Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

# Pediatric Research Institute “Città della Speranza”
## Padova

# SCIENTIFIC REPORT
## 2022-2024



# Contents

# Governance and Staff

**President** Franco Masello
**CEO** Stefano Lupi

**Scientific Director** Prof. Eugenio Baraldi
**Scientific Coordinator** Prof. Maurizio Muraca

**Board of Directors**
Guglielmo Bedeschi
Andrea Camporese
Giuseppe Dal Ben
Antonio Parbonetti
Giorgio Perilongo

**Scientific Coordinaton Commitee**
Prof. Eugenio Baraldi
Prof. Maurizio Muraca
Prof. Alessandra Biffi
Dr. Martina Piccoli

**Innovation and Technology Transfer** Davide Ederle

**Secretariat of the Scientific Direction**
Teresa Borghi
Davide Guerini

**Research Grant Officer** Maria Pisano

**Staff Direction**
Paola Passuello
Martina Esperti
Mariangela Carta
Giulia Ghedini

**Scientific Advisory Board**
**President** Prof. Andrea Biondi
**Members**
Prof. Sergio Abrignani
Prof. Ruggero De Maria
Prof. Vassilios Fanos
Prof. Graziella Pellegrini
Prof. Manuela Teresa Raimondi
Prof. Orsetta Zuffardi

# The Padua model of pediatric research


**The three pillars of the Framework Agreement**
The respective contributions of Institute of Pediatric Research, University of Padua and Padua Hospital allow to cover all bench-to-bedside steps in translational medicine.

# INTRO

From left to right: Eugenio Baraldi, *Scientific Director* - Maurizio Muraca, *Scientific Coordinator*

*"Translating research into children's health"*

## Eugenio Baraldi, *Scientific Director*
## Maurizio Muraca, *Scientific Coordinator*

With 200 investigators, the Institute is a world-leading research institute for pediatric medicine. During the three-year period 2022-24, the institute has confirmed the excellence of its scientific research, thanks to the skills and commitment of its scientists and to the fundamental support of the Fondazione Città della Speranza and its volunteers.

In agreement with the President and the CEO, the priorities of the Scientific Leadership were the development of translational research and the upgrading of the Institute's facilities with the participation of our researchers in close collaboration and synergy with the University of Padova and with the Azienda Ospedale di Padova, particularly with the Department of Women's and Children's Health. This collaboration has recently been strengthened by the signing of a new framework agreement between these institutions.

The development of translational research at the therapeutic level has reached an important milestone with the approval by the European Medicines Agency of the first in human clinical trial of an innovative experimental treatment for a serious lung disease in premature newborns, developed at our institute. An additional example of the translational impact of our research consists in the implementation of novel protocols for molecular characterization of pediatric tumors in the context of therapeutic trials conducted across Europe. Moreover, new first-in-human clinical trials are being instructed in the area of hematopoietic cell and gene therapy for the treatment of autoimmune and metabolic disorders of childhood.

The facilities of bioinformatics and imaging were enhanced with the acquisition of new equipment and particularly with the recruitment of young and selected personnel who will ensure the development and maintenance of precious technical and scientific knowledge. The certification process of the biological bank has begun, in collaboration with the Padua Hospital, and a facility for organoids is being set up, supporting the development of these increasingly important tools in biomedical research.

The researchers were involved in the Institute's programming through the establishment of working groups, which provided a fundamental contribution to establishing priorities for resource allocation. The Scientific Retreats, which took place in September 2022 and April 2024, provided an opportunity to take stock of the Institute's activity and were an important moment of exchange between the various research groups.

Collaboration with biotech and pharmaceutical companies has been promoted, which is essential both to attract new investments in research and to establish partnerships necessary to transfer the Institute's scientific results to the clinical level. The support of Fondazione CARIPARO for the growth of pediatric research is also gratefully acknowledged.

Finally, we acknowledge the daily constructive interaction with researchers and the precious contribution of the scientific secretariat and of the administrative personnel.

# Franco Masello
## *President*

*"Donating is investing in life"*

The Pediatric Research Institute today is a reality that is measured at the highest levels in the world in research and is the Italian and European reference point in particular on pediatric oncohematological diseases.

If today we have reached this level that can also be measured by the Impact Factor that places this Institute in first place in Italy in pediatric research, I must thank the Scientific Director Prof. Eugenio Baraldi, the scientific coordinator Prof. Maurizio Muraca, the Principal Investigators of the 30 research groups and all the researchers.

The Institute is a unique reality in Italy that brings together university research, private funding and the hospital where the young patients are hospitalized.

This Scientific Directorate has in fact worked to bring research to be translational, that is, closer and closer to the bedside of young patients.

This is also demonstrated by the fact that one of the patents developed within IRP has entered into phase 1 clinical trial at the Department of Pediatrics, the first case in the Padua Hospital.

This year is also the 30th anniversary of the birth of the Città della Speranza Foundation and this Institute, owned by the Foundation, has represented a very important milestone which is to unite public and private forces in the interest of the health of the child.

A job that was not easy. They are three worlds that think with different mentalities but that have nevertheless found the strength to unite by putting the child at the center.

I also thank the CEO Gen. Stefano Lupi who took office in 2023 and who has certainly made a great contribution to bringing the organization to a high level of efficiency.

A further step that will be taken in the next three years will be to make the Research Institute dialogue with private pharmaceutical companies by making available the research excellence and the numerous facilities (animal enclosure, bioinformatics, microscopy, clean rooms, etc.) always with the aim of being the first to save the lives of young patients who still do not have adequate treatment for the pathologies.

To conclude, a special thanks to the Città della Speranza Foundation which makes the Institute and research funding available, to the University of Padua (in particular the Department of Women's and Children's Health), to the Padova Hospital, and to the CARIPARO Foundation which has always been at our side in supporting research.

# Stefano Lupi
## *CEO*

*"We are the Children"*

It is with profound pride that today we can look at the reality of the Pediatric Research Institute, our reality which over time has consolidated to the point of becoming a certainty. The certainty of having exalted our values in recent years: the values of those who believe in scientific research, of those who are aware that the contamination of knowledge is the basis of scientific innovation, of those who work to transfer and transform discoveries into prevention and care for children.

The certainty that our researchers experience every day, with priceless commitment and an equal sense of duty and spirit of sacrifice, fight so that our children, our future, can grow up and become adults. A great team which, thanks to the continuous support of important supporters such as the "Città della Speranza" non-profit foundation, the "Cassa di Risparmio di Padova e Rovigo" foundation and the Veneto Region, is able to work with sophisticated and always latest generation technological tools. A team that integrates studies and expresses innovative multidisciplinary approaches, thus operating in the entire "pediatric universe" with and being able to maintain a constant dialogue with joint national and international research centers.

A team always ready to push the limits of science and to follow new and unexplored paths to find increasingly successful solutions, especially for pathologies with no cure. A team, an excellence of which we are deeply proud.

## Giovanni Paolino
## *President Fondazione Città della Speranza*

*"We want to find the promise land"*

Every day, thousands of citizens and volunteers commit time and economic resources to help our Foundation to continue its mission.

Thirty years have passed since that first thought that generated what we see today, a center of excellence where passionate and determined researchers work to complete this great journey at the end of which we want to find the promised land. A place where sick children are able to recover through less invasive therapies.

We are very confident that one step at a time this journey will reach its destination, and we find confirmation every day in the smiles of the young people who have made it.

The institute has the task of bringing us to the goal, and the continuous progress gives us hope for the future. We are therefore grateful and thankful for the work done and for what will be done, knowing that behind so much science there is also a great human passion made of commitment and competence.

## Daniela Mapelli, *Rector University of Padua*
## Antonio Parbonetti, *Prorector University of Padua*

*"Research: The Only Hope for Giving Children a Better Furure"*

Builders of a better future, for countless girls, boys, and their families. It's no coincidence that the Pediatric Research Institute "Città della Speranza" has chosen to link two elements in its name—research and hope—that are deeply intertwined. The passionate and skilled work of the IRP researchers fuels and often brings to life the hopes of young patients.

The collaboration between the University of Padua, the Padua Hospital – University, and the Città della Speranza Foundation – culminating in a new agreement that further enhanced the role of the Department of Women's and Children's Health – enables research to achieve results that can directly benefit those children, and their families, who place all their hopes in the hands of their doctors. This is also why the IRP's scientific report offers a window into the main lines of research, significant scientific results achieved, and the publications produced by the multidisciplinary work of numerous research groups at IRP, which drive the possibilities for increasingly effective treatments.

The goal is to continue along this path, strengthening collaboration among the three pillars of the "Padua model": a university renowned for its multidisciplinary approach and the high quality of its research; a hospital capable of achieving cutting-edge results each year in meeting the healthcare needs of citizens; and a foundation that annually provides substantial resources in support of research. The shared commitment is to increase children's chances of recovery and improve the quality of life for them and their families.

## Giuseppe Dal Ben
*General Manager Azienda Ospedale Università Padova*

*"Partnership: a winning model for Children's Health"*

Since 2010, the collaboration between Padua University Hospital, the University of Padua and the Pediatric Research Institute – IRP has been one of the main levers through which our Hospital promotes the activation of diagnostic, therapeutic and technological advancement processes capable of responding to the health needs of citizens who, due to the complexity, severity and rarity of the diseases they suffer from, require innovative diagnostic and therapeutic solutions resulting from the research activity.

This partnership, which has been active with significant results for about 15 years, has enabled Padua Hospital, and in particular the Department of Pediatrics, to promote new lines of research, offering researchers technologies and spaces suitable for the development of innovative solutions in a highly qualified and top-level context.

Young patients suffering from oncological diseases and rare diseases, to whom Padua Department of Pediatrics has always paid the utmost attention, both for the care commitment and for the development of new diagnostic and therapeutic opportunities, have greatly benefited.

The IRP hosts study, research - clinical, epidemiological, translational and basic - and advanced diagnostic activities dedicated mainly to childhood pathologies. The important results achieved in recent years prove the winning nature of the adopted model, underlining the importance of creating synergistic collaborations between hospitals, academia and research institutions, especially for the development of translational research.

A special mention deserves the first phase 1 study started in our Hospital related to the treatment for severe lung disease in premature infants.

The hope for the future is to further consolidate this partnership so that all institutions direct their strengths and share their resources in shared projects that can promote diagnostic and therapeutic progress aimed at offering the precision medicine that is being developed for the treatment of patients with rare and complex diseases.





# HIGHLIGHTS

**17,500 m²**
of Laboratories

**30**
Research Groups

**7**
Research Areas

**200**
Researchers

**43%**
Under 35

**75%**
Women

**530**
Publications
(2022–2023)

**17**
Top Italian Scientists
(H-index >30)

**56**
Active Research Grants

# Research Grants

IRP has participated in several calls promoted both by the Città della Speranza Foundation and other public and private institutions that support pediatric research.

### IRP Research Grants

In October 2023, Città della Speranza Onlus Foundation launched a call to support novel, cutting-edge pediatric research within the Pediatric Research Institute Città della Speranza. The call provided a budget of 3.3 million euros for clinical and translational research projects that aim to prevent pediatric pathologies and accelerate diagnosis and therapeutic innovation for childhood diseases. The call was designed to grant 3 different type of projects: "Starting Grant" to support young researchers with their own independent research program, "Consolidator Grant" aimed to help Principal Investigators to consolidate their research programs, and "Advanced Grant" to support the activities of already consolidated research groups. Of the 28 research projects presented, 14 were awarded (5 Starting, 6 Consolidator, 3 Advanced) recognizing their ground-breaking, translational and interdisciplinary approach, their applicability in the short term to clinical practice, and their strong collaboration with pediatric clinics.

### Fondazione Cariparo – Bando Ricerca Pediatrica

Cariparo Foundation has a long history of support for pediatric research. Its 2016-2022 call ended in October 2023 with the presentation of the results to the Board of Directors of the Cariparo Foundation and with the publication of a scientific report. The Cariparo foundation has therefore launched a new call in November 2023, "Bando Ricerca Pediatrica 2023" with a budget of 2.4 million euros over 3 years. We thank the Cariparo Foundation for its continuing support for the development of pediatric research.

### National and International Research Grants

Our research activities are supported by numerous national Nonprofit Organizations.

The **AIRC Foundation** has been contributing to our mission for years by funding projects of our researchers. There are currently two Investigator Grant projects and a fellowship for young researchers with PhDs.

Over the years, the Institute has been awarded as many as two projects funded by the national call for proposals of the **Just Italia Foundation**.

We received funding for a project from the **Peter Pan Association for Children with Cancer-ODV South Tyrol**, a project from the **Celeghin Foundation**, 7 fellowships from the **Umberto Veronesi Foundation**, and a project from the **Telethon Foundation**.

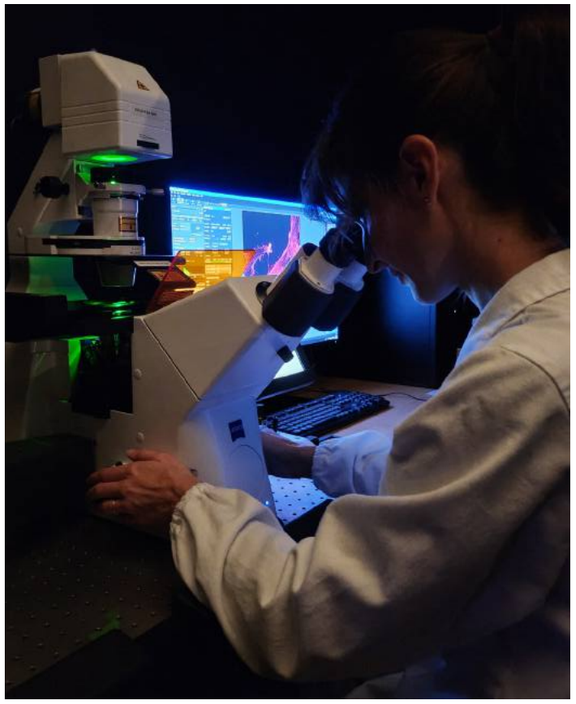
The Institute is also opening up to collaborations beyond national borders through participation in European funding programs. There is currently an ERC Starting Grant and a project funded by the **Fight Kids Cancer** program sponsored by the **European Science Foundation (ESF)**.

# FACILITIES

The Pediatric Research Institute has set up 8 internal facilities which not only offer technological support to research activities linked to IRP projects, but also play an important strategic role as they catalyse the methodological know-how developed by the research center which, in this way, it is made available to the entire research and innovation ecosystem.

## Imaging

**Facility managers** Dr. Diana Corallo, Dr. Fabio Munari



The IRP Imaging Facility is equipped with a series of microscopes listed below. A Zeiss LSM800 confocal microscope, fitted with Airyscan technology allows super-resolution imaging. The Airyscan system utilizes the scanning and optical sectioning abilities of a confocal microscope, delivering improved resolution at all imaging depths while using standard sample preparation techniques.

The Facility also hosts a Zeiss Axio Observer 7 inverted microscope, equipped with a Colibri LED light source. Additionally, the ApoTome III structured illumination system enhances contrast and resolution of biological samples. The presence of an incubator chamber allows for temperature and CO2 level adjustments, supporting the incubation of live samples and making the system ideal for long-term, live imaging under stable temperature conditions.

The Zeiss Axio Imager M1 is a basic upright motorized microscope, equipped with a color camera for imaging of histological samples and a greyscale camera for multichannel epifluorescence imaging of slide-mounted samples. This instrument also allows the acquisition of Z-stacks and tiled images.

Finally, the Leica Thunder Imager Model Organism is a fluorescence, motorized stereomicroscope that facilitates rapid and straightforward three-dimensional exploration and Z-stack acquisition through the volume of large specimens, including entire organisms or organoids. In addition, the instrument allows to get computationally cleared images directly in the live preview.

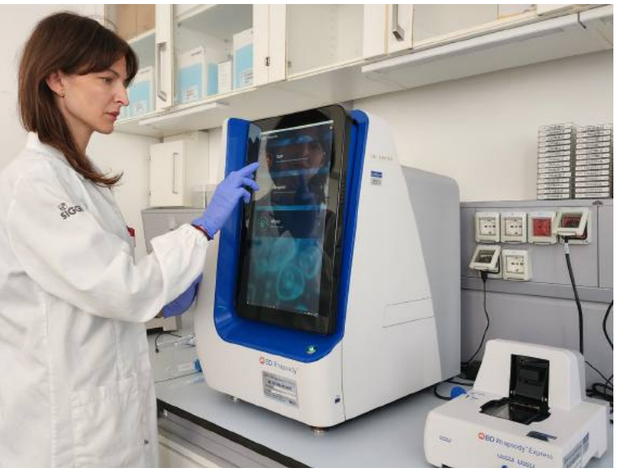
# Flow cytometry

**Facility manager** Dr. Chiara Frasson

The Flow Cytometry and Sorting Facility offers to all research's groups an efficient and personalized service. The facility provides three cytometers: a FC500-Beckman Coulter (equipped with a 488 nm laser), a Cytoflex-Beckman Coulter (equipped with three lasers: 488nm, 633nm and 405nm), a FACS Celesta -Becton Dickinson (equipped with four lasers: 488nm, 633nm, 405nm and 562nm) and two cell sorters: a MoFlo XDP (Beckman Coulter) and a FACS Aria III (Becton Dickinson). Both sorters are equipped with three lasers (MoFlo XDP: 488nm, 633nm and 355nm; FACS Aria III: 488nm, 633nm and 375nm).

# Single cell analysis

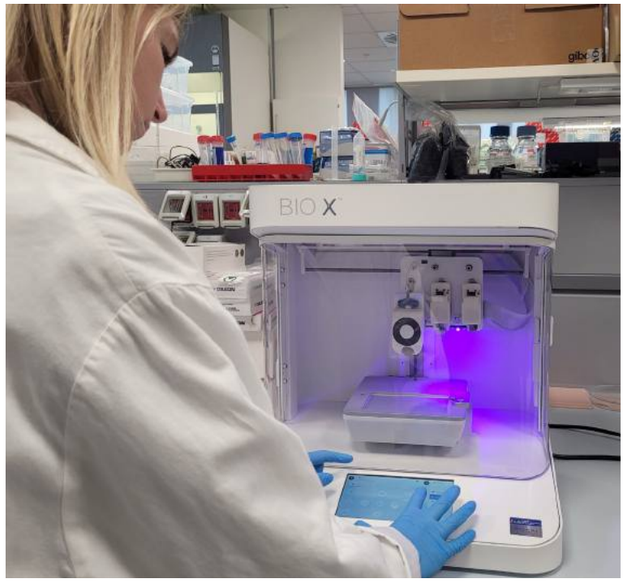
**Facility managers** Dr. Diana Corallo, Dr. Fabio Munari



The Single Cell Facility is equipped with all the instrument needed for the single cell mRNA libraries preparation workflow, including: the instrument BD Rhapsody, consisting in two Express modules for the capture and barcoding of the single cells, together with a scanner for the viability and quality check, two thermal cyclers for the synthesis of the mRNA libraries and two instruments for quantification and quality control of the libraries. Moreover, BD provides to the users of the facility free access to the platform Seven Bridges (www.sevenbridges.com), that helps the sequencing analysis with several apps and dedicated pipelines.

# 3D Bioprintring

**Facility managers** Dr. Martina Piccoli, Dr. Anna Maria Tolomeo





The 3D bioprinting facility includes the instruments listed below.

The *CellInk BioX bioprinter*, a complete standalone system that gives users great flexibility with exchangeable printheads and features. The BioX is capable of fabricating constructs containing any type of cells, enabling the fabrication of a wide range of tissue targets.

*The microfluidics-based RX1 Bioprinter*, a technology empowers tissue regeneration by:

- rapidly switching and patterning - precise placement of cells and biomaterials;
- using fibers as building blocks – microfluidic printheads extrude hydrogel fibers that can mimic features and structure of native tissue;
- bioprinting multicellular structures – inclusion of supporting cells, such as stromal or endothelial cells, can enhance the ability of a bioprinted therapeutic to promote tissue regeneration.

*The FormLab3B+ printer*, able to print a variety of surgical instruments and medical devices. This printer is suitable for:

- small to medium-sized parts requiring biocompatibility and sterilization compatibility;
- medical device prototypes, jigs, fixtures, molds, and end-use parts;
- custom anatomical models and surgical instruments for patients;
- visual aids for diagnostic and educational purposes;
- surgical planning models for diagnostic use within FDA-approved workflows;
- specialty materials research and development.

# Animal facility

**Facility manager** Dr. Giuseppe Germano

The Animal facility at the IRP provides equipment and related technologies for the accommodation and maintaining of small animals (mice and zebrafish) for biomedical research according to international (Directive 63/2010/EU) and national (D.lgs. 26/2014) legislation concerning the protection of animals used for scientific purpose.

The Mouse facility is a specific pathogen-free (SPF) animal research unit that consists of one housing room with a total capacity of 320 individually ventilated cages, three experimental procedure rooms and one fully equipped behavioral testing room.

The Zebrafish facility consists of an automatic recirculating aquaria system with the capacity to house up to 2,000 zebrafish in 160 tanks and separate lab room equipped with a variety of instrumentation for microinjection and fluorescence imaging.

# Metabolomics and Lipidomics

**Facility managers** Dr. Giuseppe Giordano, Dr. Manuela Simonato

Metabolomics provides a functional readout of the cell's biochemistry. It holds great potential to link the observed differences in the phenotype to the underlying biochemical and molecular mechanisms. The Metabolomics and Lipidomics IRP facility consists of a platform sites, provide by a mass spectrometry (MS) based metabolomic services and offer customized solutions for specific experimental setup. The site in IRP is part of the SDB Department Center for Human Metabolomics and Lipidomics and specializes in primary metabolism. By constructing a unique in house library with over 500 metabolites and over 120.000 from public web data bases of primary and secondary metabolites, the team developed a high throughput pipeline for comparative metabolic and lipidomics profiling. The introduction of Ion Mobility Mass Spectrometry and the use of advanced data processing software (including sophisticated data analysis) ensures a straightforward and high quality data output. The site also holds unique expertise in tracer (13C, 2H, etc.) based metabolomics and Lipidomics (target analysis). This technology provides crucial insights into the activity of metabolic pathways by administration of non-radioactive isotopically labeled substrates to the medium (in vitro) or organism (in vivo).

The main lines of our research are: development of projection to latent structures regression (PLS) able to explicitly include experimental design, development of new approaches to discover relevant and irrelevant features in PLS models, development of procedures to estimate the power of the PLS models and implementation of the developed procedures using the R platform. Moreover, our team supports users in the design of new experiments and offers a full service for data analysis.

Two laboratories are operational at the IRP: the Mass Spectrometry and Metabolomics Laboratory (Dr. G. Giordano) and the Pediatric Critical Care Laboratory (Prof. P. Cogo).

# Biobank

**Facility manager** Prof. Marco Agostini


The systematic and prospective storage of biological samples (tissues, blood derivatives, cells, nucleic acids) exceeding diagnostic needs is the fundamental basis for conducting scientific studies aimed at:

- contributing to the development of personalized therapies according to the genetic profile of the patient's pathology,
- identifying predictive markers of response to targeted therapies,
- promoting translational research ("from bench to bedside") in diagnostic-therapeutic pathways, in all their phases: screening, diagnosis, treatment, and follow-up.

Within the Pediatric Research Institute (IRP), there is a Pediatric Oncology Biobank (BBOP) for research purposes, with dedicated facilities and equipment for the cryopreservation of biological material from patients treated in the Pediatric units of the Azienda Ospedale-Università Padova. The BBOP-IRP Biobank contains more than 10,000 samples of pediatric onco-hematologic diseases, in the form of vital cryopreservations, pellets, and nucleic acids. BBOP-IRP is part of the strategic development project for a Unified Research Biobank under the Azienda Ospedale-Università Padova (Integrated Plan of Activities and Organization 2024–2026 – PIAO; Resolution of the General Director No. 177 of 31/01/2024), with which it will evolve to become:

- a cutting-edge biobank, both nationally and internationally,
- a biobank certified to ISO 9001:2015 and accredited to ISO 20387:2019, capable of ensuring high-quality standards for the management of biological materials and associated data.

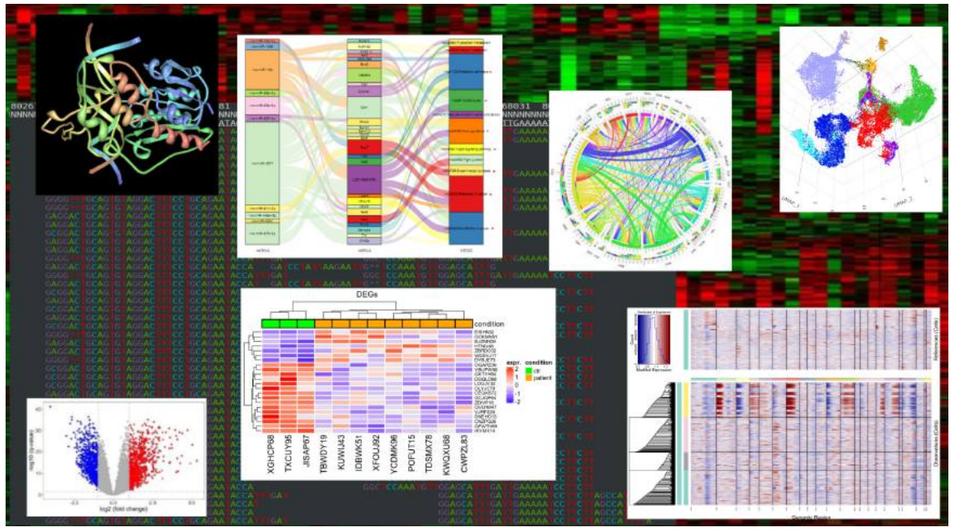
The consolidated experience in the retrieval and preservation of biological samples for research purposes, which underpins the established scientific background and support for translational research in biomedicine by various research groups, has allowed BBOP-IRP to become part of the BBMRI.it network (https://www.bbmri.it), the National Node of the European Research Infrastructure for Biobanks and Biomolecular Resources (BBMRI-ERIC), born thanks to the joint efforts of the Ministry of University and Research and the Ministry of Health. BBMRI.it is an infrastructure distributed throughout the national territory, comprising biobanks, biological resource centers, and collections located in various Italian regions. Membership in this system offers the Research Biobank operational support in various areas of development, as well as the opportunity to interact with the Italian excellence in the field.





# Bioinformatics

**Facility manager** Dr. Carlo Zanon
**Team member** Dr. Anna Corrà
**System manager member** Dr. Paolo Mazzon

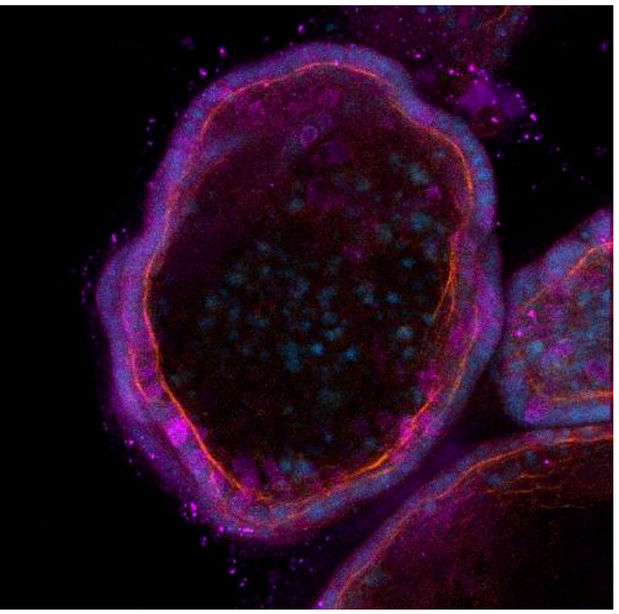



The Bioinformatics Core Service mission is to offer the IRP research community support at all stages of bioinformatics analysis, addressing a wide variety of needs ranging from experimental planning, data transfer and storage, data analysis, results interpretation, manuscript preparation and custom tools development.

The activities are based on a collaborative background and rely on a shared informatic setting for a full integration with the laboratories and the other facilities. The main activities focus on the management and analysis of big data produced by high-throughput NGS technologies from bulk and single-cell samples (Whole Genome Sequencing, Whole Exome Sequencing, Target Resequencing, Whole Transcriptome Sequencing, Barcode Tracking, Clonal Tracking, Whole Bisulfite Genome Sequencing, Reduced Representation Bisulfite Sequencing, ATAC-Sequencing). Other activities include the analyses of data produced by array based technologies, integration of omics data, mining of published datasets and data submission to public repositories. A common activity consists in coding custom scripts and developing tailored solutions to solve emerging problems.

The available hardware infrastructure includes two cluster nodes (1Tb of RAM for each node and a total of 110 computing cores), one server (64GB RAM, 16 cores), one tower workstation (64GB RAM, 16 cores) and one portable workstation (32GB RAM, 16 cores). The Core relies on a dedicated storage capacity of 40TB for its routine analyses demands.

# Organoids



A facility is being developed to derive organoid cultures from different organs and tissues including lung, intestine and various cancers. Organoids represent a breakthrough for biomedical research. They are self-organizing complex culture systems that closely resemble organs. All organoids are generated from stem cells and for this reason the analysis of organoid formation can provide valuable information about the mechanisms underlying organ development and organ regeneration. Their relevance is also widely recognized in pharmaceutical drug testing and to investigate the mechanisms of diseases at molecular level.

# Other instruments

- Autostainer 360-S2D (Ahsi)
- Freeze dryer LI5P with pump 15 m3/h (Vacuum Service)
- GentleMacs (Miltenyi)
- iBright FL1500 Imaging System (Thermo Fisher)
- Microplate reader Spark (Tecan)
- Nanosight NS300 (Malvern Panalytical)
- qNano TRPS Measurement System (iZON)
- QuantStudioTM 5 384-well Real-Time PCR System (Thermo Fisher)
- Seahorse XFe96 (Agilent)
- TissueLyser II (Qiagen)
- Ultracentrifuge Optima XE-90 (Beckman Coulter)





# SCIENTIFIC EVENTS

## IRP Retreats

Three days in which researchers come together to share their research with oral and poster presentations. Team building activities encouraged the birth of brand-new ideas and fruitful collaborations.

At the end of the meeting, the awards “*Famiglia Masello in memoria di Rita Masello e Massimo Zilio*” and “*Elisa Camporese*” were assigned to three young investigators.

**3th RETREAT IRP**
September 29th - 30th and October 1st, 2022

**4th RETREAT IRP**
April 11th - 12th - 13st, 2024

# Lectures and Seminars

31st January 2022
**Dr. Valentina Poletti**
Pediatric Research Institute
*"Early development of a viral-free ex vivo gene therapy for β-hemoglobinopathies"*

22nd April 2022
**Prof. Ivan Martin**
University of Basel, Switzerland
*"Engineered 3D culture systems as models for tissue development, regeneration and pathology"*

31st March 2022
**Prof. Manuela Teresa Raimondi**
Department of Chemistry, Materials and Chemical Engineering "G. Natta", Polytechnic University, Milan
*"Frontiers in cell modelling"*

16th May 2022
**Prof. Sergio Abrignani**
Scientific Director National Institute of Molecular Genetics "Romeo ed Enrica Invernizzi", Full Professor of Immunology and General Pathology, University of Milan
*"Mass vaccination throughout a pandemia"*

20th June 2023
**Prof. Jeffrey M. Beekman**
Department of Pediatric Pulmonology and the Regenerative Medicine Center, Utrecht
*"The (im)possibilities of airway organoids"*

27th October 2023
**Prof. Paolo Caliceti**
Full Professor of pharmaceutical Technology and Biopharmaceutics
Department of Pharmaceutical and Pharmacological Sciences, University of Padua
*"Supramulecolar multifunctional architectures for advances drug delivery"*

23rd November, 2023
**Prof. Ildiko Szabò**
Full Professor in Biochemistry at the Department of Biology, University of Padua
*"Targeting mitochondrial ion channels in cancer"*

15th December, 2023
**Prof. Marco Presta**
Full Professor in General Pathology at the Department of Molecular and Translational Medicine, University of Brescia
*"β-galactosylceramidase (GALC): from genetic diseases to cancer"*

26th January, 2024
**Prof. Giovanni Tosi**
Full Professor in Pharmaceutical Technology and Legislation at the Department of Life Sciences, University of Modena and Reggio Emilia
*"Nanomedicine: from Covid to Brain Diseases"*

23rd February, 2024
**Prof. Panagiotis Ntziachristos**
Full Professor at the Department of Biomolecular Medicine, Faculty of Medicine and Health Sciences, Ghent University
*"Dissecting and targeting transcriptome-related vulnerabilities in acute lymphoblastic leukemia"*

17th May, 2024
**Prof. Matteo Fassan**
Full Professor at the Department of Medicine, Surgical Pathology Unit, University of Padua, Veneto Institute of Oncology, IOV-IRCCS.
*"Molecular pathology, digital pathology and artificial intelligence: new actors in the clinical evolution of pathology"*

28th June, 2024
**Prof. Rudolf Oehler**
Associate Professor of the Department of General Surgery - Medical University of Vienna
*"Metastasis-Associated Fibroblasts in Peritoneal Surface Malignancies"*

1st October, 2024
**Prof. Daniel Tennant**
Professor in Biochemistry, Institutefor Metabolism and System Research (IMSR) University of Birmingham
*"Proline biosynthesis in health and disease - the importance of the cellular environment on compartmentalised metabolic networks"*

9th October, 2024
**Dr. Davide Ederle**
Innovation and Technology Tranfer
*"Patent Literature"*

# SCIENTIFIC DISSEMINATION EVENTS

## Open days

### Open Days for Secondary School Students

Every six month, IRP opens its doors to secondary schools in the Veneto region, to promote scientific culture and research to students who could be tomorrow's researchers.

### Open Days for citizens

During the past two years, the Institute received more than 2000 citizens who visited the laboratories and discussed with theinvestigators the most advanced research activities in Pediatrics.






## Youth, Health and Lifestyles

In May 22nd, 2023 more than 800 high school students from the region attended the event in the beautiful setting of Palazzo della Ragione in Padua. The event was the third stage of a journey to promote healthy lifestyles in collaboration with the Department of Women's and Children's Health, the Azienda Ospedale of Padua, the University of Padova, the Province of Padua, the City of Padua, the Veneto Region, the Salus Pueri Foundation and the Pediatric Research Institute "Città della Speranza".

"The Chart of Padua," a document intended for young people, parents, doctors, pediatricians, educators, and teachers aims at encouraging the early adoption of a harmonious set of correct lifestyles to promote health.

February 27th, 2022

## HANDS4RARE


Researches of the Institute visited several classrooms in Padua during February 2022, to spread knowledge on the importance of rare diseases and on the advencements of diagnosis and therapy in the field. The project included the round table "Diamoci una mano", meeting patients with rare diseases, and "Ricercatori per un'ora", a hands-on workshop for middle school children.

Solesino - September 24th, 2022

# Lady Run

A non-competitive charity walk to rise the importance of pediatric research.


May 7th, 2023 - April 12th,2024

# FIPAV

On Sunday 7 May, a thousand children competed in a volleyball match organized by the Regional Committee of the Italian Volleyball Federation (FIPAV). Sixty volleyball courts were set by volunteers from FIPAV and from the CdS Foundation to promote the value of sport activities among young people.

During th event, children and families visited the Research Tower accompanied by researchers to discover the laboratories and meet those who work concretely every day to find cures for pediatric diseases.





May 7th, 2023 - April 12th,2024

# UniSTEM DAY



UniStem Day is dedicated to high school students, organized by the UniStem Center since 2009. The day is intended as an opportunity for learning, discovery and discussion in the field of scientific research, particularly stem cell research. Experiences related to daily researchers' life and to the steps leading to technological and scientific progress will be covered. The event will take place in 97 universities and research institutes around the world in Australia, Denmark, France, Germany, Italy, Norway, the Netherlands, Poland, the United Kingdom, Serbia, Spain, Sweden and Hungary. In total 13 countries, 2 continents and 30,000 students: all together ready to embark on the infinite journey of scientific research.

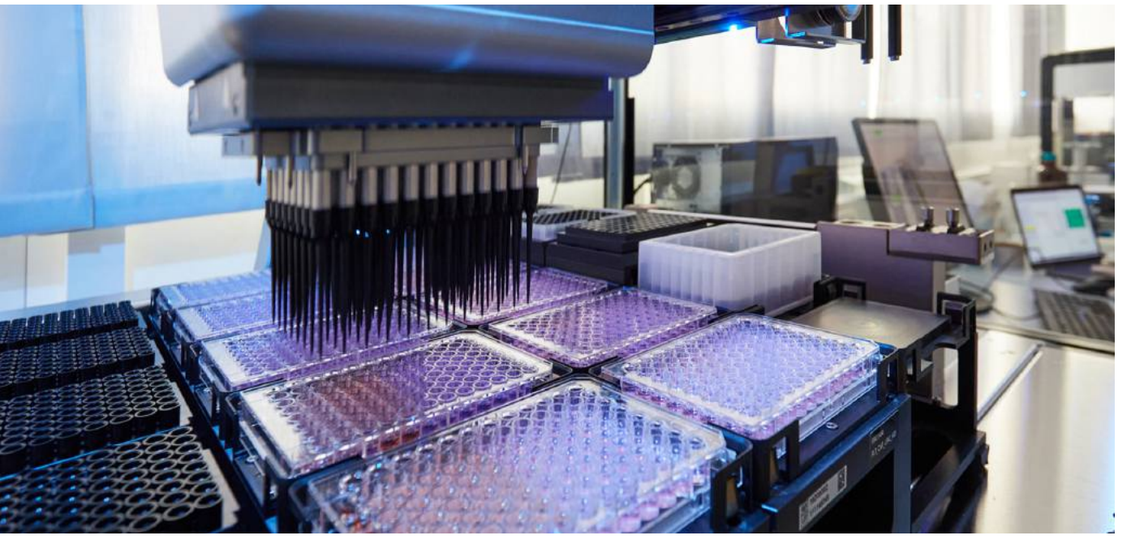
UniStem is the Coordinated Research Center on Stem Cells of the University of Milan, founded in 2006 by Elena Cattaneo, Giulio Cossu, Fabio Gandolfi and Yvan Torrente. The Center aims to integrate, coordinate and promote access to information on the study of stem cells and their application potential. UniStem Day is part of the scientific dissemination activities on stem cells promoted by the UniStem Center and celebrates its sixteenth edition this year.

# RESEARCH AREAS

RESEARCH AREA

## Pediatric Hematology, Oncology and Hematopoietic Cell&Gene Therapy

**Coordinator** Prof. Alessandra Biffi



The research area in pediatric hematology, oncology and hematopoietic cell&gene therapy belongs to the Division of Pediatric Hematology, Oncology and Stem Cell Transplant of the Padua University and Hospital and is devoted to performing cutting-edge research in:

- pediatric oncohematology, to improve diagnostics and identify novel mechanisms of tumorigenesis and new therapeutic targets;
- tumor modeling in 3D and in vivo, to enable testing of novel treatment approaches for pediatric cancer;
- hematopoietic cell and gene therapy, to develop innovative strategies for pediatric cancer, hemoglobinopathies, immune defects, neurometabolic and neurodegenerative diseases.

The mission of the group is translational in nature, with the goal of enabling therapeutic advances in pediatric hematological, oncological, metabolic diseases thanks to technology development and to a continuous dialogue between the clinical ward, the diagnostic labs and the research laboratory from bench to bedside and back.

The multidisciplinary nature of the team is at the basis of the success of the group.





**Prof. Alessandra Biffi**
*Coordinator Pediatric Hematology, Oncology and Hematopoietic Cell&Gene Therapy Area*

*Scopus ID 7003906961*

Prof. Biffi is the Chief of the Division of Pediatric Hematology, Oncology and Stem Cell Transplant at Padua University and at the Padua University Hospital since October 2018, and coordinates the Research Area on Onco-Hematology, Stem Cell Transplant and Gene Therapy at the Pediatric Research Institute in Padua. Previously, she was the Director of the Gene therapy Program and clinical attending in Stem Cell Transplant at the at Dana-Farber/Boston Children’s Cancer in Boston (2015-2018), and Head of unit at the San Raffaele Telethon Institute for Gene Therapy in Milano, where she also practiced as attending physician and head of a clinical unit in Pediatric Stem Cell Transplant and Immunohematology (up to 2015).

She trained over 60 fellows and post-doctoral fellows and numerous residents and medical students in her laboratory and clinics, the majority of whom are still in academic medicine.

She received a Consolidator ERC grant award and several other awards, including a Chair in Hematology at Boston Children’s Hospital. She has extensive clinical experience in pediatric stem cell transplant and in early phase cell therapy clinical trials. She is actively involved in advanced allogeneic transplant protocols for metabolic conditions, primary immunodeficiencies and hemoglobinopathies, as well as in gene therapy trials for neurological genetic diseases, hemoglobinopathies, immunodeficiencies and cancer.

Her preclinical and clinical research and clinical activity are dedicated at developing innovative treatment modalities for monogenic disorders based on hematopoietic stem cell (HSC) transplantation and gene therapy. Her research is devoted at enhancing the efficacy of HSC-based therapeutic approaches for disorders with severe nervous system involvement by fostering brain microglia replacement by donor cells after HSC transplantation upon detailed understanding of this phenomenon, and enhancing the potential of protein delivery to the affected nervous system by means of the gene corrected progeny of the transplanted and engineered HSCs.

Her seminal work on Metachromatic Leukodystrophy provided first obvious evidence of therapeutic efficacy of hematopoietic stem cell gene therapy for this disease and other similar disorders. This innovative treatment modality is now a registered advanced medicinal product, approved for commercialization by EMA and FDA. Similar promising data were generated in her laboratory in other neurometabolic and adult-onset neurodegenerative diseases, and new IP associated to these findings was generated and licensed. Recently, she established a program for CAR-T cell therapy development at Padua University and IRP targeting new antigens for pediatric acute myeloid leukemia, generated new IP and licensed it in the setting of a clinical development plan.

Over the past 20 years of independent translational research she received over 20M€ of funding from competitive academic grants, including prestigious grants from the European Research Council - ERC (Consolidator and Advanced grants), the European Innovation Council – EIC (Transition grant) and the NIH (R01). Moreover, she deposited multiple patents, most of which were licensed to the industry. She established proficient collaborations and sponsored research agreements with biotech companies in the gene therapy space. She also founded a biotech company, Altheia Science, spin off of the University of Padua, devoted to the development of gene therapy for autoimmune disorders and cancer.

# Advanced Diagnostics and Target Discovery in Acute Lymphoblastic Leukemia (ALL)

**Group Leaders**
Prof. Barbara Buldini - Principal Investigator
Prof. Martina Pigazzi - Co-Principal Investigator
Prof. Alessandra Biffi - Co-Principal Investigator

## Research activity

Acute lymphoblastic leukemia (ALL) is the most common cancer diagnosed in children. It has an overall survival of approximately 80%, with certain subsets experiencing greater than 98% cure rate. Incremental advances in therapy have led to marked improvements in survival since it was first treated, with these advances highlighting the importance of clinical trials through cooperative multicenter groups. Childhood ALL also serves as the paradigm for risk-based therapy. By identifying the features that have been shown to affect prognosis, patients can be classified into groups based on risk of treatment failure. Those with favorable features can be treated with less toxic regimens, whereas more aggressive regimens are reserved for those with more high-risk disease. It is therefore paramount to identify those features shown to consistently affect prognosis and, thus, influence treatment. Thus, our research aims at the identification of new genetic aberrations and mutations with prognostic value to be used as biomarkers to improve diagnosis and risk stratification of patients and their treatment response by employing tailored adapted therapies. To this goal, different advanced diagnostics and research initiatives are conducted in our group:

- **Advanced Flow Cytometry Application in Childhood Acute Leukemia**
  Prof. Barbara Buldin - Principal Investigator

- **Transcriptomics and Functional Genomics in JMML and B ALL**
  Dr. Silvia Bresolin - Principal Investigator

- **Phosphoproteomics for ALL diagnostics and research**
  Dr. Valentina Serafin - Junior Principal Investigator



# Advanced Flow Cytometry Application in Childhood Acute Leukemia

**Group Leader** Prof. Barbara Buldini - Principal Investigator

## Research activity

Acute Lymphoblastic Leukemia (ALL) is the most frequent cancer in childhood and the second cause of death for tumor in pediatric age, mainly after relapse. ALL is a heterogeneous group of neoplasms with different clinical, morphologic, immunophenotypic, and genetic features and variable response to therapy.

Improvements of immunophenotyping and response-to-therapy assessment by measurable residual disease (MRD) monitoring, together with the advancement in ALL characterization in molecular genetics by Next-Generation Sequencing (NGS) techniques, have determined a finest stratification of patients in risk-based classes. In the last decades, our Laboratory has been playing a central role in multiparametric flow cytometric application at diagnosis and MRD monitoring in pediatric ALL. One important achievement has been the publication of common guidelines for immunophenotyping at diagnosis published in 2017 after an extensive standardization and validation effort by our Lab together with other 9 referral Laboratories in Europe belonging to the AIEOP-BFM (Associazione Italiana Ematologia Oncologia Pediatrica and Berlin-Frankfurt-Munster) Flow Network.

At diagnosis, multiparametric flow cytometry (MFC) has an important role in blast cell definition for lineage assessment and identification of different subgroups. Moreover, immunophenotype may change during treatment causing a switch (SW) that consists of the appearance of a blast population with different phenotype from that detected at diagnosis.

Our group, recently described a subgroup of pediatric B-cell Precursor ALL (BCP-ALL) characterized by the aberrant expression of the myeloid marker CD371, a transient switch towards the myelomonocytic lineage during the first phase of chemotherapy, and a worse response to Induction therapy. We deeply characterized the immunophenotypic switch in order to provide standardized and reproducible operating procedure for blast identification during therapy, pointing to a proper identification of blast cells for a correct risk-based stratification of patients. We are actually running a project that aims to characterize the biological bases of the immunophenotypic switch through a multiomic single-cell application to bone marrow specimens collected at diagnosis and during therapy. Moreover, by the analysis of the genes and the surface proteins expressed in each single cell isolated from these patients at diagnosis we were able to distinguish a unique immature population never recognized with current standard technology. To better define this subgroup of patients we also adopted a very large study on genomic alteration and epigenetic modification characterizing these cells. The integration of all these omic approaches will give a complete description of this subtype of leukemia to potentially identify new targets for a tailored personalized therapy and improve the response to Induction therapy.

There is a peculiar ALL subtype associated with hypereosinophilia, in which eosinophils may be the prevalent population in the bone marrow with an amount of blast cells much less than the one required by international classifications as the blast cut-off (25%) to begin treatment. Nevertheless, the collateral effects associated with hypereosinophilia may lead patients to death, this requiring a precocious chemotherapy starting. We are actually running and leading an

international study of the Ponte di Legno consortium with the aim of refining blast cell cut-off for ALL definition in the presence of hypereosinophilia at diagnosis to initiate treatment. During treatment, MFC is critical for a fast identification of residual amounts of blast cells with a sensitivity level up to 10-5 nucleated cells for a peculiar immunophenotype. Additionally, MRD by MFC replaces quantitative PCR-MRD of IG/TR rearrangements for therapeutic risk group stratification in absence of appropriate molecular markers to be used. This phenomenon occurs more frequently in T-ALL, in which MFC-MRD by flow is particularly challenging due to the heterogeneity of blast immunophenotype at diagnosis, and possible antigen modulation during treatment. In the effort to improve MFC-MRD sensitivity, we recently described the usefulness of CD48 and CD99 markers in MFC-MRD in T-ALL. We are actually running a project with the purpose of refining the MFC-MRD panels for T-ALL with the introduction of new markers (e.g., CD371) and the adoption of personalized panels built up on the immunophenotypic features identified at diagnosis. Moreover, we are joining an international project to produce a custom combination of antibodies to be applied in the iBFM Consortium for MRD detection in T-ALL.

Antigen downregulation during therapy is a well-known phenomenon and is becoming even more frequent after the introduction of targeted immunotherapy. This phenomenon can have critical consequences on MRD assessment by flow. Indeed, a downregulation of target antigens, that are also the pivotal antigens of MFC-MRD detection (CD19 and CD7 for B- and T-ALL, respectively), requires a substantial modification of the gating strategy for leukemia associated immunophenotype (LAIP) detection. Additionally, CD19 and CD7 expression may also change in the normal regenerating counterpart of bone marrow, impacting on the leukemic cell discrimination through the "different from normal" approach in MRD detection. Therefore, it is mandatory to identify new markers to improve MFC-MRD monitoring in this setting, both for T- and B-lineage ALL. In this context, we recently described CD72 as a potential new marker for MFC-MRD monitoring in B-ALL, being expressed also in CD19 negative B-lineage ALL. We are actually carrying out a study to evaluate the role of CD72 in MFC-MRD monitoring in B-lineage ALL.

Pediatric refractory/relapsed (R/R) acute lymphoblastic leukemia (ALL) is still associated with a dismal prognosis. Traditionally, pediatric R/R ALL treatment relied on conventional chemotherapy and consolidation with hematopoietic stem cell transplant once deep remission was obtained. Regardless, modern oncology aims toward a personalized medicine approach based on disease and patient-specific molecular and cellular features. In this context, we are working on a project with the aim to develop a real-time drug response profiling (DRP) platform to screen patient-derived bone marrow (BM) and peripheral blood (PB) samples with a multi-drug library, including from 20 to 100 compounds already tested at least in phase 2 clinical trials, to have them available for patient treatment on compassionate use, if yet to be approved for pediatric use. This study may potentially offer a concrete plan of treatment for those R/R ALL pediatric cases who would not have an option to be cured so far. The real-time DRP platform will contribute to a patient-tailored treatment, limiting the use of ineffective drugs and drug-related toxicity and potentially impacting pediatric patients' quality of life and outcomes.

## PI's Biosketch

*Scopus ID 10639186000*

Barbara Buldini is an internationally recognized expert of pediatric acute leukemias laboratory at diagnosis and in the response-to-therapy monitoring.

She is Associate Professor at the University of Padova, Italy (since 2021) and serves in the managerial position of Altissima Specialità -Referente di Branca Specialistica Leukemia Diagnosis (since 2020).

She is PI of Morphology and Flow Cytometry group at the Pediatric Onco-Hematology Laboratory in Padova, the AIEOP reference Laboratory for the diagnosis and MFC-MRD assessment of childhood acute leukemias (since 2018).

She is the scientific director of immunophenotype and MFC-MRD in ALL at first diagnosis and relapse, Infant ALL, and AML (AIEOP-BFM ALL trials) (since 2012).

She coordinates and collaborates on several national and international projects/groups/associations/societies.

She leads the Padua Lab as an AIEOP-BFM partner unit in the MFC training of ALLIC Labs with limited economic resources that use essentially MFC for monitoring MRD in ALL (Argentina and Georgia).

She coordinates the AIEOP-BFM-AML Flow MRD group with Prof. Dworzak (-since 2011).

She is Member of:

AIEOP-BFM Flow Network, including the principal national reference Laboratories of BFM and AIEOP for the standardization of immunophenotype at Diagnosis and MFC-MRD in childhood ALL (since 2006).

Trial Steering Committee of AIEOP-BFM ALL (since 2018) and AIEOP-BFM AML 2020 (since 2019) protocols.

ITCC (Innovative Therapies for Children with Cancer) Hema Committee, Villejiuf, France, (since 2021).

TDC_AIEOP-BFM_ALL_genetics team (since 2023).

She is Member for AIEOP of the Federal Tumor board (since2023).

She is PI of "Immunophenotypic Plasticity of Pediatric B Acute Lymphoblastic Leukemia: a Genomic Study" project founded by Cariparo foundation in the Pediatric Research Institute "Città della Speranza, Padua (since 2020).

She is an AIEOP Member in Ponte di Legno Group (since 2018).

She is national principal investigator of two Ponte di Legno Group studies: Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) and Gamma-Delta-positive T-ALL (since 2019).

She coordinates an ongoing study for Ponte di Legno group on ALL with Hypereosinophilia (since 2023). She is member of: Gruppo di Lavoro (GdL) AIEOP "Biologia Cellulare e Molecolare" (since 2009); consultant of GdL AIEOP LAnL (Acute non-Lymphoblastic Leukemia - since 2012) and LLA (acute lymphoblastic leukemia - since 2018).

She is Tutor in "FlowInLab project" [https://elearning.unipd.it/flowinlab/], Moodle-based website developed in collaboration with DLM Office, University of Padua (since 2016).

She was Member of the iLTB (international leukemia/lymphoma target board) core team, coordinated by Princes Maxima Centrum (Utrecht), for Relapsed ALL (2021-2022).

She was Member of Board of Directors at AIEOP (2018-2021).

She participated to the 'International Project Ambi Studio' about Ambiguous Acute Leukemias, in collaboration with iBFM FLOW network (coordinated by Prof. O. Hrusak, Praga, Czech Republic), ALLIC and St. Jude's Children Hospital, Memphis, Tennessee, USA.

She performed clinical track at Clinic of Pediatric Hematology-Oncology, IRCCS Policlinico San Matteo, University of Pavia, Italy, 2005-2006.

She is co-author for WHO 5th Edition, Precursor B cell Neoplasm.

Publications on peer reviewed Journals: 89 (Scopus h-index:29; Citations:2935)

**Team members**

Prof. Barbara Buldini - Principal Investigator

Dr. Elena Varotto - Senior Scientist

Dr. Giulia Gomiero - Post Doc

## Selected publications

- Alexander TB, Gu Z, Iacobucci I, Dickerson K, Choi JK, Xu B, Payne-Turner D, Yoshihara H, Loh ML, Horan J, Buldini B, et al. The genetic basis and cell of origin of mixed phenotype acute leukemia. Nature. 2018 Oct;562(7727):373-379;
- Kimura S, Park CS, Montefiori LE, Iacobucci I, Polonen P, Gao Q, Arnold ED, Attarbaschi A, Brown A, Buldini B, Caldwell KJ, Chang Y, Chen C, Cheng C, Cheng Z, Choi J, Conter V, Crews KR, de Groot-Kruseman HA, Deguchi T, Eguchi M, Muhle HE, Elitzur S, Escherich G, Freeman BB, Gu Z, Han K, Horibe K, Imamura T, Jeha S, Kato M, Chiew KH, Khan T, Kicinski M, Kohrer S, Kornblau SM, Kotecha RS, Li CK, Liu YC, Locatelli F, Luger SM, Paietta EM, Manabe A, Marquart HV, Masetti R, Maybury M, Mazilier P, Meijerink JPP, Mitchell S, Miyamura T, Moore AS, Oshima K, Pawinska-Wasikowska K, Pieters R, Prater MS, Pruett-Miller SM, Pui CH, Qu C, Reiterova M, Reyes N, Roberts KG, Rowe JM, Sato A, Schmiegelow K, Schrappe M, Shen S, Skoczen S, Spinelli O, Stary J, Svaton M, Takagi M, Takita J, Tang Y, Teachey DT, Thomas PG, Tomizawa D, Trka J, Varotto E, Vincent TL, Yang JJ, Yeoh AE, Zhou Y, Zimmermann M, Inaba H, Mullighan CG. Biologic and clinical analysis of childhood gamma delta T-ALL identifies LMO2/STAG2 rearrangements as extremely high-risk. Cancer Discov. 2024 Jun 25. doi: 10.1158/2159-8290.CD-23-1452. Epub ahead of print. PMID: 38916500.
- Buldini B, Faggin G, Porcù E, Scarparo P, Polato K, Tregnago C, Varotto E, Rizzardi P, Rizzari C, Locatelli F, Biffi A, Pigazzi M. CD72 is a pan-tumor antigen associated to pediatric acute leukemia. Cytometry A. 2023 Dec;103(12):1004-1009. doi: 10.1002/cyto.a.24790. Epub 2023 Oct 24. PMID: 37876342.
- Buldini B, Varotto E, Maurer-Granofszky M, Gaipa G, Schumich A, Brüggemann M, Mejstrikova E, Cazzaniga G, Hrusak O, Szczepanowski M, Scarparo P, Zimmermann M, Strehl S, Schinnerl D, Zaliova M, Karawajew L, Bourquin JP, Feuerstein T, Cario G, Alten J, Möricke A, Biffi A, Parasole R, Fagioli F, Valsecchi MG, Biondi A, Locatelli F, Attarbaschi A, Schrappe M, Conter V, Basso G, Dworzak MN. CD371-positive pediatric B-cell acute lymphoblastic leukemia: propensity to lineage switch and slow early response to treatment. Blood. 2024 Apr 25;143(17):1738-1751. doi: 10.1182/blood.2023021952. PMID: 38215390.
- Campbell M, Kiss C, Zimmermann M, Riccheri C, Kowalczyk J, Felice MS, Kuzmanovic M, Kovacs G, Kosmidis H, Gonzalez A, Bilic E, Castillo L, Kolenova A, Jazbec J, Popa A, Konstantinov D, Kappelmayer J, Szczepanski T, Dworzak M, Buldini B, Gaipa G, Marinov N, Rossi J, Nagy A, Gaspar I, Stary J, Schrappe M. Childhood Acute Lymphoblastic Leukemia: Results of the Randomized Acute Lymphoblastic Leukemia Intercontinental-Berlin-Frankfurt-Münster 2009 Trial. J Clin Oncol. 2023 Jul 1;41(19):3499-3511. doi: 10.1200/JCO.22.01760. Epub 2023 May 4. PMID: 37141547.

# Transcriptomics and Functional Genomics in JMML and B-ALL

**Group Leader** Dr. Silvia Bresolin - Principal Investigator

## Research activity

The goal of the group is to identify at the genomic, transcriptomic, epigenomic and single-cell multi-omic level the networks of acquired and inherited aberrations that drive the development and the progression of B-cell Acute Lymphoblastic Leukemia (B-ALL) and Juvenile Myelomonocytic Leukemia (JMML). Indeed, the group has a strong expertise in generation, analysis and integration of data supporting the improvement of diagnosis, risk stratification and treatment of leukemia pediatric patients supported by experimental and functional studies using both in vitro and in vivo disease models. The main projects of the group are listed below.

*A multimodal integrated single cell strategy to erase Juvenile Myelomonocytic Leukemia.*

This project aims to understand the regulatory mechanisms behind JMML by analyzing single-cell transcriptome and epigenome regulation. We will explore the interactions between cancer cells and the bone marrow microenvironment. Using CRISPR/Cas9 genome wide screening, our goal will be to identify new biomarkers and therapeutic targets. The results will also be validated in patient-derived xenograft models to ensure clinical applicability.

*Dissecting Juvenile Myelomonocytic Leukemia complexity: 3D epigenomic landscape and AI-powered multi-omics patient stratification.*

We plan to investigate JMML patients' 3D chromatin architecture and leverage Artificial Intelligence (AI) based multi-omics data analysis to integrate clinical, epigenetic, genetic and transcriptome features. We will focus on modeling deeper JMML heterogeneity, discovering new biomarkers and providing a useful tool for clinicians to better classify patients, thus improving therapeutic interventions and clinical outcome.

*CircRNAs involved in mechanisms of malignant transformation and relapse in T-ALL: towards new RNA-based therapies*

This project aims to disclose new mechanisms of leukemogenesis and resistance in T-ALL focusing on circRNA roles in mechanisms of malignant transformation. CircRNAs are transcripts in which a downstream splice donor site is covalently bound to an upstream acceptor by backsplicing. The discovery of circular RNAs (circRNAs) added a new layer to our understanding of transcriptome complexity. They can act as miRNA sponges, interact with RNA-binding protein or can also be translated into peptides. The project focuses on the study of these circRNA in leukemogenesis mechanisms by in vitro model. This project is in collaboration with Prof. Bortoluzzi Stefania (DMM, University of Padova).

*Precision medicine and clonal evolution investigation on high risk B-ALL*

In the last decade a great advance in the comprehension of the genetic and biological bases of child-hood leukemia has been achieved and genomic analysis contributed to the improvement of risk stratification and patient treatment, however about 20% of BALL patients present treatment resistance and relapse; our group is involved in the identification of genomic and transcriptomic

alterations of high risk BALL patients and in particular of therapy resistant and relapsed patients; we characterize by different omics approaches (exome, RNA-seq, gene expression profiling) driver mutations in BALL patients to identify drug resistance mechanisms and clonal structure and track clonal dynamicity between diagnosis and relapse.

*Advanced diagnostics in JMML*

We leverage Next Generation Sequencing (NGS) to identify mutations and track their evolution during treatment and follow up, in order to provide clinicians with a precise tool to guide therapeutic interventions.

## PI's Biosketch

*Scopus ID 36027490300*

Dr. Bresolin obtained her degree in Medical Biotechnology in 2007 at University of Padua and her PhD in Medicina dello Sviluppo e Scienze della Programmazione, Indirizzo Immunologia, Ematologia-Oncologia, Genetica at the University of Padova in 2012. During her PhD she attended the laboratory of Oncohematology where she focused her research on advancement of new molecular technologies aiming at the improvement of the prognosis and diagnosis of pediatric patients with myelodysplasia and juvenile myelo-monocytic leukemia. Since 2007, she works in the Laboratory of Pediatric Oncohematology focusing on the molecular characterization and development of an in vitro and in vivo model of myelodysplastic and myeloproliferative diseases. In 2012 she attended the laboratory directed by Prof. Weiss at Children Hospital of Philadelphia, PA, USA working on the generation and maintenance of leukemia iPS cells. She is member of the Italian AIEOP working group on JMML and MDS and member of the Europe-an Working Group of MDS and SAA (EWOG-MDS-SAA). Her scientific research is also focused on the molecular and genetic characterization of leukemia and myeloproliferative disease with "omics" technologies at both genomic and transcriptome levels by means of gene expression profiling and next generation sequencing. She is also involved in the genetic diagnosis and management of patients with hematological disease and cancer predisposition. Dr. Silvia Bresolin is part of several national and international collaborations for "omics" data generation and analysis. She is actively involved in projects on the functional characterization of circRNA in MLL rearranged leukemia and on the genetic variations predisposing to leukemia in childhood. From 2018 to 2022, she was Assistant Professor at the Woman and Child's health Department, Division of Pediatric Hematology, Oncology and Stem Cell Transplant of the Padua University and Hospital. At present, she is a PI of Pediatric Research Institute. She is author of more than 60 publications in peer reviewed international journals.

### Team members

Dr. Silvia Bresolin - Principal Investigator
Dr. Alice Cani - Post Doc
Dr. Alberto Peloso - PhD Student
Dr. Ilaria Stefani - Research Fellow
Dr. Martina Volgger - Research Fellow

## Selected publications


- Cani A, Tretti Parenzan C, Frasson C, Rampazzo E, Scarparo P, Francescato S, Caicci F, Barbieri V, Rosato A, Cesaro S, Zecca M, Micalizzi C, Sainati L, Pigazzi M, Biffi A, Buldini B, Locatelli F, Persano L, Masetti R, te Kronnie G, Bresolin S. Long-term proliferation of immature hypoxia-dependent JMML cells supported by a 3D in vitro system. Blood Adv. 2023;7(8):1513-1524. doi:10.1182/bloodadvances.2021006746
- Tretti Parenzan C, Dal Molin A, Longo G, Gaffo E, Buratin A, Cani A, Boldrin E, Serafin V, Guglielmelli P, Vannucchi A M, Cazzaniga G, Biondi A, Locatelli F, Meyer L H, Buldini B, te Kronnie G, Bresolin S, Bortoluzzi S. Functional relevance of circRNA aberrant expression in pediatric acute leukemia with KMT2A::AFF1 fusion. Blood Adv. 2024;8(5):1305-1319. doi:10.1182/bloodadvances.2023011291
- Fazio G, Bresolin S, Silvestri D, Quadri M, Saitta C, Vendramini E, Buldini B, Palmi C, Bardini M, Grioni A, Rigamonti S, Galbiati M, Mecca S, Savino AM, Peloso A, Tu JW, Bhatia S, Borkhardt A, Micalizzi C, Lo Nigro L, Locatelli F, Conter V, Rizzari C, Valsecchi MG, Te Kronnie G, Biondi A, Cazzaniga G. PAX5 fusion genes are frequent in poor risk childhood acute lymphoblastic leukaemia and can be targeted with BIBF1120. EBioMedicine. 2022 Sep;83:104224. doi:10.1016/j.ebiom.2022.104224. Epub 2022 Aug 16. PMID: 35985167; PMCID: PMC9403348

# Phosphoproteomics for ALL Diagnostics and Research

**Group Leader** Dr. Valentina Serafin - Junior Principal Investigator

## Research activity

The group is in charge of the Reverse Phase Protein Arrays (RPPA) facility that allows the identification of new disease biomarkers and new therapeutic targets in oncological and non-oncological diseases through phosphoproteomic profiling. To do so we dispose of a library of more than 130 validated antibody belonging to the most deregulated pathways in cancer and inflammation, and a full equipped facility for sample preparation, slides printing and staining. We believe that monitoring the activation status of signal transduction pathways will be key to identify patient subgroups that can benefit from the use of specific kinase inhibitors and to point out proteins suitable for patient risk stratification and targeted therapy. In recent years through RPPA we identified and then validated new potential biomarkers and therapeutic targets for B and T Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML) and T cell Acute Lymphoblastic Lymphoma pediatric patients (T LBL).

In particular, on-going projects include identification of new glucocorticoid resistance mechanisms in T ALL pediatric patients and identification of new biomarkers useful to recognize patients that at relapse could benefit of novel targeted therapeutic approaches.

*Identification of new glucocorticoid resistance mechanisms in T ALL pediatric patients*

In the past five years our research mainly focused on the study of molecular mechanisms responsible of glucocorticoid resistance in T ALL pediatric patients. Indeed, we observed the hyperactivation of LCK kinase in patients more resistant to glucocorticoids and in ETP T ALL, a subset of patients highly resistant to therapy (Serafin V. et al, Blood 2017). The pharmacological inhibition or the specific gene silencing of this kinase in glucocorticoid resistant cells turn them sensitive to corticosteroids both in vitro and in vivo (Serafin V. et al, Leukemia 2017). Starting from these evidences we identified among the molecular mechanisms underlying the glucocorticoid resistance in T-ALL patients the involvement of NFATs family transcription factors. Thus, we are now designing and testing novel inhibitors able to suppress the NFAT family transcription factor activity (Supported by AIRC "My First AIRC Grant" and FUV).

*Identification of new biomarkers useful to recognize patients that at relapse could benefit of novel targeted therapeutic approaches*

Nowadays relapse represents the major obstacle in pediatric T-acute lymphoblastic leukemia (T-ALL) patients' treatment, since for relapse patients the prognosis remains dismal due either to the drug resistance or to the high toxicity for chemotherapy intensification. Thus, by performing the phosphoproteomic profile of poor and good prognosis relapsed T-ALL pediatric patients we aim to identify new biomarkers useful to recognize patients that at relapse could benefit of novel targeted therapeutic approaches, thus allowing the setting-up of novel therapeutic options to prevent therapy resistance occurrence and excessive toxicities (Supported by AIRC fellowship to Giulia Veltri PhD).

## Jr PI's Biosketch

*Scopus ID 27268039100*

Dr. Serafin graduated in Medical Biology at the University of Padua (Italy) in 2008, after a year spent at the University of Wuerzburg (Germany) where her commitment to cancer research started. In 2012 she completed her PhD in Oncology and Surgery Oncology at the Department of Surgery, Oncology and Gastroenterology at the University of Padova where she studied the role of Notch3 signaling both in colorectal cancer and in leukemia. (Serafin V. et al, Journal of Pathology 2011, Pastò A. et al, Cancer Research 2014, Ghisi M. et al, Blood 2011). During her PhD she had the opportunity to collaborate with the Oncohaematology Laboratory headed by Prof. Basso where afterward she started her post doctoral fellowship under the supervision of Dr. Accordi. During this period, she has been supported for three years by the Fondazione Italiana per la Ricerca sul Cancro (FIRC) and two years by the Fondazione Umberto Veronesi (FUV). She published 13 scientific papers in peer-reviewed international journals regarding phosphoproteomic profiling of pediatric Acute Leukemias and other not pediatric diseases. Among them, she initiated and developed a project regarding glucocorticoid resistance in pediatric patients affected by T ALL. She observed a hyperactivation of LCK kinase in patients more resistant to glucocorticoids and in ETP T-ALL, a subset of patients highly resistant to therapy (Serafin V. et al, Blood 2017, Serafin V. et al, Leukemia 2017). These evidences have an important clinical relevance since this pathway can be pharmacologically inhibited to sensitize cells and turn patients responsive to the action of glucocorticoids. Focusing on the characterization of molecular mechanisms underlying the glucocorticoid resistance in 2019 she won the My First AIRC Grant, which allowed her to start her independent career as junior principal investigator at the Pediatric Research Institute "Città della Speranza". In 2019 she also had the opportunity to spend a period of time at the Northwestern University in Chicago where she improved her molecular basic science competence in order to carry on this ambitious project. In the last 4 years, thanks to AIRC, FUV, AIL and IRP support she increased her autonomy as scientist and developed her own field of research. Dr. Serafin's scientific production is based on the publication of 34 original papers, 1 review and 1 editorial, being in 8 of them the first, last or corresponding author with an average IF of 8.7 and an H-index=17.

**Team members**

Dr. Valentina Serafin - Junior Principal Investigator

Dr. Giulia Veltri - Post Doc

## Selected publications


- Minuzzo S, Agnusdei V, Pinazza M, Amaro AA, Sacchetto V, Pfeffer U, Bertorelle R, Spinelli O, Serafin V, Indraccolo S. Targeting NOTCH1 in combination with antimetabolite drugs prolongs life span in relapsed pediatric and adult T-acute lymphoblastic leukemia xenografts. Exp Hematol Oncol. 2023 Sep 4;12(1):76. doi: 10.1186/s40164-023-00439-6. PMID: 37667380; PMCID: PMC10476325.
- Veltri G, Silvestri C, Gallingani I, Sandei M, Vencato S, Lovisa F, Cortese G, Pillon M, Carraro E, Bresolin S, Biffi A, Basso G, Accordi B, Mussolin L, Serafin V. Ruxolitinib as a Novel Therapeutic Option for Poor Prognosis T-LBL Pediatric Patients. Cancers (Basel). 2021 Jul 24;13(15):3724. doi: 10.3390/cancers13153724. PMID: 34359628; PMCID: PMC8345121.

• Zhou Y, Han C, Wang E, Lorch AH, Serafin V, Cho BK, Gutierrez Diaz BT, Calvo J, Fang C, Khodadadi-Jamayran A, Tabaglio T, Marier C, Kuchmiy A, Sun L, Yacu G, Filip SK, Jin Q, Takahashi YH, Amici DR, Rendleman EJ, Rawat R, Bresolin S, Paganin M, Zhang C, Li H, Kandela I, Politanska Y, Abdala-Valencia H, Mendillo ML, Zhu P, Palhais B, Van Vlierberghe P, Taghon T, Aifantis I, Goo YA, Guccione E, Heguy A, Tsirigos A, Wee KB, Mishra RK, Pflumio F, Accordi B, Basso G, Ntziachristos P. Posttranslational Regulation of the Exon Skipping Machinery Controls Aberrant Splicing in Leukemia. Cancer Discov. 2020 Sep;10(9):1388-1409. doi: 10.1158/2159-8290.CD-19-1436. Epub 2020 May 22. PMID: 32444465; PMCID: PMC7483384.





# Advanced Diagnostics and Target Discovery in Rare Pediatric Solid Tumors

**Group Leader** Prof. Gianni Bisogno - Principal Investigator

## Research activity

The laboratory works in close collaboration with the Pediatric Haemato-Oncology Unit in Padua. Over the years, it has developed a particular interest in diagnostics, basic and translational research for children with soft tissue sarcomas and other rare tumors.

The main activities are as follows.

*Collection, processing and storage of biological samples from patients with solid tumors*

A pediatric soft tissue biobank has been established since 1995 and every year the laboratory analyses almost 150 new cases collected from more than 30 pediatric oncology centers belonging to the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP), performing about 1000 molecular analyses with diagnostic and prognostic value.

*Provide the necessary molecular biology studies to support the diagnosis of sarcomas*

The laboratory is involved in the investigation of new diagnostic and prognostic biomarkers in children with soft tissue sarcomas (STS). In many of these malignancies, we have demonstrated the presence of several new genetic abnormalities, including point mutations, deletions, amplifications and chromosomal translocations, which have improved our ability to identify the best treatment option for each patient, both reducing the side effects of conventional treatments and identifying high-risk patients suitable for novel therapeutic approaches.

*Supporting research projects dedicated to the study of the biological characteristics of pediatric soft tissue sarcomas (STS) and biomarkers that may have direct clinical application*

A series of studies using liquid biopsies have also been carried out by our group to identify novel biomarkers predictive of cancer resistance and metastasis, through a comprehensive cellular and molecular analysis of peripheral blood, bone marrow and plasma samples from children with STS. Liquid biopsies are non-invasive blood tests for the detection of circulating tumor cells (CTCs), cell-free tumor DNA (ctDNA) and cancer-associated antigens/autoantibodies shed into the bloodstream by the primary tumor and metastases, which can help identify tumors capable of spreading to distant organs and develop better strategies to predict the risk of relapse. Our group has demonstrated that 1) CTCs are found in the bloodstream of children with soft tissue sarcomas, 2) CTCs and cfDNA samples carry pre-treatment tumor mutations that may be important for assessing disease progression and response to treatment, and 3) autoantibodies against tumor antigens exist and induce an immune response that distinguishes affected children from healthy subjects, as well as patients belonging to different risk groups.

Given our disease-focused preclinical and clinical research interests, the major advances in the treatment of children and adolescents with sarcoma being pursued by our group are: preclinical drug testing and repositioning in non-responder patient cells, and antibody-based immunotherapy in those at risk of relapse. These two topics are currently represented by the PRESTO study and the PANDORA project. The PRESTO study (Personalized Drug Sensitivity Profiling Program

for Progressive and Relapsed Pediatric Sarcomas) proposes a coordinated approach that translates next-generation sequencing (NGS) and drug sensitivity profiling (DSP) data into the selection of new therapeutic compounds using patient-derived 2D primary cell cultures (PDCs) or 3D tumoroids as preclinical test models. The PANDORA project (Development of Patient-Derived Neutralising Antibodies for Pediatric Cancer Therapy) aims to develop, characterize and test patient-derived monoclonal antibodies (moAbs) and single-chain variable fragments (scFvs) against oncoantigens of therapeutic value. This is to be achieved using a single memory B cell cloning strategy and phage display technology.

## PI's Biosketch

*Scopus ID 7003672935*

Gianni Bisogno is Full Professor in Pediatrics and Consultant Pediatric Oncologist, Coordinator of the Solid Tumor Program and the "Laboratory for the advanced diagnostic and target discovery in rare solid tumors of children "Department of Woman's and Child's Health in Padova. His clinical and research activities focus mainly on childhood solid tumors. He has been Coordinator of the Italian Soft Tissue Sarcoma Committee (STSC) since 2007. Over the years, he has succeeded in empowering the group's activities, promoting the launch of several clinical trials, and creating a multidisciplinary team that supports the activities of all Italian pediatric oncology centers by providing services for central diagnostic reviews, molecular biology characterization, real-time advice on difficult cases, and treatments not available in other Italian (or foreign) centers. As a result, nearly 100% of Italian children with Rhabdomyosarcoma (RMS) have been registered in the protocols coordinated by the STSC (AIEOP Registry) and the 5-year survival of patients with localized tumors has increased from 62% (RMS79 study) to over 80% (RMS2005 study). Professor Bisogno was one of the founders of the European Pediatric Soft tissue Sarcoma Study Group (EpSSG) in 2004, the largest European organization devoted to promote research for children with soft tissue sarcoma and PI of the European trial on localized rhabdomysarcoma (RMS2005), which recently changed the RMS standard treatment demonstrating the effectiveness of maintenance chemotherapy (Selected for ASCO Plenary Section, June 2018). Experience gained with such rare tumors as pediatric sarcomas was pivotal in establishing a new collaborative scheme in 2000 called the TREP (Tumori rari in età pediatrica) project. This has been the most successful initiative in favor of children with very rare tumors (nasopharyngeal carcinoma, pediatric melanoma, pleuropulmonary blastoma, and others) that have tended to be rather neglected by the pediatric oncology community. A national network has been established, guidelines for diagnosis and treatment have been drawn up, and clinical data have been collected. A molecular biology research program has also been organized. The model created for the TREP project was subsequently adopted by other European countries and this led to the foundation of the EXPeRT (European Cooperative Study group for Pediatric rare tumors) in Padova, June 2008. All these studies have been paralleled by an effort to promote basic and translational research with a strong clinical impact. Therefore, the search for new diagnostic tools has led to the first identification of new genetic alterations like VGLL2 related fusion in spindle cells rhabdomyosarcoma, and other different prognostic biomarkers (miR-26a, IGFBP2 and others). Prof. Bisogno is also responsible for the pediatric clinical trial office dedicated to manage studies on new drugs. He has been leader or partner in several projects supported by different institutions, including European funded projects such as EPOC, ENCCA and EXPORNET. He is the coordinator of the recently approved PARTNER, an EU funded project dedicated to create a European Registry for children with very rare tumors. His activity in the field of pediatric oncology is demonstrated by his publications, which include more than 300 international papers (total IF > 1800; H index 50) and 12 book chapters.

### Team members

Prof. Gianni Bisogno - Principal Investigator
Dr. Paolo Bonvini - Senior Scientist
Dr. Lucia Tombolan - Senior Scientist
Dr. Elena Poli - Post Doc
Dr. Daniela Di Carlo - Research Fellow
Dr. Sara Provesi - Research Fellow
Dr. Elena Turolla - Research Fellow

### Selected publications


- Tombolan L, Rossi E, Binatti A, Zin A, Manicone M, Facchinetti A, Lucchetta S, Carmen Affinita M, Bonvini P, Bortoluzzi S, Zamarchi R, Bisogno G. Clinical significance of circulating tumor cells and cell-free DNA in pediatric rhabdomyosarcoma. MOL ONCOL 2022 May; 16: 2071
- Ruhen O, Lak NSM, Stutterheim J, Danielli SG, Chicard M, Iddir Y, Saint-Charles A, Di Paolo V, Tombolan L, Gatz SA, Aladowicz E, Proszek P, Jamal S, Stankunaite R, Hughes D, Carter P, Izquierdo E, Wasti A, Chisholm JC, George SL, Pace E, Chesler L, Aerts I, Pierron G, Zaidi S, Delattre O, Surdez D, Kelsey A, Hubank M, Bonvini P, Bisogno G, Di Giannatale A, Schleiermacher G, Schà¤fer BW, Tytgat GAM, Shipley J. Molecular Characterization of Circulating Tumor DNA in Pediatric Rhabdomyosarcoma: A Feasibility Study. JCO Prec Oncol 2022 Oct; 6: e2100534
- Poli E, Barbon V, Lucchetta S, Cattelan M, Santoro L, Zin A, Milano GM, Zanetti I, Bisogno G, Bonvini P. Immunoreactivity against fibroblast growth factor 8 in alveolar rhabdomyosarcoma patients and its involvement in tumor aggressiveness. ONCOIMMUNOLOGY 2022; 11: 2096349
- Poli E, Cattelan M, Zanetti I, Scagnellato A, Giordano G, Zin A, Bisogno G, Bonvini P. Autoantibody profiling of alveolar rhabdomyosarcoma patients unveils tumor-associated antigens with diagnostic and prognostic significance. ONCOIMMUNOLOGY 2021; 10: 1954765
- Bonvini P, Rossi E, Zin A, Manicone M, Vidotto R, Facchinetti A, Tombolan L, Affinita MC, Santoro L, Zamarchi R, Bisogno G. Case Report: Circulating Tumor Cells as a Response Biomarker in ALK-Positive Metastatic Inflammatory Myofibroblastic Tumor. FRONT PEDIATR 2021; 9: 652583

# Biology of CNS Pediatric Tumors

**Group Leader** Dr. Luca Persano - Principal Investigator

## Research activity

The Biology of CNS Pediatric Tumors laboratory is currently involved in different studies aimed at the characterization of the molecular basis of pediatric brain tumor aggressiveness and resistance to treatments. In particular, we recently generated and characterized both at the molecular and functional levels different chemotherapy-resistant models of medulloblastoma cells, describing the most relevant pathways potentially involved in sustaining therapy resistance. Accordingly, we are currently investigating the mechanisms of emergence, during the chemotherapy stimulus, of particular drug-resistant cell subpopulations (i.e. persister cells) through a single-cell multi-omic approach.

In parallel, it's many years that our group is interested in characterizing the several microenvironmental cues that sustain brain tumor aggressiveness and therapy resistance, by investigating the role played by the hypoxic microenvironment and the extracellular matrix composition on brain tumor phenotype, with particular interest in the so called "cancer stem cells" subpopulation.

## PI's Biosketch

*Scopus ID 8693588700*

Dr. Persano graduated in Pharmaceutical Biotechnology in 2005 at the University of Padua. Since his PhD studentship in Oncology and Surgical Oncology Dr. Persano's studies have been focused on dissecting the molecular pathways underlying cancer progression and resistance to therapy, with particular focus on the role played by tumor microenvironment. He achieved his PhD in 2009 with a project committed to study the process of tumor angiogenesis and exploit its potential inhibition as a therapeutic strategy in different tumors including prostate, ovarian, esophageal and colon cancers. In 2009 Dr. Persano moved to the Laboratory of Pediatric Oncohematology, in which he focused his interests in brain tumor biology, here obtaining the position of Assistant Professor. Dr. Persano's research interests are now focused in unraveling the molecular mechanisms by which the activation of specific intracellular circuits, coupled with stimulation from the tumor microenvironment, can modulate the differentiation status, phenotype, and treatment response of brain tumor cells.

In this context, it is almost 15 years since Dr. Persano focused his research activities on the role played by the hypoxic tumor microenvironment in sustaining a stem-like phenotype of medulloblastoma (MB) and glioblastoma (GBM) cells and the relationship existing between the hypoxic sensor HIF-1α, their cancer stem cell (CSC) phenotype, and a series of fundamental developmental signaling pathways, including Notch and PI3K in MB, and BMP and Wnt in GBM. Importantly, in 2010 Dr. Persano's research group was able to finely characterize the GBM mass as composed by multiple layers of cancer cells endowed with different stem cell properties, phenotype, and response to treatments, according to the intratumoral hypoxic gradient and CSC distribution. Starting from these studies, in the following years, his research group characterized the role of the BMP pathway and the Wnt signaling as main potential targets to promote GBM cell differentiation, sensitize them to standard treatments, and even impact on tumor angiogenesis and stem-like cell trans-differentiation properties.

In the last years, Dr. Persano's group established a strong and fruitful collaboration with the research group headed by Dr. Mariateresa Mancuso, Division of Health Protection Technologies, Italian National Agency for Energy New Technologies and Sustainable Economic Development (ENEA) for the study of peculiar electromagnetic fields as potential novel therapeutics against GBM and MB stem-like cells.

More importantly, in the most recent years, he obtained specific grants from the Rally Foundation for Childhood Cancer Research and CARIPARO Foundation for the study of the mechanisms of therapy resistance in MB, which allowed to investigate the emergence of peculiar drug-resistant cell populations in response to cycling chemotherapy in MB. These most recent studies are still ongoing in the laboratory.

## Team members

Dr. Luca Persano - Principal Investigator

Dr. Elena Rampazzo - Post Doc

## Selected publications


- Mariotto E, Rampazzo E, Bortolozzi R, Rruga F, Zeni I, Manfreda L, Marchioro C, Canton M, Cani A, Magni R, Luchini A, Bresolin S, Viola G, and Persano L. Molecular and functional profiling of chemotolerant cells unveils nucleoside metabolism-dependent vulnerabilities in medulloblastoma. Acta Neuropathol Commun. 2023 Nov 17;11(1):183. doi: 10.1186/s40478-023-01679-7.
- Cescon M, Rampazzo E, Bresolin S, Da Ros F, Manfreda L, Cani A, Della Puppa A, Braghetta P, Bonaldo P and Persano L. Collagen VI sustains cell stemness and chemotherapy resistance in glioblastoma. Cell Mol Life Sci. 2023 Jul 28;80(8):233. doi: 10.1007/s00018-023-04887-5.
- Porcù E, Maule F, Manfreda L, Mariotto E, Bresolin S, Cani A, Bortolozzi R, Della Puppa A, Viola G, Rampazzo E and Persano L. Identification of Homoharringtonine as a potent inhibitor of glioblastoma cell proliferation and migration. Transl Res. 2023 Jan; 251:41-53. doi: 10.1016/j.trsl.2022.06.017. Epub 2022 Jul 3.
- Boso D, Rampazzo E, Zanon C, Bresolin S, Maule F, Porcù E, Cani A, Della Puppa A, Trentin L, Basso G and Persano L. HIF-1α/Wnt signaling-dependent control of gene transcription regulates neuronal differentiation of glioblastoma stem cells. Theranostics. 2019 Jul 9;9(17):4860-4877. doi: 10.7150/thno.35882. eCollection 2019.
- Rampazzo E, Persano L, Karim N, Hodgking G, Pinto R, Casciati S, Tanori E, Zambotti A, Bresolin S, Cani A, Pannicelli A, Davis I, Hancock C, Palego C, Viola G, Mancuso M, and Merla C. On the effects of 30 GHz sinusoidal wave exposure on glioblastoma organoids. Frontiers in Oncology 2024. Accepted for Publication

# Experimental Pharmacology

**Group Leader** Prof. Giampietro Viola - Principal Investigator

## Research activity

The Experimental Pharmacology lab is involved in the study of new strategies in cancer therapeutics following the main research lines listed below.

*Identification of new therapeutic target in medulloblastoma resistance*

Medulloblastoma (MB) is the most common brain tumor in the pediatric age and is very aggressive and characterized by low survival and high incidence of relapse. Our group is actively involved in the characterization of the molecular basis of MB aggressiveness and resistance by modeling the chemotherapy-induced evolution of MB cells in vitro by applying conventional chemotherapy. The -omics characterization of MB resistant cells will provide novel therapeutic opportunities potentially able to reduce risk of relapse and to increase survival rates.

*Study of BAG interactome and design of new BAG inhibitors*

The resistant medulloblastoma cells express high level of BAG protein family, a class of antiapoptotic proteins that possess the ability to prevent tumor cell death. Our goal is to identify the predominant BAG member that may be responsible for sustaining therapy resistance and relapse in MB, together with all its interacting proteins. In addition we are studying the possibility of designing novel BAGs inhibitors by Fragment-Based Drug Discovery approach. In this context we avail ourselves of the collaboration of Prof. M. Sturlese (University of Padua) regarding the screening of fragments which is performed using NMR techniques.

*Cancer metabolism and REDOX homeostasis*

We have recently demonstrated that cancer cells can regulate the Nrf2 pathway as a pro-survival response against drug treatments. Nrf2 is the major regulator of redox homeostasis and defense against oxidative stress. So far, our results show that starting from early exposure of chemotherapy drugs; MB cells induce Nrf2 expression and its transcriptional activation, supporting the involvement of Nrf2 pathway in MB response to chemotherapeutic treatment. More interestingly, we demonstrated that the upregulation of this detoxifying system induces a metabolic switch of MB cells and sustains resistance to chemotherapy. By combining metabolomics analysis (collaboration with Prof. D. Tennant University of Birmingham UK) with transcriptomic and proteomic data of MB resistant cells, our group aim to deepen the metabolic rewiring that occur during the onset of chemotherapy resistance, with the final goal to uncover specific metabolic vulnerability of drug resistant MB cells to be exploited as therapeutic opportunities.

*Drug Discovery*

In collaboration with various medicinal chemists in Italy and Europe, the laboratory deals with the screening and study of the mechanism of action of potential molecules with anti-tumor activity. studies are conducted using different tumor cell lines. For the identified lead compounds, further evaluations can be performed in in vivo animal models in collaboration with Prof. Ronca of the University of Brescia

## PI's Biosketch

*Scopus ID 7006633082*

Giampietro Viola graduated in Pharmacy (1988) and Chemistry and Pharmaceutical Technology (1995) at the University of Padua. In 1999 he obtained a PhD in Pharmaceutical Sciences. He started his career as researcher at FIDIA Research Laboratories s.p.a. and then joined the University of Padua as research assistant. He spent a large number of periods in qualified research structures abroad. Actually, he is Associate Professor at the University of Padua in the Department of Women's and Child's Health (UOC of Oncohematology). Since 2017 he has got the Italian Scientific National Habilitation to Full Professor of Medicinal Chemistry. His main research lines are the identification of new therapeutic targets in medulloblastoma (MB) resistance. He is actively involved in the characterization of the molecular basis of MB aggressiveness and resistance by modelling the chemotherapy-induced evolution of MB cells in vitro by applying conventional chemotherapy. The -omics characterization of MB resistant cells will provide novel therapeutic opportunities potentially able to reduce risk of relapse and to increase survival rates.

Active projects in this area include the study of BAG interactome, cancer metabolism and Redox homeostasis and drug discovery.

*The study of BAG interactome*

The resistant medulloblastoma cells express high levels of BAG protein family, a class of antiapoptotic proteins that possess the ability to prevent tumor cell death. Our goal is to identify the predominant BAG member that may be responsible for sustaining therapy resistance and relapse in MB, together with all its interacting proteins. By using the innovative technique of "molecular painting", we will identify novel potential BAG-partner hotspots to be drugged to achieve a more efficient clearance of residual cancer cells after standard treatments.

*Cancer metabolism and Redox homeostasis*

Recently we and others have demonstrated that cancer cells can regulate the Nrf2 pathway as a pro-survival response against drug treatments. Nrf2 is the major regulator of redox homeostasis and defense against oxidative stress. So far, its expression and transcriptional activation, support the involvement of the Nrf2 pathway in MB response to chemotherapeutic treatment. Interestingly we demonstrated that the upregulation of this detoxifying system induces a metabolic switch of MB cells and sustains resistance to chemotherapy. By study of Nrf2 involvement in the metabolic changes that occur in the onset of chemotherapy resistance, we aim to give further insight on the characterization of MB resistance and the identification of novel druggable targets.

*Drug discovery*

In collaboration with various medicinal chemists in Italy and Europe, the laboratory deals with the screening and study of the mechanism of action of potential molecules with anti-tumor activity. He is author of more than 190 scientific papers in qualified peer review journals (H index=43, 12.635 citations, from Scopus; Total IF= 974.628). It was responsible of the unit of the University of Padova in the context of SUMCASTEC project-Horizon 2020 that deals with the development of a medical device able to separate cancer stem cells in MB and glioblastoma through the application of electromagnetic fields. Moreover, it was also granted in the last three years by

AIRC (Investigator Grant), Fondazione JUST and participated as team members in several projects (Cariparo pediatrico, Rally Foundation, Ministerio de Innovacion y Ciencia, Spain). He currently serves as reviewer for many journals in the field of medicinal chemistry and pharmacology and he is Associate Editor of Biochemical Pharmacology and Editorial Board Member of Cancers. He is holder of many patents concerning the synthesis and antitumor application of new potential antitumoral molecules.

**Team members**

Prof. Giampietro Viola - Principal Investigator
Dr. Elena Mariotto - Junior Principal Investigator
Dr. Roberta Bortolozzi - Senior Scientist
Dr. Franco Breda - Research Fellow
Dr. Ignacio Reales Prieto - Research Fellow
Dr. Lorenzo Manfreda - PhD Student
Dr. Chiara Marchioro - PhD Student
Dr. Martina Canton - PhD Student

**Selected publications**


- Mariotto E., Rampazzo E., Bortolozzi R., Fatlum Rruga F., Zeni I., Manfreda L., Marchioro C., Canton M., Cani A., Magni R., Luchini A., Bresolin S., VIOLA G., Persano L. Molecular and functional profiling of chemotolerant cells unveils nucleoside metabolism-dependent vulnerabilities in medulloblastoma. Acta Neuropathologica Communications (2023), 11:183.
- Romagnoli R., Baraldi P.G., Prencipe F., Oliva P., Baraldi S., Ortega Schiaffino S., Kimatrai Salvador M., Lopez Cara L.C., Brancale A., Ferla S., Hamel E., Ronca R., Bortolozzi R., Mariotto E., Mattiuzzo E., VIOLA G. Design, Synthesis and biological evaluation of 6-substituted Thieno[3,2-d]pyrimidine analogues as dual EGFR kinase and microtubule inhibitors J. Med. Chem. (2019), 62, 1274-1290.
- Romagnoli R., Oliva P., Maria Kimatrai Salvador M., Encarnacion Camacho M., Padroni C, Raveglia L, Brancale A., Ferla S., Hamel E, Ronca R., Grillo E., Bortolozzi R, Rruga F., Mariotto E., VIOLA G. Design, Synthesis and Biological Evaluation of Novel Vicinal Diaryl-Substituted 1H-Pyrazole Analogues of Combretastatin A-4 as Highly Potent Tubulin Polymerization Inhibitors Eur J. Med. Chem (2019), 181, 111577.
- Roncato F, Rruga F., Porcu E., Casarin E., Ronca R., Realdon R., Basso G., Alon R., Basso G., VIOLA G. Morpurgo M. Improvement and extension of anti-EGFR targeting in breast cancer therapy by integration with the Avidin-Nucleic-Acid-Nano Assemblies Nat. Commun. (2018) 9(1):4070
- Bortolozzi R., Mattiuzzo E., Trentin L., Accordi B., Basso G., VIOLA G. Ribociclib, a CDK4/CDK6 kinase inhibitor, enhance glucocorticoid sensitivity in B-acute lymphoblastic leukemia (B-ALL). Biochem Pharm. (2018), 153, 230-241.


**Subarea**

## High-Throughput Drug Screening for Precision Oncology

**Group Leader** Dr. Elena Mariotto - Junior Principal Investigator

**Research activity**

The High-Throughput Drug Screening for Precision Oncology unit is devoted to two major on-going projects listed below.

*Establishing more representative in vitro 3D model to study cancer cell adaptation to the hostile tumor microenvironment (e.g. hypoxia, starvation, acidosis) in Glioma and Medulloblastoma pediatric brain tumor to unveil new targetable metabolic vulnerabilities (funded by Supporting TAlent in ReSearch Starting Grant, University of Padova, 2022-2024).*

*Drug Sensitivity Profiling (DSP) as promising tool to predict the response to chemotherapy in oncological pediatric patients in order to* i) achieve effective therapeutic strategies for relapsed/refractory patients lacking actionable genetic alterations, ii) avoid unnecessary toxicity, and iii) prevent drug resistance and tumor relapse (funded by IRP-Starting Grant, Pediatric Research Institute, 2024-2027).

The High-Throughput Screening (HTS) facility consist of a semi-automated liquid handling platform (Microlab STAR 96-CORE, Hamilton), dedicated CO2 incubator and 1.5% O2 hypoxic cabinet (H35 Hypoxystation, Don Whitley Scientific), and a multimodal plate reader (Spark, Tecan). The facility enables to perform large drug repositioning/repurposing screening with in-house drug libraries (>3500 compounds) for anticancer drug discovery, dose-response sensitivity profiling and complex drug synergism studies to accelerate the identification and validation of new treatment options for pediatric cancers.

In collaboration with Prof. Giampietro Viola, I have been actively involved in the study of Bcl2-Associated AthanoGene (BAG) protein family in sustaining tumor relapse and chemotherapy resistance in pediatric tumors. Thanks to this project, we successfully designed a novel BAG3 inhibitor able to sensitize cancer cell to chemotherapy (in partnership with Evotec pharmaceutical company, Aptuit Srl, Verona, Italy).

**JrPI's Biosketch**

*Scopus ID 0000-0002-3960-8561*

Since the beginning of my career, my research interests have focused on a comprehensive understanding of key molecular pathways that sustain tumor drug resistance and the identification of promising anticancer drugs for pediatric cancers. My academic training and research experience have provided me with an excellent background in multiple biological disciplines including molecular and cell biology, as well as preclinical drug screening across different tumor types. I graduated in 2013 in both Health Biology (University of Padova, Italy) and in Genetics (Paris Diderot University, France) within the European Double degree program, and then I obtained the Ph.D. in Developmental Medicine and Health Planning Sciences, Pediatric Oncohematology Unit - Dept. of Women's and Children's Health (University of Padova, 2017). During my training, I have been the opportunity to work in different environment, such as the Institut Universitaire d'Hematologie (IUH) (Paris, France), the Centro di Riferimento Oncologico (CRO) (Aviano, Italy),

the Pediatric Research Institute (IRP) and the University of Padova (UNIPD, Italy), resulting in a solid national and international network of collaborations.

The last five years of my post doctoral career have been focused on fighting Medulloblastoma chemotherapy resistance by establishing a novel ex vivo model of drug resistance by exposing patient-derived Medulloblastoma (PD-MB) cells to high-dose chemotherapy, funded in part by the Associazione Italiana per la Ricerca sul Cancro (AIRC, Italy - 2020-2022) and the Rally Foundation for Childhood Cancer Research (US, 2021-2022). Moreover, the strong pharmacological background that I cultivated during my Post doctoral training encouraged me to develop from scratch an up-to-date High-Throughput drug Screening (HTS) platform to test more than 3500 drugs within the Academic research context (Mariotto E. et al. Acta Neuropathol Commun, 2023). I am currently Junior PI of the High-Throughput Drug Screening for Precision Oncology Lab at the Pediatric Research Institute thanks to i) the competitive Supporting TAlent in ReSearch (STARS@UNIPD) Starting Grant (University of Padova, 2022-2024) to explore more realistic culturing conditions to closely mimic the peculiar brain tumor microenvironment to unveil hidden metabolic synthetic lethal drugs, and ii) the intramural IRP-Starting Grant (Pediatric Research Institute, 2024-2027) for a personalized medicine approach to test the sensitivity to chemotherapy of patient-derived cancer cells collected at the University Hospital of Padova.

### Team members

Dr. Elena Mariotto - Junior Principal Investigator
Dr. Chiara Marchioro - PhD Student
Dr. Martina Canton - PhD Student
Dr. Martina Portaluri - Undergraduate fellow

### Selected publications


- Mariotto E, Rampazzo E, Bortolozzi R, Rruga F, Zeni I, Manfreda L, Marchioro C, Canton M, Cani A, Magni R, Luchini A, Bresolin S, Viola G, and Persano L. Molecular and functional profiling of chemotolerant cells unveils nucleoside metabolism-dependent vulnerabilities in medulloblastoma. Acta Neuropathol Commun, 11 (2023) 183. https://doi.org/10.1186/s40478-023-01679-7
- Budassi F, Marchioro C, Canton M, Favaro A, Sturlese M, Urbinati C, Rusnati M, Romagnoli R, Viola G, and Mariotto E. Design, synthesis and biological evaluation of novel 2,4-thiazolidinedione derivatives able to target the human BAG3 protein. Eur J Med Chem, 261 (2023) 115824. https://doi.org/10.1016/j.ejmech.2023.115824
- Mariotto E, Corallo D, Pantile M, Giarin E, Pigazzi M, Basso G, Viola G, Aveic S. BAG1 down-regulation increases chemo-sensitivity of acute lymphoblastic leukaemia cells. J Cell Mol Med. 2021;25(18):9060-5. https://doi.org/10.1111/jcmm.16822
- Mariotto E, Viola G, Zanon C, Aveic S. A BAG's life: Every connection matters in cancer. Pharmacology & Therapeutics, 107498 (2020). https://doi.org/10.1016/j.pharmthera.2020.107498
- Mariotto E, Bortolozzi R, Volpin I, Carta D, Serafin V, Accordi B, Basso G, Navarro PL, Lopez-Cara LC, Viola, G. EB-3D a novel choline kinase inhibitor induces deregulation of the AMPK-mTOR pathway and apoptosis in leukemia T-cells. Biochem Pharmacol. 2018 Sep;155:213-223. https://doi.org/10.1016/j.bcp.2018.07.004


# Molecular Diagnostic of Non Hodgkin Lymphoma

**Group Leader** Prof. Lara Mussolin - Principal Investigator

### Research activity

Our research area is dedicated mainly to the study and characterization of Non-Hodgkin lymphoma of childhood. The general approach includes the analysis of molecular mechanisms of tumorigenesis with a translational approach aimed to transfer biological results from the bench to clinical trials. This lab makes part of an international consortium with the aim to identify novel therapies and biomarkers with a particular focus regarding the role of liquid biopsy in these hematological malignancies.

### PI's Biosketch

*Scopus ID 8886437100*

Prof. L. Mussolin is an Associate Professor of the Department of Woman and Child Health in Padova, Italy. She is a member of the scientific committee of Pediatric Research Institute Città della Speranza. She is responsible for molecular diagnosis of non-Hodgkin lymphomas (NHLs) of childhood for all national AIEOP (Associazione Italiana di Emato-Oncologia Pediatrica) centres. Prof. Mussolin has throughout her research career focused on the childhood NHL biological studies. She coordinates a research group aimed to identify new prognostic markers and biological criteria for risk stratification in pediatric lymphoma. She is involved in several international collaborative efforts; she is member of the EICNHL (European InterGroup of Non-Hodgkin Lymphoma) Group and in 2023 she was elected chair of Biology of this international network. She is member of European Research Initiative of ALK-related malignancies (ERIA). The main task of ERIA is to strengthen cooperation and partnership of research groups focused on the role of ALK in cancer. A consortium, together with some of these members, was recently founded with the aim to train and educate talented researchers. Prof. Mussolin is the Italian national PI of this consortium

She is invited to numerous national and international conferences as a speaker. Prof. Mussolin's activity in the field of pediatric oncology is demonstrated by her publications, which include more than 80 international papers, book chapters and more than 300 abstracts for poster/oral presentations at national and international meetings.

### Team members

Prof. Lara Mussolin - Principal Investigator
Dr. Gaia Martire - PhD Student
Dr. Lavinia Ferrone - Post Doc
Dr. Carlotta Caterina Damanti - Post Doc
Dr. Matteo Marzi - PhD Student
Dr. Rebekka Salzmann - PhD Student
Dr. Alessia Danieli - PhD Student
Dr. Domenico Rizzato - Research Fellow
Dr. Elisa Tosato - Senior Technologist/Research Associates

## Selected publications


- Martire G, Lovisa F, Carraro E, Rizzato D, Cesaro S, Mura RM, Tondo A, Bertolin C, Boaretto F, Salviati L, Biffi A, Pillon M, Mussolin L. TP53 DNA binding domain mutational status and rituximab-based treatment are independent prognostic factors for pediatric Burkitt lymphoma patients stratification. Haematologica. 2024 Feb 22. doi: 10.3324/haematol.2023.284868. Epub ahead of print. PMID: 38385281.
- Damanti CC, Ferrone L, Gaffo E, Garbin A, Tosato A, Contarini G, Gallingani I, Angioni R, Molon B, Borile G, Carraro E, Pillon M, Scarmozzino F, Dei Tos AP, Pizzi M, Ciscato F, Rasola A, Biffi A, Bortoluzzi S, Lovisa F, Mussolin L. Plasma small-extracellular vesicles enriched in miR-122-5p promote disease aggressiveness in pediatric anaplastic large-cell lymphoma. Cancer Commun (Lond). 2023 May;43(5):630-634. doi: 10.1002/cac2.12415. Epub 2023 Apr 4. PMID: 37014813; PMCID: PMC10174085.
- Damm-Welk C, Lovisa F, Contarini G, Lüdersen J, Carraro E, Knörr F, Förster J, Zimmermann M, Sala A, Vinti L, Tondo A, Pillon M, Woessmann W, Mussolin L. Quantification of Minimal Disease by Digital PCR in ALK-Positive Anaplastic Large Cell Lymphoma: A Step towards Risk Stratification in International Trials? Cancers (Basel). 2022 Mar 27;14(7):1703. doi: 10.3390/cancers14071703. PMID: 35406475; PMCID: PMC8996924.
- Garbin A, Lovisa F, Holmes AB, Damanti CC, Gallingani I, Carraro E, Accordi B, Veltri G, Pizzi M, d'Amore ESG, Pillon M, Biffi A, Basso K, Mussolin L. miR-939 acts as tumor suppressor by modulating JUNB transcriptional activity in pediatric anaplastic large cell lymphoma. Haematologica. 2021 Feb 1;106(2):610-613. doi: 10.3324/haematol.2019.241307. PMID: 32299901; PMCID: PMC7849582.
- Lovisa F, Binatti A, Coppe A, Primerano S, Carraro E, Pillon M, Pizzi M, Guzzardo V, Buffardi S, Porta F, Farruggia P, De Santis R, Bulian P, Basso G, Lazzari E, d'Amore ESG, Bortoluzzi S, Mussolin L. A high definition picture of key genes and pathways mutated in pediatric follicular lymphoma. Haematologica. 2019 Sep;104(9):e406-e409. doi: 10.3324/haematol.2018.211631. Epub 2019 Feb 28. PMID: 30819919; PMCID: PMC6717562.


# Research and discovery in Hematopoietic Cell&Gene Therapy

**Group Leader** Prof. Alessandra Biffi - Principal Investigator

## Research activity

The activity of the group is devoted to generating novel therapeutic paradigms and clinical translation of new platforms based on the use of genetically engineered hematopoietic cells in multiple disease areas.

*Inherited neurometabolic diseases of childhood*

Our group is historically focused on the development of novel treatment approaches based on the use of genetically engineered hematopoietic stem and progenitors cells (HSPCs) for the treatment of inborn errors of metabolism severely affecting the central nervous system (CNS). The therapeutic potential of HSPC gene therapy in these conditions is based on their ability to repopulate the myeloid compartment of the recipient, including microglia in the CNS, with a transplant-derived, mature progeny able to express therapeutic transcripts and exert neuro-immunomodulatory and neuroprotective functions. The transplant progeny in the CNS recapitulates most of microglia functional features and can: i) attenuates or even reverse the detrimental effects of microglia activation response on other CNS cell types, such as neurons, thus mitigating CNS pathology; ii) restores a physiological myeloid cell/microglia function, including efficient scavenging, within the CNS with the potential to clear accumulated debris and undegraded molecules, including storage material; iii) provides a new effective source of therapeutic molecules, such as lysosomal enzymes for LSDs, within the CNS through genetic engineering; the functional protein could also be secreted and provided to neighboring cells. The curative potential of this approach can be further enhanced by the use of optimized protocols for rapid reconstitution of myeloid cell populations in the brain and spinal cord by the transplanted cells/their progeny that are being explored and developed in the laboratory, based on innovative routes for the delivery of the engineered HSPCs or on genetic modification of the HSPCs to provide them with an in vivo repopulation advantage in the CNS. These novel approaches are exploited for developing novel treatment opportunities for neurometabolic disorders of childhood with unmet medical need.

*Acquired neurodegenerative diseases*

The knowledge and technology developed by our group towards the treatment of neurometabolic disorders of childhood is now exploited for pioneering research in the area of adult-onset neurodegenerative disorders that share with the former conditions many pathogenic mechanisms. Among others, we are working on Frontotemporal Dementia (FTD) with the working hypothesis that achieving brain replacement of the lysosomal protein progranulin (GRN) is a rational therapeutic approach for familial FTD forms associated to GRN mutations, and on Alzheimer's disease associated to Trem2 mutations, to assess the therapeutic potential of a TREM2-directed microglia engineering. To ensure the successful application of HSC gene therapy to these conditions, we are also focused on more basic studies aimed at further unraveling the mechanisms of microglia maintenance in physiological and pathological conditions, as well as the modalities of myeloid cell/microglia replacement following HSPC transplantation. Goal is the development of translational protocols allowing selective and specific targeting and transplantation of populations with the potential of contributing

to microglia turnover. Moreover, in this same setting regulated expression of therapeutic genes in mature microglia is being pursued by gene transfer and gene editing approaches. Industrial alliances partially support these research projects.

*Autoimmune disorders*

The use of autologous hematopoietic cell transplantation (HCT) has been widely explored in the context of randomized multicenter clinical trials as a treatment option for autoimmune diseases, such as multiple sclerosis (MS) and type 1 diabetes (T1D). The aim behind this approach resides within the immunoregulatory properties of HSPCsand it is based on the possibility to permanently reprogram an auto-aggressive immune system towards a de novo self-tolerant immune repertoire by HSPC transplantation. Indeed, the expansion and reinfusion of autologous HSPCs was demonstrated to be a potent therapy in reverting hyperglycemia in T1D patients, while several data demonstrate that adoptive transfer of HSPCs, either in pharmacologically conditioned or unconditioned MS subjects allows the homing of immune-modulatory cells in the affected areas of the CNS. In this context, the evidence on the role of the immune-regulatory factor programmed death-ligand 1 (PD-L1) in HSPC immunobiology has opened the possibility to establish novel therapeutic strategies based on PD-L1 expression in HSPCs to treat autoimmune diseases as MS and T1D.Considering both the common autoimmune mechanisms underlying T1D and MS, and the role of the PD-1/PD-L1 axis in both diseases, the improvement of immuno-regulatory properties of HSPCs by expression of PD-L1 may result in a therapeutic benefit in MS and T1D upon transplantation. Our data in T1D and MS animal models provide support for this concept. In the setting of Sponsored research programs with Altheia Science, a spin-off of the University of Padua, we are developing this concept in the setting of translational studies towards clinical testing.

*Pediatric cancer collaborative projects have been established within our Division combining the local unique expertise in pediatric oncology, advanced diagnostics and target discovery with the hematopoietic cell gene transfer and gene therapy expertise of the Biffi's group*

These projects are devoted to exploring the possibility of identifying and targeting unique surface tumor antigens by cell-based immunotherapy. The most advanced project is focused on acute myeloid leukemia and exploits newly discovered AML-specific antigens for the development of a Chimeric Antigen Receptor (CAR) T cell approach. Available proof of concept data demonstrates not only the feasibility but also the efficacy of the proposed strategy, and the most promising targets and CARs are being exploited for translational purposes. Industrial alliances partially support these research projects.

*β-hemoglobinopathies, namely β-Thalassemia and Sickle Cell Disease*

HSPC gene therapy is an extremely promising approach for these conditions. Dr. Poletti in our laboratory is exploring an original, forward-looking gene therapy strategy for β-globin defects based on a non-viral approach, with a solid potential for clinical efficacy and translation, with a strategic design to maximize accessibility to the multitude of neglected children born with these lethal and devastating diseases in the poorest, and most affected, countries. See link to subarea.

## PI's Biosketch

See Area Coordinator

## Team members

Prof. Alessandra Biffi - Principal Investigator
Dr. Valentina Poletti - Junior Principal Investigator
Dr. Rita Milazzo - Senior scientists
Dr. Annita Montepeloso - Senior scientists
Dr. Yuri Ciervo - Senior Technologist/Research Associates
Dr. Giulia Santinon - Senior Technologist/Research Associates
Dr. Silvia Spadini - Post-doctoral Fellows
Dr. Linda Bucciarelli - PhD Student
Dr. Pietro Rigoni - PhD Student
Dr. Anna Tognon - PhD Student
Dr. Raffaele Mattera - PhD Student
Dr. Linda Rossini - PhD Student
Dr. Davide Mattioli - Post-graduate Fellow
Dr. Martina Lombi - Post-graduate Fellows
Dr. Massimo Accardo - Lab manager
Dr. Veronica Favero - Animal behavior technician

## Selected publications


- Das S, Rruga F, Montepeloso A, Dimartino A, Spadini S, Corre G, Patel J, Cavalca E, Ferro F, Gatti A, Milazzo R, Galy A, Politi LS, Rizzardi GP, Vallanti G, Poletti V, Biffi A. An empowered, clinically viable hematopoietic stem cell gene therapy for the treatment of multisystemic mucopolysaccharidosis type II. Mol Ther. 2024 Mar 6;32(3):619-636;
- Poletti V, Montepeloso A, Pellin D, Biffi A. Prostaglandin E2 as transduction enhancer affects competitive engraftment of human hematopoietic stem and progenitor cells. Mol Ther Methods Clin Dev. 2023 Oct 11;31:101131;
- Buldini B, Faggin G, Porcù E, Scarparo P, Polato K, Tregnago C, Varotto E, Rizzardi P, Rizzari C, Locatelli F, Biffi A*, Pigazzi M* (*co-last authors). CD72 is a pan-tumor antigen associated to pediatric acute leukemia. Cytometry A. 2023 Dec;103(12):1004-1009;
- Peviani M, Das S, Patel J, Jno-Charles O, Kumar R, Zguro A, Mathews TD, Cabras P, Milazzo R, Cavalca E, Poletti V, Biffi A. An innovative hematopoietic stem cell gene therapy approach benefits CLN1 disease in the mouse model. EMBO Mol Med. 2023 Apr 11;15(4):e15968;
- Fumagalli F, Calbi V, Natali Sora MG, Sessa M, Baldoli C, Rancoita PMV, Ciotti F, Sarzana M, Fraschini M, Zambon AA, Acquati S, Redaelli D, Attanasio V, Miglietta S, De Mattia F, Barzaghi F, Ferrua F, Migliavacca M, Tucci F, Gallo V, Del Carro U, Canale S, Spiga I, Lorioli L, Recupero S, Fratini ES, Morena F, Silvani P, Calvi MR, Facchini M, Locatelli S, Corti A, Zancan S, Antonioli G, Farinelli G, Gabaldo M, Garcia-Segovia J, Schwab LC, Downey GF, Filippi M, Cicalese MP, Martino S, Di Serio C, Ciceri F, Bernardo ME, Naldini L, Biffi A*, Aiuti A*(*co-last authors). Lentiviral haematopoietic stem-cell gene therapy for early-onset metachromatic leukodystrophy: long-term results from a non-randomised, open-label, phase 1/2 trial and expanded access. Lancet. 2022 Jan 22;399(10322):372-383.

- Esrick EB*, Lehmann LE*, Biffi A*, Achebe M, Brendel C, Ciuculescu MF, Daley H, MacKinnon B, Morris E, Federico A, Abriss D, Boardman K, Khelladi R, Shaw K, Negre H, Negre O, Nikiforow S, Ritz J, Pai SY, London WB, Dansereau C, Heeney MM, Armant M, Manis JP, Williams DA (*co-first authors). Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease. N Engl J Med. 2021 Jan 21;384(3):205-215;
- Kim J, Hu C, Moufawad El Achkar C, Black LE, Douville J, Larson A, Pendergast MK, Goldkind SF, Lee EA, Kuniholm A, Soucy A, Vaze J, Belur NR, Fredriksen K, Stojkovska I, Tsytsykova A, Armant M, DiDonato RL, Choi J, Cornelissen L, Pereira LM, Augustine EF, Genetti CA, Dies K, Barton B, Williams L, Goodlett BD, Riley BL, Pasternak A, Berry ER, Pflock KA, Chu S, Reed C, Tyndall K, Agrawal PB, Beggs AH, Grant PE, Urion DK, Snyder RO, Waisbren SE, Poduri A, Park PJ, Patterson A, Biffi A, Mazzulli JR, Bodamer O, Berde CB, Yu TW. Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease. N Engl J Med. 2019 Oct 24;381(17):1644-1652;
- Pellin D, Loperfido M, Baricordi C, Wolock SL, Montepeloso A, Weinberg OK, Biffi A, Klein AM, Biasco L. A comprehensive single cell transcriptional landscape of human hematopoietic progenitors. Nat Commun. 2019 Jun 3;10(1):2395;
- Wu Y, Zeng J, Roscoe BP, Liu P, Yao Q, Lazzarotto CR, Clement K, Cole MA, Luk K, Baricordi C, Shen AH, Ren C, Esrick EB, Manis JP, Dorfman DM, Williams DA, Biffi A, Brugnara C, Biasco L, Brendel C, Pinello L, Tsai SQ, Wolfe SA, Bauer DE. Highly efficient therapeutic gene editing of human hematopoietic stem cells. Nat Med. 2019 May;25(5):776-783;
- Biffi A. Gene Therapy as a Curative Option for β-Thalassemia. N Engl J Med. 2018 Apr 19;378(16):1551-1552;
- Esrick EB, Manis JP, Daley H, Baricordi C, Trébéden-Negre H, Pierciey FJ, Armant M, Nikiforow S, Heeney MM, London WB, Biasco L, Asmal M, Williams DA, Biffi A. Successful hematopoietic stem cell mobilization and apheresis collection using plerixafor alone in sickle cell patients. Blood Adv. 2018 Oct 9;2(19):2505-2512;


## Subarea

## Gene therapy for hemoglobinopathies and neurometabolic diseases caused by β-Galactosidase deficiency

**Group Leader** Dr. Valentina Poletti - Junior Principal Investigator

### Research activity

Valentina Poletti's laboratory is focused on the development of innovative, safe and efficient GT strategies for hemoglobinopathies and neurometabolic diseases caused by β-Galactosidase deficiency. β-Thalassemia and Sickle Cell Disease are the most common monogenic diseases world-wide, with >317,000 newborn/year, caused by defects of β-globin expression or structure. Ex vivo Hematopoietic Stem/Progenitor Cells (HSPCs) gene therapy (GT), i.e. autologous transplantation of HSCs modified by lentiviral (LV) gene-transfer to express a therapeutic protein, is an extremely promising approach for patients not eligible to allogeneic HSPC transplantation. Only partial clinical efficacy has been achieved so far by the only approved GT for β-thalassemia, i.e. Zynteglo®, mainly due to suboptimal Hb production and inner LV restraints. Here we propose to develop an original, forward-looking GT strategy for β-globin defects to overcome these limitations, based on 1) gene-cassette optimization for enhanced production of a potent anti-sickling β-globin; 2) the usage of novel hyper-functional transposon-vectors to safely integrate multiple copies of complex gene-cassettes into HSPCs; 3) the implementation of a new, cost-effective, scalable and standardized vector-production generating ready-to-use minicircle DNAs. Another focus of the lab. is the generation of novel synthetic hyper-functional enzymes for GT by molecular evolution. In particular, the use of a novel synthetic hyper-functional human β-Galactosidase (β-Gal) could enable the HSC GT for two lysosomal storage diseases caused by β-Gal deficiency, i.e. GM1 Gangliosidosis and Mucopolysaccharidosis type IVB, with an increased efficacy and reduced dose and cost. This molecular evolution approach could set a new modality for the development of novel therapies for orphan genetic diseases.

### JrPI's Biosketch

*Scopus ID 25655343700*

Valentina Poletti is Assistant Professor of Genetics in the Department of Woman's and Child's Health of University of Padova and Jr group leader at the Pediatric Research Institute "Città della Speranza" (Padua, Italy). She is a scientist at the boundary between basic and applied science, with a deep knowledge of basic molecular and cell biology (transcriptional regulation, provirus-genome interaction, design of regulatory elements and vectors, human HSC homeostasis and differentiation) and a significant experience acquired over more than 15 years in ex vivo GT applied to multiple genetic diseases (skin, immunodeficiencies, hemoglobinopathies and lysosomal storage diseases). During her years of work in Italy, France (Genethon) and USA (Dana Farber/Harvard University) she developed competences in hematology, Hematopoietic Stem Cell (HSC) transplantation and animal models of disease, and contributed to Clinical Trial Authorization (CTA)-enabling studies of GT that enabled two clinical trials in USA (X-linked SCID) and France (sickle-cell disease). She has been Faculty member of Harvard Medical School from 2019 to 2023, and she was awarded of a prestigious Marie Skłodowska-Curie fellowship in 2020 to establish her laboratory in Italy.

She has an H-index of 13 (Scopus), 20 high-rank publications among original articles and reviews from 2008 to date, in the top 25% journals, including Molecular Therapy and family journals (Methods and Clinical Development, Nucleic Acids), EMBO Molecular Medicine, Human Gene Therapy and Scientific Reports. She is co-inventor of two patented regulated promoters to express therapeutic genes.

Her laboratory is focused on the development of innovative, safe and efficient GT strategies for hemoglobinopathies and neurometabolic diseases caused by β-Galactosidase deficiency.

### Team members

Dr. Valentina Poletti - Junior Principal Investigator

Dr. Linda Bucciarelli - PhD Student

Dr. Elena Scarabello - Senior Technologist/Research Associates

### Selected publications


- Poletti V., Montepeloso A., Pellin D, Biffi A. Prostaglandin E2 as transduction enhancer affects competitive engraftment of human hematopoietic stem and progenitor cells. Molecular Therapy Methods and Clinical Development, 2023, 31, 101131
- Poletti V. and Mavilio F. Designing lentiviral vectors for gene therapy of genetic diseases. Viruses, 2021, 13(8), 1526

- Poletti V. and Mavilio F. Interactions between Retroviruses and the Host Cell Genome. Molecular Therapy Methods and Clinical Development, 2018, 8, pp. 31–41
- Poletti V., Charrier S., Corre G., Gjata B., Vignaud A., Zhang F., Rothe M., Schambach A., Gaspar H. B., Thrasher A.J., Mavilio F. Preclinical Development of a Lentiviral Vector for Gene Therapy of X-Linked Severe Combined Immunodeficiency. Molecular Therapy Methods and Clinical Development, 2018, 9, pp. 257–269
- Poletti V., Urbinati F., Charrier S., Corre G., Hollis R.P., Campo Fernandez B., Martin S., Rothe M., Schambach A., Kohn D.B., Mavilio F. Pre-clinical Development of a Lentiviral Vector Expressing the Anti-sickling βAS3 Globin for Gene Therapy for Sickle Cell Disease. Molecular Therapy Methods and Clinical Development, 2018, 11, pp. 167–179






# Target discovery and biology of acute myeloid leukemia

**Group Leader** Prof. Martina Pigazzi - Principal Investigator

## Research activity

Leukemias account for approximately one third of all pediatric malignancies and remain a leading cause of morbidity and mortality in children and adolescents. Acute myeloid leukemia (AML) in children is one of the most genetically heterogeneous diseases among pediatric cancers. With the current therapeutic approaches, the event-free survival rate ranges between 55 and 70%, with one out of three children experiencing a refractory or relapsed disease. Market authorization of targeted and immune therapies emerged as salvage treatment for other diseases, whereas in AML, they are unfrequently used, with intensive chemotherapy and stem cell transplantation still representing the main treatment options. The actual dearth of new agent approval for pediatric AML underlines that performing AML underlined mechanisms and robust preclinical studies are mandatory for pediatric drug advance. Our research aims at the identification of new genetic aberrations and mutations with prognostic value to be used as biomarkers to improve diagnosis and risk stratification of patients and their treatment response by employing tailored adapted therapies. We apply NGS at diagnosis of leukemia to find relevant prognostic mutations and design new quantitative molecular assays for monitoring minimal molecular disease, increasing the ability to stratify patients at diagnosis, during treatment and at follow up. For most aggressive AML where cytogenetic aberrancies or mutations are present, we set up functional studies deepening into the mechanism of leukemogenesis. We deep into the intimate connection between the dysregulation of gene expression and malignant transformation and highlight the importance of investigating key players in the regulation of gene expression to shed light on transforming events to define new targeted therapeutic approaches. We employed RNA and DNA sequencing and proteome status of AML and to be studied in vitro we recapitulated an innovative in vitro three-dimensional (3D) model, and in vivo models' patient-derived xenografts (PDXs). We established >30 AML PDXs through sequential engraftment of primary AML cells in NSG mice, representing most of the14 high-risk genetic subtypes. AML-PDXs robustly resembling the original AML in terms of immunophenotype, genomic, and transcriptomic profiles offer a comprehensive view of the disease complexity, useful for tailor therapies. The whole-exome sequencing characterization evidenced a high intra-tumoral heterogeneity of the disease giving the opportunity to trace disease evolution and thus select and test new drugs. The use of PDX models to perform unconventional trials such as umbrella, basket and platform trials will accelerate the use of preclinical studies toward personalized medicine approaches, in compliance with clinical needs. The 3D model is created for mimicking bone extracellular matrix and is composed of 70 wt% hydroxyapatite/30 wt% collagen type I where different cell types of the stroma of the bone marrow are cultured with leukemia cells or healthy hematopoietic stem cells. The 3D system allows proliferating long-term co-cultures up to 21 days and maintains the cell features. Our research delved into the intricate interactions between primary mesenchymal stromal cells (MSCs) and acute myeloid leukemia cells in the bone marrow niche and produced evidence that this contact is generating several changes inducing leukemia microenvironment (TME) and inflammation. These models are helping in the understanding of cancer transformation mechanisms for the developing of promising alternatives or complementary therapeutics including target and immune therapies to current chemotherapy,

particularly for the AML that undergoes to relapse. A patent for a drug selective for one aggressive AML subtype has been generated and we are developing a sub-area for developing novel drugs and testing to be successfully advanced in clinical trials. More recently, we are dissecting the leukemia stem cell metabolism and mitochondrial characterization and targeting. We are actively participating to the Children's Liver Tumor European Research Network creating PDX for an innovative anticancer drug for the treatment of patients with high-risk hepatoblastoma facing disease recurrence or chemotherapeutic treatment-failure. Our research aims to introduce a novel translational dimension to the field of conventional approaches towards novel applications based on scientific and biological evidence with the final aim to improve blood diseases cure.

## PI's Biosketch

*Scopus ID 23010194100*

Martina Pigazzi is Associate Professor of the Department of Women's and Children's Health at the University-Hospital of Padua. From her graduation in 2000 she attended the pediatric oncology-hematology laboratory of the pediatric clinic-hospital of Padova. In 2004 she Specialized in Medical Genetics at the Faculty of Medicine of the University of Verona; in 2009 she became Doctor of Philosophy at the School in Developmental Medicine - oncology, hematology and immunology area - of the Faculty of Medicine of the University of Padova. Since 2009 she is the Geneticist responsible for the genetic diagnosis of all Italian patients affected by acute myeloid leukemia enrolled in the national protocols of the Italian Association of Pediatric Hemato-Oncology (AIEOP), providing genetic counseling at diagnosis and during patient treatment and follow-up. Since 2018 she is responsible for the Genetics Unit at the Onco-Hematology laboratory, Department of Women's and Children's Health, Division of Pediatric Hematology, Oncology and Stem Cell Transplantation of the University Hospital of Padova, which acts as a reference laboratory Italian for the diagnosis of pediatric tumors. Since 2010 she is member of various national and international groups covering the role of expert in leukemia. Since 2009 she leads an independent laboratory carrying out research for the genetic characterization of leukemias, evaluating the prognostic impact and functional role of associated mutations with the aim of finding new drugs and new therapeutic strategies. Since 2016 she is member of international commissions whose main objectives are to improve therapies in the field of pediatric leukemia and accelerate the opening of trials for the clinical testing of new drugs (PeDAL, ACCELERATE, i-BFM, ITCC-P4). Since 2013 she is Principal Investigator at the Pediatric Research Institute (IRP) and leads a research group of 4 post docs, 3 doctoral students, 2 technicians and several students who attend to carry out their degree thesis. She won several research projects funded by national agencies (AIRC, AIL, IRP, CARIPARO, FUV), from the Ministry of Health (PRIN), and from european calls (Horizon 2020, FKC). She owns 2 international patents. Pigazzi's bibliography counts more than 80 original manuscripts published in the main international cancer, hematology and cancer research journals, some chapters in scientific books; she received national and international awards and prizes. As professor she teaches at the University of Padova at Medicine courses and at the associated degrees for Nurses as well as for the Doctoral School in Developmental Medicine, and in the Specialty school of Medical Genetics. She mentored more than 40 students for their thesis degree in medicine, biology, biotechnology, or for the specialties and doctorates thesis in the medical-oncology or genetic fields.

### Team members

Prof. Martina Pigazzi - Principal Investigator
Dr. Claudia Tregnago - Senior Scientist
Dr. Maddalena Benetton - Post Doc
Dr. Ambra Da Ros - Post Doc
Dr. Giorgia Longo - PhD Student
Dr. Sara Perpinello - PhD Student
Dr. Silvia Merlini - PhD Student
Dr. Diego Calvetti - Senior Technologist/Research Associates
Dr. Katia Polato - Senior Technologist/Research Associates

## Selected publications


- Buldini B, Faggin G, Porcù E, Scarparo P, Polato K, Tregnago C, Varotto E, Rizzardi P, Rizzari C, Locatelli F, Biffi A, Pigazzi M. CD72 is a pan-tumor antigen associated to pediatric acute leukemia. Cytometry A. 2023 Dec;103(12):1004-1009. doi: 10.1002/cyto.a.24790. Epub 2023 Oct 24.PMID: 37876342
- Benetton M, Merli P, Walter C, Hansen M, Da Ros A, Polato K, Tregnago C, Abrahamsson J, Strocchio L, Sonneveld E, Fogelstrand L, Von Neuhoff N, Reinhardt D, Hasle H, Pigazzi M, Locatelli F. Molecular Measurable Residual Disease Assessment before Hematopoietic Stem Cell Transplantation in Pediatric Acute Myeloid Leukemia Patients: A Retrospective Study by the I-BFM Study Group. Biomedicines. 2022 Jun 28;10(7):1530. doi: 10.3390/biomedicines10071530.PMID: 35884834
- Tregnago C, Benetton M, Da Ros A, Borella G, Longo G, Polato K, Francescato S, Biffi A, Pigazzi M. Novel Compounds Synergize With Venetoclax to Target KMT2A-Rearranged Pediatric Acute Myeloid Leukemia. Front Pharmacol. 2022 Jan 27;12:820191. doi: 10.3389/fphar.2021.820191. eCollection 2021.PMID: 35153769
- Porcù E, Benetton M, Bisio V, Da Ros A, Tregnago C, Borella G, Zanon C, Bordi M, Germano G, Manni S, Campello S, Rao DS, Locatelli F, Pigazzi M. The long non-coding RNA CDK6-AS1 overexpression impacts on acute myeloid leukemia differentiation and mitochondrial dynamics. iScience. 2021 Oct 26; 24(11):103350. doi: 10.1016/j.isci.2021.103350. eCollection 2021 Nov 19.PMID: 34816103
- Borella G, Da Ros A, Borile G, Porcù E, Tregnago C, Benetton M, Marchetti A, Bisio V, Montini B, Michielotto B, Cani A, Leszl A, Campodoni E, Sandri M, Montesi M, Bresolin S, Cairo S, Buldini B, Locatelli F, Pigazzi M. Targeting the plasticity of mesenchymal stromal cells to reroute the course of acute myeloid leukemia. Blood. 2021 Aug 19; 138(7):557-570. doi: 10.1182/blood.2020009845.PMID: 34010415

# Target Discovery and Biology of Neuroblastoma

**Group Leader** Dr. Sanja Aveic - Principal Investigator

## Research activity

Research in Aveic's laboratory focuses on the genetic and epigenetic mechanisms associated with phenotypic plasticity of drug-resistant neuroblastoma cells. By combining bioengineering and biomedicine the group is exploring novel targeted approaches against aggressive (metastatic) neuroblastoma phenotypes, and, in collaboration with clinicians, testing the feasibility of translating the results into medical applications. The group is currently pursuing projects in three key areas.

*Target discovery*

By linking neuroblastoma transcriptomics and epigenomics, the group aims to understand how the tumor develops and comprehend its heterogeneity in order to impede drug resistance, and the occurrence of metastases.

Oncopharmacology

Applying chemistry, 2D and 3D cell cultures, and animal models of neuroblastoma, ongoing research is focused on testing new targeted strategies against pro-metastatic molecular and epigenetic events while seeking their optimal combinations with chemotherapy and immunotherapy.

*Bioengineering*

Using engineered synthetic or natural biomaterials that interact with the cells, the group simulates 3D tumor models of neuroblastoma and uses them to test the efficacy of proposed therapeutic advances.

## PI's Biosketch

*Scopus ID 20435413800*

Dr. Aveic graduated from the University of Belgrade, Serbia, from the Department of Molecular Biology and Physiology, Division of Experimental Biomedicine. She received her PhD in 2010 from the University of Padua and worked as a Post Doc for 4 years at the Department of Pediatric Oncohematology in Padua. Her main fields of interest are pediatric neoplasms, including leukemias and neuroblastomas. Since 2014, she has been studying the molecular aspects of neuroblastoma genesis to seek more effective personalized therapeutic regimens. In 2018, Dr. Aveic become group leader of the Laboratory of Target Discovery and Biology of Neuroblastoma at the Pediatric Research Institute Città della Speranza Foundation. Since February 2019, she is also a cell lab leader at the RWTH Aachen University Hospital in the Department of Dental Materials and Biomaterials. Dr. Aveic has been a reviewer for numerous scientific journals in the field of pediatric oncology and biomaterials. She is a member of the International Society of Pediatric Oncology Europe Neuroblastoma (SIOPEN) and the European Society for Biomaterials (ESB). As part of the Italian Neuroblastoma Working Group, composed of physicians, pathologists, bioinformaticians, and biologists, Dr. Aveic participates in the preclinical evaluation of new protocols for neuroblastoma-targeted therapies. Her scientific output has more than 50 original articles published in peer-reviewed high impact journals, being the first, last and/or corresponding author in more than half of them.

## Team members

Dr. Sanja Aveic - Principal Investigator
Dr. Diana Corallo - Post Doc
Dr. Marcella Pantile - Senior Technologist/Research Associates
Dr. Sara Menegazzo - PhD Student

## Selected publications


- Corallo D, Pastorino F, Pantile M, Mariotto E, Caicci F, Viola G, Ponzoni M, Tonini GP, Aveic S. Autophagic flux inhibition enhances cytotoxicity of the receptor tyrosine kinase inhibitor ponatinib.
  J Exp Clin Cancer Res (IF: 7.04; Q1). 2020 Sep 22;39(1):195. doi: 10.1186/s13046-020-01692-x.
- Corallo D, Donadon M, Pantile M, Sidarovich V, Cocchi S, Ori M, De Sarlo M, Candiani S, Frasson C, Distel M, Quattrone A, Zanon C, Basso G, Tonini GP, Aveic S. LIN28B increases neural crest cell migration and leads to transformation of trunk sympathoadrenal precursors. Cell Death Differ (IF: 15.83; Q1). 2020 Apr;27(4):1225-1242. doi: 10.1038/s41418-019-0425-3.
- Aveic S, Janßen S, Nasehi R, Seidelmann M, Vogt M, Pantile M, Rütten S, Fischer H. A 3D printed in vitro bone model for the assessment of molecular and cellular cues in metastatic neuroblastoma. Biomater Sci (IF: 6.84; Q1). 2021 Mar 10;9(5):1716-1727. doi: 10.1039/d0bm00921k.
- Nasehi R, Abdallah AT, Pantile M, Zanon C, Vogt M, Rütten S, Fischer H, Aveic S. 3D geometry orchestrates the transcriptional landscape of metastatic neuroblastoma cells in a multicellular in vitro bone model.
  Mater Today Bio (IF: 7.35; Q1). 2023 Feb 28;19:100596. doi: 10.1016/j.mtbio.2023.100596
- Capasso M, Brignole C, Lasorsa VA, Bensa V, Cantalupo S, Sebastiani E, Quattrone A, Ciampi E, Avitabile M, Sementa AR, Mazzocco K, Cafferata B, Gaggero G, Vellone VG, Cilli M, Calarco E, Giusto E, Perri P, Aveic S, Fruci D, Tondo A, Luksch R, Mura R, Rabusin M, De Leonardis F, Cellini M, Coccia P, Iolascon A, Corrias MV, Conte M, Garaventa A, Amoroso L, Ponzoni M, Pastorino F. From the identification of actionable molecular targets to the generation of faithful neuroblastoma patient-derived preclinical models. J Transl Med (IF: 4.12; Q1). 2024 Feb 13;22(1):151. doi: 10.1186/s12967-024-04954-w.

RESEARCH AREA

# Experimental Cardiology

**Coordinators** Prof. Giovanni Di Salvo, Prof. Gino Gerosa


Currently the experimental cardiology area includes two labs.

- The lab "Pediatric cardiomyopathies" conducts basic science studies to investigate the mechanisms underlying congenital cardiomyodistrophies in cardiomyocytes derived from induced pluripotent stem cells obtained from patients. Animal models are used to characterize the pathogenesis of the diseases for new therapies.
- The CARE-MED lab aims at developing novel techniques for myocardial repair, based on tissue engineering and on the use of extracellular vesicles, natural nanoparticles stimulating tissue regeneration. These nanoparticles will also be used as experimental therapy for congenital pediatric cardiomyopathies, establishing a collaboration and integration within the research area.

**Prof. Giovanni Di Salvo**
*Coordinator Experimental Cardiology Area*

*Scopus ID 7003610825*

Prof. Di Salvo received his Medical Degree cum Laude in 1996 and completed his residency in Cardiology Magna cum Laude in 2000. He also earned MSc Master Degree in Medical Imaging Magna cum Laude from Katholieke University of Leuven, Belgium in 2002 and PhD in Cardio-Respiratory Physiopathology and Associated Biotechnologies Magna cum Laude in 2004. He was appointed as Assistant Professor in Pediatric Cardiology since 2006 to Jannuary 2012 at the Second University of Naples, Italy, and as Adjunct Full Professor Pediatric Cardiology at Al Faisal University, Riyadh, Saudi Arabia, were he has been Director of Heart Center Research Unit and Consultant Pediatric Cardiology – Structural Heart Disease from June 2014 to 2016.

Prof. Di Salvo was appointed as Consultant, Lead Pediatric Echocardiography, at Royal Brompton, London, UK, from September 2016 to September 2019, as Lead Pediatric Cardiology Research at Royal Brompton, London, UK from July 2017 to September 2019 and as Honorary Senior Lecturer at the Imperial College of London, from October 2016 to September 2019. He awarded of the qualification for Full Professor in Cardiology in 2018. Since 2019 he is r Full Professor in Pediatric cardiology and Congenital Heart Disease at University of Padua. Since then, he is the only full professor in pediatric cardiology in Italy.

Since 2020 he is chair of the European Task force on Adult CHD.

He has been executive board member of the European Association of Cardiovascular Imaging (EACVI) and is actually holding the position of Treasurer at EACVI.

Since 2022 is the Chair of the Italian Working Group on Congenital Heart Diseases of the Italian Society of Cardiology.

He is now president elect of the Italian Society of Echocardiography and CardioVascular Imaging (SIECVI).

**Prof. Gino Gerosa**
*Coordinator Experimental Cardiology Area*

*Scopus ID 7005516149*

Full Professor of Cardiac Surgery at the Department of Cardio-Thoracic-Vascular Sciences and Public Health of the University of Padova; Director of the Cardiac Surgery Unit & Heart Transplant and MCS Programme, Padova University Hospital; and, in charge for the LIFE4HUB Research Program. Past President of the Italian Society of Cardiovascular Surgery (2018-2020), President of the Italian College of Professor of Cardiac Surgery, he has been one of the invited Speaker at G7 Health on July 2024 in Genova. Prof. Gerosa is founder and director of the Cardiovascular Regenerative Medicine Laboratory at University of Padova. He is co-inventor of 2 international patents: i) "Method for detecting a xenoantigen in fixed tissues used as bioprosthetic replacements"; ii) "Artificial Heart".

Prof. Gerosa has performed the first implant in Italy of a Total Artificial Heart on November 2007 and the first DcD heart transplant in the world with a FWIT > 45 m' on 23rd May, 2024.

He has been honoured with the title of Commendatore in 2017 and Grande Ufficiale in 2024 of the Order of Merit of the Italian Republic by the President of the Republic.

# Cardiovascular Regenerative Medicine (CARE-MED)

## Group Leaders

Prof. Gino Gerosa - Principal Investigator,
Dr. Anna Maria Tolomeo – Junior Principal Investigator

## Research activity

The Laboratory of Cardiovascular Regenerative Medicine (CARE-MED) directed by Prof. Gino Gerosa (PI) and Dr. Anna Maria Tolomeo (Junior PI) studies the cellular and molecular mechanisms underlying cardiac pathologies involving the different functional structures of cardiac tissue, from genome to cellular microenvironment. Their alteration can lead to heart failure and thus a reduction in the heart's ability to perform its normal contractile function, resulting in irreversible damage and the need for transplantation. In this scenario the CARE-MED researchers' activities are divided into two main research strands: i) developing patient-specific bioengineered cardiac structures, capable of replacing the organ by reducing the patient's use of immunosuppressants; ii) determining the cardiac and immune cellular components involved in the onset of various cardiac diseases and studying the plasma of these patients, to identify the molecules responsible for cardiac damage. These will represent a novelty among all emerging technologies, as they will combine strategies based on regenerative medicine techniques to achieve highly specialized personalized medicine.

*Projects*

Development of an ex-vivo three-dimensional (3D) cardiac tissue platform for clinical use as a pace-maker or laboratory use for drug testing (BIOPACE).

Generation of a total bioengineered heart and its different components.

Determination of the biological age of the transplanted hearts compared to their chronological age and the subsequent evolution once transplanted in a recipient of different age.

Generation in-vivo (porcine) models of "Donation after Circulatory Death"(DCD), for the recovery of hearts suitable for transplantation.

Evaluation of cutting-edge drugs useful for preserving the heart's functionality during transport to the recipient site after organ harvesting.

Evaluation of cutting-edge technologies from genetically-modified pigs.

## PI's Biosketch

Prof. Gino Gerosa

See Area Coordinator

### Team members

Prof. Gino Gerosa - Principal Investigator
Dr. Anna Maria Tolomeo - Junior Principal Investigator
Dr. Saima Imran - Post Doc
Dr. Federica Serra - Post Doc
Dr. Nicola Pradegan - PhD Student
Dr. Alice Tomas - Research Fellow
Dr. Agnese Lauroja - Research Fellow

### Selected publications


- Palmosi T*, Tolomeo AM*, Cirillo C, Sandrin D, Sciro M, Negrisolo S, Todesco M, Caicci F, Santoro M, Dal Lago E, Marchesan M, Modesti M, Bagno A, Romanato F, Grumati P, Fabozzo A, Gerosa G. Small intestinal submucosa-derived extracellular matrix as a heterotopic scaffold for cardiovascular applications. Front Bioeng Biotechnol. 2022 Dec 12;10:1042434. doi: 10.3389/fbioe.2022.1042434. PMID: 36578513; PMCID: PMC9792098.
- Tolomeo AM, Fabozzo A, Malvicini R, De Lazzari G, Bisaccia P, Gaburro G, Arcidiacono D, Notarangelo D, Caicci F, Zanella F, Marchesan M, Yannarelli G, Santovito G, Muraca M, Gerosa G. Temperature-Related Effects of Myocardial Protection Strategies in Swine Hearts after Prolonged Warm Ischemia. Antioxidants (Basel). 2022 Feb 28;11(3):476. doi: 10.3390/antiox11030476. PMID: 35326125; PMCID: PMC8944743.
- Faggioli M, Moro A, Butt S, Todesco M, Sandrin D, Borile G, Bagno A, Fabozzo A, Romanato F, Marchesan M, Imran S, Gerosa G. A New Decellularization Protocol of Porcine Aortic Valves Using Tergitol to Characterize the Scaffold with the Biocompatibility Profile Using Human Bone Marrow Mesenchymal Stem Cells. Polymers (Basel). 2022 Mar 17;14(6):1226. doi: 10.3390/polym14061226. PMID: 35335556; PMCID: PMC8949722.
- Pavanello S, Campisi M, Fabozzo A, Cibin G, Tarzia V, Toscano G, Gerosa G. The biological age of the heart is consistently younger than chronological age. Sci Rep. 2020 Jul 1;10(1):10752. doi: 10.1038/s41598-020-67622-1. PMID: 32612244; PMCID: PMC7329913.
- Dal Sasso E, Menabò R, Agrillo D, Arrigoni G, Franchin C, Giraudo C, Filippi A, Borile G, Ascione G, Zanella F, Fabozzo A, Motta R, Romanato F, Di Lisa F, Iop L, Gerosa G. RegenHeart: A Time-Effective, Low-Concentration, Detergent-Based Method Aiming for Conservative Decellularization of the Whole Heart Organ. ACS Biomater Sci Eng. 2020 Oct 12;6(10):5493-5506. doi: 10.1021/acsbiomaterials.0c00540. Epub 2020 Sep 10. PMID: 33320567; PMCID: PMC8011801.


### Subarea

## Extracellular Vesicles as Biomarkers and Tools for Personalised Medicine (EXO-MED)

### Group Leader

Dr. Anna Maria Tolomeo – Junior Principal Investigator

### Research activity

The sub-area "Extracellular Vesicles as Biomarkers and Tools for Personalized Medicine", of the CARE-MED lab, studies the plasma secretome of patients with cardiovascular and gastrointestinal pathologies, to identify the molecules responsible for tissue damage. An important component of the secretome are extracellular vesicles (EVs), circular structures released by all cells that mediate cross-talk between different components of a tissue, both under physiological and pathological conditions. The study of their composition and function in the propagation of tissue damage, coupled with their use as exogenous vectors for the 'delivery' of healing molecules, are two important research topics of the lab. In this context, the laboratory is working on the development of two dynamic three-dimensional bioprinted microfluidic models able to in-vitro mimic the complex structure of tissues and to explore the use of different substrates to more closely represent the morphology of the organs to study its functionality in acute and chronic patient-specific diseases. These will represent a novelty among all emerging technologies, since they will combine strategies based on regenerative medicine techniques to achieve highly specialized personalized medicine.

*Projects*

Role of EVs in diagnosis, in patients with organ failure, and their function as biomarkers of treatment outcome.

Development of an ex-vivo three-dimensional (3D) cardiac and gut tissue models for the "delivery" of therapeutic molecules as a potential treatment of chronic damage using and engineered EVs.

Generation in-vitro models of "Donation after Circulatory Death"(DCD), for the recovery of organs suitable for transplantation.

Evaluation of cutting-edge drugs useful for preserving the transplantable organ's functionality during transport to the recipient site after harvesting.

Study the complex interplay between Environmental Pollution and Cardiovascular and Gastrointestinal Diseases to develop new treatments to make organs more resistant to PFAS contamination.

### JrPI's Biosketch

*Scopus ID 56764354400*

Anna Maria Tolomeo is Junior Principal Investigator at the Pediatric Research Institute "Città della Speranza" and Principal Scientist and Lab Coordinator at the University of Padova. Graduated in Evolutionary Biology at the University of Padova (Italy) in 2014, Anna Maria earned the Ph.D. in Developmental Medicine at the same University of Padova in 2018. She is Scientific Coordinator of Unit on Food and Drug Administration (FDA)-certified Nanoparticles manufacturing for Typeone Biomaterials Srl (Lecce, Italy) and she was Co-Supervisor of Unit on Extracellular Vesicles (EVs) Quality Assurance for Bronchopneumodysplasia treatment, for Exo Biologics SA (Neil, Belgium). In July 2023 European Medicines Agency (EMA) authorized the 'first in man' study. Since 2023, she is Scientific Coordinator of two Research Units on Organ Reconditioning and 3D-bioprinted-scaffolds development, for Life4Hub (Living Innovative Fully Engineered for HUman Bioreplacement) national center, focused on patient-specific medicine. With a strong expertise in the study of EVs as therapeutic tools, in 2019, she collaborated on the patent: "Extracellular vesicles isolated from genetically modified cells over-expressing SerpinB3 for use in medicine".

**Team members**

Dr. Anna Maria Tolomeo - Junior Principal Investigator

Dr. Alice Tomas - Research Fellow

Dr. Agnese Lauroja - Research Fellow

### Selected publications


- Malvicini R, De Lazzari G, Tolomeo AM, Santa-Cruz D, Ullah M, Cirillo C, Grumati P, Pacienza N, Muraca M, Yannarelli G. Influence of the isolation method on characteristics and functional activity of mesenchymal stromal cell-derived extracellular vesicles. Cytotherapy. 2024 Feb;26(2):157-170. doi: 10.1016/j.jcyt.2023.11.001. Epub 2023 Dec 8. PMID: 38069981.
- Mimmi S, Zimbo AM, Rotundo S, Cione E, Nisticò N, Aloisio A, Maisano D, Tolomeo AM, Dattilo V, Lionello R, Fioravanti A, Di Loria A, Quirino A, Marascio N, Russo A, Trecarichi EM, Matera G, Quinto I, Torti C, Iaccino E. SARS CoV-2 spike protein-guided exosome isolation facilitates detection of potential miRNA biomarkers in COVID-19 infections. Clin Chem Lab Med. 2023 Mar 27;61(8):1518-1524. doi: 10.1515/cclm-2022-1286. PMID: 36972680.
- Tolomeo AM, Zuccolotto G, Malvicini R, De Lazzari G, Penna A, Franco C, Caicci F, Magarotto F, Quarta S, Pozzobon M, Rosato A, Muraca M, Collino F. Biodistribution of Intratracheal, Intranasal, and Intravenous Injections of Human Mesenchymal Stromal Cell-Derived Extracellular Vesicles in a Mouse Model for Drug Delivery Studies. Pharmaceutics. 2023 Feb 7;15(2):548. doi: 10.3390/pharmaceutics15020548. PMID: 36839873; PMCID: PMC9964290.
- Tolomeo AM, Quarta S, Biasiolo A, Ruvoletto M, Pozzobon M, De Lazzari G, Malvicini R, Turato C, Arrigoni G, Pontisso P, Muraca M. Engineered EVs for Oxidative Stress Protection. Pharmaceuticals (Basel). 2021 Jul 21;14(8):703. doi: 10.3390/ph14080703. PMID: 34451800; PMCID: PMC8399368.
- Tolomeo AM, Castagliuolo I, Piccoli M, Grassi M, Magarotto F, De Lazzari G, Malvicini R, Caicci F, Franzin C, Scarpa M, Macchi V, De Caro R, Angriman I, Viola A, Porzionato A, Pozzobon M, Muraca M. Extracellular Vesicles Secreted by Mesenchymal Stromal Cells Exert Opposite Effects to Their Cells of Origin in Murine Sodium Dextran Sulfate-Induced Colitis. Front Immunol. 2021 Apr 13;12:627605. doi: 10.3389/fimmu.2021.627605. PMID: 33927713; PMCID: PMC8076641.


# Pediatric Cardiomyopathies

**Group Leader**

Prof. Giovanni Di Salvo - Principal Investigator

### Research activity

The experimental cardiology laboratory studies pediatric cardiomyopathies, congenital heart diseases, and pediatric heart failure. Pediatric heart failure is the most lethal pediatric disease. The research spans from cellular evaluation to the study of cardiac mechanics and the impact of new therapies on cardiac function.

The project is carried out in collaboration with Dr. Nina Kaludercic from the Neuroscience Institute of the CNR in Padua. Dr. Kaludercic's is a basic research scientist and her research is focused on mitochondria, redox signaling and autophagy in relation to cardiovascular diseases. Her expertise with in vivo models of cardiac dysfunction and in vitro models (i.e. iPSC-derived cardiomyocytes) will be essential for the successful outcome of this project. The PI and Dr. Kaludercic actively collaborate and interact together in relation to the research proposed.

The employed personnel are from both University of Padua and the Azienda Ospedale Università Padova, as well as personnel paid on IRP grants.

### PI's Biosketch

See Area Coordinator

**Team members**

Prof. Giovanni Di Salvo - Principal Investigator

Prof. Jolanda Sabatino - Associate Professor

Prof. Biagio Castaldi – MD, Clinical Scientist

Dr. Irene Cattapan - Research Fellow

Prof. Nina Kaludercic - Full Professor

Dr. Ruth Jepchirchir Arusei - PhD Student

### Selected publications


- Kaludercic N, Arusei RJ, Di Lisa F. Recent advances on the role of monoamine oxidases in cardiac pathophysiology. Basic Res Cardiol. 2023 Oct 4;118(1):41. doi: 10.1007/s00395-023-01012-2. PMID: 37792081; PMCID: PMC10550854.
- Di Sante M, Antonucci S, Pontarollo L, Cappellaro I, Segat F, Deshwal S, Greotti E, Grilo LF, Menabà[2] R, Di Lisa F, Kaludercic N. Monoamine oxidase A-dependent ROS formation modulates human cardiomyocyte differentiation through AKT and WNT activation. Basic Res Cardiol. 2023 Jan 20;118(1):4. doi: 10.1007/s00395-023-00977-4. PMID: 36670288; PMCID: PMC9859871.
- Sabatino J, Budts W, Di Salvo G. Charting new frontiers in pediatric cardiomyopathies: lessons from the ESC EORP Cardiomyopathy and Myocarditis Registry in pediatric age. Eur Heart J. 2024 Apr 21;45(16):1455-1457. doi: 10.1093/eurheartj/ehae143. PMID: 38592468.

- Castaldi B, Cuppini E, Fumanelli J, Di Candia A, Sabatino J, Sirico D, Vida V, Padalino M, Di Salvo G. Chronic Heart Failure in Children: State of the Art and New Perspectives. J Clin Med. 2023 Mar 30;12(7):2611. doi: 10.3390/jcm12072611. PMID: 37048694; PMCID: PMC10095364.
- Dedja A, Cattapan C, Di Salvo G, Avesani M, Sabatino J, Guariento A, Vida V. A Rodent Model of The Ross Operation: Syngeneic Pulmonary Artery Graft Implantation in A Systemic Position. J Vis Exp. 2022 Apr 1;(182). doi: 10.3791/63179. PMID: 35435912.






RESEARCH AREA

# Genetics and Rare Diseases

**Coordinator** Prof. Leonardo Salviati

The “Genetics and Rare Diseases” research area is comprised of six groups, all involved in the research on rare diseases affecting pediatric patients. Although, the research fields are diverse, some common features are shared: all the groups have a long-standing tradition in their respective fields, they combine research and diagnostic activities, which are often tightly linked, and they are all directly involved in the European Reference Networks (ERN) such as ITHACHA (congenital malformations and intellectual disability), GENTURIS (rare genetic tumors), ERKnet (renal diseases), and MetabERN (metabolic diseases).

They employ personnel from both University of Padua and the Azienda Ospedale Università Padova, as well as personnel paid on IRP grants. The laboratories host several PhD students as well as the residents of the medical genetics program.

**Prof. Leonardo Salviati**
*Coordinator Genetics and Rare Diseases Area*

*Scopus ID 6602836082*

Prof. Salviati received his MD degree cum Laude from the University of Padua in 1995 and completed his residency in Pediatrics in 1999. From 1999 to 2002, he worked as a clinical research fellow in the laboratories of Eric A. Schon and Billi Di Mauro at the Houston-Merritt Center for Inherited Myopathies and Mitochondrial Diseases at the Dept. of Neurology, Columbia University, New York, focusing his research on mitochondrial disorders in particular on the biogenesis of Cytochrome c Oxidase. He received his PhD in Developmental Biology from the University of Padua in 2004. In 2005, he was appointed assistant Professor of Medical Genetics at the University of Padua, then in 2014 he became associate Professor of Medical Genetics and in 2019 he was appointed full Professor and Director of the Clinical Genetics Unit, and of the molecular diagnostics laboratory of Azienda Ospedale-Università Padova. Prof. Salviati has started his own research group in 2005 focusing on mitochondrial and neurometabolic disorders, in particular on Coenzyme Q deficiency. The group has joined IRP at the end of 2013. Prof. Salviati is the recipient of national and international grants for more than 3M €. Overall, he has co-authored over 180 peer-reviewed articles with more than 1100 citations and an h-index of 53 (according to Scopus). Total impact factor is over 1200 points.

# Clinical Genetics and Epidemiology

## Group Leader

Prof. Leonardo Salviati - Principal Investigator

## Research activity

The group has been active since 2005. Our research was originally focused on mitochondrial diseases and later expanded to include a whole series of other genetic disorders.

Currently the group has two main lines of research. The first concerns the biogenesis of the mitochondrial respiratory chain, and in particular of Coenzyme Q and disorders of intermediate metabolism. Our goals are to identify and characterize human genes involved in the biogenesis of the mitochondrial respiratory chain, identify mutations in patients with these diseases, to develop simple models to characterize these mutations, to study their pathophysiology, and test new therapeutic approaches. In recent years we have expanded our scope of research to include other types of metabolic diseases, as well as genetic disorders unrelated to cellular metabolism.

The second field of research concerns genetic diseases in general. The expansion of our diagnostic service has provided us with an incredible amount of genetic data (we have analyzed more than 10,000 patients with NGS, for a variety of different conditions). The main limitation of this approach is that it is often very difficult to establish the pathogenicity of variants identified by diagnostic tests. In recent years, we have developed several models (hybrid minigenes, yeast, CRISPR-CAS9-edited human cells, and the nematode C. elegans) that have allowed us to validate many new variants and establish genotype-phenotypic correlates for several diseases and to identify new genes associated with human diseases.

The main results obtained in the last year are the identification of a specific role for frataxin, the deficient protein in Friedreich's Ataxia, in the biogenesis of complex I of the respiratory chain, and the identification of the oxidative decarboxylase that catalyzes the first step of the biosynthesis of coenzyme Q ring, an enzyme that has eluded researchers for over 30 years.

## PI's Biosketch

See Area Coordinator

**Team Members**

Prof. Leonardo Salviati - Principal Investigator
Dr. Maria Andrea Desbats - Senior Scientist
Dr. Cinzia Bertolin - Senior Scientist
Dr. Francesca Boaretto - Senior Scientist
Dr. Alessandra Friso - Senior Scientist
Dr. Monica Forzan - Senior Scientist
Dr. Chiara Rigon - Senior Scientist
Dr. Sara Zanchetti - Senior Scientist
Dr. Giuseppe Castello - Senior Scientist
Prof. Matteo Cassina - Senior Scientist
Dr. Carlotta Liccardi - Senior Technologist/Research Associates
Dr. Carmen Manolio - Senior Technologist/Research Associates
Dr. Sofia Tessarolo - Senior Technologist/Research Associates
Dr. Elena Fabiani - Senior Technologist/Research Associates
Dr. Addolorata Cordella - Senior Technologist/Research Associates
Dr. Elisa Baschiera - Post Doc
Dr. Cristina Calderan - Post Doc
Dr. Marco Marchi - Post Doc
Dr. Agata Valentino - PhD Student

**Selected publications**


- Calderan C, Sorrentino U, Persano L, Trevisson E, Sartori G, Salviati L, Desbats MA. A yeast based assay establishes the pathogenicity of novel missense ACTA2 variants associated with aortic aneurysms. Eur J Hum Genet. 2024 Mar 15. doi: 10.1038/s41431-024-01591-1.
- Pelosi L, Morbiato L, Burgardt A, Tonello F, Bartlett AK, Guerra RM, Ferizhendi KK, Desbats MA, Rascalou B, Marchi M, Vázquez-Fonseca L, Agosto C, Zanotti G, Roger-Margueritat M, Alcázar-Fabra M, García-Corzo L, Sánchez-Cuesta A, Navas P, Brea-Calvo G, Trevisson E, Wendisch VF, Pagliarini DJ, Salviati L, Pierrel F. COQ4 is required for the oxidative decarboxylation of the C1 carbon of coenzyme Q in eukaryotic cells. Mol Cell. 2024 Mar 7;84(5):981-989.e7. doi: 10.1016/j.molcel.2024.01.003.
- Doni D, Cavion F, Bortolus M, Baschiera E, Muccioli S, Tombesi G, d'Ettorre F, Ottaviani D, Marchesan E, Leanza L, Greggio E, Ziviani E, Russo A, Bellin M, Sartori G, Carbonera D, Salviati L, Costantini P. Human frataxin, the Friedreich ataxia deficient protein, interacts with mitochondrial respiratory chain. Cell Death Dis. 2023 Dec 8;14(12):805. doi: 10.1038/s41419-023-06320-y.
- Baschiera E, Sorrentino U, Calderan C, Desbats MA, Salviati L. The multiple roles of coenzyme Q in cellular homeostasis and their relevance for the pathogenesis of coenzyme Q deficiency. Free Radic Biol Med. 2021 Apr;166:277-286.
- Favaro G, Romanello V, Varanita T, Andrea Desbats M, Morbidoni V, Tezze C, Albiero M, Canato M, Gherardi G, De Stefani D, Mammucari C, Blaauw B, Boncompagni S, Protasi F, Reggiani C, Scorrano L, Salviati L, Sandri M. DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. Nat Commun. 2019 Jun 12;10(1):2576.


# Mitochondrial DNA maintenance disorders

**Group Leader**

Dr. Mara Doimo - Junior Principal Investigator

**Research activity**

Mitochondria are key organelles as they are responsible for supplying the proper form of energy necessary to the cell to exert all its functions. Unlike other cellular organelles, mitochondria possess their own DNA (mtDNA). In humans, the mitochondrial genome is comprised of several copies of a 16 kilobases (kb), double strand DNA (dsDNA) circular molecule. The genes encoded by this small genome are essential for the biosynthesis of the mitochondrial respiratory chain.

The maintenance of mtDNA requires a set of nuclear genes involved in mtDNA replication, in controlling the mitochondrial nucleotide pools, and in mediating mitochondrial fusion and fission. Mutations in these genes are associated with several pathological conditions, hereby referred to as mtDNA maintenance defects (MDMD). Patients with MDMD presents with different phenotypic manifestations, including neuropathy, myopathy, hepatopathy and encephalopathy. The most severe conditions, characterized by depletion of mtDNA (i.e. the reduction of mtDNA copy number in cells), manifest during the neonatal period or early infancy. Although in the majority of the cases the genetic cause triggering the disease is known, the mechanistic process linking the genetic defect to the loss of mtDNA integrity is still poorly understood. Nonetheless, we recently showed that specific DNA secondary structures, called G-quadruplexes (G4s), can form at the mtDNA and interfere with the process of replication leading to mtDNA instability (Doimo et al, 2023). Our recently established group aims at elucidating the molecular mechanisms regulating the maintenance of the mitochondrial genome and particularly how DNA secondary structures and epigenetic modifications impact these processes.

We are developing novel genomic, proteomic and single molecules methodologies and apply them in cell models and patient's derived tissues. In addition, we are establishing in vivo models of disease that will allow us to explore therapies that can counteract the mtDNA genomic instability and block the progression of the disease.

**JrPI's Biosketch**

*Scopus ID 24278714700*

I earned my PhD in Medical Development (Curriculum: Genetics) at the University of Padova, Italy in 2012. During my research I worked on inborn errors of mitochondrial metabolism. By employing yeast genetics, I elucidated the mechanism behind the onset of primary Coenzyme Q (CoQ) deficiencies and urea cycle disorders, and explored new therapeutic routes, specifically to bypass the impairment of CoQ biosynthesis. In 2008-2009 I was a visiting researcher at the University of Geneva, Switzerland in the laboratory of Prof. Scorrano where I expanded my knowledge about mitochondrial dynamics and cell biology.

Following my PhD, I was awarded the Marie-Slowdoska-Curie Individual Fellowship and the Wenner-Gren fellowship and moved to Umea University, Sweden, to study the processes of DNA replication and repair. My research in Umea focused on the function of non-canonical secondary

structures, called G-quadruplexes (G4s), in the mitochondrial DNA (mtDNA) and their role in genome instability. During this time, I also had the opportunity to collaborate on a multidisciplinary project aimed at developing novel chemical compounds that can target G-quadruplexes (G4s). The project led to the development of a patent aimed at developing novel anti-cancer therapies. In 2022 I spent a year at the Chemical Biology Consortium Sweden (CBCS) where I was developing HTS screening for drug discoveries.

In 2022 I was awarded the PNRR-YOUNG RESEARCHER MSCA grant to start my own research group and I moved back to Italy at the Pediatric Research Institute "Città della Speranza". More recently, I was awarded the IRP Starting Grant.

My research topic focuses on the study of the mechanisms that cause mtDNA instability, ultimately leading to mitochondrial DNA maintenance defects. My final goal is to contribute to develop therapies against these pathologies whose most severe forms manifest at birth or at early infancy with an extremely poor outcome for the patients.

### Team Members

Dr. Mara Doimo - Junior Principal Investigator
Dr. Ilaria Cestonaro - Research Fellow
Dr. Caterina Giovine - Research Fellow

### Selected publications


- Doimo M #, Chaudhari N, Abrahamsson S, L'Hôte V, Nguyen TVH, Berner A, Ndi M, Abrahamsson A, Das RN, Aasumets K, Goffart S, Pohjoismäki JLO, López MD, Chorell E, Wanrooij S. # Enhanced mitochondrial G-quadruplex formation impedes replication fork progression leading to mtDNA loss in human cells. Nucleic Acids Res. 2023 Aug 11;51(14):7392-7408. PMID:37351621
- Prasad B*, Doimo M*, Andréasson M, L'Hôte V, Chorell E, Wanrooij S. A complementary chemical probe approach towards customized studies of G-quadruplex DNA structures in live cells. Chem Sci. 2022 Feb 1;13(8):2347-2354. doi: 10.1039/d1sc05816a. eCollection 2022 Feb 23. PMID: 35310480
- Jamroskovic J*, Doimo M*, Chand K, Obi I, Kumar R, Brännström K, Hedenström M, Nath Das R, Akhunzianov A, Deiana M, Kasho K, Sulis Sato S, Pourbozorgi PL, Mason JE, Medini P, Öhlund D, Wanrooij S, Chorell E, Sabouri N. Quinazoline Ligands Induce Cancer Cell Death through Selective STAT3 Inhibition and G-Quadruplex Stabilization. J Am Chem Soc. 2020 Feb 12;142(6):2876-2888. doi: 10.1021/jacs.9b11232. Epub 2020 Jan 28. PMID: 31990532
- Berner A, Das RN, Bhuma N, Golebiewska J, Abrahamsson A, Andréasson M, Chaudhari N, Doimo M, Bose PP, Chand K, Strömberg R, Wanrooij S, Chorell E.G4-Ligand-Conjugated Oligonucleotides Mediate Selective Binding and Stabilization of Individual G4 DNA Structures. J Am Chem Soc. 2024 Mar 13;146(10):6926-6935. doi: 10.1021/jacs.3c14408. Epub 2024 Mar 2. PMID: 38430200
- Doimo M, Pfeiffer A, Wanrooij PH, Wanrooij S. Chapter 1 - mtDNA replication, maintenance, and nucleoid organization. In: The Human Mitochondrial Genome. Editor(s): Giuseppe Gasparre, Anna Maria Porcelli. Academic Press. 2020. Pages 3-33


# Model Organisms and Rare Diseases

### Group Leader

Prof. Eva Trevisson - Principal Investigator

### Research activity

Our focus is the characterization of new genes involved in ultrarare diseases and the functional analysis of gene variants. The main interest is related to neurometabolic diseases (in particular the development of innovative treatments for specific mitochondrial disorders) and developmental disorders (such as ciliopathies), as well as the role of genes with unknown function in the etiology of pediatric-onset disorders. For this purpose we employ different models, including multicellular organisms, such as the nematode C. elegans, Zebrafish and mouse models.

### PI's Biosketch

*Scopus ID 8922220000*

My research has focused on the study of the genetic bases and the pathogenesis of different genetic diseases, including inherited metabolic disorders (isolated cytochrome c oxidase defect, primary coenzyme Q deficiency) and urea cycle defects.

My group has set up different systems to validate pathogenic mutations and to establish genotype-phenotype correlations, using distinct approaches in both yeast and mammalian cells. To unravel the function of novel genes, I also employ multicellular organisms, including C. elegans and Zebrafish.

As a clinical geneticist, I am involved in cancer genetics and tumor predisposition syndromes. I am particularly interested in mechanisms driving cancers associated with a genetic predisposition (neurocutaneous disorders such as neurofibromatoses and schwannomatosis). I am employing the same organisms to model germline mutations in oncosuppressors/oncogenes identified in rare tumor predisposing syndromes in order to elucidate pathogenetic mechanisms and for drug screening.

I have co-authored 89 Medline peer-reviewed publications and 6 book chapters, with a total impact factor of 338 (Journal of Citation reports 2024), H-index 34 and total citations 4,102 (Scopus 2024).

I teach Molecular Genetics and Medical Genetics in different academic courses (including the Medical School and Medical biotechnologies), masters, medical residency programs and in the PhD program in Developmental Medicine and Programming Sciences at the University of Padova. I have supervised a number of undergraduate theses, more than 10 theses for residency programs and four PhD thesis.

### Team Members

Prof. Eva Trevisson - Principal Investigator
Dr. Cristina Cerqua - Post Doc
Dr. Valeria Morbidoni - Post Doc
Dr. Elena Tacchetto - PhD Student
Dr. Giulia Toffanin - PhD Student
Dr. Chiara Canciani - Research Fellow

## Selected publications


- Franco-Romero A, Morbidoni V, Milan G, Sartori R, Wulff J, Romanello V, Armani A, Salviati L, Conte M, Salvioli S, Franceschi C, Buonomo V, Swoboda CO, Grumati P, Pannone L, Martinelli S, Jefferies HB, Dikic I, van der Laan J, Cabreiro F, Millay DP, Tooze SA, Trevisson E, Sandri M. C16ORF70/Mytho promotes healthy aging in C. elegans and prevents cellular senescence in mammals. J Clin Invest. 2024 Jun 13:e165814. doi: 10.1172/JCI165814.
- Morbidoni V, Agolini E, Slep KC, Pannone L, Zuccarello D, Cassina M, Grosso E, Gai G, Salviati L, Dallapiccola B, Novelli A, Martinelli S, Trevisson E. Biallelic mutations in the TOGARAM1 gene cause a novel primary ciliopathy. J Med Genet. 2021;58(8):526-533. doi: 10.1136/jmedgenet-2020-106833.
- Morbidoni V, Baschiera E, Forzan M, Fumini V, Ali DS, Giorgi G, Buson L, Desbats MA, Cassina M, Clementi M, Salviati L, Trevisson E. Hybrid Minigene Assay: An Efficient Tool to Characterize mRNA Splicing Profiles of NF1 Variants. Cancers (Basel). 2021;13(5):999. doi: 10.3390/cancers13050999.
- Gambarotto L, Metti S, Chrisam M, Cerqua C, Sabatelli P, Armani A, Zanon C, Spizzotin M, Castagnaro S, Strappazzon F, Grumati P, Cescon M, Braghetta P, Trevisson E, Cecconi F, Bonaldo P. Ambra1 deficiency impairs mitophagy in skeletal muscle. J Cachexia Sarcopenia Muscle. 2022;13(4):2211-2224.
- Dentici ML, Niceta M, Lepri FR, Mancini C, Priolo M, Bonnard AA, Cappelletti C, Leoni C, Ciolfi A, Pizzi S, Cordeddu V, Rossi C, Ferilli M, Mucciolo M, Colona VL, Fauth C, Bellini M, Biasucci G, Sinibaldi L, Briuglia S, Gazzin A, Carli D, Memo L, Trevisson E, Tartaglia M. Loss-of-function variants in ERF are associated with a Noonan syndrome-like phenotype with or without craniosynostosis. Eur J Hum Genet 2024. doi: 10.1038/s41431-024-01642-7.


# Diagnosis and Therapy of Lysosomal Disorders

## Group Leader

Dr. Rosella Tomanin - Principal Investigator

## Research activity

The Laboratory focuses its research on Mucopolysaccharidoses (MPSs), a cluster of 12 Lysosomal Storage Disorders, due to the deficit of the enzymes catabolizing mucopolysaccharides (GAG) inside lysosomes, thus causing their severe accumulation within lysosomes and in the extracellular matrix. MPSs generally affect most organ-systems, including CNS, in more than 70% of the cases.

For many years, only symptomatic therapies were available for MPSs, while haematopoietic stem cell transplantation has been so far successfully applied only to MPS I, since its application to other MPSs has shown controversial results. More recently, some MPSs benefit from Enzyme Replacement Therapy (ERT), the weekly administration of the functional enzyme. ERT shows some peripheral efficacy, but is mostly ineffective on the CNS disease, since enzymes cannot cross the BBB. Furthermore, brain pathogenesis remains quite obscure, while its understanding would be extremely helpful to monitor patients' prognosis and detect new therapeutic targets.

Important objectives of our research are therefore the comprehension of MPS brain pathology and its possible treatment, by using in vitro and in vivo models.

We also conduct research activity on the clinical side, with the follow-up of MPS patients under ERT, to monitor disease progression and treatment efficacy. Moreover, in the last 6 years we conducted three projects of Mutation Update on the genes ARSB, GALNS and IDS, causing MPS VI, MPS IVA and MPS II respectively, performing a complete molecular classification of all variants described for the 3 genes. In the last decade the laboratory has published 30 peer-reviewed articles, 1 manuscript is at the moment under revision and 3 in preparation.

*Projects of the research group*

The comprehension of the brain pathogenesis and its progression in MPS II has been completed in the mouse model for the disease (manuscript in preparation), while it is still under analysis in the Drosophila model. These in vivo studies should help to elucidate the onset of the neurological impairment during the disease progression and the molecular alterations implicated.

We focused on the identification of the potentially altered pathways resulting in the severe neuronopathic forms of MPS. To this aim we used in vitro human neural models. We generated induced pluripotent stem cells starting from human primary fibroblasts [Casamassa et al 2022, PMID:35759972]. Moreover, in collaboration with the Dept. of Molecular Medicine, UNIPD, a second in vitro model was generated, an IDS knockout human neuronal cell line [Badenetti et al 2023, PMID:37357221]. Preliminary findings allow to hypothesize that neuronal differentiation might be significantly affected by IDS functional impairment.

In the last years, we started a collaboration with the Dept. of Pharmaceutical and Pharmacological Sciences, UNIPD, aiming at generating/characterizing Drosophila models of mucopolysaccharidoses. A Drosophila knock-down model of MPS I, showed to be a good model of the disease [De Filippis et al 2021, PMID:35011691]. An MPS II fly model, generated in collaboration with the

LIMES Institute in Bonn (Germany), was used to test the antioxidant efficacy of Vitamin E and the improvement of the lysosomal/mitochondrial impairment [Rigon et al, unpublished].

We conducted an international study which has completed the "mutation update" of the GALNS gene (whose mutations cause MPS IVA), through a re-classification of all published variants and the description of 68 novel ones [Zanetti et al 2021, PMID:34387910]. In the last two years, this analysis has been extended to the gene IDS (whose variants cause MPS II) [Zanetti, D'Avanzo and Tomanin, submitted].

A search of molecular biomarkers allowing a faster diagnostic procedure and a monitoring of ERT efficacy was performed by analyzing urine samples obtained from suspected MPS patients [D'Avanzo et al 2023, PMID:36979466], and from IDS knock-out mice under ERT (Maccari et al 2022, PMID:35816218). Both investigations conducted through a Mass Spectrometry analysis.

In the last 3 years we published 3 reviews: on MPS VI [D'Avanzo et al 2021, PMID:34948256], on the treatment of Neurometabolic Diseases [Begley et al 2024, PMID:38963225] and on the brain-targeting therapeutic approaches in MPS II [Zanetti and Tomanin, 2024, PMID:39177874].

The Laboratory also performed biochemical and molecular analyses related to MPS diagnosis, and follow-up of MPS patients under ERT.

## PI's Biosketch

*Scopus ID 6603451357*

Dr. Rosella Tomanin (SCOPUS ID: 6603451357, H-index: 24; total citations according to Scopus: 1900) is a Biologist and a Medical Geneticist, Head of the Laboratory of Diagnosis and Therapy of Lysosomal Disorders (Dept. of Women's and Children's Health of the University of Padova and Pediatric Research Institute Città della Speranza).

Graduated at the University of Padova in 1985, she has a PhD in Scienze dello Sviluppo (Pediatrics) and a Specialization in Medical Genetics. In 35 years' laboratory experience, Dr. Tomanin has been involved in numerous projects in the fields of genetics, molecular and cellular biology and pediatric diseases. She spent 4 years at McMaster University (Hamilton, Ontario, Canada), at first as a post-doc and next as a Visiting Scientist, working on oncogenic adenoviruses and generation of recombinant adenoviral vectors for gene transfer and gene therapy applications.

Dr. Tomanin is co-author of 68 publications on peer-reviewed International Journals, 5 book chapters, and some publications in Italian Journals. She served as a reviewer for several International Journals, among which: PlosOne, Gene, Cytotherapy, Medicine, Orphanet J Rare Dis, Comput Struct Biotechnol, J Genet Mol Biol, Int J Mol Sci, Mol Genet Metab Rep.

*Awards and Acknowledgements*

Jan 1986-Dec 1989: Fellowships, Centro Regionale di Alta Specializzazione in Cancerogenesi Ambientale, Istituto Veneto di Scienze, Lettere ed Arti, Venezia.

Jan 1990-Aug 1991: Post doctoral Fellowship in Molecular Biology, Cancer Research Group, Health Science Centre, McMaster University, Hamilton, Ontario, Canada

Mar 1993-Apr 1995: Visiting Scientist, Lab of Molecular Virology, Dept. of Biology, McMaster University, Hamilton, Ontario, Canada

Jan 1994-June 1994: Fellowship, Canadian Government-International Council for Canadian Studies
Mar 1998-Mar 1999: Senior Scientist Fellowship, Istituto per lo Studio e la Cura dei Tumori, Milano
Apr 1999-Mar 2001: Post-doctoral Fellowship, Facoltà di Medicina, Università di Padova

### Team Members

Dr. Rosella Tomanin - Principal Investigator
Dr. Alessandra Zanetti - Senior Technologist/Research Associates
Dr. Francesca D'Avanzo - Post Doc
Dr. Concetta De Filippis - Research Fellow

### Selected publications


- Zanetti A, D'Avanzo F, AlSayed M, Brusius-Facchin AC, Chien YH, Giugliani R, Izzo E, Kasper DC, Lin HY, Lin SP, Pollard L, Singh A, Tonin R, Wood T, Morrone A, Tomanin R. Molecular basis of mucopolysaccharidosis IVA (Morquio A syndrome): A review and classification of GALNS gene variants and reporting of 68 novel variants. Hum Mutat. 2021 Nov;42(11):1384-1398. doi: 10.1002/humu.24270. Epub 2021 Aug 23. PMID: 34387910
- D'Avanzo F, Rigon L, Zanetti A, Tomanin R. Mucopolysaccharidosis Type II: One Hundred Years of Research, Diagnosis, and Treatment. Int J Mol Sci. 2020 Feb 13;21(4):1258. doi: 10.3390/ijms21041258. PMID: 32070051
- Rigon L, Salvalaio M, Pederzoli F, Legnini E, Duskey JT, D'Avanzo F, De Filippis C, Ruozi B, Marin O, Vandelli MA, Ottonelli I, Scarpa M, Tosi G, Tomanin R. Targeting Brain Disease in MPSII: Preclinical Evaluation of IDS-Loaded PLGA Nanoparticles. Int J Mol Sci. 2019 Apr 24;20(8):2014. doi: 10.3390/ijms20082014. PMID: 31022913
- Tomanin R, Karageorgos L, Zanetti A, Al-Sayed M, Bailey M, Miller N, Sakuraba H, Hopwood JJ. Mucopolysaccharidosis type VI (MPS VI) and molecular analysis: Review and classification of published variants in the ARSB gene. Hum Mutat. 2018 Dec;39(12):1788-1802. doi: 10.1002/humu.23613. Epub 2018 Sep 17. PMID: 30118150

# Immunopathology and molecular Biology of the Kidney

## Group Leader

Dr. Elisa Benetti - Principal Investigator

## Research activity

The Laboratory of Immunopathology and Molecular Biology of the Kidney is part of the Pediatric Nephrology Unit, Dept. of Women's and Children's Health, Padua University Hospital. The Unit is a center of excellence and reference, where professionals collaborate in a multidisciplinary and comprehensive approach to take care of children with renal diseases. The Unit takes also part to international networks for diagnosing and treating rare kidney diseases, such as the European Reference Network (ERN) ERKNet and TransplantChild. The Laboratory of Immunopathology and Molecular Biology of the Kidney provides an analysis pattern for the immune-histological classification of primary and secondary pediatric kidney and urinary tract diseases and the follow-up of pediatric kidney transplant recipients. Furthermore, the Laboratory preserves a remarkable biobank of renal tissues from transplanted and native kidneys. The Pediatric Nephrology Unit and the Laboratory carry on important scientific studies, including clinical trials as well as translational research projects, concerning kidney transplantation, acute kidney injury, dialysis, congenital abnormalities of the kidney and urinary tract (CAKUT), nephrotic syndrome and other pediatric kidney diseases.

The current main field of research of the Laboratory is the study of factors affecting the survival of kidney transplantation in the pediatric population. To date, the survival rate of a graft is about 10-20 years, a period that is too short for pediatric patients. Therefore, an effort should be made to identify factors that can negatively impact graft survival as early as possible to treat them before they disrupt renal function. To this purpose, the Laboratory collaborates with several other research groups in IRP and is committed on many topics, such as the role and the prognostic value of intrarenal positivity of viruses and the characterization of extracellular vesicles (EVs) isolated from serum and urine of transplanted children. These EVs could be useful to identify novel non-invasive biomarkers predictive of rejection before the damage is detectable in the kidney. Other two huge research projects are being carried on in collaboration with the Transplantation Immunology Lab (Prof. Cozzi) and are aimed to study the role of non-HLA antibodies in antibody-mediated rejection, one of the main causes of graft loss. More in detail, the projects are aimed at assessing the possible causative role of anti-AT1R (Angiotensin 1 Receptor) and anti-ETAR (Endothelin 1 Receptor) immunity in pediatric renal transplant failure using an innovative approach to study whether anti-HLA antibodies are associated with a particular metabolomics and lipidomics signature in key cells involved in antibody mediated rejection. The Lab is also engaged in the ORCHESTRA project, funded by the European Union and involving 10-European and 9 non-European countries. This international project is aimed at delivering scientific evidence to improve the prevention and treatment of the infections caused by SARS-nCoV2. The main outcome of ORCHESTRA is the creation of a new pan-European cohort including SARS-CoV-2 infected and non-infected individuals of all ages and conditions, including transplanted individuals.

Since 2024, the Laboratory is also involved in a research project (STANGA study, PI Prof. Enrico Vidal, director of Pediatric Nephrology Unit) focused on acute kidney injury (AKI) and extracorporeal supportive therapies. This 3-years prospective cohort study aims to develop a risk score for predicting AKI in the first week of life for infants admitted to the Neonatal Intensive Care unit. AKI affects approximately 20% of infants admitted to the NICU. The purpose of the study is to enhance the ability to predict AKI by integrating clinical data with urinary biomarkers, such as NGAL, TIMP-2 and IGFBP-7, and CCL-14. This comprehensive data collection process will leverage artificial intelligence systems to identify the most effective combinations of clinical data and biomarkers. Early identification of affected patients is crucial to promptly discontinue nephrotoxic medications, to optimize fluid therapy, to monitor renal function as a surrogate perfusion index to adjust hemodynamic support, and to reduce the occurrence of unrecognized AKI cases, ensuring that all affected infants receive necessary follow-up.

## PI's Biosketch

*Scopus ID 6602437925*

Dr. Benetti is the PI of the Laboratory of Immunopathology and Molecular Biology of the Kidney, where she has been working since 2004.

Dr. Elisa Benetti, MD, PhD, is a pediatric nephrologist who graduated from the Medical School of the Padua University in 2004. In 2010, she obtained her specialization as a Pediatrician with a specific focus on Pediatric Nephrology, and a PhD in Developmental Medicine and Planning Science in 2014 at the same University. Since 2014, she has been coordinating the Pediatric Renal Transplant program of Padua. Currently, she is actively involved in the European Reference Network (ERN) transplantation (TransplantChild), being the Director of the Clinical Audits, and is also a member of the ERN for rare kidney diseases (ERKNet). She also is the Coordinator of the Veneto Regional Registry of Nephrotic Syndrome.

Since 2004, her clinical and research activity has been focused mainly on rare renal diseases, renal transplantation, nephrotic syndrome, and renal developmental abnormalities. She has published more than 100 papers, as an author or co-author, on renal genetic diseases, nephrotic syndrome, and renal transplantation. She is also author of several book chapters. She also acts as a reviewer for several international peer-review journals.

Dr. Benetti has been taking up a teaching position at the Pediatric Surgery Residency Program (since 2018) and at the Pediatrics Residency Program since (2021) of the University of Padua. Furthermore, she has been coordinating the Second level University Master in Pediatric Nephrology of the University of Padua since 2019.

Dr. Benetti is a past vice-president and counselor of the Italian Society for Pediatric Nephrology (SINePe) and is a member of many national and international scientific societies and registries on renal disease and kidney transplantation, including the European Society of Pediatric Nephrology, the European Renal Association-European Dialysis and Transplant Association, the European Society for Organ Transplantation, the Italian Society for Organ Transplant, the Italian Society of Nephrology, the Italian Society of Pediatrics, the CERTAIN (Cooperative European Pediatric Renal Transplant Initiative) research network, the ESCAPE Study Group, and PODONET Consortium. In 2010, she successfully completed the International Summer School of Renal Pathology of ERA-EDTA and was thus eligible to be admitted to the Renal Pathology Society (RPS).

During her career, Dr. Benetti has promoted and attended many national and international meetings and conferences and has been the recipient of several awards for her research. Over the years, she has been the local principal investigator or co-investigator of many national and international research projects and clinical trials in the field of pediatric kidney transplantation, pediatric primary glomerular diseases, nephrotic syndrome, and rare renal genetic disorders.

### Team Members

Dr. Elisa Benetti - Principal Investigator
Dr. Susanna Negrisolo – Lab Coordinator
Dr. Diana Marzenta – Laboratory Technician
Dr. Nicola Bertazza Partigiani – MD, Clinical Scientist
Dr. Maria Sangermano – MD, Clinical Scientist
Prof. Enrico Vidal – MD, Clinical Scientist, PI of the STANGA Research Project
Dr. Aurora Toffanin – Research Fellow
Dr. Benedetta Antoniello - PhD Student
Dr. Giovanni Ceschia – PhD Student

### Selected publications


- Sangermano M, Negrisolo S, Antoniello B, Vadori M, Cozzi E, Benetti E. Use of Tocilizumab in the treatment of chronic active antibody-mediated rejection in pediatric kidney transplant recipients. Hum Immunol. 2024 Sep;85(5):111088. doi: 10.1016/j.humimm.2024.111088.
- Carraro A, De Gaspari P, Antoniello B, Marzenta D, Vianello E, Bussolati B, Tritta S, Collino F, Bertoldi L, Benvenuto G, Vedovelli L, Benetti E, Negrisolo S. New Insights into Pediatric Kidney Transplant Rejection Biomarkers: Tissue, Plasma and Urine MicroRNAs Compared to Protocol Biopsy Histology. Int J Mol Sci. 2024 Feb 5;25(3):1911. doi: 10.3390/ijms25031911.
- Bertazza Partigiani N, Negrisolo S, Carraro A, Marzenta D, Manaresi E, Gallinella G, Barzon L, Benetti E. Pre-Existing Intrarenal Parvovirus B19 Infection May Relate to Antibody-Mediated Rejection in Pediatric Kidney Transplant Patients. Int J Mol Sci. 2023 May 23;24(11):9147. doi: 10.3390/ijms24119147.
- Negrisolo S, Benetti E. PAX2 and CAKUT Phenotypes: Report on Two New Variants and a Review of Mutations from the Leiden Open Variation Database. Int J Mol Sci. 2023 Feb 19;24(4):4165. doi: 10.3390/ijms24044165.
- Giannella M, Righi E, Pascale R, Rinaldi M, Caroccia N, Gamberini C, Palacios-Baena ZR, Caponcello G, Morelli MC, Tamè M, Busutti M, Comai G, Potena L, Salvaterra E, Feltrin G, Cillo U, Gerosa G, Cananzi M, Piano S, Benetti E, Burra P, Loy M, Furian L, Zaza G, Onorati F, Carraro A, Gastaldon F, Nordio M, Kumar-Singh S, Abedini M, Boffetta P, Rodríguez-Baño J, Lazzarotto T, Viale P, Tacconelli E, On Behalf Of The Orchestra Study Group Workpackage. Evaluation of the Kinetics of Antibody Response to COVID-19 Vaccine in Solid Organ Transplant Recipients: The Prospective Multicenter ORCHESTRA Cohort. Microorganisms. 2022 May 12;10(5):1021. doi: 10.3390/microorganisms10051021.


# Molecular Genetics of Neurodevelopmental Disorders

### Group Leader

Prof. Alessandra Murgia - Principal Investigator

### Research activity

The Laboratory of Molecular Genetics of Neurodevelopment (GNS Lab) is a leading center for the molecular diagnostics of Fragile X Disorders: Fragile X Syndrome (FXS), Fragile X-associated Neuropsychiatric Conditions (FXANC), Fragile X-associated Primary Ovarian Insufficiency (FXPOI), Fragile X-Associated Tremor Ataxia Syndrome (FXTAS).

The laboratory members have a long and well established experience in the development and validation of protocols for genomic and transcriptomic analysis in rare pediatric neurodevelopmental disorders, and work in synergy with the pediatric Neurology and Neurophysiology unit of the Department of Women's and Children's Health.

The Laboratory is a part of the X-Fragile Multidisciplinary Network of Padua (http://www.sdb.unipd.it/centro-x-fragile).

The main research objective is to understand the molecular mechanisms and correlation with the clinical manifestations of Fragile X Syndrome and FMR1 premutation-related diseases (FXPAC), with two main goals:

- identifying molecular biomarkers that can represent better tools for evaluating the clinical course of these disorders and possibly monitor the efficacy of new drugs;
- identifying possible new specific pathways that can be target of innovative therapies.

### PI's Biosketch

*Scopus ID 7004130427*

Prof. Murgia obtained the MD degree in 1981 and specialized in Endocrinology (1984) and Pediatrics (1995) at the School of Medicine, University of Padua. She also obtained a PhD in Developmental Sciences. In 1985 Prof. Murgia moved to Philadelphia where she worked as Post-Doctoral Fellow at the Dept.s of Internal Medicine and Human Genetics, University of Pennsylvania School of Medicine until the end of 1991, before coming back to Padova.

Prof. Murgia is Associate Professor of Pediatrics, Coordinator of the Fragile X Center of Padova and Principal Investigator of the Molecular Genetics of Neurodevelopment Laboratory.

She teaches Child Neuropsychiatry at the Schools of Medicine and School of Psychology. Prof. Murgia has authored 90 full papers published in international peer-reviewed and indexed scientific journals.

During her academic career, Prof. Murgia's laboratory has been supported by National and EU Research Grants as a Primary Investigator/collaborator, most notably AIRC; Ricerca Sanitaria Finalizzata Regione Veneto; COFIN/PRIN; (GENDEAF); EuroRett Project: European Consortium for the study of Rett Syndrome and Fondazione Cariplo; Telethon; FRAXA Research Foudation as well as IRP and SDB grants.

She is sitting in the scientific committee of the Italian Fragile X Association and board of advisors

of the International Fragile X Association; she is member of the advisory board of Shionogi. Prof. Murgia is a member of the American Society of Human Genetics (ASHG); European Society of Human Genetics (ESHG); Italian Society of Human Genetics (SIGU).

**Team Members**

Prof. Alessandra Murgia - Principal Investigator
Dr. Roberta Polli - Senior Technologist/Research Associates
Dr. Elisa Bettella - Senior Technologist/Research Associates
Dr. Marilena Cameran - Senior Technologist/Research Associates

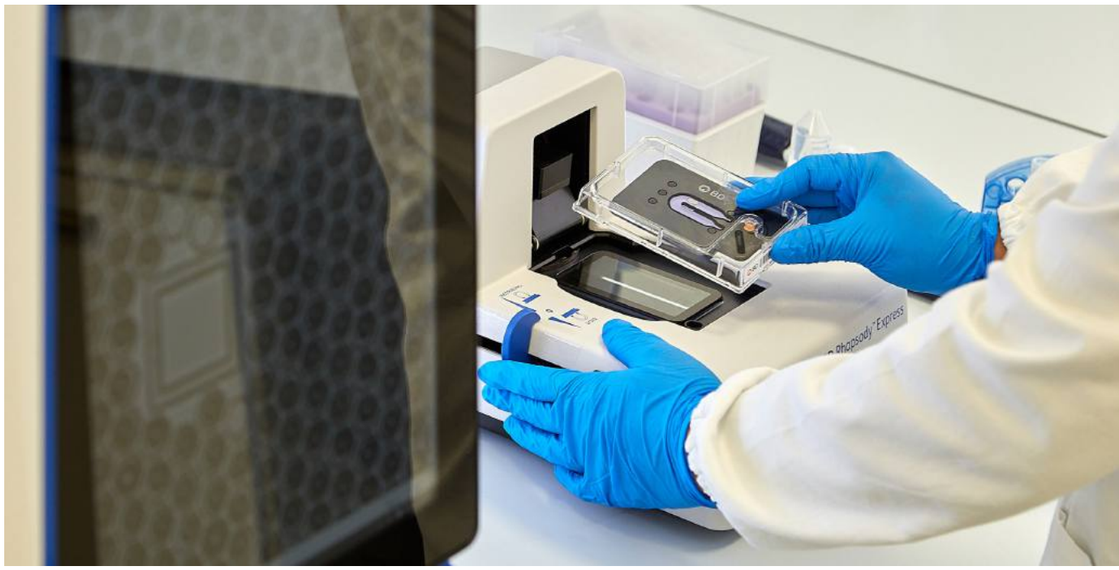
**Selected publications**


- Protic D, Polli R, Hwang YH, Mendoza G, Hagerman R, Durbin-Johnson B, Hayward BE, Usdin K, Murgia A, Tassone F. Activation Ratio Correlates with IQ in Female Carriers of the FMR1 Premutation. Cells. 2023 Jun 24;12(13):1711.
- Leonardi E, Aspromonte MC, Drongitis D, Bettella E, Verrillo L, Polli R, McEntagart M, Licchetta L, Dilena R, D'Arrigo S, Ciaccio C, Esposito S, Leuzzi V, Torella A, Baldo D, Lonardo F, Bonato G, Pellegrin S, Stanzial F, Posmyk R, Kaczorowska E, Carecchio M, Gos M, Rzońca-Niewczas S, Miano MG, Murgia A.Expanding the genetics and phenotypic spectrum of Lysine-specific demethylase 5C (KDM5C): a report of 13 novel variants. Eur J Hum Genet. 2023 Feb;31(2):202-215.
- Sawacha Z, Spolaor F, Piątkowska WJ, Cibin F, Ciniglio A, Guiotto A, Ricca M, Polli R, Murgia A. Feasibility and Reliability Assessment of Video-Based Motion Analysis and Surface Electromyography in Children with Fragile X during Gait. Sensors (Basel). 2021 Jul 12;21(14):4746
- Vos N, Reilly J, Elting MW, Campeau PM, Coman D, Stark Z, Tan TY, Amor DJ, Kaur S, StJohn M, Morgan AT, Kamien BA, Patel C, Tedder ML, Merla G, Prontera P, Castori M, Muru K, Collins F, Christodoulou J, Smith J, Zeev BB, Murgia A, Leonardi E, Esber N, Martinez-Monseny A, Casas-Alba D, Wallis M, Mannens M, Levy MA, Relator R, Alders M, Sadikovic B. DNA methylation episignatures are sensitive and specific biomarkers for detection of patients with KAT6A/KAT6B variants. Epigenomics. 2023 Mar;15(6):351-367
- Guo H, Bettella E, Marcogliese PC, Zhao R, Andrews JC, Nowakowski TJ, Gillentine MA, Hoekzema K, Wang T, Wu H, Jangam S, Liu C, Ni H, Willemsen MH, van Bon BW, Rinne T, Stevens SJC, Kleefstra T, Brunner HG, Yntema HG, Long M, Zhao W, Hu Z, Colson C, Richard N, Schwartz CE, Romano C, Castiglia L, Bottitta M, Dhar SU, Erwin DJ, Emrick L, Keren B, Afenjar A, Zhu B, Bai B, Stankiewicz P, Herman K; University of Washington Center for Mendelian Genomics; Mercimek-Andrews S, Juusola J, Wilfert AB, Abou Jamra R, Büttner B, Mefford HC, Muir AM, Scheffer IE, Regan BM, Malone S, Gecz J, Cobben J, Weiss MM, Waisfisz Q, Bijlsma EK, Hoffer MJV, Ruivenkamp CAL, Sartori S, Xia F, Rosenfeld JA, Bernier RA, Wangler MF, Yamamoto S, Xia K, Stegmann APA, Bellen HJ, Murgia A, Eichler EE. Disruptive mutations in TANC2 define a neurodevelopmental syndrome associated with psychiatric disorders. Nat Commun. 2019 Oct 15;10(1):4679


RESEARCH AREA

# Immunology and Neuroimmunology

**Coordinator** Prof. Emanuele Cozzi



The "Immunology and Neuroimmunology" research area is coordinated by Prof. Emanuele Cozzi and comprises of four distinct yet deeply connected units. The research priority of the area is to uncover key molecular mechanisms driving infection, inflammatory role and immune-mediated disorders and to translate these findings from bench to bedside.

Our research is currently aimed at investigating immune-related pathogenic mechanisms in many disorders, including acquired autoimmune demyelinating syndromes, pediatric multiple sclerosis, perinatal stroke, pediatric epilepsies and encephalopathy with seizures, paraneoplastic and auto-immune neurological syndromes. A relevant activity within this research area is dedicated to the study of the antibody mediated rejection mechanisms following pediatric kidney transplantation. Currently, antibody mediated rejection continues to be one of the most critical immune barriers in renal transplantation, significantly affecting graft survival.

Our approach combines pre-clinical and clinical studies in order to identify new early disease biomarkers and novel therapeutic targets.

The staff involved includes academic researchers from various Departments of the University of Padova (Biomedical Sciences, the Department of Women's and Children's Health and the Depart-ment of Cardio-Thoraco-Vascular Sciences and Public Health) as well as clinicians from the Padua University Hospital, building up an effective multidisciplinary team for improving research outcomes.

**Prof. Emanuele Cozzi**
*Coordinator Immunology and Neuroimmunology Area*

*Scopus ID 7006230073*

Prof. Emanuele Cozzi, MD, PhD, is a clinical immunologist who graduated from the Medical School of the Padua University in 1984. In 1987, he obtained his specialization as an allergist and clinical immunologist in the same University and in 2000 a PhD from the University of Cambridge.

From 1988 until 1991, he has worked as a Research Fellow in the Department of Microbiology, New York Medical College, Valhalla, New York. There, he was mainly involved in clinical trials regarding the use of monoclonal antibodies in the treatment of autoimmune diseases.

From 1993 until 2001, Prof. Cozzi has worked as a Clinical Research Associate in the Department of Surgery of the University Cambridge, where he has contributed to both clinical and preclinical activities in the field of solid organ transplantation.

In September 2001, Prof. Cozzi joined the Padua Medical Center where he directs the Unit of Transplantation Immunology. Furthermore, since January 2020 he is Full Professor at the Department of Cardio-Thoraco-Vascular Sciences and Public Health at the University of Padua.

Prof. Cozzi has published extensively in the field of transplantation and is author of more than 230 manuscripts in international journals (Scopus citations: 8.547; h-index: 46). His areas of expertise encompass clinical transplantation immunology, with particular regard to humoral rejection, and preclinical research in the field of solid organ and neural cell transplantation. He has led several multicenter research initiatives and was Coordinator of the EU-funded XENOME Project. He has also coordinated the multicenter EU-funded TRANSLINK Project, whose objective was to define the role of immune events in the premature valve failure in patients receiving animal-derived bioprosthetic heart valves. Prof. Cozzi is a reviewer for several major funding agencies, including the National Institutes of Health and National Institutes of Allergy and infectious Disease (NIH/NIAID), Bethesda, Maryland (USA) and for the PHRC Programme, Ministère des Solidarités et de la Santé, Paris (France).

Prof. Cozzi is a past-president of the International Xenotransplantation Association (IXA) and a member of the IXA Ethics Committee; he has served as vice-president of the Italian Society of Organ Transplantation. He is the National Expert at the National Transplant Center (CNT-Rome) for issues related to transplantation in incompatible patients, organ trafficking and for international affairs. He is the Italian Delegate acting as Transplant Expert at the Council of Europe and a past President of "European Committee on Organ Transplantation" (CD-P-TO), at the Council of Europe in Strasbourg. Furthermore, he is Consultant for the Vatican for issues related to xenotransplantation; member of the Vatican Pontifical Academy for Life (PAV) and President of the "Morgagni Foundation" (Padua).

# Immunity, Inflammation & Angiogenesis

## Group Leaders

Prof. Marcella Canton - Co-Principal Investigator
Prof. Barbara Molon - Co-Principal Investigator

## Research activity

Immunity, Inflammation & Angiogenesis Laboratory studies signals modulating angiogenesis, inflammation and immunity in various physiopathological conditions.

*Exploiting MSC-derived EVs to fight cancer*

Pathological angiogenesis is a hallmark of several conditions including eye diseases, inflammatory diseases, and cancer. Stromal cells play a crucial role in regulating angiogenesis through the release of soluble factors or direct contact with endothelial cells. We analyzed the properties of extracellular vesicles (EVs) released by bone marrow mesenchymal stromal cells (MSCs) and explored the possibility of using them to therapeutically target angiogenesis. We demonstrated (Angioni et al. JEV 2020) that in response to pro-inflammatory cytokines, MSCs produce EVs that are enriched in TIMP-1, CD39 and CD73 and inhibit angiogenesis targeting both extracellular matrix remodelling and endothelial cell migration. Our final goal is to exploit the anti-angiogenic EVs to target pathological tumor associated angiogenesis.

Collaborators: Maurizio Muraca, University of Padua & IRP.

*Fostering Cancer Immunotherapy for the treatment of pediatric Lymphomas*

The current standard-of-care for pediatric B non-Hodgkin Lymphomas (B-NHL) with standard-risk and high-risk disease results in more than 94% 5-year EFS. Nonetheless, even today, the long-term survival for patients with relapsed or refractory disease is poor. CAR T therapy has come out as one of the most effective options in the treatment of B-cell acute lymphoblastic leukemia and adult B-cell lymphomas and a multicentric trial has been recently approved in pediatric patients with relapsed or refractory B-NHLs. Even so, the available clinical experience has shown that 30-60% patients relapse after treatment, probably due to CAR T incomplete responses, downregulation of tumor-related antigens and immune escape mechanisms in the hosts, which is a great challenge for disease control. Therefore, understanding the mechanisms that underlie post-CAR T relapse and establishing corresponding prevention and treatment strategies is of paramount urgency. Of note, the induction of a tolerogenic environment made up by myeloid immunosuppressive cell subsets so far is one of the major obstacles that CAR T cells need to circumvent to fully accomplish their job. The metabolic landscape of the tumor microenvironment (TME) has recently turned out as a key determinant of anti-tumor immunity and therapy effectiveness. Our research priority will be the investigation of the metabolic crosstalk within B-NHL environment in children with the specific aim of identifying leading metabolic checkpoints that can hamper CAR T cell responses. As state-of-the-art approach, we will investigate myeloid regulatory cell immunosuppressive signature at single-cell level.

Collaborators: Lara Mussolin Unipd& IRP, Elisa Cimetta Unipd&IRP, Alessandra Castegna University of Bari

*Multiple sclerosis and acquired autoimmune demyelinating syndromes*

Acquired demyelinating diseases of the central nervous systems (CNS) constitute a broad spectrum of highly disabling inflammatory and neurodegenerative diseases. Multiple sclerosis (MS) and the so-called Neuromyelits Optica-Spectrum Disorders (NMO-SD), whose incidence and prevalence are dramatically increasing worldwide, may have a pediatric onset, characterized by severe clinical and neuroradiological pictures. The aim of our project is the discovery of still unknown immunopathogenic mechanisms in pediatric MS through: i) a comprehensive, single-cell multi-omics analysis of the pathogenic cell populations; ii) the identification of possible target antigens, and iii) the identification of novel antigens through T cell receptor (TCR) and HLA profiling.

Collaborators: Paolo Gallo, Stefano Sartori, University of Padua & IRP

*Monoamine Oxidases in Innate Immunity*

Reactive oxygen species (ROS) are fundamental for macrophages to kill invasive microorganisms. Moreover, they have a key role in regulating signal transduction pathways, gene expression and differentiation. Besides NADPH oxidase, mitochondria are gaining increasing relevance as a source of ROS in immune cells, although the exact sites of formation are still unclear. Monoamine oxidase (MAO) is a relevant source of hydrogen peroxide in mitochondria, generated by oxidative deamination of amines. Since this enzyme has been scarcely characterized in phagocytic cells, we aimed at clarifying its role in innate immunity and in the activation of the NLRP inflammasome. The uncontrolled activation of the inflammasome drives progression of inflammatory, metabolic, and neurodegenerative disorders. However, it is still unclear what is the specific role of mitochondrial ROS in NLRP3 triggering. We show that oxidative stress induced by MAO activity plays a crucial role in inflammasome activation in acute and chronic inflammation. Mechanistically, MAO-B-dependent ROS formation caused mitochondrial dysfunction and NF-kB induction, resulting in NLRP3 and pro-IL-1b overexpression. Both in vitro and in vivo, MAO-B inhibition by rasagiline prevented IL-1b secretion and MAO-B deficient mice showed impaired response to LPS-mediated endotoxemia. Importantly, in a Duchenne dystrophy model, rasagiline administration reduced inflammasome activation in muscle-infiltrating macrophages, along with muscle performance recovery. Our findings identify MAO-B as a specific producer of mitochondrial ROS fuelling NLRP3 inflammasome, thereby providing the basis for repurposing MAO-B inhibitors to treat inflammasome-mediated pathologies. Thus, we are currently investigating whether clinical-grade monoamine oxidase inhibitors can be viable candidates in the treatment of autoinflammatory and autoimmune disorders.

Collaborators: Rosella Tomanin IRP, Libero Vitiello Unipd, Bert Blaauw Unipd, Wolfgang Jerolimek, Pharmaxis, Australia

## PI's Biosketch

**Marcella Canton**

*Scopus ID 7004910913*

During my PhD training, I began my research on mitochondrial physiology and ion transport under the supervision of G.F. Azzone, one of the founding Fathers of Bioenergetics. My education in Cellular and Molecular Biology was completed with a long-term stay under the supervision of F. Di Lisa, where I characterized mitochondrial function in intact cells in anoxic conditions. I provided evidence of the mechanisms underlying the PUVA therapy (psoralen +UVA) and highlighted the central role of mitochondria. In parallel, my research interest has been focused on the study of the oxidative modifications of myofibrillar proteins. I demonstrated that the oxidation of myofibrillar proteins correlates linearly with contractile dysfunction. This study has been extended to the oxidative modifications of skeletal muscle in muscular dystrophy. I provided evidence that the excess of reactive oxygen species (ROS) observed in two different murine models of muscular dystrophy and in myoblasts from patients affected by collagen VI myopathies is mainly due to monoamine oxidase (MAO) overactivation. Moreover, I highlighted a causal link between MAO-dependent ROS production and contractile impairment thereby providing the rationale for a translational study of MAO inhibitors for treatment of muscle dystrophy. To this aim, I have been funded and I coordinated my own group within Di Lisa's one, strictly collaborating with Dr. Vitiello and Dr. Blaauw (Padova University). A few years ago, I started a collaboration with A. Viola's and B. Molon's team (Pediatric Research Institute "Città della Speranza", Padova, IT), that have a long-standing and outstanding expertise in immunology. Thanks to their synergistic collaboration, my group is currently focused at characterizing the role of MAOB in the innate immune system and in inflammatory diseases.

*Positions, Scientific Appointments and Honors Positions and Employment*

*Current Positions*

01/04/2022: Associate Professor of Biochemistry (05/E1 - BIO/10), University of Padova, Dept of Biomedical Sciences, Padova, Italy.

01/01/2017: PI at the Pediatric Research Institute "Città della Speranza" leading the "Monoamine oxidases in innate immunity" lab.

*Previous Positions*

01/01/2006-31/03/2021: Tenure-track Assistant Professor of Biochemistry (05/E1 - BIO/10), University of Padova, Dept. of Biomedical Sciences, Padova, Italy.

*Supervision of Graduate Students and Post-doctoral Fellows*

7 post-doctoral fellows, 6 PhD students and 105 master students at the Dept of Biological Chemistry, and at the Dept. of Biomedical Sciences, University of Padova, Italy.

**Barbara Molon**

*Scopus ID 6506737265*

Dr. Molon started her academic career with a degree in Biology in 2003 followed by Degree of Specialist in Clinical Pathology in 2010 at the University of Padova. At the beginning of her career Prof. Molon's research was focused on the characterization of key-pathways supporting T cell-activation (supervisor Prof. Viola, Venetian Institute of Molecular Medicine, VIMM, Padova) and subsequently centered on tumor immunology investigating novel mechanisms of tumor immune escape and tolerance (supervisor Prof. Bronte Istituto Oncologico Veneto, IRCCS, Padova).

In 2011, she was appointed as Junior PI he scientific at the Venetian Institute of Molecular Medicine (VIMM) in Padova. Her research activity focused on myeloid cell commitment and functions in cancer and in other pathological scenarios.

Prof. Molon is currently PI of the "Immunity, Inflammation & Angiogenesis" laboratory at the Pediatric Research Institute, investigating cellular and molecular mechanisms that regulate the immune response in different psychopathological conditions, including pediatric cancers and neuroinflammatory disorders with the final goal to translate these findings from bench to bedside.

*Current Position*

Associate Professor, Department of Biomedical Sciences, University of Padua.

*Previous Positions*

2019-2022: Assistant Professor (Ricercatore a tempo determinato L.230/05, Tenure Track). Department of Biomedical Sciences, University of Padua

2015-2018: Assistant Professor (Ricercatore a tempo determinato). Immunology Section Department of Biomedical Sciences, University of Padua

2012-2018: Junior Principal Investigator at the Venetian Institute of Molecular medicine, VIMM, Padua, Italy.

*Institutional Responsibilities*

2019-present: Faculty Committee, PhD school in Biomedical Sciences, University of Padova;

2019-present: Faculty Committee, school of Clinical pathology and clinical biochemistry, Medical schools, University of Padova

2012-2018: Member of the Scientific board of the Veneto Institute of Molecular Medicine;

2016-present: Faculty Committee, School of Nursing, University of Padova.

Reviewing Activities

2012- present: Grant reviewer: Wellcome Trust UK

Ad hoc reviewer: The Journal of Immunology, Immunology; Journal of Leukocyte Biology; Communications Biology

*Funding*

Project: Raising Metabolism against suppressive microenvironment: new immunometabolic targets to improve CAR T cell fitness for Childhood B Non-Hodgkin Lymphoma Treatment. IRP-Fondazione Città della Speranza. 2021-2023. Role: PI-Coordinator;

Project: Chemokine control of nuclear plasticity in metastatic cells". Progetti di ricerca dipartimentali SID. 2017–2019Università degli studi di Padova Role: PI-Coordinator

Project: Post-translational modifications induced by reactive species in tumor microenvironment: a new pathway to tumor immune escape BANDO GIOVANI RICERCATORI 2009- Ministero della Salute – Direzione Generale dellaRicerca Scientifica e Tecnologica. GR-2009-1558698, Role: PI-Coordinator

**Team Members**

Prof. Marcella Canton - Co-Principal Investigator

Prof. Barbara Molon - Co-Principal Investigator

Dr. Roberta Angioni - Senior Scientist

Dr. Francisca Venegas Celedón - Post Doc

Dr. Alessandra Maria Testa - Research Fellow

Dr. Annachiara Marin - PhD Student

Dr. Elena Baldisseri - PhD Student

**Selected Publications**


- Angioni R, Bonfanti M, Caporale N, Sánchez-Rodríguez R, Munari F, Savino A, Pasqualato S, Buratto D, Pagani I, Bertoldi N, Zanon C, Ferrari P, Ricciardelli E, Putaggio C, Ghezzi S, Elli F, Rotta L, Scardua A, Weber J, Cecatiello V, Iorio F, Zonta F, Cattelan AM, Vicenzi E, Vannini A, Molon B, Villa CE, Viola A, Testa G. RAGE engagement by SARS-CoV-2 enables monocyte infection and underlies COVID-19 severity. Cell Rep Med. 2023 Nov 21;4(11):101266. doi: 10.1016/j.xcrm.2023.101266. Epub 2023 Nov 8. PMID: 37944530; PMCID: PMC10694673.
- Angioni R, Sánchez-Rodríguez R, Munari F, Bertoldi N, Arcidiacono D, Cavinato S, Marturano D, Zaramella A, Realdon S, Cattelan A, Viola A, Molon B. Age-severity matched cytokine profiling reveals specific signatures in Covid-19 patients. Cell Death Dis. 2020 Nov 6;11(11):957. doi: 10.1038/s41419-020-03151-z. PMID: 33159040; PMCID: PMC7646225.
- Venegas FC, Sánchez-Rodríguez R, Luisetto R, Angioni R, Viola A, Canton M. Oxidative Stress by the Mitochondrial Monoamine Oxidase B Mediates Calcium Pyrophosphate Crystal-Induced Arthritis. Arthritis Rheumatol. 2024 Feb;76(2):279-284. doi: 10.1002/art.42697. Epub 2023 Dec 21. PMID: 37695218.
- Damanti CC, Ferrone L, Gaffo E, Garbin A, Tosato A, Contarini G, Gallingani I, Angioni R, Molon B, Borile G, Carraro E, Pillon M, Scarmozzino F, Dei Tos AP, Pizzi M, Ciscato F, Rasola A, Biffi A, Bortoluzzi S, Lovisa F, Mussolin L. Plasma small-extracellular vesicles enriched in miR-122-5p promote disease aggressiveness in pediatric anaplastic large-cell lymphoma. Cancer Commun (Lond). 2023 May;43(5):630-634. doi: 10.1002/cac2.12415. Epub 2023 Apr 4. PMID: 37014813; PMCID: PMC10174085.
- Sánchez-Rodríguez R, Tezze C, Agnellini AHR, Angioni R, Venegas FC, Cioccarelli C, Munari F, Bertoldi N, Canton M, Desbats MA, Salviati L, Gissi R, Castegna A, Soriano ME, Sandri M, Scorrano L, Viola A, Molon B. OPA1 drives macrophage metabolism and functional commitment via p65 signaling. Cell Death Differ. 2023 Mar;30(3):742-752. doi: 10.1038/s41418-022-01076-y. Epub 2022 Oct 28. PMID: 36307526; PMCID: PMC9984365.

# Immune-Mediated Nervous System Disease study group

## Group Leader

Prof. Stefano Sartori - Principal Investigator

## Research activity

The Neuroimmunology Group focuses on unraveling the role of immunity and inflammation in pediatric-onset epilepsies, encephalopathy with seizures, adult and pediatric paraneoplastic and autoimmune neurological syndromes. The main aims also include the identification of potential diagnostic and prognostic biomarkers that represent fundamental tools in the clinical settings. Our laboratory works in synergy with the clinical work, in particular with the pediatric and adult neurology department of Azienda Ospedale Università Padova (Dr. Margherita Nosadini, Dr. Marco Puthenparampil, Prof. Stefano Sartori, Prof. Paolo Gallo) and the neurobiology laboratory. Moreover, long-standing collaborations have been nurtured with the Multiple Sclerosis Center of the Dept. of Neurology of Vicenza Hospital (Dr. Luigi Zuliani), the Neuroimmunology Laboratory, IRCCS Mondino Foundation, Pavia (Dr. Matteo Gastaldi), and the Depart. of Pediatric Neurology, Children's Hospital Datteln, University Witten/Herdecke, Datteln, Germany (Prof. Kevin Rostásy). Our group received funding to support a project on pediatric-onset multiple sclerosis (pedMS). PedMS is an immune demyelinating disorder of childhood characterized by chronic inflammation that leads to progressive brain degeneration. Globally, its incidence and prevalence are about 0.87 per 100,000 individuals annually and 8.11 per 100,000 individuals, respectively. The clinical presentation of PedMS at onset could overlap with recurrent or multiphasic acute disseminated encephalomyelitis (ADEM), neuromyelitis optica spectrum disorders (NMOSD), or clinically isolated syndromes. Importantly, some of these disorders are distinct from MS, as their pathogenic mechanism is predominantly antibody-mediated. The most important characterized antigens are present on glial cells: aquaporin-4 (AQP4), a water channel mainly present on astrocytes, is the most important target in NMOSD, Glial Fibrillary Acidic Protein (GFAP) are targeted in a more recently described autoimmune astrocytopathy most frequently presenting as meningoencephalitis, while Myelin Oligodendrocyte Glycoprotein (MOG) is the main target in pediatric ADEM and other syndromes that have clinical-radiological overlap with MS. Up to now, these CNS inflammatory demyelinating disorders and their outcomes are distinguished based on major clinical and radiologic features; however, more reliable biologic markers need to be identified. The aims of our project are: i) the identification of novel imaging and biological markers in children with pedMS; ii) a comprehensive, single-cell multi-omics analysis of the pathogenic cell populations; ii) the identification of possible target antigens, and iii) the identification of novel antigens through T cell receptor (TCR) and HLA profiling; iii) and iii) eventually, the identification of novel therapeutic target molecules. This project is conducted in close collaboration with the "Translational Immunology" Laboratory in IRP directed by Prof. Barbara Molon. The following researchers are actively involved in this shared project: Dr. Alessandra Maria Testa, Dr. Francisca Venegas Celedón, and Dr. Annachiara Marin.

Other ongoing projects of the Neuroimmunology Group include the characterization of GFAP astrocytopathy through a systematic literature review, with focus on clinical-radiological features in children, and the description of the Italian cohorts of pediatric NMDAR encephalitis and MOG associated disorders.

## PI's Biosketch

*Scopus ID 8655110800*

Following a MD degree at the University of Padua in 2001, Prof. Stefano Sartori specialized in Pediatrics, with a specific focus on Pediatric neurology in 2006. Prof. Sartori subsequently enrolled in a PhD programme in Pediatric Neurology (2007 - 2009) followed by advanced higher education courses on Epilepsy (2011, University of Ferrara) and in Children Movement Disorders (2017, University of Rome). Since 2010, Prof. Sartori has been researching in the field of Neuroimmunology with a focus on immune-mediated encephalitis and epilepsies.

Prof. Sartori was co-coordinator of the Study Group on Autoimmune Epilepsies of the Italian League Against Epilepsy from 2014 to 2021, and was part of the Neuroimmunology Study Group of the Italian Society of Pediatric Neurology since 2015 to 2021.

Currently, Prof. Sartori works at the Dept. of Women's and Children's Health, University of Padua, where he coordinates the Pediatric Neurology and Neurophysiology programme.

Prof. Sartori is also a Professor at Medicine and Surgery degree course and at the training programmes in Pediatrics, Child Neuropsychiatry and Rehabilitation of the University of Padua, where he is course leader in Pediatric Neurology and Epilepsy.

Following a MD degree at the University of Padua in 2001, Prof. Stefano Sartori specialized in Pediatrics, with a specific focus on Pediatric neurology in 2006. Prof. Sartori subsequently enrolled in a PhD programme in Pediatric Neurology (2007 - 2009) followed by advanced higher education courses on Epilepsy (2011, University of Ferrara) and in Children Movement Disorders (2017, University of Rome). Since 2010, Prof. Sartori has been researching in the field of Neuroimmunology with a focus on immune-mediated encephalitis and epilepsies.

Prof. Sartori was co-coordinator of the Study Group on Autoimmune Epilepsies of the Italian League Against Epilepsy from 2014 to 2021, and was part of the Neuroimmunology Study Group of the Italian Society of Pediatric Neurology since 2015 to 2021.

Currently, Prof. Sartori works at the Dept. of Women's and Children's Health, University of Padua, where he coordinates the Pediatric Neurology and Neurophysiology programme. Prof. Sartori is also a Professor at Medicine and Surgery degree course and at the training programmes in Pediatrics, Child Neuropsychiatry and Rehabilitation of the University of Padua, where he is course leader in Pediatric Neurology and Epilepsy.

During his career, Prof. Sartori has co-authored over 160 indexed peer-reviewed articles and over 200 proceedings in Pediatrics, Pediatric Neurology, Epilepsy and Neuroimmunology.

### Team Members

Prof. Stefano Sartori - Principal Investigator
Dr. Margherita Nosadini - Senior Scientist
Dr. Marco Puthenparampil - Senior Scientist
Dr. Alessandra Maria Testa - Post Doc
Dr. Annachiara Marin - PhD Student
Dr. Francisca Venegas Celedón – Post Doc
Dr. Luigi Zuliani – MD, Clinical Scientist

## Selected publications


- Puthenparampil M, Gaggiola M, Miscioscia A, Mauceri VA, De Napoli F, Zanotelli G, Anglani M, Nosadini M, Sartori S, Perini P, Rinaldi F, Gallo P. Alemtuzumab following natalizumab is more effective in adult-onset than pediatric-onset multiple sclerosis. Ther Adv Neurol Disord. 2023 Oct 6;16:17562864231177196. doi: 10.1177/17562864231177196. PMID: 37808246; PMCID: PMC10559704.
- Nosadini M, Eyre M, Giacomini T, Valeriani M, Della Corte M, Praticò AD, Annovazzi P, Cordani R, Cordelli DM, Crichiutti G, Di Rosa G, Dolcemascolo V, Fetta A, Freri E, Gallo P, Gastaldi M, Granata T, Grazian L, Iorio R, Lombardini M, Margoni M, Mariotto S, Matricardi S, Melani F, Nardocci N, Papetti L, Passarini A, Pisani F, Po' C, Puthenparampil M, Ragona F, Savasta S, Siliquini S, Toldo I, Tozzo A, Turco EC, Varone A, Vogrig A, Zuliani L, Bugin S, Rossato S, Orsini A, Cantalupo G, Mancardi MM, Ferilli MAN, Foiadelli T, Sartori S. Early Immunotherapy and Longer Corticosteroid Treatment Are Associated With Lower Risk of Relapsing Disease Course in Pediatric MOGAD. Neurol Neuroimmunol Neuroinflamm. 2022 Nov 29;10(1):e200065. doi: 10.1212/NXI.0000000000200065. PMID: 36446614; PMCID: PMC9709714.
- Gastaldi M, Foiadelli T, Greco G, Scaranzin S, Rigoni E, Masciocchi S, Ferrari S, Mancinelli C, Brambilla L, Mancardi M, Giacomini T, Ferraro D, Della Corte M, Gallo A, Di Filippo M, Benedetti L, Novi G, Versino M, Banfi P, Iorio R, Moiola L, Turco E, Sartori S, Nosadini M, Ruggieri M, Savasta S, Colombo E, Ballante E, Jarius S, Mariotto S, Franciotta D; NINA study group. Prognostic relevance of quantitative and longitudinal MOG antibody testing in patients with MOGAD: a multicentre retrospective study. J Neurol Neurosurg Psychiatry. 2023 Mar;94(3):201-210. doi: 10.1136/jnnp-2022-330237. Epub 2022 Dec 2. PMID: 36460438.
- Margoni M, Villani U, Finos L, Franciotta S, Rubin M, Nosadini M, Sartori S, Anglani MG, Causin F, Perini P, Rinaldi F, Bertoldo A, Gallo P. Neurite orientation dispersion and density imaging discloses early changes in the normal-appearing white matter in pediatric multiple sclerosis. J Neurol Neurosurg Psychiatry. Epub 2021 Jul 16. 2022 Mar;93(3):332-334. doi: 10.1136/jnnp-2021-326355.. PMID: 34272345.
- Nosadini M, Toldo I, Tascini B, Bien CG, Parmeggiani L, De Gaspari P, Zuliani L, Sartori S. LGI1 and CASPR2 autoimmunity in children: Systematic literature review and report of a young girl with Morvan syndrome. J Neuroimmunol. 2019 Oct 15;335:577008. doi: 10.1016/j.jneuroim.2019.577008. Epub 2019 Jul 18. PMID: 31352183.


# Neuronal circuits in developmental disorders

## Group Leader

Dr. Manuela Allegra - Junior Principal Investigator

## Research activity

In our laboratory, the main research interest is centered on the field of neuroplasticity. We study the neural mechanisms underlying the capability of the brain to rewire itself in response to environmental pressures or being the key mechanism for neurorehabilitation in case of injury and for the prevention of neurodegenerative disorders later in life. We focus on the hippocampus and neocortex, and our experimental approach includes, among others, in vivo recording techniques (extracellular LFP recordings and single- photon calcium imaging) and neuronal activity manipulation (optogenetics and pharmacogenetics) in behaving animals, combined with anatomical tracing.

In collaboration with Prof. Antonella Viola and Prof. Matteo Caleo, we are currently developing a mouse model for perinatal stroke to study how the microglia function within the neuronal milieu surrounding the lesion may affect the neuroplasticity underlying the spontaneous functional recovery.

## JrPI's Biosketch

*Scopus ID 54985377400*

Manuela Allegra graduated in Neurobiology at the University of Pisa in 2009, with a thesis on the role of the inhibitory GABAergic system on hippocampal hyperexcitability in a mouse model for the autism spectrum disorders (Sgadò et al., 2013). She received her Ph.D. at the Scuola Normale Superiore (Pisa), where the main focus of her research activity was the study of neuroplasticity mechanisms in physiological and pathological conditions in rodents. Under the supervision of Prof. Matteo Caleo, she used the rodent visual system as a functional and anatomical model for experience-dependent plasticity (Allegra et al., 2014; Deidda, Allegra et al., 2015), she consolidated her expertise on the rodents visual system (Cappello et al., 2012; Tonazzini et al., 2020), and she built a solid background on the processes of adult hippocampal neurogenesis and hippocampal hyperexcitability (Cerri et al., 2016; Allegra et al., 2017; Busti, Allegra et al., 2020). In 2017, Dr. Allegra moved to Paris and joined the laboratory of Dr. Christoph Schmidt-Hieber at the Institut Pasteur. Here she was awarded with the Marie Curie individual fellowship and her research interest was focused on the hippocampal function in memory encoding and recall (Allegra et al., 2020; Zhang et al., 2021). In 2020 she was appointed to a permanent research position by the CNR (Italy) and she joined the Department of Biomedical Sciences of the University of Padua. Dr. Allegra has started her own research group with a starting grant from Fondazione CaRiPaRo (Moving Researchers for Pediatrics), in collaboration with Prof. Antonella Viola and Prof. Matteo Caleo.

**Team Members**

Dr. Manuela Allegra - Junior Principal Investigator
Dr. Livia Vignozzi - Post Doc
Dr. Gianmarco Cuboni - PhD Student
Dr. Emanuela Beretta - PhD Student
Dr. Giacomo Vecchieschi - Research Fellow
Dr. Catalina Campuzano - Research Fellow

### Selected publications


- Gandit B, Posani L, Zhang CL, Soham S, Ortiz C, Allegra M*, Schmidt-Hieber C*. Transformation of spatial representations along hippocampal circuits. iScience, 2024 (accepted for publication)
- Allegra M, Posani L, Gómez-Ocádiz R, Schmidt-Hieber C. Differential Relation between Neuronal and Behavioral Discrimination during Memory Encoding. Neuron Vol 108, issue 6, P1103-1112.E6, Dec 23, 2020
- Busti I*, Allegra M*, Spalletti C, Panzi C, Restani L, Billuart P, Caleo M. ROCK/PKA Inhibition Rescues Hippocampal Hyperexcitability and GABAergic Neuron Alterations in a Oligophrenin-1 Knock-Out Mouse Model of X-Linked Intellectual Disability. J Neurosci. 2020 Mar 25;40(13):2776-2788


- Busti I*, Allegra M*, Spalletti C, Panzi C, Restani L, Billuart P, Caleo M. ROCK/PKA Inhibition Rescues Hippocampal Hyperexcitability and GABAergic Neuron Alterations in a Oligophrenin-1 Knock-Out Mouse Model of X-Linked Intellectual Disability. J Neurosci. 2020 Mar 25;40(13):2776-2788


# Transplant Immunology

### Group Leader

Prof. Emanuele Cozzi - Principal Investigator

### Research activity

The Transplant Immunology Laboratory headed by Prof. Emanuele Cozzi is an acknowledged center of excellence in the national and international transplantation landscape. It carries out translational research activities in the context of both pediatric and adult transplantation.

At this stage, it is well-known that, following transplantation, a kidney graft will not function indefinitely. Indeed, the estimated half-life of a pediatric transplant kidney is around 15-20 years only. This means that within barely 20 years following transplantation, half of the transplant recipients will lose their graft, return to dialysis and, where possible, will be listed for a second transplant. Clearly, the incapacity to ensure indefinite survival of the graft following transplantation represents a serious burden that inevitably aggravates the initial scarce availability of kidneys accessible for transplantation. Intense investigations have been conducted especially in the last two decades to clarify the reasons underlying the premature failure of transplanted kidneys. These studies have demonstrated that both immunological and non-immunological events underlie such an untimely organ failure. In this context, the studies conducted by the Transplant Immunology Laboratory in collaboration with a multidisciplinary team that includes pediatricians, pediatric nephrologists, pathologists and scientists are aimed at clarifying the role of a particular subtype of antibodies (the non-HLA antibodies) as a possible cause of premature failure of pediatric renal transplants.

### PI's Biosketch

See Area Coordinator

**Team Members**

Prof. Emanuele Cozzi - Principal Investigator
Dr. Marta Vadori – Senior Scientist

### Selected publications

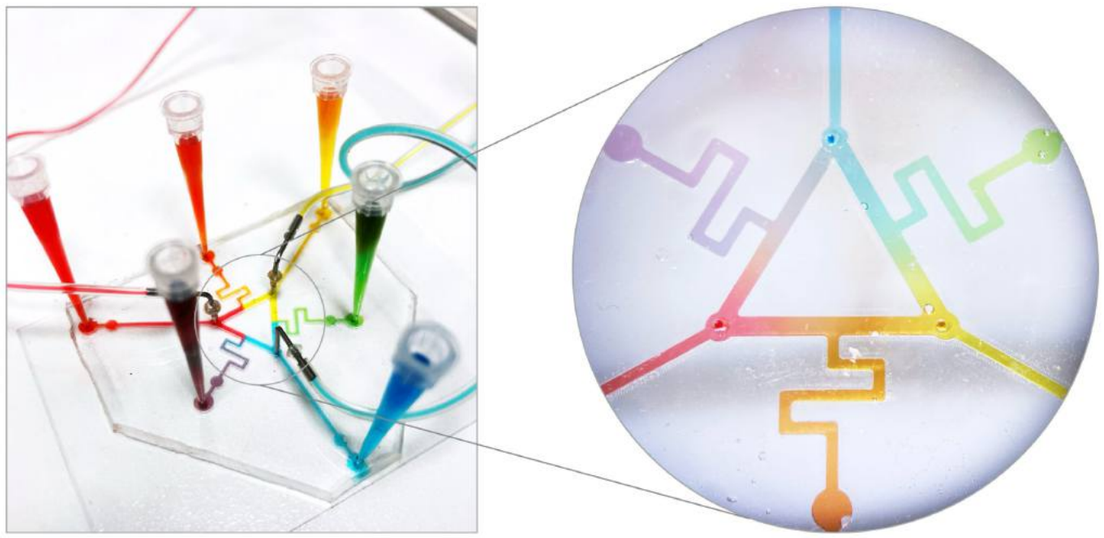

- Tona F*, Vadori M*, Civieri G, Masiero G, Iop L, Antonelli G, Perazzolo Marra M, Bianco F, Cecere A, Lorenzoni G, Naumova N, Bernava G, Basso D, Plebani M, Cozzi E, Iliceto S. Association of autoantibodies targeting endothelin type-A receptors with no-reflow in ST-elevation myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2023 Dec 21;12(12):856-861. (T.F.* and V.M.* equally contributed)
- Civieri G*, Vadori M*, Masiero G, Iop L, Tansella D, Pergola V, Cozzi E, Iliceto S, Tona F. Spontaneous coronary artery dissection in women with acute myocardial infarction: is there a new role for autoimmunity? Eur Heart J Acute Cardiovasc Care. 2023 Dec 21;12(12):856-861. (C.G.* and V.M.* equally contributed)
- Senage T, Paul A, Le Tourneau T, et al. The role of antibody responses against glycans in bioprosthetic heart valve calcification and deterioration. Nat Med. 2022 Feb;28(2):283-294.

- Pezzuto F, Lunardi F, Vadori M, Zampieri D, Casiraghi F, Azzollini N, Vuljan S E, Mammana M, Vedovelli L, Schiavon M, Gregori D, Cozzi E, Rea F, Calabrese F. Chronic lung allograft pathology lesions in two rat strain combinations. J Thorac Dis. 2021 May;13(5):2833-2843.
- Aron Badin R, Bugi A, Williams S, Vadori M, Michael M, Jan C, Nassi A, Lecourtois S, Blancher A, Cozzi E, Hantraye P, Perrier A L. MHC matching fails to prevent long-term rejection of iPSC-derived neurons in non-human primates. Nat Commun. 2019 Sep 25;10(1):4357.


RESEARCH AREA

# Medical Biotechnology

**Coordinator** Prof. Marco Agostini



The Medical Biotechnology research area comprises two groups involved in the research on the development and use of advanced technologies: patients derived tissue 3D model, microfluidics, nanofabrication, additive manufacturing, 3D bioprinting, development of biosensor platforms, optimization of microscopy techniques for biomedical applications.

Although the search fields are different, the common characteristics are shared and the translational nature of technological approaches aims to offer innovative solutions to other IRP research areas to enable therapeutic advances in pediatric oncological diseases.

The staff involved are part of different departments of the University of Padua (Industrial Engineering, Physics and Astronomy and Surgery, Oncology and Gastroenterology) as well as personnel paid on IRP grants, whose technical application experience is based on a real translation of the approaches in clinical practice.

**Prof. Marco Agostini**
*Coordinator Medical Biotechnology Area*

*Scopus ID 7005322173*

Prof. Agostini earned his degree in Biological Sciences and a PhD in Oncologic and Surgical Sciences from the University of Padua, Italy.

His thesis research focused on genetic and molecular characterization of cancer, with a concentration on the genetic pathways underlying the progression and outcome of colon cancer as well as drug delivery system modulation. As a Post-Doctoral fellow, he studied the molecular basis of hereditary colorectal cancer syndromes, the multidisciplinary treatment of colorectal rectal cancer, and the genetics and oncology of gastrointestinal tumors. After moving to the Netherlands in 2005 for a Post-Doctoral fellowship in the Dept. of Pathology at the Josephine Nefkens Institute in the Erasmus University Medical Center, Prof. Agostini was appointed Assistant Professor in the Dept. of Oncology and Surgical Sciences at the University of Padua, in 2006, where he conceived the groundwork for two major projects: the discovery of new molecular markers for the non-invasive early detection of cancer and the identification of the roles of molecular markers in pathologic tumor responses and rectal cancer patient outcome after receiving preoperative chemoradiotherapy. Since then, Prof. Agostini has been leading these projects in collaboration with other investigators from multiple institutions. These collaborations have established strong translational research relationships within the community of molecular and oncology medicine, which support the ongoing clinical translation of research innovations. Most recently, Prof. Agostini's research activity is focused on the application of nanotechnology and decellularization techniques to the field of molecular biology, proteomics and 3D culture model in relation to cancer research.

His aims are to respectively improve cancer detection and predict patient's response to chemotherapy, by identifying specific cancer biomarkers.

Grant: from 2012 a total budget of 2.523.000,00 euros of which 1.863.000,00 euros as Coordinator and/or Principal Investigator.

Official h-index and publications: 33 (according to Scopus). He has published 136 articles in peer reviewed scientific journals.

# Biomedical Applications of Multiscale Engineering Technologies (BIAMET)

## Group Leader

Prof. Elisa Cimetta - Principal Investigator

## Research activity

The laboratory's main research interests focus on the application of engineering principles to biological studies. In particular, the BIAMET laboratory (Biomedical Applications of Multiscale Engineering Technologies) specializes in the design and development of advanced microscale technologies and microbioreactor platforms for the in vitro culture of cells. The ultimate goal of the research is the optimization of biological models and cell cultures for their application in clinical settings for a better understanding of the mechanisms of the diseases and develop and test novel drugs and therapeutic strategies.

## PI's Biosketch

*Scopus ID 15847798700*

After graduating and receiving her PhD in Chemical Engineering at the University of Padua, she joined the laboratory of Prof. Gordana Vunjak-Novakovic at Columbia University in New York as an Associate Research Scientist. During her time at Columbia University, she won a prestigious scholarship awarded by the New York Stem Cell Foundation and earned a postgraduate certification in business and administration. Dr. Cimetta is a co-founder of EpiBone Inc., a company dedicated to the production of engineered bone and patient-specific osteochondral tissues with ongoing Phase I-II clinical trials with the FDA. In 2016 she was appointed Associate Professor at the Department of Industrial Engineering (DII), University of Padua. In 2017 she was awarded the ERC Starting Grant by the European Union which led to her recently awarded Proof of Concept proposal. In 2018 she was promoted to Associate Professor. Her BIAMET laboratory has two locations, one at the DII and one at the Pediatric Research Institute (IRP, Città della Speranza in Padua). With more than 15 years of experience in the field of bioreactor and microreactor development, she has been a pioneer in the development of tools, technologies and devices aimed at studying biological systems and bringing them closer to new clinical and therapeutic applications.

## Team members

Prof. Elisa Cimetta - Principal Investigator
Dr. Sara Micheli - Research Fellow
Dr. Pina Fusco - Research Fellow
Dr. Veronica Zingales - Research Fellow
Dr. Anna Fietta - PhD Student
Dr. Eleonora Zanre - PhD Student
Dr. Federico Maggiotto - PhD Student
Dr. Eva Dalla Valle - PhD Student

### Selected publications


- Fusco P, Fietta A, Esposito MR, Zanella L, Micheli S, Bastianello A, Bova L, Borile G, Germano G, Cimetta E. miR-210-3p enriched extracellular vesicles from hypoxic neuroblastoma cells stimulate migration and invasion of target cells. Cell & Bioscience, 2023 May;13:1,89. 10.1186/s13578-023-01045-z
- Micheli S, Mocellin P, Sorgato M, Bova L, Cimetta E. Modeling-based design specifications for microfluidic gradients generators for biomedical applications. Biochemical Engineering Journal 181 (2022) 108415. 10.1016/j.bej.2022.108415
- Bova L, Maggiotto F, Micheli S, Giomo M, Sgarbossa P, Gagliano O, Falcone D, Cimetta E. A porous gelatin methacrylate-based material for 3D cell-laden constructs. Macromolecular Bioscience, 2023 Feb;23(2):e2200357. 10.1002/mabi.202200357
- Zingales V, Esposito MR, Quagliata M, Cimetta E, Ruiz MJ. "Comparative Study of Spheroids (3D) and Monolayer Cultures (2D) for the In Vitro Assessment of Cytotoxicity Induced by the Mycotoxins Sterigmatocystin, Ochratoxin A and Patulin". Foods 2024, 13(4), 564; https://doi.org/10.3390/foods13040564
- Micheli S, Piunti C, Sorgato M, Lucchetta G, Cimetta E. "Cancer-on-a-Chip Platform to Study Metastatic Microenvironments". Chemical Engineering Transactions 2022, 93, 10.3303/CET2293036


# NanoInspired Biomedicine

### Group Leader

Prof. Marco Agostini - Principal Investigator

### Research activity

NanoInspired biomedicine lab focuses on:

- study of the genetic and molecular characterization of cancer with a concentration on the genetic pathway involved in the progression and outcome of colon cancer as well as drug delivery system modulation;
- discovery of new molecular markers for the non-invasive (liquid biopsies) early detection of cancer, and identifying the roles of circulating molecular markers in pathologic tumor responses and rectal cancer patient outcome after receiving preoperative chemoradiotherapy (i.e. circulating cell free DNA (cfDNA), circulating miRNA, proteins, metabolites, circulating cell free RNA);
- decellularized colorectal cancer matrix as bioactive microenvironment for in vitro 3D cancer research. Aims: i) to standardize a decellularization protocol for the healthy colonic ECM and CRC counterpart, able to eliminate the cellular component but simultaneously maintains its structure, biochemical composition and biological properties; ii) to characterize the decellularized healthy colonic mucosa and CRC ECM by analyzing the main structural components, its three-dimensional organization and the proteome and secretome composition; iii) to verify whether the CRC ECM possesses different biological properties compared with healthy colonic mucosa by means of recellularization experiments with stabilized CRC cell lines;
- application of biomimetic proteolipid vesicles, called Leukosomes, for targeting inflamed tissues. Explored the application of these biomimetic particles to several diseases that share an inflammatory background, such as inflamed bowel disease, atherosclerosis, primary and metastatic cancer, and autoimmune diseases.

### PI's Biosketch

See Area Coordinator

### Team members

Prof. Marco Agostini - Principal Investigator
Dr. Edoardo D'Angelo - Senior Technologist/Research Associates
Dr. Francesca Sensi - Post Doc
Prof. Piero Traldi – Senior Scientist
Dr. Asia Marangio - PhD Student
Dr. Anna De Rossi - PhD Student

**Selected publications**

- D'Angelo E, Pastrello C, Biccari A, Marangio A, Sensi F, Crotti S, Fassan M, Jurisica I, Pucciarelli S, Agostini M. An integrated multiomics analysis of rectal cancer patients identified POU2F3 as a putative druggable target and entinostat as a cytotoxic enhancer of 5-fluorouracil. Int J Cancer. 2023 Jul 15;153(2):437-449. doi: 10.1002/ijc.34478. Epub 2023 Mar 23. PMID: 36815540.
- Sensi F, D'angelo E, Biccari A, Marangio A, Battisti G, Crotti S, Fassan M, Laterza C, Giomo M, Elvassore N, Spolverato G, Pucciarelli S, Agostini M. Establishment of a human 3D pancreatic adenocarcinoma model based on a patient-derived extracellular matrix scaffold. Transl Res. 2023 Mar;253:57-67. doi: 10.1016/j.trsl.2022.08.015. Epub 2022 Sep 10. PMID: 36096350.
- Giordano F, Lenna S, Baudo G, Rampado R, Massaro M, De Rosa E, Ewing A, Kurenbekova L, Agostini M, Yustein JT & Taraballi F. Tyrosine kinase inhibitor-loaded biomimetic nanoparticles as a treatment for osteosarcoma.
- Cancer Nanotechnology volume 13, Article number: 40 (2022)
- Rampado R, Crotti S, Caliceti P, Pucciarelli S, Agostini M. Recent Advances in Understanding the Protein Corona of Nanoparticles and in the Formulation of "Stealthy" Nanomaterials. Front Bioeng Biotechnol. 2020 Apr 3;8:166. doi: 10.3389/fbioe.2020.00166. PMID: 32309278; PMCID: PMC7145938.
- D'Angelo E, Natarajan D, Sensi F, Ajayi O, Fassan M, Mammano E, Pilati P, Pavan P, Bresolin S, Preziosi M, Miquel R, Zen Y, Chokshi S, Menon K, Heaton N, Spolverato G, Piccoli M, Williams R, Urbani L, Agostini M. Patient-Derived Scaffolds of Colorectal Cancer Metastases as an Organotypic 3D Model of the Liver Metastatic Microenvironment. Cancers (Basel). 2020 Feb 5;12(2):364. doi: 10.3390/cancers12020364. PMID: 32033473; PMCID: PMC7072130.

RESEARCH AREA

# Predictive Medicine

**Coordinator** Prof. Eugenio Baraldi

The overall goal of predictive medicine in pediatric medicine is to flag risk factors so that physicians can work to reduce the chances of future problems for patients. Our research team, composed of clinicians, biostatisticians, biologists and chemist, develops advanced predictive models in the field of disease risk prediction and prevention and follow-up.

The Pediatric Critical Care Project (PCare) is conducting studies on lung surfactant metabolism and drug administration, neonatal nutrition, and respiratory and neurological outcomes of congenital heart disease.

Metabolome and lipidomics are of interest for expanding our understanding of complex biological systems in the context of function, since metabolites are downstream of all other levels of biological regulation. Therefore, the metabolome reflects the cumulative changes resulting from processes involving the genome, transcriptome and proteome, as well as their interactions with the environment. The metabolome therefore directly reflects the phenotype of a given biological system at the molecular metabolic level. In other words, while genomics, transcriptomics, and proteomics together provide a model of what might be happening within a biological system, metabolomics provides a snapshot of phenotypic traits, revealing what is currently happening or has happened as a result of these other levels of biological regulation.

**Prof. Eugenio Baraldi**
*Coordinator Predictive Medicine Area*

*Scopus ID 7006821460*

Prof. Baraldi obtained his MD degree (1982) and subsequently specialized in Pediatrics (1986) and Allergy and Immunology (1990) at the University of Padua. In 1990, he joined the Dept. of Pediatrics, Harbor UCLA, USA, as a Research Fellow, before returning to Italy where he became firstly Associate Professor (2005) and then Full Professor of Pediatrics (2010) and Director of the School of Pediatrics, at the University of Padua (2012-2016 and 2020-2024). Prof. Baraldi has also been President of the "Italian Society of Pediatric Respiratory Diseases" (2010-2014) and founder and executive member of ReSViNET, an international network supporting research in the RSV field.

Since 2014, Prof. Baraldi is serving as Director of the Neonatology-Neonatal Intensive Care Unit and Director of the Master in Neonatology and Neonatal Intensive Care (2015-2023), University of Padova. Since 2022 he is director of the Women's and Children's Health Department, University of Padova, and Scientific Director Pediatric Research Institute "Città della Speranza".

He is Coordinator of the project "Metabolomics in Pediatrics".

Prof. Baraldi is reviewer of research programs for the Italian Ministry for the University and Research (MIUR), the Guidance Committee for the Assessment of Research (CIVR), the Netherlands Asthma Foundation, the Asthma Foundation of Western Australia, Swiss National Science Foundation and HORIZON-HLTH-Diasease-03 (CLARITY Project).

Prof. Baraldi published more than 350 full papers in international journals (h-index 76, > 21.000 citations, source Google Scholar); including NEJM, Lancet, JAMA and AJRCCM. He is included in the list of "Top Italian Scientist" (www.topitalianscientists.org).

# Mass Spectrometry and Metabolomics

## Group Leaders

Prof. Eugenio Baraldi - Co-Principal Investigators
Dr. Giuseppe Giordano – Co-Principal Investigators

## Research activity

### Metabolomics and Lipidomics: A New Frontier for Research in Pediatrics

Metabolomics is the most recent of the "omic" sciences. Metabolomics and Lipidomics can be defined as the quantitative analysis of all the metabolites (small molecules) of a biological sample aiming to the investigation of the multiparametric metabolic response of a living system to pathophysiological stimuli or genetic modifications. A metabolic profile consists of the set of metabolites reflecting enzyme expression and activity, and includes the building blocks and breakdown products of the DNA, RNA, proteins, and cellular components. Also, it is affected by several factors unrelated to the genome, such as interactions with commensal microorganisms, nutritional factors, environmental agents, and any exposure to drugs or toxic substances resulting in discordance between genotype and phenotype. In many fields of medicine, there is a growing interest in characterizing diseases at molecular level with a view to developing an individually tailored therapeutic approach. Metabolomics and Lipidomics is a novel area that promises to contribute significantly to the characterization of various disease phenotypes and to the identification of personal metabolic features that can predict response to therapies. Based on analytical platforms such as mass spectrometry or NMR spectroscopy, the metabolomics approach enables a comprehensive overview of the metabolites, leading to the characterization of the metabolic fingerprint of a given sample. These metabolic fingerprints can then be used to distinguish between different disease phenotypes and to predict a drug's effectiveness and/or toxicity. Several studies published in the last few years applied the metabolomic approach in the field of pediatric medicine. Being a highly informative technique that can be used on samples collected non-invasively (e.g. urine or exhaled breath condensate), metabolomics has appeal for the study of pediatric diseases.

### Summary of ongoing research and main recent results achieved

### When Time is Brain: A Multi-Omic Approach for Rapid Diagnosis and Prognostic Characterization of Hypoxic-Ischemic Encephalopathy

Hypoxic-ischemic encephalopathy (HIE) is characterized by inadequate oxygen supply to vital organs and peripheral tissues in newborns, resulting in tissue hypoxia and injury. HIE may lead to adverse early outcomes like neonatal death as well as long-term consequences, including cerebral palsy, epilepsy, behavioral disorders, cognitive impairment, and neurodevelopmental disability. Currently, therapeutic hypothermia stands as the sole approved neuroprotective treatment for HIE. Our research on this topic began in 2015, involving untargeted metabolomic analysis of urine samples from 53 asphyctic newborns, 21 exhibiting HIE signs at brain MRI and 32 without. Key metabolites—L-alanine, L-lysine, creatine, and L-3-methylhistidine—differentiated the HIE group from the non-HIE group. Further analysis of samples from HIE newborns and healthy controls revealed three impacted metabolic pathways during HIE: steroidogenesis, lysine degradation, and carnitine synthesis. To explore the predictive potential of the urinary metabolome at birth, we conducted a longitudinal follow-up of our cohort up to 7 years, revealing correlations between birth levels of pipecolic acid, L-pyroglutamic acid, gamma-aminobutyric acid, and L-3-methyl-

histidine, and long-term neurological outcomes. Our future work until 2026 is encapsulated in the "MultiOmicAsphyxia" study, integrating diverse -omic techniques and miRNA analysis for a comprehensive understanding of HIE. The objectives of this project include validating prior findings through targeted metabolomics, advancing comprehension of HIE pathophysiology with multi-modal -omic techniques and miRNA analysis, analyzing data for early biomarkers and metabolic targets, and developing a bedside device using low-field NMR for early metabolome characterization in each HIE newborn, to provide tailored care and assess specific outcomes efficiently. (Ongoing research project)

**Urinary metabotypes of newborns with perinatal asphyxia undergoing therapeutic hypothermia**

Perinatal asphyxia (PA) still occurs in about three to five per 1,000 deliveries in developed countries; 20% of these infants show hypoxic-ischemic encephalopathy (HIE) on brain magnetic resonance imaging (MRI). The aim of our study was to apply metabolomic analysis to newborns undergoing therapeutic hypothermia (TH) after PA to identify a distinct metabotype associated with the development of HIE on brain MRI. We enrolled 53 infants born at >35 weeks of gestation with PA: 21 of them showed HIE on brain MRI (the "HIE" group), and 32 did not (the "no HIE" group). Urine samples were collected at 24, 48 and 72 hours of TH. Metabolomic data were acquired using high-resolution mass spectrometry and analyzed with univariate and multivariate methods. Considering the first urines collected during TH, untargeted analysis found 111 relevant predictors capable of discriminating between the two groups. Of 35 metabolites showing independent discriminatory power, four have been well characterized: L-alanine, Creatine, L-3-methylhistidine, and L-lysine. The first three relate to cellular energy metabolism; their involvement suggests a multimodal derangement of cellular energy metabolism during PA/HIE. In addition, seven other metabolites with a lower annotation level (proline betaine, L-prolyl-L-phenylalanine, 2-methyl-dodecanedioic acid, S-(2-methylpropionyl)-dihydrolipoamide-E, 2,6 dimethylheptanoyl carnitine, Octanoylglucuronide, 19-hydroxyandrost-4-ene-3,17-dione) showed biological consistency with the clinical picture of PA. Moreover, 4 annotated metabolites (L-lysine, L-3-methylhistidine, 2-methyl-dodecanedioic acid, S-(2-methylpropionyl)-dihydrolipoamide-E) retained a significant difference between the "HIE" and "no HIE" groups during all the TH treatment. Our analysis identified a distinct urinary metabotype associated with pathological findings on MRI, and discovered 2 putative markers (L-lysine, L-3-methylhistidine) which may be useful for identifying neonates at risk of developing HIE after PA. (PLoS One 2016 Oct 18;11(10):e0164211)

**Unravelling the role of lipids in ZIKA virus Infection and traversing of the Placenta**

ZIKA virus (ZIKV) is a member of the Flaviviridae family. Infection of pregnant women might cause variable combinations of neurological and developmental disorders, known as the congenital ZIKA syndrome (CZS). Little is known about ZIKA's ability to infect and cross the placenta. Likewise, we ignore the direct and indirect pathological effects associated with placental infection. This gap in knowledge seriously hampers our ability to diagnose and treat infected patients. Only recently, lipidomics has offered invaluable insights into the field of flavivirology, describing a fascinating interplay, in which viruses lead to a profound rearrangement of the host lipid metabolism. Interestingly, for some flaviviruses, severity and incidence of the disease is worsened in overweight populations, suggesting a role of nutrition in the regulation of infection. Our project aims to investigate: 1) how ZIKA infections affect the lipid metabolism of the placenta and the associated fetal sequelae. 2) How lipid composition of the viral envelope defines placental tropism. 3) How nutrition can determine susceptibility to infection and vertical transmission (VT). Liquid chromatography and mass spectrometry will be applied for the lipidomic analyses. Generated data will: 1) improve our understanding of vertical transmission, indicating potential markers of placental infection; 2) create the basis for the development of antivirals selectively targeting the placenta; 3) inform cohort retrospective studies to verify the role of nutrition as risk factor for CZS, during pregnancy. (submitted)

**Inborn errors of bile acid synthesis (BASD) are rare genetic disorders of liver metabolism that cause chronic liver diseases**

Inborn errors of bile acid synthesis are rare genetic disorders of liver metabolism that cause chronic liver diseases, fat malabsorption, and fat-soluble vitamin deficiency during child-hood. These defects, due to a defective functioning of enzymes, are characterized by a failure to produce normal bile acids (BAs) and an accumulation of unusual BAs and BAs intermediates. BAs are potent digestive surfactants that promote the absorption of cholesterol, lipids and fat-soluble vitamins acting as emulsifiers. They provide the primary driving force for the promotion and secretion of bile and are essential for the development of the biliary excretory route for the elimination of endogenous and exogenous toxic substances, including bilirubin, xenobiotics, and drug metabolites. Inborn errors in the BA biosynthetic pathways include cerebrotendinous xanthomatosis (CTX, sterol 27-hydroxylase deficiency), 3β-hydroxysteroid- $\Delta^5$-C27-steroid dehydrogenase deficiency (HSD3B7), $D_4$-3-oxosteroid-5β-reductase deficiency, oxysterol 7α-hydroxylase deficiency, cholesterol 7α-hydroxylase deficiency, α-methylacyl-CoA racemase (AMACR), peroxisomal β-oxidation, and amino acid N-acyltransferase. The most useful screening test is the analysis of urinary bile acids and bile alcohols by flow injection analysis (FIA) electrospray ionization-tandem mass spectrometry (ESI–MS/MS). The screening procedures indicate that inborn errors of BA metabolism probably account for 1% to 2% of the cases of liver disease in infants, children, and adolescents, making this an important and specific category of metabolic liver disease. Early diagnosis of inborn errors of BAs synthesis is important because if the disorder remains untreated, progressive liver disease, together with neurologic disease, may develop and lead to death or require liver transplantation. The treatment of these defects is based on the oral administration of primary bile acids as cholic acid and chenodeoxycholic acid. However, their use was associated with an increased risk of serious possible adverse events, and treatment needs to be accurately monitored. (Chem Phys Lipids. 2017 Apr; 204:43-56. doi: 10.1016/j.chemphyslip.2017.03.004) (Ongoing research project)

## PI's Biosketch

**Eugenio Baraldi**

See Area Coordinator

**Giuseppe Giordano**

*Scopus ID 7202918601*

*Qualifications and work experience*

Head of the Mass Spectrometry and Metabolomics unit at the Women's and Children's Health Department, University of Padova

From 2006-2010 he was Seconded National Expert Joint Research Center of the European Commission; Institute for Health & Consumer Protection (IHCP); Physical & Chemical Exposure Unit (PCE); ISPRA (VA), Italy

Head of the Mass Spectrometry Lab at the Women's and Children's Health Department he

works on: Biochemical diagnosis of Inborn Error of Metabolism acylcarnitines and amino acids profiling of blood spots using ESI-MS/MS. Postmortem diagnosis of fatty acid oxidation disorders. Biochemical screening in urine of disorders of bile acids metabolism by ESI-MS/MS. Measurement of palmitate and linoleate turnover in critically ill infants by monitoring stable isotope labeled tracers in vivo. by GC/MS and GC-(combustion)(pirolysis) -IRMS for the elements $^{13}C$, $^{15}N2$, $^{2}H_2$. Measurement of acylcarnitine metabolism in fibroblasts, by monitoring stable isotope labeled tracers in vitro, by the used of HPLC ESI-MS/MS. Oxidative stress, by LC-MS/MS exhaled nitrotyrosine on asthmatic children.

He has been involved in research projects for the application of metabolomics approach by NMR and mass spectrometry.

*Education*

Research Doctorate Degree in Developmental Sciences (1990)

Doctoral Degree in Biological Sciences, (1984)

*Teaching activities*

2011 Appointed as Professor. Master in methodology and application of mass spectrometry in clinical chemistry. Faculty of Sciences Catania University, Italy

2013 Appointed as Professor. Master in methodology and application of mass spectrometry in clinical chemistry. Faculty of Sciences Catania University, Italy Membership of Research Network

2000-2016 SIMMESN, Italian Society for the Study of Hereditary Metabolic Diseases and Neonatal Screening. (founder member and from 2004 to 2010 Board member)

2006-2016 Member of the Metabolomics Society

2013-2016 Member of the IMaSS Italian Mass Spectrometry Society (founder member and Board member from 2013-2015) Organization of scientific meetings

2014 Workshop on "The Metabolomics Approaches: Advanced Analytical Tools for Future, challenges, perspectives and applications". In charge of the Scientific Committee, Italy.

2016 Workshop on "Wandering across pediatric cholestatic liver disease: focus on bile acid synthesis defects". In charge of the Scientific Committee, Italy

*Grants*

Project IRP-AdG 2024-26 MultiOmicAsphyxia Study - Unravelling early biomarkers and therapeutic targets in hypoxicischemic encephalopathy following perinatal asphyxia by use of multi-omic technology (Giuseppe Giordano, Enrico Valerio)

European Project "REspiratory Syncytial virus Consortium in EUrope" (acronimo "RESCEU"), di seguito "il Progetto", finanziato dalla Commissione Europea nell'ambito del Programma IMI2 (2017-2022) descritto nel Grant Agreement N. 116019

Regione Veneto GR-2019-12368539 "Tight glycemic control and Regional Brain Oxygenation In Very Preterm Infants: A Randomized Controlled Trial To Test The Effect of Continuous Glucose Monitoring (CGM) on the Brain Functional Response and its Metabolomic Footprints, combining liquid-chromatography-mass spectrometry (LC-MS) analysis and brain diffuse optic tomography (DOT)" (Alfonso Galderisi)

IRP-StG-2021 NeoGluControl Study - Neonatal brain oxygenation and Glucose control: metabolomic fingerprints of hypoglycemia (Alfonso Galderisi)

Ministero della Salute 2011-15 Pre-natal and early life origin of chronic obstructive lung respiratory diseases: identification of novel diagnostic biomarkers and therapeutic targets by applying the metabolomic approach. (Eugenio Baraldi)

Università di Padova Dipartimento-SDB 2017-19 Metabolomics applied to eosinophilic esophagitis in children: identification of novel biomarkers for diagnosis and non-invasive monitoring (Silvia Carraro)

Project IRPPenta 18/07 Novel approaches to diagnosis and therapy of sepsis. (Eugenio Baraldi)

**Team Members**

Prof. Eugenio Baraldi – Co-Principal Investigators
Dr. Giuseppe Giordano – Co-Principal Investigators
Prof. Silvia Carraro - Associate Professor
Dr. Alfonso Galderisi - MD, Clinical Scientist
Dr. Enrico Valerio - MD, Clinical Scientist
Dr. Luca Bonadies - MD, Clinical Scientist
Dr. Paola Pirillo – Post Doc
Dr. Gabriele Poloniato – Post Doc
Dr. Matteo Stocchero – Senior Scientist
Dr. Elena Ferramosca - Research Fellow

## Selected publications


- The Impact of Antenatal Corticosteroids on the Metabolome of Preterm Newborns: An Untargeted Approach. Valerio E, Meneghelli M, Stocchero M, Galderisi A, Visentin S, Bonadies L, Pirillo P, Poloniato G, Giordano G, Baraldi E. Int J Mol Sci. 2024 May 28;25(11):5860. doi: 10.3390/ijms25115860.
- The genomic evolutionary dynamics and global circulation patterns of respiratory syncytial virus. Langedijk AC, Vrancken B, Lebbink RJ, Wilkins D, Kelly EJ, Baraldi E, Mascareñas de Los Santos AH, Danilenko DM, Choi EH, Palomino MA, Chi H, Keller C, Cohen R, Papenburg J, Pernica J, Greenough A, Richmond P, Martinón-Torres F, Heikkinen T, Stein RT, Hosoya M, Nunes MC, Verwey C, Evers A, Kragten-Tabatabaie L, Suchard MA, Kosakovsky Pond SL, Poletto C, Colizza V, Lemey P, Bont LJ; INFORM-RSV Study Group. Nat Commun. 2024 Apr 10;15(1):3083. doi: 10.1038/s41467-024-47118-6.
- Neurosteroid pathway derangement in asphyctic infants treated with hypothermia: an untargeted metabolomic approach. Valerio E, Stocchero M, Pirillo P, D'Errico I, Bonadies L, Galderisi A, Giordano G, Baraldi E. EBioMedicine. 2023 Jun;92:104636. doi: 10.1016/j.ebiom.2023.104636. Epub 2023 May 29
- The Metabolome and the Gut Microbiota for the Prediction of Necrotizing Enterocolitis and Spontaneous Intestinal Perforation: A Systematic Review. Moschino L, Verlato G, Duci M, Cavicchiolo ME, Guiducci S, Stocchero M, Giordano G, Fascetti Leon F, Baraldi E. Nutrients. 2022 Sep 18;14(18):3859. doi: 10.3390/nu14183859.
- The Metabolome and the Gut Microbiota for the Prediction of Necrotizing Enterocolitis and Spontaneous Intestinal Perforation: A Systematic Review. Moschino L, Verlato G, Duci M, Cavicchiolo ME, Guiducci S, Stocchero M, Giordano G, Fascetti Leon F, Baraldi E. Nutrients. 2022 Sep 18;14(18):3859. doi: 10.3390/nu14183859.
- Early Biomarkers of Bronchopulmonary Dysplasia: A Quick Look to the State of the Art. Bonadies L, Moschino L, Valerio E, Giordano G, Manzoni P, Baraldi E. Am J Perinatol. 2022 Dec;39(S 01):S26-S30. doi: 10.1055/s-0042-1758867. Epub 2022 Dec 5.

- Urinary metabotypes of newborns with perinatal asphyxia undergoing therapeutic hypothermia. Valerio E, Mardegan V, Stocchero M, Cavicchiolo ME, Pirillo P, Poloniato G, D'Onofrio G, Bonadies L, Giordano G, Baraldi E. PLoS One. 2022 Aug 16;17(8):e0273175. doi: 10.1371/journal.pone.0273175. eCollection 2022.
- Breathomics in Asthmatic Children Treated with Inhaled Corticosteroids. Ferraro VA, Carraro S, Pirillo P, Gucciardi A, Poloniato G, Stocchero M, Giordano G, Zanconato S, Baraldi E. Metabolites. 2020 Sep 29;10(10):390. doi: 10.3390/metabo10100390.
- Metabolomic profiling of intrauterine growth-restricted preterm infants: a matched case-control study. Priante E, Verlato G, Stocchero M, Giordano G, Pirillo P, Bonadies L, Visentin S, Moschino L, Baraldi E. Pediatr Res. 2023 May;93(6):1599-1608. doi: 10.1038/s41390-022-02292-5. Epub 2022 Sep 9.
- Untargeted and Targeted Metabolomic Profiling of Preterm Newborns with EarlyOnset Sepsis: A Case-Control Study. Mardegan V, Giordano G, Stocchero M, Pirillo P, Poloniato G, Donadel E, Salvadori S, Giaquinto C, Priante E, Baraldi E. Metabolites. 2021 Feb 18;11(2):115. doi: 10.3390/metabo11020115.


# Pediatric Critical Care (PCare)

## Group Leader

Prof. Paola Cogo - Principal Investigator

## Research activity

Care Laboratory has 30 years of experience of translational medicine in critical care-related diseases (including animal models, infants and adults). The multidisciplinary team of PCare lab is made up of professionals dealing with a wide variety of fields such as health professionals, biologist, chemist, and biotechnologist. The focus of our research is the development of stable isotope and high-resolution mass spectrometry-based techniques (targeted and untargeted) to improve the understanding of human biology on a cellular and whole-organism level.

Studies are conducted with an analytical chemistry approach involving isolation, quantitation, and in vivo tracking of target molecules through stable isotopes tracing, using isotope-ratio mass spectrometry and high-resolution mass spectrometry. This approach allow the development and application of extremely sensible methods to various diseases, including respiratory distress syndrome, brain injury and to drug delivery, exploiting the potential of complex matrices like tracheal aspirates, urine, blood, tissues and cells. The laboratory owns 3 gas-chromatographers (Agilent 6890N) coupled with: a flame-ion detector; a single quadrupole mass spectrometer (5973i); two isotope-ratio mass spectrometers (Thermo Delta V), and a thermal combustion/elemental analyzer (Thermo TC/EA). Moreover, an UHPLC (Thermo Ultimate 3000) coupled with a high-resolution mass spectrometer (Thermo Q Exactive) for targeted and untargeted femtogram metabolite and protein analysis.

In recent years, we have focused on the neurological and pulmonary injuries occurring during pediatric cardiac surgery for congenital heart defects (CHD). The latter are the most common congenital disease affecting about 1% of all births. Improved surgical techniques have reduced operative mortality to 3% and major concerns are now focused on the long-term outcome, especially on neurological and neurodevelopmental disorders along with lung injuries. We are trying to define a panel of biomarker that correlates with the neurological and pulmonary outcome of children undergoing open-heart surgery for complex CHD. We recently described how the minimum temperature reached during cardiac surgery is the most important factor that influences the rise of a brain injury neuromarker (GFAP) and how the type of cardiac diseases is linked to a specific pulmonary surfactant status. Moreover, we linked the plasmatic amount of GFAP, along with other surgical parameters, to children's neurodevelopmental outcome assessed 18 months after surgery. Neurodevelopmental follow-up showed that GFAP was associated with impaired communication skills. Up to 52% of CHD children reported neuropsychological impairments mainly related to the domain of the social and affective perception. Moreover, our laboratory applied an untargeted metabolomics approach to elucidate the mechanism underlying CHD phenotype and to identify the metabolite signatures of early brain damage.

Pulmonary surfactant status in critical diseases, both acute and chronic, is also a key topic of the laboratory. We used stable isotope tracers and high resolution-mass spectrometry techniques to describe lipids and metabolites profile and surfactant specific proteins metabolism and amounts in different disease in children and animal models.

Studies on preterm and newborn infants' nutrition (focused on parenteral nutrition) are also ongoing with important results obtained describing the metabolism of lipids components and their relationship with the diseases. Lipidomics and metabolomics studies are planned to study lipids metabolism in the fetus and during pregnancy complicated by intrauterine growth restriction.

Our research team collaborates with several laboratories and with industries for the development of methods for targeted and untargeted analysis of both small molecules and proteins.

## PI's Biosketch

*Scopus ID 6603967151*

After the Medical Degree in 1984 and a Medical Residency in Pediatrics in 1989 at the University of Padua, Prof. Paola Cogo worked as a Clinical fellow in Critical Care Medicine at the Children's Hospital of Philadelphia (1992-93) and as Research Fellow at the Neonatology Dept. of the Sophia Children's Hospital in Rotterdam (1993). Prof. Cogo then returned to Italy where she obtained her Medical Residency in Neonatology at the University of Padua, before being awarded research grants and fellowships to establish her own independent laboratory. Prof. Cogo is the PI of the Pediatric Critical Care Lab (PCare) of the Dept. of Women's and Children's Health (University of Padua) at the Fondazione Pediatric Research Institute, "Città della Speranza" and Chair of the Division of Pediatrics, University Hospital, ASUFC - Udine (University of Udine). She is Full Professor in Anaesthesiology (MED41) and in Pediatrics (MED 38).

She chairs the research section on Congenital Heart disease, ESPNIC Society, and she is part of the international committee in Pediatric Cardiac Intensive Care Society.

Paola Cogo has performed translational research in critical care for the last 30 years. One of her main research topics concerns the mechanism and prevention of acute organ damage associated with cardiac surgery in infants with congenital heart diseases. Her research team developed new methods to study in vivo metabolic pathways, using stable isotopes, and high-resolution mass spectrometry in humans. This approach permitted to assess mechanisms of acute organ injury and drug delivery and pharmacokinetics, by exploiting complex matrices like tracheal aspirates, urine, and blood.

Prof. Cogo is author of 245 Scopus-indexed documents (5178 citations) with an h-index of 37.

### Team Members

Prof. Paola Cogo - Principal Investigator
Dr. Manuela Simonato - Senior Scientist
Dr. Anna Sartori - PhD Student
Dr. Ambra Bertocco - PhD Student
Dr. Veronica Longo - Research Fellow
Prof. Virgilio Paolo Carnielli - Full Professor
Dr. Alessio Correani - Senior Scientist
Dr. Giovanna Verlato – MD, Clinical Scientist
Prof. Aldo Baritussio - Associate Professor

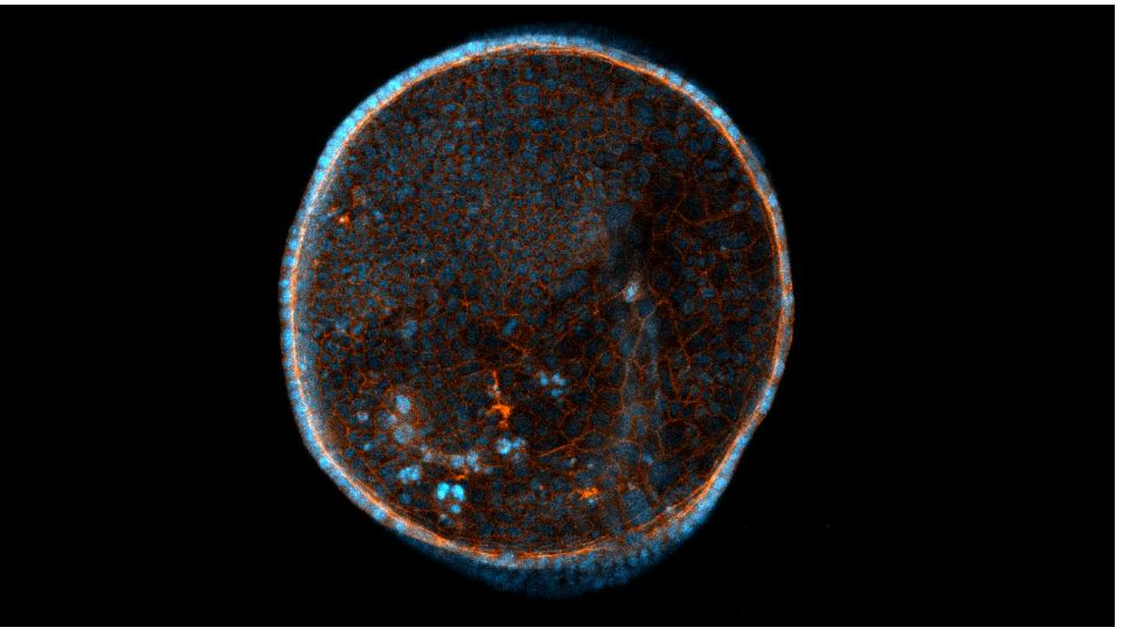
## Selected publications


- Arslanbaeva L, Tosi G, Ravazzolo M, Simonato M, Tucci FA, Pece S, Cogo P, Santoro MM. UBIAD1 and CoQ10 protect melanoma cells from lipid peroxidation-mediated cell death. Redox Biol. 2022;51:102272. doi:10.1016/j.redox.2022.102272
- Simonato M, Visentin S, Verlato G, Cosmi E, Correani A, Cogo P, Carnielli VP. DHA turnover in pregnant women using the natural abundance variation of 13C: a pilot study. Br J Nutr. 2022 Apr 11:1-19. doi: 10.1017/S0007114522001088. PMID: 35403583
- Simonato M, Dall'Acqua S, Zilli C, Sut S, Tenconi R, Gallo N, Sfriso P, Sartori L, Cavallin F, Fiocco U, Cogo P, Agostinis P, Aldovini A, Bruttomesso D, Marcolongo R, Comai S, Baritussio A. Tryptophan Metabolites, Cytokines, and Fatty Acid Binding Protein 2 in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. Biomedicines vol. 9,11 1724. 19 Nov. 2021, doi:10.3390/biomedicines9111724
- Ricci F, Bresesti I, LaVerde PAM, Salomone F, Casiraghi C, Mersanne A, Storti M, Catozzi C, Tigli L, Zecchi R, Franceschi P, Murgia X, Simonato M, Cogo P, Carnielli V, Lista G. Surfactant lung delivery with LISA and InSurE in adult rabbits with respiratory distress. Pediatr Res. 2021 Sep;90(3):576-583. doi: 10.1038/s41390-020-01324-2. Epub 2021 Jan 15.
- Simonato M, Fochi I, Vedovelli L, Giambelluca S, Carollo C, Padalino M, Carnielli VP, Cogo P. Urinary metabolomics reveals kynurenine pathway perturbation in newborns with transposition of great arteries after surgical repair. Metabolomics. 2019 Oct 28;15(11):145. doi: 10.1007/s11306-019-1605-3. PMID: 31659512; PMCID: PMC6817811. Journal cover

RESEARCH AREA

# Regenerative Medicine

**Coordinator** Prof. Maurizio Muraca



The Regenerative Medicine area includes four distinct yet deeply intertwined lines of research. By joining the expertise in tissue engineering, physiology and pathophysiology, the area aims at:

- studying the application of extracellular vesicles as therapeutic tools in inflammatory and autoimmune diseases involving organs such as the lung and the intestine;
- creating in vitro 3D model to study the cross-talk between cells and extracellular matrix in muscle such as rhabdomyosarcoma, the most common and aggressive soft tissue sarcoma in childhood;
- developing a biological ink, starting from the decellularized diaphragm extracellular matrix, mixed with the cells that constitute the tissue under physiological conditions, to apply a personalized regenerative medicine treatment for congenital diaphragmatic hernia by 3D bioprinting approach;
- generating 3D models of human skeletal muscle equipped with neuronal network, by combining human induced pluripotent stem cell technology and biomaterial science, for the identification of cellular and molecular players involved in neuromuscular disorders, including muscular dystrophies.

**Prof. Maurizio Muraca**
*Coordinator Regenerative Medicine Area*

*Scopus ID 7006578223*

After a MD at the University of Padua in 1976, Prof. Muraca specialized in Internal Medicine (1981) and in Gastroenterology (1985) while also pursuing a PhD in Biochemistry (1985) at the Catholic University of Leuven, Belgium. Since 1982, Prof. Muraca has been working at the University of Padua, firstly as Assistant Professor at the Dept. of Internal Medicine, then as Associate Professor of Clinical Medicine, University of Padua since 1993. From 1999 to 2004, Prof. Muraca was Head of the Hepatology and Liver Transplant Group of the 1st Medical Clinic, University of Padua, and in 2004 he moved to Rome as Head of Clinical Chemistry and Microbiology Lab and as Coordinator of the research area in Regenerative Medicine, at the Pediatric Research Hospital "Bambino Gesù". In 2014 he returned to Padua as Associate Professor of Clinical Medicine at Dept. of Women's and Children's Health. After his retirement in 2022, he became Co-director of the Pediatric Research Institute "Città della Speranza".

From 1997 to 2001 he coordinated the first clinical study in Italy on the treatment of liver failure with a bioartificial liver as part of an international trial controlled by the Food and Drug Administration. In 2000 he performed the first clinical hepatocyte transplant in Europe after a series of studies in animal models (Lancet 2002; 359: 317).

He is author and co-author of 169 original articles in peer-reviewed international journals, 3 international books in English, 2 books in Italian, 16 chapters in international books, 51 original articles and book chapters in Italian. He is inventor of five patents in cell therapy. H-Index: 44 (Scopus) – 48 (Research Gate).

*Awards*

10/11/2006: International Award "Giuseppe Sciacca", section Medicine

18/07/2011: Award "Gentlemen d'Italia" for scientific activity

# Neuromuscular Engineering

## Group Leader

Dr. Anna Urciuolo - Junior Principal Investigator

## Research activity

Our group results from the multidisciplinary integration of different expertise in skeletal muscle and stem cell biology, extracellular matrix and biomaterial engineering. Our current research project aims at generating 3D models of human skeletal muscle equipped with a neuronal network. To do so, we integrate stem cell biology and extracellular matrix/biomaterial engineering to derive in the lab patient-specific multicellular microtissues and organoids that reproduce the human neuromuscular system. Such 3D in vitro models are used to mimic the structural/functional properties of human skeletal muscle and neuromuscular junction, for the identification of cellular and molecular players involved in neuromuscular genesis and maintenance in healthy and disease, with particular interest in neuromuscular disorders, dystrophies and cancer-induced muscle cachexia.

## JrPI's Biosketch

*Scopus ID 6508342717*

I was trained as medical biotechnologist in 2007 and then in 2011 I concluded my PhD program in genetics and developmental biology at the University of Padova (Italy). During my post-doctoral career in Italy and UK (University College of London, UCL) I have had the opportunity to join and experience different research environments, embracing and incorporating into my research line basic biology and animal models, together with stem cell technology and bioengineering approaches. My studies have been mainly focused on the role exerted by the environment and the extracellular matrix (ECM) to control stem cells and skeletal muscle in health and disease. After a first post-doc experience at the University of Padova, which studies were focused on muscular dystrophies associate to collagen VI mutations and skeletal muscle regeneration, I moved in UCL where I specialized in tissue engineering strategies (decellularized muscles) for the development of regenerative medicine strategies. In 2016 I moved back in Italy at the Department of Industrial Engineering of the University of Padova, where I developed a novel 3D bioprinting technology, named intravital 3D bioprinting. In 2018 I won as PI the STARS@ UNIPD grant (intramural grant supporting talented scientists) with the Dept. of Woman's and Children's Health of the University of Padova, and I started my independent career as PI. During this time I focused on human induced pluripotent stem cell technology coupled with organoid and biomaterial science. Since the end of 2019 I'm leading the Neuromuscular Engineering lab at the Pediatric Research Institute "Città della Speranza" (Italy). Since 2021, I have been working at the University of Padua as Associate Professor at the Dept. of Molecular Medicine and I'm holding an honorary Lecturer position at the UCL (UK).

I have published 34 research articles of which 11 as first author (2 reviews and 8 original articles); 7 as corresponding author (3 review and 4 original articles). Overall, I published on top international scientific journals in the field of stem cell and bioengineering, holding an h-index of 20 and 3032 citations (Scopus).

## Team Members

Dr. Anna Urciuolo Junior - Principal Investigator
Dr. Beatrice Auletta - PhD Student
Dr. Pietro Chiolerio - Research Fellow
Dr. Luigi Sartore - Research Fellow

## Selected publications


- Anna Urciuolo*#, Giovanni Giuseppe Giobbe*, Yixiao Dong*, Federica Michielin, Luca Brandolino, Michael Magnussen, Onelia Gagliano, Giulia Selmin, Valentina Scattolini, Paolo Raffa, Paola Caccin, Soichi Shibuya, Dominic Scaglioni, Xuechun Wang, Ju Qu, Mako Nikolic, Marco Montagner, Gabriel L. Galea, Hans Clevers, Monica Giomo, Paolo De Coppi, Nicola Elvassore. Hydrogel-in-hydrogel live bioprinting for guidance and control of organoids and organotypic cultures. Nature Communications, Dec 2023.
- Elisa Cesare*, Anna Urciuolo*, Hannah T. Stuart*, Erika Torchio, Alessia Gesualdo, Cecilia Laterza, Onelia Gagliano, Sebastian Martewicz, Meihua Cui, Anna Manfredi, Lucio Di Filippo, Patrizia Sabatelli, Stefano Squarzoni, Irene Zorzan, Riccardo M. Betto, Graziano Martello, Davide Cacchiarelli, Camilla Luni, Nicola Elvassore. 3D ECM-Rich Environment Sustains the Identity of Naïve Human iPSCs. Cell Stem Cell, 2022.
- Paolo Raffa, Valentina Scattolini, Mattia FM Gerli, Silvia Perin, M Cui, Paolo De Coppi, Nicola Elvassore, Paola Caccin, Camilla Luni, Anna Urciuolo#. Decellularized skeletal muscles display neurotrophic effects in 3D organotypic cultures. Stem Cells Translational Medicine, 2020.
- Anna Urciuolo*, Poli, I., Brandolino, L., Raffa, P., Scattolini, V., Laterza, C., Giobbe, G.G., Zambaiti, E., Selmin, G., Magnussen, M., Brigo, L., De Coppi, P., Salmaso, S., Giomo, M., Elvassore, N. Intravital 3D bioprinting. Nature Biomedical Engineering, 2020.
- Urciuolo, A.*, Urbani, L.*, Perin, S., Maghsoudlou, P., Scottoni, F., Gjinovci, A., Collins-Hooper, H., Loukogeorgakis, S., Tyraskis, A., Torelli, S., Germinario, E., Fallas, M.E.A., Julia-Vilella, C., Eaton, S., Blaauw, B., Patel, K., De Coppi, P. Decellularised skeletal muscles allow functional muscle regeneration by promoting host cell migration. Scientific Reports, 2018.

# Stem Cells and Regenerative Medicine

## Group Leader

Prof. Michela Pozzobon - Principal Investigator

## Research activity

The research activity of the lab is focused on the development of in vivo and in vitro 3D constructs for regenerative medicine/tissue engineering approaches, using the expertise in skeletal muscle, stem cell biology and extracellular matrix engineering. A second line of research concerns the use of cellular vesicles from stromal mesenchymal cells as a therapeutic tool.

The research topics of the lab are the following.

*Extracellular vescicles derived from mesenchymal stem/stromal cells as therapeutic tool*

During the last decade, MSCs have been studied as a promising tool for regenerative medicine and anti-inflammatory tool. MSCs were originally described as stem cells, but it was later recognized that data supporting such a functional designation are still lacking, so the term stromal has been recommended by the International Society for Cellular Therapy. MSCs are plastic-adherent cells with a multipotent differentiation capacity in vitro. They can be isolated from a variety of tissues, including bone marrow, fat, cord blood and tissue, and placenta. MSCs are the most used cells in regenerative medicine because of their regulatory effect on the body's immune response and their ability to protect tissues against a variety of injuries while enhancing regeneration and repair. MSCs were initially believed to differentiate and repopulate injured sites with tissue-specific cell phenotypes, but it soon became evident that the therapeutic effects observed were mediated by paracrine signals regulating immune response, counteracting apoptosis, limiting fibrogenesis, stimulating endogenous stem cells and thus resulting in tissue repair. In the last decade, accumulating evidence has shown that such signals are conveyed mainly by various membrane vesicles, including exosomes and microvesicles, collectively named extracellular vesicles (EVs). EVs are complex biological machines secreted by all cell types and ranging from 0.03 to 1 micron in size. They are distinguished mainly by their biogenesis, since they exhibit overlapping physical and chemical characteristics. EVs carry a variety of proteins, lipids and nucleic acids, with profound effects on the metabolism - and even on the phenotype - of the target cell. MSC-derived EVs have shown immune regulating effects in vitro and revealed a remarkable anti-inflammatory and pro-regenerative capacity in several animal models of disease. Our research group has developed a series of in vitro and ex vivo 3D models (like organoids of lung and intestine) to study Specific projects include:

- mesenchymal stem/stromal cell-derived extracellular vesicles to prevent and revert the development of Bronchopulmonary Dysplasia; the disease affects premature babies.
- mesenchymal stem/stromal cell-derived extracellular vesicles as therapeutic tool for acute respiratory distress syndrome and to prevent the development of interstitial lung fibrosis
- mesenchymal stem/stromal cell-derived extracellular vesicles as therapeutic tool for Inflammatory Bowel Diseases;
- evaluation of the role of different proteins expressed on the surface of MSC-EVs with known immune modulatory properties, by knockout or hyperexpression approaches. Indeed, although the EV RNA cargo is a possible mediator of signals influencing the metabolism of target cells, it is now appreciated that probably most of the EV-mediated signals take place at the level of plasma membrane, while following internalization EVs are generally targeted to lysosomes where their content is degraded.

*Pathophysiology of extracellular matrix and tissue engineering*

Extracellular vesicles in regenerative medicine and muscle dysfunction models. The need of new biomaterials to replenish the loss of muscle mass is currently a challenge. Indeed, after congenital malformations, trauma or tumor surgery the volume mass loss can be filled with synthetic materials already used in the clinical practice but the regain of function is still very difficult to reach. Nowadays the decellularization of tissues allows the obtainment of the highest biocompatible scaffold without the genetic material, such as the extracellular matrix (ECM). This biomaterial retains the biomechanical properties, proteins and biochemical factors that characterized the native tissue. ECM obtained removing the cellular components from the native tissue by means of decellularization, represents the optimal 3D support for cell culture since the in vivo microenvironment is recapitulated. We already demonstrated that engineered ECM actively integrate by inducing vascularization, cell recruitment and ECM production. On the other hand, adverse events such as foreign body response and fibrosis are prevented. With the aim to study the EV mechanism, the focus of the project is as follows.

- Muscle decellularized tissue will be engineered with human muscle precursor cells and macrophages. After damage, the EVs will be administered, and specific molecular pathways will be investigated. EVs from different cell sources (muscle precursor cells, amniotic fluid stem cells, cord blood mesenchymal stem cells, endothelial cells) will be considered and their biological activities evaluated.
- The presence of retained EVs in the decellularized matrix will be verified, and their biological activities in vitro will be evaluated.

Studies on the extracellular matrix of rhabdomyosarcoma. Rhabdomyosarcoma (RMS) is the most common and aggressive soft tissue sarcoma in childhood, quite often present in muscle tissue. Recently, the study of the tumor microenvironment and specifically of the extracellular matrix (ECM), highlighted the valuable role of the cross-talk between cells and their niche linking alteration of ECM composition to pathological outcomes. The interest in this new aspect starts widening the understanding of tumor progression and opens new avenues for developing innovative therapies.

We have already dissected the ECM protein composition and now we are focusing on the glycoproteins present in the intercellular part with specific focus on glypicans. A three-dimensional model hydrogel based with different cell population will be developed to study tumor behavior and to establish a platform for drug screening.

## PI's Biosketch

*Scopus ID 6602197182*

Michela Pozzobon is associate professor in Applied Biology, at the Department of Women's and Children's Health, University of Padova.

She graduated in Pharmaceutical Chemistry at the University of Padua in 2000 and then joined the Dept. of Pharmaceutical Chemistry on an 18 month-fellowship for an EU project on polymer synthesis. Prof. Pozzobon spent 3 years (2002-2004) at the University of Oxford as a Research Assistant in the Oncology Group (Nuffield Dept. of Clinical Sciences, UK) under the supervision of Prof. David Y. Mason. In 2004, she joined the lab of Stem Cells and Regenerative

Medicine, working with Dr. Paolo De Coppi, and she has coordinated the laboratory since 2010. During this time, Dr. Pozzobon enrolled in the PhD program in Tissue Engineering and Regenerative Medicine at the University of Padua, which was completed in 2008. Her research activity is focused on the study of the tissue biology and its application in regenerative medicine (cell therapy and tissue engineering), with a specific attention to the pediatric field and muscle related problems. Prof. Pozzobon is actively involved in national and international projects related to regenerative medicine and tumor microenvironment. She is member of the scientific advisory board of the italian group of stem-mesenchymal cells (GISM), Managing Committee member of the COST European project SPRINT on perinatal stem cells (2019-2023). Her expertise combines the training in oncology and the research on regenerative medicine using scaffolds, stem cells and extracellular vesicles.

She currently was awarded two projects (PRIN and UNIMPRESA) focused on the investigation of the mechanism of action of extracellular vesicles toward tissue regeneration.

Prof. Pozzobon is author of over 100 abstracts selected for poster or oral presentation; her scientific production counts 97 publications on peer review journals, in 34 of them she is the first, last or corresponding author. Google Scholar H index: 37. Scopus H index: 34.

### Team Member

Prof. Michela Pozzobon - Principal Investigator
Dr. Paola Bisaccia - Post Doc
Dr. Alice Zaramella - Post Doc
Dr. Agner Enrique Dorigo Hochuli - PhD Student
Dr. Raquel Moll Diaz - PhD Student
Prof. Mara Cananzi – MD, Clinical Scientist
Prof. Francesco Fascetti – MD, Clinical Scientist
Dr. Lorenzo Zanetto – MD, PhD Student

### Selected publications


- Bisaccia P, Magarotto F, D'Agostino S, Dedja A, Barbon S, Guidolin D, Liboni C, Angioni R, De Lazzari G, Caicci F, Viola A, Jurga M, Kundrotas G, Stevens D, Mancuso D, Gramegna E, Seitaj B, Kashyap R, De Vos B, Macchi V, Baraldi E, Porzionato A, De Caro R, Muraca M, Pozzobon M. Extracellular Vesicles From Mesenchymal Umbilical Cord Cells Exert Protection Against Oxidative Stress and Fibrosis in a Rat Model of Bronchopulmonary Dysplasia. Stem Cells Transl Med. 2023 Nov 15:szad070. doi: 10.1093/stcltm/szad070. PMID: 37963808
- Saggioro M, D'Agostino S, Veltri G, Bacchiega M, Tombolan L, Zanon C, Gamba P, Serafin V, Muraro MG, Martin I, Pozzobon M. A perfusion-based three-dimensional cell culture system to model alveolar rhabdomyosarcoma pathological features. Sci Rep. 2023 Jun 9;13(1):9444. doi: 10.1038/s41598-023-36210-4. PMID: 37296184
- Puttock EH, Tyler EJ, Manni M, Maniati E, Butterworth C, Burger Ramos M, Peerani E, Hirani P, Gauthier V, Liu Y, Maniscalco G, Rajeeve V, Cutillas P, Trevisan C, Pozzobon M, Lockley M, Rastrick J, Läubli H, White A, Pearce OMT. Extracellular matrix educates an immunoregulatory tumor macrophage phenotype found in ovarian cancer metastasis. Nat Commun. 2023 May 15;14(1):2514. doi: 10.1038/s41467-023-38093-5. PMID: 37188691
- Saggioro M, D'Agostino S, Gallo A, Crotti S, D'Aronco S, Corallo D, Veltri G, Martinez G, Grigoletto A, Tolomeo AM, Tafuro G, Agostini M, Aveic S, Serafin V, Semenzato A, Pasut G, Pozzobon M. A rhabdomyosarcoma hydrogel model to unveil cell-extracellular matrix interactions. Biomater Sci. 2021 Nov 19. doi: 10.1039/d1bm00929j. PMID: 34796888
- Magarotto F, Sgrò A, Dorigo Hochuli AH, Andreetta M, Grassi M, Saggioro M, Nogara L, Tolomeo AM, Francescato R, Collino F, Germano G, Caicci F, Maghin E, Piccoli M, Jurga M, Blaauw B, Gamba P, Muraca M, Pozzobon M. Muscle functional recovery is driven by extracellular vesicles combined with muscle extracellular matrix in a volumetric muscle loss murine model. Biomaterials. 2021 Feb;269:120653. doi: 10.1016/j.biomaterials.2021.120653. Epub 2021 Jan 7.PMID: 33461058

# Tissue Engineering

## Group Leader

Dr. Martina Piccoli - Principal Investigator

## Research activity

The group is born from the joint experience of applied biology and biomaterials engineering. Our group mainly studies skeletal muscle regeneration to develop new tissue engineering approaches for treating muscle diseases, defects and malformations. Examples of technical skills developed by our group are tissue decellularization and recellularization, 3D printing and bioprinting, 2D and 3D in vitro culture systems, and the design and fabrication of bioreactors for different tissue culture applications. Currently, our research is supported by project grants whose purpose is to regenerate in vitro different tissue engineered constructs as both in vitro models and in vivo tissue substitutes. Our experience ranges from the use of commercially available and clinically approved biomaterials to the production of extracellular matrix scaffolds, from the use of immortalized cell lines to the isolation of stem and progenitor cells from tissue biopsies, from the characterization of simple cell cultures to the functional analysis of 3D constructs obtained using bioreactors and computerized mechanical systems.

## PI's Biosketch

*Scopus ID 15127681100*

Martina Piccoli has a background in biological sciences, with specific training and expertise acquired in pediatric and developmental research. Her research includes the characterization of stem cells and the development of biomaterials for tissue engineering purposes. During her post-graduate training, she developed technical skills in the field of tissue engineering and in particular on decellularization methods to produce different formulations of natural biomaterials. Since 2017 she has been the principal investigator of the Tissue Engineering laboratory of the Pediatric Research Institute Città della Speranza. Her lab is focused on the production of 3D constructs with different approaches and techniques.

## Team Members

Dr. Martina Piccoli - Principal Investigator
Dr. Edoardo Maghin - Post Doc
Dr. Elena Merotto - PhD Student
Dr. Sara Manzoli - Research Fellow
Dr. Matilde Anna Corbetta - Senior Scientist

## Selected publications


- Maghin E, Carraro E, Boso D, Dedja A, Giagante M, Caccin P, Barna RA, Bresolin S, Cani A, Borile G, Sandrin D, Romanato F, Cecchinato F, Urciuolo A, Sandonà D, De Coppi P, Pavan PG, Piccoli M. Customized bioreactor enables the production of 3D diaphragmatic constructs influencing matrix remodeling and fibroblast overgrowth. NPJ Regen Med. 2022 Apr 25;7(1):25. doi: 10.1038/s41536-022-00222-x.
- Boso D, Carraro E, Maghin E, Todros S, Dedja A, Giomo M, Elvassore N, De Coppi P, Pavan PG, Piccoli M. Porcine Decellularized Diaphragm Hydrogel: A New Option for Skeletal Muscle Malformations.
Biomedicines. 2021 Jun 22;9(7):709. doi: 10.3390/biomedicines9070709.
- Trevisan C, Fallas MEA, Maghin E, Franzin C, Pavan P, Caccin P, Chiavegato A, Carraro E, Boso D, Boldrin F, Caicci F, Bertin E, Urbani L, Milan A, Biz C, Lazzari L, De Coppi P, Pozzobon M, Piccoli M. Generation of a Functioning and Self-Renewing Diaphragmatic Muscle Construct. Stem Cells Transl Med. 2019 Aug;8(8):858-869. doi: 10.1002/sctm.18-0206. Epub 2019 Apr 10.
- Trevisan C, Maghin E, Dedja A, Caccin P, de Cesare N, Franzin C, Boso D, Pesce P, Caicci F, Boldrin F, Urbani L, De Coppi P, Pozzobon M, Pavan P, Piccoli M. Allogenic tissue-specific decellularized scaffolds promote long-term muscle innervation and functional recovery in a surgical diaphragmatic hernia model. Acta Biomater. 2019 Apr 15;89:115-125. doi: 10.1016/j.actbio.2019.03.007. Epub 2019 Mar 6.
- de Cesare N, Trevisan C, Maghin E, Piccoli M, Pavan PG. A finite element analysis of diaphragmatic hernia repair on an animal model. J Mech Behav Biomed Mater. 2018 Oct;86:33-42. doi: 10.1016/j.jmbbm.2018.06.005. Epub 2018 Jun 15.

**Photo credits** Nicola Maioli, Matteo Sandi, Teresa Borghi
**Progetto grafico** paolofontana.design
**Stampa** Berchet ingegneria di stampa

*Novembre 2024*


**Fondazione**
**Istituto di Ricerca Pediatrica**
**Città della Speranza**

Corso Stati Uniti, 4
35127 Padova
+39 049 9640111
info@irpcds.org
www.irpcds.org