Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

2020 - 2021

Scientific Report

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA
Città della Speranza

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Governance

President	**Franco Masello** (November 2021 – present)
CEO	**Dr. Luca Primavera** (October 2020 - present)
Scientific Director	**Prof. Antonella Viola** (September 2017 – October 2022)
Scientific Director	**Prof. Eugenio Baraldi** (November 2022 - present)
Scientific Coordinator	**Prof. Maurizio Muraca** (November 2022 - present)

Scientific Advisory Board
(January 2020 – present)

President

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University of Milano-Bicocca, Milan, Italy

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Prof. Orsetta Zuffardi
University of Pavia, Pavia, Italy

IRP is a world-leading research institute, home to reference laboratories for pediatric medicine and scientific research. In 2021, with its 282 scientific publications with a mean impact factor of 7.8, IRP ranks among the very first research centers in Italy.

There are seven main research areas at the Institute: Genetics and rare disease; Immunology and neuroimmunology; Medical biotechnology; Onco-hematology, stem cell transplant and gene therapy; Predictive medicine; Regenerative medicine; Experimental cardiology. Through a multidisciplinary approach, the Institute brings together the expertise in biomedicine, bioengineering, biochemistry and material sciences. This broad view on research is made possible by the strong collaboration with the University of Padova and, in particular, with the Department of Women's and Children's Health (SDB), which shares with IRP not only resources and facilities, but also goals and overall vision. Moreover, IRP works collaboratively with national and international University Hospitals and Research Institutes, providing an exceptional training environment in pediatric research to students and researchers, promoting innovation and technology transfer and also facilitating the dialogue with the public through conferences and outreach activities.

IRP applies the principles of precision medicine with the specific aim of translating science into children's health. We aim to integrate the complexity of science with the need for acceleration in the search for effective therapies.

Antonella Viola
Scientific Director

"We aim to integrate the complexity of science with the need for acceleration in the search for effective therapies.

Luca Primavera

In 2012, when the Research Tower was inaugurated and the laboratories became operational, IRP was little more than a dream. The dream of providing the scientific world and the community with an effective tool to combat pediatric diseases before they occurred in children. Until that time the Città della Speranza Foundation, founding member of the Institute, had mainly focused on financing the care and assistance to families. Ten years later we can proudly say IRP has grown steadily in results, in the recruitment of the best Italian and foreign scientists, in fundraising. The almost 200 researchers who occupy the 11 floors of the Research Tower every day no longer deal only with oncological pathologies, but study the entire child universe, with a multidisciplinary approach that brings together expertise and best practices.

For these reasons, today we can easily say that a young researcher or an established scientist finds in IRP the best conditions to operate, with modern, adequate and equipped spaces, high-level technology (more than 500,000 euros per year just to increase, implement and improve the facilities equipment) and the possibility of living in close contact with similar research groups.

All this is made possible thanks to the commitment of the researchers, the administrative staff, and the financial supporters of the Institute. Among these I mention three, alongside IRP since its inception: the Cassa di Risparmio di Padova and Rovigo Foundation, which recently renewed its commitment, the Veneto Region and the Città della Speranza Foundation, which invests almost 6 million euros a year in research, diagnostics and infrastructure. Without them, that dream that began in 2012 would not have come true.

Andrea Camporese

On 8 June 2022 we celebrated the 10th anniversary of the Paediatric Research Institute 'Città della Speranza'. An Institute strongly desired, conceived and realised by our non-profit organisation to provide answers to paediatric oncological diseases and in general to help children suffering from serious pathologies.

In a university city such as Padua, which has just commemorated the 800th anniversary of its foundation, where there is a Paediatric Hospital of excellence, in an extraordinary region in terms of culture, entrepreneurship, beautiful landscapes and unique cities of art, in an area where solidarity and willingness to help others are rooted in the community, to create a unique place where high-level research can be carried out and attract the world's best excellence seemed to us a logical consequence. Reserving a precise role for our Foundation: to do its part, with its own values, to contribute to the social, scientific and economic development of the country. A role that we see growing every day and which fills us with pride and satisfaction.

This is why I extend my endless thanks to the volunteers and donors, who allow us to continue to dream and to be at the side of researchers every day. In this way, by helping them realise their dreams as scientists, we help the children and families being treated in the Paediatric Oncology and Paediatrics departments.

Giorgio Perilongo

The partnership between the Pediatric Research Institute "Città della Speranza" (IRP) and the Department of Woman's and Child's Health of the University Hospital of Padua is going through a progressive consolidation process. Ultimately, a more "mature" model of collaboration between a private foundation– IRP – and a public hospital - "The Pediatrics of Padua"- is going to be established. The ultimate goal of this partnership is to provide to the children and their families who seek for their cure the "Pediatrics of Padua", with the outcome of modern translational research activity directed to investigate into the intimate genetic and biological mechanisms underscoring the development of many pediatric diseases, in order to refine their diagnostic and treatment approaches and thus, ultimately, to improve their cure rate. The "Pediatrics of Padua" with its own 248 beds, its own 15 Clinical Divisions and 8 Departmental Clinical Services, its own 11 Regional Pediatric Specialized Referral Centers and 3 Pediatric Regional Clinical Networks for which it functions as the HUB, with its own 100.000 hospital admissions and almost 1000.000 out-patient visits per year, with its own participation, as a Health Care Provider, to 19 of the 24 European References Networks - the European virtual networks launched by the European Commission in 2017 connecting the centers of excellence for rare diseases - represents the main pediatric institution of the entire Nord Est region of Italy and one of the main Italian Pediatric academic Hospitals. The "Pediatrics of Padua" is indeed an official member of the Association of the Italian Children's Hospitals. Furthermore, thanks also to the resources provided by the IRP, this year the "Pediatrics of Padua" has been acknowledged as one of the National Center of Excellence for clinical and translational research. In the last three years it has produced 850 peer reviewed scientific publications for a total Impact Factor of 4.600. Finally, in line with its own academic profile and mandate, the "Pediatrics of Padua" runs 6 post graduate residency programs in the field of Pediatrics, 8 masters and a PhD school. Thus, within this scenario, it is conceivable to predict that the IRP and the ""Pediatrics of Padua", in consideration of the partially overlapping respective "vision and mission", of a more and more actual synergistic actions secondary to a better alignment of the respective research developmental plans and finally, of a more effective sharing of the respective relevant resources, are expected to be major protagonists of the present international efforts to improve the cure rate of many pediatric diseases as the impressive increases in scientific research technologies and knowledge, experienced in these last decades, are expected to produce.

Highlights

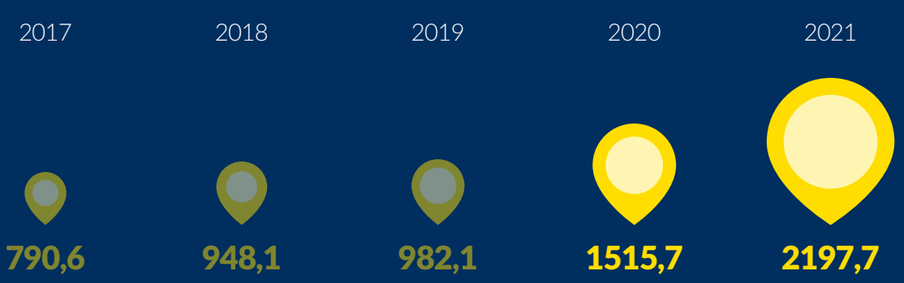
7 Research areas

29 Research groups

197 Researchers

Publications

Total IF



Average IF

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IRP researchers publish articles on peer-reviewed journals every year, with a constantly increasing trend both in terms of number of publications and average impact factor. The number of research grants and scientific prizes awarded by national and international entities has also increased over the years. IRP researchers are regurarly invited to be part of evaluation panels (e. g. EU, AIEOP, CNR) and to give talks or lectures at major national and international conferences.

2020-2021

19 Scientific Awards

155 Evaluation Panel

40 Research Grants

151 Invited Talks / Lectures

IRP Facilities

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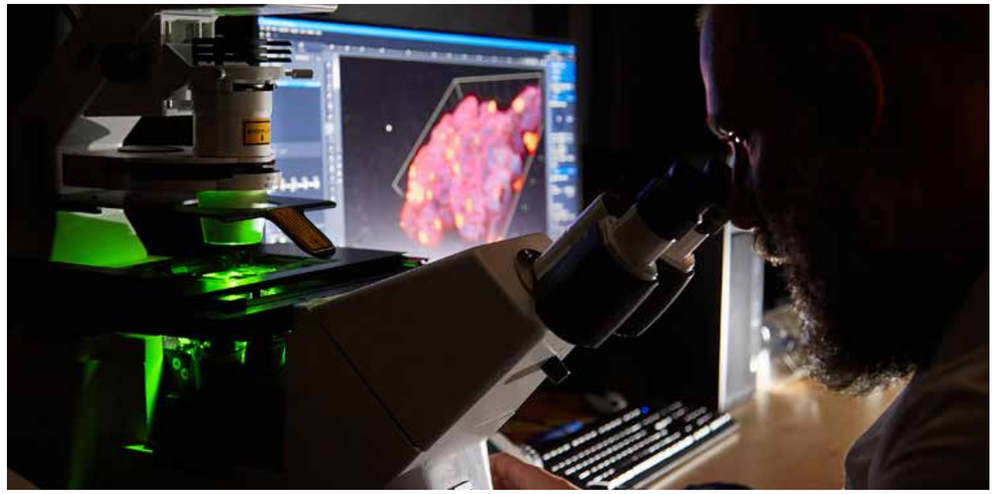
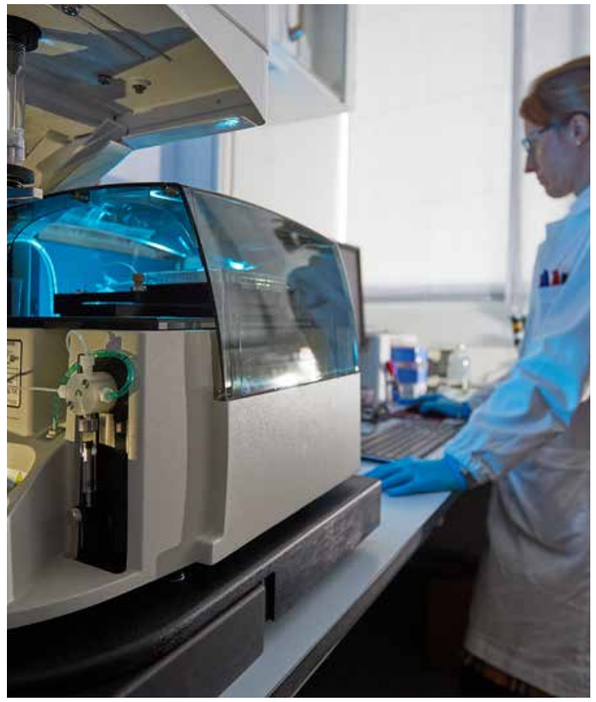
Microscopy

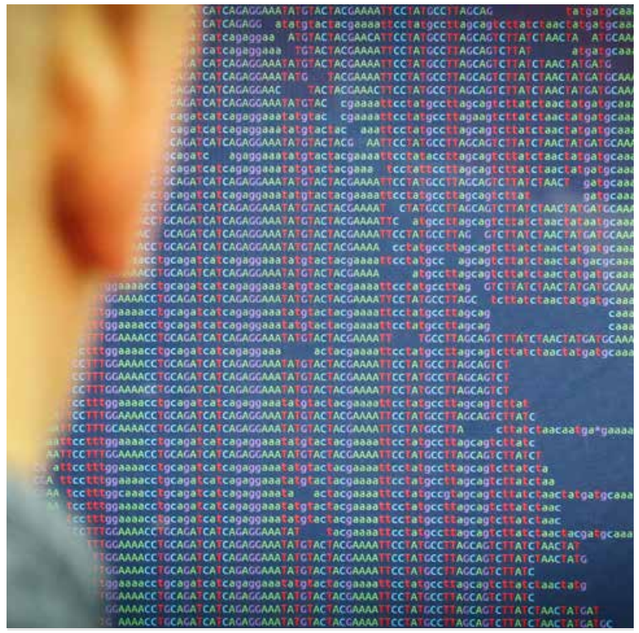
The Microscopy facility is equipped with a ZEISS LSM 800 confocal microscope, with Airyscan technology for superresolution imaging. There are also three other fluorescence microscopes including the newest ZEISS Axio Observer equipped with LED lamp, coupled with the structured illumination system ApoTome3, provided also with a CO2 incubator for live imaging experiments.





Flow cytometry

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 488 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).

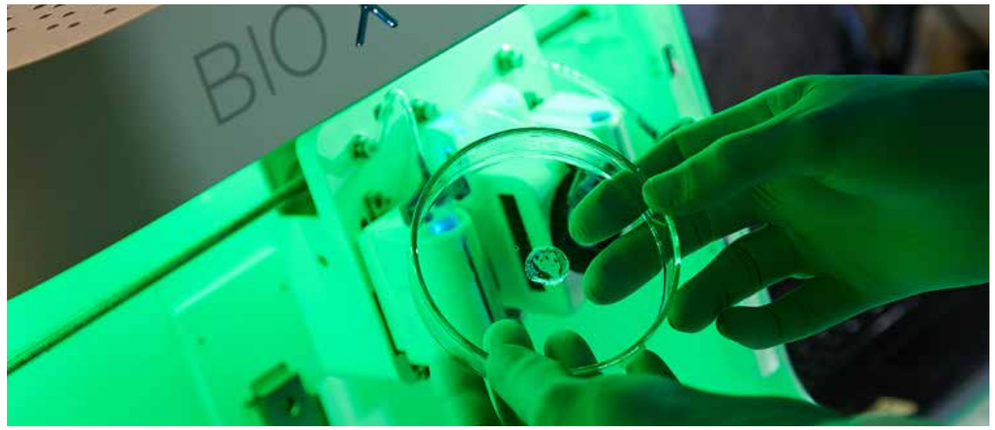


Bioinformatics

The Bioinformatics Unit supports the research community needs at the Istituto di Ricerca Pediatrica with a variety of services ranging from planning genomic experiments to high throughput data analysis and interpretation using and developing computational methods.

Single cell analysis

The Single Cell Facility is equipped with all the instrument needed for the single cell mRNA libraries preparation workflow, including: the new instrument BD Rhapsody, consisting in two Express modules for the capture and barcoding of the single cells, together with a scanner for the vitality 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 gives to the users of the facility free access to the platform Seven Bridges (www.sevenbridges.com), that will help the sequencing analysis with several apps and dedicated pipelines.



3D Bioprintring

The 3D bioprinting facility is composed of 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 types of cells, enabling the fabrication of any tissue target.

Model Organism Core

The Model Organism Core provides small vertebrate zebrafish and the mouse model.
The Zebrafish facility consists of 2 racks of an automatic recirculation housing system that can maintain more than 2,000 adult zebrafish and a separate injection room with two injection stations.
The Mouse facility is a modified barrier animal unit holding specific pathogen-free (SPF) mice only, and consists of one large mouse holding room with 4 vented racks, three molecular biology lab and a behaviour suite. The animal care is managed by the Organismo Preposto al Benessere degli Animali (OPBA) according to the articles 25 and 26 of the Italian D.lgs 26/2014 in compliance of the European Directive 63/2010 UE.

Lipidomics/Metabolomics/Proteomics

Triple-quadrupole API4000 (AbSciex), with ESI and APCI sources. Quantitative analysis of known compounds. The compounds that can be analyzed are medium / high polarity molecules and sizes between 100-1500 Da. Instrumental sensitivity at ppb levels.
qTOF XEVO-G2-XS (Waters), with ESI source. Instrument suitable for qualitative analysis (identification of unknown compounds) and quantity of known compounds. The compounds that can be analyzed are medium/high polarity molecules and sizes between 100-1500 Da. It is possible to analyze intact proteins of medium/small size and digested proteins. Instrumental sensitivity to ppb/ppt levels (dependent compound).
UHPLC-Q Exactive™ mass spectrometer (Thermo Scientific), with ESI source, is suited to untargeted or targeted screening with high-confidence confirmation but is equally capable of a broad range of qualitative and quantitative applications. Resolution of 140,000 at m/z 200 and <1ppm of mass accuracy. Mass range 50-6000 m/z.
5973 inert GC-MS (Agilent), GC coupled to a single quadrupole, both electronic impact and chemical ionisation mode. Quantitative analysis of small organic molecules. Mass range: 1.6-800 Da.
GC coupled to two high-resolution magnetic sectors (IRMS, Thermo Scientific) with electronic impact ionization source. IRMS instrument is used to measure precisely small differences in the abundances of isotopes such as 13C/12C, 2H/1H and 18O/16O, even at the same time, of organic molecules (fatty acid, organic acid and amino acid).
GC with FID detector, for quantitative determination of small known molecules.

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
- QuantStudioTM 5 384-well Real-Time PCR System (Thermo Fisher)
- Seahorse XFe96 (Agilent)
- TissueLyser II (Qiagen)
- Ultracentrifuge Optima XE-90 (Beckman Coulter)

EVENTS 2020-2021

25 **Internal webinars**

5 **Invited speaker webinars**

Viaggio al centro della scienza

Viaggio al centro della scienza/ Journey to the centre of the science 2020 - 3rd edition “Five dialogues on Covid-19”

Viaggio al centro della scienza/ Journey to the centre of the science 2021 - 4th edition “Four dialogues on vaccines”

SAB site visit 2021

Due to the pandemic, the SAB visit planned for December 2020 was cancelled and, in agreement with the SAB members, it was decided to carry out an evaluation of the institute’s work remotely. In May 2021, the scientific reports of the research groups were sent to the SAB members, together with the report of the Scientific Directorate. Online interviews between the SAB members, the Scientific Directorate and the Coordinators of the various IRP Research Areas took place between September and October.

At the end of the evaluation process, the SAB produced a report that, on the one hand, expressed an extremely positive opinion of the work carried out by the individual Research Areas and the Scientific Directorate and, on the other, produced a number of useful suggestions for the future.

Research area

Experimental Cardiology

Coordinator: **Prof. Giovanni Di Salvo**

Currently the experimental cardiology area is represented by the lab "Experimental cardiology in congenital and structural heart diseases and cardiomyopathies" that works on both clinical and basic science approaches. Clinical studies are carried out in pediatric patients with DCM vs children with DCM and LVNC. Basic sciences studies are conducted in order to investigate the underlying mechanisms in iPSC-derived cardiomyocytes obtained from the same patients enrolled in the clinical study. Finally, mdx mice are be used to characterize LVNC pathogenesis and test for therapeutic strategies in vivo.

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.

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 Ph.D 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 Paediatric 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 and for Full Professor in Paediatric cardiology and Congenital Heart Disease in 2019. He was appointed as Director of Paediatric cardiology at the University of Padua, Italy since September 2019.

Experimental Cardiology in Congenital and Structural Heart Diseases and Cardiomyopathies

PI: **Giovanni Di Salvo**

Team members

Giovanni Di Salvo
Principal Investigator

Nina Kaludercic
CNR Researcher

Jolanda Sabatino
Senior Postdoctoral Researcher, Assistant Professor

Angela Di Candia
Postdoctoral Fellow

Irene Cattapan
Postdoctoral Fellow

Ruth Jepchirchir Arusei
Predoctoral Fellow

Karim Rahhali
Predoctoral Fellow

Research activity

Experimental Cardiology Laboratory studies causes and mechanisms that contribute to hypertrophic growth and cardiac remodeling in different congenital and structural heart diseases

Cardiac mechanics in children with left ventricular non-compaction and dilated cardiomyopathy:
In the pediatric population, left ventricular non-compaction (LVNC) is the third most common cardiomyopathy. Based on the current morphological diagnostic criteria, differentiating LVNC from dilated cardiomyopathy (DCM) alone is challenging. Our group aims at assessing and characterizing cardiac mechanics in children with DCM vs children with DCM and LVNC by advanced imaging techniques to understand whether abnormalities in cardiac mechanics, observed in children with LVNC, could be used to discriminate LVNC and DCM from isolated DCM. In addition, using cardiomyocytes differentiated from induced pluripotent stem cells (iPSCs) derived from LVNC and DCM patients, we are studying molecular signals that lead to cardiomyocyte dysfunction in these patients.
Understanding the mechanisms underlying cardiac remodeling in congenital cardiomyopathies:
Duchenne muscular dystrophy (DMD) is a progressive myopathic disorder caused by a recessive mutation in the dystrophin gene on the X chromosome. With the advances of respiratory and other therapies, the leading cause of death for DMD patients is cardiovascular disease. Indeed, DMD is associated with dilated cardiomyopathy, conduction abnormalities and widespread fibrosis of the left ventricle. Considering that there is no consensus for the treatment of DMD cardiomyopathy, our group is using mouse models of DMD as well as cardiomyocytes differentiated from hiPSCs derived from DMD patients to investigate whether targeting mitochondria and mitochondrial sources of oxidative stress may represent potential novel treatments options with a therapeutic value.
Collaborator: Fabio Di Lisa, University of Padova

PI's biosketch

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 Ph.D 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 Paediatric 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 and for Full Professor in Paediatric cardiology and Congenital Heart Disease in 2019. He was appointed as Director of Paediatric cardiology at the University of Padua, Italy since September 2019.

Selected publications

Pergola V, Di Salvo G, Fadel B, Galzerano D, Al-Shaid M, Al-Admawi M, Al Amri M, Al-Ahmadi M, Al-Halees Z. The long term results of the Ross procedure: The importance of candidate selection. Int J Cardiol. 2020 Jul 14:S0167-5273(20)33457-4. doi: 10.1016/j.ijcard.2020.07.009. Online ahead of print. PMID: 32679140.

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Di Salvo G, et al. 2016–2018 EACVI Scientific Documents Committee. Imaging the adult with congenital heart disease: a multimodality imaging approach-position paper from the EACVI. Eur Heart J Cardiovasc Imaging. 2018 Aug 6. doi: 0.1093/ehjci/jey102. [Epub ahead of print] PubMed PMID: 30084968.

Deshwal S, Forkink M, Hu CH, Buonincontri G, Antonucci S, Di Sante M, Murphy MP, Paolocci N, Mochly-Rosen D, Krieg T, Di Lisa F, Kaludercic N. Monoamine oxidase-dependent endoplasmic reticulum-mitochondria dysfunction and mast cell degranulation lead to adverse cardiac remodeling in diabetes. Cell Death Differ. 2018 Feb 19.

Kaludercic N, Takimoto E, Nagayama T, Lai EW, Feng N, Bedja D, Shih JC, Chen K, Gabrielson KL, Pacak K, Blakely RD, Kass DA, Di Lisa F, Paolocci N. Monoamine oxidase A mediated enhanced catabolism of norepinephrine contributes to adverse remodeling and pump failure in hearts with pressure overload. Circ Res. 2010 Jan 8;106(1):193-202.

Research area

Genetics and Rare Diseases

Coordinator: **Prof. Leonardo Salviati**

The “Genetics and Rare Diseases” research area comprises of five 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. University personnel include one full professor, three associate professors, and one assistant professor, as well as seven technicians. Hospital staff includes one attending physician, four biologists, and one technician. The laboratories host several PhD students as well as the residents of the program in medical genetics.

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 DiMauro 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, Azienda Ospedale Università Padova. Since 2015, he is the director of the Medical Genetics Residency program. He is also the supervisor of the molecular diagnostics laboratory. Prof. Salviati has started his own research group in 2005 focusing on mitochondrial and neurometabolic disorders, with particular focus on Coenzyme Q deficiency. The group has joined IRP since the end of 2013. Prof. Salviati is the recipient of national and international grants for more than 3M€. Overall, he has co-authored over 150 peer-reviewed articles with more than 8000 citations and an h-index of 50 (according to Scopus). Total impact factor is over 1000 points (ISI 2020).

Clinical Genetics and Epidemiology

PI: **Leonardo Salviati**

Team members

Leonardo Salviati
Principal Investigator

Matteo Cassina
Associate professor of Medical genetics

Daniela Bettio
Assistant professor of Medical genetics

Maria Andrea Desbats
Post Doctoral Fellow

Elisa Baschiera
Post Doctoral Fellow

Cristina Calderan
PhD Student

Marco Marchi
PhD Student

Agata Valentino
PhD Student

Alessandra Friso
Biologist

Cinzia Bertolin
Biologist

Francesca Boaretto
Biologist

Monica Forzan
Biologist

Chiara Rigon
Biologist

Adina Cordella
Technician

Davide Garbo
Administrative staff

Research activity

The group is active since 2005 and its research was originally focused mainly on mitochondrial diseases. Our aims were to identify and characterize human genes involved in the biogenesis of the mitochondrial respiratory chain (RC), identify mutations in patients with RC defects, and to develop simple tools to characterize these mutants and to study the pathophysiology of these disorders and test novel therapeutic approaches. In the past years we have expanded our research focus to include also other metabolic diseases, as well as genetic disorders unrelated to cellular metabolism. Regarding the latter, we have developed a novel diagnostic strategy for genetic disorders based on NGS technology. Using a 2-level approach we now have the capability to provide a molecular diagnosis for over 90% of known genetic diseases in a clinical setting.

Based on our previous work we have developed three main lines of research, which we are currently pursuing.

The first line of research deals with the biogenesis of the RC, in particular of Coenzyme Q biosynthesis and its regulation. Using CRISPR-CAS9 we have generated several KO cell lines for genes which are (or are presumed to be) involved in these processes. These cells were instrumental to determine the correct sequence of reactions in the CoQ biosynthetic pathway in humans.

The second one involves metabolic disorders, in particular Urea Cycle defects, diosorders of lipid metabolism, and defects of vitamin B6-dependent enzymes such as ornithine aminotransferase (OAT). We are currently studying the molecular pathogenesis of these conditions and possible therapeutic approaches.

The last field of research deals with genetic diseases in general. The expansion of our diagnostic service has provided us with an incredible amount of genetic data (we have analyzed >3000 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 identified variants, especially of synonymous and missense exonic changes and of intronic variants outside the canonical splicing consensus. The reliability of prediction software is still problematic in a diagnostic setting; hence there is a clear necessity for experimental tools to validate these mutations. In the past years, we have developed several models from hybrid minigenes, to yeast, CRISPR-CAS9 edited human cells, and C. elegans that have allowed us to validate (or to dismiss ass neutral polymorphisms) many novel variants, and to establish genotype-phenotype correlations for different diseases. We have employed these strategies to identify new genes associated with human diseases.

PI's biosketch

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 DiMauro 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, Azienda Ospedale Università Padova. Since 2015, he is the director of the Medical Genetics Residency program. He is also the supervisor of the molecular diagnostics laboratory. Prof. Salviati has started his own research group in 2005 focusing on mitochondrial and neurometabolic disorders, with particular focus on Coenzyme Q deficiency. The group has joined IRP since the end of 2013. Prof. Salviati is the recipient of national and international grants for more than 3M €. Overall, he has co-authored over 150 peer-reviewed articles with more than 8000 citations and an h-index of 50 (according to Scopus). Total impact factor is over 1000 points (ISI 2020).

Selected publications

Matte A, Federti E, Kung C, Kosinski PA, Narayanaswamy R, Russo R, Federico G, Carlomagno F, Desbats MA, Salviati L, Leboeuf C, Valenti MT, Turrini F, Janin A, Yu S, Beneduce E, Ronseaux S, Iatcenko I, Dang L, Ganz T, Jung CL, Iolascon A, Brugnara C, De Franceschi L. The pyruvate kinase activator mitapivat reduces hemolysis and improves anemia in a β-thalassemia mouse model. J Clin Invest. 2021 May 17;131(10):e144206.

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Diagnosis and Therapy of Lysosomal Disorders

PI: **Rosella Tomanin**

Team members

Rosella Tomanin
Principal Investigator

Alessandra Zanetti
Researcher

Francesca D'Avanzo
Researcher

Concetta De Filippis
Junior Fellow

Research activity

Since 1993, the Laboratory focuses its research interests on Mucopolysaccharidoses (MPSs), a cluster of Lysosomal Storage Disorders, including 11 different pathologies due to the deficit of the enzymes normally catabolizing mucopolysaccharides (or glycosaminoglycans) inside the lysosomal compartment. Being these enzymes coded by housekeeping genes, MPSs generally affect most, if not all, organ-systems, including the brain in more than 70% of the cases. Although still incurable, in the last 10- 15 years some MPSs have taken advantage of the availability of the Enzyme Replacement Therapy (ERT), consisting of weekly administrations of the functional enzyme. ERT has shown so far some peripheral efficacy, while unfortunately it has not shown to be of any help to the CNS disease, due to the inability of the recombinant enzymes infused to cross the blood-brain barrier. In addition, brain pathogenesis remains quite obscure for these diseases, while the understanding of its origin and progression would be extremely helpful to both monitor patients' prognosis and detect new potential therapeutic targets. Main objectives of the research team are therefore the comprehension of the brain pathogenesis in these disorders and the evaluation of innovative treatments, by using different in vitro and in vivo models. In addition, the Laboratory is committed in evaluating and classifying the genomic variants underlying these disorders.

PI's biosketch

Dr. Rosella Tomanin graduated in Biology at the University of Padua, progressed her studies with a PhD in Developmental Sciences (Pediatrics) and a Specialization in Medical Genetics. In 35 years of laboratory experience, Dr. Tomanin has been involved in numerous projects in the fields of genetics, molecular and cellular biology and pediatric diseases. Dr. Tomanin spent 4 years at McMaster University (Hamilton, Ontario, Canada), at first as a PostDoc and next as a Visiting Scientist, working on oncogenic adenoviruses and generation of recombinant adenoviral vectors for gene transfer and gene therapy applications.
Currently she is head of the Laboratory of Diagnosis and Therapy of Lysosomal Disorders, Istituto di Ricerca Pediatrica (IRP) Città della Speranza and Dept. of Women's and Children's Health, University of Padova - Italy.
Dr. Tomanin is co-author of 66 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, and as a project reviewer for some International Disease Foundations.

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: Postdoctoral Fellowship in Molecular Biology, Cancer Research Group, Health Science Centre, McMaster University, Hamilton, Ontario, Canada
Mar 1993 - Apr 1995: Visiting Scientist, Laboratory of Molecular Virology, Dept. of Biology, McMaster University, Hamilton, Ontario, Canada
Jan 1994 - June 1994: Fellowship of the Canadian Government – International Council for Canadian Studies
Mar 1998 - Mar 1999: Senior Scientist Fellowship, Istituto per lo Studio e la Cura dei Tumori di Milano
Apr 1999 - Mar 2001: PostDoctoral Fellowship, School of Medicine, University of Padua.

Projects of the research group:

- In the last 10 to 12 years, the Laboratory has been largely involved in the evaluation and application, in vitro and in vivo, of a nanoparticle-based approach, functionalized to obtain drug brain- targeting, as a potential non-invasive therapeutic approach for MPS brain disease. We first successfully delivered Albumin, as a model high MW molecule, to the brain of MPS I and MPS II mouse models (Salvalaio et al. PLoS One 2016 May 26;11(5):e0156452). Next, we successfully delivered the recombinant form of the enzyme iduronate 2-sulfatase to the brain of the MPS II mouse model (Rigon et al. Int J Mol Sci 2019 Apr 24; 20(8):2014). Optimization of the NPs-mediated strategy was conducted during 2020-2021. In vitro experiments were extremely promising. In vivo experiments were re-set a couple of times,

by progressively reducing the albumin content of NPs; these last evaluations still need to be completed.

- In the last 4 years, we have conducted a collaboration with the Dept. of Pharmaceutical and Pharmacological Sciences, of the University of Padova, aiming at generating and characterizing Drosophila models of mucopolysaccharidoses. Within this collaboration we have generated a Drosophila knock-down model of MPS I, whose characterization was completed in 2021, showing that the fly may represent a good model of the disease. The animal characterization was recently published (De Filippis et al, Cells 2022, 11(1):129). Drosophila is a fast and valuable model to study pathogenesis issues and a good tool for screening of therapeutic molecules. The model is now under further evaluation, including the analysis of several molecular pathways, with the aim to identify possible metabolic alterations.
- With the aim to understand the onset of brain pathogenesis and its progression in MPSs, we are completing the characterization of the brain parenchyma of the MPS II mouse model by evaluating different biomarkers at several progressive age of the animal. This will also represent a useful tool to monitor the therapeutic efficacy of brain-targeted approaches. We are now concluding this analysis.
- In 2019, we started a new project aiming at identifying potentially altered pathways resulting in the severe forms of MPS, leading to neuro- cognitive and behavioral impairment. This project is conducted by generating induced pluripotent stem cells (iPSC) starting from human primary fibroblasts.
- Within the Advanced Diagnostics interests of the Lab, we have set-up and validated a targeted panel for the contemporary molecular analysis of 50 Lysosomal Storage Disorders (Zanetti et al. J Mol Diagn 2020 Apr; 22(4): 488-502). Following the completion of the "mutation update" of the ARSB gene (whose mutations cause the MPS VI), through a re-classification of all published variants (Tomanin et al. Hum Mutat 2018 Dec;39(12):1788-1802), during 2020-2021, this analysis, was extended to the GALNS gene, whose mutations are responsible of the MPS IVA. This analysis was completed and recently published (Zanetti et al. Hum Mutat 2021 Nov;42(11):1384-1398). We are now reclassifying the genomic variants of the IDS gene, whose mutations cause the Hunter Syndrome or MPS II.

Diagnostic activity: the laboratory also performs some biochemical and molecular analyses related to the diagnosis of Mucopolysaccharidoses.

Selected publications

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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.

Immunopathology and Molecular Biology of Kidney

PI: **Elisa Benetti**

Research activity

The Laboratory of Immunopathology and Molecular Biology of the Kidney is part of the Pediatric Nephrology Dialysis and Transplant Unit of the Dept. of Women's and Children's Health of Padua University Hospital. The Unit is a center of excellence and reference for Pediatric Nephrology and rare kidney diseases. It is also part of international registries and networks (i.e. ERKNet; Certain; Transplantchild) for the diagnosis and treatment of kidney rare diseases. Furthermore, it works in close collaboration with various specialists to follow children from prenatal diagnosis to kidney transplantation, using a multidisciplinary and comprehensive approach. The Laboratory provides an analysis pattern for the immune-histological classification of primary and secondary pediatric renal diseases and for the follow-up of pediatric kidney transplantation recipients. It also coordinates the management of molecular tests for genetic kidney diseases. Furthermore, the laboratory has a remarkable biobank of renal tissues from transplanted or native kidneys. Pediatric Nephrology Unit and the Laboratory coordinate important scientific studies, from the clinical trials to the translational research, concerning kidney transplantation, congenital abnormalities of the kidney and urinary tract (CAKUT), and rare kidney diseases, including nephrotic syndrome, hereditary glomerulopathy and tubulopathy.

The main field of interest of our Laboratory is the study of factors affecting the survival of kidney transplantation in the pediatric population. To date, the survival graft rate is about

Team members

Elisa Benetti
Principal Investigator

Susanna Negrisolo
Senior PostDoctoral Researcher; Lab Manager

Andrea Carraro
PostDoctoral Researcher

Diana Marzenta
Lab Technician

Benedetta Antoniello
Student

PI's biosketch

Scopus ID: 6602437925
Dr. Benetti is the P.I. 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 specialisation 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 Center of Padua. Currently, she also is a representative of Padua University Hospital in the European Reference Network (ERN) for Rare Kidney diseases (ERKNet) and Transplantation (TransplantChild). 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 70 papers, as an author or co-author, on renal genetic diseases, nephrotic syndrome and renal transplantation. She is also author of three 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 of the University of Padua since 2018 and at the Pediatrics Residency Program of the same University since 2021. Furthermore, she has been acting as a tutor in the Pediatrics Residency Program and in the Second level University Master in Pediatric Nephrology of the University of Padua since 2011.
Dr. Benetti is a past vice-president and counselor of the Italian Society for Pediatric Nephrology 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 (ESPN), the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA), the Italian Society of Nephrology (SIN), the Italian Society for Organ Transplant (SITO), the Italian Society of Paediatrics (SIP), Certain registry, ESCAPE Study Group, PODONET Consortium. In 2010, she successfully completed the International Summer School of Renal Pathology of the European Renal Association-European Dialysis and Transplant Association (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 approximately 20 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.

15-20 years, a too short period for a pediatric patient. The gold standard in the diagnosis of renal allograft failure (the main cause of kidney rejection) is the biopsy and the Pediatric Renal Transplant Centre of Padua, which also resides at the Pediatric Nephrology Unit, is a pioneer of protocol biopsy. Protocol biopsy allows to identify rejection before the onset of clinical features. Three main topics that are on study are: 1. the prognostic value of infiltrate cell phenotyping in renal transplant biopsies; 2. the relevance and the prognostic value of intrarenal positivity of viruses; 3. the dosage and the role of antibody against the donor (HLA and non-HLA). We are collaborating with Transplantation Immunology Lab (Prof. Cozzi) to explore the impact of non -HLA antibodies anti AT1R and ETAR and to assess the possible causative role of anti-ETAR immunity in premature pediatric renal transplant failure. Furthermore, we are collaborating with many IRP groups with different expertise, to characterize the extracellular vesicles (EVs) isolated from serum and urine of our transplanted children. These EVs act as a message delivery system of the graft, and their cargo could be useful to identify novel non-invasive biomarkers predictive of rejection, to personalize the treatment of children with a suspicious of subclinical graft rejection before the damage is detectable in the kidney.

The Lab is also involved in the ORCHESTRA project. ORCHESTRA is funded by the European Union's Horizon 2020 research and innovation programme under the ERAvsCORONA Action Plan which was developed jointly by Commission services and national authorities. ORCHESTRA is a European 3-years project (2020-2023) aimed to deliver scientific evidence to improve the prevention and treatment of the infections caused by SARS-CoV-2. The project builds up on existing and new large scale population cohorts in Europe (France, Germany, Spain, Italy, Belgium, Romania, Netherlands, Portugal, Luxemburg and Slovakia) and in 9 non-European countries. 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. The cohort involves four different populations: members of the general population, COVID-19 patients, fragile individuals (children, elderly, transplanted, oncological, HIV infected individuals, and those with Parkinson disease and rheumatologic disease). This cohort will apply homogenous protocols for data collection, data sharing, sampling, and follow-up to rapidly advance the knowledge on the control and management of COVID-19.

Furthermore, the Lab is in involved in many studies on genetic renal diseases (e.g., primary hyperoxaluria, genetic nephrotic syndrome, Bartter syndrome and primary tubulopathies) that can lead to chronic kidney disease and renal transplantation during childhood.

Selected publications

Vaisitti T, Peritore D, Magistroni P, Ricci A, Lombardini L, Gringeri E, Catalano S, Spada M, Sciveres M, Di Giorgio A, Limongelli G, Varrenti M, Gerosa G, Terzi A, Pace Napoleone C, Amodeo A, Ragni L, Dello Strologo L, Benetti E, Fontana I, Testa S, Peruzzi L, Mitrotti A, Abbate S, Comai G, Gotti E, Schiavon M, Boffini M, De Angelis D, Bertani A, Pinelli D, Torre M, Poggi C, Deaglio S, Cardillo M, Amoroso A; Italian Pediatric Transplant Centers. The frequency of rare and monogenic diseases in pediatric organ transplant recipients in Italy. Orphanet J Rare Dis. 2021 Sep 4;16(1):374. doi: 10.1186/s13023-021-02013-x.

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Baker A, Frauca Remacha E, Torres Canizales J, Bravo-Gallego LY, Fitzpatrick E, Alonso Melgar A, Muñoz Bartolo G, Garcia Guereta L, Ramos Boluda E, Mozo Y, Broniszczak D, Jarmużek W, Kalicinski P, Maecker-Kolhoff B, Carlens J, Baumann U, Roy C, Chardot C, Benetti E, Cananzi M, Calore E, Dello Strologo L, Candusso M, Lopes MF, Brito MJ, Gonçalves C, Do Carmo C, Stephenne X, Wennberg L, Stone R, Rascon J, Lindemans C, Turkiewicz D, Giraldi E, Nicastro E, D'Antiga L, Ackermann O, Jara Vega P. Current Practices on Diagnosis, Prevention and Treatment of Post-Transplant Lymphoproliferative Disorder in Pediatric Patients after Solid Organ Transplantation: Results of ERN TransplantChild Healthcare Working Group Survey. Children (Basel). 2021 Jul 29;8(8):661. doi: 10.3390/children8080661.

Sahbani D, Strumbo B, Tedeschi S, Conte E, Camerino GM, Benetti E, Montini G, Aceto G, Procino G, Imbrici P, Liantonio A. Functional Study of Novel Bartter's Syndrome Mutations in ClC-Kb and Rescue by the Accessory Subunit Barttin Toward Personalized Medicine. Front Pharmacol. 2020 Mar 17;11:327. doi: 10.3389/fphar.2020.00327.

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Model Organisms and Rare Diseases

PI: **Eva Trevisson**

Research activity

The main interest of our laboratory has been the development of models to study the bases of rare genetic diseases and to dissect their pathogenesis.

Since the introduction of next generation sequencing technologies, interpretation of genomic variants, their validation and predictions of possible effects on gene products have acquired a crucial importance and become one of the most relevant challenges in human genetics. What we have been mostly interested in is the setting up of simple models that allow to validate novel variants identified in patients affected by rare genetic diseases, but also to establish genotype-phenotype correlations and to analyze the molecular pathogenesis of these conditions. In this regard, we have successfully employed S. cerevisiae to analyze missense mutations in genes affecting fundamental cellular processes or mammalian cells in order to test the effects of genomic variants on transcript maturation.

More recently, we have moved to multicellular models, including C. elegans, D. rerio and M. musculus.

C. elegans and D. rerio represent simple highly prolific organisms with a rapid life cycle that display organized tissues and organs with a high genetic similarity with humans. In our laboratory, we use C. elegans as model to study genes whose function is unknown and potentially involved in genetic diseases. Moreover, we have successfully employed this nematode to functionally validate new variants found in a novel disease gene in patients affected by a severe neurological condition. We are now employing C. elegans and D. rerio as model organisms in order to study the mechanisms driving pediatric cancers associated with a genetic predisposition and to set up simple models for drug screening.

Finally, we are also employing mouse models to investigate novel therapeutic approaches for primary CoQ10 deficiency testing the effects of ring analogues supplementation and gene replacement in a commercially available model of CoQ deficiency.

Team members

Eva Trevisson
Principal Investigator

Cristina Cerqua
PostDoctoral Researcher

Valeria Morbidoni
PostDoctoral Researcher

Lisa Buson
PostDoctoral Researcher

Elena Tacchetto
PhD student

PI's biosketch

Scopus ID: 8922220000

Prof. Trevisson obtained her Medical Degree at the University of Padua and completed her residency program in Medical Genetics at the University of Siena. She earned her PhD in Rare Diseases (PhD School in Developmental Medicine, University of Padua). During her PhD program, supervised by Prof. Salviati, she set up models of neurometabolic conditions, mainly mitochondrial disorders and urea cycle defects.

Prof. Trevisson had also the opportunity to visit as fellow an excellent center of developmental biology in Spain (Centro Andaluz de Biologìa del Desarrollo, Seville) in the Lab. of Prof. Navas, where she could work with C. elegans, that has been widely used in most research fields and she established a knockdown model of a mitochondrial disorder.

Since 2014, she leads a group in the laboratory of Genetics at the Istituto di Ricerca Pediatrica (IRP), where she is following on modeling human genetic diseases in C. elegans, D. rerio and M. musculus and she has started a novel research line on cancer predisposition syndromes.

Since October 2020, she has been working as Associate Professor of Medical Genetics at the University of Padova.

Being a medical geneticist, she also performs genetic consultations at the Clinical Genetics Unit of the Azienda Ospedale Università Padova, where she is in charge of the neurofibromatoses outpatient clinic, a referral centre for these conditions. This clinical practice gave her the opportunity to deal with inherited tumor predisposing syndromes.

She teaches Molecular Genetics and Medical Genetics in different academic courses, medical residency programs and in the PhD program in Developmental Medicine at the University of Padua.

She has co-authored 80 Medline publications and five book chapters, with a total impact factor of 416,5 (Journal of Citation reports 2020), h-index 29, total citations 2828 (Scopus).

Selected publications

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 Feb 27;13(5):999. doi: 10.3390/cancers13050999.

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 Aug;58(8):526-533. doi: 10.1136/jmedgenet-2020-106833.

Cerqua C, Casarin A, Pierrel F, Vazquez Fonseca L, Viola G, Salviati L, Trevisson E. Vitamin K2 cannot substitute Coenzyme Q10 as electron carrier in the mitochondrial respiratory chain of mammalian cells. Sci Rep. 2019; 9:6553. doi: 10.1038/s41598-019-43014-y.

Trevisson E, Morbidoni V, Forzan M, Daolio C, Fumini V, Parrozzani R, Cassina M, Midena E, Salviati L, Clementi M. The Arg1038Gly missense variant in the NF1 gene causes a mild phenotype without neurofibromas. Mol Genet Genomic Med. 2019; 7:e616. doi: 10.1002/mgg3.616.

Cerqua C, Morbidoni V, Desbats MA, Doimo M, Frasson C, Sacconi S, Baldoin MC, Sartori G, Basso G, Salviati L, Trevisson E. COX16 is required for assembly of cytochrome c oxidase in human cells and is involved in copper delivery to COX2. Biochim Biophys Acta Bioenerg. 2018; 1859:244-252. doi: 10.1016/j.bbabio.2018.01.004.

Neurodevelopmental Molecular Genetics Laboratory

PI: **Alessandra Murgia**

Research activity

The Molecular Genetics of Neurodevelopment Laboratory has been, until December 2020, a referral center for molecular diagnostics of neurodevelopmental conditions.

The diagnostic activity of the laboratory covered Intellectual Disability/Autism spectrum disorders, Developmental epilepsies Cerebral Palsy and Infantile Movement Disorders and Hereditary Sensorineural Hearing Loss.

The laboratory members have a long and well established experience in the development and validation of molecular strategies and protocols for genetic analysis of rare pediatric disorders and work in close collaboration with the clinical component of the Women's and Children's Health Department (SDB).

Several translational research programs have been carried with the aim of studying the biological bases of neurodevelopmental conditions known for their clinical and genetic heterogeneity, and in particular intellectual disability associated with autism and early onset epilepsy.

Conventional methods of molecular genetic analysis have been replaced in the laboratory, since 2011, by the application of Next Generation Sequencing technology, in order to develop new and more efficient diagnostic tools. The laboratory has provided NGS analysis of the following customized targeted gene panels: EIEE/developmental epilepsy; ID/ASD; SNHL and Tuberous Sclerosis, Cerebral Palsy- Infantile Movement disorders.

Team members

Alessandra Murgia
Principal Investigator

Roberta Polli
Lab manager, Quality Control Manager

Emanuela Leonardi
Senior PostDoctoral Researcher

Elisa Bettella
Senior PostDoctoral Researcher

Maria Cristina Aspromonte
PhD student

Marilena Cameran
Lab Technician

PI's biosketch

Scopus ID 7004130427
Prof. Murgia obtained the MD degree in 1981 and specialized in Endocrinology (1984) and in 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 PostDoctoral Fellow at the Dept. of Human Genetics, University of Pennsylvania School of Medicine until 1991 before coming back to Padova where she is Principal Investigator of the Molecular Genetics of Neurodevelopment Laboratory and Associate Professor of Pediatrics, Department SDB, School of Medicine. Her primary scientific interest is on neurodevelopmental disorders.
Prof Murgia teaches Child neuropsychiatry at the School of Medicine, School of Psychology and Speech Therapy University of Padua.

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.
Prof. Murgia and her team are collaborators of a Telethon and ERC projects, both centered on Fragile X Syndrome, led by Prof. Nicola Elvassore (University of Padua)
Prof. Murgia holds memberships of the following scientific societies: American Society of Human Genetics (ASHG); European Society of Human Genetics (ESHG); Italian Society of Human Genetics (SIGU).

Areas of interest: Neurodevelopmental disorders, Intellectual Disability/Autism Spectrum Disorders, Early Onset Epilepsy; Cerebral Palsy and Infantile movement disorders, Hereditary Deafness.

Fragile X program:
the Laboratory is a nationally recognized center for Fragile X molecular testing. The molecular diagnostic activity of the Azienda Ospedaliera-Università di Padova for Fragile X associated conditions (FXS, FXPOI, FXANC, FXTAS, FXVAC) is currently centralized at this laboratory.
Prof. Alessandra Murgia has coordinated one of three Italian centers ever involved in international pharmacological trials for Fragile X Syndrome (AFQ, Novartis). The laboratory is currently collaborating at two projects (Telethon and ERC funded) for the study of the pathogenic mechanisms of Fragile X associated conditions.
In January 2014, the "Multidisciplinary Fragile X Padua Network" has been established, as the first and only Italian initiative of integrated clinical activity for Fragile X Syndrome and Fragile X- Associated conditions (http://www.sdb.unipd.it/centro-x-fragile).

The activity of Molecular diagnostics is therefore paralleled by an intense clinical activity coordinated and carried out by Prof. Murgia, assisted by a team of child neuropsychiatrists and neuropsychologists, and with the close collaboration of a network of multidisciplinary pediatric specialists of the SDB Department.
The SDB Fragile X Center is also involved in a collaboration with the Department of Information Engineering (Prof. Zimi Sawacha) for the study of motor components of the Fragile X Phenotypes.
The Multidisciplinary Fragile X Padua Network is officially recognized as center of excellence by the Italian Fragile X Syndrome Association; it is member of the International FXTAS Consortium.
Prof. Alessandra Murgia (vice-representative for Padua AOP) and the laboratory staff participate in the ERN-ITHACA (European Reference Network For Rare Congenital Malformations and Intellectual Disability).
Major Lab Equipment:
NGS platform: Ion Torrent PGM/S5; Ion GeneStudio S5 System; 20 Terabite Archive drive. Automatic sequencer ABI PRISM 3130 Genetic Analyzer; Nucleic Acids automated extractor Promega Maxwell 16 IVD. GelDOC Biorad.

Selected publications

H Guo, E Bettella, PC Marcogliese, R Zhao, J C Andrews, TJ Nowakowski, M A Gillentine1, K Hoekzema, T Wang, H Wu, S Jangam, C Liu, H Ni, MH Willemsen, BW van Bon, T Rinne,, SJC Stevens, T Kleefstra, HG Brunner, HG Yntema, M Long, W Zhao, Zhengmao Hu, C Colson, N Richard, CE Schwartz, C Romano, L Castiglia, M Bottitta, SU Dhar, DJ Erwin, L mrick, B Keren, A Afenjar, B Zhu, B Bai, P Stankiewicz, K Herman, University of Washington Center for Mendelian Genomics, S Mercimek-Andrews, J Juusola, AB Wilfert, R AbouJamra, B Büttner, HC Mefford, AM Muir, I EScheffer, BM Regan, S Malone, J Gecz, J Cobben, MM Weiss, Q Waisfisz, EK Bijlsma, MJ V Hoffer, CAL Ruivenkamp, S Sartori, F Xia, J A Rosenfeld,RA Bernier, MF Wangler, S Yamamoto,K Xia, APA Stegmann, HJ Bellen, A Murgia, Evan E Eichler. Disruptive mutations in TANC2 define a neurodevelopmental syndrome associated with psychiatric disorders. Nat Commun. 2019;10(1):4679.

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Research area

Immunology and Neuroimmunology

Coordinator: **Prof. Antonella Viola**

The "Immunology and Neuroimmunology" research area is coordinated by Prof. Antonella Viola and comprises of three distinct yet deeply connected units.

The research priority of the area is to uncover key molecular mechanisms driving infection and inflammatory disorders and to translate these findings from bench to bedside.

Our research is currently aimed at investigating immune-related pathogenetic mechanisms in multiple disorders, including acquired autoimmune demyelinating syndromes, pediatric multiple sclerosis, perinatal stroke, neonatal bronchopulmonary dysplasia and arrhythmogenic cardiomyopathy. Our approach combines pre-clinical and clinical studies in order to identify new early disease biomarkers and novel therapeutic targets.

The staff involve academic researchers from Departments of the University of Padova (Biomedical Sciences and the Department of Women's and Children's Health) as well as clinicians from the University Hospital of Padova, building up an effective multidisciplinary team for improving research outcomes.

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA
Città della Speranza

Prof. Antonella Viola

Coordinator Immunology and Neuroimmunology Area

Scopus ID: 7005414761

Following a degree in Biological Sciences (1991) and a PhD in Evolutionary Biology (1995) at the Dept. of Biological Sciences of the University of Padua, Italy, Prof. Viola joined the Basel Institute of Immunology (Basel, Switzerland) where she worked as a Scientific Member for 4 years In 1999, thanks to a prestigious EMBO fellowship Prof. Viola returned to Italy at the European Molecular Biology Laboratory (EMBL) - Monterotondo (Rome), and in 2001 joined the Venetian Institute of Molecular Medicine (VIMM) - Padova, where she established her research group in immunology (2001-2007). Between 2006 and 2014, Prof. Viola also led her research group at the Humanitas Research Hospital (Rozzano, Milan).

Since 2015, Antonella Viola has been a Full Professor of General Pathology at the Dept. of Biomedical Sciences of the University of Padua. Prof. Viola was also the Deputy Director of the VIMM from 2015 until 2017, when she was appointed Scientific Director of the Fondazione Istituto di Ricerca Pediatrica Città della Speranza. Prof. Viola has coordinated several national and international research projects aimed at investigating the immune system. Over the years, Prof. Viola has attended several events and conferences in research institutes all around the world as an invited speaker, most notably at the Imperial College (London, UK), the Institut Pasteur (Paris, France), the Harvard Medical School (Boston, USA), Oxford University (Oxford, UK), the Medical Research Council (MRC - Cambridge, UK) and the Jefferson University (Philadelphia, USA).

During her career, Prof. Viola has been a member of the Italian Association for Cancer Research (AIRC) and scientific grant reviewer for several national and international funding agencies, including the European Research Council (ERC) Scientific excellence grants. For her outstanding contribution to the field of immunology, Prof. Viola was the recipient of several awards such as the Roche Prize for Immunology in 1997, the Cancer Research Institute Investigator Award (New York) in 2005 and the Chiara d'Onofrio Foundation award in 2008. In 2006, Prof. Viola was honoured with the titles 'EMBO young investigator' and subsequently with the title 'EMBO member' in 2016, first woman at the University of Padua and in the entire Northeast Italy to receive such an achievement. Over the years, Prof. Viola has secured several research grants, among which the prestigious ERC Advanced Investigator grant.

Immunity, Inflammation & Angiogenesis

PI: **Antonella Viola**

Research activity

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

Team members

Antonella Viola
Principal Investigator

Barbara Molon
Senior PostDoctoral Researcher, Assistant Professor

Roberta Angioni
PostDoctoral Researcher

Ricardo Sanchéz-Rodriguez
PostDoctoral Researcher

Fabio Munari
Lab Manager

Nicole Bertoldi
Lab Manager

Fransisca Carolina Venegas
PhD student

Alessandra Maria Testa
PhD student

Chiara Cioccarelli
PhD student

Gloria Orlando
PhD student

A) Exploiting Mesenchymal Stromal Cell-derived Extracellular Vesicles 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 analysed 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 pediatric pathological tumor associated angiogenesis.

Collaborators: Maurizio Muraca, University of Padua & IRP. Diana Corallo, Post-doctoral Fellows IRP.

B) Multiple sclerosis and acquired autoimmune demyelinating syndromes

PAcquired 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 pedMS 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.

PI's biosketch

Scopus ID: 7005414761

Following a degree in Biological Sciences (1991) and a PhD in Evolutionary Biology (1995) at the Dept. of Biological Sciences of the University of Padua, Italy, Prof. Viola joined the Basel Institute of Immunology (Basel, Switzerland) where she worked as a Scientific Member for 4 years In 1999, thanks to a prestigious EMBO fellowship Prof. Viola returned to Italy at the European Molecular Biology Laboratory (EMBL) - Monterotondo (Rome), and in 2001 joined the Venetian Institute of Molecular Medicine (VIMM) - Padova, where she established her research group in immunology (2001-2007). Between 2006 and 2014, Prof. Viola also led her research group at the Humanitas Research Hospital (Rozzano, Milan).

Since 2015, Antonella Viola has been a Full Professor of General Pathology at the Dept. of Biomedical Sciences of the University of Padua. Prof. Viola was also the Deputy Director of the VIMM from 2015 until 2017, when she was appointed Scientific Director of the Fondazione Istituto di Ricerca Pediatrica Città della Speranza.

Prof. Viola has coordinated several national and international research projects aimed at investigating the immune system. Over the years, Prof. Viola has attended several events and conferences in research institutes all around the world as an invited speaker, most notably at the Imperial College (London, UK), the Institut Pasteur (Paris, France), the Harvard Medical School (Boston, USA), Oxford University (Oxford, UK), the Medical Research Council (MRC - Cambridge, UK) and the Jefferson University (Philadelphia, USA).

During her career, Prof. Viola has been a member of the Italian Association for Cancer Research (AIRC) and scientific grant reviewer for several national and international funding agencies, including the European Research Council (ERC) Scientific excellence grants. For her outstanding contribution to the field of immunology, Prof. Viola was the recipient of several awards such as the Roche Prize for Immunology in 1997, the Cancer Research Institute Investigator Award (New York) in 2005 and the Chiara d'Onofrio Foundation award in 2008. In 2006, Prof. Viola was honoured with the titles 'EMBO young investigator' and subsequently with the title 'EMBO member' in 2016, first woman at the University of Padua and in the entire Northeast Italy to receive such an achievement. Over the years, Prof. Viola has secured several research grants, among which the prestigious ERC Advanced Investigator grant.

C) Covid-19: understanding the role of inflammation and immunity

Covid-19 has overwhelmed the sanitary system worldwide. The triggering and tuning of the immune response in patients have immediately come out as a burning issue to be addressed to clinically manage the disease and the patients' outcome. The identification of key immune signatures and their contribution to SARS-Cov2 infection and pathology represent the forefront of COVID-19 research. Our projects take up this hard challenge by performing a comprehensive analysis of the immune contexture in COVID-19 patients by exploiting novel, state of the art approaches. In this line, we defined circulating factors that positively correlate with older age, longer hospitalization, and a more severe form of the disease and may thus represent the leading signature in critical COVID-19 patients. Furthermore, in a cohort of vaccinated patients, we revealed a significant reduction in the levels of IL-1ß and DAMPs (danger associated molecular pattern molecules) as compared to non-vaccinated ones. COVID-19 vaccination indeed prevents severe clinical manifestations in patients and limits the release of systemic danger signals in SARS-CoV-2 infected people. From a mechanistic point of view, we indicated that the host inflammatory milieu favors SARS-CoV-2 infection by directly increasing the host transmembrane protease/serine subfamily 2 (TMPRSS2) expression that cleaves the Spike protein to facilitate membranes fusion. We unveiled the molecular mechanism that regulates this process and that can be therapeutically advantageously targeted.

Collaborators: Annamaria Cattelan – Azienda Ospedaliera di Padova, Giuseppe Testa – Human Technopole (Milano), Paolo Rossi, Carlo Giaquinto.

D) Mitochondrial dynamics drive macrophage metabolism

Macrophages are essential players for the host response against pathogens, regulation of inflammation, and tissue regeneration. The wide range of macrophage functions rely on their heterogeneity and plasticity that enable a dynamic adaptation of their responses according to the surrounding environmental cues. Recent studies suggest that metabolism provides synergistic support for macrophage activation and elicitation of desirable immune responses; however, the metabolic pathways orchestrating macrophage activation are still under scrutiny. Optic atrophy 1 (OPA1) is a mitochondria-shaping protein controlling mitochondrial fusion, cristae biogenesis and respiration; clear evidence shows that the lack or dysfunctional activity of this protein triggers the accumulation of metabolic intermediates of the TCA cycle. We found that OPA1 has a crucial role in macrophage activation. Selective OPA1 deletion in myeloid cells impairs M1-macrophage commitment. Mechanistically, Opa1 deletion leads to TCA cycle metabolite accumulation and defective NF-κB signaling activation. In an in vivo model of muscle regeneration upon injury, Opa1 knock-out macrophages persist within the damaged tissue, leading to excess collagen deposition and impairment in muscle regeneration.

Collaborators: Luca Scorrano –University of Padova, Marco Sandri – University of Padova, Alessandra Castegna - University of Bari.

Selected publications

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Monoamine Oxidases in Innate Immunity

PI: **Marcella Canton**

Research activity

Reactive oxygen species (ROS) are well known to be fundamental for macrophages to kill invasive microorganisms. Moreover, they have an important 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 only partially elucidated. Monoamine oxidase (MAO) is a relevant source of hydrogen peroxide in mitochondria, generated by oxidative deamination of biogenic amines. Since this enzyme has been scarcely characterized in phagocytic cells, we aimed at clarifying whether it plays a role in the differentiation and activation of macrophages. Our findings show that oxidative stress induced by MAOB overactivation plays a crucial role in inflammasome activation in several preclinical models of acute and chronic inflammation. Inflammasomes are protective weapons against pathogens and cellular damage, though their uncontrolled activation drives progression of inflammatory, metabolic, and neurodegenerative disorders. Several signals activate the NLRP3 inflammasome and a few studies reported that mitochondrial reactive oxygen species (ROS) are involved in this process. However, it is still unclear what is the specific role of mitochondrial ROS in NLRP3 triggering and, most importantly, which is their specific source. Mechanistically, MAO-B-dependent ROS formation caused mitochondrial dysfunction and NF-kB activation, resulting in NLRP3 and pro-IL-1ß overexpression. Both in vitro and in vivo, targeting MAO-B with clinical-grade inhibitors (rasagiline and safinamide) prevented IL-1ß secretion and MAO-B deficient mice showed impaired response to LPS-mediated endotoxemia. Importantly, in a Duchenne dystrophy murine model, rasagiline administration reduced inflammasome activation in muscle-infiltrating macrophages, along with muscle performance recovery. In collaboration with Dr. Piccoli, we are currently developing a three-dimensional model of dystrophic muscle with hiPSCs from Duchenne patients as a model for in vitro drug screening.
In addition, MAO-B inhibition blunts many other pro-inflammatory cytokines, such as IL6, and chemokines, such as CXCL1, in crystal-induced arthropathies. Our findings identify MAO-B as a specific producer of mitochondrial ROS fueling an inflammatory response, thereby providing the basis for repurposing MAO-B inhibitors to treat inflammatory diseases. Thus, we are currently investigating whether clinical-grade MAOB inhibitors can be viable candidates in the treatment of autoinflammatory and autoimmune disorders (including gout, pseudogout, metabolic arthropathies and multiple sclerosis) in preclinical models and in biopsies from patients.

Team members

Marcella Canton
Principal Investigator

Eugenia Carraro
PhD student

Francisca Carolina Venegas
PhD student

Marta De Lorenzi
Undergraduate student

Sofia Barosco
Undergraduate student

PI's biosketch

Scopus ID: 7004910913
Prof. Canton started her academic career with a degree in Pharmaceutical Chemistry and Technology (summa cum Laude in 1991) followed by a PhD in Molecular and Cellular Biology and Pathology (1995). At the beginning of her career Prof. Canton's research was focused on mitochondrial pathophysiology (supervisor Prof. Azzone) and subsequently centered on investigating the mechanisms underlying cardiac dysfunction (supervisor Prof. Di Lisa) focusing on identifying sources and targets of oxidative stress in the myocardium in pathologic conditions, highlighting a causal link between oxidation of myofibrillar proteins and contractile impairment. Her group then moved to the study of skeletal muscle, providing the rationale for a translational study of Monoamine Oxidase (MAO) inhibitors for treatment of muscular dystrophy. Prof. Canton is currently PI of the Monoamine oxidases in innate immunity laboratory at the Istituto di Ricerca Pediatrica, focusing on the role of MAO in the redox signaling in innate immune system.

Selected publications

Canton M, Sánchez-Rodríguez R, Spera I, Venegas FC, Favia M, Viola A, Castegna A (2021) Reactive Oxygen Species in Macrophages: Sources and Targets Front Immunol https://doi.org/10.3389/fimmu.2021.734229.

Sánchez-Rodríguez R, Munari F, Angioni R, Venegas F, Agnellini A, Castro-Gil MP, Castegna A, Luisetto R, Viola A, Canton M (2020) Targeting monoamine oxidase to dampen NLRP3 inflammasome activation in acute and chronic inflammation. Cell Mol Immunol doi: 10.1038/s41423-020-0441-8.

Castegna A, Gissi R, Menga A, Montopoli M, Favia M, Viola A, Canton M (2020) Pharmacological targets of metabolism in disease: opportunities from macrophages Pharmacol. Ther 210:107521. doi: 10.1016/j.pharmthera.2020.107521.

Costiniti V, Spera I, Menabo R, Palmieri EM, Menga A, Scarcia P, Porcelli V, Gissi R, Castegna A, Canton M (2018) Monoamine oxidase-dependent histamine catabolism accounts for post-ischemic cardiac redox imbalance and injury. Biochim Biophys Acta Mol Basis Dis, 1864:3050-9.

Canton M, Menazza S, Sheeran FL, Polverino De Laureto P, Di Lisa F, And Pepe S (2011) Oxidation of myofibrillar proteins in human heart failure. J Am Coll Cardiol, vol. 57 (3); p. 300-309, ISSN: 0735-1097.

Neuroimmunology

PI: **Stefano Sartori**

Research activity

The Neuroimmunology Laboratory focuses on unraveling the role of immunity and inflammation in paediatric onset epilepsies, encephalopathy with seizures, adult paraneoplastic and autoimmune neurological syndromes. The laboratory actively works at both diagnostic and research levels. The major aims are the identification of possible diagnostic and prognostic biomarkers that are fundamental tools in the clinical settings. We are one of the few national reference centers research laboratories for the diagnosis of this type of neurological diseases.

Team members

Stefano Sartori
Principal Investigator

Piera De Gaspari
PostDoctoral Researcher

Margherita Nosadini
Clinical Researcher

Marco Zoccarato
Clinical Researcher

Luigi Zuliani
Clinical Researcher

Medical Diagnostic Activities

Each year, we receive paediatric and adult patients' samples (both CSF and serum) from all over Italy. The expertise of the laboratory, acquired over the years, and the collective experience in international laboratories, enable us to accurately diagnose paediatric and adult autoimmune neurological syndromes based on the detection of autoantibodies. Our laboratory works in synergy with the clinic, in particular with the pediatric and adult neurology department of Azienda Ospedale Università Padova, the neurobiology laboratory and the Dept. of Neurology of Vicenza Hospital (Dr. Luigi Zuliani) and monthly we guarantee the analysis and diagnosis for samples of patients suspected having autoimmune neurological syndromes. We are also reference laboratory for second opinion and doubtful cases for Euroimmun Italy, a diagnostic company, leader in the neuroimmunology field and located here in IRP, too.

We can guarantee the accurate screening and detection of antibodies direct to intracellular protein (e.g. Hu, Yo, Ma2, CV2/CRMP5, Ri, amphiphysin, GAD), surface neuronal antigens (NMDAR, CASPR2, LGI1. GABAb, DPPX) and glia targets (e.g. AQP4, MOG). The presence of antibodies in both the serum and cerebrospinal fluid (CSF) of patients is reached using different techniques such as: indirect immunochemistry, indirect immunofluorescence,

PI's biosketch

Scopus ID 8655110800
Following a MD degree at the University of Padua in 2001, Prof. Stefano Sartori specialised in Paediatrics, with a specific focus on Paediatric neurology in 2006.
Prof. Sartori subsequently enrolled in a PhD programme in Paediatric 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, and in collaboration with Prof. Giometto, Dr. Zuliani, Dr. Zoccarato, Dr. De Gaspari, and Dr. Nosadini has been contributing to establishing a Neuroimmunology research group in Veneto.
Prof. Sartori has been the coordinator of the Study Group on Autoimmune Epilepsies of the Italian League Against Epilepsy since 2014, and has been part of the Neuroimmunology Study Group of the Italian Society of Pediatric Neurology since 2015. Currently, Prof. Sartori works at the Dept. of Women's and Children's Health, University of Padua, where he coordinates the Paediatric Neurology and Neurophysiology programme. Prof. Sartori is also a Professor at the training programmes in Paediatrics, Child Neuropsychiatry and Rehabilitation of the University of Padua, where he is course leader in Paediatric Neurology and Epilepsy.
During his career, Dr. Sartori has co-authored over 125 indexed peer-reviewed articles and over 200 proceedings in Paediatrics, Paediatric Neurology, Epilepsy and Neuroimmunology.

immunoblot and cell-based assay (CBA).
Regarding the spectrum of autoimmune neurological disorders, despite the increasing spectrum of antibody reactivity already described, some subjects affected by suspected autoimmune encephalopathies (AE) still test negative for onconeural, GAD and NsAbs (i.e. "seronegative" autoimmune encephalitis patients). This negativity does not necessarily imply the absence of antibody markers, rather possible novel uncharacterized auto-antibodies. Further studies are thus warranted to find new antigens related to the immunological response in neurological patients with possible autoimmune disorders. Based on this hypothesis, our idea is to identify new proteins responsible for the autoimmune response in "seronegative" patients and to investigate the prevalent pathogenic mechanisms and possibly the pathways activated by antibodies.
Furthermore, our group received fundings to support a project regarding Pediatric 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 (Yan K. et al., 2020). PedMS clinical presentation at onset could overlap with recurrent or multiphasic acute disseminated encephalomyelitis (ADEM), neuromyelitis optica (NMO), 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. Acquaporin-4 (AQP4), a water channel mainly present on astrocytes, is the most important target in NMO, while Myelin Oligodendrocyte Glycoprotein (MOG) is the main target in pediatric ADEM and other syndromes that 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) to identify novel imaging and biological markers in pedMS children; ii) to understand the type of immune cells involved in the pathogenesis of this disease and iii) eventually find novel therapeutic target molecules.

Selected publications

Nosadini M, Eyre M, Molteni E, Thomas T, Irani SR, Dalmau J, Dale RC, Lim M; International NMDAR Antibody Encephalitis Consensus Group, Anlar B, Armangue T, Benseler S, Cellucci T, Deiva K, Gallentine W, Gombolay G, Gorman MP, Hacohen Y, Jiang Y, Lim BC, Muscal E, Ndondo A, Neuteboom R, Rostásy K, Sakuma H, Sartori S, Sharma S, Tenembaum SN, Van Mater HA, Wells E, Wickstrom R, Yeshokumar AK. Use and Safety of Immunotherapeutic Management of N-Methyl-d-Aspartate Receptor Antibody Encephalitis: A Meta-analysis. JAMA Neurol. 2021 Nov 1;78(11):1333-1344. doi: 10.1001/jamaneurol.2021.3188. PMID: 34542573; PMCID: PMC8453367.

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Gastaldi M, Mariotto S, Giannoccaro MP, Iorio R, Zoccarato M, Nosadini M, Benedetti L, Casagrande S, Di Filippo M, Valeriani M, Ricci S, Bova S, Arbasino C, Mauri M, Versino M, Vigevano F, Papetti L, Romoli M, Lapucci C, Massa F, Sartori S, Zuliani L, Barilaro A, De Gaspari P, Spagni G, Evoli A, Liguori R, Ferrari S, Marchioni E, Giometto B, Massacesi L, Franciotta D. Subgroup comparison according to clinical phenotype and serostatus in autoimmune encephalitis: a multicenter retrospective study. Eur J Neurol. 2020 Apr;27(4):633-643. doi: 10.1111/ene.14139. Epub 2020 Jan 14. PMID: 31814224.

Neuronal Circuits in Developmental Disorders

Junior PI: **Manuela Allegra**

Team members

Manuela Allegra
Junior Principal Investigator

Gabriele Deidda
Assistant Professor

Stefano Varani
Post-doctoral Researcher

Livia Vignozzi
PhD student

Alessandra Maria Testa
PhD student

Research activity

In our laboratory, the main research interest is centered on the field of neuroplasticity, converging towards its behavioral consequences, namely learning and memory. We study the neural mechanisms underlying the capability of the brain to rewire itself in response to environmental pressures, as this plasticity of the nervous system is crucial throughout the lifespan, refining sensory systems during development, mediating learning and memory and 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 (who recently passed away), 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.

PI's biosketch

Scopus ID: 54985377400
Dr. 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. She then received the Ph.D. in Neuroscience 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, she consolidated her expertise on the rodents visual system and she built a solid background on the processes of adult hippocampal neurogenesis and hippocampal hyperexcitability. 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. 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 now started her own research group with a starting grant from Fondazione CaRiPaRo (Moving Researchers for Pediatrics), in collaboration with Prof Antonella Viola.

Selected publications

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.

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Allegra M, Spalletti C, Vignoli B, Azzimondi S, Busti I, Billuart P, Canossa M, Caleo M. Pharmacological rescue of adult hippocampal neurogenesis in a mouse model of X-linked intellectual disability. Neurobiology of Disease, 2017 Apr;100:75-86.

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Transplantation Immunology

PI: **Emanuele Cozzi**

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 paediatric and adult transplantation. In particular, its research is mainly focused on overcoming immunological incompatibilities that limit access of many patients to transplantation. The Transplantation Immunology group is dedicated to the following lines of research:

Team members

Emanuele Cozzi
Principal Investigator

Marta Vadori
Senior Post Doctoral Researcher

Elisa Cuciz
Research Fellow

Edi Lanza
Project Manager

Role of non-HLA antibodies in the premature failure of pediatric renal transplants

Renal transplantation represents the ideal treatment option for children in terminal kidney failure. However, in half of the cases graft survival will not reach 20 years. Several immunological and non-immunological reasons underlie such a premature organ failure and antibody-mediated rejection is the first cause of graft loss after the first year. Whilst anti-HLA antibodies are the primary cause of antibody-mediated rejection, recently other, non-HLA antibodies have been identified. At this stage, the detrimental role on graft survival of anti-AT1R antibodies has been demonstrated both in pediatric and adult renal transplantation, whilst the potential harmful role of anti-ETAR antibodies remains purely speculative. In this light, using biological specimens (blood samples and biopsies) from a unique cohort of >150 patients from two major pediatric transplant centers, this translational study will unequivocally assess the possible causative role of anti-ETAR immunity in premature pediatric renal transplant failure.

Contributors: Elisa Benetti, Luisa Murer, Marco Spada, Luca dello Strologo, Padua University Hospital, Bambino Gesù Hospital (Rome) and IRP.

PI's biosketch

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 specialisation as an allergist and clinical immunologist in the same University and a PhD from the University of Cambridge in 2000.

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 utilisation 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 200 manuscripts in international journals (citations: 7.311; h-index: 40). 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 lead several multicentre 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 since 2020 he is 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).

Role of non-HLA antibodies in cardiovascular diseases

Non-HLA antibodies, such as anti-AT1R and anti-ETAR, are functional agonist autoantibodies which are able to activate their target receptors and mediate vasoconstriction, extracellular matrix remodelling and activate proinflammatory cascades. The latter activities could play a central role in many cardiac diseases. However, to date there are only very few data suggesting that these non-HLA antibodies may constitute an independent risk factor for adverse outcomes in cardiovascular diseases. Currently the Transplantation Immunology lab is involved in the measurement of non-HLA antibodies and in the evaluation of their functional activities in samples from different cohorts of patients with coronary diseases. These data will clarify the possible pathophysiological role for non-HLA autoantibodies and may lead to important therapeutic interventions.

Contributors: Prof Francesco Tona, Prof Sabino Iliceto, Prof Laura Iop. Padua University Hospital.

Cell transplantation therapies in the treatment of neurodegenerative disorders

Chronic neurological disorders encompass a number of diseases affecting the functionality of various areas of the central nervous system, whose incidence is expected to rise with increased life expectancy. A potentially promising approach to the treatment of such diseases is represented by the application of cell transplantation therapies with the aim to replace, repair or regenerate injured areas, or to modulate and dim the inflammatory processes possibly associated. Over the years our group has developed a tremendous expertise in this research area, particularly in the context of both Parkinson's and Huntington's disease (HD). We have contributed to the progress in this field with the development of challenging preclinical models in primates and have set up assays to enable the fine monitoring of the cellular and humoral immune responses following neural cell transplantation in such models. Currently we are also involved in the design of a new UK-based clinical trial regarding transplantation of human neural precursors in a cohort of patients with HD.

Contributors: Philippe Hantraye, Romina Aron Badin, Padua University Hospital and CEA Neurodegenerative Diseases Laboratory, Fontenay-aux-Roses (Parigi)

Immune responses and Covid-19

Our group has always been very interested in the management of the immune response following solid organ transplantation. Recently we have been involved in the care of transplant recipients who developed Covid-19 and we were able to report in the literature 2 of the first 3 cases of SARS-CoV-2 driven pneumonia in lung transplant recipients. At this stage, our group has developed a specific research interest in this area and we are contributing to research initiatives aimed at in increasing the insight into the role of immune responses on the outcome of transplant recipients with Covid-19.

Contributors: Fiorella Calabrese, Federico Rea, Anna Maria Cattelan, Daniele Lilleri, Padua University Hospital and Hospital of Pavia.

Selected publications

Cozzi E, Schneeberger S, Bellini MI, Berglund E, Böhmig G, Fowler K, Hoogduijn M, Jochmans I, Marckmann G, Marson L, Neuberger J, Oberbauer R, Pierson RN 3rd, Reichart B, Scobie L, White C, Naesens M; ESOT Workstream 1 of the TLJ (Transplantation Learning Journey) project. Organ transplants of the future: planning for innovations including xenotransplantation. Transpl Int. 2021 Nov; 34:2006-2018.

Deml L, Hüber CM, Barabas S, Spindler T, Cozzi E, Grossi P. Stimulatory Effect of CMV Immunoglobulin on Innate Immunity and on the Immunogenicity of CMV Antigens. Transplant Direct. 2021 Oct; 22;7:e781.

Aubert O, Yoo D, Zielinski D, Cozzi E, Cardillo M, Dürr M, Domínguez-Gil B, Coll E, Da Silva MI, Sallinen V, Lemström K, Midtvedt K, Ulloa C, Immer F, Weissenbacher A, Vallant N, Basic-Jukic N, Tanabe K, Papatheodoridis G, Menoudakou G, Torres M, Soratti C, Hansen Krogh D, Lefaucheur C, Ferreira G, Silva HT Jr, Hartell D, Forsythe J, Mumford L, Reese PP, Kerbaul F, Jacquelinet C, Vogelaar S, Papalois V, Loupy A. COVID-19 pandemic and worldwide organ transplantation: a population-based study. Lancet Public Health. 2021 Oct; 6:e709-e719.

Salam Bashir, Leopold K Fezeu, Shani Leviatan Ben-Arye, Sharon Yehuda, Eliran Moshe Reuven, Fabien Szabo de Edelenyi Imen Fellah-Hebia, Thierry Le Tourneau, Berthe Marie Imbert-Marcille, Emmanuel B Drouet, Mathilde Touvier, Jean-Christian Roussel, Hai Yu, Xi Chen, Serge Hercberg, Emanuele Cozzi, Jean-Paul Soulillou, Pilar Galan, Vered Padler-Karavani. Association between Neu5Gc carbohydrate and serum antibodies against it provides the molecular link to cancer: French NutriNet-Santé study. BMC Med. 2020 Sep;18:262.

Cozzi E, Faccioli E, Marinello S, Loy M, Congedi S, Calabrese F, Romagnoli M, Cattelan AM, Rea F.COVID-19 pneumonia in lung transplant recipients: report of two cases. Am J Transplant. 2020 Oct; 20; 2933-2937.

Research area

Medical Biotechnology

Coordinator: **Prof. Marco Agostini**

The Medical Biotechnology research area comprises three groups, all 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 Gastronterology) 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 PostDoctoral 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 tumours. After moving to the Netherlands in 2005 for a PostDoctoral 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 tecniques 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 2.399.000,00 euros of which 1.563.000,00 euros as Coordinator and/or Principal Investigator.

Official h-index and publications: 29 (according to Scopus). He has published 126 articles in peer reviewed scientific journals.

Biamet

PI: **Elisa Cimetta**

Team members

Elisa Cimetta
Principal Investigator

Maria Rosaria Esposito
Lab Manager

Pina Fusco
PostDoctoral Researcher

Veronica Zingales
Visiting PostDoctoral Researcher

Francisco Juan Marti Quijal
Visiting PhD Student

Sara Micheli
PhD Student

Luca Zanella
PhD Student

Noemi Torriero
PhD Student

Anna Fietta
PhD Student

Caterina Piunti
PhD Student

Federico Maggiotto
Research Associate

Eleonora Zanré
Research Associate

Research activity

Prof. Cimetta's main research interests focus on the application of engineering principles to biological studies. Prof. Cimetta's laboratory specializes in the design and development of advanced microscale technologies and microbioreactor platforms (uBR) 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 to develop and test novel drugs, therapies and therapeutic strategies.
Classically used cell culture approaches do not reproduce the complexity of the in vivo interaction between cells and the dynamic microenvironment (uEnv), limiting the understanding of its precise role in biology. An engineering approach to biological studies brings several advantages over existing techniques since it allows exerting a precise and versatile control over the parameters characterizing a biological system. Microscale systems possess intrinsic characteristics such as small transport distances, small volumes handling, and the ability to introduce and measure fast dynamic changes in the soluble environment. Transport phenomena become more easily predicted and mathematically described and are amenable to computational modeling. These characteristics ultimately translate into the capability of allowing precise control and fine-tuning of variables in a large parameter space, thus providing rational approaches to the optimization of culture conditions and reducing the intrinsic variability of biological phenomena.
The need for innovative approaches capturing the complexity of the interactions between cells and their uEnv is particularly felt in the field of tumor biology, as tumors are extremely heterogeneous and capable of conditioning both the local uEnv and that of distant organs. Exosomes, small vesicles secreted by the tumor, are a fundamental form of communication between cancer and the surrounding environment, influencing a host of target cells locally and at a distance. In one type of cancer, Neuroblastoma (NB), exosomes seem to be particularly important; yet, current approaches to study their role in NB communication with local and distant µEnv fall short of providing clear answers.
Supported by an ERC Starting Grant, the main focus of the laboratory is now on the design and development of advanced microscale technologies and µBRs as in vivo-like systems to probe the role of Neuroblastoma-derived extracellular vesicles (EVs) in cancer dissemination. The laboratory develops and implements advanced platforms, powerful means to enable highthroughput screening of environmental effectors of tumor behavior in a more realistic setting. From fundamental data gathered through "traditional" biological studies, we highlighted peculiar characteristics of vesicles secreted by NB cells cultured in hypoxic environments. Evidence of increased EVs-mediated aggressive behaviors were observed both in naïve cancer cells and in target cell types, representative of the most frequent metastatic NB sites. We are now using our know-how in advanced technological solutions to deepen our knowledge of such phenomena. Key studies aim at:

PI's biosketch

Prof. Cimetta graduated in Chemical Engineering and obtained her PhD in Industrial Engineering at the University of Padua. In 2010, after a 1 year PostDoc in Padova under the supervision of Prof. Elvassore, she won an international selection for a position of Associate Research Scientist (ARS) at the Biomedical Engineering Dept. at Columbia University, New York, NY, USA. She held this position between 2010 and 2013 at the "Laboratory for Stem Cells and Tissue Engineering" under the supervision of Prof. Vunjak-Novakovic. During the same time span, the New York Stem Cell Foundation awarded her one of their prestigious fellowships. In 2011, Prof. Cimetta obtained a certification for the successful completion of the Postbaccalaureate Business Program at Columbia University. She is a co-founder of EpiBone, a Columbia University spinoff that aims at generating custom-shaped bone and osteochondral tissues starting from the patients' own stem cells.
In 2016, she won a selection for an Assistant Professor (RTDb) position at the Dept. of Industrial Engineering of the University of Padua. She is now Associate Professor in the same department. In 2017, she was granted an ERC Starting Grant from the European Union, the most prestigious research award for young scientists in Europe. During the same year, she also joined the IRP, an opportunity that enabled to greatly strengthen the biological aspects and significance of her laboratory's research.

- testing gradients of NB-derived EVs on microstructured 2D and 3D cultures under different oxygen conditions to better define how hypoxia influences physicochemical properties of EVs and of target cultures
- using 3D spheroids (formed by NB cells alone and multiple cell populations) to evaluate the behavior of core vs shell portions and tie it to both oxygen partial pressures and exposure to defined EVs concentrations
- testing long-range effects of NB derived EVs on target tissues, metastatic sites of NB
- loading non-cancer EVs with selected drugs
- 3D bioprinting of vascularized tissue mimics cellularized with endothelial, mesenchymal and NB cells to better study and evaluate their interactions

These technologies can bridge the gap between standard in vitro techniques and in vivo biological phenomena, providing a novel tool to understand certain aspects of cancer biology and ultimately advancing our understanding of NB.

The laboratory is also actively engaged in collaborative projects such as:

- development of advanced tumor microenvironment-on-a-chip: a microfluidic multi-cellular model based on patient-specific ECM-derived hydrogels
- development of co-culture enabling devices to study the interactions between the immune and the tumor environments
- effect of the composition of bioactive bone-like scaffolds as therapeutic agents.

Selected publications

Zingales V, Torriero N, Zanella L, Fernández-Franzón M, Ruiz MJ, Esposito MR, Cimetta E. "Development of an in vitro neuroblastoma 3D model and its application for sterigmatocystin-induced cytotoxicity testing". Food and Chemical Toxicology. Volume 157, November 2021 Article number 112605.

Fusco P, Mattiuzzo E, Frasson C, Viola G, Cimetta E, Esposito MR, Tonini GP. "Verteporfin induces apoptosis and reduces the stem cell-like properties in Neuroblastoma tumour-initiating cells through inhibition of the YAP/TAZ pathway". Eur J Pharmacol. 2020 Dec 18;893:173829.

Bova L, Billi F, Cimetta E. "Mini-review: advances in 3D bioprinting of vascularized constructs". Biol Direct. 2020; Nov; 15(1).

Fusco P, Parisatto B, Rampazzo E, Persano L, Frasson C, Di Meglio A, Leslz A, Santoro L, Cafferata B, Zin A, Cimetta E, Basso G, Esposito MR, Tonini GP. Patient-derived organoids (PDOs) as a novel in vitro model for neuroblastoma tumours. BMC Cancer. 2019 Oct 21;19(1):970.

Cimetta E, Sirabella D, Yeager K, Davidson K, Simon J, Moon RT, Vunjak-Novakovic G. 2013. "Microfluidic bioreactor for dynamic regulation of early mesodermal commitment in human pluripotent stem cell". Lab on a Chip, 13(3): 355-64.

NanoInspired Biomedicine

PI: **Marco Agostini**

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;
- 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;
- study the role of tumour extracellular matrix (ECM) in affecting CRC cell behaviour and identify patient-specific sensitivity to chemotherapy agents, a step towards a personalized approach to predict response to treatment superior to conventional 2D cancer cell cultures.
- combination of already established technologies for patient-derived organoid culture of primary cancer cells (PDOs) and same-patient ECM-derived scaffolds obtained through decellularization.

Team members

Marco Agostini
Principal Investigator

Edoardo D'Angelo
PostDoctoral Researcher

Francesca Sensi
PostDoctoral Researcher

Asia Marangio
PhD student

Andrea Biccari
Fellow Researcher

Pietro Traldi
Emeritus Professor

PI's biosketch

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 PostDoctoral 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 tumours. After moving to the Netherlands in 2005 for a PostDoctoral 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 tecniques 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 2.399.000,00 euros of which 1.563.000,00 euros as Coordinator and/or Principal Investigator.
Official h-index and publications: 29 (according to Scopus). He has published 126 articles in peer reviewed scientific journals.

- 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;
- bio-analytical methods development and validation following FDA/EMA guidelines for targeted analysis of lipids, proteins/peptides, drugs (PK and TDM), and metabolites by mass spectrometry.

Selected publications

Gentilin E, D'Angelo E, Agostini M(*) and Astolfi L. (* Corrisponding Authors)
Decellularized normal and cancer tissues as tools for cancer research.
Cancer Gene Therapy. 2021.

Coletta S, Lonardi S, Sensi F, D'Angelo E, Fassan M, Pucciarelli S, Valzelli A, Biccari A, Vermi W, Della Bella C, Barizza A, D'Elios MM, de Bernard M, Agostini M (*) and Codolo G. (*Corrisponding Authors) Tumor Cells and the Extracellular Matrix Dictate the Pro-Tumoral Profile of Macrophages in CRC. Cancers. 2021, 13(20), 5199

Nanodelivery Systems Face Challenges and Limitations in Bone Diseases Management.
Giordano F, Lenna S, Rampado R, Brozovich A, Hirase T, Tognon MG, Martini F, Agostini M, Yustein JT, Taraballi F. Advanced Therapeutics. 2021.

Recellularized Colorectal Cancer Patient-derived Scaffolds as in vitro Pre-clinical 3D Model for Drug Screening. Sensi F, D'Angelo E, Piccoli M, Pavan P, Mastrotto F, Caliceti P, Biccari A, Corallo D, Urbani L, Fassan M, Spolverato G, Riello P, Pucciarelli S, Agostini M.
Cancers (Basel). 2020

Patient-Derived Scaffolds of Colorectal Cancer Metastases as an Organotypic 3D Model of the Liver Metastatic Microenvironment. 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.
Cancers (Basel). 2020.

Research area

Pediatric Hematology, Oncology and Hematopoietic Cell&Gene Therapy

Coordinator: **Prof. Alessandra Biffi**

The research area in pediatric hematology, oncology and hematopoietic cell and gene therapy belongs to the Division of Pediatric Hematology, Oncology and Stem Cell Transplant of the Azienda Ospedale Università Padova 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 and oncological 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.

Fondazione ISTITUTO DI RICERCA PEDIATRICA

Prof. Alessandra Biffi

Coordinator Pediatric hematology, oncology and hematopoietic cell&gene therapy Area

Scopus ID: 7003906961

Prof. Biffi is head of the Pediatric Oncohematology and Stem Cell Transplant Division (clinics, diagnostic and research laboratories) at the Azienda Ospedale Università Padova since October 2018 and she coordinates the research area on Oncohematology, Stem Cell Transplant and Gene Therapy at the Istituto di Ricerca Pediatrica. Previously, Prof. Biffi 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 Milan, where she also practiced as attending physician and head of a clinical unit in Pediatric Stem Cell Transplant and Immunohematology (up to 2015). Prof. Biffi has trained over 40 fellows and PostDoctoral fellows as well as numerous residents and medical students in her laboratory and clinics, the majority of whom are still in academic medicine. Prof. Biffi has published over 120 peer-reviewed manuscripts and textbook chapters. She has extensive clinical experience in pediatric stem cell transplant and in early phase cell and gene therapy clinical trials. Prof. Biffi's preclinical and clinical research is dedicated to enhancing the efficacy of Hemopoietic Stem Cells (HSC)-based therapeutic approaches for inherited disorders with severe nervous system involvement by: i) fostering brain microglia replacement by donor cells after HSC transplantation upon detailed understanding of this phenomenon (Capotondo et al., PNAS 2012), and ii) enhancing the potential of enzyme delivery to the affected nervous system by means of the gene corrected progeny of the transplanted, lentiviral vector (LV)-transduced HSCs (Biffi et al., Science 2013; Sessa et al., Lancet 2016). Additional research activities comprise of novel exploratory projects on the therapeutic role of engineered microglia in neurodegenerative diseases, HSC gene therapy application to autoimmune disorders and novel exploratory projects on targeted cancer therapy in collaboration with local PIs. She is actively collaborating with biotech companies in the gene therapy field, she is one of the founders and scientific advisors of Altheia Science s.r.l., a spinoff company of the University of Padua that established sponsored research agreements with the Dept. of Women's and Children's Health.

Advanced Diagnostics and Target Discovery in all Immunophenotypic Plasticity of Pediatric B Acute Lymphoblastic Leukemia: a Genomic Study

PI: **Barbara Buldini**

Research activity

Acute leukemia is the most common cancer in childhood, accounting for about 30% of all neoplastic diseases under 20 years of age. Although primary disease outcomes are significantly improved in acute lymphoblastic and myeloid leukemia in the last few decades, relapse still occurred in a non-negligible proportion of patients and is associated with a dismal prognosis. MFC plays a central role in pediatric ALs diagnostics through immunophenotyping and DNA-index assessment at diagnosis and MFC-MRD monitoring during therapy. To note, MFC-MRD is referral technique for MRD monitoring in pediatric AML and ALL. Our group has several aims: first, to improve blast characterization at diagnosis, identify early AL subtypes associable with specific biological behaviors and, consequently, specific risk groups and modulate therapy intensity. Second, to study and characterize those leukemias whose lineage is not clearly identifiable, to address them to the proper therapeutic regimen. Third, to identify and describe anomalous immunophenotypic behavior like transient lineage switch or antigen downregulation: this may hamper MFC-MRD assessment and have a significant impact on therapy choice and intensity. By defining the immunophenotypic changes, creating standard operating procedures to analyze them, finding alternative and stable markers, and discovering the biology subtending these phenomena, we aim to better risk assignment and tailor therapy.

Team members

Barbara Buldini
MD, PhD, Principal Investigator

Elena Varotto
MD, PhD

Silvia Bresolin
PhD

Chiara Frasson
PhD

Giulia Gomiero
PhD student

PI's biosketch

Scopus ID: 10639186000
Prof. Barbara Buldini, MD, PhD, is an internationally recognized expert of pediatric acute leukemias (AL) laboratory diagnosis and response-to-therapy monitoring. The main topic of her pluriannual diagnostic and research activity is multiparametric flow-cytometry (MFC) application to pediatric AL blasts characterization at diagnosis, relapse and measurable residual disease (MRD) assessment during therapy. Thanks to her expertise in the field and numerous national and international connections, she has profoundly impacted on pediatric ALs diagnostics and prognosis: she improved AL subgroups definition, which is necessary for leading to the proper therapy administration; she produced standard operating procedures for immunophenotyping and MFC-MRD monitoring; she described specific immunophenotype-genotype correlations for identifying potential genetic alterations from blast immunophenotypic patterns precociously; she investigated the biological basis of peculiar immunophenotypic behaviors like lineage-switch; she worked on antigen discovery to better MFC-MRD monitoring and identified potential new targets for immunotherapy. Additionally, she is one of the most recognized experts on pediatric AML MFC-MRD and strongly contributed to its adoption in AML therapeutic protocols.
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 the PI (Principal Investigator) of the Morphology and Flow Cytometry group at the Pediatric Onco-Hematology Laboratory in Padova (ISO9001:2015 Certified Lab), the AIEOP reference laboratory for the diagnosis (immunophenotype and DNA-index) and MFC-MRD assessment of childhood acute leukemias (since 2018), with about 1500 and 1000 reports per year, respectively.
She is the scientific director of immunophenotype and MFC-MRD in ALL at first diagnosis (AIEOP-BFM ALL trials), relapse (InterALL 2010), Infant ALL and AML (AIEOP LAM 2013, since 2012).
She is member of numerous national and international groups/associations/societies. She coordinates the AIEOP-BFM-AML Flow MRD group with Professor Dworzak (standardization of MFC-MRD in pediatric AML-since 2011).

Fourth, to improve MFC-MRD accuracy. Indeed, there are still some immunophenotypic subtypes, especially T-ALL and AML, for those MFC-MRD monitoring reaches a suboptimal sensitivity due to blast immunophenotype overlapping with those of normal precursor cells. The discovery of new markers through transcriptomics and proteomics application to specific subtypes of blast cells and normal lineage-related precursors may potentially overcome these limits. Fifth, MFC immunophenotype and MRD are still hampered by operator-dependent interpretation and variability in the absence of a rigorous standardization on the whole procedure (from sample preparation to data interpretation), expert operators, and continuous

She has been delegated with Professor Dworzak by the European Pediatric AML Study Group (BFM/NOPHO/LAME/AIOEP/DCOG/BSPHO/UK) for defining "Response-to-treatment/MRD Immunophenotyping" in AML (since 2012). She is member of the Trial Steering Committee of AIEOP-BFM ALL (since 2018) and AIEOP-BFM AML 2020 (since 2019) protocols.
She is the PI for AIEOP of the Ponte di Legno projects on Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) and Gamma-Delta-positive T-ALL (since 2019) and of the Ambi Study, Rreport on Acute Leukemia of Ambigous LineageLeucemie Acute di Lineage Ambiguo in collaboration with con iBFM FLOW network (coordinated by Prof. O. Hrusak, Prague, Repubblica Ceca), ALLIC e St. Jude's Children Hospital, Memphis, Tennessee, USA.
She is involved in the writing of n.4 chapters of the WHO (World Health Organization) since 2021.
She is member of the ITCC Hema Committee for discussion on innovative therapies for children with Cancer and the iLTB core team for sharing and discussion of cases with resistant Acute Lymphoblastic Leukemia, since 2021. She leads the Padua Lab as AIEOP-BFM partner unit in the training by MFC of ALLIC Labs, groups with limited economic resources that use essentially MFC for monitoring MRD in ALL treatment. She is Tutor in the "FlowInLab project" [https://elearning.unipd.it/flowinlab/], Moodle-based platform developed in collaboration with DLM Office, University of Padua as a Flow Cytometry training service.
She coordinates and collaborates to several national and international projects.
Publications on peer reviewed Journals: 68 (Scopus h-index:21; Citations:1957)

inter-and intra-laboratory quality control and quality assurance processes. Our group, together with iBFM laboratories, focuses on continuous training of minor laboratories both in ALL and AML.

Selected publications

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Maurer-Granofszky M, Schumich A, Buldini B, Gaipa G, Kappelmayer J, Mejstrikova E, Karawajew L, Rossi J, Suzan AÇ, Agriello E, Anastasiou-Grenzelia T, Barcala V, Barna G, Batinić D, Bourquin JP, Brüggemann M, Bukowska-Strakova K, Burnusuzov H, Carelli D, Deniz G, Dubravčić K, Feuerstein T, Gaillard MI, Galeano A, Giordano H, Gonzalez A, Groeneveld-Krentz S, Hevessy Z, Hrusak O, Iarossi MB, Jáksó P, Kloboves Prevodnik V, Kohlscheen S, Kreminska E, Maglia O, Malusardi C, Marinov N, Martin BM, Möller C, Nikulshin S, Palazzi J, Paterakis G, Popov A, Ratei R, Rodríguez C, Sajaroff EO, Sala S, Samardzija G, Sartor M, Scarparo P, Sędek Ł, Slavkovic B, Solari L, Svec P, Szczepanski T, Taparkou A, Torrebadell M, Tzanoudaki M, Varotto E, Vernitsky H, Attarbaschi A, Schrappe M, Conter V, Biondi A, Felice M, Campbell M, Kiss C, Basso G, Dworzak MN, On Behalf Of I-Bfm-Flow-Network. An Extensive Quality Control and Quality Assurance (QC/QA) Program Significantly Improves Inter-Laboratory Concordance Rates of Flow-Cytometric Minimal Residual Disease Assessment in Acute Lymphoblastic Leukemia: An I-BFM-FLOW-Network Report. Cancers (Basel). 2021 Dec 6;13(23):6148. doi: 10.3390/cancers13236148.

Advanced Diagnostics and Target Discovery in all Gene Expression Profiling and Next Generation Sequencing in Jmml and B all

PI: **Silvia Bresolin**

Research activity

The goal of our group is to identify at genomic and transcriptomic level the networks of acquired and inherited aberrations that drive the development and the progression of hematological disorders. Especially, 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.

The main projects of the group are:

Biology of somatogenetic architecture and clonal evolutionof Juvenile Myleomonocytic Leukemia

Juvenile Myelomonocytic Leukemia (JMML) is a rare form of pediatric hematological disease, characterized by excessive proliferation of monocytic and granulocytic cells, an aggressive disease course and a high risk of treatment failure. This project investigates the somatogenetic architecture of JMML to understand the grade of tumor heterogeneity in the bone marrow of patients and the sequential acquisition of mutations and transcriptomic alterations in the hematopoietic cell hierarchy to reconstruct the clonal phylogeny, its correlation within clinically distinct groups of JMML patients and the impact of mutational signature as imply in JMML etiology. The study also focuses on the development of an in vitro and in vivo model able to recapitulate key pathophysiology features of JMML disease, to trace the clone's dynamicity and to sustain the proliferation of JMML propagating cells.

Circular RNA (circRNA) in acute lymphoblasticleukemia

Circular RNAs (circRNAs) are an emerging class of stable transcriptome members that participate in circuits competing for binding of miRNAs, RNA-binding proteins (RBPs) or translation initiation and are part of key oncogenic axes. RNA-seq identified thousands of circRNAs with developmental stage- and tissue-specific expression showing that circRNAs are abundantly expressed in thehematopoietic compartment. The project efforts in the identification and validation of specific circRNAs dysregulated in acute leukemia and predict circRNA functions

Team members

Silvia Bresolin
Principal Investigator

Alice Cani
Post Doctoral researcher

Caterina Tretti
PhD student

Alberto Peloso
Graduate student

Cristina Borin
Gradute student

PI's biosketch

Dr. Bresolin started her academic career with a Medical Biotechnology degree in 2007 at University of Padua. She obtained her PhD in Developmental Medicine and Programming Sciences - Immunology, Hematology-Oncology, Genetics in 2012. During her PhD, she attended the laboratory of Oncohematology where she focused her research on advancement of new molecular technologies aiming to improve the prognosis and diagnosis of pediatric patients with Myelodyspalsia and Juvenile Myelomonocytic 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, Dr. Bresolin attended the laboratory directed by Prof. Weiss at Children's Hospital of Philadelphia, PA, USA working on the generation and maintenance of leukemia iPS cells. She is member of the Italian Association of pediatric oncohematology (AIEOP) working group on Juvenile myelomonocytic leukemia (JMML) and Myelodysplastic syndromes (MDS) and she is a member of the European Working Group of MDS and Severe Aplastic Anemia (SAA) (EWOG-MDS-SAA). Dr. Bresolin's 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 approaches, also at single cell level. She is also involved in the genetic diagnosis and management of patients with hematological disease and cancer predisposition. Dr. Bresolin is involved in several national and international collaborations for omics data generation and analysis. She is actively involved in projects on the functional characterization of circRNA in Mixed lineage leukemia (MLL) rearranged leukemia and on the genetic variations predisposing to leukemia in childhood. From 2018, she is Research Associate at the Oncohematology Clinic and Laboratory at the Dept. of Women's and Children's Health Azienda Ospedale Università Padova. Dr. Bresolin is author of more than 60 publications in peer reviewed international journals.

and interactions by computational analysis and experimental studies. Ongoing studies as part of a collaborative study with Prof. Stefania Bortoluzzi, are characterizing the circome of these patients with respect also to normal hematopoiesis to better understand their origin and role in the disease to be further integrated with known disease associated molecular networks.

Role of exosomes in treatment response kinetics and microenvironment modulation in B-ALL leukemia

The group is using different approaches to identify novel diagnostic and prognostic markers to improve diagnosis, prognosis and patients' follow-up so to facilitate the introduction of tailored treatment regimens in selected groups of patients. Circulating exosomes represent a promising source of biomarkers and evidence suggests their influence in the crosstalk with tumor andmicroenvironment cells. We are now studying by RNA-seq the small RNA cargo from isolated from plasmatic exosomes at diagnosis and during follow-up in a cohort of B-ALL and lymphoma patients as part of a collaborative study. Furthermore, we will investigate exosome-mediated sRNAs transfer from the tumor microenvironment and leukemia cells and vice versa using an in vitro model.

Characterization and clonal evolution of high risk B-ALL

In the last decade a great advance in the comprehension of the genetic and biological bases of childhood leukemia has been achieved. Genomic analysis has improved the risk stratification and therapy but about 20% of B-Cell Acute Lymphoblastic Leukemia (B-ALL) patients present treatment resistance and relapse. Our group is involved in the identification of genomic and transcriptomic alterations of high-risk B-ALL patients and in particular of therapy and relapsed patients. We characterize by different omics approach (exome, RNA-seq, gene expression profiling) drive mutations in B-ALL patients to identify drug resistance mechanisms and clonal structure to trace clonal dynamicity between diagnosis and relapse. These findings are functional validated by different modelling to guide leukemia re-search to direct patients care in the oncohematology ward.

Selected publications

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Advanced Diagnostics and Target Discovery in all Phosphoproteomics for all Diagnostics and Research

Junior PI: **Valentina Serafin**

Research activity

The Phosphoproteomics for ALL diagnostics and research group oversees the Reverse Phase Protein Arrays (RPPA) facility that allows the identification of new disease biomarkers and new therapeutic targets in oncological and non-oncological disease through phosphoproteomic profiling. To do so we dispose of a library of more than 130 validated antibodies belonging to the most commonly 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 have 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). Specifically, in T-ALL 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. The inhibition or the specific gene silencing of this kinase in glucocorticoid resistant cells turn them sensitive to corticosteroids mainly by inhibiting the downstream NFAT transcription factors family. This evidence open a new field of research related to the role of NFATs transcription factors in glucocorticoid resistance in T-ALL. Moreover, this study led to strengthening the collaboration with Prof. Ntziachristos of Ghent University (Belgium) with who we are currently deeply investigatingtranscriptional and post-translational mechanisms controlling acute leukemia initiation, maintenance and resistance to therapy.

Team members

Valentina Serafin
Junior Principal Investigator

Katharina Simon
PhD

Giulia Veltri
PhD student

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. During her PhD she had the opportunity to collaborate with the Oncohaematology Laboratory headed by Prof. Basso where afterward she started her postdoctoral fellowship under the supervision of Dr. Accordiregarding phosphoproteomic profiling of pediatric Acute Leukemias and other not pediatric diseases.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).
In 2019 focusing on the characterization of molecular mechanisms underlying the glucocorticoid resistance in T-ALL she won the My First AIRC Grant, which allowed her to start her independent career as junior principal investigator at the Institute of Pediatric Research "Cittàdella Speranza".
Dr. Serafin's scientific production is based on the publication of 28 original papers, being in 9 of them the first, corresponding or last author with an average IF of 8.6 and an H-index=13.

Finally, thanks to the collaboration with Prof. Sandra Marmiroli of the University of Modena and Reggio Emilia we are also investigating the metabolic profile of specific T-ALL pediatric subgroups and studying a combined metabolic and signaling inhibition approach for the treatment of these patients.
Another field of study of our group regards the research of new potential biomarkers and therapeutic targets in the Graft versus Host Disease (GvHD). Specifically, we focused on the study of the Chronic (cGvHD) form since the diagnosis is based only on physical examination and, when possible, histopathological confirmation. The finding of novel biomarkers for the diagnosis of cGvHD could be used together with standard criteria to significantly improve the diagnosis of this disease.
The group is involved in several national and international collaborative studies focused on the phospho-proteomic profiling of different neoplastic and non-neoplastic diseases.

Selected publications

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Advanced Diagnostics and Target Discovery in Rare Pediatric Solid Tumors

PI: **Gianni Bisogno**

Research activity

The research activity of our group mainly focuses on childhood solid tumors, and is related to i) the molecular characterization and diagnosis of soft-tissue sarcomas (STS); ii) the use of liquid biopsies for prognosis and non invasive disease monitoring of children with soft-tissue and bone sarcomas; and iii) the humoral immuno response activation and activity via autoantibodies production and release. In this context, the team has contributed to identify novel genetic alterations in common and rare pediatric soft-tissue sarcomas, demonstrate novel implications of known negative prognostic factors in children with high-risk sarcomas, and find new markers for disease detection, monitoring and drug response in the blood and bone marrow of cancer patients. In particular our group has led to the omic characterization of low and high aggressive rhabdomyosarcoma tumors by applying high-throughput genomic and proteomic expression approaches, and, in collaboration with other groups of the Istituto di Ricerca Pediatrica (IRP) Città della Speranza, has performed early studies on rhabdomyosarcoma extracellular matrix dissection and manipulation (PMCID: PMC7878542).

Team members

Gianni Bisogno
Principal Investigator

Paolo Bonvini
Senior Researcher

Angelica Zin
Senior Research Assistant

Lucia Tombolan
Senior Research Assistant

Elena Poli
PostDoctoral Researcher

Silvia Lucchetta
PhD student

i) 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 new genetic abnormalities during these the last two years, including point mutations, deletions, amplifications and

PI's biosketch

Scopus ID 7003672935
Prof. Bisogno obtained his MD in 1988, specialized in Pediatrics in 1992 and pursued a PhD in Pediatric Oncology in 1997 at the University of Padua. Prof. Bisogno is Full Professor in Pediatrics at the University of Padua and works at the Division of Oncohematology – Azienda Ospedale Università Padova as Consultant Pediatric Oncologist (1998-current) and Responsible for Solid Tumor Program (2011-current).
His clinical and research activities focus mainly on childhood solid tumors.
Prof. Bisogno is the coordinator of the Italian Soft Tissue Sarcoma Committee (STSC). 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 elsewhere in Italy. He is co-founder of the European pediatric Soft tissue Sarcoma Group (2004) and PI of the EpSSG RMS2005 trial, which has registered patients from 150 European centers and concluded two different randomized studies that have reshaped the treatment of children with rhabdomyosarcoma proving the effectiveness of maintenance treatment,
He is also leader or partner in several projects supported by different institutions, including European Community (EPOC, ENCCA, EXPORNET projects) and coordinator of PARTNER, a recently EU funded project that has allowed the establishment of a European Registry for children with very rare tumors.
Prof. Bisogno is Responsible for the Clinical Trial Office and coordinator of the Ph.D. Program of the Department of Women's and Children's Health, University of Padova, Padova Italy.

Publications: 289 papers with a total impact factor of 1952,223. Citations: h-index of 47 (according to Scopus) – update May 2022

Grants (1999-2021): 12 grants as principal investigator and 10 as co-investigator, including 3 Ricerca Fi-nalizzata, 1 AIRC, 5 European calls.

Memberships: Board member of EpSSG (European paediatric Soft tissue sarcoma Study Group) and EXPeRT (European Cooperative Study on Pediatric Rare Tumors), Scientific Board of the Deutsche Forschungsgemeinschaft (German Research Foundation); representative of the European clinical trial Groups in the Board of the International Society of Pediatric Oncology – Europe (SIOPE) (2013-2018); member of the Innovative Therapy for Children with Cancer Consortium for new drugs research in pediatric oncology; representative for the Hospital of Padua in the Pediatric Cancer European Reference Network (PAEDCAN ERN) (since 2017); member of Associazione Italiana di Ematologia e OncologiaPediatrica (AIEOP).

chromosomal translocations. Up to now, 42 different genetic markers are routinely used by our group to diagnose 18 pediatric cancers, including the most aggressive and less curable rhabdomyosarcoma, Ewing sarcoma and osteosarcoma tumors. In this respect, we've recently contributed to identify a novel variant of EWS-WT1 fusion transcript in a case of desmoplastic small round cell tumor (DSRCT), an extremely rare and aggressive sarcoma that despite the administration of multimodal therapy (surgery, chemotherapy, radiotherapy) remains characterized by a five-year survival rate not exceeding 15% (PMCID: PMC8003219).
Similarly, we have described the first pediatric cases of Salivary Secretory Carcinoma (SSC) with the ETV6-RET fusion (PMCID: PMC8385095). Likewise pediatric and adult SSC carrying the classic ETV6–NTRK3 translocation, ETV6-RET patientsmay benefit from the use of specific small-molecule compound inhibitors in some clinical circumstances, supporting the concept that fusion genes are suitable not only to perform diagnosis but also to tailor novel potential targeted therapies. Finally, we investigated the clinicopathologic features of patients with tissue undifferentiated round cell sarcomas (URCS) harboring BCOR ITD or YWHAE fusions (PMCID: PMC7483745). We succeeded to correlate BCOR and YWHAE genomic aberrations with cancer aggressiveness, providing novel insights in the pathogenesis of this malignancy, while offering new targets for therapeutic consideration.

ii) Our laboratory has gathered experience in blood biomarker research as well, contemplating detection, enumeration and molecular characterization of circulating tumor cells (CTC) in blood, and measurement of circulating tumor DNA/RNA (ctDNA/miRNA) in plasma1. In this respect, we recently analyzed pediatric soft tissue sarcomas for the expression of the cell surface epithelial cell adhesion molecule (EpCAM), demonstrating that EpCAM antigen may be considered a novel marker for sarcoma CTCs enrichment2. We provided evidence that live CTCs are detectable in the peripheral blood of pediatric patients when express markers of both epithelial (EpCAM) and mesenchymal (Desmin, DES) origin. Patients with metastasis at diagnosis have detectable double-positive CTCs (EpCAM+/DES+), as patients with localized disease who experience cancer-related events afterward. Based on theseresults, we applied our approach to patients on therapy. First, we described a case of a boy with ALK-positive inflammatory myofibroblastic tumor (IMT) successfully treated with the ALK inhibitor entrectinib and monitored longitudinally for the presence of CTCs in the peripheral blood. We provided the evidence that CTCs can be detected at diagnosis, and changes in their level over the course of treatment correlate with patient's clinical condition and final outcome3. Second, we compared two renal cell carcinoma (RCC) pediatric patients, at stage I and IV (localized vs. metastatic, respectively)4. Baseline CTCs level and changes during follow-up were consistent with patients' outcome. In case 1 (stage I), CTCs turned negative during therapy, no further treatment was administered and the patient reached complete remission. Conversely, in case 2 (stage IV), CTCs number changed from positive to negative on treatment, turning back to positive over the follow-up period. The child experienced disease progression, developed cerebral metastases and had a fatal outcome. Most importantly, changes in CTCs level preceded clinical manifestations of disease worsening.

iii) Finally, results herein reported refer to the Immune Response Biomarker Profiling (IRBP) analysis we've recently performed in children with aggressive alveolar rabdomyosarcoma (ARMS), namely the autoantibody repertoire assessment in ARMS patients' and age-matched healthy subjects. Differentially immunoreactive antigens were identified using high-throughput protein microarray technology, and patients were distinguished from healthy subjects, as well as patients belonging to different clinical risk groups. According to IRBP analysis, tumor antigens found to be differently immunoreactive in localized and metastatic tumors were used for diagnostics and prognostics purposes. Among novel ARMS antigens, some were selected for expression and immunoreactivity validation analysis in vitro (RMS cell lines) and in vivo (larger cohort of RMS patients). For some antigens, autoantibodies titer correlated with the presence of metastasis at diagnosis, resulting independent predictor of recurrence in patients with poor outcome. Oncoantigens identified by our study opened new avenues for exploitation of promising novel targets of successful immunotherapy approaches.

Selected publications

Tombolan L, Millino C, Pacchioni B, Cattelan M, Zin A, Bonvini P, Bisogno G. Circulating miR-26a as Potential Prognostic Biomarkers in Pediatric Rhabdomyosarcoma. Front Genet. 2020 Dec 10;11:606274. doi: 10.3389/fgene.2020.606274. PMID: 33362864; PMCID: PMC7758343.

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Biology of CNS Pediatric Tumors

PI: **Luca Persano**

Research activity

Current research in Glioblastoma

Glioblastoma (GBM) is the highest-grade glioma, characterized by a rapid growth rate and an extensive infiltration into the surrounding brain tissue. In this context, in the last years the research group focused their interest in unveiling the mechanisms by which GBM tumor microenvironment (i.e. hypoxia) influences the activation of many developmental pathways and how their modulation impacts GBM biology and response to drugs.

In the last years, GBM tumors have been reported to hold a complex landscape of genetic and epigenetic aberrations and to be characterized by the presence of hypoxic and vascular niches where cancer stem cells (CSCs) and bulk cells are stuck at different stages of differentiation within the same mass. Moreover, heterogeneity of GBM tumors is further raised by the extensive infiltration of heterotypic cells into the tumor bulk, in particular endothelial (EC), stromal and immune system cells, but also the composition of extracellular matrix (ECM). Indeed, ECM composition impacts tumor microenvironment and also participates in modulating tumor invasiveness and response to therapy. In this context, in collaboration with the group of Prof. Bonaldo from the Department of Molecular Medicine (University of Padova) we are exploring the expression of ECM components in GBM tumors and already identified Collagen 6 (COL6) as directly correlated with GBM aggressiveness and stem-like features. Our more recent studies in the field are devoted to explore the role of COL6 in controlling GBM cell stemness/differentiation and how its modulation can potentially impact on GBM cell response to chemotherapy.

In addition, it's an already established knowledge that many cancers, including GBM, are driven by tumor-initiating cells and rely on complex interactions with the tumor microenvironment. Standard cell culture conditions fail to recapitulate the original tumor architecture or microenvironmental gradients and are not designed to retain the cellular heterogeneity of parental tumors. For these reasons, we are developing three-dimensional culture systems supporting the long-term growth and expansion

Team members

Luca Persano
Principal Investigator

Elena Rampazzo
Postdoctoral Researcher

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 at University of Padua, 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. In the Laboratory of Molecular Immunology and Gene Therapy directed by Dr. Stefano Indraccolo, in which he achieved his PhD in 2009, his project was 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 directed by Prof. Basso, in which he focused his interests on brain tumor biology with particular emphasis in unveiling the mechanisms by which brain tumor microenvironment (i.e. hypoxia) influences the activation of many developmental pathways, including Bone Morphogenetic Proteins, Wnt and Notch signaling and how they cooperatively affect brain tumor biology, aggressiveness and phenotype. In this context, in recent years the Brain Tumors Team developed a multilayer model of Glioblastoma in which they characterized the activation of the hypoxic signaling and its crosstalk with Glioblastoma cancer stem cells. Dr. Persano's studies on Glioblastoma have been awarded by the University of Padua (Young Investigator Grant 2010) for the development of a two-year project as an independent PI with full scientific and financial responsibilities. In particular, the project was committed to the study of BMP2 as a reliable pro-differentiating molecule to induce glioblastoma cell differentiation. Lastly, Dr. Persano is also in charge of the coordination of the activities of the Brain Tumors Unit in the Laboratory of Pediatric Oncohematology, University of Padua, currently located at the Institute of Pediatric Research, where he obtained the position of Research Associate in 2018. The Consolidator IRP Grant, awarded for the two-year period 2018-2019, allowed Dr. Persano to study the mechanisms of therapy resistance in pediatric medulloblastoma tumors. This laid the foundations for the recent achievement of individual grants from the Rally Foundation for Childhood Cancer Research and CARIPARO Foundation, the former being devoted to carry out a drug screening and the latter involving the study of the molecular basis of drug resistance in recently developed medulloblastoma multidrug-resistant models.
Dr. Persano's scientific production is based on the publication of 48 original papers, being in 19 of them the first/co-first, last or corresponding author (H-index=21).

of GBM organotypic cultures derived from GBM primary cells, together with their phenotypic and molecular characterization. These organoids are being subjected to a deep histological and morphological characterization, combined with the evaluation of their cellular heterogeneity based on a series of phenotypic markers generally expressed by neural progenitor cells at different stages of differentiation.

Current research in Medulloblastoma
Medulloblastoma (MB) is the most common malignant brain tumor of childhood. Although survival has slowly increased in the past years, the prognosis of these patients remains unfavorable, with most of them suffering from high morbidity due to the high-dose chemotherapy/radiotherapy regimens they are subjected to. In the past years, our research on pediatric MB has been focused on the study of intracellular signaling pathways, commonly activated during embryonic cerebellar development, which could have a role in sustaining MBcell biology including Notch signaling (Pistollato et al. Stem Cells 2010) and the PI3K/AKT/mTOR axis (Frasson et al. Biomed Res Int 2015). More recently, our group established novel in vitro models of MB drug resistance, which offer an invaluable opportunity for identifying actionable targets, which may have been masked in the original untreated cells. Based on this knowledge, a recent research line supported by the Rally Foundation for Childhood Cancer Research is devoted to exploit these drug-resistant MB models for a high-throughput screening of multiple FDA-approved compound libraries with the final aim of prioritizing clinically approved agents for the use in therapy-resistance MB cells and their future clinical development (manuscript in preparation). In this context, through the recent achievement of a pediatric research grant from CARIPARO Foundation we are molecularly deepen the process of drug resistance acquisition in MB tumors by the single-cell analysis of their transcriptional dynamics during the acquisition of a chemotherapy resistant phenotype. Moreover, through the use of reliable models of chemotherapy resistance, coupled with the most recent single-cell analysis technologies, we are analyzing the molecular events contributing to MB drug resistance and integrate transcriptomic data with epitope mapping to perform backtracking of cell population dynamics and provide multi-omics-based risk assessment and prognostication of treatment response potentially even at MB diagnosis.

Selected publications

Lambert E, Manczak R, Barthout E, Saada S, Porcù E, Maule F, Bessette B, Viola G, Persano L, Dalmay C, Lalloué F and Pothier A. Microfluidic Lab-On-a-Chip based on UHF-Dielectrophoresis for Stemness Phenotype Characterization and Discrimination among Glioblastoma Cells. Biosensors (Basel). 2021 Oct 13;11(10):388.

Casciati A, Tanori M, Manczak R, Saada S, Tanno B, Giardullo P, Porcù E, Rampazzo E, Persano L, Viola G, Dalmay C, Lalloué F, Pothier A, Merla C and Mancuso M. Human medulloblastoma cell lines: investigating on cancer stem cell-like phenotype. Cancers (Basel). 12(1). pii:226.

Fusco P, Parisatto B, Rampazzo E, Persano L, Frasson C, Di Meglio A, Leslz A, Santoro L, Cafferata B, Zin A, Basso G, Esposito MR and Tonini GP. Patient-Derived Organoids (PDOs) as a preclinical model for neuroblastoma tumours. BMC Cancer. 2019 Oct 21;19(1):970.

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.

Porcù E, Maule F, Boso D, Rampazzo E, Barbieri V, Zuccolotto G, Rosato A, Frasson C, Viola G, Della Puppa A, Basso G and Persano L. BMP9 counteracts the tumorigenic and pro-angiogenic potential of glioblastoma. Cell Death Differ. 2018 Nov;25(10):1808-1822.

Experimental Pharmacology

PI: **Giampietro Viola**

Research activity

The experimental pharmacology group is involved in the study of new strategies in cancer therapeutics following these main research lines:

Identification of new therapeutic target in medulloblastoma resistance

Medulloblastoma (MB) is the most common brain tumor in the pediatric age and is a 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.

Active projects:

Study of BAG interactome

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 collaboration with Prof. Alessandra Luchini (George Mason University, USA) and by using the innovative technique of “molecular painting”, we will identify of 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, in accordance with the work conducted by other groups, we have demonstrated that cancer cells can regulate Nrf2 pathway as a pro-survival response against drug treatments. Nrf2 is the major regulator of redox homeostasis and defense against oxidative stress.

Team members

Giampietro Viola
Principal Investigator

Roberta Bortolozzi
PostDoctoral Researcher

Elena Mariotto
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Lorenzo Manfreda
PhD student

Chiara Marchioro
PhD student

PI's biosketch

Scopus ID: 7006633082
Prof. Viola graduated in Pharmacy and Chemistry and Pharmaceutical technology at the University of Padua. During his career, Prof. Viola has had the opportunity to attend qualified research structures abroad. He is currently Associate Professor at the Università Padova in the Dept. of Women's and Children's Health (UOC of Oncohematology). **Since 2017 he has got the Italian Scientific National Habilitation to Full Professor of Medicinal Chemistry.** His research interests concern the study of new molecules endowed with antiproliferative activity both in vitro and in vivo and in particular of the mechanisms that lead to cell death. In the last years, his research efforts have been devoted to the identification of new targets in childhood acute lymphoblastic leukemia and in pediatric brain cancers with particular attention to drug resistance. He is author of more than 180 scientific paper in specialized peer review journals. It has been responsible of the unit of the University of Padua in the context of SUMCASTEC project-Horizon 2020 that deals with the development of a medical device able to separate cancer stem cells in medulloblastoma and glioblastoma through the application of electromagnetic fields. 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 application of new potential antitumoral molecules.

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 PCARE laboratory) 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 cellsto be exploited as therapeutic opportunities.

High-throughput Drug screening
Exploiting our in vitro models of MB drug resistance, we extend this approach on multiple tumors in order to perform high-throughput drug screening (HTS) on human patient-derived (PD) tumor cells, cultivated in reliable microenvironment-controlled conditions. These screening is carried out on both 2D and 3D (organoids) cell models. Drug libraries composed by both clinically approved drugs and novel promising small molecule (more than 3550 drugs) will be tested on our in vitro model, alone and in combination with standard chemotherapy. Information retrieved from HTS, will allow the identification of new compounds able to successfully integrate into the common therapeutic schedules improving tumor eradication while reducing chemotherapy-induced side effects. In addition through a combination matrix approach we can explore hundreds of drugs-drug pairs for potential synergy. The potential of this study fits has the aim of identifying new therapeutic approaches to be used in combination, to anticipate that tumor evolution that today leads to an almost inevitable poor prognosis. The ambitious goal of this research is to be able to prevent the rapid progression of this disease in a patient-specific manner and at the same time improve the quality of life of those children who may be most affected by the collateral toxicity of current therapies. This will ensure a fast identification of clinically relevant drug candidates with a timeline consistent with a therapeutic decision-making strategy, and will be of undeniable value for both children and adults with cancer, or any other type of disease.

Drug discovery
Our group is actively involved in the design and development of new anticancer agents:
- new NOTCH1 ligands by computer-aided design. In collaboration with Prof. Brancale (University of Cardiff, UK), performing a virtual screening of a library of 3*106 small molecules, we identified 60 compounds to potentially target two different NOTCH1 binding sites;
- new FLT3 inhibitors in collaboration with Prof. Barraja (Università degli studi di Palermo);
- dual Epidermal Growth Factor Receptor Kinase and Microtubule Inhibitors in collaboration with Prof. Romagnoli (Università di Ferrara).

Selected publications

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.

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.

Mariotto E., VIOLA G., Ronca R., Bhujwalla Z.M., Accordi B., Serafin V., Persano L., Lopez Cara L.C., Bortolozzi R. The novel choline kinase alpha inhibitor EB-3D induces cellular senescence reduce tumor growth and metatastic dissemination in breast cancer Cancers. (2018), 10, 391.

Mariotto E., Bortolozzi R., Volpin I., Carta D., Serafin V., Accordi B., Navarro Luque P., Lopez Cara L.C., Basso G., 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), 155, 213-223.

Bortolozzi R., Bresolin S., Rampazzo E., Paganin M., Maule F., Mariotto E., Boso D., Minuzzo S., VIOLA G., Indraccolo S., Cazzaniga G., Basso G., Persano L. AKR1C enzymessustain therapy resistance in pediatric T-ALL. Br. J. Cancer. (2018), 118, 985-994.

Molecular Diagnostic of non Hodgkin Lymphoma

PI: **Lara Mussolin**

Research activity

Non-Hodgkin Lymphoma (NHL) is a heterogeneous group of lymphoid malignancies and it is the fourth most common malignancy across the pediatric age spectrum. Our research area of interest is dedicated mainly to the study and characterization of NHL of childhood. The general approach includes the analysis of molecular mechanisms of tumourigenesis with a translational approach aimed at transferring biological results from the bench to clinical trials. This includes also the study of new tumour specific markers for the diagnosis and the prognosis of various malignancies and the study of liquid biopsies.

Ongoing projects:

Identifying and targeting metabolic liabilities in the crosstalk between childhood and B-cell lymphomas and their microenvironment

Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) account for most cases of non-Hodgkin lymphomas in childhood. The probability of survival for refractory and relapsed patients is very poor. The identification of novel therapeutic approaches that efficiently target B cell lymphoma is still needed and requires a deeper comprehension of the gene signature along with of the changes in malignant biological behaviors. We propose the innovative hypothesis that a metabolic characterization of the crosstalk between BL/DLBCL cells and tumor microenvironment (TME) offers unprecedented therapeutic opportunities. We aim at integrating a metabolic characterization of BL/DLBCL that eludes conventional chemotherapy with a charting of TME influence in tuning their bioenergetic pitch.

Identification of cell-of-origin of B lymphomas through a genome-wide single-cell RNA profiling

This project aims to investigate the cell-of-origin (COO) of B lymphomas to understand the non-neoplastic counterpart of these tumours and the basis of their heterogeneous clinical responses to therapy and analyse their metabolic profiles, integrating them with their RNAseq signatures and with their clinical and prognostic features. This project is conducted in collaboration with Prof. Basso and Prof. Dalla Favera (Columbia University, NY USA), two of the leading international experts in the molecular characterization of normal and malignant germinal center(GC) B cells. In the last year, Prof. Basso has optimized a protocol for isolation and scRNA-seq analysis of normal GC B cells toward the dissection of GC B cell heterogeneity. Her results provide a unique framework to analyze and dissect NHL B cell COO that will be studied here in this project.

Team members

Lara Mussolin
Principal Investigator

Federica Lovisa
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Lavinia Ferrone
PostDoctoral Researcher

Carlotta Caterina Damanti
PhD student

Ilaria Gallingani
PhD student

Anna Garbin
PhD student

Anna Tosato
PhD student

Elisa Tosato
Lab Technician

Domenico Rizzato
Postgraduate level student

Giorgia Contarini
Young research fellow

PI's biosketch

Scopus ID: 8886437100
Dr. Mussolin graduated in Biology with 110/110 at the School of Biological Sciences, University of Padua in 1999, followed by a PhD in Oncological Sciences of the Childhood, University of Padua, in 2004. Dr. Mussolin then specialized in Clinical Pathology with 70/70 with honours at the School of Medicine, University of Padua. From 2001, Dr. Mussolin is head of the molecular diagnosis of Non-Hodgkin Lymphomas (NHL) of childhood in the laboratory that centralize samples from all national AIEOP (AssociazioneItaliana di Emato-OncologiaPediatrica) centres. She is a member of the EICNHL (European InterGroup of Non-Hodgkin Lymphoma) Group for the study of Minimal Residual Disease in NHL of childhood, member of the NHL working Group, the Biology working Group and the Hodgkin lymphoma working Group of AIEOP Association. Dr. Mussolin is involved in laboratory research aimed at identifying new prognostic markers in NHL through modern molecular techniques (i.e. microarray, RNAseq). She is routinely invited to numerous national and international conferences as a speaker. Dr. Mussolin is currently teaching at the University Master's Degree in Pediatric Hematology organized biannually by the Faculty of Medicine of the University of the Studies of Rome La Sapienza. Author of over 70 publications on international journals and of over 100 abstracts selected for poster or oral presentations to international and national scientific meetings, Dr. Mussolin has an h-index >20; number of citations> 1500. During her career, she has received numerous awards, namely the travel awards for young investigators sponsorized by "Swiss Lega against Cancer" (2008) and travel award for young investigators sponsorized by American Society of Hematology (2009); the "Guido Berlucchi" award for young investigators sponsored by "Fondazione Guido Berlucchi" for cancer research (2010) and the "Mario Moroni" award sponsored by Gilead Sciences (2019).

OMICS-driven characterization of pediatric CD30 positive lymphomas: from tumour biopsy to tumour microenvironment

Hodgkin lymphoma (HL) and Anaplastic Large Cell Lymphoma (ALCL) are both CD30 positive lymphomas accounting for ~10% and 3% of all cases of lymphoid neoplasms, respectively. A field of interest that has grown exponentially in the last few years, impacting various areas of research, is the extracellular vescicles (EVs) study, in particular of small EVs formed inside endosomal compartments (i.e., exosomes). Exosomes are actively released vesicles (carrying RNA, DNA and protein) that can function as inter-cellular messengers. The principal aims of this project are: i) to characterize the exosomes at the transcriptional level, comparing them with transcriptional data obtained from primitive tumour cells; ii) to provide insights on communication mechanisms between cancer cells and the microenvironment and iii) to perform an extensive research on the proteomic composition of cancer cell-derived exosomes, to define their role in disease progression and/or resistance.

Some recent activities:

Garbin A et al, Haematol 2020

We demonstrated that miR-939 expression could contribute to PDGFRB inhibition, a crucial driver for ALCL lymphomagenesis, via JUNB downregulation.

Lovisa F et al, Front Oncol 2020

Our analysis disclosed that non-miRNA derived sRNAs constitute the prominent fraction of sRNA loaded in exosomes and identified 180 sRNAs significantly more abundant in exosomes of ALCL patients compared to controls. YRNA fragments, accounting for most of exosomal content and being significantly increased in ALCL patients, were prioritized for further investigation. Quantification of RNY4 fragments and full-length sequences disclosed that the latter are massively loaded into exosomes of ALCL patients with more advanced and aggressive disease. We discussed the role of RNY4 in the modulation of tumor microenvironment.

Di Battista et al, Front Oncol 2021

Characterization of miRNA expression in cases that relapsed after first line therapy disclosed a significant association between miR-214-5p down-regulation and aggressive non-common histology. Our results suggest that miR-214-5p level may help to refine the prognostic stratification of pediatric ALK-positive ALCL.

Selected publications

Minimal Disease Monitoring in Pediatric Non-Hodgkin's Lymphoma: Current Clinical Application and Future Challenges.Mussolin L, Damm-Welk C, Pillon M, Woessmann W.Cancers, 2021.

Minimal residual disease analysis in childhood mature B-cell leukaemia/lymphoma treated with AIEOP LNH-97 protocol with/without anti-CD20 administration. Mussolin L, Lovisa F, Gallingani I, Cavallaro E, Carraro E, Damanti CC, Vinti L, Sala A, Micalizzi C, Santoro N, Piglione M, Cellini M, Buffardi S, Buldini B, D'Amore ESG, Biffi A, Pillon M. Br J Haematol, 2020.

A high definition picture of key genes and pathways mutated in pediatric follicular lymphoma. 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. Haematologica, 2019.

NPM-ALK expression levels identify two distinct subtypes of pediatric anaplastic large cell lymphoma. Pomari E, Basso G, Bresolin S, Pillon M, Carraro E, d'Amore ES, Viola G, Frasson C, Basso K, Bonvini P, Mussolin L. Leukemia, 2017.

An aberrant microRNA signature in childhood T-cell lymphoblastic lymphoma affecting CDKN1B expression, NOTCH1 and growth factor signaling pathways. Mussolin L, Holmes AB, Romualdi C, Sales G, D'Amore ES, Ghisi M, Pillon M, Rosolen A, Basso K. Leukemia, 2014.

Research and Discovery in Hematopoietic Cell&Gene Therapy

PI: **Alessandra Biffi**
Junior PI: **Valentina Poletti**

Team members

Alessandra Biffi
Principal Investigator

Valentina Poletti
Junior Principal Investigator

Rita Milazzo
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Yuri Ciervo
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Fatlum Rruga
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Giulia Santinon
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PhD Student

Annagiada Toniolo
Post-graduate fellow

Mohammed Kiyemba
Post-graduate fellow

Camilla Fabris
Post-graduate fellow

Research activity

Prof. Biffi's main research interest is on the development of novel Hematopoietic Stem Cell (HSC)-based gene therapy approaches for inherited and, more recently, acquired disorders affecting the central nervous system (CNS). Engineered HSCs progeny in the CNS of myeloablated transplant recipients represent a vehicle for therapeutic molecule delivery across the blood-brain barrier. Prof. Biffi has already proven the efficacy of this innovative approach in animal models and patients affected by lysosomal storage disorders (LSDs) and her group is currently pursuing research aimed at enhancing the therapeutic potential of this strategy and broaden its application. This research area, which has been funded by a Consolidator ERC grant to Prof. Biffi, is now part of an alliance with industry aimed at the clinical development of the new approach in novel indications and of novel academic grants, including a POC and an Advanced ERC, aiming also at the development of targeted gene addition in rare LSDs. Among the ongoing projects, a Phase I/II clinical trial of HSC gene therapy for a severe combined immunodeficiency that is funded by IRP and is being activated to patients' enrollment. Moreover, the lab hosts research sponsored by Altheia Science, a spinoff company of the University of Padua founded by Prof. Biffi and dedicated to the development of HSC gene therapy for new autoimmune indications. The laboratory operates in the context of international collaborations such as with Boston Children's Hospital and Harvard Medical School.

Dr. Poletti's current research activity is focused on the development of innovative GT strategies for orphan and rare genetic diseases, with a synergistically basic and translational approach based on the study of human hematopoietic stem cell homeostasis, lentiviral vector design, non-viral transgenesis, and interaction between integrating genetic elements and the human genome.

PI's biosketch

Alessandra Biffi
Scopus ID: 7003906961

Prof. Biffi is head of the Pediatric Oncohematology and Stem Cell Transplant Division (clinics, diagnostic and research laboratories) at the Azienda Ospedale Università Padova since October 2018 and she coordinates the research area on Oncohematology, Stem Cell Transplant and Gene Therapy at the Istituto di Ricerca Pediatrica. Previously, Prof. Biffi 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 Milan, where she also practiced as attending physician and head of a clinical unit in Pediatric Stem Cell Transplant and Immunohematology (up to 2015). Prof. Biffi has trained over 40 fellows and PostDoctoral fellows as well as numerous residents and medical students in her laboratory and clinics, the majority of whom are still in academic medicine. Prof. Biffi has published over 120 peer-reviewed manuscripts and textbook chapters. She has extensive clinical experience in pediatric stem cell transplant and in early phase cell and gene therapy clinical trials. Prof. Biffi's preclinical and clinical research is dedicated to enhancing the efficacy of Hemopoietic Stem Cells (HSC)-based therapeutic approaches for inherited disorders with severe nervous system involvement by: i) fostering brain microglia replacement by donor cells after HSC transplantation upon detailed understanding of this phenomenon (Capotondo et al., PNAS 2012), and ii) enhancing the potential of enzyme delivery to the affected nervous system by means of the gene corrected progeny of the transplanted, lentiviral vector (LV)-transduced HSCs (Biffi et al., Science 2013; Sessa et al., Lancet 2016). Additional research activities comprise of novel exploratory projects on the therapeutic role of engineered microglia in neurodegenerative diseases, HSC gene therapy application to autoimmune disorders and novel exploratory projects on targeted cancer therapy in collaboration with local PIs. She is actively collaborating with biotech companies in the gene therapy field, she is one of the founders and scientific advisors of Altheia Science s.r.l., a spinoff company of the University of Padua that established sponsored research agreements with the Dept. of Women's and Children's Health.

Valentina Poletti
Scopus: 25655343700
The lab also hosts a Junior PI, Dr Valentina Poletti, who is currently Marie Skłodowska-Curie fellow at Woman and Child Health Department of University of Padova, and affiliated Faculty at Harvard Medical School(Boston, MA, USA). She has been working in developing ex vivo gene therapy (GT) strategies for monogenic diseases, including skin and cornea diseases, immunodeficiencies, hemoglobinopathies and LSDs, since 2005. She obtained her PhD in Molecular Medicine at the University Vita-Salute San Raffaele (Milan, Italy) in 2012. Afterwards, she worked as staff scientist at Genethon (Evry, France), a French institute dedicated to translational research in gene therapy for rare diseases. She led several preclinical IND-enabling studies of GT for immunodeficiencies and hemoglobinopathies, instrumental for the approval of three clinical trials by the American medicinal Agency FDA (ClinicalTrials.govIdentifier#NCT03311503, #NCT02247843) and the French medicinal agency ANSM (ClinicalTrials.gov Identifier #NCT03964792), which were published in multiple manuscripts in 2018 and 2019 (Poletti V. et al. June 2018; Poletti V. et al. Dec. 2018; Weber L. et al 2018; Urbinati F. et al. 2018;Liddonici M.R. et al 2018; Charrier et al. 2019). She set up a research team for the study of the interaction between integrated viral vectors and the human genome (Poletti V. et al.,2017), and contributed to the investigation of the mechanism of integration of Sleeping Beauty transposon-vector in human hematopoietic stem/progenitor cells (Holstein et al. 2018). In 2018 she joined the Gene Therapy Program of Dana-Farber/Boston Children's Cancer and Blood Disorders Center/ Harvard Medical School (Boston, MA, USA) as an Instructor in pediatrics, contributing to multiple basic and translational studies of GT for LSDs. She was awarded with a Marie Skłodowska-Curie grant/fellowship by the European Commission to come back to Italy, and a CARIPARO grant to establish her research activity at Istituto di Ricerca Pediatrica Città Della Speranza. Her publishing track record includes 16 peer-reviewed papers and a textbook chapter from 2008 to date, on high-impact factor scientific journals specialized in human molecular genetics and gene therapy, and she actively collaborates with many international academic and industrial partners.

Selected publications

Metachromatic leukodystrophy: A single-center longitudinal study of 45 patients. Fumagalli F, Zambon AA, Rancoita PMV, Baldoli C, Canale S, Spiga I, Medaglini S, Penati R, Facchini M, Ciotti F, Sarzana M, Lorioli L, Cesani M, Natali Sora MG, Del Carro U, Cugnata F, Antonioli G, Recupero S, Calbi V, Di Serio C, Aiuti A, Biffi A, Sessa M. J Inherit Metab Dis. 2021 Sep;44(5):1151-1164. doi: 10.1002/jimd.12388.

Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease. 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. N Engl J Med. 2021 Jan 21;384(3):205-215. doi: 10.1056/NEJMoa2029392.

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Target Discovery and Biology of Acute Myeloid Leukemia

PI: **Martina Pigazzi**

Research activity

Leukemias account for approximately one third of all pediatric malignancies and remain a leading cause of death in children and adolescents. Acute myeloid leukemia (AML) accounts for about 20% of acute leukemia cases in childhood and, although children's outcomes have significantly improved over the past 30 years reaching about 70% survival, most of these improvements have not been achieved with the introduction of new drugs. Despite complete remission rates of 85-90%, AML recurrence is still the leading cause of treatment failure. AML is a phenotypically and genetically heterogeneous hematological neoplasm. Our lab spent two decades in identifying most of the recurrent lesions and mutations, and the most recent extensive sequencing efforts, have mapped the genomic landscape of pediatric AML revealing a huge number of biologically and prognostically relevant driver lesions. In addition to the identification of the recurrent genetic aberrations, our group want to delineate the complex mechanisms by which they contribute to the onset and evolution of the disease, with the aim of facilitating the development of new targeted therapies to improve the cure rate of AML. To move in this direction, the three-dimensional (3D) and murine models of AML are considered indispensable research tools because 1) they mirror specific genetic subtypes of human AML, 2) define the cellular-intrinsic and extrinsic mechanisms of the disease, 3) allow the interaction between cells with coexisting genetic lesions, 4) consider the role of microenvironment thus supporting that the testing of new therapeutic approaches in these predictive models would increase the number of new drugs for AML children that will be successfully introduced in the market.

In these years, we generated a 3D model by using a hydroxyapatite and collagen scaffolds able to mimic the bone marrow niche, and several patient-derived xenograft models (PDX) to test and validate new treatment opportunities. This translational research approach strives to understand cancer transformation mechanisms, with particular interest in driver oncogenes and the identification of

Team members

Martina Pigazzi
Principal Investigator

Claudia Tregnago
Post Doc

Giulia Borella
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Maddalena Benetton
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Ambra Da Ros
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Giorgia Longo
PhD student

Katia Polato
Technician

Olivia Marini
Post Doc

Giovanna Gioachin
Post-graduation fellowship

Alessia Strapazzon
Post-graduation fellowship

PI's biosketch

Scopus ID: 23010194100
Prof. Pigazzi Martina, Geneticist, PhD in Oncology, is Associate Professor at Woman and Child health Department, Onco-Hematology Clinic -University of Padova- and the Head of the Genetic Unit at the Onco-Hematology laboratory serving as Italian reference laboratory for pediatric hematological disease diagnosis. From 2008 she is in charge as Responsible Geneticist of all the Italian patients with acute myeloid leukemia enrolled in the Associazione Italiana Emato-Oncologia Pediatrica (AIEOP) for the AML2002/01 (enrollment of 482 patients) and AML 2013/01 clinical trials (ongoing-EudraCT 2014-000652-28). For the national trial the genetic consultancy is given at diagnosis and during the follow-up of patients by the study of residual disease after treatment blocks and before and after the hematopoietic bone marrow transplantation. She is the member of the AIEOP LAnL (acute non lymphoblastic leukemia) Group, being the delegate at the International BFM Study Group for the international committee "I-BFM MRD-AML Task Force". She is Chair of the EU-task-force group "Molecular MRD inAML" for the international BFM study group. From 2004 she is the Group Leader of the "Molecular Biology of the Acute myeloid leukemia"at the Onco-Hematology lab - Woman and child health Department- working mainly at the identification of new genetic aberrations and mutations with prognostic value used as biomarker at diagnosis definition, forrisk stratification of patients and tailored therapy. She improves functional role of new unknown mutations in leukemia at diagnosis, creates assays of minimal molecular disease monitoring during treatment for preemptively recognize a disease recurrence and refine patients' clinical management. From 2013 she is Group leader at Pediatric Research Institute. Her research deals with the understanding of leukemogenesis, with particular interest in driver oncogenes and activated pathways for testing of promising alternatives or complementary therapeutics to current chemotherapy by using innovative in vitro and in vivo models. She's author and coauthor of 58 manuscripts published in main influent peer reviewed journals of oncology-hematology-cancer research. Her independent research is based on several national and European grants. She is co-inventor of two patents. She is worldwide recognized as pediatric AML geneticist and included in the Steering committee member of the International Leukemia Target Board (iLTB), Pediatric consortia for treating acute relapsed leukemia, the PeDALand Eupal; she is active in the international ACCELERATE and i-BFM groups. She is panel member of the biological-basic and translational initiative ITCC-P4 for improving treatment of children with leukemia.

activated pathways, for promising alternatives or complementary therapies to current chemotherapy, to be used particularly in children suffering from relapse. We are using the drug repositioning strategy by testing commercial libraries of FDA-approved compounds in high-throughput drug screenings, as well as newly proposed targeted therapies for adult AML, or drugs suggested by the international consortia of the Leukemia and Lymphoma Society (LLS) and academic groups (EuPal) program currently guiding scientists to perform preclinical studies for new drugs that have high commitment to be included in the future clinical trials. Thanks to our 3D model, we underpinned the role of mesenchymal stromal cells (MSCs) and their pro-oncogenic role toward the niche. Our idea of a dual targeting, the stroma and the blasts, was successful, and now we are dissecting MSCs characteristics, including their electro-physiological properties, in order to generate novel strategies to reduce their protective role and improve leukemia clearance to reduce the risk of disease re-emergence. This latter consideration, recently guides our interest in the characterization of the leukemia stem cells (LCSs), the cell population that is considered to be responsible of the relapse occurrence, with particular interest toward LSCs metabolism and the mitochondrial targeting as novel opportunities to eradicate this critical leukemia component. Recently, tumor specific antigens definition for the generation of novel CAR-T cell therapy specific for AML have been also identified and pre-clinical testing of new CAR constructs isunder development.

The AML group research deals with the idea of providing knowledge in AML initiation and treatment, with the long term aim of saving all children with AML, reduce treatment toxicity and support a safer adulthood.

Selected publications

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.

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Tregnago C, Benetton M, Padrin D, Polato K, Borella G, Da Ros A, Marchetti A, Porcù E, Del Bufalo F, Mecucci C, Locatelli F, Pigazzi M. NPM1 Mutational Status Underlines Different Biological Features in Pediatric AML. Cancers (Basel). 2021 Jul 10;13(14):3457.

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Target Discovery and Biology of Neuroblastoma

PI: **Sanja Aveic**

Research activity

Neuroblastoma (NB) is a pediatric tumor originating from the neural crest-derived sympathoadrenal progenitors. It shows wide-ranging clinical and biological heterogeneity and hence may require tailored therapies to reach a more successful cure rate. Long-term survival for high-risk (HR) patients is still below 50% and various metastatic sites are commonly observed in this clinical group. Dissemination of tumor cells to the bone marrow (BM) is the most frequent event in HR patients with NB and accounts for about 70% of all metastases. Once BM metastasis is detected, the disease progresses rapidly due to the intense growth of NB cells, while patients suffer from extensive bone pain. Therefore, an aggressive course of chemotherapy is often mandatory although its efficiency is low due to the acquired chemotherapy resistance of NB cells. The lack of standardized in vitro and in vivo models of metastatic NB introduces difficulties in the investigation of the molecular background of the aggressive phenotypes and the selection of new therapeutic strategies. In this context, our main research is focused on the adaptation and enhancement of the innovative 3D in vitro, and zebrafish and murine in vivo experimental models of NB that will allow us to study the biology of cell spreading within the BM niche. This approach will grant a more comprehensive assessment of the molecular and cellular events that sustain metastasis occurrence and resistance to the currently proposed therapy options.

Team members

Sanja Aveic
Principal Investigator

Diana Corallo
PostDoctoral Researcher

Sara Mengazzo
PhD Student

Marcella Pantile
Lab Technician

Pharmacology

The research activities of the Laboratory of Target Discovery and Biology of Neuroblastoma are focused on elucidating the biological processes that sustain drug resistance in NB leading to tumor cells' spreading and disease progression. We are particularly interested in assessing the possibilities of targeting cytoprotective autophagy to revert resistant NB cell phenotypes. Towards this goal, we are employing 2D and 3D in vitro, and zebrafish and murine PDX in vivo models of NB as the pre-clinical approaches to decode novel therapeutic window for treating metastatic disease with autophagy inhibitors.

Basic research

At a molecular level, we are investigating the role of Lin28B gene in the neural crest cell migration during early phases of embryonal development using advantages of zebrafishin vivo model. We have reported a failure of Lin28B overexpressing embryos to develop functional peripheral sympathetic nervous system leading to NB. The Lin28B overexpressing NB cells are highly invasive and motile implying its association with pro-metastatic phenotypes. Comprehensive molecular analyses are involved in deciphering mechanisms of Lin28B dependent cell migration and invasion leading to the metastatic spread of NB cells.

Innovations

In collaboration with national and international research groups, we are interested in the generation of 3D in vitro models of NB that will allow more extensive studies of biological pathways in BM metastatic niche. We are fabricating 3D tumor models by combining cell lines and ex-vivo primary cells with a biomimetic scaffolding system to recapitulate the heterogeneity of NB tumors. These 3D bioengineered NB models will be explored as drug screening platforms in pre-clinical research.

PI's biosketch

Scopus ID: 20435413800
Dr. Aveic graduated at the University of Belgrade, Serbia, at the Dept. of Molecular Biology and Physiology, of the Experimental Biomedicine section. She completed her PhD in 2010 at the University of Padua and worked as a PostDoctoral Researcher for 4 years in the Dept. of Pediatric Oncohematology. Her main fields of interest are pediatric malignant neoplasms, including leukemias and neuroblastomas. Since 2014, Dr. Aveic has been studying the molecular aspects of neuroblastoma genesis to seek more effective tailored therapeutic regimens. In 2018, Dr. Aveic became PI of the Laboratory of Target Discovery and Biology of Neuroblastoma at the Pediatric Research Institute Fondazione Città della Speranza. Since February 2019, she is also a cell lab leader at the University Hospital of Aachen, at the Dept. of Dental Materials and Biomaterials. Dr. Aveic served as a reviewer for numerous scientific journals and international projects in the field of pediatric oncology and biomaterials. Since 2018 Dr. Aveic is an active member of the International Society of Pediatric Oncology Europe Neuroblastoma (SIOPEN) and, since 2019, she is a member of the European Society for Biomaterials (ESB). Dr. Aveic is also part of the Italian Neuroblastoma working group composed of medical doctors, pathologists, bioinformaticians, and biologists currently assessing new protocols for targeted therapies. The main research interests of her team focus on the characterization of the core molecular and cellular events associated with the metastatic neuroblastoma phenotypes.

Selected publications

Corallo D, Zanon C, Pantile M, Tonini GP, Zin A, Francescato S, Rossi B, Trevisson E, Pinato C, Monferrer E, Noguera R, Aliño SF, Herrero MJ, Biffi A, Viscardi E, Aveic S. Integrated CGH/WES Analyses Advance Understanding of Aggressive Neuroblastoma Evolution: A Case Study. Cells. 2021 Oct 9;10(10):2695. doi: 10.3390/cells10102695.

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. 2021 Mar 10;9(5):1716-1727. doi: 10.1039/d0bm00921k.

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 Sep;25(18):9060-9065. doi: 10.1111/jcmm.16822. *co-first authors.

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. 2020 Apr;27(4):1225-1242. doi: 10.1038/s41418-019-0425-3.

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. 2020 Sep 22;39(1):195. doi: 10.1186/s13046-020-01692-x.

Research area

Predictive Medicine

Coordinator: **Prof. Eugenio Baraldi**

The overall aim of predictive medicine in pediatric medicine is to flag risk factors so that physicians and patients can work together to reduce the chances of future problems. Our group of researchers, consisting of clinicians, biostatistician and biologists, develops advanced predictive models in the field of disease risk prediction and prevention.

The Pediatric Critical Care Project (PCare) is running studies on pulmonary surfactant metabolism and drug delivery, neonatal nutrition and respiratory and neurological outcomes of congenital heart diseases.

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ISTITUTO DI RICERCA
PEDIATRICA
città della speranza

Prof. Eugenio Baraldi

Coordinator Predictive Medicine Area

Scopus ID: 7006821460

Prof. Baraldi obtained his MD degree (1982) and susequently specialised 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 (2005) and then Full Professor of Pediatrics (2010) and Director of the School of Pediatrics, at the University of Padua (2012-2016). 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 - Azienda Ospedale -Università Padova.

Prof. Baraldi has also been President of the "Italian Society of Pediatric Respiratory Diseases" (2010-2014).

He is Coordinator of the project "Metabolomics in Pediatrics" and Coordinator of the respiratory followup of a cohort of children with bronchopulmonary dysplasia, since 1990 (JAMA 2009;302:1418-20).

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 and the Asthma Foundation of Western Australia.

Prof. Baraldi published more than 180 full papers in international journals (h-index 52, total IF=1030); including NEJM, Lancet, JAMA and AIRCCM.

Prof. Baraldi is included in the list of "Top Italian Scientist"
(www.topitalianscientists.org).

Mass Spectrometry and Metabolomics

PIs: **Eugenio Baraldi, Giuseppe Giordano**

Team members

Eugenio Baraldi
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Giuseppe Giordano
Co-Principal Investigator

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Paola Pirillo
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Elena Priante
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Research activity

Metabolomics and Lipidomics is the most recent of the omic sciences. Metabolomics and Lipidomics can be defined as the quantitative analysis of all the metabolites (small molecules <1.5 kDa) 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 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

PI's biosketch

Eugenio Baraldi
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Prof. Baraldi obtained his MD degree (1982) and susequently specialised 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 (2005) and then Full Professor of Pediatrics (2010) and Director of the School of Pediatrics, at the University of Padua (2012-2016). 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 - Azienda Ospedale -Università Padova.
Prof. Baraldi has also been President of the "Italian Society of Pediatric Respiratory Diseases" (2010-2014).
He is Coordinator of the project "Metabolomics in Pediatrics" and Coordinator of the respiratory followup of a cohort of children with bronchopulmonary dysplasia, since 1990 (JAMA 2009;302:1418-20).
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 and the Asthma Foundation of Western Australia.
Prof. Baraldi published more than 180 full papers in international journals (h-index 52, total IF=1030); including NEJM, Lancet, JAMA and AIRCCM.
Prof. Baraldi is included in the list of "Top Italian Scientist" (www.topitalianscientists.org).

Giuseppe Giordano
Scopus ID 7202918601
Dr. Giordano has achieved his academic career with a degree in Biological Sciences, followed by a PhD in Developmental Sciences (1990). Between 1989-1993, Dr. Giordano worked as Associate Research Scientist at the Dept. of Molecular Biophysics and Biochemistry, Yale University School of Medicine (USA), before pursuing a specialization in Clinical Pathology and Biochemistry. -In 2006-2010 he served as Seconded National Expert Joint Research Center of the European Commission; Institute for Health & Consumer Protection (IHCP); Physical & Chemical Exposure Unit (PCE); ISPRA (VA), Italy.
Co-Principal Investigator of the Mass Spectrometry Lab, Dr. Giordano 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 13C, 15N2, 2H2; 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.
Membership:
- 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)
- 2019-2021 Member of The "SIBioC" Società Italiana di Biochimica Clinica.

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.

Risk of bronchopulmonary dysplasia in preterm neonates

Preterm delivery (PTD) is a major challenge in the field of obstetrics and neonatology. Since 2006 preterm birth rates have been declining both in the United States and in European countries. Nevertheless, prematurity remains a major cause of morbidity and mortality worldwide, which exceed those of infants born full-term. Preterm neonates are at increased risk of both short- and long-term pathological outcomes and, among these, bronchopulmonary dysplasia (BPD) accounts for the vast majority of cases of chronic lung disease after premature birth. Metabolomics allows simultaneous characterization of low molecular weight compounds and may provide a picture of such a complex condition (PLoS One 2016 Oct 18;11(10):e0164211).

Metabolic perturbations in children with type 1 diabetes

Despite a considerable reduction in diabetes-related complications, including end-stage renal disease, over the past 30 years, individuals with type 1 diabetes (T1D) still exhibit a nearly 3-fold excess mortality as compared with the general population without diabetes, cardiovascular diseases being the leading cause of death in young adults with T1D. While the incidence of T1D is steeply increasing, the age at onset is progressively lowering. An early onset of the disease has been associated to marked morphological alterations of brain morphology and growth. The mechanisms underlying such alterations still remain unexplained. We enrolled children with T1D aged 6-15 years, and healthy peers to investigate the differences in the urine metabolome and to explore the contribution of HbA1c and clinical features to the observed differences (Pediatr Diabetes. 2017 Apr 12. doi: 10.1111/pedi.12524).

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. Untargeted analysis found 111 relevant predictors capable of discriminating between the two groups. Of 35 metabolites showing independent discriminatory power, five have already been well characterized: L-alanine, Creatine, L-3-methylhistidine, L-lysine, and Theobromine. The first three relate to cellular energy metabolism; their involvement suggests a multimodal derangement of cellular energy metabolism during PA/HIE. Our analysis identified a distinct urinary metabotype associated with pathological findings on MRI, which may be useful for identifying neonates at risk of developing HIE after PA. (submitted).

Unravelling the role of lipids in ZIKA virus Infection and traversing of the Placenta (submitted)

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). For objective 1, infection in vitro of placental cells will be followed by lipidomic and transcriptomic analyses to characterize the response of cells. For objective 2, viruses with different envelope compositions will be compared for their ability to replicate in placental cells in vitro. For objective 3, pregnant mice on different dietary regimes will be infected and VT and fetal malformations will be assessed. 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 are rare genetic disorders 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. 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.

Selected publications

Carraro S, Ferraro VA, Maretti M, Giordano G, Pirillo P, Stocchero M, Zanconato S, Baraldi E. Metabolomic profile at birth, bronchiolitis and recurrent wheezing: a 3-year prospective study. Metabolites 11 (2021) 825.

Mardegan V, Giordano G, Stocchero M, Pirillo P, Poloniato G, Donadel E, Salvadori S, Giaquinto C, Priante E, Baraldi E. Untargeted and targeted metabolomic profiling of preterm newborns with early onset sepsis: a case-control study. Metabolites 11 (2021) 115.

Ferraro VA, Carraro S, Pirillo P, Gucciardi A, Poloniato G, Stocchero M, Giordano G, Zanconato S, Baraldi E. Breathomics in asthmatic children treated with inhaled corticosteroids. Metabolites 10 (2020) 390.

Zaramella P., Munari F., Stocchero M., Molon B., Nardo D., Priante E., Tosato F., Bonadies L., Viola A., Baraldi E. Innate immunity ascertained from blood and tracheal aspirates of preterm newborn provides new clues for assessing bronchopulmonary dysplasia. PloS ONE 14 (2019) e0221206.

Barlotta A., Pirillo P., Stocchero M., Donato F., Giordano G., Bont L., Zanconato S., Carraro S., Baraldi E. Metabolomic profiling of infants with recurrent wheezing after bronchiolitis. The Journal of Infectious Diseases (2018), https://doi.org/10.1093/infdis/jiy659.

Pediatric Critical Care

PI: **Paola Cogo**

Research activity

Team members

Paola Cogo
Principal Investigator

Manuela Simonato
PostDoctoral Researcher

Anna Sartori
PhD student

Ambra Bertocco
Green PhD student (shared with the Department of Biomedical Science)

Alessio Correani
Assistant professor

Virgilio Carnielli
Clinical Researcher

Aldo Baritussio
Clinical Researcher

Giovanna Verlato
Clinical Researcher

Chiara Righetti
Master student

Lorenzo Gentile
Master student

Elena Scarparo
Master student

Francesco Di Napoli
Master student

Our research team has more than 30 years of experience in translational medicine of acute lung diseases (including animal models, newborns, and adults) and in genetic mutations of lung surfactant-specific proteins.
The focus of our research is the development of stable isotope and high-resolution mass spectrometry-based approaches (targeted and untargeted) to improve the understanding of human biology on a cellular and whole-organism levels.
In recent years, we have focused on the neurological and pulmonary injuries occurring during pediatric cardiac surgery for congenital heart defects. 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 biomarkers that correlates with the neurological and pulmonary outcome of children undergoing open-heart surgery for congenital heart diseases. We recently described how the minimum temperature reached during children 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 GFAP, along with other surgical parameters, to children's

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) and Chair of the Division of Pediatrics (University of Udine). Recently she was promoted 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. Prof. Cogo is author of 211 Scopus-indexed documents (3639 citations) with an h-index of 31.

neurodevelopmental outcome at 18 months after surgery. Neurodevelopmental follow up showed that up to 52% of CHD children reported neuropsychological impairments mainly related to the domain of the social and affective perception. Cognitive, neuropsychological, and psychopathological functioning were also assessed 5.7 ± 2.2 years after surgery. The peak GFAP plasma level, especially if greater than 0.49 ng/mL, resulted as a significant risk factor for abnormal neurodevelopment at long-term follow-up especially in the social and behavioral area. Moreover, our laboratory applied an untargeted metabolomic approach to elucidate the mechanism underlying CHD phenotype and to identify the metabolite signatures of early brain damage.
Pulmonary surfactant status in critical diseases is also a key topic of the laboratory. We use stable isotope tracers and high resolution-mass spectrometry techniques to describe lipids and surfactant specific proteins amounts and kinetics and polar metabolites profile in different acute and chronic disease in children, adults and animal model.
Studies on preterm and newborn infants' nutrition (focused on parenteral nutrition) are also ongoing with important results obtained describing the metabolism of lipid components and their relationship with the diseases. Lipidomic studies are planned to study lipids metabolism in the fetus and during pregnancy complicated by intrauterine growth restriction.
Untargeted and targeted lipidomic and metabolomic studies are in progress to characterize the small molecules profile of urine extracellular vesicles to identify potential biomarkers of early kidney rejection.

Selected publications

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Vedovelli L, Padalino M, Suppiej A, Sartori S, Falasco G, Simonato M, Carnielli VP, Stellin G, Cogo P: Cardiopulmonary-Bypass Glial Fibrillary Acidic Protein Correlates With Neurocognitive Skills. Ann Thorac Surg 2018; 106: 792-798.

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 the development of 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 cells, decellularized matrix and 3D bioprinting, for the identification of cellular and molecular players involved in neuromuscular genesis.

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 and as Coordinator of the research area in Regenerative Medicine at the Istituto di Ricerca Pediatrica "Città della Speranza". He is author and co-author of 167 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: 39 (Scopus) – 44 (Google Scholar).

Awards:

- 10/11/2006: International Award "Giuseppe Sciacca", section Medicine
- 18/07/2011: Award "Gentlemen d'Italia" for scientific activity

Extracellular Vesicles as Therapeutic Tool

PI: **Maurizio Muraca**

Research activity

Mesenchymal stem/stromal cell-derived extracellular vesicles (MSC-EVs) primed with pro-inflammatory cytokines as therapeutic tool for Inflammatory Bowel Disease

This project represents the translational development of a patent submitted with Prof. Antonella Viola on the anti-angiogenic activity of MSC-EVs primed with a cocktail of pro-inflammatory cytokines (PCT/IB2016/057608). The work is performed in collaboration with the biotech company Exo Biologics (Liege, Belgium), which has acquired the license of the above patent from the University of Padova and from the Fondazione Città della Speranza. In a recent publication, we compared the effects of murine MSC-EVs primed with pro-inflammatory cytokines (pEVs) with "naïve" non-primed EVs (nEVs) in a murine model of DSS-induced colitis. Only mice treated with PEVs showed clinico-pathological improvement, decreased intestinal fibrosis and angiogenesis and a striking increase in intestinal expression of Mucin 5ac, suggesting improved epithelial function. Moreover, treatment with pEVs resulted in the polarization of intestinal macrophages towards and anti-inflammatory phenotype and in an increased Treg/Teff ratio at the level of the intestinal lymph node. More recently, we started testing clinical-grade pEVs from human origin (Cord tissue) manufactured by Exo Biologics. In a macrophage polarization assay, pEVs showed 50% higher ability to suppress pro-inflammatory macrophage polarization compared with nEVs.

Our next experimental plan to characterize these clinical-grade human pEVs include the following:

- Biochemical markers of angiogenesis
- Proteomic evaluation of primed vs. naïve Evs
- ELISA assays for TIMP-1 and CD73

(anti-angiogenic markers for pEVs) in vitro assays

- InnoZyme MMP2/MMP9 activity assay kit
- ATPase activity
- Tube formation assay with HUVEC / SVEC
- LPS/TNF alpha -stimulated CaCo2: trans-epithelial electrical resistance / cytokine secretion
- LPS/TNF alpha-stimulated intestinal organoids (under development) in vivo assays
- Zebrafish (angiogenesis)
- DSS-induced colitis in mice

Mesenchymal stem/stromal cell-derived extracellular vesicles to prevent the development of Bronchopulmonary Dysplasia

We have recently submitted an Investigative Medicinal Product Dossier to the Regulatory Authorities asking permission to perform a first-in-man study on the use of MSC-EVs for the prevention of Bronchopulmonary Dysplasia in preterm newborns at risk. The dossier included pharmacodynamics, pharmacokinetics and toxicity studies performed in collaboration with the biotech company Exo Biologics. To further characterize the EV product, and in view of a possible phase II-III study, we will now focus on investigating the mechanism of action of these nanoparticles. As a first working hypothesis, we will evaluate the involvement of the CD69 pathway on lymphocytes and on macrophage cell lines, based on our successful establishment of potency assays (accepted for publication).

Team members

Maurizio Muraca
Principal Investigator

Giada De Lazzari
PhD student

Fabio Magarotto
PhD student

Ricardo Malvicini
Visiting PhD student - Argentina

PI's biosketch

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 and as Coordinator of the research area in Regenerative Medicine at the Istituto di Ricerca Pediatrica "Città della Speranza". He is author and co-author of 167 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: 39 (Scopus) – 44 (Google Scholar).

Awards:

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

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

Selected publications

Tolomeo, A.M., 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 (2021) Pharmaceuticals, 14 (8), art. no. 703.

Porzionato, A., Zaramella, P., Dedja, A., Guidolin, D., Bonadies, L., Macchi, V., Pozzobon, M., Jurga, M., Perilongo, G., de Caro, R., Baraldi, E., Muraca, M. Intratracheal administration of mesenchymal stem cell-derived extracellular vesicles reduces lung injuries in a chronic rat model of bronchopulmonary dysplasia. (2021) American Journal of Physiology - Lung Cellular and Molecular Physiology, 320 (5), pp. L688-L704.

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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 (2021) Frontiers in Immunology, 12, art. no. 627605.

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Neuromuscular Engineering

Junior PI: **Anna Urciuolo**

Team members

Anna Urciuolo
Junior Principal Investigator

Francesca Cecchinato
PostDoctoral Researcher

Beatrice Auletta
PostGraduated Fellow

Gilda Barbato
Master Student

Agnese Lauroja
Master Student

Pietro Chiolerio
Master Student

Martina Maino
Master Student

Former Members

Paolo Raffa
PostDoctoral Researcher

Valentina Scattolini
PostDoctoral Researcher

Maria Easler
Master Student

Marco Braggion
Master Student

Research activity

Conventional in vitro models used to study human tissue pathophysiology often fail to mimic in vivo relevant cell behavior. However, the development of novel in vitro models that can overtake such limitations are needed to move research from animal models to patients. This is particularly evident for skeletal muscle, where myofiber contraction and homeostasis is also guaranteed by the combination of a proper myofiber 3D organization, the extracellular environment and by the action of the nervous system. Skeletal muscle can be functionally compromised due to dysfunction of muscular, extracellular and/or of neuronal components, as in neuromuscular diseases.

Our recently established group results from the multidisciplinary integration of different expertise in skeletal muscle and stem cell biology, extracellular matrix and biomaterial engineering. The general lab interest is in understanding how the extracellular environment can guide and instruct different cell types toward their integrated function proper of an organ or tissue. Our main research project aims in studying healthy and diseased human neuromuscular tissue by using 3D models of skeletal muscle equipped with a neuronal network. Such 3D in vitro models is used to mimic the structural/functional properties

PI's biosketch

Scopus ID 6508342717

During the master's degree in Medical Biotechnologies at the University of Padua (2007, Italy) Dr. Urciuolo acquired molecular biology skills with particular focus on extracellular matrix (ECM), analysis of animal and in vitro models, and on skeletal muscle. During her PhD and her first PostDoctoral experience at the Dept. of Molecular Medicine, Dr. Urciuolo began and developed a new research line in Prof. Paolo Bonaldo's lab focused on the role of ECM (and in particular collagen VI) in preserving muscle stem cell niche, muscle regeneration and skeletal muscle mechanical properties. After this experience, in 2014, Dr. Urciuolo entered the field of tissue engineering and regenerative medicine in the lab of Prof. Paolo De Coppi at UCL-Institute of Child Health in London. Together with the extraordinary personal and scientific international experience, Dr. Urciuolo had the possibility to implement her surgery skills in murine models and to reach high stages of competence in tissue engineering by using decellularised organs. In particular, she combined decellularized organs with multiple cell types to develop tissue engineering constructs to be applied in regenerative medicine strategies in diseased animal models, with particular focus on skeletal muscle. In 2016, Dr. Urciuolo joined Prof. Nicola Elvassore's lab at the University of Padua, where she implement her specialization with tissue engineering, human induced pluripotent stem cells (hiPSCs) derivation and differentiation, microfluidics, biomaterials and 3D bioprinting. In collaboration with Bioera Lab (University of Padua) and UCL-Institute of Child Health, Dr. Urciuolo developed a new technology, named intravital three-dimensional (i3D) bioprinting, which allows hydrogel fabrication inside pre-existing 3D environment, both in vitro and in live animal models. The i3D technology offers an unprecedented opportunity to study cell biology and physiology in 3D environments, where the internal structure and organization can be modified according to biological needs, and allow i3D bioprinting of skeletal muscles in live animals.

From 2018, thanks to the UniPD STARS-Starting grant, Dr. Urciuolo is a Junior Principal Investigator at the Istituto di Ricerca Pediatrica. From 2021 she is Assistant Professor in the Department of Molecular Medicine at the University of Padova and Honorary Lecturer in the Department of Developmental Biology at the UCL-Institute of Child Health (London, UK).

The current research line of her lab results from the multidisciplinary integration of cell biology, extracellular matrix, and biomaterial engineering. Particular interest of her research team resides in the development and the study of 3D models of human skeletal muscle equipped with neuronal network, by combining hiPSC-derived cells, decellularised matrix and 3D bioprinting.

Dr. Urciuolo authored 27 publications with a total number of citations equal to 2195 and h-index of 16 (according to Scopus).

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.

Selected publications

Selmin, G., Gagliano, O., De Coppi, P., Serena E., Urciuolo, A., Elvassore, N. MYOD modified mRNA drives direct on-chip programming of human pluripotent stem cells into skeletal myocytes. Biochemical and Biophysical Research Communications, 2021, 560, pp. 139–145.

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Raffa P, Scattolini V, Gerli M F, Perin S, Cui M, De Coppi P, Elvassore N, Caccin P, Luni C, Urciuolo A. Decellularized skeletal muscles display neurotrophic effects in 3D organotypic cultures. STEM CELLS Translational Medicine (2020).

Urciuolo A, Serena E, Ghua R, Zatti S, Giomo M, Mattei N, Vetralla M, Selmin G, Luni C, Vitulo N, Valle G, Vitiello L, Elvassore N. Engineering a 3D in vitro model of human skeletal muscle at the single fiber scale. PLoS One (2020).

Urciuolo A, Poli I, Brandolino L, Raffa P, Scattolini V, Laterza C, Giobbe GM, Zambaiti E, Selmin G, Magnussen M, De Coppi P, Brigo L, Salmaso S, Giomo M, Elvassore N. Intravital 3D bioprinting. Nature Biomedical Engineering (2020).

Stem Cells and Regenerative Medicine

PI: **Michela Pozzobon**

Research activity

Team members

Michela Pozzobon
Principal Investigator

Stefania D'Agostino
PostDoctoral Researcher

Fabio Magarotto
PhD Student

Paola Bisaccia
PhD Student

Maira Bacchiega
Master Student

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. The lab is developing the following two main projects:

Muscle regeneration in pediatric skeletal muscle defects: study of the mechanism of action of human extracellular vesicles in a three-dimensional extracellular matrix model.

Defects of abdominal wall closure in pediatric patients (as gastroschisis and omphalocele, or abdominal wall weakness) are rare muscle deficiencies with an unmet clinical need. Implant of synthetic patches, that do not grow with the baby, is the standard of care. To avoid multiple surgery, biological scaffolds from decellularized muscles (muscle extracellular matrix, mECM) are a promising therapeutic tool. These organ-specific scaffolds possess pro-regenerative factors and native ECM structure, that drive tissue reconstruction by attraction and invasion of the recipient cells. However, implanted mECM needs additional stimuli to counteract fibrosis, enhance cell migration and regain contractility of the skeletal muscle. Extracellular vesicles (EVs) are nanoparticles produced by cells, conveying a variety of biological signals. In particular, EVs secreted by mesenchymal stromal cells are able to improve tissue regeneration by counteracting cell apoptosis, improving angiogenesis, modulating inflammation and fibrosis. We demonstrated in a murine model of volumetric muscle loss, that the implantation of mECM (as biological scaffold) loaded with EVs (as biochemical stimuli), improves muscle regeneration and functionality in respect to the untreated mECM. Starting from this ground, this project aims to:

- investigate EV mechanism of action by integrating the native mECM environment of skeletal muscle with the key cellular players involved during regeneration.

In particular, 3D in vitro models that will include decellularized muscles, muscle precursor cells, macrophages, neuronal cells and EVs, will be setup.

Development of tridimensional model of rhabdomyosarcoma to mimic physiological cell-extracellular matrix interaction

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood accounting for over half of all cases. It is typical of the pediatric age spanning from very young children to adolescents. It develops from immature mesenchymal cells committed to skeletal muscle differentiation and can arise anywhere in the body. At diagnosis, approximately 20-25% of

PI's biosketch

Scopus ID 6602197182
Dr. Pozzobon 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.
Dr. 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.
In 2020 she got a tenure track assistant professor position (RTDb) in Applied Biology, at the Department of Women's and Children's Health, University of Padova.
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.
Dr. Pozzobon is actively involved in national and international projects related to regenerative medicine and tumor microenvironment and is a MC 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. In 2020 she was awarded the IRP-Consolidator grant to investigate the mechanism of action of extracellular vesicles in muscle regeneration.
Dr Pozzobon is author of over 100 abstracts selected for poster or oral presentation; her scientific production counts 84 publications on peer review journals, in 26 of them she is the first, last or corresponding author. Google Scholar H index: 35. Scopus H index: 31.

cases present metastasis that occur especially in lung and bone marrow, which strongly suggests that the microenvironment contribute significantly to the growth potential of this malignancy. Studies of ECM support the important role of the cross-talk between transformed cells and their niche, linking ECM composition with pathological conditions. The interest in this new aspect starts widening the understanding of tumor progression and opens new avenues for developing innovative therapies.

Our aim is two folds:
- to investigate the best 3D model to mimic RMS microenvironment and the metastatic migration of cancer cells;
- to investigate of the role of the highly expressed GPC3 proteoglycan, in RMS tumor growth and progression, in 3D.

Selected publications

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. Online ahead of print. PMID: 34796888.

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Bertin E, Piccoli M, Franzin C, Spiro G, Donà S, Dedja A, Schiavi F, Taschin E, Bonaldo P, Braghetta P, De Coppi P, Pozzobon M. First steps to define murine amniotic fluid stem cell microenvironment. Sci Rep. 2016 Nov 15;6:37080. doi: 10.1038/srep37080.

Pozzobon M, Bollini S, Iop L, De Gaspari P, Chiavegato A, Rossi CA, Giuliani S, Fascetti Leon F, Elvassore N, Sartore S, De Coppi P. Human bone marrow-derived CD133(+) cells delivered to a collagen patch on cryoinjured rat heart promote angiogenesis and arteriogenesis. Cell Transplant. 2010;19(10):1247-60. Epub 2010 May 4.

Tissue Engineering

PI: **Martina Piccoli**

Research activity

Congenital Diaphragmatic Hernia (CDH) is a neonatal malformation that occurs during the diaphragmatic muscle development. Despite the advancement in the techniques of CDH treatment, the common use of synthetic implants (i.e. Gore-Tex) to repair the hernia is often followed by significant side effects, such as limited elasticity and lack of growth with the child, leading to subsequent muscle tears and implant failure. Hence, young patients need to undergo multiple surgeries, increasing each time the risk of complications and additional side effects. In the recent years, tissue engineering has brought significant improvements in the treatment of defects and congenital malformations in general. Unfortunately, the clinical application of biological materials obtained from tissues different from the skeletal muscle did not allow a clear improvement in the treatment of CDH, if compared to the common use of synthetic implants.

Our research group has shown that the use of a biological implant obtained through decellularization of the diaphragmatic muscle greatly improves CDH treatment in an animal model, avoiding an extensive scar formation, and consequently limiting recurrences. Moreover, the addition of muscle cells to the decellularized diaphragm through the use of a customized bioreactor, allows to generate a mature and functional diaphragm in vitro. Despite these important results, this classic tissue engineering approach requires long preparation times, depends on organ donation and cannot be used

Team members

Martina Piccoli
Principal Investigator

Edoardo Maghin
PostDoctoral Researcher

Andrea Roberto Calore
PostDoctoral Researcher

Eugenia Carraro
PhD Student

Lucia Rossi
Research Fellow

Elena Merotto
Master Student

PI's biosketch

Scopus ID: 15127681100
Dr. Piccoli graduated in Biological Sciences at the University of Padua in 2004 developing a cellular and molecular study in the Pediatric Dept. laboratory. In 2009, Dr. Piccoli completed a postgraduate degree in Clinical Pathology working on a project focused on the study of amniotic fluid stem cells, their phenotypical characterization and in vivo use in different mouse models before completing her PhD period in 2013 in the same field. Dr. Piccoli has a broad background in biological science and clinical pathology, with specific training and expertise in development and pediatric research. Her research includes fetal and adult stem cell characterization and production of biological scaffolds for tissue engineering purposes. During her post-graduate training, Dr. Piccoli laid the groundwork for the current research by developing technical skills in tissue engineering field: in particular, in decellularization methods to produce natural bioscaffolds and cell culture techniques to regenerate in vitro 3D tissue-like structures. In addition, she is the principal investigator of different granted projects and is co-inventor of two patents.

for large-scale production. The manufacture of a product that is standardized and identical for all patients is therefore not feasible.
As a research team, starting from the decellularized diaphragm extracellular matrix, the group aims at developing a biological ink mixed with cells that constitute the tissue under physiological conditions. This bioink is used for 3D printing of a construct through a printer developed for this purpose. The construct is then grown and matured inside a specific and homemade bioreactor. It is necessary, in fact, to stimulate the arrangement and alignment of the cells within the printed construct to obtain, at the end of the process, a diaphragm that resembles the original tissue as much as possible. The main objective is to obtain a specific and always identical biomaterial, to make its large-scale production and the manufacture of the construct finely tunable and automated through 3D printing, aiming at an even more personalized regenerative medicine treatment.

Selected publications

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