smooth and skeletal muscle development and could differentiate to multiple mesodermal lineages *in vitro*. When transplanted into the muscle of wild type or dystrophic mice, these cells could contribute to post-natal muscle regeneration. Later on, I further demonstrated that treatment with nitric oxide donors can enhance the endogenous contribution of VE-Cad⁺ cells to skeletal muscle (Tirone et al., *Plos One* 2016). Moreover, my research also helped characterizing the identity of adult mesoangioblasts, which we found to be instead associated with muscle pericytes. These non-canonical myogenic progenitors contributed to postnatal muscle growth in an unperturbed setting, and this contribution increased during acute or chronic muscle damage (Dellavalle et al., *Nature Communications*, 2011). This seminal work has been cited more than 350 times so far.

1. Cossu G, Tonlorenzi R, Brunelli S, Sampaolesi M, Messina G, **Azzoni E** et al., "Mesoangioblasts at 20: From the embryonic aorta to the patient bed", *Front Genet.* 2023 Jan 4;13:1056114. doi: 10.3389/fgene.2022.1056114.
2. **Azzoni E**, Conti V, Campana L, Dellavalle A, Adams RH, Cossu G and Brunelli S "Hemogenic endothelium generates mesoangioblasts that contribute to several mesodermal lineages *in vivo*", *Development* 2014 141:1821-1834; doi:10.1242/dev. 103242
3. Tirone M, Conti V, Manenti F, Nicolosi P, D'Orlando C, **Azzoni E***, Brunelli S*, " Nitric Oxide Donor Molsidomine positively modulates myogenic differentiation of embryonic endothelial progenitors", *Plos One*, 2016 Oct 19;11(10):e0164893. doi: 10.1371/journal.pone.0164893. *:corresponding author
4. Dellavalle A, Maroli G, Covarello D, **Azzoni E**, Innocenzi A, Perani L, Antonini S, Sambasivan R, Brunelli S, Tajbakhsh S and Cossu G "Pericytes resident in post-natal skeletal muscle differentiate into muscle fibres and generate satellite cells" *Nature Communications* 2011 Oct 11;2:499.