Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

2017 -2019

Scientific Report

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

2017 -2019

Scientific Report

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

Contents

Intro

Research Areas

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

Governance

President	**Prof. Antonio Parbonetti** (January 2019 – present)
Scientific Director	**Prof. Antonella Viola** (September 2017 – present)
CEO	**Dr. Andrea Camporese** (September 2017 - October 2020)
	Dr. Luca Primavera (since October 2020)

Scientific Advisory Board
(November 2017 – December 2019)

President

Prof. Lorenzo Moretta
Ospedale Pediatrico Bambino Gesù, Rome, Italy

Prof. Andrea Biondi
University of Milano-Bicocca, Milan, Italy

Prof. Ilaria Capua
One Health Center of Excellence for Research and Training,
University of Florida, USA

Scientific Advisory Board
(since January 2020)

President

Prof. Lorenzo Moretta
Ospedale Pediatrico Bambino Gesù, Rome, Italy

Prof. Andrea Biondi
University of Milano-Bicocca, Milan, Italy

Prof. Ruggero De Maria
Università Cattolica del Sacro Cuore, Rome, Italy

Prof. Vassilios Fanos
University of Cagliari, Cagliari, Italy

Prof. Jan-Henning Klusmann
Martin Luther University Halle-Wittenberg, Halle, Germany

Prof. Graziella Pellegrini
University of Modena and Reggio Emilia /
Centre for Regenerative Medicine, Modena, Italy

Prof. Manuela Teresa Raimondi
Politecnico di Milano, Milan, Italy

Prof. Orsetta Zuffardi
University of Pavia, Pavia, Italy

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

The Istituto di Ricerca Pediatrica (IRP) is a dynamic and multidisciplinary environment that has experienced an important development and growth in the last years, thanks to the partners, the investors, and especially to the researchers who, with passion and dedication, work hard to improve living conditions or prospects of healing children.

This dynamism is the result of the collaboration between the Fondazione Città della Speranza, the Azienda Ospedaliera, the University of Padua and the different Academic Departments within University (Department of Women's and Children's Health; Department of Biomedical Sciences; Department of Surgical, Oncological and Gastroenterological Sciences; Department of Industrial Engineering and Department of Physics). In the last three years, the number of departments and researchers involved in IRP has increased sharply, this testify the quality of services and facilities that researchers can use, the quality of research environment that facilities sharing new ideas, thus making us confident about future growth opportunities. Attracting talents is one of our core goals, because it is fundamental for high quality research.

The new governance resulting from the new statute of the IRP allow us to look forward to the future with confidence. The IRP need to exploit the intellectual property and the knowledge and the cutting edge research to improve diagnostics, therapies and guarantee a sustainable growth. The knowledge accumulated over the last years represents our core resource to improve quality of life of children and their families.

In accordance with the mission of the IRP and its strategic approach, we hope that there will be a growing trend for clinical trials in order to create a stronger connection between research and therapy. In this vein, the board of directors has established the Patent Committee in order to focus the attention on how the intellectual properties can be used.

Attracting talents is one of our core goals, because it is fundamental for high quality research

Furthermore, it is necessary to implement actions that increase the visibility and reputation of the IRP nationally and internationally. IRP is already organizing numerous initiatives involving key stakeholders, schools and the society as all. Particularly, IRP organizes cultural events and invites regularly keynote researchers to deliver speeches targeting hot topic issues. We think that research should improve the quality of life and IRP is strongly committed to increase and spread knowledge: This is part of our long-term strategy

Finally, I would like to thank the funders and volunteers who have placed their trust in the IRP. We feel the responsibility to make effective and efficient use of the resources they provide. A special thanks also goes to Fondazione Città della Speranza, the Scientific Director Prof. Antonella Viola, Dr. Andrea Camporese and Prof. Giorgio Perilongo for their hard and valuable work..

Antonio Parbonetti
President


città della speranza
Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

The Istituto di Ricerca Pediatrica (IRP) Città della Speranza has become a true centre of excellence over the last eight years, since its opening.

High tech laboratory facilities, a multidisciplinary environment for clinical and preclinical research, the advanced diagnostic, training activities and the strong connection between the public and the private sector are the key features of its success, that goes beyond any expectations for what concerns both acquired expertise and the economic sustainability.

In the last three years, we have massively invested in the expansion of the institute. If we consider only the 2018-19 period, Fondazione Città della Speranza has invested 2.5 mil Euros to improve and upgrade infrastructure and facilities, and to implement new IT platforms and services, necessary to also maintain and ensure the workplace's safety.

As part of our ambitious plans for growth, we need to strengthen our lasting relationship with the University of Padua and the Azienda Ospedaliera, as well as with the territory. It is paramount to pursue the achievement of important results for all of us.

The partnership between public research and private industry does not just mean having more available resources, but also being able to attract external funds, by participating in national and international calls for proposals. Furthermore, talented early career researchers could be encouraged to continue their studies and career thanks to the support of the private sector.

All these aspects must be implemented, just like investing in time, resources and effort in order to raise the public's scientific awareness and engagement through outreach campaigns, following the path outlined by our Scientific Director. With this purpose, every year we host about 2000 visitors and students from primary, middle and high schools. The latter are part of the "Adopt a researcher" initiative, which aims at creating a bond between the fundraising campaigns and the scientific projects and researchers to be supported.

This scientific report is a clear testimony of the effort and work of an entire community

This scientific report is a clear testimony of the effort and work of an entire community. I would like to thank our Scientific Director Prof. Antonella Viola for her enormous dedication in this challenging and crucial time in the life of the Institute. She is the right person to lead us through it. The achievements reported in this document are the reason why we can now guarantee a further 10 mil Euros to support research for the next 3 years, notably thanks to the generous contribution of the Fondazione Città della Speranza and the Fondazione Cariparo, through their calls for proposals dedicated to the pediatric research carried out in IRP.

A special acknowledgement goes to the Fondazione Città della Speranza and its volunteers that work along side us in this incredible adventure with the sole goal of protecting and caring for sick children.

Andrea Camporese
CEO 2017 - 2020


Fondazione
ISTITUTO DI RICERCA
PEDIATRICA
città della speranza

The Department of Women's and Children's Health (DWCH) is an academic medical center providing state-of-the-art patient care to children of any age, teaching and training in Pediatrics and promoting research. It encompasses a university and a hospital component; the former is part of the University of Padua, the latter of the General Hospital of Padua. From a clinical point of view, it is a 210-bed hospital facility functioning as a Children's Hospital (within a Hospital). It is composed of eighteen medical and surgical pediatric divisions and, as a such, it is the main pediatric institution of the entire North Est of Italy, operating as a Hub for a variety of pediatric specialty regional networks. As a whole, almost 700 people work in the Department. The DWCH is a full member of the Italian Association of Children's Hospitals. It joins (being, the General Hospital, the official "Health Care Provider - HCP") eighteen European Excellent Networks (ERN) on rare diseases (of the twenty-one of which the General hospital is HCP). The DWCH is also among the very few Italian pediatric institutions covering the entire spectrum of hematopoietic stem cells and solid organ transplant programs in children (but the intestine). Slightly annually more than 10.000 are the hospital admissions, 3.000 the newborns (delivered in the DWCH) and 25.000 the emergency room visits. The university personnel of the Hospital are involved in teaching and training in almost all the pre- and post-graduate courses of the School of Medicine of the University of Padua. The DWCH runs five residency programs (Pediatrics, Child Neuropsychiatric, Pediatric Surgery, Community Medicine and Genetics), a PhD school in Pediatrics and a large series of post-graduates courses devoted to Continuous Medical Education. The DWCH works in close synergy with the Istituto di Ricerca Pediatrica to generate its research activity. The two institutions together, by sharing human resources, knowledge, competence, ideas and technical equipment and above all making effective a continuous flow of data, information and ideas from the various laboratories sitting in the institution and the pediatric clinical wards of the DWCH, represent the largest reality running basic and clinical research in pediatric in Italy and one of the largest in Europe.

The IRP and the DWCH together represent the largest reality running basic and clinical research in pediatrics in Italy and one of the largest in Europe

Giorgio Perilongo
Director of the Dept. of Women's and Children's Health - University of Padua

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

IRP is a world-leading research institute, home to reference laboratories for pediatric medicine and scientific research. There are six 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. Through a multidisciplinary approach, the Institute brings together the expertise in biomedicine, bioengineering, biochemistry and material sciences; moreover the Institute and the Department of Women's and Children's Health (SDB) - University of Padua have built a long lasting strategic partnership and now share not only resources and facilities, but also their goals and overall vision.

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.

The quality of research institutes depends on two factors: people and technology. One of the biggest challenges that Italian research has to face is how to be competitive in a scenario of world-renowned organisations that offer a wide array of services and facilities, required to support the activity of Principal Investigators and their teams. During the 2017-2019 time span, the Institute has grown extensively adding more research areas, incrementing and establishing new facilities (such as single cell transcriptomic analysis, microscopy, flow cytometry), creating and strengthening a collaborative environment to make our research more time- and cost-effective. As such, the cohabitation of private and public research realities within the Tower of Research (Istituto Oncologico Veneto-IOV, LifeLab Program and others), has proven to be a major advantage in establishing new collaborative projects, promoting knowledge exchange and sharing of expertise. Additionally, IRP has tied a collaboration with the Penta Foundation, which will allow us to expand toward the field of infectious diseases in pediatrics.

Modern medicine is often described with the 4Ps: Personalised, Predictive, Preventive and Participatory; and this is even more fitting when thinking about medicine specifically dedicated to children, from neonatology to oncology and genetic diseases. IRP applies these principles to precision medicine with the specific aim of translating science into children's health.

> **IRP applies the principle of the 4Ps with the specific aim of translating science into children's health**

Antonella Viola
Scientific Director

6
Research areas

26
Research groups

161
Researchers

Publications

2017
N° **160**
IF **790,63**
AV IF **4,94**

2018
N° **172**
IF **948,07**
AV IF **5,51**

2019
N° **177**
IF **982,08**
AV IF **5,55**

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

IRP researchers publish about 160-170 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.

2017-2019

29 Scientific Awards

81 Evaluation Panel

75 Research Grants

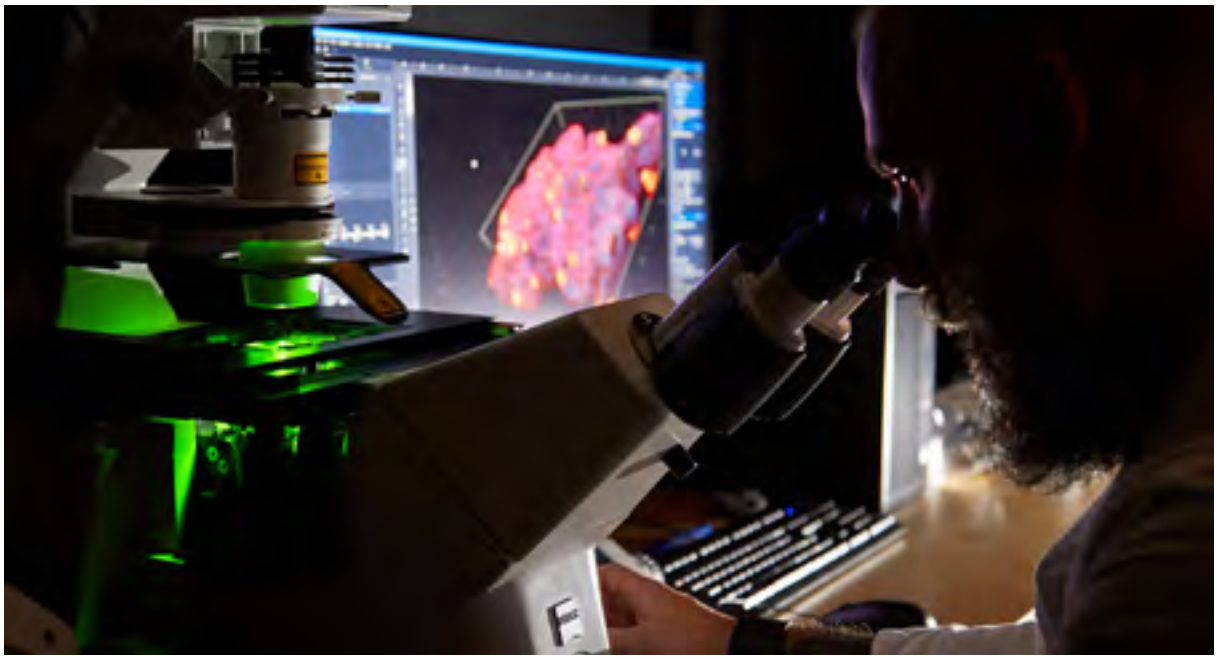
141 Invited Talks / Lectures

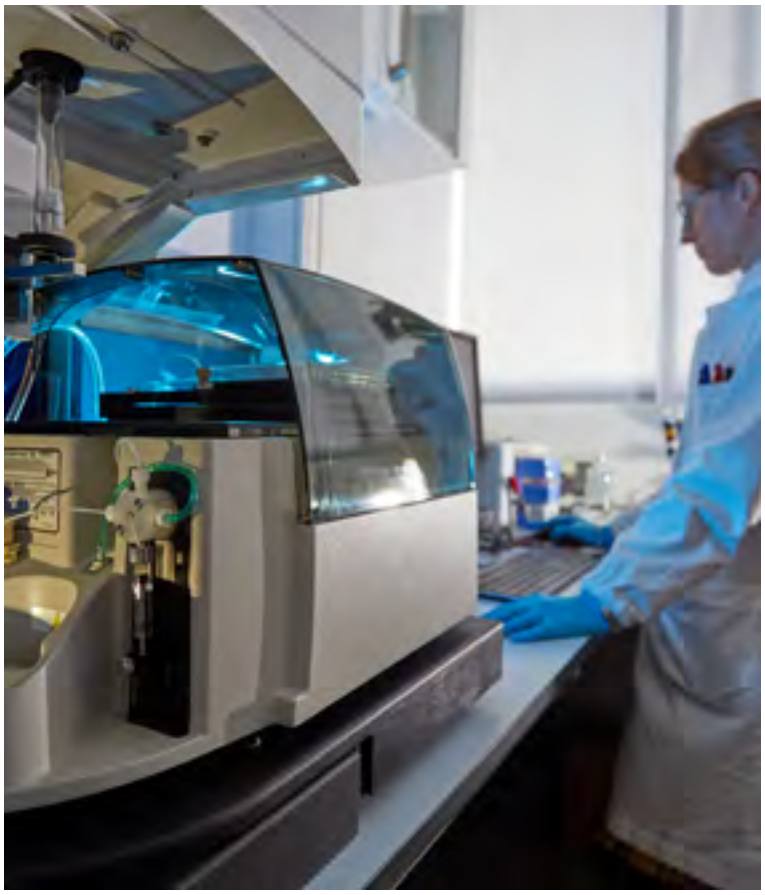
IRP Facilities

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

Microscopy

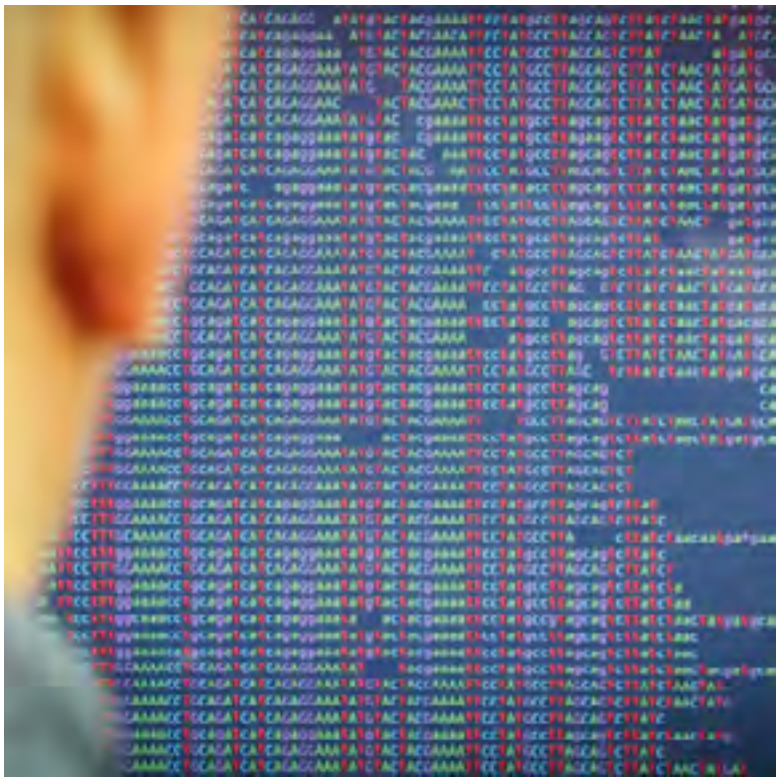
The Microscopy facility is equipped with the last generation ZEISS LSM 800 confocal microscope, with Airyscan technology for superresolution imaging. There are also three other fluorescence microscopes including the newest ZEISS Axio Observer with LED technology, provided with a CO2 incubator for live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





Flow cytometry

The Flow Cytometry and Sorting Facility offers to all research's groups an efficient and personalized service. The facility provides two cytometers, a FC500 and a Cytoflex, and three cell sorters, a MoFlo XDP, a FACS Aria III and a FACS Celesta.

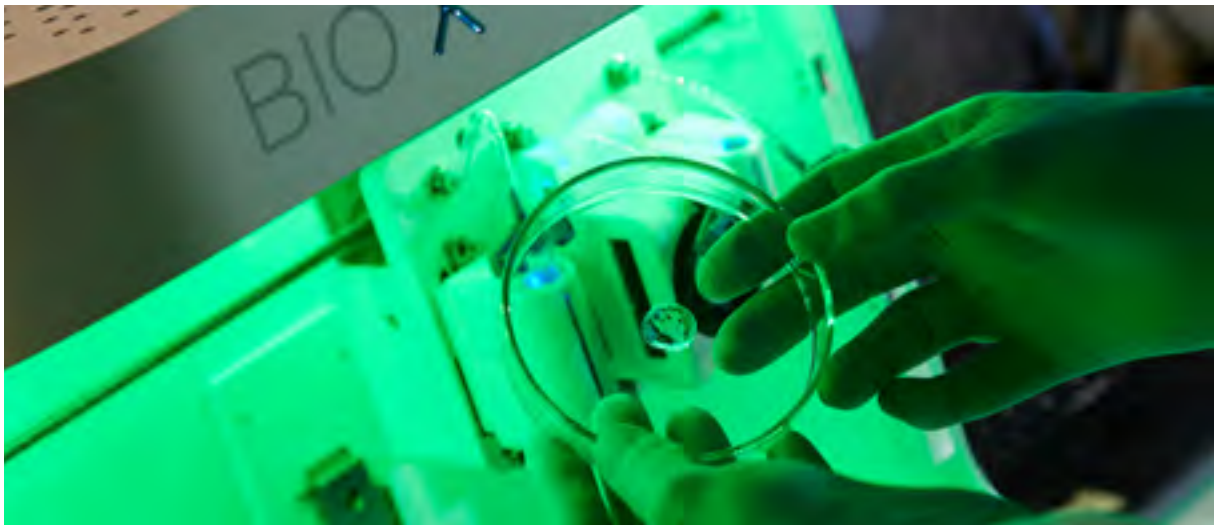


Bioinformatics

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

EVENTS 2017-2019

32 **PhD student progress reports**

25 **Internal seminar**

16 **Invited speaker seminars**

4 **Invited speaker Lectures**

On the top

1st SAB site visit

December 2018

On the right

1st Annual Retreat

April 2018

Dr. Sonia Giambelluca and Dr. Caterina Trevisan were awarded the prize "Famiglia Masello in memoria di Rita Masello e Massimo Zilio" for the two best oral communications.

Dr Cristina Calderan, Dr. Diana Corallo and Dr. Elena Mariotto were awarded the prizes "Elisa Camporese" and "Matteo Fochesato" for the three best poster presentations.

On the top

2nd Annual Retreat

October 2019

Dr. Roberta Angioni and Dr. Edoardo D'Angelo were awarded the prize "Famiglia Masello in memoria di Rita Masello e Massimo Zilio" for the two best oral communications.
Dr. Giulia Borile, Dr. Bianca Calì and Dr. Anna Garbin were awarded the prizes "Elisa Camporese" and "Matteo Fochesato" for the three best poster presentations.

Below

Prof Elena Cattaneo

on May 10, 2019

Elena Cattaneo, Full Professor at the University of Milan and Senator for Life in the Italian Senate since 2013, visited the Institute urging researchers to act in the public sphere to completely fulfill their role as scientists.

From the top

Course: "Introduction to Translational medicine: from benchside to bedside to the community"

January 14-18, 2019

Organized by the George Mason University, Dept. of Women's and Children's Health-Azienda Ospedaliera-Università di Padova and Istituto di Ricerca Pediatrica.

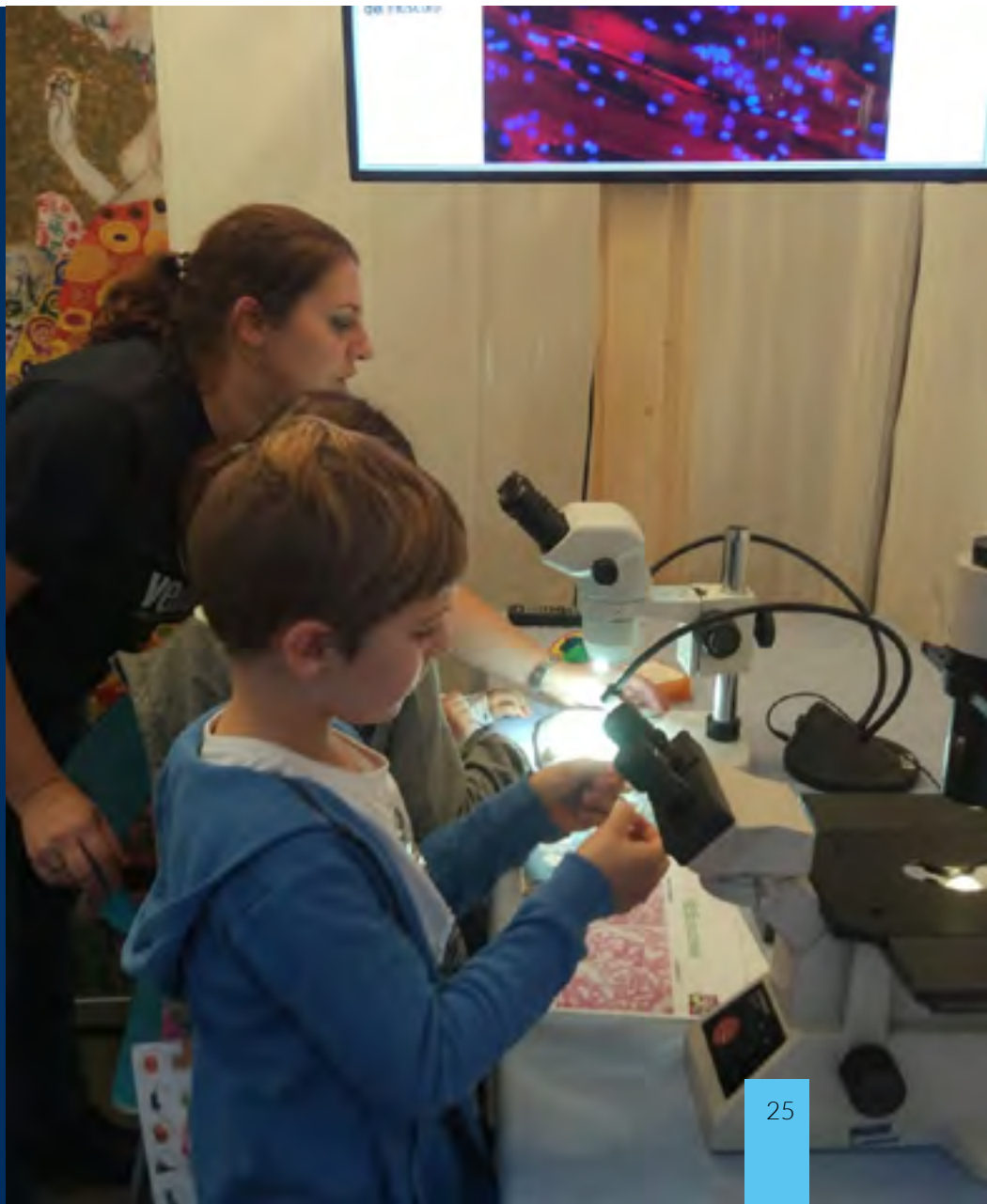
First edition of conference series

"Viaggio al centro della scienza – Journey to the centre of the science"

May 2018

On the top
Second edition of
conference series
“Viaggio al centro della scienza – Journey to the centre of the science”
May 2019

On the right
European Researchers’ Night
2017-2018-2019



Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

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.


Fondazione
ISTITUTO DI RICERCA
PEDIATRICA
città della speranza

Prof. Leonardo Salviati

Coordinator Genetics and Rare Diseases Area

Scopus ID: 6602836082

Prof. Salviati received his MD degree cum Laude from the University of Padua in 1995 and completed his residency in Pediatrics in 1999. From 1999 to 2002, he worked as a clinical research fellow in the laboratories of Eric A. Schon and Billi Di Mauro at the Houston-Merritt Center for Inherited Myopathies and Mitochondrial Diseases at the Dept. of Neurology, Columbia University, New York, focusing his research on mitochondrial disorders in particular on the biogenesis of Cytochrome c Oxidase. He received his PhD in Developmental Biology from the University of Padua in 2004. In 2005, he was appointed assistant Professor of Medical Genetics at the University of Padua, then in 2014 he became associate Professor of Medical Genetics and in 2019 he was appointed full Professor and Director of the Clinical Genetics Unit, 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 2M €. Overall, he has co-authored 143 peer-reviewed articles with more than 6000 citations and an h-index of 44 (according to Scopus). Total impact factor is over 800 points (ISI 2019).

Clinical Genetics and Epidemiology Laboratory

PI: **Leonardo Salviati**

Team members

Leonardo Salviati
Principal Investigator

Matteo Cassina
Associate professor of Medical genetics

Daniela Bettio
Assistant professor of Medical genetics

Chiara Rigon
Technician

Cristina Calderan
PhD Student

Elisa Baschiera
PhD Student

Alessandra Friso
Biologist

Cinzia Bertolin
Biologist

Francesca Boaretto
Biologist

Monica Forzan
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, wich 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,

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 Di Mauro at the Houston-Merritt Center for Inherited Myopathies and Mitochondrial Diseases at the Dept. of Neurology, Columbia University, New York, focusing his research on mitochondrial disorders in particular on the biogenesis of Cytochrome c Oxidase. He received his PhD in Developmental Biology from the University of Padua in 2004. In 2005, he was appointed assistant Professor of Medical Genetics at the University of Padua, then in 2014 he became associate Professor of Medical Genetics and in 2019 he was appointed full Professor and Director of the Clinical Genetics Unit, 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 2M €. Overall, he has co-authored 143 peer-reviewed articles with more than 6000 citations and an h-index of 44 (according to Scopus). Total impact factor is over 800 points (ISI 2019).

in particular vitamin B6-dependent enzymes such as ornithine aminotransferase (OAT). We are currently studying the molecular pathogenesis of OAT deficiency and therapeutic role of vitamin B6 and its vitamers in patients.
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 three new genes associated with human diseases: MCM5, ADCK2, and TOGARAM.

Selected publications

Vanillic Acid Restores Coenzyme Q Biosynthesis and ATP Production in Human Cells Lacking COQ6. Acosta Lopez MJ, Trevisson E, Canton M, Vazquez-Fonseca L, Morbidoni V, Baschiera E, Frasson C, Pelosi L, Rascalou B, Desbats MA, Alcázar-Fabra M, Ríos JJ, Sánchez-García A, Basso G, Navas P, Pierrel F, Brea-Calvo G, Salviati L. Oxid Med Cell Longev. 2019 Jul 10;2019:3904905.

DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. Favaro G, Romanello V, Varanita T, Andrea Desbats M, Morbidoni V, Tezze C, Albiero M, Canato M, Gherardi G, De Stefani D, Mammucari C, Blaauw B, Boncompagni S, Protasi F, Reggiani C, Scorrano L, Salviati L, Sandri M. Nat Commun. 2019 Jun 12;10(1):2576.

Mutations in COQ8B (ADCK4) found in patients with steroid-resistant nephrotic syndrome alter COQ8B function. Vazquez Fonseca L, Doimo M, Calderan C, Desbats MA, Acosta MJ, Cerqua C, Cassina M, Ashraf S, Hildebrandt F, Sartori G, Navas P, Trevisson E, Salviati L. Hum Mutat. 2018 Mar;39(3):406-414.

Age-Associated Loss of OPA1 in Muscle Impacts Muscle Mass, Metabolic Homeostasis, Systemic Inflammation, and Epithelial Senescence. Tezze C, Romanello V, Desbats MA, Fadini GP, Albiero M, Favaro G, Ciciliot S, Soriano ME, Morbidoni V, Cerqua C, Loefler S, Kern H, Franceschi C, Salvioli S, Conte M, Blaauw B, Zampieri S, Salviati L, Scorrano L, Sandri M. Cell Metab. 2017 Jun 6;25(6):1374-1389.

Mitochondrial dysfunction in inherited renal disease and acute kidney injury.Emma F, Montini G, Parikh SM, Salviati L. Nat Rev Nephrol. 2016 May;12(5):267-80.

Diagnosis and Therapy of Lysosomal Disorders Laboratory

PI **Rosella Tomanin**

Research activity

We mainly focus our research interests on Mucopolysaccharidoses (MPSs), a cluster of Lysosomal Storage Disorders, due to the deficit of the enzymes normally catabolizing mucopolysaccharides (or glycosaminoglycans) inside the lysosomal compartment. Being lysosomal 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. The treatment though has shown so far some peripheral efficacy, but unfortunately does not help the CNS disease, due to the inability of the enzymes 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 relative possible treatments, by using different *in vitro* and *in vivo* models.

Team members

Rosella Tomanin
Principal Investigator

Alessandra Zanetti
Research Assistant

Francesca D'Avanzo
PostDoctoral Researcher

Concetta De Filippis
PhD student

Projects of the research group

- In the last decade, 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 have 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). We have also successfully delivered the recombinant form of the enzyme iduronate 2-sulfatase to

PI's biosketch

Scopus ID: 6603451357

Dr. Tomanin graduated at the University of Padua in 1985, 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.

Dr. Tomanin is co-author of 60 publications on peer-reviewed International Journals, 5 book chapters, and some publications in Italian Journals. She served as a reviewer for several International Journals, among which: PlosOne, Gene, Cytotherapy, Medicine, Orphanet J. Rare Dis., Comput. Struct. Biotechnol. J, Genet Mol Biol, Int J Mol Sci, Mol Genet Metab Rep.

AWARDS and ACKNOWLEDGEMENTS:

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

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

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

Jan 1994 - June 1994: Fellowship 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.

the brain of the MPS II mouse model (Rigon *et al*. Int J Mol Sci 2019 Apr 24; 20(8):2014). We have recently optimized the NPs-mediated strategy, by increasing the enzyme stability and its encapsulated amount, though maintaining efficiency and efficacy, as shown by the *in vitro* results obtained (unpublished), while the *in vivo* delivery is ongoing.

- With the aim of understanding the onset of brain pathogenesis in these disorders and also its progression, we are characterizing the brain parenchyma of the MPS II mouse model by evaluating different biomarkers at several progressive age of the animal, which will be also useful to monitor therapeutic efficacy of brain-targeted approaches at different animal ages. We are at the moment completing the analysis performed so far by evaluating the very early stages of life.
- In the last couple of years we have started a new project in collaboration with the Dept. of Pharmaceutical and Pharmacological Sciences, University of Padova, aiming at generating and characterizing Drosophila models of mucopolysaccharidoses. Our PhD student has generated a Drosophila knock-down model of MPS I, whose characterization is almost

complete, showing that the fly may represent a good model of the disease. Drosophila is a fast and valuable model to study pathogenesis issues and also a good tool for screening of therapeutic molecules.

- Our interest in the MPS brain pathogenesis is also focused on the identification of the 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 issue of Advanced Diagnostics, we have recently 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). We have also completed the "mutation update" of the ARSB gene, through a re-classification of all published variants, according to ACMG (American College of Medical Genetics and Genomics) (Tomanin *et al*. Hum Mutat 2018 Dec;39(12):1788-1802); in the last few months, this analysis, helping to perform a correct molecular diagnosis, has been extended to other 2 genes of interest, one of which is under completion.

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

Selected publications

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.

Zanetti A, D'Avanzo F, Bertoldi L, Zampieri G, Feltrin E, De Pascale F, Rampazzo A, Forzan M, Valle G, Tomanin R. Setup and Validation of a Targeted Next-Generation Sequencing Approach for the Diagnosis of Lysosomal Storage Disorders. J Mol Diagn. 2020 Apr;22(4):488-502.

Rigon L, Salvalaio M, Pederzoli F, Legnini E, Duskey JT, D'Avanzo F, De Filippis C, Ruozi B, Marin O, Vandelli MA, Ottonelli I, Scarpa M, Tosi G, Tomanin R. Targeting Brain Disease in MPSII: Preclinical Evaluation of IDS-Loaded PLGA Nanoparticles. Int J Mol Sci. 2019 Apr 24;20(8):2014.

Tomanin R, Karageorgos L, Zanetti A, Al-Sayed M, Bailey M, Miller N, Sakuraba H, Hopwood JJ. Mucopolysaccharidosis Type VI (MPS VI) and Molecular Analysis: Review and Classification of Published Variants in the ARSB Gene. Hum Mutat. 2018 Dec;39(12):1788-1802.

Bellesso S, Salvalaio M, Lualdi S, Tognon E, Costa R, Braghetta P, Giraudo C, Stramare R, Rigon L, Filocamo M, Tomanin R, Moro E. FGF Signaling Deregulation Is Associated With Early Developmental Skeletal Defects in Animal Models for Mucopolysaccharidosis Type II (MPSII). Hum Mol Genet. 2018 Jul 1;27(13):2262-2275.

Immunopathology and Molecular Biology of Kidney Laboratory

PI **Luisa Murer**

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 the Azienda Ospedale Università Padova. The Unit is a center of excellence and reference for Pediatric Nephrology and Rare kidney disease. It is also part of the international registries and networks (i.e. ERKNet) for the diagnosis and treatment of kidney rare diseases. Furthermore, it works in close collaboration with various specialists to follow children patients 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 paraffin and frozen renal tissues of transplanted pediatric patients. The Pediatric Nephrology Di-alysis and Transplant unit together with the laboratory conduct and coordinate important scientific studies, from the clinical trials to the translational research, in particular concerning kidney trans-plantation, congenital abnormalities of the kidney and urinary tract (CAKUT), nephrotic syndrome, acute kidney injury (AKI) and dialysis in childhood.

Concerning the CAKUT, one of the most severe phenotypes is renal hypodysplasia (RHD), which is a defect in the number and/or normal

Team members

Luisa Murer
Principal Investigator

Susanna Negrisolo
Senior PostDoctoral Researcher; Lab Manager

Andrea Carraro
PostDoctoral Researcher

Irene Alberici
Clinical Researcher

Elisa Benetti
Clinical Researcher

Nicola Bertazza Partigiani
Clinical Researcher

Maria Sangermano
Collaborator, Clinical Researcher

Diana Marzenta
Lab Technician

Elisabetta Bettin
Master student

PI's biosketch

Scopus ID: 7004133262
Dr. Murer studied at the University of Padua. She obtained the Medical Doctor degree in 1986, the Residency in Pediatrics School in 1990, the PhD in Developmental Sciences in 1994 and the Residency in Pediatric Nephrology in 1995. She moved to the UK in 1987 for a fellowship in the Pediatric Research Unit Guy's Hospital, London. Since 2001, she is Adjunct Professor of many Specialization Schools at the Dept. of Women's and Children's Health Dept.at the University of Padua. Since 2007, Dr. Murer is the Head of the Pediatric Nephrology, Dialysis and Transplant Unit and the PI of the laboratory of Immunopathology and Molecular Biology of Kidney at the Dept. of Women's and Children's Health (Azienda Ospedale Università Padova). Currently, she is President of the Italian Pediatric Nephrology Society (SINePe) and Representative of the European Reference Network for rare renal disease (ERKNet). Since 1990, she belongs to the Italian Society of Pediatric Nephrology and the European Society of Pediatric Nephrology (ESPN), in which she is part of the Kidney Transplantation Working Group, the Nephrotic Syndrome Working group and the CAKUT Working Group. Furthermore, Dr. Murer is also member of national and international societies and registries of renal disease: Italian Society of Nephrology (SIN), Italian Society for Organ Transplant (SITO), Certain registry, ESCAPE, International Society of Pediatric Nephrology (IPNA), and European Renal Association and European Dialysis and Transplant Association (EDTA). She is principal investigator of national and international projects, and she is also reviewer of the Journal of Nephrology, Kidney International, Pediatric Nephrology, Pediatric Transplantation, Clinical Kidney Journal; European Journal of Pediatrics; PLOS One; Clinical Chemistry and Laboratory Medicine; Nephrology Dialysis Transplantation; Italian Journal of Pediatrics; BMC Nephrology.

differentiation of nephronic units with a subsequent impair-ment of kidney function. Even though mutations of at least 17 genes involved in the early stages of kidney development have been associated with RHD, the majority of patients remains without a genetic diagnosis. In the last three years, we have been participating in the University of Padua's Strategic project "Bioinfogen" with the aim to create new bioinformatics tools to facilitate NGS data analysis in Mendelian diseases. Our unit is investigating RHD genetic causes in 20 patients and their healthy relatives, with whole exome sequencing. To date, we identified a new candidate gene for isolated bilateral RHD (INVS) and validation studies are still ongoing. Furthermore, we highlighted a variant in a candidate gene in 25% of the analysed cases. These results will permit to lay the basis for setting up a perspective RHD diagnostic panel.
Another 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 15 years, un-acceptable for pediatric patients. The gold standard in the diagnosis of renal allograft failure (the main cause of kidney rejection) is the biopsy and the Pediatric Transplant Centre of Padua

is a pioneer of protocol biopsy, that allows recognizing the rejection when there are no clinical features. In our lab, we are evaluating the prognostic value of infiltrate cell phenotyping, of the intrarenal presence of the virus, of the dosage of antibody against the donor. Now we are collaborating with many IRP groups with different expertise, to characterize the extracellular vesicles (EVs) isolated from se-rum 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.

Selected publications

Iatropoulos P, Daina E, Curreri M, Piras R, Valoti E, Mele C, Bresin E, Gamba S, Alberti M, Breno M, Perna A, Bettoni S, Sabadini E, Murer L, Vivarelli M, Noris M, Remuzzi G; Registry of Membranoproliferative Glomerulonephritis/C3 Glomerulopathy; Nastasi. 2018. Cluster Analysis Identifies Distinct Pathogenetic Patterns in C3 Glomerulopathies/Immune Complex-Mediated Membranoproliferative GN. Journal of the American Society of Nephrology, 29(1), pp. 283-294.

Negrisolo, S., Carraro, A., Fregonese, G., Benetti E., Schaefer F., Alberti M., Melchionda S., Fischetto R., Giordano M, Murer L. 2018 Could the interaction between LMX1B and PAX2 influence the severity of renal symptoms? European Journal of Human Genetics 26, pp.1708–1712.

Giglio S, Provenzano A, Mazzinghi B, Becherucci F, Giunti L, Sansavini G, Ravaglia F, Roperto R, Farsetti S, Benetti E, Rotondi M, Murer L., Lazzeri E, Lasagni L, Materassi M, Romagnani P. 2015. Heterogeneous genetic alterations in sporadic nephrotic syndrome associate with resistance to immunosuppression. Journal of The American Society of Nephrology, vol. 26; p. 230-236.

Negrisolo S, Benetti E, Centi S, Della Vella M, Ghirardo G, Zanon GF, Murer L, Artifoni L. PAX2 gene mutations in pediatric and young adult transplant recipients: Kidney and urinary tract malformations without ocular anomalies. 2011. Clinical Genetics 80(6), pp. 581-585.

Grenda R. Watson A. Trompeter R. Tönshoff B. Jaray J. Fitzpatrick M. Murer L. Vondrak K. Maxwell H. Van Damme R. Lombaerts Loirat C. Mor E. Cochat P. Milford D. V Brown M. Webb N. J. A. A randomized trial to assess the impact of early steroid withdrawal on growth in pediatric renal transplantation: The twist study. 2010.

Barzon L*- Murer L*, Pacenti M, Biasolo M, Della Vella M, Benetti E, Zanon G, Palù G. 2009. Investigation of intrarenal viral infections in kidney transplant recipients unveils an association between parvovirus B19 and chronic allograft injury. The Journal of Infectious Diseases, VOL. 199, P. 372-380.

Model Organisms and Rare Diseases Laboratory

PI **Eva Trevisson**

Research activity

The main interest of our laboratory has been the development of models to study the genetic bases and the pathophysiology of inherited neurometabolic disorders.

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* and *D. rerio*. These organisms represent simple highly prolific organisms with a rapid life cycle that display organized tissues and organs and allow studying the effects of novel variants on nervous system development.

Our interest is now moving to cancer genetics, and particularly to the study of the mechanisms driving pediatric cancers associated with a genetic predisposition. We are employing the same organisms to model germline mutations in oncosuppressors/oncogenes identified in rare tumor predisposing syndromes in order to deeply analyze cancerogenesis and to set up simple models for drug screening.

Team members

Eva Trevisson
Principal Investigator

Cerqua Cristina
PostDoctoral Researcher

Morbidoni Valeria
PostDoctoral Researcher

Buson Lisa
PostDoctoral Researcher

PI's biosketch

Scopus ID: 8922220000

Dr. Trevisson obtained her Medical Degree at the University of Padua and certification 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.

Dr. Trevisson had also the opportunity to visit as fellow an excellent center of developmental biology in Spain (Centro Andaluz de Biologia 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 also established a knockdown model of a mitochondrial disorder.

Since March 2011, she works as Assistant Professor of Medical Genetics at the Dept. of Women's and Children's Health of the University of Padua and since 2014 she leads a group in the laboratory of Genetics at the Istituto di Ricerca Pediatrica (IRP), where she is following on with modeling human genetic diseases in C. elegans and D. rerio and she has started a novel research line on cancer genetics.

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 neurofibromatosis outpatient clinic. 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 neurosciences at the University of Padua.

She has co-authored 68 Medline publications and five book chapters, with a total impact factor of 278 (Journal of Citation reports 2016), h-index 22, total citations 1932 (according to Scopus).

Selected publications

Morbidoni V, Agolini E, Slep KS, Pannone L, Zuccarello D, Cassina M, Grosso E, Gai G, Salviati L, Dallapiccola B, Novelli A, Martinelli S, Trevisson E. Biallelic mutations in TOGARAM1 gene cause a novel primary ciliopathy. Journal of Medical Genetics. In press. 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. Epub.

Cassina M, Cerqua C, Rossi S, Salviati L, Martini A, Clementi M, Trevisson E. A synonymous splicing mutation in the SF3B4 gene segregates in a family with highly variable Nager syndrome. Eur J Hum Genet. 2017;25(3):371-375.

Neurodevelopmental Molecular Genetics Laboratory

PI **Alessandra Murgia**

Research activity

The Laboratory is a referral center, for the North-East Italian territory, for molecular diagnostics of neurodevelopmental conditions.

In particular, the diagnostic activity covers Intellectual Disability/Autism spectrum disorders, Epileptic encephalopathies of the first year of life/childhood epilepsy, 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. Several translational research programs have been carried on and are currently ongoing with the aim at studying the biological bases of neurodevelopmental genetically heterogeneous conditions in particular intellectual disability associated with autism and early onset epilepsy.

Conventional methods of molecular genetic analysis have been replaced in the laboratory by the application of Next Generation Sequencing technology, in order to develop new and more efficient diagnostic tools. The laboratory provides NGS analysis of the following customized targeted gene panels: EIEE/early epilepsy; ID/ASD ; SNHL and Tuberous Sclerosis. A Cerebral Palsy- Infantile Movement disorders targeted panel has been developed and is in the process of being implemented in clinical activity.

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

Team members

Alessandra Murgia
Principal Investigator

Roberta Polli
Lab manager;
Quality Control Manager

Emanuela Leonardi
Senior PostDoctoral Researcher

Elisa Bettella
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 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 Medical Genetics, Pediatrics, Schools of Medicine, Speech Therapy and Psychology.

Prof. Murgia has authored 58 full papers that have been 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.
Memberships scientific societies: American Society of Human Genetics (ASHG); European Society of Human Genetics (ESHG); Italian Society of Human Genetics (SIGU).

Major Lab Equipment:
NGS platform: Ion Torrent PGM/S5; 20 Terabite Archive drive. Automatic sequencer ABI PRISM 3130 Genetic Analyzer; Quantitative PCR ABI PRISM 7000, Nucleic Acids automated extractor Promega Maxwell 16 IVD. GelDOC Biorad.
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).
Fragile X program: the Laboratory is a nationally recognized center for Fragile X molecular testing. Prof. Alessandra Murgia, has coordinated one of three Italian centers ever involved in international pharmacological trials for Fragile X Syndrome (AFQ, Novartis).
In January 2014, the "Multidisciplinary Fragile X Padua Network" has been founded, 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 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.

Selected publications

Bettella E, Di Rosa G, Polli R, Leonardi E, Tortorella G, Sartori S, Murgia A. Early-onset epileptic encephalopathy in a girl carrying a truncating mutation of the ARX gene: rethinking the ARX phenotype in females. Clin Genet. 2013 Jul;84(1).

Yrigollen C, Martorell L, Durbin-Johnson B, Naudo M, Genoves J, Murgia A, Polli R, Zhou L, Barbouth D, Rupchock A, Finucane B, Latham GJ, Hadd A, Berry-Kravis E, Tassone F. AGG interruptions and maternal age effect on FMR1 CGG allele stability during transmission. Journal of Neurodevelopmental Disorders 2014 J NeurodevDisord. 2014;6(1):24.

Aspromonte MC, Bellini M, Gasparini A, Carraro M, Bettella E, Polli R, Cesca F, Bigoni S, Boni S, Carlet O, Negrin S, Mammi I, Milani D, Peron A, Sartori S, Toldo I, Soli F, Turolla L, Stanzial F, Benedicenti F, Marino-Buslje C, Tosatto SCE, Murgia A, Leonardi E. Characterization of Intellectual disability and Autism comorbidity through gene panel sequencing. Hum Mutat. 2019 Jun 17.

Randi Hagerman, Sebastien Jacquemont, Elizabeth Berry-Kravis, Vincent Des Portes, Andrew Stanfield, Barbara Koumaras, GerdRosenkranz, Alessandra Murgia, Christian Wolf, George Apostol, and Florian von Raison. Mavoglurant in Fragile X Syndrome: Results of two open-label, extension trials in adults and adolescents. Scientific Reports 2018 Nov 19;8(1):16970. doi: 10.1038/s41598-018-34978-4.

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.

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

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

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 Laboratory

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

Cristina Liboni
PhD Student

Elisabetta Marcuzzi
PhD student

Alessandra Maria Testa
PhD student

Chiara Cioccarelli
PhD student

Gloria Orlando
PhD student

Fransisca Venegas
PhD student

Fabio Munari
Lab technician

Nicole Bertoldi
Lab techinican

The Cardiac microenvironment in ACM

Cardiovascular diseases are the worldwide leading cause of death. Among them, arrhythmogenic cardiomyopathy (ACM) is a major cause of sudden death in the young and in athletes. ACM is a rare (1:2000-1:5000) congenital disease with an autosomal-dominant trait. ACM-causing genes mostly encode major components of the cardiac desmosome and up to 50% of ACM patients harbor mutations in one of them. The structural substrate of ACM consists of progressive myocardial dystrophy with fibro- fatty replacement in the ventricular walls, starting from the subepicardium. Our hypothesis is that mesenchymal stromal cells (MSC) play a crucial role in the disease. Taking advantage of recently established mouse models of ACM, we study the role of MSC and infiltrating cells in the pathogenesis of ACM.

Collaborators: Cristina Basso, Marco Mongillo, Tania Zaglia, University of Padua.

Exploiting MSC-derived EVs to fight cancer

Pathological angiogenesis is a hallmark of several conditions including eye diseases, inflammatory diseases, and cancer. Stromal cells play a crucial role in regulating angiogenesis through the release

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.

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 pathological tumor associated angiogenesis.

Collaborators: Maurizio Muraca, University of Padua & IRP.

Multiple sclerosis and acquired autoimmune demyelinating syndromes

Acquired demyelinating diseases of the central nervous systems (CNS) constitute a broad spectrum of highly disabling inflammatory and neurodegenerative diseases. Multiple sclerosis (MS) and the so-called Neuromyelits Optica-Spectrum Disorders (NMO-SD), whose incidence and prevalence are dramatically increasing worldwide, may have a pediatric onset, characterized by severe clinical and neuroradiological pictures. The aim of our project is the discovery of still unknown immunopathogenic mechanisms in 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.

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.

Collaborators: Annamaria Cattelan, Giuseppe Testa, Paolo Rossi, Carlo Giaquinto

Selected publications

R.L. Contento, S. Campello, A.E. Trovato, E. Magrini, F. Anselmi and A. Viola. 2010. Adhesion shapes T cells for prompt and sustained T cell receptor signaling. EMBO Journal, 29:4035-47.C.

Mazzon, A. Anselmo, J. Cibella, C. Soldani, A. Destro, N. Kim, M. Roncalli, S.J. Burden, M.L. Dustin, A. Sarukhan and A. Viola. 2011. The critical role of agrin in the hematopoietic stem cell niche.. Blood, 118(10): 2733-2742.

Molon B, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D, De Palma A, Mauri PL, Monegal A, Rescigno M, Savino B, Colombo P, Jonjic N, Pecanic S, Lazzarato L, Fruttero R, Gasco A, Bronte V, Viola A. 2011. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. The Journal of Experimental Medicine 208(10):1949-62.

Kallikourdis M, Trovato AE, Anselmi F, Sarukhan A, Roselli G, Tassone L, Badolato R, Viola A. 2013. The CXCR4 mutations in WHIM syndrome impair the stability of the T cell immunological synapse. Blood 122(5):666-73.

Wang CM, Ploia C, Anselmi F, Sarukhan A, Viola A. 2014. ATP acts as a paracrine signaling molecule to reduce the motility of T cells. EMBO J 33(12):1354-64.

Monoamine Oxidases in Innate Immunity Laboratory

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 MAO activity plays a crucial role in inflammasome activation in acute and chronic inflammation. Inflammasomes represent 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 induction, resulting in NLRP3 and pro-IL-1B overexpression. Both *in vitro* and *in vivo*, MAO-B inhibition by rasagiline prevented IL-1B secretion and MAO-B deficient mice showed impaired response to LPS-mediated endotoxemia. Importantly, in a Duchenne dystrophy model, rasagiline administration reduced inflammasome activation in muscle-infiltrating macrophages, along with muscle performance recovery. Our findings identify MAO-B as a specific producer of mitochondrial ROS fuelling NLRP3 inflammasome, thereby providing the basis for repurposing MAO-B inhibitors to treat inflammasome-mediated pathologies.

Team members

Marcella Canton
Principal Investigator

Ricardo Sanchez-Rodriguez
PostDoctoral Researcher

Eugenia Carraro
PhD student

Giorgia Contarini
Undergraduate student

PI's biosketch

Scopus ID: 7004910913
Dr. 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. Dr. 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 signalling in innate immune system.

Thus, we are currently investigating whether clinical-grade monoamine oxidase inhibitors can be viable candidates in the treatment of autoinflammatory and autoimmune disorders.

Selected publications

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.

Menazza S, Blaauw B, Tiepolo T, Toniolo L, Braghetta P, Spolaore B, Reggiani C, Di Lisa F, Bonaldo P, Canton M (2010) Oxidative stress by monoamine oxidases is causally involved in myofiber damage in muscular dystrophy. Hum Mol Genet, vol. 19; p. 4207-4215.

Neuroimmunology Laboratory

PI: **Stefano Sartori**

Research activity

The Neuroimmunology Laboratory focuses on unraveling the role of immunity and inflammation in pediatric 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.

Medical Diagnostic Activities

Each year, we receive pediatric 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 pediatric 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

Team members

Stefano Sartori
Principal Investigator

De Gaspari Piera
PostDoctoral Researcher

Nosadini Margherita
Clinical Researcher

Zoccarato Marco
Clinical Researcher

Zuliani Luigi
Clinical Researcher

PI's biosketch

Scopus ID 8655110800
Following a MD degree at the University of Padua in 2001, Dr. Sartori specialised in Pediatrics, with a specific focus on Pediatric neurology in 2006. Dr. Sartori subsequently enrolled in a PhD programme in Pediatric Neurology (2007-2009) followed by advanced higher education courses on Epilepsy (2011, University of Ferrara) and in Children Movement Disorders (2017, University of Rome). Since 2010, Dr. Sartori has been doing research 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, Dr. Suppiej and Dr. Nosadini has been contributing to establishing a Neuroimmunology research group in Veneto. Dr. 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, Dr. Sartori works at the Dept. of Women's and Children's Health, University of Padua, where he coordinates the Pediatric Neurology and Neurophysiology programme. Dr. Sartori is also a Professor at the training programmes in Pediatrics, Child Neuropsychiatry and Rehabilitation of the University of Padua, where he is course leader in Pediatric Neurology and Epilepsy. During his career, Dr. Sartori has co-authored over 90 indexed peer-reviewed articles and over 200 proceedings in Pediatrics, Pediatric Neurology, Epilepsy and Neuroimmunology.

techniques such as: indirect immunochemistry, indirect immunofluorescence, 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

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.

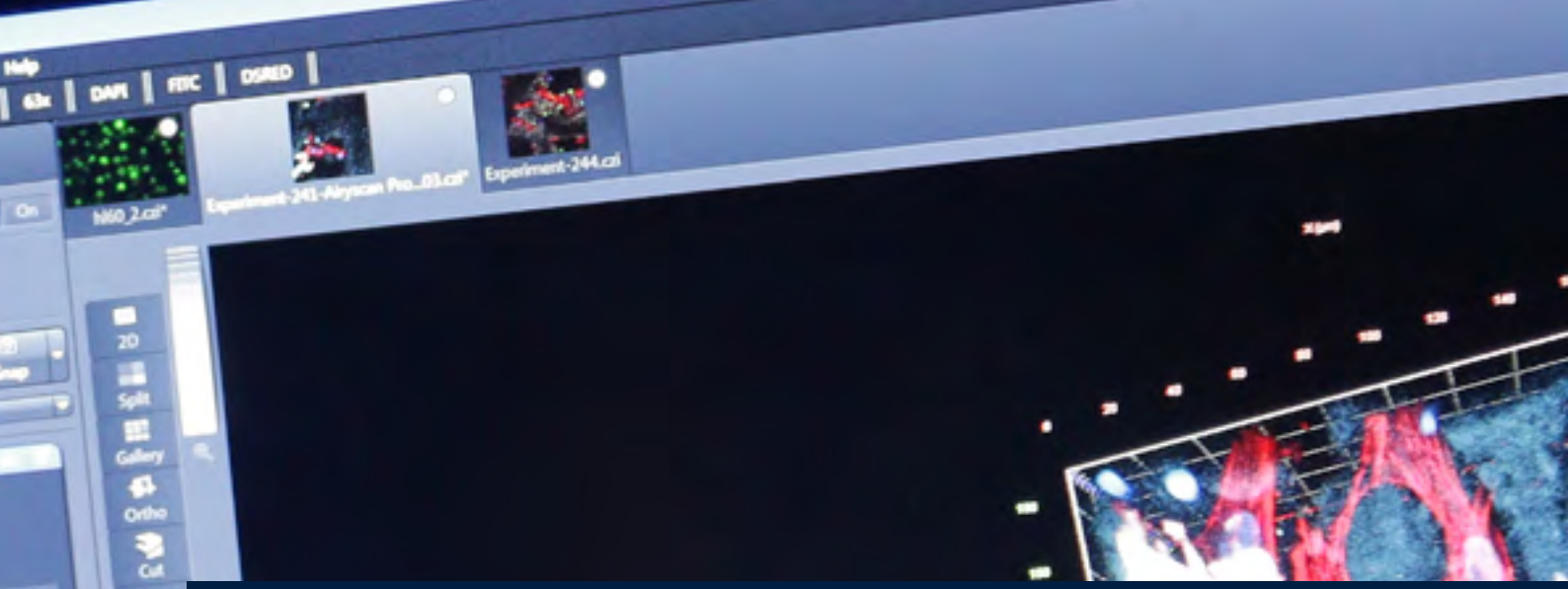
Zuliani L, Nosadini M, Gastaldi M, Spatola M, Iorio R, Zoccarato M, Mariotto S, De Gaspari P, Perini F, Ferrari S, Evoli A, Sartori S, Franciotta D, Giometto B. Management of antibody-mediated autoimmune encephalitis in adults and children: literature review and consensus-based practical recommendations. Neurol Sci. 2019 Oct;40(10):2017-2030. doi: 10.1007/s10072-019-03930-3. Epub 2019 Jun 3. PMID: 31161339.

Nosadini M, Toldo I, Tascini B, Bien CG, Parmeggiani L, De Gaspari P, Zuliani L, Sartori S. LGI1 and CASPR2 autoimmunity in children: Systematic literature review and report of a young girl with Morvan syndrome. J Neuroimmunol. 2019 Oct 15;335:577008. doi: 10.1016/j.jneuroim.2019.577008. Epub 2019 Jul 18. PMID: 31352183.

Mariotto S, Gajofatto A, Zuliani L, Zoccarato M, Gastaldi M, Franciotta D, Cantalupo G, Piardi F, Polo A, Alberti D, Sartori S, Zanusso G, Agrò L, Demurtas R, Sechi G, Sechi E, Monaco S, Ferrari S. Serum and CSF neurofilament light chain levels in antibody-mediated encephalitis. J Neurol. 2019 Jul;266(7):1643-1648. I.F.: 4.204.

Suppiej A, Nosadini M, Zuliani L, Pelizza MF, Toldo I, Bertossi C, Tison T, Zoccarato M, Marson P, Giometto B, Dale RC, Sartori S. Plasma exchange in pediatric anti-NMDAR encephalitis: A systematic review. Brain Dev. 2016 Aug;38(7):613-22. doi: 10.1016/j.braindev.2016.01.009. Epub 2016 Feb 28. PMID: 26926399.

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA



Research area

Medical Biotechnology

Coordinator: **Dr. 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.


Fondazione
ISTITUTO DI RICERCA
PEDIATRICA
città della speranza

Dr. Marco Agostini

Coordinator Medical Biotechnology Area

Scopus ID: 7005322173

Dr. 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, Dr. 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, Dr. 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, Dr. 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: 24 (according to Scopus). He has published 112 articles in peer reviewed scientific journals.

Optics and Bioimaging Laboratory

PI: **Filippo Romanato**

Research activity

The research activities of the Optics and Bioimaging Group are focused on the development of micro- and nano-platforms and devices for different applications: from nano-optics to telecom, from holography to microscopy and astronomy, from biology to materials science. Nanotechnology development and application rely on the intersection of expertise from many science and engineering disciplines. The Optics and Bioimaging Group at IRP is structured synergistically to foster integration among the physical, biological, and clinical sciences. Our bioimaging, optical and lab-on-chip systems intend to overcome these limitations following innovative technological solutions that conjugate physics with biology and medicine.

Team members

Filippo Romanato
Principal Investigator

Giulia Borile
PostDoctoral Researcher

Gianluca Ruffato
PostDoctoral Researcher

Pietro Capaldo
PostDoctoral Researcher

Deborah Sandrin
PostDoctoral Researcher

Andrea Vogliardi
Graduated student

Bioimaging

The group is highly specialized in bioimaging, from experimental design, to labelling protocols, data acquisition and elaboration.

In the Optics and Bioimaging Group, we have designed and assembled a custom microscope capable of performing Two-Photon Microscopy, Label-Free Microscopy that is currently being upgraded for STED super-resolution. Two-Photon Microscopy (TPM) takes advantage of the quasi-simultaneous absorption of two photons by a molecular receptor in a single quantum event. Despite the need for very intense light sources, the NIR wavelengths used in TPM are significantly less prone to scattering and absorption from thick specimens and, therefore, exhibit longer penetration depths. Additionally, TPM has an inherent capability of performing axial sectioning and confines the photo-bleaching effect inside a very limited volume. When specimens are very susceptible to photo-bleaching and photo-damages or when any staining process must be avoided in order to preserve the

PI's biosketch

Scopus ID: 7003449626
Prof. Romanato obtained his PhD in Physics at the University of Padova in 1994, then he was visitor scientist at MIT (1996), senior scientists at ESRF synchrotron (1997-8) and Associate Professor at the School of Materials Engineering of NTU (Nanyang Technological University) of Singapore (2005-9), where he founded a group for the development of plasmonic bio-sensors. Prof. Romanato is Associate Professor at the Dept. of Physics and Astronomy "G. Galilei" at the University of Padua where he teaches Physics and Material Sciences courses and is the coordinator of the Nanodevices Group. He is director of the Laboratory for Nanofabrication and Nanodevices (LaNN), in Padua, which he founded and developed, and which is currently managed by the EcamRicert group. He is the Principal Investigator of the group of Optics and Bioimaging Group at the Istituto di Ricerca Pediatrica. Since June 1998, he is in charge of the beam line for the X-ray lithography and founder of the nanofabrication LILIT group at the National TASC INFM-CNR laboratory based at the synchrotron Elettra in Trieste. Nowadays the full lithography facility is constituted of 4 clean rooms and a complete set of nanofabrication tools. He is co-founder of 4 spin-offs related to nanofabrication, biosensing and telecom. His research activity is focused on nanofabrication, telecom, biosensing and nonlinear microscopy. He has been coordinator and principal investigator of several national and international projects for a total of 9 M €, among which, unit-responsible in an FP7 project and in several national projects aimed at the development of nanodevices for nanoptics and lab-on-chip. He is co-author of 10 issued patents, 233 scientific reviewed papers h-index 31 (according to Scopus).

specimens in their original conditions, the use of Label-Free Microscopy (LFM) is preferable. LFM can be applied to study samples with no need for exogenous fluorescent probes, while keeping the main benefits of TPM.
The group is also collaborating in confocal and live microscopy projects to optimize data acquisition and elaboration.

Optics
The generation and control of complex light beams, i.e. beams with unusual distribution of amplitude and phase, requires the manipulation of the wavefront at the micrometric scale. This is achievable by realizing specific 3-dimensional micro-structured patterns on a transparent material, which act locally on the incident light in order to transfer specific phase distributions to the electromagnetic field. Our activities cover the design, the fabrication and characterization of nanostructures and nano-objects. The applications of these optical devices range from the telecommunications world (OAM-mode division multiplexing for enhanced information capacity), to microscopy (high-resolution optical microscopes).
The Optics and Bioimaging Group has developed a high-sensitivity SPR-based biosensor for applications in the biomedical field. A phase-interrogation setup based on polarization scan

allows a high miniaturization and integration of the plasmonic sensor in lab-on-a-chip platforms. The detection setup (patented) is simple, cost effective and guarantees high sensing speeds and performances.

Selected publications

A Comprehensive Comparison of Bovine and Porcine Decellularized Pericardia: New Insights for Surgical Applications. Zouhair S, Sasso ED, Tuladhar SR, Fidalgo C, Vedovelli L, Filippi A, Borile G, Bagno A, Marchesan M, Giorgio R, Gregori D, Wolkers WF, Romanato F, Korossis S, Gerosa G, Iop L.Biomolecules. 2020 Feb 28;10(3):371. doi: 10.3390/biom10030371.

Multiplication and division of the orbital angular momentum of light with diffractive transformation optics. Ruffato G, Massari M, Romanato F. Light Sci Appl. 2019 Dec 5;8:113. doi: 10.1038/s41377-019-0222-2. eCollection 2019.

Label-free, real-time on-chip sensing of living cells via grating-coupled surface plasmon resonance. Borile G, Rossi S, Filippi A, Gazzola E, Capaldo P, Tregnago C, Pigazzi M, Romanato F. Biophys Chem. 2019 Nov;254:106262. doi: 10.1016/j.bpc.2019.106262. Epub 2019 Sep 3.

Total angular momentum sorting in the telecom infrared with silicon Pancharatnam-Berry transformation optics. Ruffato G, Capaldo P, Massari M, Mafakheri E, Romanato F.Opt Express. 2019 May 27;27(11):15750-15764. doi: 10.1364/OE.27.015750. PMID: 31163766.

Multimodal label-free *ex vivo* imaging using a dual-wavelength microscope with axial chromatic aberration compensation. Filippi A, Dal Sasso E, Iop L, Armani A, Gintoli M, Sandri M, Gerosa G, Romanato F, Borile G. J Biomed Opt. 2018 Mar;23(9):1-9. doi: 10.1117/1.JBO.23.9.091403.

NanoInspired Biomedicine Laboratory

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

Team members

Marco Agostini
Principal Investigator

Sara Cotti
PostDoctoral Researcher

Edoardo D'Angelo
PostDoctoral Researcher

Francesca Sensi
PostDoctoral Researcher

Laura Moracci
Research Assistant

Riccardo Rampado
PhD student

Pietro Traldi
Emeritus Professor

PI's biosketch

Scopus ID: 7005322173

Dr. 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, Dr. 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, Dr. 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, Dr. 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: 24 (according to Scopus). He has published 112 articles in peer reviewed scientific journals.

- study of the interaction between 5 fluorouracil and catechins (epicatechin, epicatechin 3-gallate, epigallocatechin and epigallocatechin gallate) present in green tea extracts (GTEs) by mass spectrometry to identify possible natural substances as adjuvants to classic chemotherapy treatments in CRC patients;
- 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

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 Mar 13;12(3):681. doi: 10.3390/cancers12030681.

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, Mam-mano 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 Feb 5;12(2):364. doi: 10.3390/cancers12020364.

Tryptophan Metabolism as Source of New Prognostic Biomarkers for FAP Patients. Crotti S, Bedin C, Bertazzo A, Digito M, Zuin M, Urso ED, Agostini M. Int J Tryptophan Res. 2019 Nov 20;12:1178646919890293. doi: 10.1177/1178646919890293.

Reduced Plasma Levels of Very-Long-Chain Dicarboxylic Acid 28:4 in Italian and Brazilian Colorec-tal Cancer Patient Cohorts. Wood PL, Donohue MM, Cebak JE, Beckmann TG, Messias MCF, Cre-didio L, Coy CSR, Carvalho PO, Crotti S, D'Aronco S, Urso EDL, Agostini M.Metabolites. 2018 Dec 6;8(4):91. doi: 10.3390/metabo8040091.

Engineered biomimetic nanovesicles show intrinsic anti-inflammatory properties for the treatment of inflammatory bowel diseases. Corbo C, Cromer WE, Molinaro R, Toledano Furman NE, Hartman KA, De Rosa E, Boada C, Wang X, Zawieja DC, Agostini M, Salvatore F, Abraham BP, Tasciotti E. Nanoscale. 2017 Oct 5;9(38):14581-14591. doi: 10.1039/c7nr04734g.

NBTECH Laboratory

PI: **Elisa Cimetta**

Research activity

Prof. Cimetta's main research interests focus on the application of engineering principles to biological studies. In particular, 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 the cells and the dynamic microenvironment (uEnv), limiting the understanding of its precise role in biology. The 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

Team members

Elisa Cimetta
Principal Investigator

Maria Rosaria Esposito
Lab technician

Pina Fusco
PostDoctoral Researcher

Lorenzo Bova
PhD Student

Sara Micheli
PhD Student

Luca Zanella
PhD Student

Noemi Torriero
PhD Student

Gaia Santi
Research Associate

PI's biosketch

Scopus ID: 15847798700
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.

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 exosomes in cancer dissemination.

The laboratory will develop and implement advanced platforms, powerful means to enable highthroughput screening of environmental effectors of tumor behavior in a more realistic setting. Specifically, we will test gradients of NB derived exosomes on 3D cultures under different oxygen conditions aiming at defining how hypoxia influences physicochemical properties of exosomes and of target cultures. Finally, we will test long-range effects of NB derived exosomes on target tissues, metastatic sites of NB tumor. Notably, current members of the laboratory also acquired strong expertise in the establishment of NB organoids (3D culture) from tumor biopsies of the patients.

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.

Selected publications

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.

Reilein A, Cimetta E, Tandon NM, Kalderon D and Vunjak-Novakovic G . 2008. Live imaging of stem cells in the germarium of the Drosophila ovary using a reusable gas-permeable imaging chamber. Nature Protocols 14(8), pp. 2598.

Esposito MR, Binatti A, Pantile M, Coppe A, Mazzocco K, Longo L, Capasso M, Lasorsa VA, Luksch R, Bortoluzzi S, Tonini GP. Somatic mutations in specific and connected sub-pathways are associated to short neuroblastoma patients' survival and indicate proteins targetable at onset of disease. International Journal of Cancer. 2018 Nov 15;143(10):2525-2536.

Fusco P, Esposito MR, Tonini GP. Chromosome instability in neuroblastoma. Oncol Lett. 2018 Dec;16(6):6887-6894.

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.

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

Research area

Onco-Hematology, Stem Cell Transplant and 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 Onco-hematology, stem cell transplant and 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 80 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, and of Genething s.r.l., a gene therapy consulting company, also a spinoff of the University of Padua.

Brain Tumors Laboratory

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. The recent identification of Annexin A2 as a crucial mediator of GBM cell migration and proliferation (Maule *et al.*, Oncotarget 2016), allowed to transcriptionally characterize the behavior of glioblastoma cells upon Annexin 2A knock-down or inhibition and to screen a selection of compounds able to resemble these Annexin 2A-dependent transcriptional effects. In particular, through an *in silico* screening of drug-matched transcriptional profiles, the inhibitor of protein synthesis Homoharringtonine (HHT) has shown a promising efficacy in blocking glioblastoma cell invasiveness *in vitro*. HHT is now undergoing a wide evaluation of its effects in primary glioblastoma cells, together with a deep characterization of the expected additional molecular mechanisms underlying its anti-cancer action in our models. In addition, based on our recent publication describing how the molecular crosstalk between HIF-1α and Wnt pathways is able to control GBM cell phenotype and aggressiveness (Boso *et al.*, Theranostics 2019), our more recent studies in the field are devoted to explore the pharmacological inhibition of the β-catenin co-factor TCF4 as a reliable tool to induce glioblastoma cell differentiation and sensitization to chemotherapy. In particular, we identified the histone deacetylase inhibitors of the hydroxamate class Trichostatin-A (TSA) and suberoylanilide hydroxamic acid (SAHA) as potent inhibitors of TCF4 levels in glioblastoma cells. These are being characterized for the effects on cell phenotype, the response to additional drug used for glioma therapy such as temozolomide and the molecular mechanisms potentially involved in these processes.

Team members

Luca Persaro
Principal Investigator

Elena Rampazzo
PostDoctoral Researcher supported by the Umberto Veronesi Foundation

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.

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 unfavourable, 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 MB

cell 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. In this context, the recent achievement of a pediatric research grant from CARIPARO Foundation will allow to get a deep molecular insight in the process of drug resistance acquisition of these tumors through 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 will analyze 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

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.

Bortolozzi R, Bresolin S, Rampazzo E, Paganin M, Maule F, Mariotto E, Boso D, Minuzzo S, Agnusdei V, Viola G, Cazzaniga G, Basso G and Persano L. AKR1C enzymes sustain therapy resistance in pediatric T-ALL. Br J Cancer. 2018 Apr;118(7):985-994.

Rampazzo E, Persano L, Pistollato F, Moro E, Frasson C, Porazzi P, Della Puppa A, Bresolin S, Battilana G, Indraccolo S, Te Kronnie G, Argenton F, Tiso N, Basso G. Wnt activation promotes neuronal differentiation of glioblastoma. Cell Death Dis. 2013 Feb 21;4:e500.

Persano L, Pistollato F, Rampazzo E, Della Puppa A, Abbadi S, Frasson C, Volpin F, Indraccolo S, Scienza R, Basso G. BMP2 sensitizes glioblastoma stem-like cells to Temozolomide by affecting HIF-1α stability and MGMT expression. Cell Death Dis. 2012 Oct 18;3:e412.

Experimental Pharmacology Laboratory

PI **Giampietro Viola**

Research activity

The experimental pharmacology group is involved in the study of new strategies in cancer therapeutics following three 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

Team members

Giampietro Viola
Principal Investigator

Roberta Bortolozzi
PostDoctoral Researcher

Elena Mariotto
PostDoctoral Researcher supported by AIRC Foundation for Cancer Research

Fatlum Rruga
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 Azienda Ospedale Università Padova in the Dept. of Women's and Children's Health (UOC of Oncohematology). 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 about 160 scientific paper in specialized peer review journals. It is 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.

is the major regulator of redox homeostasis and defense against oxidative stress. So far, our results show that starting from early exposure of chemotherapy drugs; MB cells induce Nrf2 expression and its transcriptional activation, supporting the involvement of Nrf2 pathway in MB response to chemotherapic 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 studying the involvement of Nrf2 in the metabolic changes that occur in the onset of chemotherapy resistance, our research group aim to give furhter insight on the characterization of MB resistance and the identification of novel druggable targets.

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. Drug libraries composed by both clinically approved drugs and novel promising small molecule 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.
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 enzymes sustain therapy resistance in pediatric T-ALL. Br. J. Cancer. (2018), 118, 985-994.

Gene Expression (GEP) – NGS Laboratory

PIs: **Silvia Bresolin / Geertruy te Kronnie**

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 evolution of 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 and its correlation within clinically distinct groups of JMML patients. The study also focuses on the development of an *in vitro* and *in vivo* model able to recapitulate key pathophysiology features of JMML disease and to trace the clone's dynamicity.

Circular RNA (circRNA) in normal hematopoiesis and in high risk B-ALL

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 the

Team members

Silvia Bresolin
Co-Principal Investigator

Geertruy te Kronnie
Co-Principal Investigator

Alice Cani
PostDoctoral Researcher

Caterina Tretti
PhD student

PI's biosketch

Silvia Bresolin

Scopus ID: 36027490300

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. 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 50 publications in peer reviewed international journals.

Geertruy te Kronnie

Scopus ID: 6603953611

Since 2000 Dr. te Kronnie has been working in the field of hematology at Dept. of Women's and Children's Health, Azienda Ospedale Università Padova. As senior scientist, Dr. te Kronnie joined the laboratory of Oncohematology in Padua to explore the genomics of healthy and leukemic cells using new omic approaches. Omics approaches that promised to boost both diagnostics and more basic research in hematology. At the time, the center was the pioneer in the field and its services were on much demand. Dr. te Kronnie established an international network that leads the way for a growing number of national and international collaborations that investigated the molecular status of malignant blood cells. Dr. te Knronnie's web of international collaborations resulted most recently in a COST Action, a network of haematologists and geneticists from more than 30 counties that focus on genetic predisposition to leukemia and lymphoma [https://www.legend-cost.eu].

hematopoietic compartment. The project efforts in the identification and validation of specific circRNAs dysregulated in high risk leukemia and predict circRNA functions 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.

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 identi-fy 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.

Predisposition to Inherited Leukemia

Clinicians have known since decades that families with ALL in subsequent generations may point to hereditary genetic components, even if ALL in the vast majority of the cases is considered an acquired disease of somato-genetic origin. Some point mutations in PAX5, ETV6 and IKZF1 were recently reported to be present in families with recurrent ALL and confer an increased risk to ALL development. These studies provide the rationale to identify disease related mutations in families with recurrent ALL. Inherited or *de novo* acquired constitutional mutations in genes are likely to impact the phenotype of the leukemia altering the biology of the aberrant cells, the systemic response to current therapies and the long term disease free survival of patients. Thus, therapies may require adjustments and/or provide opportunities to develop specific therapies for these cases. Being inherited ALL a rare variant of a common disorder, the only way to meaningfully address these issues is to establish direct collaborations. Since November 2017, we joined a European program (COST-Action –CA16223) that joins 24 countries across Europe in a joint effort to improve the understanding of leukemia predisposition in pediatric patients, to improve patient care and enable genetic counseling of patients and families. Ongoing studies are exanimating genetic basis that predispose to leukemia as well as other hematological malignancies.

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' followup 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 and

microenvironment 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.

Selected publications

Dal Molin A, Bresolin S, Gaffo E, Tretti C, Boldrin E, Meyer LH, Guglielmelli P, Vannucchi AM, Te Kronnie G, Bortoluzzi S. CircRNAs Are Here to Stay: A Perspective on the MLL Recombinome Front Genet. 2019 Feb 13;10:88. doi: 10.3389/fgene.2019.00088.

Churchman ML, Qian M, Te Kronnie G, Zhang R, Yang W, Zhang H, Lana T, Tedrick P, Baskin R, Verbist K, Peters JL, Devidas M, Larsen E, Moore IM, Gu Z, Qu C, Yoshihara H, Porter SN, Pruett-Miller SM, Wu G, Raetz E, Martin PL, Bowman WP, Winick N, Mardis E, Fulton R, Stanulla M, Evans WE, Relling MV, Pui CH, Hunger SP, Loh ML, Handgretinger R, Nichols KE, Yang JJ, Mullighan CG. Germline Genetic IKZF1 Variation and Predisposition to Childhood Acute Lymphoblastic Leukemia. Cancer Cell. 2018 May 14;33(5):937-948.e8. doi: 10.1016/j.ccell.2018.03.021. Epub 2018 Apr 19. PMID: 29681510 Free PMC article.

Stanulla M, Dagdan E, Zaliova M, Möricke A, Palmi C, Cazzaniga G, Eckert C, Te Kronnie G, Bourquin JP, Bornhauser B, Koehler R, Bartram CR, Ludwig WD, Bleckmann K, Groeneveld-Krentz S, Schewe D, Junk SV, Hinze L, Klein N, Kratz CP, Biondi A, Borkhardt A, Kulozik A, Muckenthaler MU, Basso G, Valsecchi MG, Izraeli S, Petersen BS, Franke A, Dörge P, Steinemann D, Haas OA, Panzer-Grümayer R, Cavé H, Houlston RS, Cario G, Schrappe M, Zimmermann M; TRANSCALL Consortium; International BFM Study Group. IKZF1plus Defines a New Minimal Residual Disease-Dependent Very-Poor Prognostic Profile in Pediatric B-Cell Precursor Acute Lymphoblastic Leukemia. J Clin Oncol. 2018 Apr 20;36(12):1240-1249. doi: 10.1200/JCO.2017.74.3617.

Helsmoortel HH, Bresolin S, Lammens T, Cavé H, Noellke P, Caye A, Ghazavi F, de Vries A, Hasle H, Labarque V, Masetti R, Stary J, van den Heuvel-Eibrink MM, Philippé J, Van Roy N, Benoit Y, Speleman F, Niemeyer C, Flotho C, Basso G, Te Kronnie G, Van Vlierberghe P, De Moerloose B. LIN28B overexpression defines a novel fetal-like subgroup of juvenile myelomonocytic leukemia. Blood. 2016 Mar 3;127(9):1163-72. doi: 10.1182/blood-2015-09-667808.

Bresolin S, Zecca M, Flotho C, Trentin L, Zangrando A, Sainati L, Stary J, de Moerloose B, Hasle H, Niemeyer CM, Te Kronnie G, Locatelli F, Basso G. Gene expression-based classification as an independent predictor of clinical outcome in juvenile myelomonocytic leukemia. J Clin Oncol. 2010 Apr 10;28(11):1919-27. doi: 10.1200/JCO.2009.24.4426.

HSC Gene Therapy Laboratory

PI: **Alessandra Biffi**

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) (Biffi, JCI 2004 and 2006; Visigalli, Blood 2010; Biffi, Science 2013; Sessa, Lancet 2016) and her group is currently pursuing research aimed at enhancing the therapeutic potential of this strategy (Capotondo, PNAS 2012; Capotondo, Sci Advances 2017; Peviani, Biomaterial 2019) and broaden its application. This research area, which has been funded by an Advanced 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. 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.

Team members

Alessandra Biffi
Principal Investigator

Rita Milazzo
Senior Researcher - Altheia Science

Yuri Ciervo
PostDoctoral Researcher

Chiara Rigobello
PostDoctoral Researcher

Laura Rigon
PostDoctoral Researcher

Giulia Santinon
PostDoctoral Researcher

Annalisa Trenti
PostDoctoral Researcher

Martina Clematide
Lab Technician

Lisa Menegazzo
Lab Manager, Lab Technician

Silvia Spadini
PhD student

PI's biosketch

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 80 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, and of Genething s.r.l., a gene therapy consulting company, also a spinoff of the University of Padua.

Selected publications

Biodegradable polymeric nanoparticles administered in the cerebrospinal fluid: Brain biodistribution, preferential internalization in microglia and implications for cell-selective drug release. Peviani M, Capasso Palmiero U, Cecere F, Milazzo R, Moscatelli D, Biffi A. Biomaterials. 2019 Jul;209:25-40. doi: 10.1016/j.biomaterials.2019.04.012.

Pellin D. *et al.* A comprehensive single cell transcriptional landscape of human hematopoietic progenitors. Nat Commun. 2019 Jun 3;10(1):2395.

Intracerebroventricular delivery of hematopoietic progenitors results in rapid and robust engraftment of microglia-like cells. Capotondo A, Milazzo R, Garcia-Manteiga JM, Cavalca E, Montepeloso A, Garrison BS, Peviani M, Rossi DJ, Biffi A. Sci Adv. 2017 Dec 6;3(12):e1701211. doi: 10.1126/sciadv.1701211.

Sessa, M. *et al.* Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. Lancet 388, 476-487 (2016).

Biffi, A. *et al.* Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy. Science 341, 1233158–1233158 (2013).

Molecular Genetic of Pediatric Leukemias Laboratory

PI **Martina Pigazzi**

Research activity

Leukemias account for approximately one third of all pediatric malignancies and remains a leading cause of acute leukemia-related death in children and adolescents. Acute myeloid leukemia (AML) represents around 20% of cases of acute leukemia in childhood, and even if the outcome of children has significantly improved over the past 30 years, reaching up to 60% of survival at 8 years in most of recent concluded clinical trials, these improvements are not related to the introduction of new drugs. Notwithstanding complete remission rates of 85–90%, disease recurrence still represents the main cause of treatment failure. Our clinical research aims at the identification of new genetic aberrations and mutations with prognostic value to be used as biomarkers to improve diagnosis definition and risk stratification of patients for moving toward a tailored adapted therapy. We played a main role in this field and characterized a large number of new unknown mutations in AML at diagnosis with prognostic role thanks to large retrospective studies, and we also established new quantitative molecular assays for the monitoring of minimal molecular disease, increasing the ability to look after patients during their follow up with relevant implications in disease management, specifically before bone marrow transplantation. For most of the identified new mutations we also set up functional studies deepening into the mechanism of leukemogenesis. The identification of structural alterations, such as inter- and intra-chromosomal rearrangements, remains the most frequent mechanism of mutagenesis occurring in pediatric AML, but for a large proportion of patients (up to 50%) a genetic biomarker is not present at diagnosis. Thus, we interpret the intimate connection between the dysregulation of gene expression and malignant

Team members

Martina Pigazzi
Principal Investigator

Claudia Tregnago
Senior PostDoctoral Researcher

Giulia Borella
PostDoctoral Researcher

Elena Porcù
PostDoctoral Researcher

Maddalena Benetton
PhD student

Ambra Da Ros
PhD student

Katia Polato
Lab Technician

Barbara Montini
Lab Manager

Anna Marchetti
Graduated student

PI's biosketch

Scopus ID: 23010194100

From 2008 Dr. Pigazzi is the Head 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 followup of patients by studying the 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". Dr. Pigazzi is Chair of the EU-task-force group "Molecular Genetics of AML" for the international BFM study group. From 2004, she is the Group Leader of the "Molecular Biology of the Acute myeloid leukemia" at the Oncohematology lab – Dept. of Women's and Children's Health - working mainly at the identification of new genetic aberrations and mutations with prognostic value used as biomarkers at diagnosis definition, for risk stratification of patients and tailored therapy. Dr. Pigazzi improves the functional role of new unknown mutations in leukemia at diagnosis, creates assays of minimal molecular disease monitoring during treatment for preemptively recognizing a disease recurrence and refine patients' clinical management. From 2013 Dr. Pigazzi is Group leader at the Istituto di Ricerca Pediatrica. 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 is author and co-author of more than 45 manuscripts published in oncohematological peer reviewed journals. She is PI of several national and European grants with main interest in drug screenings, using three-dimensional and animal disease models as patient-derived xenografts (PDXs). She is worldwide recognized as pediatric AML geneticist and included in the board of several international panels with main clinical (PeDAL, ACCELERATE, i-BFM) and biological aims (ITCC-P4) for improving the outcome of children with leukemia.

transformation, and highlight the importance of investigating key players in the regulation of gene expression to shed light to leukemogenesis and to define new therapeutic approaches. Our work is based on coding and non coding genes, such as lncRNAs and microRNAs, to dissect the key pathogenetic mechanisms of disease onset and recurrence, looking for new targets. We employed integrated high-throughput approaches looking at the DNA sequencing and methylation, transcriptome of coding and non coding genes, and proteome status of patients to be then recapitulated in several *in vitro*, 2D and 3D, and *in vivo* models, patient-derived xenograft (PDX), in order to create new treatment opportunities. This translational research approach commits to understand cancer transformation mechanisms, with particular interest in driver oncogenes and activated pathways identification, for promising alternatives or complementary therapeutics to current chemotherapy particularly in children who experience relapse. We use the repositioning strategy of old drugs by using high-throughput drug screening of commercial library with FDA-approved compounds, as well as targeted

therapy approaches in humanized xenograft mice models (PDXs) following main international Consortia of the Leukemia and Lymphoma Society for novel drugs included in clinical trials. We are also involved in experimentations including cancer cell metabolism and mitochondrial targeting, the use of nanoparticles for non coding RNAs delivery, and tumor specific antigens definition for the generation of novel CAR-T cell therapy specific for AML.
Recently, we start a collaboration with Dr. Stefano Cairo on the Children's Liver Tumour European Research Network – ChiLTERN which is a H2020 project for creating innovative *in vivo* models to identify new anticancer drugs or combinations of drugs for the treatment of patients with high-risk hepatoblastoma facing disease recurrence or chemotherapeutic treatment-failure. This activity would provide proof of concepts for patients enrolled in the PHITT trial which is a collaborative study involving major clinical European groups running pediatric liver tumor trials.
Finally, our research deals with the idea of destroying cancer by understanding tumor initiation to provide knowledge to definitively cure more children with cancer, expose fewer children to toxic chemotherapy and ensure their safe growth

Selected publications

Zampini M, Tregnago C, Bisio V, Simula L, Borella G, Manara E, Zanon C, Zonta F, Serafin V, Accordi B, Campello S, Buldini B, Pession A, Locatelli F, Basso G, Pigazzi M. Epigenetic heterogeneity affects the risk of relapse in children with t(8;21)RUNX1-RUNX1T1-rearranged AML. Leukemia. 2018 May;32(5):1124-1134. doi: 10.1038/s41375-017-0003-y. Epub 2018 Feb 2. PMID: 29472719.

Noort S, Zimmermann M, Reinhardt D, Cuccuini W, Pigazzi M, Smith J, Ries RE, Alonzo TA, Hirsch B, Tomizawa D, Locatelli F, Gruber TA, Raimondi S, Sonneveld E, Cheuk DK, Dworzak M, Stary J, Abrahamsson J, Arad-Cohen N, Czogala M, De Moerloose B, Hasle H, Meshinchi S, van den Heuvel-Eibrink M, Zwaan CM. Prognostic impact of t(16;21)(p11;q22) and t(16;21)(q24;q22) in pediatric AML: a retrospective study by the I-BFM Study Group. Blood. 2018 Oct 11;132(15):1584-1592.

de Rooij JD, Branstetter C, Ma J, Li Y, Walsh MP, Cheng J, Obulkasim A, Dang J, Easton J, Verboon LJ, Mulder HL, Zimmermann M, Koss C, Gupta P, Edmonson M, Rusch M, Lim JY, Reinhardt K, Pigazzi M, Song G, Yeoh AE, Shih LY, Liang DC, Halene S, Krause DS, Zhang J, Downing JR, Locatelli F, Reinhardt D, van den Heuvel-Eibrink MM, Zwaan CM, Fornerod M, Gruber TA. Pediatric non-Down syndrome acute megakaryoblastic leukemia is characterized by distinct genomic subsets with varying outcomes. Nat Genet. 2017 Mar;49(3):451-456. doi: 10.1038/ng.3772. Epub 2017 Jan 23. PMID: 28112737; PMCID: PMC5687824.

Tregnago C, Manara E, Zampini M, Bisio V, Borga C, Bresolin S, Aveic S, Germano G, Basso G, Pigazzi M. CREB engages C/EBPδ to initiate leukemogenesis. Leukemia. 2016 Sep;30(9):1887-96.

Palanichamy JK, Tran TM, Howard JM, Contreras JR, Fernando TR, Sterne-Weiler T, Katzman S, Toloue M, Yan W, Basso G, Pigazzi M, Sanford JR, Rao DS. RNA-binding protein IGF2BP3 targeting of oncogenic transcripts promotes hematopoietic progenitor proliferation. J Clin Invest. 2016 Apr 1;126(4):1495-511.

Neuroblastoma Laboratory

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 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 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

Marcella Pantile
Lab Technician

Pharmacology

The research activities of the Neuroblastoma Laboratory are focused on elucidating thebiological 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

PI's biosketch

Scopus ID: 20435413800
Dr. Aveic graduated at the University of Belgrade, Serbia, at the Dept. of Molecular Biology and Physiology, in 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 Neuroblastoma Laboratory at the Istituto di Ricerca Pediatrica. Since February 2019, she is also a lab manager at the University Hospital of Aachen, at the Dept. of Dental Materials and Biomaterials. Dr. Aveic served as a reviewer for numerous scientific journals 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.

employing 2D and 3D *in vitro*, and zebrafish and murine PDX *in vivo* models of NB as the pre-clinical approaches that should help us in decoding novel therapeutic window for treating metastatic disease.

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 zebrafish. We have recently found that Lin28B overexpressing embryos fail to develop functional peripheral sympathetic nervous system and develop NB. The Lin28B overexpressing NB cells are highly invasive and motile implying its association with pro-metastatic phenotypes. Currently, we are surveying Lin28B dependent protein network to distinguish the main promoters of sustained cell migration and invasion.

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.

Selected publications

Mariotto E, Viola G, Zanon C, Aveic S. A BAG's life: Every connection matters in cancer. Pharmacol Ther. 2020 May;209:107498. doi: 10.1016/j.pharmthera.2020.107498.

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.

Aveic S, Davtalab R, Vogt M, Weber M, Buttler P, Tonini GP, Fischer H. Calcium phosphate scaffolds with defined interconnecting channel structure provide a mimetic 3D niche for bone marrow metastasized tumor cell growth. Acta Biomater. 2019 Apr 1;88:527-539. doi: 10.1016/j.actbio.2019.02.030.

Duarte Campos DF, Bonnin Marquez A, O'Seanain C, Fischer H, Blaeser A, Vogt M, Corallo D, Aveic S. Exploring Cancer Cell Behavior *In Vitro* in Three-Dimensional Multicellular Bioprintable Collagen-Based Hydrogels. Cancers (Basel). 2019 Feb 5;11(2):180. doi: 10.3390/cancers11020180.

Sidarovich V, De Mariano M, Aveic S, Pancher M, Adami V, Gatto P, Pizzini S, Pasini L, Croce M, Parodi F, Cimmino F, Avitabile M, Emionite L, Cilli M, Ferrini S, Pagano A, Capasso M, Quattrone A, Tonini GP, Longo L. A High-Content Screening of Anticancer Compounds Suggests the Multiple Tyrosine Kinase Inhibitor Ponatinib for Repurposing in Neuroblastoma Therapy. Mol Cancer Ther. 2018 Jul;17(7):1405-1415. doi: 10.1158/1535-7163.MCT-17-0841.

Non Hodgkin Lymphoma - Molecular Diagnostic Laboratory

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:

Identification of new biomarkers in liquid biopsy of pediatric patients with Burkitt Lymphoma (BL)

BL is the most frequent subtype of NHL occurring in children and adolescents, representing more than half of childhood lymphomas. Probability of survival for refractory and relapse is very poor. Novel biomarkers are required to better understand the disease heterogeneity and the mechanism of relapse/progression. The aim of this study is to elucidate the prognostic role of liquid biopsy in particular to evaluate cell free DNA as marker of disease progression, and to characterize cells that constitute Minimal Residual Disease, in terms of side population, with gene expression analysis and single cell sequencing.

Team members

Lara Mussolin
Principal Investigator

Federica Lovisa
PostDoctoral Researcher

Lavinia Ferrone
PostDoctoral Researcher

Carlotta Damani
PhD student

Piero Di Battista
PhD student

Ilaria Gallingani
PhD student

Anna Garbin
PhD student

Elisa Tosato
Lab Technician

Lisa Quartesan
Graduated student

Anna Giada Toniolo
Graduated student

Anna Tosato
Graduated student

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 specializated 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 (Associazione Italiana di Emato-Oncologia Pediatrica) 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 50 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> 1000. 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.

Metabolic Profiling of diffuse large B-cell lymphomas: bench-to-bedside characterization of TRAP1 oncogenic role

Diffuse large B-cell lymphomas (DLBCLs) are a heterogeneous group of aggressive lymphoid

neoplasms. The present study will address the many open issues concerning the metabolic reprogramming of DLBCL and its clinical, prognostic and therapeutic implications. Pre-clinical and pathological studies will specifically consider the oncogenic role of TRAP1, an emerging master regulator of tumor cell metabolism.

Recent activities:

Genomics-driven identification of molecular mechanismsand biomarkers in pediatric follicular lymphoma

Follicular lymphoma (FL) is the second most common histological subtype of non-Hodgkin lymphoma (NHL) of adulthood; pediatric FL (pFL) has recently been recognized as a novel variant of FL in the World Health Organization classification of lymphoma, accounting for not more than 2-3% of NHLs in this age group. We analyzed the mutational landscape of pFL by exome sequencing of a series of tumour biopsies and our network analysis considerably extended previous data on the mutational landscape of FL of pediatric age, further indicating the signaling pathways of possible pathogenic relevance in these malignancies (Lovisa F, Haematol 2019).

Characterization of miRNA Profiles associated with ALCL primary tumours

We demonstrated that miR-939 expression could contribute to PDGFRB inhibition, a crucial driver for ALCL lymphomagenesis, via JUNB downregulation (Garbin A, Haematol 2020).

Selected publications

miR-939 acts as tumor suppressor by modulating JUNB transcriptional activity in pediatric anaplastic large cell lymphoma. Garbin A, Lovisa F, Holmes AB, Damanti CC, Gallingani I, Carraro E, Accordi B, Veltri G, Pizzi M, d'Amore ESG, Pillon M, Biffi A, Basso K, Mussolin L. Haematologica, 2020.

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.

Minimal disseminated disease in high-risk Burkitt's lymphoma identifies patients with different prognosis. Mussolin L, Pillon M, d'Amore ES, Conter V, Piglione M, Lo Nigro L, Garaventa A, Buffardi S, Aricò M, Rosolen A. J Clin Oncol. 2011.

Phosphoproteomic Laboratory

PI: **Benedetta Accordi**

Research activity

The Phosphoproteomics group is in charge of the Reverse Phase Protein Arrays (RPPA) facility that allows the identification of new disease biomarkers and new therapeutic targets in oncological and non oncological 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. Finally, thanks to a 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.

Team members

Benedetta Accordi
Principal Investigator - until Jan 2020

Valentina Serafin
PostDoctoral Researcher
Junior Principal Investigator - since February 2020

Giulia Veltri
PhD student

PI's biosketch

Scopus ID: 57190391373.
Dr. Accordi graduated in 2002 in Biological Sciences at the University of Padua. In 2006, Dr. Accordi received her PhD in Biologia dello Sviluppo e Scienze della Programmazione. During her PhD, she attended the Laboratory of Pediatric Oncohematology, Azienda Ospedale Università Padova , where she focused her research on the molecular biology of pediatric B-Acute Lymphoblastic Leukemia (B-ALL).
In 2005-06, Dr. Accordi attended the Laboratory headed by Dr. L. A. Liotta and Dr. E. Petricoin III at the Center for Applied Proteomics and Molecular Medicine at George Mason University (Manassas, VA, USA), where she was trained on the Reverse Phase Protein Arrays technique. She worked on the phosphoproteomic profiling of pediatric B-ALL supported by a grant from the Istituto Superiore di Sanità (Rome, Italy).
From October 2006, she is working at the Laboratory of Pediatric Oncohematology, Azienda Ospedale Università Padova and at the Istituto di Ricerca Pediatrica, where she has set up the Phosphoproteomics facility. Her research topic focuses on phosphoproteomic profiling of pediatric leukemias, and she is involved in several national and international collaborations focused on the phosphoproteomic profiling of neoplastic and non-neoplastic pediatric and adult diseases.

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

Zhou Y, Han C, Wang E, *et al.* Posttranslational regulation of the exon skipping machinery controls aberrant splicing in leukemia [published online ahead of print, 2020 May 22]. Cancer Discov. 2020;CD-19-1436. doi:10.1158/2159-8290.CD-19-1436.

Jin Q, Martinez CA, Arcipowski KM, *et al.* USP7 Cooperates with NOTCH1 to Drive the Oncogenic Transcriptional Program in T-Cell Leukemia. Clin Cancer Res. 2019;25(1):222-239. doi:10.1158/1078-0432.CCR-18-1740.

De Smedt R, Peirs S, Morscio J, *et al.* Pre-clinical evaluation of second generation PIM inhibitors for the treatment of T-cell acute lymphoblastic leukemia and lymphoma. Haematologica. 2019;104(1):e17-e20. doi:10.3324/haematol.2018.199257.

Serafin V, Capuzzo G, Milani G, *et al.* Glucocorticoid resistance is reverted by LCK inhibition in pediatric T-cell acute lymphoblastic leukemia. Blood. 2017 Dec 21;130(25):2750-2761. doi: 10.1182/blood-2017-05-784603.

Serafin V, Lissandron V, Buldini B, *et al.* Phosphoproteomic analysis reveals hyperactivation of mTOR/STAT3 and LCK/Calcineurin axes in pediatric early T-cell precursor ALL. Leukemia. 2017;31(4):1007-1011. doi:10.1038/leu.2017.13.

Research in Pediatric Solid Tumors Laboratory

PI: **Gianni Bisogno**

Research activity

The laboratory works in close collaboration with the Pediatric Oncohematology Division in Padua and over the years has developed a particular interest in diagnostics and basic and translational research for children with soft tissue sarcomas and other rare tumors. The main activities are: i) to collect, handle and store biological samples from patients with solid tumors; ii) to provide the necessary molecular biology investigations to support the diagnosis of sarcoma; iii) to promote research projects dedicated to the study of biological characteristics of pediatric soft tissue sarcomas (STS) and biomarkers that can have a direct clinical application.

- A pediatric soft tissue biobank has been established since 1995 and every year the laboratory analyze almost 150 new cases collected from more than 30 pediatric oncology centers part of the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP), performing more than 1000 molecular analyses with diagnostic and prognostic significance.
- The laboratory is involved in the investigation of new diagnostic and prognostic biomarkers in children with soft tissue sarcomas (STS). In many of these malignancies we have demonstrated the presence of several new genetic abnormalities, including point mutations, deletions, amplifications and chromosomal translocations that have improved our ability to identify the best treatment option for each patient, both reducing the side effects of the conventional treatments as well as identifying high-risk patients suitable for novel therapeutic approaches. During these years, the number of assessed genetic alterations have progressively improved,and it now includes more than 30 specific abnormalities, like the most recent VGLL2-CITED2 and VGLL2-NCOA2 in sclerosing and spindle cell RMS (ScRMS and SRMS, respectively), TEAD1-NCOA2 or SRF-NCOA2 gene fusions in ScRMS and SRMS also, BCOR exon

Team members

Gianni Bisogno
Principal Investigator

Paolo Bonvini
Senior Research

Lucia Tombolan
Senior Research Assistant

Angelica Zin
Senior Research Assistant

Elena Poli
PostDoctoral Researcher

Silvia Lucchetta
PhD student

PI's biosketch

Scopus ID 7003672935
Prof. Bisogno obtained his MD in 1988 and progress into specializing in Pediatrics with a Clinical Residency in 1992 before pursuing a PhD in Pediatric Oncology in 1997 at the University of Padua. Prof. Bisogno is Associate 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 Head of the Solid Tumor Unit (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 have been registering patients from 150 European centers and includes two different randomized studies.
He is also leader or partner in several projects supported by different institutions, including European Community such as EPOC, ENCCA and EXPORNET and coordinator of PARTNER, a recently funded project dedicated to the creation of a European Registry for children with very rare tumors.
Prof. Bisogno is Responsible for the Trial Office of the Pediatric Dept. of Padua.
Publications: 232 papers with a total impact factor of 1.194,857. Citations: h-index of 42 (according to Scopus)
Grants (1999-2018): 12 grants as principal investigator and 10 as co-investigator, including 3 Ricerca Fi-nalizzata, 1 AIRC, 5 European calls.
Memberships: Scientific Board of the Deutsche Forschungsgemeinschaft (German Research Foundation) (2008-2010); 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) (2017); member of Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP); member of the review board on rare diseases for the Agency for Health Quality and Assessment of Catalonia (2020).

16 ITD and YWHAE-NUTM2B fusions in soft tissue undifferentiated round cell sarcomas (URCS) and primitive myxoid mesenchymal tumor of infancy (PMMTI), CIC-DUX4 and BCOR-CCNB3in a subset of pediatric undifferentiated (UND) sarcomas and translocation-negative Ewing sarcomas (ES), in addition to the more common ones routinely tested. For instance, in collaboration with the European Pediatric Soft Tissue Sarcoma Study Group (EpSSG), we have demonstrated that the presence of fusion transcript PAX-FOXO1 in the

blood and bone marrow of children with alveolar rhabdomyosarcoma tumors (and lymph node involvement) has a strong impact on prognosis.

- A series of studies utilizing liquid biopsies have also been carried out by our group, in order to identify novel biomarkers predictive of cancer resistance and metastasis, throughout a comprehensive cellular and molecular analysis of peripheral blood, bone marrow and plasma samples of children with STS. Liquid biopsies are non-invasive blood tests for the detection of circulating tumor cells (CTCs), cell-free tumor DNA (ctDNA) and cancer-associated antigens shed into the bloodstream by the primary tumor and metastasis, which can help to identify tumors able to spread to distant organs and develop better strategies to predict risk of relapse. The laboratory focuses on the immunoaffinity capturing and magnetic sorting set up of CTCs in children with STS (in collaboration with the Veneto Institute of Oncology, IOV), coupled with ultra-deep NGS sequencing and gene expression profiling of both CTCs and ctDNA. We also performed proteome-scale examination of STS patients' plasma, to assess the presence of tumor antigens and matching tumor-associated autoantibodies that may help to detect the presence of tumor at diagnosis and classify patients belonging to different risk groups. By doing so, we have observed that: i) CTCs are found in the bloodstream of children with soft tissue sarcomas; ii) CTCs andcfDNA samples harbor pre-treatment tumor mutations that may be important to assess disease evolution and treatment response; iii) autoantibodies against STS tumor antigens do exist and evoke an immune response that distinguishes affected children from healthy subjects, likewise patients belonging to different risk groups. Nonetheless, a main research interest of our group remains the identification of driver genes and proteins in childhood cancer targetable by novel anticancer drug therapeutics. During the past years, we have paid particular attention to small-molecule inhibitors, based on the concept that tumors carrying deregulated enzymes (i.e. tyrosine kinases) are particularly susceptible to their inhibition. We have tested several target-specific and group-selective agents, using heterogeneous and oncogene-addicted *in vitro* models of pediatric STS and inhibitor compounds from either research institutes or pharmaceutical companies. In collaboration with several research groups, we contributed to demonstrate: i) the potential role of ALK tyrosine kinase in tumor progression and metastasis of pediatric RMS; ii) the anti-differentiation and tumorigenic function of ZNF521 in MLL-rearranged cells; iii) the prognostic value of miR-223 in pediatric T-Cell lymphoblastic lymphoma; iv) the differential expression level of NPM-ALK fusion gene in distinct subtypes of ALCL (anaplastic large cell lymphoma) patients; v) the preclinical activity of new tyrosine kinase inhibitors in pediatric tumors harboring ALK and TRK gene rearrangements.

Selected publications

Poli E, Zin A, Cattelan M, Tombolan L, Zanetti I, Scagnellato A, Bonvini P§ and Bisogno G§. Prognostic Value of Circulating IGFBP2 and Related Autoantibodies in Children with Metastatic Rhabdomyosarcoma. Diagnostics. 2020 Feb 20;10(2). pii: E115. doi: 10.3390/diagnostics10020115. PMID: 32093404. (§ co-last authors).

Bisogno G, De Salvo GL, Bergeron C, Gallego Melcón S, Merks JH, Kelsey A, Martelli H, Minard-Colin V, Orbach D, Glosli H, Chisholm J, Casanova M, Zanetti I, Devalck C, Ben-Arush M, Mudry P, Ferman S, Jenney M, Ferrari A; European pediatric Soft tissue sarcoma Study GroupVinorelbine and Continuous Low-Dose Cyclophosphamide as Maintenance Chemotherapy in Patients With High-Risk Rhabdomyosarcoma (RMS 2005): A Multicentre, Open-Label, Randomised, Phase 3 Trial. Lancet Oncol. 2019 Nov;20(11):1566-1575. doi: 10.1016/S1470-2045(19)30617-5. Epub 2019 Sep 24. PMID: 31562043.

Bisogno G, Jenney M, Bergeron C, Gallego Melcón S, Ferrari A, Oberlin O, Carli M, Ste-vens M, Kelsey A, De Paoli A, Gaze MN, Martelli H, Devalck C, Merks JH, Ben-Arush M, Glosli H, Chisholm J, Orbach D, Minard-Colin V, De Salvo GL; European pediatric Soft tis-sue sarcoma Study Group. Addition of dose-intensified doxorubicin to standard chemother-apy for rhabdomyosarcoma (EpSSG RMS 2005): a multicentre, open-label, randomised con-trolled, phase 3 trial.Lancet Oncol. 2018 Aug;19(8):1061-1071.

Tombolan L, Poli E, Martini P, Zin A, Romualdi C, Bisogno G, Lanfranchi G. NELL1, whose high expression correlates with negative outcomes, has different methylation pat-terns in alveolar and embryonal rhabdomyosarcoma.Oncotarget. 2017 May 16;8(20):33086-33099.

Bonvini P, Zin A, Alaggio R, Pawel B, Bisogno G, Rosolen A. High ALK mRNA expression has a negative prognostic significance in rhabdomyosarcoma.Br J Cancer. 2013 Dec 10;109(12):3084-91. doi: 10.1038/bjc.2013.653. Epub 2013 Oct 22.PubMed PMID: 24149177; PubMed Central PMCID: PMC3859940.

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

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.

The Pediatric Metabolomics Project is running studies in the field on neonatology (bronchopulmonary dysplasia (BPD), sepsis, perinatal asphyxia, glucose monitoring), prevention of BPD with extracellular vesicles and early origins of chronic respiratory diseases (bronchiolitis, asthma). The results of these projects have been published in high impact factor journals such as J Pediatrics, Metabolomics, Lipids, Clinical Nutrition, Pediatric Research, J Control Release, J Infection Disease, PLoS One

Fondazione
ISTITUTO DI RICERCA
PEDIATRICA

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 Laboratory

PIs: **Eugenio Baraldi, Giuseppe Giordano**

Research activity

Metabolomics is the most recent of the omic sciences. Metabolomics can be defined as the quantitative analysis of all the metabolites (small molecules <1 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 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

Team members

Eugenio Baraldi
Co-Principal Investigator

Giuseppe Giordano
Co-Principal Investigator

Matteo Stocchero
Biostatistician

Paola Pirillo
PostDoctoral Researcher

Gabriele Poloniato
PhD student

Mauro Naturale
Lab Technician

Silvia Carraro
Associate Professor, Clinician

Alfonso Galderisi
PostDoctoral Researcher, Clinician

PI's biosketch

Eugenio Baraldi

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).

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)

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).

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, Baraldi E, Giordano G, Pirillo P, Stocchero M, Houben M, Bont L.. 2018. Metabolomic Profile of Amniotic Fluid and Wheezing in the First Year of Life-A Healthy Birth Cohort Study.. J Pediatr, 196 : 264-269 doi: 10.1016 /j.jpeds.2018.01.012.

Galderisi A, Pirillo P, Moret V, Stocchero M, Gucciardi A, Perilongo G, Moretti C, Monciotti C, Giordano G, Baraldi E.. 2017. Metabolomics reveals new metabolic perturbations in children with type 1 diabetes. Pediatr Diabetes , doi: 10.1111/pedi.12524.

Donazzolo E, Gucciardi A, Mazzier D, Peggion C, Pirillo P, Naturale M, Moretto A, Giordano G.. 2017. Improved synthesis of glycine, taurine and sulfate conjugated bile acids as reference compounds and internal standards for ESI-MS/MS urinary Profiling of inborn errors of bile acid synthesis.. Chem Phys Lipids.

Baraldi E, Giordano G, Stocchero M, Moschino L, Zaramella P, Tran MR, Carraro S, Romero R, Gervasi MT. 2016. Untargeted Metabolomic Analysis of Amniotic Fluid in the Prediction of Preterm Delivery and Bronchopulmonary Dysplasia. PLoS One , 18;11(10):e0164211.

Carraro S, Giordano G, Reniero F, Perilongo G, Baraldi E . 2009. Metabolomics- a new frontier for pediatric research. J Pediatr, 154: 638-644.

Pediatric Critical Care Laboratory

PIs: **Paola Cogo**

Research activity

Our research team has 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 has 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 one or more easily assayed biomarker 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 and how the type of cardiac diseases is linked to a specific pulmonary surfactant status. Moreover, we linked a specific marker of brain injury (GFAP), along with other surgical parameters, to children's neurodevelopmental outcome at 18 months after surgery. Neurodevelopmental followup in 45 of these CHD children showed that GFAP was associated with impaired communication skills. Up to 52% of CHD children

Team members

Paola Cogo
Principal Investigator

Manuela Simonato
PostDoctoral Researcher

Alessio Correani
PostDoctoral Researcher

Virgilio Carnielli
Clinical Researcher

Aldo Baritussio
Clinical Researcher

Giovanna Verlato
Clinical Researcher

Nicola Faganello
Master student

Laura Mezzalira
Master student

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. 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 187 Scopus-indexed documents (2383 citations) with an h-index of 29.

reported neuropsychological impairments mainly related to the domain of the social and affective perception. 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, both acute and chronic, is also a key topic of the laboratory. We used stable isotope tracers and high resolution-mass spectrometry techniques to describe lipids and metabolites profile and surfactant specific proteins metabolism and amounts in different disease in children and adults.
We are also studying a protective ventilation protocol to be used during cardiac surgery in CHD patients.

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

Along with clinical studies, cellular and molecular studies are in progress to characterize the response of brain cell (especially glial cells) to hypothermia, hypoxia, and rewarming.

Selected publications

Simonato M, Fochi I, Vedovelli L, Giambelluca S, Carollo C, Padalino M, Carnielli VP, Cogo P: Urinary metabolomics reveals kynurenine pathway perturbation in newborns with transposition of great arteries after surgical repair. Metabolomics 2019; 15: 145.

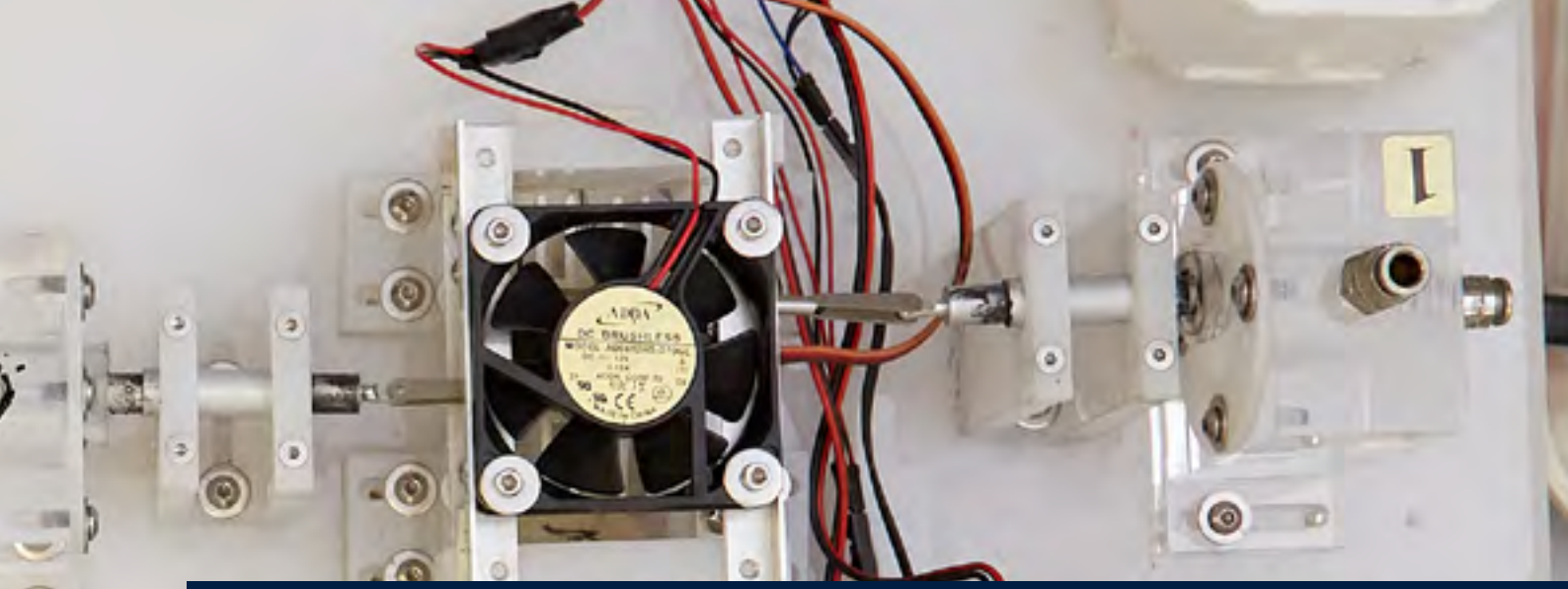
Giambelluca S, Ricci F, Simonato M, Vedovelli L, Traldi U, Correani A, Casiraghi C, Storti M, Mersanne A, Cogo P, Salomone F, Carnielli VP: Tracing exogenous surfactant *in vivo* in rabbits by the natural variation of (13)C. Respir Res 2019; 20: 158.

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.

Simonato M, Baritussio A, Carnielli VP, Vedovelli L, Falasco G, Salvagno M, Padalino M, Cogo P: Influence of the type of congenital heart defects on epithelial lining fluid composition in infants undergoing cardiac surgery with cardiopulmonary bypass. Pediatr Res 2018.

Correani A, Visentin S, Cosmi E, Ponchia E, D'Aronco S, Simonato M, Vedovelli L, Cogo P, Carnielli VP: The maternal-fetal gradient of free and esterified phytosterols at the time of delivery in humans. Clin Nutr 2018; 37: 2107-2112.


1
3
2
PERFUSOR
secura



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.


Fondazione
ISTITUTO DI RICERCA
PEDIATRICA
città della speranza

Prof. Maurizio Muraca

Coordinator Regenerative Medicine Area

Scopus ID 7006578223

After a MD at the University of Padua in 1976, Prof. Muraca specialised in Internal Medicine (1981) and subsequently 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 acted as the Head of the Hepatology and Liver Transplant Group of the 1st Medical Clinic, University of Padua, and subsequently moved to Rome to work as Head of the Clinical Chemistry and Regenerative Medicine Research, at the Pediatric Research Hospital "Bambino Gesù" until 2014, before returning to Padua where he has been working as Associate Professor of Clinical Medicine, Dept. of Women's and Children's Health, University of Padua and Principal Investigator of the Stem Cell and Regenerative Medicine Lab, at the Istituto di Ricerca Pediatrica. Prof. Muraca 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: 35 (Scopus) – 41 (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 Laboratory

PI: **Maurizio Muraca**

Research activity

Mesenchymal stem/stromal cell-derived extracellular vesicles as therapeutic tool for Inflammatory Bowel Disease

Our recent experimental work clearly showed the limitations of the DSS model of colitis. In particular, this model is not lymphocyte-mediated and suffers from a considerable variability probably due to environmental factors. Our preliminary experience with a more chronic model (colitis induced by TNBS) was not encouraging, showing again a large variability in response. Moreover, our previous *in vitro* work showed that MSC-EVs involved both Treg and IL-10 production as possible mediators of the therapeutic effect. Therefore, alternative animal models such as the cell transfer model (lacking Treg) and the IL-10 knockout model could be unfeasible to test the effects of our product. For these reasons, we are developing an *ex vivo* assay using intestinal samples obtained at surgery from patients with Crohn's disease (Prof. Imerio Angriman, General Surgery, Padua). This method would keep the sample viable under hyperoxic culture conditions for 24 hours, allowing testing the effects of drugs directly on the patient's mucosa, by evaluating the expression and release of cytokines. Using this model, we will evaluate the effects of both "naïve" and An5-bound MSC-EVs.

Biodistribution studies are a requirement of Regulatory Authorities to allow first-in-man studies. Fluorescent-labeled MSC-EVs will be administered either via enema or IV both to helthy mice and to mice with DSS-induced colitis to follow the absorption and distribution of the nanoparticles in the different organs (in coll. with Antonio Rosato).

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

Following our recently published positive results *in vivo* (Porzionato *et al.* 2018), we will administer both MSC-EVs and MSC-EV-An5 in the same animal

Team members

Maurizio Muraca
Principal Investigator

Anna Maria Tolomeo
PostDoctoral Researcher

Giada De Lazzari
PhD student

Ricardo Malvicini
Visiting PhD student - Argentina

Marco Brugnaro
Master student

PI's biosketch

Scopus ID 7006578223
After a MD at the University of Padua in 1976, Prof. Muraca specialised in Internal Medicine (1981) and subsequently 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 acted as the Head of the Hepatology and Liver Transplant Group of the 1st Medical Clinic, University of Padua, and subsequently moved to Rome to work as Head of the Clinical Chemistry and Regenerative Medicine Research, at the Pediatric Research Hospital "Bambino Gesù" until 2014, before returning to Padua where he has been working as Associate Professor of Clinical Medicine, Dept. of Women's and Children's Health, University of Padua and Principal Investigator of the Stem Cell and Regenerative Medicine Lab, at the Istituto di Ricerca Pediatrica. Prof. Muraca 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: 35 (Scopus) – 41 (Google Scholar).
Awards:
10/11/2006: International Award "Giuseppe Sciacca", section Medicine
18/07/2011: Award "Gentlemen d'Italia" for scientific activity

model of BPD with a dose escalation approach. Moreover, since it has been reported that the beneficial effects of MSC-EVs are mediated by systemic effects on the immune system, we will evaluate the modifications of both innate and adaptive immune cell lineages in thymus, spleen and lung lymph nodes in treated groups compared to controls. Finally, we will also compare the effects of local vs. systemic administration.
Tests *in vitro* will include the effects of MSC-EVs on both innate and adaptive immunity in human cells. These tests could also help verify the activity of different EV batches for clinical application. Moreover, the same tests will help identify the mechanisms of action underlying the observed effects on different immune cell populations. To this end, we will evaluate the role of different proteins expressed on the surface of MSC-EVs with known immune modulatory properties, by knockout or hyperexpression approaches. Indeed, although the EV RNA cargo is a possible mediator of signals influencing the metabolism of target cells, it is now appreciated that probably most of the EV-mediated signals take place at the level of plasma membrane, while following internalization EVs are generally targeted to lysosomes where their content is degraded (Van Niel, D'Angelo and Raposo, 2018).
Biodistribution studies will help understand the effects on target organs. Fluorescent-labeled MSC-EVs will be administered either IT or IV to helthy (normoxic) newborn rats and to (hyperoxic) rats developing BPD to follow the absorption and distribution of nanoparticles in the different organs (in coll. with Prof. Antonio Rosato).

Role of extracellular vesicles in bone tumor pathogenesis: implications for therapy

In this project, we aim at understanding to what extent EVs are involved in the development of bone malignancies. More precisely, we will identify the pathways involved in EV tumorigenic functions and exploit their specific tropism to target cells and create new methods to fight cancer-induced bone diseases. Therefore, our project will be developed according to the following three main tasks:

- Investigate the involvement of EVs in the transfer of RANKL to target cells and in the activation of osteoclast bone resorption and angiogenesis during bone tumorigenesis: i) isolate, quantify and characterise RANKL-positive EVs released by osteoblasts; ii) treat osteoblasts with tumour cell conditioned media and isolate, quantify and characterise their RANKL-positive EV release; iii) investigate the effect of tumour-cell induced RANKL-positive EVs release by osteoblasts on osteoclast bone resorption and angiogenesis *in vitro* and *in vivo*.
- Characterise tumour-induced osteoblast EV RNA and protein profiles to identify molecular pathways and biological processes deregulated by bone malignancies.
- Employ osteoblast EVs loaded with chemotherapeutics to antagonise bone tumour growth.

Selected publications

Budoni M, Fierabracci A, Luciano R, Petrini S, Di Ciommo V, Muraca M. The immunosuppressive effect of mesenchymal stromal cells on B lymphocytes is mediated by membrane vesicles. CELL TRANSPLANTATION 2013, vol. 22, p. 369-379.

Del Fattore A, Luciano R, Saracino R, Battafarano G, Rizzo C, Pascucci L, Alessandri G, Pessina A, Perrotta A, Fierabracci A, Muraca M (2014). Differential effects of extracellular vesicles secreted by mesenchymal stem cells from different sources on glioblastoma cells. EXPERT OPINION ON BIOLOGICAL THERAPY 2014; vol. 14, p. 495-504.

Porzionato, A., Zaramella, P., Dedja, A., Guidolin, D., Van Wemmel, K., Macchi, V., Jurga, M., Perilongo, G., De Caro, R., Baraldi, E., Muraca, M. Intratracheal administration of clinical-grade mesenchymal stem cell-derived extracellular vesicles reduces lung injury in a rat model of bronchopulmonary dysplasia. American Journal of Physiology - Lung Cellular and Molecular Physiology 2019; 316 (1), pp. L6-L19.

Börger V, Weiss DJ, Anderson JD, Borràs FE, Bussolati B, Carter DRF, Dominici M, Falcón-Pérez JM, Gimona M, Hill AF, Hoffman AM, de Kleijn D, Levine BL, Lim R, Lötvall J, Mitsialis SA, Monguió-Tortajada M, Muraca M, Nieuwland R, Nowocin A, O'Driscoll L, Ortiz LA, Phinney DG, Reischl I, Rohde E, Sanzenbacher R, Théry C, Toh WS, Witwer KW, Lim SK, Giebel B. ISEV and ISCT statement on EVs from MSCs and other cells: considerations for potential therapeutic agents to suppress COVID-19. Cytotherapy 2020 May 16. doi: 10.1016/j.jcyt.2020.05.002. Online ahead of print.

Muraca M, Pessina A, Pozzobon M, Dominici M, Galderisi U, Lazzar L, Parolini L, Lucarelli E, Perilongo G, Baraldi E. Mesenchymal stromal cells and their secreted extracellular vesicles as therapeutic tools for COVID-19 pneumonia? Journal of Controlled Release, Available online 3 July 2020. Doi: https://doi.org/10.1016/j.jconrel.2020.06.036.

Neuromuscular Engineering Laboratory

PI **Anna Urciuolo**

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 and by the action of the nervous system. Skeletal muscle can be functionally compromised due to dysfunction of muscular and/or of neuronal components, as in neuromuscular diseases.

Team members

Anna Urciuolo
Junior Principal Investigator

Paolo Raffa
PostDoctoral Researcher

Maria Easler
Master Student

Our recently established group results from the multidisciplinary integration of different expertise in skeletal muscle and stem cell biology, extracellular matrix and biomaterial engineering. Our current research project aims at generating 3D models of human skeletal muscle equipped with a neuronal network. Such 3D *in vitro* models will be used to mimic the structural/functional properties of human skeletal muscle and neuromuscular junction, for the identification of cellular and molecular players involved in neuromuscular genesis and maintenance in healthy and disease.

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 3D (i3D) bioprinting, which allows hydrogel fabrication inside pre-existing 3D environment, both in vitro and in vivo, based on the implementation of multiphoton microscopes and photo-sensitive biopolymers. 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. The current research line of her lab results from the multidisciplinary integration of cell biology and extracellular matrix/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.

From 2018, thanks to the STARS-Starting grant, Dr. Urciuolo is a Junior Principal Investigator at the Istituto di Ricerca Pediatrica, and from 2020 she is a visiting Research Associate at the Stem Cell and Regenerative Medicine Section of the UCL-Institute of Child Health (London, UK).

Dr. Urciuolo authored 22 publications with a total number of citations equal to 1539 and h-index of 14 (according to Scopus).

Selected publications

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, 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). doi: 10.1038/s41551-020-0568-z. Impact factor: 17.135.

Urciuolo A, *et al*. Decellularised skeletal muscles allow functional muscle regeneration by promoting host cell migration. Scientific Reports. (2018) 8:8398.

Urciuolo A, *et al*. Collagen VI regulates satellite cell self-renewal and muscle regeneration. Nat Commun, (2013) 4:1964.

Grumati P, *et al*. Autophagy is defective in collagen VI muscular dystrophies and its reactivation rescues myofiber degeneration. Nat Med, (2010) 16(11), 1313-20.

Stem Cells and Regenerative Medicine Laboratory

PI: **Michela Pozzobon**

Research activity

Team members

Michela Pozzobon
Principal Investigator

Stefania D'Agostino
PostDoctoral Researcher

Fabio Magarotto
Research Associate

Anna Chiara Moscato
Master student

Matteo Barel
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 developed expertise in skeletal muscle, stem cell biology and extracellular matrix engineering. The lab is developing the following two main projects:

Muscle extracellular muscle engineered with Extracellular Vesicles for muscle reconstruction

The need of new biomaterials to replenish the loss of muscle mass is currently a challenge. Indeed, after congenital malformations, trauma or tumor surgery the volume mass loss can be filled with synthetic materials already used in clinical practice, but the regain of function is still very difficult to reach. Nowadays the decellularization of tissues allows the obtainment of the highest biocompatible scaffold without the genetic material, such as the extracellular matrix (ECM). This biomaterial, that retains the biomechanical properties, proteins and biochemical factors that characterized the native tissue, induces vascularization and new cell recruitment, but fibrosis and gain of function are still problems to face. It is known that there are intercellular signals mediating tissue repair conveyed via biologically active nanoparticles secreted by cells, called extracellular vesicles (EVs). With the aim to improve the muscle functional regeneration, the project focuses on:

- functionalization of the muscle ECM with EVs, naïve or modified, from different cell sources (muscle precursor cells, amniotic fluid stem cells, cord blood mesenchymal stem cells, endothelial cells);
- fibrosis and functional muscle recovery evaluation in a well-set model of volumetric muscle loss. In perspective, in other muscle disease models (such as dystrophy) the effect of "naïve" and engineered EV activity will be evaluated.

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 with an 18 months fellowship on an EU project on polymer synthesis. Dr. Pozzobon spent 3 years (2002-2004) at the University of Oxford as Research Assistant in the Oncology Group (Nuffield Dept. of Clinical Sciences, UK) under the supervision of Prof David Y. Mason. Since 2004, she joined the lab of Stem Cells and Regenerative Medicine, working with Dr. Paolo De Coppi which she has been coordinating since 2010. During this time, Dr. Pozzobon enrolled in the PhD program in Tissue Engineering and Regenerative Medicine at the University of Padua which was completed in 2008. Her research activity is focused on the study of the tissue biology and the application in regenerative medicine (cell therapy and tissue engineering), specifically in the pediatric field with focus on muscle related problems. Dr. Pozzobon is actively involved in national and international projects, and is MC member of the COST European project SPRINT on perinatal stem cells (2019-2021). Her expertise combines the training in oncologic field and the research on regenerative medicine. Since 2004, her studies on stem cells from amniotic fluid stem cells (fetal stem cells) and from skeletal muscle (adult stem cells) used with regenerative medicine/tissue engineering approaches have been applied on murine models of muscle disease or defects. In 2016, Dr. Pozzobon obtained the senior research fellowship number GRIC15AIPF of the University of Padua and in 2019 was awarded the IRP-Synergy grant 2019 to investigate the interaction of extracellular vesicles and extracellular matrix.

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 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 process of integrin-mediated cell motility responsible for the early metastatic migration of cancer cells using a protein tuned hydrogel, HA based, as tridimensional (3D) model;
- to investigate of the role of the highly expressed GPC3 proteoglycan, in RMS tumor growth and progression, in 3D.

Selected publications

Trevisan C, Fallas MEA, Maghin E, Franzin C, Pavan P, Caccin P, Chiavegato A, Carraro E, Boso D, Boldrin F, Caicci F, Bertin E, Urbani L, Milan A, Biz C, Lazzari L, De Coppi P, Pozzobon M, Piccoli M. Generation of a Functioning and Self-Renewing Diaphragmatic Muscle Construct. Stem Cells Transl Med. 2019 Apr 10. doi: 10.1002/sctm.18-0206.

Pozzobon M, Saggioro M, D'Agostino S, Bisogno G, Muraca M, Gamba P. Alveolar Rhabdomyosarcoma Decellularization. Methods Mol Biol. 2018;1577:317-325. doi: 10.1007/7651_2017_45.

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.

Piccoli M, Urbani L, Alvarez-Fallas ME, Franzin C, Dedja A, Bertin E, Zuccolotto G, Rosato A, Pavan P, Elvassore N, De Coppi P, Pozzobon M. Improvement of diaphragmatic performance through orthotopic application of decellularized extracellular matrix patch. Biomaterials. 2015 Oct 9;74:245-255. doi: 10.1016/j. Biomaterials. 2016 Jan;74:245-55. doi: 10.1016/j. biomaterials.2015.10.005. Epub 2015 Oct 9.].

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 Laboratory

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 recently shown that the use of a biological implant obtained through the decellularization of the diaphragmatic muscle greatly improves CDH treatment in an animal model, avoiding an extensive scar formation, and consequently limiting recurrences. Despite these important results, this classic tissue engineering approach requires long preparation times, depends on organ donation and cannot be used for large-scale production. Manifacturing a product that is standardized and identical for all patients is therefore not feasible.

Team members

Martina Piccoli
Principal Investigator

Daniele Boso
PostDoctoral Researcher

Edoardo Maghin
PhD Student

Eugenia Carraro
PhD 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 cell, their phenotypical characterization and in vivo use in different mouse models before completing her PhD period in 2013 in the same field and the study was developed with cellular and biomolecular techniques. 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 cells 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 tissue-like structures. In addition, she successfully contributed in the achievement of specific tasks in several projects, taking part also at the management side (e.g. staffing, research protections, budget); several peer-reviewed publications from each project have been produced.

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

Generation of a Functioning and Self-Renewing Diaphragmatic Muscle Construct. Trevisan C, Fallas MEA, Maghin E, Franzin C, Pavan P, Caccin P, Chiavegato A, Carraro E, Boso D, Boldrin F, Caicci F, Bertin E, Urbani L, Milan A, Biz C, Lazzari L, De Coppi P, Pozzobon M, Piccoli M. Stem Cells Transl Med. 2019 Aug;8(8):858-869. doi: 10.1002/sctm.18-0206.

Allogenic tissue-specific decellularized scaffolds promote long-term muscle innervation and functional recovery in a surgical diaphragmatic hernia model. Trevisan C, Maghin E, Dedja A, Caccin P, de Cesare N, Franzin C, Boso D, Pesce P, Caicci F, Boldrin F, Urbani L, De Coppi P, Pozzobon M, Pavan P, Piccoli M. Acta Biomater. 2019 Apr 15;89:115-125. doi: 10.1016/j.actbio.2019.03.007.

A finite element analysis of diaphragmatic hernia repair on an animal model. de Cesare N, Trevisan C, Maghin E, Piccoli M, Pavan PG. J Mech Behav Biomed Mater. 2018 Oct;86:33-42. doi: 10.1016/j.jmbbm.2018.06.005.

Mouse Skeletal Muscle Decellularization. Piccoli M, Trevisan C, Maghin E, Franzin C, Pozzobon M. Methods Mol Biol. 2018;1577:87-93. doi: 10.1007/7651_2017_28.

Improvement of diaphragmatic performance through orthotopic application of decellularized extracellular matrix patch. Piccoli M, Urbani L, Alvarez-Fallas ME, Franzin C, Dedja A, Bertin E, Zuccolotto G, Rosato A, Pavan P, Elvassore N, De Coppi P, Pozzobon M. Biomaterials. 2016 Jan;74:245-55. doi: 10.1016/j.biomaterials.2015.10.005.

Contacts

Fondazione
Istituto di Ricerca Pediatrica
Città della Speranza

Corso Stati Uniti, 4
35127 Padova
Phone: +39 049 9640111
Fax: +39 049 9640101
Email: info@irpcds.org