Research Report 2023-2024

Molecular Biotechnology Center "Guido Tarone"
@ University of Torino

Centro di Biotecnologie Molecolari "Guido Tarone"
@ Università di Torino

Università di Torino
M B C
Molecular Biotechnology Center
Guido Tarone

Dedicated to Lorenzo Silengo,
whose drive and vision led us
where we are.

WELCOME

to the vibrant world of the Molecular Biotechnology Center at the University of Turin. As the director of this institution, it is with great pleasure that I present this comprehensive booklet, encapsulating the diverse and groundbreaking research activities undertaken by more than 30 principal investigators within our center. This compilation serves as a testimony to our commitment to advancing knowledge and finding innovative solutions to the pressing health challenges of our time.

Our research spans a wide array of themes including cancer, immunology, cardiovascular diseases, metabolic disorders and neurodegeneration, with an overarching goal of generating novel diagnostic and therapeutic approaches that can contribute to address the health concerns that accompany the aging population of our planet. The challenge we embrace is clear – to improve the quality of life, particularly for the elderly, through cutting-edge biotechnological research.

The Molecular Biotechnology Center is a unique hub, fostering collaboration and synergy among researchers with diverse scientific interests and distinct affiliations. In addition to the founder Department of Molecular Biotechnology, other prominent University Departments such as those of Neuroscience, Oncology, Medical Sciences and Life Sciences and Systems Biology contribute to this endeavor.

Our center operates on the principle that collaboration as well as cross-pollination of ideas and skills breeds success. By providing a shared environment where energies, knowledge, facilities, and instruments are seamlessly integrated, we aim to enhance productivity and facilitate scientific breakthroughs. This collaborative spirit extends across disciplines, encouraging interdisciplinary research that transcends traditional boundaries.

All participating departments have generously contributed state-of-the-art equipment as shared resources, which are meticulously maintained by specialized personnel. This enables the MBC to host a comprehensive array of cutting-edge technical facilities, including bioimaging, high-content microscopy, histology, transcriptomics, cell sorting, genomics, and mass sequencing. Furthermore, the center houses a state-of-the-art cell factory dedicated to advancing cell-based therapies, along with some of the University of Torino's largest facilities for preclinical modeling. These shared resources not only serve as key assets driving our research towards high international standards but also contribute to cost reduction and environmental impact mitigation.

As a hub for aspiring scientists, the Molecular Biotechnology Center places a strong emphasis on supporting the career development and promotion of young group leaders. We provide the resources and mentorship needed for these emerging researchers to thrive, contributing to the advancement of their respective fields. In this vein, in collaboration with the Italian Society of Biophysics and Molecular Biology (SIBBM), the MBC will soon offer in-depth courses on research topics dedicated to young researchers, such as basic molecular biology techniques and advanced optical microscopy.

Our success is reflected in the increasing number of researchers funded by the European Research Council (ERC) choosing to join our ranks, and in the steady rise of publications in top-tier journals such as Nature and Science, which reinforce our position not only in the local but also in the international arena of biomedical, biotechnological research.

The Center also serves as a catalyst for industrial innovation and economic development, actively fostering collaboration with biotechnology and pharmaceutical companies, providing a dynamic environment where academia and industry can converge, accelerating the translation of research findings into practical applications and contributing to the broader goal of advancing science, technology and health care for the benefit of society.

To accommodate our growing community and ambitions, the Molecular Biotechnology Center is expanding physically with the inauguration of new buildings. This expansion not only symbolizes our commitment to facilitating a larger critical mass but also underlines our dedication to fostering the best translational research in the Italian northwest.

In conclusion, the Molecular Biotechnology Center stands as a beacon of innovation, collaboration, and excellence. This booklet highlights the remarkable achievements of our principal investigators and emphasizes the collective impact of our research on the global scientific landscape. As we continue to grow, evolve, and push the boundaries of scientific discovery, we invite you to join us on this exciting journey towards a healthier and sustainable future.

Thank you for your interest in the Molecular Biotechnology Center at the University of Turin.

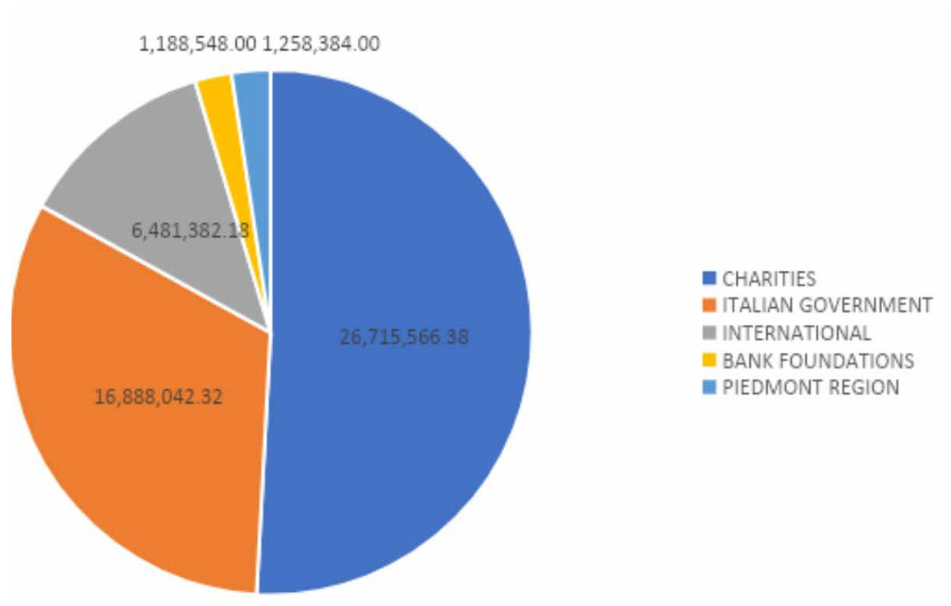
Emilio Hirsch
Director, Molecular Biotechnology Center
University of Turin

Università di Torino

M B C

Molecular Biotechnology Center
Guido Tarone

FUNDING OBTAINED BY RESEARCHERS AT THE MBC IN THE YEARS 2019-2023



MOLECULAR BIOTECHNOLOGY CENTER CORE FACILITIES

Thanks to the advanced instrumentation shared by all Departments, the Molecular Biotechnology Center is able to provide several Core Facilities, which offer high level technical support and cutting-edge skills and technologies. The services are accessible both to members of the University of Turin and to external user groups and companies.

The MBC Core Facilities enhance research efficiency and cost-effectiveness, enhancing the achievement of ambitious research goals.

Currently, the MBC's Core Facilities cover the following areas:

- → The Zebrafish facility
- → Rodent animal model facility
- → Advanced Optical Microscopy - Open Lab of Advanced Microscopy
- → High resolution echocardiography for small rodents
- → Molecular Imaging Center
- → NGS facility
- → Gene Transfer Service
- → Mass spectrometry
- → Separative techniques - High Performance Liquid Chromatography HPLC
- → Bioinformatics and Genomics core-lab (BGcore)
- → Cell Factory for Regenerative Medicine
- → Histology Service Core

The technical component of the center actively collaborates in the research activities of the MBC and the management of the Core Facilities.

The names of the affiliated personnel are listed below:

Research Technicians affiliated within MBC

- → Aigotti Riccardo
- → Alberti Diego
- → Anselmi Francesca
- → Argoub Saaid
- → Arigoni Maddalena
- → Astolfi Monica
- → Azzolino Ornella
- → Balmas Elisa
- → Balzac Fiorella
- → Baroni Simona
- → Bondesan Paola
- → Bonello Lisa
- → Capozza Martina
- → Cena Annamaria
- → Corino Daniele
- → Costamagna Costanzo
- → Cravero Tiziana
- → Di Gaetano Cornelia
- → Donna Daniela
- → Drandi Daniela
- → Durando Giancarlo
- → Gai Marta
- → Genuardi Elisa
- → Krepelova Anna
- → Lamba Simona Elena
- → Logrand Federica
- → Lorenzato Annalisa
- → Marengo Stefano
- → Merighi Irene Fiore
- → Ortolan Erika
- → Patrucco Enrico
- → Rachele Stefania
- → Raouf Abdelmajid
- → Rubinetto Cristina
- → Ruggeri Marina
- → Vallaro Maura
- → Viale Alessandra

IT Technicians affiliated within MBC

- → Margaglione Salvatore Leonardo
- → Lentini Antonio

Index

→ FIORELLA ALTRUDA 12
→ CHIARA AMBROGIO 16
→ ISAIA BARBIERI 20
→ ALBERTO BARDELLI 26
→ ALESSANDRO BERTERO 32
→ ANGELO BIFONE 36
→ MARA BRANCACCIO 42
→ BENEDETTO BRUNO 48
→ ALFREDO BRUSCO 54
→ BENEDETTA BUSSOLATI 58
→ ENZO CALAUTTI 62
→ RAFFAELE A CALOGERO 66
→ CANCER METABOLISM AND CACHEXIA (CAMECA) 70
→ PAOLA CAPPELLO 270
→ GIULIA CARON 76
→ FEDERICA CAVALLO 204
→ DEBORAH CHIABRANDO 80
→ ROBERTO CHIARLE 174
→ LAURA CONTI 208
→ MARTA COSCIA 84
→ CLAUDIA CURCIO 272
→ SILVIA DEAGLIO 88
→ PAOLA DEFILIPPI 94
→ DANIELA DELLI CASTELLI 98
→ ENZA DI GREGORIO 189
→ GIACOMO DONATI 102
→ GIUSEPPE ERMONDI 106
→ SHARMILA FAGOONEE 112
→ GIUSEPPE FERRAUTO 188
→ ADA FUNARO 116
→ ALESSANDRA GHIGO 120
→ ELIANA GIANOLIO 124
→ ANDREA GRAZIANI 128
→ FEDERICO GULLUNI 132
→ EMILIO HIRSCH 136
→ DARIO LIVIO LONGO 142
→ MARINA MARCHISIO CONTE 146
→ MIRIAM MARTINI 150
→ MASSIMO MASSAIA 154
→ CLAUDIO MEDANA 160
→ ALESSIO MENGA 75
→ VALERIA MENCHISE 164
→ GIORGIO ROBERTO MERLO 168
→ MOLECULAR AND TRANSLATIONAL ONCOLOGY 172
→ MOLECULAR IMAGING AND NANOBIOTECHNOLOGIES 182
→ ALESSANDRO MOROTTI 190
→ FRANCESCO NERI 194
→ FRANCESCO NOVELLI 268
→ SALVATORE OLIVIERO 198
→ ONCOIMMUNOLOGY LAB 202
→ ROBERTO PIVA 218
→ VALERIA POLI 222
→ PAOLO E. PORPORATO 74
→ VALENTINA PROSERPIO 226
→ ELENA QUAGLINO 212
→ FRANCESCA REINERI 230
→ CHIARA RIGANTI 234
→ GIUSEPPE SAGLIO 238
→ RICCARDO TAULLI 242
→ DANIELA TAVERNA 246
→ ENZO TERRENO 252
→ EMANUELA TOLOSANO 258
→ TUMOR IMMUNOLOGY LAB 262
→ TIZIANA VAISITTI 274
→ SONJA VISENTIN 280
→ CLAUDIA VOENA 178

photo credit: Pia, Unito, Unsplash

FIORELLA ALTRUDA

Professor Emeritus Molecular Biotechnology Center “Guido Tarone”, University of Torino

BIOGRAPHICAL SKETCH

1994-2022	Full Professor of Molecular Genetics, Department of Molecular Biotechnology and Health Sciences, University of Torino
From 2022	President of the Molecular Biotechnology Center “Guido Tarone”, University of Torino
From 2017	Site Manager of the Cell Factory, University of Torino
Form 2015	Affiliate to the CNR, Istituto di Biostrutture e Bioimmagini, Napoli
2013-2022	Director of the Molecular Biotechnology Center “Guido Tarone”, University of Torino
2012-2016	Member of the Senate of the University of Torino
2013-2020	Member of the board of Director of the Consorzio Italiano Biotecnologie and representative member of the University of Torino

MAIN GROUP MEMBERS
Innovative Cell-Based Therapy for Human Diseases

Sharmila Fagoonee *Researcher IBB CNR*
Giorgia Ammirata *PhD student in Molecolar Medicine*
Valentina Fonsato *Qualified Person Cell Factory*
Chiara Pasquino *Quality Assurance Cell Factory*
Veronica Dimuccio *Production Manager Cell Factory*

EDUCATION AND TRAINING

- November 1976-December 1977: Postdoctoral fellow- Dept. of Molecular Biophysics and Biochemistry, Yale University, USA
- January-April 1981: Visiting fellow- Dept. of Animal Embryology, University of Geneva, Switzerland
- January 1982-December 1982: Postdoctoral fellow, European Molecular Biology Laboratory, Heidelberg, Germany
- October-December 1984: Visiting fellow- European Molecular Biology Laboratory, Heidelberg, Germany
- July-September 1989: Visiting scientist, Scripps Clinic and Research Laboratory, La Jolla, SA

OTHER EXPERIENCE AND PROFESSIONAL MEMBERSHIPS

- From 2022: CEO of EuremAb srl
- From 2021: President of the Incubator 2i3T University of Torino
- From 2021: Member of the Accademia delle Scienze, Torino
- From 2017: Member of the Director Board of DiaSorin S.p.A
- From 2015: President of the Bioindustry Park S.p.A. Colleretto Giacosa (Torino)
- From 2013: Member of the Accademia di Medicina, Torino
- From 2012: Member of the Scientific Board of CentroScienza Foundation

RESEARCH ACTIVITY

Research on murine starch on murine stem cells

During the last 2 decades, there has been a significant interest in exploring the developmental plasticity of pluripotent germ cells. Spermatogonial stem cells have been successfully isolated from both human and murine testis. These pluripotent stem cells, known as germ line cell-derived pluripotent stem cells (GPSCs) have been induced to differentiate into various cell types, including cardiomyocytes and neurons, among others. This exciting potential opens up new avenues for research and holds promise for future applications in regenerative medicine and tissue engineering.

Hepatic differentiation

We successfully generated hepatocytes from mouse GPSCs with high efficiency and thoroughly characterized their functionality. Moreover, we demonstrated that these differentiated cells have the capability to engraft within the mouse liver following partial hepatectomy. This exciting discovery opened up new possibilities for using GPSC-derived hepatocytes in potential regenerative therapies and liver-related research (PMID: 26323094).

Renal differentiation

We also showed that GPSCs can undergo differentiation, resulting in the development of functional renal tubular-like cells in vitro (GTCs). Through an in vivo model of kidney ischemia, our research showed that GTCs offer remarkable protection against both acute and chronic kidney damage. These findings hold significant promise for potential therapeutic applications in treating kidney-related conditions and promoting renal regeneration (PMID: 24136918).

Research on human stem cells - Therapy for human metabolic diseases

Crigler Najjar Syndrome type I (CNSI) is a rare recessive disorder caused by mutations in the Ugt1a1 gene. Unfortunately, there is currently no permanent cure for this condition, and liver transplantation remains the main treatment option despite its limitations. As an alternative approach, stem cell-based therapy holds promise for addressing this disorder. To explore this potential, we conducted experiments using human liver stem cells (HLSC) in immune-compromised NOD SCID Gamma (NSG)/Ugt1-/- mice, which closely resemble the pathological manifestations found in CNSI patients.

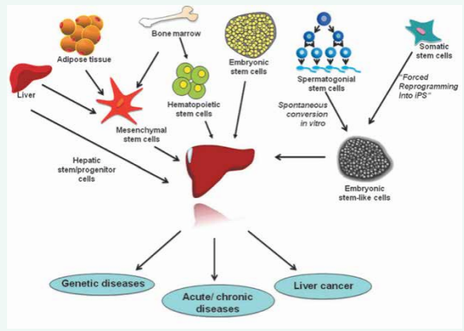


Figure 1.
Various sources of adult stem cells can be differentiated into hepatic-like cells for potential liver disease treatment. Mesenchymal stem cells can be induced to differentiate into hepatocyte-like cells. Additionally, hepatic stem cells and pluripotent stem cells derived from spermatogonial stem cells or reprogrammed somatic cells can also be guided to differentiate into hepatocyte-like cells

We showed that the HLSC-injected mutant mice exhibited remarkable recovery from brain damage in comparison to the controls. These findings provide a compelling proof-of-concept, demonstrating that HLSC therapy can effectively treat CNSI in this animal model. These promising results pave the way for potential therapeutic applications of HLSC-based treatments in patients with CNSI (PMID: 31965023).

FUTURE RESEARCH PLANS

Cancer immunotherapy using chimeric antigen receptor (CAR) T cells is the first approved genetically modified cellular therapy and has become a standard treatment for many hematological malignancies showing great efficacy and safety.

However off-target toxicity has been observed mainly due to organ or tissue damage caused by massive inflammatory citokines release induced by the treatment.

To explore T cell location following the infusion of the modified cells imaging tools will be used in collaboration with the Eurobioimaging team of the MBC. These data could be very important to improve the success of the therapy and to avoid off-target toxicity.

FUNDING ID (PAST 5 YERS)

- Telethon 2017-2018 N° GGP14020 Terapia cellulare per la sindrome di Crigler-Najjar di tipo I con cellule staminali epatiche umane 312.400 euro
- FESR 2021 Regione Piemonte: codice domanda 378-20-Sviluppo di test molecolari su campioni salivari per la diagnosi di SARS-Cov-2 € 508.384,00
- POR FESR 2014/2020 - Bando Piattaforma tecnologica Salute e Benessere – progetto “Terapie avanzate per processi fibritici cronici (EVER)” € 3.527.891
- Erasmus+, Key Action 2, 2019: Cooperation for innovation and the exchange of good practices (Grant Agreement n. 2019-2146/001-001) Modernisation de la formation en Biotechnologie en Tunisie et developpement de l’employabilite des diplomes 72.728,00 euro
- National Project ALISEI: CTN01_00177_88744 460 IRMI: Italian Infrastructure for Regenerative Medicine 500.000 euro
- 2022WFXCWM PRIN 2022 pilot in vitro and in vivo study for molecular characterization of epidermal stem cells and quality and safety assessment of epidermal cultures for combined cell and gene therapy 324.000 euro

SELECTED PUBLICATION

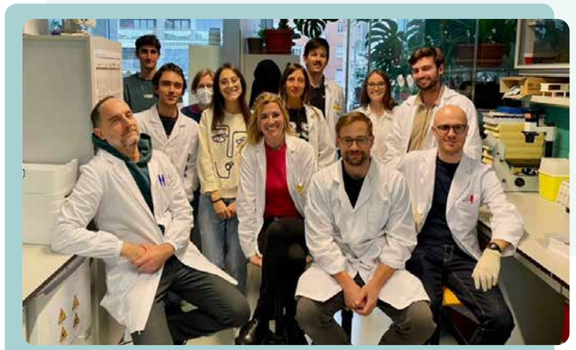
- Fagoonee S, Arigoni M, Manco M, Olivero M, Bizzaro F, Magagnotti C, Andolfo A, Miniscalco B, Forni M, Todeschi S, Tolosano E, Bocchietto E, Calogero R, Altruda F. Circulating Extracellular Vesicles Contain Liver-Derived RNA Species as Indicators of Severe Cholestasis-Induced Early Liver Fibrosis in Mice. Antioxid Redox Signal. 2022 Jan 4. doi: 10.1089/ars.2021.0023.
- Petrillo S, Manco M, Altruda F, Fagoonee S, Tolosano E Liver Sinusoidal Endothelial Cells at the Crossroad of Iron Overload and Liver Fibrosis..Antioxid Redox Signal. 2021 Aug 20;35(6):474-486. doi: 10.1089/ars.2020.8168. Epub 2020 Aug 27
- Petrillo S, Carrà G, Bottino P, Zanotto E, De Santis MC, Margaria JP, Giorgio A, Mandili G, Martini M, Cavallo R, Barberio D, Altruda F A Novel Multiplex qRT-PCR Assay to Detect SARS-CoV-2 Infection: High Sensitivity and Increased Testing Capacity. .Microorganisms. 2020 Jul 17;8(7):1064. doi: 10.3390/microorganisms8071064.
- Famulari ES, Navarro-Tableros V, Herrera Sanchez MB, Bortolussi G, Gai M, Conti L, Silengo L, Tolosano E, Tetta C, Muro AF, Camussi G, Fagoonee S, Altruda F.Human liver stem cells express UGT1A1 and improve phenotype of immunocompromised Crigler Najjar syndrome type I mice. Sci Rep. 2020 21;10(1):887. doi: 10.1038/s41598-020-57820-2.PMID: 31965023
- De Chiara L, Famulari ES, Fagoonee S, van Daalen SKM, Buttiglieri S, Revelli A, Tolosano E, Silengo L, van Pelt AMM, Altruda F.Characterization of Human Mesenchymal Stem Cells Isolated from the Testis. Stem Cells Int. 2018; 2018:4910304. doi:10.1155/2018/4910304.
- Fusella F, Seclì L, Busso E, Krepelova A, Moiso E, Rocca S, Conti L, Annaratone L, Rubinetto C, Mello-Grand M, Singh V, Chiorino G, Silengo L, Altruda F, Turco E, Morotti A, Oliviero S, Castellano I, Cavallo F, Provero P, Tarone G, Brancaccio The IKK/NF-κB signaling pathway requires Morgana to drive breast cancer metastasis. Nat Commun. 2017 Nov 21;8(1):1636. doi: 10.1038/s41467-017-01829-1.
- Fagoonee S, Famulari ES, Silengo L, Camussi G, Altruda F. Prospects for Adult Stem Cells in the Treatment of Liver Diseases. Stem Cells Dev. 2016, 25(20):1471-1482. doi: 10.1089/scd.2016.0144.
- De Chiara L, Fagoonee S, Ranghino A, Bruno S, Camussi G, Tolosano E, Silengo L, Altruda F.J Renal cells from spermatogonial germline stem cells protect against kidney injury. Am Soc Nephrol. 2014 Feb;25(2):316-28. doi: 10.1681/ASN.2013040367.
- Chiabrando D, Marro S, Mercurio S, Giorgi C, Petrillo S, Vinchi F, Fiorito V, Fagoonee S, Camporeale A, Turco E, Merlo GR, Silengo L, Altruda F, Pinton P, Tolosano E. The mitochondrial heme exporter FLVCR1b mediates erythroid differentiation. J Clin Invest. 2012 Dec;122(12):4569-79. doi: 10.1172/JCI62422.
- Hobbs RM, Fagoonee S, Papa A, Webster K, Altruda F, Nishinakamura R, Chai L, Pandolfi PP. Functional antagonism between Sall4 and Plzf defines germline progenitors. Cell Stem Cell. 2012 Mar 2;10(3):284-98. doi: 10.1016/j.stem.2012.02.004.

CHIARA AMBROGIO

Molecular oncology

BIOGRAPHICAL SKETCH

She has worked extensively in the area of tumor initiation processes, translational cancer research and pre-clinical evaluation of novel drugs for the treatment of lung cancer. After training with Mariano Barbacid at the CNIO (2009-2016) and Pasi Janne at the Dana Farber Cancer Institute (2016-2019), she established her Molecular Oncology Laboratory at the Molecular Biotechnology Center (MBC)-Department of Biotechnology and Health Sciences in 2020 (https://ambrogiolab.com) thanks to prestigious grants from the ERC and the Harvard Armenise Foundation.



MOLECULAR ONCOLOGY LAB GROUP MEMBERS

Chiara AMBROGIO *PI*
Enrico PATRUCCO *Lab Director*
Alessia MIRA *Postdoc*
Maximilian KRAMER-DRAUBERG *Postdoc*
Cristina CAFFARRA MALVEZZI *Postdoc*
Rossella SCARDACI *Postdoc*
Ewa BERLINSKA *PhD student*
Sandra VIETTI MICHELINA *PhD student*
Pietro SCAPARONE *PhD student*
Ilenia SAVINELLI *Research Technician*
Haiyun WANG *Visiting Professor*
Ettore PETRINI *undergraduate student*
Riccardo GRIBAUDO *undergraduate student*
Mirco RICCIATO *undergraduate student*
Emilia BERARDELLI *undergraduate student*
Roberto MIGNACCO *undergraduate student*

RESEARCH ACTIVITY

The RAS oncogene family consists of four 21-kDa GTPases isoforms (KRAS4A, KRAS4B, HRAS, and NRAS) that are among the most frequently mutated genes in human cancer. Very recently, two direct KRAS inhibitors targeting the G12C mutations have been approved for clinical treatment with modest results due to rapid development of resistance; moreover, there are no effective therapies to specifically treat cancers expressing KRAS mutations other than G12C (Figure 1). As a consequence, an urgent need remains for innovative therapies to improve outcomes for KRAS mutant cancer patients.

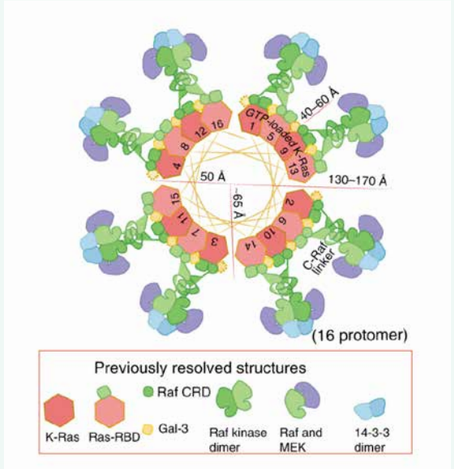


Figure 1.
The KRAS pathway (from Di Federico et al, Current Oncology Reports, 2023)..

One intriguing and still largely unexplored aspect of KRAS biology is the relevance of membrane dynamics for downstream signaling and drug sensitivity. KRAS clustering at the membrane dictates the activation of KRAS signaling. We recently described that KRAS dimerization is essential both for the oncogenic activity of the mutant allele and for the tumor suppressive function of the wild-type allele, but the detailed mechanistic understanding and potential therapeutic value of these findings are still uncovered.

Our laboratory is devoted to the in vitro and in vivo characterization of the “KRAS signalosome”, defined as the functional protein complex engaging KRAS and related effectors, modulators and adaptors at the cell membrane (Figure 2).

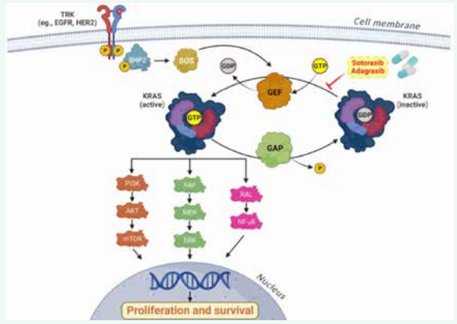


Figure 2.
Schematic representation of the signalosome model with a helical wheel representing the Ras assembly at the center. (from Mysore*, Ambrogio* et al, Nat Struct Mol Biol, 2021).

Future research plans:

In parallel to the projects focused on the characterization of the KRAS signalosome and the role of wild-type KRAS as tumor suppressor, we are actively working on other lines of investigation dealing with understanding of the relevance of RAF kinases localization in cancer cells in vivo, the discovery of therapeutic vulnerabilities of specific KRAS isoforms and the characterization of on-target and off-target mechanisms of resistance to KRAS-G12C inhibitors using PROTAC approaches to degrade KRAS.

FUNDING ID (PAST 5 YERS)

- 2023-2028: MIUR FARE (Italian Ministry of University and Research)
- 2023-2024: SRA (Scientific Research Agreement) Roche2022-2023: Kaerton Foundation-Cancer INNOVA program
- 2022-2027: AIRC (Italian Association for Cancer Research)
- 2021-2022: SRA (Scientific Research Agreement) Verastem
- 2021-2026: ERC Consolidator Grant
- 2021-2023: SRA (Scientific Research Agreement) Revolution Medicines
- 2020-2023: Harvard-Armenise Career Development Grant

SELECTED PUBLICATIONS

- Ambrogio C*, Gómez-López G, Falcone M, Villanueva A, Crosetto N, Blasco R, Sánchez-Céspedes M, Ren X, Wang Z, Ding K, Serrano M, Hidalgo M, Santamaría D*, Barbacid M*. Combined inhibition of Ddr1 and Notch signaling as an effective therapeutic strategy for K-Ras-driven/p53-null lung adenocarcinomas. Nat Med. 2016 Feb 8. *Co-corresponding author.
- Ambrogio C*, Barbacid M, Santamaría D*. In vivo oncogenic conflict triggered by co-existing KRAS and EGFR activating mutations in lung adenocarcinoma. Oncogene. 2016 Oct 24. *Co-corresponding author.
- Nieto P, Ambrogio C, de Esteban L, Gómez-López G, Blasco MT, Yao Z, Marais R, Rosen N, Chiarle R, Pisano DG, Barbacid M, Santamaría D. A B-Raf kinase inactive mutant induces lung adenocarcinoma. Nature. 2017 Aug 2.
- Ambrogio C*, Köhler J, Zhou ZW, Wang H, Paranal R, Li J, Capelletti M, Caffarra C, Li S, Lv Q, Gondi S, Hunter JC, Lu J, Chiarle R, Santamaría D, Westover KD, Jänne PA*. KRAS dimerization impacts MEK inhibitor sensitivity and oncogenic activity of mutant KRAS. Cell. 2018 Jan 4. *Co-corresponding author.
- Wang H, Lv Q, Xu Y, Cai Z, Zheng J, Chen X, Dai Y, Jänne PA, Ambrogio C* and Köhler J*. An integrative pharmacogenomics analysis identifies CK2 alpha as a promising therapeutic target in KRAS(G12C) mutant lung cancer. EBioMedicine. 2019 Nov;49:106-117. doi: 10.1016/j.ebiom.2019.10.012. Epub 2019 Oct 23. *Co-last author.
- Zhou Z*, Ambrogio C*, Bera AK, Li Q, Li X, Li L, Son J, Gondi S, Li J, Campbell E, Jin H, Okoro JJ, Xu C, Jänne PA, Westover KD. KRASQ61H signals through MAPK in a RAF dimer-dependent manner in non-small cell lung cancer. Cancer Res. 2020 Jun 30. *equally contributed.
- Nokin MJ, Darbo E, Travert C, Drogat B, Lacouture A, San José S, Cabrera N, Turcq B, Prouzet-Mauleon V, Falcone M, Villanueva A, Wang H, Herfs M, Mosteiro M, Jänne PA, Pujol JL, Maraver A, Barbacid M, Nadal E, Santamaría D and Ambrogio C. Inhibition of DDR1 enhances in vivo chemosensitivity in KRAS-mutant lung adenocarcinoma. JCI Insight. 2020 Aug 6.
- Kramer-Drauberg M and Ambrogio C. Discoveries in the redox regulation of KRAS. The International Journal of Biochemistry & Cell Biology, 2020 Dec 10.
- Mysore VP*, Zhou Z*, Ambrogio C*, Wang Q, Okoro J, Jänne PA, Westover KD, Shan Y, Shaw DE. Structural model of a Ras-Raf signalosome. Nat Struct Mol Biol. 2021 Oct;28(10):847-857. doi: 10.1038/s41594-021-00667-6. Epub 2021 Oct 8. *equally contributed.
- Ricciuti B, Son J, Okoro JJ, Wang X, Paranal R, Wang H, Eum Y, Lin M, Haikala HM, Li J, Xu Y, Alessi JV, Chhoeu C, Mira A, Patrucco E, Redig AJ, Köhler J, Richard E, Nokin MJ, Santamaria D, Gokhale PC, Awad MM, Jänne PA, Ambrogio C. Comparative analysis and isoform-specific therapeutic vulnerabilities of KRAS mutations in non-small cell lung cancer. Clinical Cancer Research, 2022 January.

ISAIA BARBIERI

Laboratory of cancer epistranscriptomics

BIOGRAPHICAL SKETCH

2022-present	AIRC Start-up Research Group leader, Molecular Biotechnology Center, University of Turin.
2018- 2022	Junior research group leader, funded by CRUK Cambridge centre, Department of Pathology, University of Cambridge.
2017- 2018	Post-Doctoral research associate funded by the Kay Kendall Leukaemia Fund. The Gurdon Institute, University of Cambridge.
2013-2016	Post-Doctoral research associate funded by Leukaemia & Lymphoma research, The Gurdon Institute, University of Cambridge.
2012-2013	Post-Doctoral research associate funded by CRUK, The Gurdon Institute, University of Cambridge.
2008-2012	Ph. D. in Molecular Medicine, University of Turin, Italy.

MAIN GROUP MEMBERS

Dr Roberta Chiavetta *post-doctoral research associate*
Dr Giorgio Cinque *post-doctoral research associate*
Niccoló Chiapasco *Biotechnology master student*
Domenico Ignoti *Biotechnology master student*
Maxim Bouvet *Biotechnology master student*

RESEARCH ACTIVITY

The discovery of chemical modifications of histone proteins and DNA brought the field of epigenetics to the forefront, highlighting their impact on gene expression. Epigenetic marks are orchestrated by three classes of proteins: writers, erasers, and readers and are ultimately responsible for cell type specification during development and its maintenance in adult individuals. It is therefore unsurprising that many epigenetic modifications are dysregulated in multiple cancer types.

Similarly, more than 100 different types of post-synthesis modifications have been discovered on RNA. All four RNA bases and the ribose sugar can be targets for these modifications (Figure 1). Ribosomal RNA (rRNA) and transfer RNA (tRNA) are notably the most extensively modified. It is worth noting that most research efforts have historically been centred on protein and DNA modifications, leaving modifications of RNA largely unexplored.

The excitement surrounding this field is fuelled by the untapped biological insights related to the modifications themselves and their therapeutic potential. Multiple lines of evidence now suggest that the dysregulation of epitranscriptomic pathways plays a role in the development of human diseases, including cancer. Consequently, several biotechnology companies have emerged with the goal of identifying pharmacological agents that target RNA epigenetic pathways.

In our laboratory, we are characterizing RNA methyltransferases as potential targets in haematological malignancies and solid tumours. Our focus lies in both uncovering new biologically significant functions with clinical relevance and identifying new epitranscriptomic molecular mechanisms. Specifically, we are keen on exploring the functional interplay between epitranscriptomic and traditional epigenetic mechanisms.

Our ultimate goal is to develop small molecule inhibitors targeting RNA enzymes catalytic activity and develop new potential therapeutic approaches for cancer treatment.

Currently, the laboratory is dedicated to two primary lines of research:

1. Targeting the m6A pathway in ALK-driven Anaplastic Large Cell lymphoma.

Recently the m6A RNA modification pathway was implicated in several pathological contexts, including cancer (Figure 2). In particular, several studies showed that the m6A writers METTL3 and METTL16 are essential for the maintenance of Acute Myeloid Leukaemia (Figure 3). These finding sparked the interests in the generation of small molecule inhibitors targeting the m6A pathway.

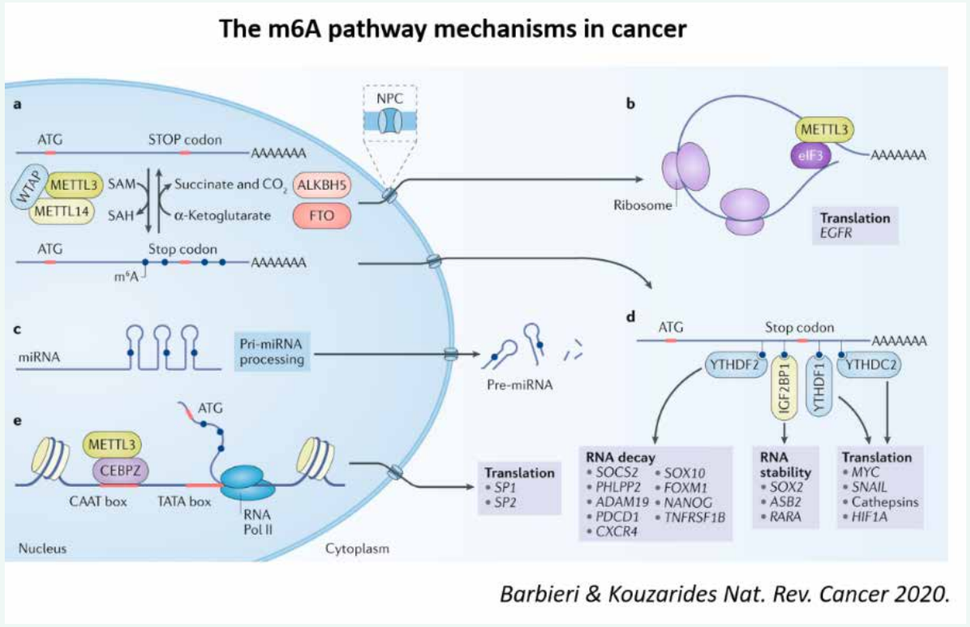
We are testing the effects of both genetic inactivation of METTL3 and small molecule METTL3 inhibitors in ALK-driven Anaplastic large cell lymphoma (ALCL) and characterizing the molecular mechanisms responsible for their effects. Our interest sparked from the observation that ALK signalling enhance the m6A machinery through upregulating the transcription of METTL3.

We are testing newly developed METTL3 inhibitors as a potential strategy to inhibit ALK+ ALCL cell proliferation both in vitro and in vivo. We are studying their effects both alone and in combination with the ALK-inhibitor Crizotinib in different ALCL cell lines and patient-derived xenograft cells grown in vitro. Additionally, we are investigating the potential use of METTL3 inhibitors as a new approach to overcome primary and/or acquired Crizotinib resistance in both cellular models of acquired resistance and in patient-derived xenografts (PDX) established from pediatric Crizotinib-resistant lymphoma samples.

2. Characterize the role of the mRNA methyltransferase TGS1 in cancer.

We identified Tgs1 as one of the top targets required for the proliferation of RN2C mouse acute myeloid leukemia cells through a dropout CRISPR-CAS9 screening approach Figure 3). This enzyme is responsible for converting 7-methylguanosine 5’-cap (m7G) into 2,2,7 trimethylguanosine (m2,2,7G) on specific cellular RNAs.

High levels of TGS1 correlate with a poor prognosis in Aggressive childhood leukemia and TGS1 exhibits high expression in AML compared to other cancer types. TGS1



Barbieri & Kouzarides Nat. Rev. Cancer 2020.

Figure 1.

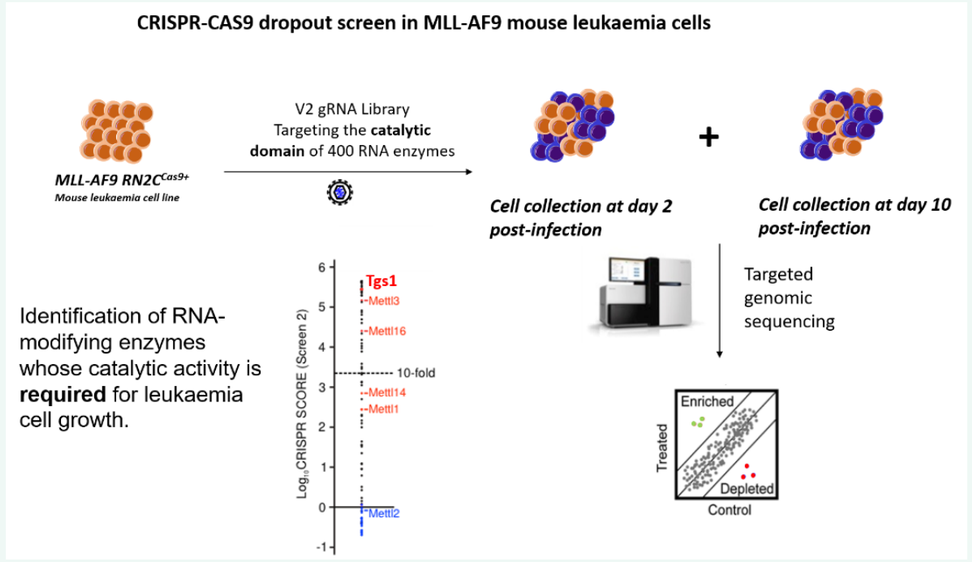


Figure 2.

is responsible for di-methylating the 5' cap of small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA), thereby regulating their nuclear localization. Additionally, we discoverd that, in acute myeloid leukaemia cells, TGS1 can modify the cap of a specific group of 500 mRNAs encoding specifically for proteins involved in cellular metabolism such as selenoproteins and mitochondrial factors. Mechanistically the m2,2,7G cap modification increases the translational rate of these crucial metabolic factors.

RNA-seq experiment in two TGS1-depleted AML cell lines and revealed that TGS1 silencing induces the reshaping of cellular oxygen metabolism pathways, as indicated by gene ontology analysis. This observation supports the involvement of selenoproteins and mitochondrial proteins, which primarily function is protecting against oxidative stress and regulating oxygen metabolism, respectively.

Crucially, TGS1 is overexpressed in numerous cancer types. In the future we intend to explore the function of this enzyme and the m2,2,7G modification in these cancer types, with a particular focus on its impact on metabolic processes.

Future perspectives

In the next few years we aim to develop the current projects and move our findings towards more translational models such as patient derived xenograft and mouse models, both in vitro and in vivo. Apart from this, we aim further identify and characterize epitranscriptomic factors in cancer. In particular, we will focus on performing CRISPR-CAS9 synthetic lethality screens, in the context of drug resistance, and screens to identify RNA enzymes involved in tumour progression mechanisms such as extravasation, angiogenesis and metastasis. Our findings will shed light on both epitranscriptomic mechanisms and their therapeutic potential in cancer.

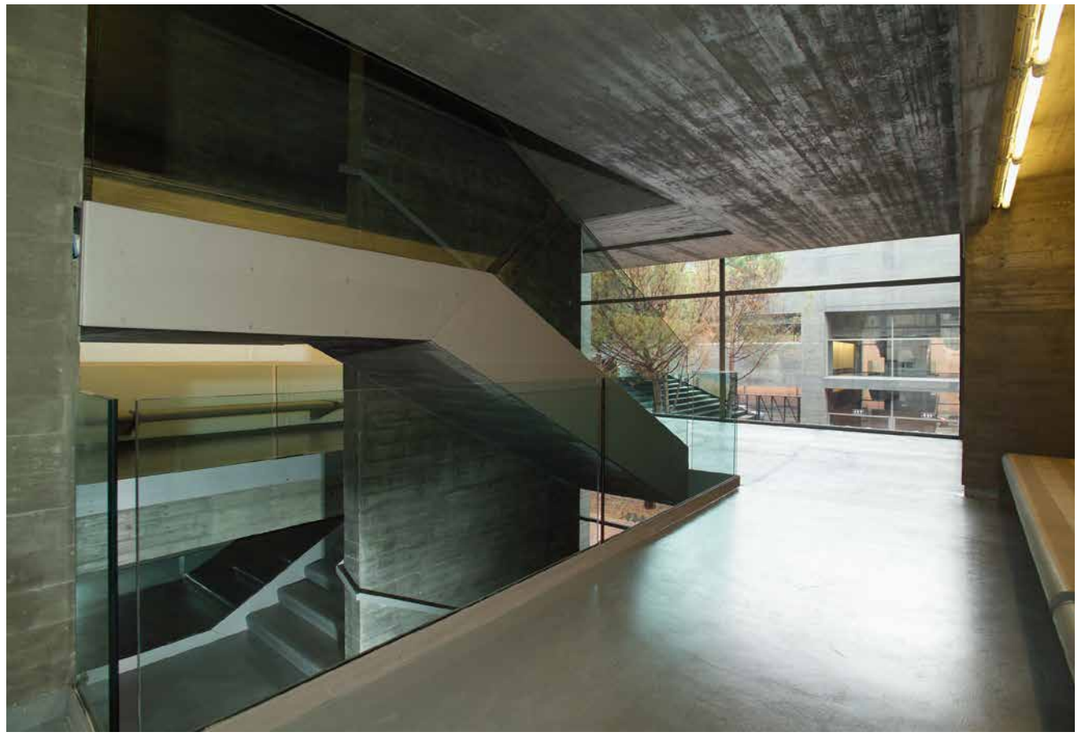
FUNDING ID

- → 2022-2027 "Investigating RNA methylation in cancer" AIRC Start-Up grant, Italy
- → 2021-2022 "METTL3 inhibition as a novel therapeutic approach in ALK-driven ALCL both sensitive and resistant to ALK inhibition" Little princess trust project grant (UK)
- → 2019-2020 "RNA methylation in SDH deficient Gastrointestinal stromal tumours"Gist support UK (GSUK)/ Pathological society UK
- → 2018-2022 "RNA methylation in Paediatric cancers" Start-up package Cancer research UK Cambridge centre, paediatric program. (UK)
- → 2017-2020 "Targeting m6A RNA methylation in AML." The Kay Kendall Leukaemia Fund. (UK)

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=57193984639

- Dunsmore L et al., Controlled masking and targeted release of redox-cycling ortho-quinones via a C–C bond-cleaving 1,6-elimination. Nature Chemistry volume 14, pages754–765 (2022) doi: 10.1038/s41557-022-00964-7
- Leger A, Amaral PP, et al., RNA modifications detection by comparative Nanopore direct RNA sequencing. Nat Commun. 2021 Dec 10;12(1):7198. doi: 10.1038/s41467-021-27393-3.
- Miano V, Codino A, Pandolfini P and Barbieri I., The non-coding epitranscriptome in cancer. Briefings in Functional Genomics, Volume 20, Issue 2, March 2021, Pages 94–105, doi.org/10.1093/bfgp/elab003
- Prokoph N, et al., IL10RA Modulates Crizotinib Sensitivity in NPM1-ALK-positive Anaplastic Large Cell Lymphoma. Blood (2020) 136 (14): 1657–1669. doi: 10.1182/blood.2019003793.
- Barbieri I & Kouzarides T. , Role of RNA modifications in cancer. Nat Rev Cancer 20, 303–322 (2020). doi.org/10.1038/s41568-020-0253-2
- Pandolfini L, Barbieri I et al., METTL1 Promotes let-MicroRNA Processing via m7G Methylation, Mol Cell. 2019 Jun 20;74(6):1278-1290.e9. doi: 10.1016/j.molcel.2019.03.040.
- Tzelepis K, et al., SRPK1 maintains acute myeloid leukemia through effects on isoform usage of epigenetic regulators including BRD4. Nat Commun. 2018 Dec 19;9(1):5378. doi: 10.1038/s41467-018-07620-0.
- Barbieri I, Tzelepis K, Pandolfini L, et al., Promoter-bound METTL3 maintains myeloid leukaemia via m6A-dependent translation control. Nature. 2017 Dec 7;552(7683):126-131. doi: 10.1038/nature24678
- Wyspianska BS, Bannister AJ, Barbieri I et al., BET protein inhibition shows efficacy against JAK2V617F-driven neoplasms. Leukemia. 2014 Jan;28(1):88-97. doi: 10.1038/leu.2013.234.
- Barbieri I, Pensa S, et al., Constitutively active Stat3 enhances Neu-mediated migration and metastasis in mammary tumours via upregulation of Cten. Cancer Research, 2010 Mar 15;70(6):2558-67. doi: 10.1158/0008-5472.CAN-09-2840



ALBERTO BARDELLI

Genomics of Cancer and Targeted Therapies

BIOGRAPHICAL SKETCH

Since 2016	Full Professor, Dept. of Oncology, University of Torino, Italy.
Since 2022	Scientific Director, IFOM ETS – The AIRC Institute of Molecular Oncology.
2004-2022	Director, Laboratory of Molecular Oncology, Candiolo Cancer Institude IRCCS.
2021-pres.	Scientific Advisory Board member, Oncode Institute, Utrecht (The Netherlands).
2021	ERC Advanced Grant
2020	Guido Venosta Award, FIRC AIRC, Presidenza della Repubblica Italiana.
2019-pres.	The Johns Hopkins Society of Scholars, Baltimore (USA)
2018-pres.	Scientific Advisory Board member, MD Anderson Moon Shots Program, Houston (USA)
2018-pres.	Scientific Advisory Board member, Neophore, Cambridge (UK).
2018-2020	President, EACR (European Association for Cancer Research)
2017-pres.	Scientific Advisory Board member, Cancer Research UK Manchester Institute, Manchester (UK)
2017	European Society for Medical Oncology (ESMO) Translational Research Award
2017-pres.	Fellow of European Molecular Biology Organization (EMBO)
2016	Grant for Oncology Innovation Research Project
2015-pres.	Board Member, European Association for Cancer Research (EACR)
2015	Fellow of the Turin Academy of Sciences
2013-pres.	Scientific Committee, Pezcoller Foundation
2013-pres.	Scientific Committee, AIRC (Italian Association for Cancer Research)
2004	The Alfred Blalock Research Award - Johns Hopkins Medical School, Baltimore USA
1999-2004	Postdoctoral fellow, The Johns Hopkins University; Baltimore MD USA. Supervisor: Prof. Bert Vogelstein.
1996-1999	Postdoctoral Fellow, IRCC and the University of Torino, School of Medicine
1991-1996	PhD degree in Biochemistry and Molecular Biology, University College London. Supervisor: Prof. M. Waterfield

MAIN GROUP MEMBERS

Mariangela Russo *Assistant Professor and co-PI*
Giovanni Germano *Assistant Professor*
Elisa Mariella, Alberto Sogari *Postoctoral Fellows*
Gaia Grasso, Martina Miotto *PhD students*
Mariachiara Mammone *Post Graduate fellows*
Simona Elena Lamba, Annalisa Lorenzato *Research Technicians*
Julie Bonetto, Anna Micaletto, Emma Tassanelli, Asia Calà *Students*
Simona Destefanis *Adminstrative Staff:*

Group Members at IFOM ETS - The AIRC Institute of Molecular Oncology:

Vito Amodio *Postoctoral Fellows*
Sharon Scardellato, Eleonora Piumatti, Pietro Paolo Vitiello, Federico Lazzarini, Vittorio Battaglieri, Paolo Battuello, Gianluca Mauri, Giorgio Patelli *PhD students*
Giovanni Crisafulli, Rosaria Chilà *Staff Scientists*

RESEARCH ACTIVITY

Colorectal (CRC) cancer is the second worldwide cancer leading cause of death. Treatment of metastatic colorectal cancer (mCRC) has improved over the last 15 years since the introduction of EGFR-targeted therapy, antiangiogenic agents, and the use of intensive triplet chemotherapy regimens based on fluoropyrimidines, oxaliplatin, and irinotecan.

Our research is focused specifically on precision oncology for CRC, in particular on the characterization of tumor heterogeneity and mechanisms of tumor evolution during therapy administration, with the final aim to identify novel vulnerabilities and therapeutic strategies to prevent or delay the onset of resistance, thus improving survival of cancer patients.

By using CRC 2D cell lines, 3D patients-derived organoids (PDO) and xenopatients (PDX) we defined the mechanisms of primary and secondary resistance to targeted therapies, including how metastatic CRC escape from EGFR, BRAF, TRK, and HER2 inhibition (Fig. 1). Our preclinical findings defining the effectiveness of combinatorial EGFR-BRAF therapy led to practice changes of the clinical guidelines for mCRC carrying BRAF mutations (https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-encorafenib-combination-cetuximab-metastatic-colorectal-cancer-braf-v600e-mutation) (Fig. 1).

We developed new therapeutic approaches for CRC patients which led to clinical trials in which our laboratory was actively engaged. The HERACLES (https://clinicaltrials.gov/ct2/show/NCT00490841) and ARETHUSA (https://clinicaltrials.gov/ct2/show/NCT03519412) trials are paradigmatic example (Fig. 1).

Our studies in the liquid biopsies field demonstrated that analysis of circulating tumor DNA (ctDNA) can detect mechanisms of resistance to targeted therapies in mCRC and can be used for the clinical follow up of patients. The clinical trial CHRONOS (https://clinicaltrials.gov/ct2/show/NCT03227926) represents the first time interventional liquid biopsy to direct EGFR therapy in CRC patients (Fig. 1).

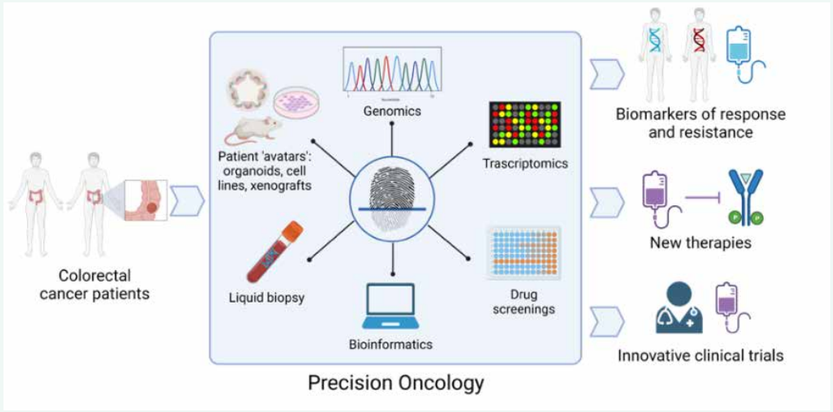


Figure 1.

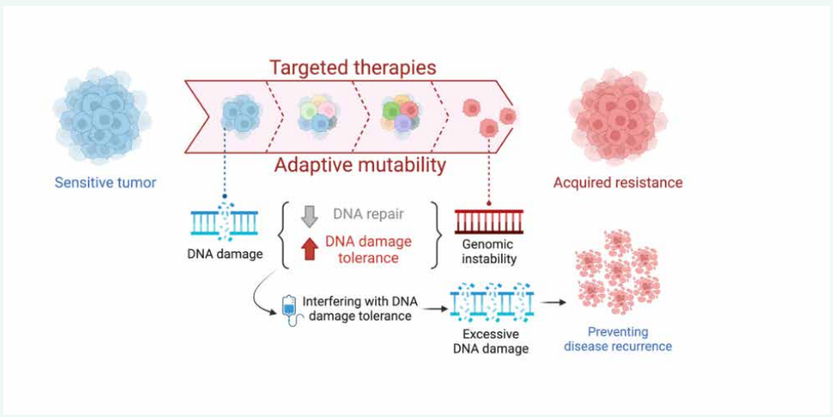


Figure 2.

We recently unveiled that cancer cells, alike bacteria in response to antibiotic stress, adaptively down-modulate DNA mismatch repair (MMR) and homologous recombination (HR) proteins, and switch to an error prone-mediated DNA replication process in presence of increased DNA damage when exposed to targeted therapy (Fig. 2). This adaptive mutability stress-response, in turn, leads to genetic instability and a transient increase of the mutation rate of surviving cancer persister cells (Fig.2), thus favoring the probability that mutations conferring fitness advantage in the presence of the drug (i.e., conferring drug resistance) eventually emerge (Fig. 2).

Additionally, immune checkpoint inhibitors have been shown to induce durable responses in a subset of approximately 5% patients with mCRC that carry defective mismatch repair (MMRd) or are microsatellite unstable (MSI). We discovered that inactivation of MMR genes in microsatellite stable (MSS) immune refractory CRCs leads to immune surveillance and response to immune therapy, and proposed that this could be pursued for therapeutic purposes (Fig. 3). These results led to the ongoing clinial trial ARETHUSA.

On the same line, aiming at converting MSS non-immunogenic “cold” CRC to immunogenic “hot” tumors sensitive to immunotherapy, we generated preclinical models of heterogeneous tumors in which cold components (mismatch repair proficient, MMRp) and hot counterparts (mismatch repair deficient, MMRd) coexist in the same shared microenvironment. We observed that the modulation of tumor composition by pharmacological enrichment of MMRd component could enhance immune surveillance towards heterogeneous murine tumors (Fig. 3).

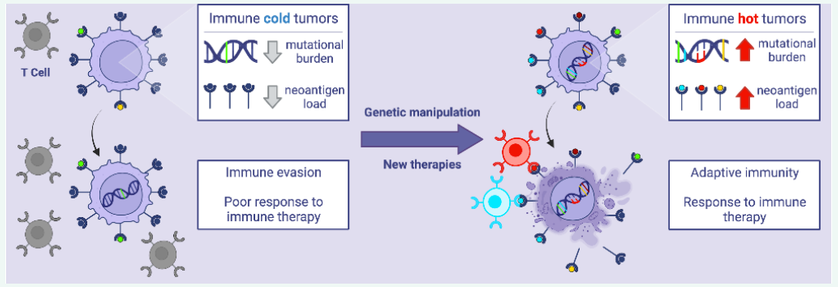


Figure 3.

Future research plans

The evidence that target therapies induce DNA damage in cancer cells and that, cancer cells switch from oncogenic dependencies to DNA damage tolerance (DDT) dependency, in order to create a permissive milieu for the emergence of mutations, uncover new vulnerabilities that could be therapeutically exploit. We plan to use molecular, pharmacological and CRISPR-CAS9 genetic screenings to characterize markers and features of cancer persister cells and to investigate the role of DNA Damage Response (DDR) in the evolution of tumor recurrence aiming at identifying new therapeutic regimens for CRC patients. Additioanally, our future goals are based on the discovery and validation of new targets and therapeutic regimens that are able to increase the immunogenicity of otherwise “cold” and immune refractory CRCs.

FUNDING ID (PAST 5 YERS)

→ 2021 PI, Funder European Commission ERC-2020-ADG. Proposal no.: 101020342: “TARGET: Targeting DNA repair pathways, sparking”.
→ 2021 Participant, Funder-European Commission and European Federation of Pharmaceutical Industries and Associations. H2020-JTI-IMI2-2020-20 Tumour Plasticity: “PERSIST-SEQ: Building a reproducible single-cell experimental workflow to capture tumour drug persistence”.
→ 2018 Principal Investigator, Funder-AIRC-Italian Association for Cancer Research. Special Program 5 per mille Metastases Project n. 21091: “Insights into the evolving heterogeneity of metastatic colorectal cancer: from mechanisms to therapies”.
→ 2019 Principal Investigator, Funder-AIRC-Italian Association for Cancer Research. AIRC IG2018 Project n. 21923: “Inactivation of DNA repair to improve cancer immune surveillance”.
→ 2018 Participant, Funder-AIRC - CRUK - FC AECC. Accelerator Award Project n. 22795: “ACRCelerate: Colorectal Cancer Stratified Medicine Network”.
→ 2018 Participant, Funder-Ministero dell’Istruzione, dell’Università e della Ricerca. PON ARS01_00492: “Biopsie liquide per la Gestione Clinica dei Tumori”.

SELECTED PUBLICATIONS

- Amodio V, et al., and Bardelli A. Genetic and pharmacological modulation of DNA mismatch repair heterogeneous tumors promotes immune surveillance. Cancer Cell. 2023 Jan;41(1):196-209. doi: 10.1016/j.ccell.2022.12.003, https://www.scopus.com/record/display.uri?eid=2-s2.0-85145997702&origin=resultslist
- Sartore-Bianchi A, et al., and Bardelli A. Circulating tumor DNA to guide rechallenge with panitumumab in metastatic colorectal cancer: the phase 2 CHRONOS trial. Nat Med. 2022 Aug;28(8):1612-1618. doi: 10.1038/s41591-022-01886-0 https://www.scopus.com/record/display.uri?eid=2-s2.0-85135266993&origin=resultslist
- Crisafulli G, et al., and Bardelli A. Temozolomide treatment alters mismatch repair and boosts mutational burden in tumor and blood of colorectal cancer patients. Cancer Discov. 2022 Jul 6;12(7):1656-1675. doi: 10.1158/2159-8290.CD-21-1434 https://www.scopus.com/record/display.uri?eid=2-s2.0-85131820295&origin=resultslist
- Di Nicolantonio F, et al, and Bardelli A Precision oncology in metastatic colorectal cancer - from biology to medicine. Nat Rev Clin Oncol. 2021 Aug;18(8):506-525 doi: 10.1038/s41571-021-00495-z https://www.scopus.com/record/display.uri?eid=2-s2.0-85104762834&origin=resultslist
- Amodio V, et al., and Bardelli A*, Misale S* (* Shared last authorship) EGFR blockade reverts resistance to KRAS G12C inhibition in colorectal cancer. Cancer Discov. 2020 Aug;10(8):1129-1139 doi: 10.1158/2159-8290.CD-20-0187 https://www.scopus.com/record/display.uri?eid=2-s2.0-85088308512&origin=resultslist
- Russo M, et al., and Bardelli A. Adaptive mutability of colorectal cancers in response to targeted therapies. Science. 2019 Dec 20;366(6472):1473-1480bdoi: 10.1126/science.aav4474 https://www.scopus.com/record/display.uri?eid=2-s2.0-85077106191&origin=resultslist
- Siravegna G, et al., and Bardelli A Radiologic and genomic evolution of individual metastases during HER2 blockade in colorectal cancer. Cancer Cell. 2018 Jul 9;34(1):148-162 doi: 10.1016/j.ccell.2018.06.004 https://www.scopus.com/record/display.uri?eid=2-s2.0-85048975846&origin=resultslist
- Germano G, et al, and Bardelli A. Inactivation of DNA repair triggers neoantigen generation and impairs tumor growth. Nature. 2017 Dec 7;552(7683):116-120 doi: 10.1038/nature24673 https://www.scopus.com/record/display.uri?eid=2-s2.0-85037841016&origin=resultslist
- Misale Set al., and Bardelli A. Emergence of KRAS mutations and acquired resistance to anti EGFR therapy in colorectal cancer Nature. 2012 Jun 28;486(7404):532-6 doi: 10.1038/nature11156 https://www.scopus.com/record/display.uri?eid=2-s2.0-84862999938&origin=resultslist
- Bardelli A, et al., Mutational Analysis of the Tyrosine Kinome in Colorectal Cancers . Science. 2003 May 9;300(5621):949 doi: 10.1126/science.1082596 https://www.scopus.com/record/display.uri?eid=2-s2.0-0038670241&origin=resultslist

ALESSANDRO BERTERO

HEART ENGINEERING & DEVELOPMENTAL GENOMICS

BIOGRAPHICAL SKETCH

Alessandro Bertero is a group leader at the Molecular Biotechnology Center of the University of Turin, and an Associate Professor in the Department of Molecular Biotechnology and Health Sciences. He began his training with the late Guido Tarone at the University of Turin in Italy, where he investigated the Melusin signalling pathway in cardiac hypertrophy and obtained a BSci (2009) and an MSci (2011). Having being awarded a British Heart Foundation Graduate Fellowship, he moved to the University of Cambridge in the UK. Working with Ludovic Vallier he obtained an MRes (2012) and a PhD (2016) by studying the mechanisms by which TGF beta signalling controls early differentiation of human pluripotent stem cells. Alessandro performed his postdoctoral training with Chuck Murry at the University of Washington in the US with the support of an EMBO Long-Term Fellowship (2017). During this time he determined the role of three dimensional chromatin organization dynamics during human cardiogenesis and in inherited dilated cardiomyopathy. Alessandro launched his group at the UW Institute for Stem Cell and Regenerative Medicine in 2019. In 2021, he relocated the lab to his alma mater in Italy thanks to the Giovanni Armenise-Harvard Foundation Career Development Award. He now leads the Armenise-Harvard Laboratory of Heart Engineering & Developmental Genomics et al (HEDGe lab).

HEART ENGINEERING & DEVELOPMENTAL GENOMICS ET AL. (HEDGe lab)

Kirsten Snijders *PhD, Postdoc*
Łukasz Truszkowski *PhD, Postdoc*
Federica Sozza *MSci, PhD Student*
Sveva Bottini *MSci, PhD Student*
Giulia Savorè *MSci, PhD Student*
Silvia Becca *MSci, PhD Student*
Maria Luisa Ratto *MSci, PhD Student*
Michelle Guichardaz *BSci Master Student*
Martina Terenzi *BSci Master student*
Martina Arricale *Bachelor Student*
Sara Bianchi *Bachelor Student*

RESEARCH ACTIVITY

Our long term vision is improving human sustainable wellbeing. We work to achieve this goal through the integrative application of stem cell biology, gene editing, genomics, and bioengineering to: (1) elucidate the genetic underpinnings of cardiac disease, the #1 killer worldwide; (2) develop regenerative medicine therapy for congenital heart disease, the most common life-threatening malformation in newborns; and, last but not least, (3) provide a cell-based alternative to factory farming, the main cause of biodiversity loss and a central contributor to climate change. These seemingly distinct aspects are actually deeply interconnected: elucidating the gene regulatory mechanisms behind cardiac development and disease provides the knowledge needed to develop cells and tissues for heart remuscularization, which in turn can be produced in even larger scale from animal cells for human consumption. Overall, our work has the potential to improve human life on earth from a holistic perspective: cradle to table and all the way to rocking chair.

Our key achievements to date in these three major areas include: (1) elucidating the role of cis and trans nuclear architecture dynamics during both normal heart development and in the pathogenesis of inherited disease (Bertero et al, Nat Commun 2019; Bertero et al JCB 2019); (2) reducing the arrhythmogenic risk of cardiac remuscularization with human pluripotent stem cell-derived cardiomyocytes (patent pending; collaboration between the UW Heart Regeneration Program and Sana Biotechnology); and (3) developing a technology to efficiently and cheaply reprogram pluripotent stem cells into differentiated progenies (patent WO2018096343A1), enabling applications both in the drug screening and cell therapy fields (i.e. bit.bio), and in cellular agriculture (i.e. Meatable).

Our current focus is the role of three-dimensional chromatin organization in heart development and disease. We are also building novel genetic tools to probe the structure-function relationship of chromatin compartmentalization. Our work mainly relies on genetic engineering with CRISPR/Cas9, differentiation of human pluripotent stem cells into cardiomyocytes (hPSC-CMs), generation of 3D-engineered heart tissues,determination of nuclear architecture and function with genomic and imaging assays, and analysis of cardiac physiology.

Why the et al.? We are immensely worried about the future of our planet in the face of rapidly advancing climate and ecological disasters due to unsustainable human activities. Besides striving for a more sustainable way to do research (i.e recycle and reuse) and travel (i.e. minimize and carbon offset emissions), we place what know-how we have to the service of climate defense. In this light, we wish to try helping combat climate change through cellular agriculture.

SOURCES OF FUNDING:

- → 2023 – 28 ERC Starting Grant (PI) | European Research Council
- → 2023 – 25 PRIN PNRR 2022 & PRIN 2022 (PI & co-PI) | Ministry for University and Research, IT
- → 2022 – 25 FEBS Excellence Award (PI) | Federation of European Biochemical Societies
- → 2022 – 25 AV Single Ventricle Research Fund (PI) | Additional Ventures, USA
- → 2021 – 26 GAHF Career Development Award (PI) | Giovanni Armenise-Harvard Foundation, USA
- → 2020 – 24 NIH R01 (co-I then sub-awardee) | National Institutes of Health, USA

SELECTED PUBLICATIONS:

(*co-first; #co-senior; @correspondence; Bertero lab members)

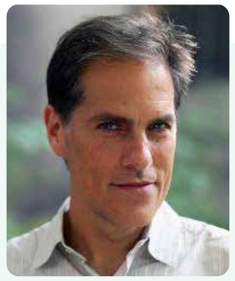
- Marchiano S, … [26 authors], Bertero A#, Murry CE#,@ (2023). Gene editing to prevent ventricular arrhythmias associated with cardiomyocyte cell therapy. Cell Stem Cell 30, 396-414
- Balmas E, Sozza F, Bottini S, Ratto ML, Savorè G, Becca S, Snijders KE#, Bertero A#,@ (2023). Manipulating and studying gene function in human pluripotent stem cell models (review). FEBS Letters doi:10.1002/1873-3468.14709
- Bottini S*, Fuoco C*, Schiavo N*, Bertero A#,@, Biressi S#,@, Conti L#,@, Gargioli C#,@ (2023). A call for an 'Asilomar' for cultivated meat and seafood (perspective). Nature Biotechnology doi:10.1038/s41587-023-01849-x
- Bertero A, … [10 authors], Murry CE@ (2019). Chromatin compartment dynamics in a haploinsufficient model of cardiac laminopathy. Journal of Cell Biology 218, 2919-44
- Bertero A*, Fields PA*, ... [7 authors], Murry CE@ (2019). Dynamics of genome reorganization during human cardiogenesis reveal an RBM20-dependent splicing factory. Nature Communications 10, 1538
- Bertero A*, Brown S*, .. [14 authors], Vallier L@ (2018). The SMAD2/3 interactome reveals that TGFβ controls m6A mRNA methylation in pluripotency. Nature 555, 256–9
- Bertero A*,@, Pawlowski M*, Ortmann D*, … [15 authors], Vallier L@ (2016). Optimized inducible shRNA and CRISPR/Cas9 platforms for in vitro studies of human development using hPSCs. Development 143, 4405–18

PATENTS:

- Murry CE, Marchianò S, Reinecke H, Bertero A (17th June 2021). Electrophysiological modification to suppress arrhythmias . International Patent Application: PCT/US2022/027382. Assignee: University of Washington
- Vallier L, Kotter M, Pawlowski M, Bertero A, Ortmann D (24th November 2016). Controllable transcription. European Patent: EP3545079B1; US Patent: US11697823B2. Assignee: Cambridge Enterprise Limited

ANGELO BIFONE

Neurobioimaging Lab



BIOGRAPHICAL SKETCH

Master of Science (MSc) in Physics from the University of Rome “La Sapienza,” a PhD in Physics from Scuola Normale Superiore in Pisa, and a Master in Business Administration (MBA) from the Business School of the University of Bologna.

My career has encompassed roles in both academia and industry R&D. I started my professional journey at the University of Berkeley in California and subsequently at the University of Leiden in the Netherlands, as a Postdoctoral researcher. I served as a Lecturer at the Institute of Cancer Research in the UK from 1996 to 2001, where I attained the status of Teacher at the University of London. Subsequently, I founded and led for nine years the Neuroimaging Department at the Medicines Research Center of Glaxo Smith-Kline, a prominent research-based pharmaceutical company located in Verona, Italy, and Harlow, UK. In 2010, I joined the Istituto Italiano di Tecnologia, where I assumed roles of increasing responsibility. These positions included serving as the Director of the Center for Nanotechnology Innovation in Pisa and later as the Director of the Center for Neuroscience and Cognitive Systems in Rovereto (Tn).

Since 2019, I am Full Professor of Physics as Applied to Medicine and Biology at the University of Torino.

MAIN GROUP MEMBERS

Eleonora Cavallari *RTD-A*
Federico Gorrini *PostDoc*
Anna Macula *PhD*

RESEARCH ACTIVITY

Our research activity is focused primarily on the development of biomedical imaging techniques, with an emphasis on Magnetic Resonance Imaging (MRI) and its application to the neurosciences. We strive to improve sensitivity, resolution and specificity of MRI, and to push the boundaries of this technique as a diagnostic and research tool to study brain structure and function.

A distinctive aspect of our research is the use of imaging agents and advanced image-analysis methods to improve functional MRI, a method to map hemodynamic responses in the brain as a surrogate for changes in the underlying neuronal activity. Typically, fMRI relies on an intrinsic contrast mechanism, the Blood Oxygen Level-Dependent (BOLD) effect, which yields relatively modest MR signal changes, often in the range of just a few percent, at best. The use of exogenous agents can dramatically increase the sensitivity of fMRI, thus improving spatial resolution and making it possible to resolve the brain functional architecture at a finer level. We have extensively applied these methods, dubbed phMRI, in models of human neurological and psychiatric disease, like autism and schizophrenia, with the goal to identify imaging endophenotypes (i.e., biological markers of disease traits) and functional markers of response to treatment. PhMRI has proven particularly impactful in the drug discovery process, providing a powerful translational tool to accelerate progression of novel therapeutic agents towards the clinic.

More recently, we have explored the combination of MR Imaging and transgenic technology to study the neurophysiological basis of brain connectivity and its derangements in neurological and psychiatric disease, with a particular focus on drug addiction. Techniques like chemo-genetics can be used to reversible modulate the activity and connectivity of specific brain circuits, thus making it possible to test causal relationships between aberrant connectivity pathways and behavior. In combination with imaging methods, this approach has contributed to elucidating the key role of specific regions and circuits, like the Anterior Insular Cortex and the Salience Network, in the dependence on alcohol, heroin and other addictive substances in murine models (Fig.1). The use of MRI, a translational approach par excellence, has been instrumental to validate preclinical findings in humans, where the same circuits appear to play an homologous role (Fig.2). The notable implications of this research encompass the identification of new therapeutic targets and methodologies, among them Transcranial Magnetic Stimulation (TMS).

On a more foundational level, our focus lies in the exploration of innovative imaging techniques and agents, positioning our group at the forefront of a dynamically evolving field. Presently, we are engaged in research concerning the utilization of nanodiamonds and other nanostructured materials as imaging probes, as well as their application in hyperpolarizing nuclear spins for the production of hyperpolarized agents for in vivo cellular and tissue imaging.

Lastly, we recognize the transformative role that Artificial Intelligence is poised to assume in the fields of radiology and bioimaging. With its unparalleled ability to process extensive datasets and discern subtle patterns, AI holds the potential to dramatically enhance the efficacy and efficiency of contrast agents in diagnostic imaging. By way of example, we have recently demonstrated the ability of AI, in combination with Gadolinium-based contrast agents, to increase detectability of small, low-enhancing lesions (Fig. 3), thus improving sensitivity and diagnostic power. We are committed to explore the synergy between AI and contrast agents to revolutionize diagnostic imaging and improve patient outcomes.

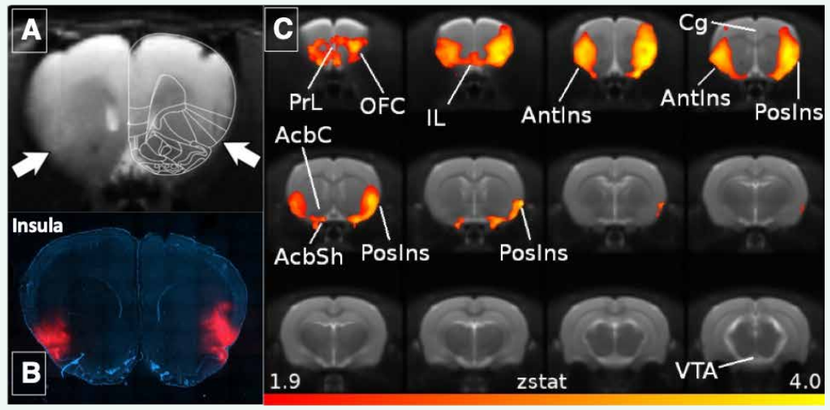


Figure 1.
(A) Anatomical detail of the rat brain, indicating the location of the Anterior Insular Cortex (AIC); (B) expression of artificial receptors in the AIC that focally and reversibly activate or inhibit neuronal activity upon administration of a non-endogenous ligand; (C) functional MRI of the patterns of downstream activation triggered by chemo-genetic stimulation of the Anterior Insula. Behaviorally, stimulation of the AIC resulted in a strong reduction in drug-seeking and -taking behaviors in animal models of Alcohol Use Disorder. Adapted from Haaranen et al., 2020.

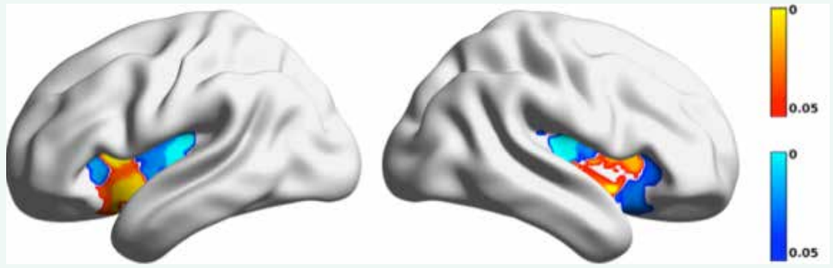


Figure 2.
Activity and connectivity of the Anterior Insular Cortex, as measured by MRI, showed significant differences between healthy subjects and Alcohol Use Disorder (AUD) patients. The Anterior Insula represent the gateway of the interoceptive effects of drugs, and these data demonstrate an excessively integrative role of this brain area in controlling drug seeking behaviors in AUD. Adapted from Bordier et al., 2022.

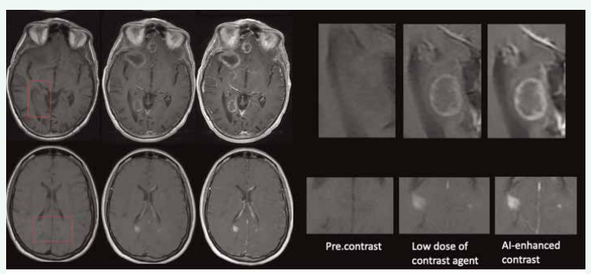


Figure 3.
Enhanced detection of small and weakly enhancing lesions through AI-driven contrast augmentation. Adapted from Fringuello-Mingo, 2023

FUTURE RESEARCH PLANS:

Studying the early stages of neurodevelopment presents a considerable challenge. Recently, we have made significant advancements by using MRI in conjunction with activity-sensitive contrast agents to map brain function in chick embryos in ovo. This study has produced the first direct evidence that brain lateralization is influenced by exposure to sensory stimuli already at the embryonic level, a mechanism that could be relevant for all vertebrates, including humans. While these findings are preliminary, they pave the way to tackling numerous unresolved issues in the field of developmental biology. We will leverage these methods to investigate the neurochemical factors underpinning both typical and atypical brain development. Moreover, we will deploy novel molecular imaging techniques to unravel the metabolic pathways involved at different stages of development, as well as the metabolic mechanisms that support rapid changes in brain activity induced by sensory or pharmacological stimuli. Ultimately, we intend to apply these groundbreaking methodologies to explore and validate pharmacological approaches aimed at rescuing aberrant brain connectivity and miswiring, as observed in various neurodevelopmental disorders.

FUNDING ID (PAST 5 YERS)

- → POC NODES 2023 – Spoke 2 Green and sustainable technologies: ROSEWATER (Reduction of contrast agents in wastewater), Principal Investigator (starting December 2023).
- → EC Horizon 2020: GA 8581492, FET-OPEN AlternativesToGd (Alternatives to Gadolinium) 1/10/2019 - 30/9/2023, Principal Investigator.
- → EC Horizon 2020: GA 76642, ITN- ZULF (Zero and Ultra-Low Field MRI), 1/1/2018 - 30/6/2022, Principal Investigator.
- → CRT Foundation: RF 2019.0610. Alcohol dependence and brain connectivity, 1/1/2020 - 30/6/2022, Principal Investigator.
- → EC Horizon 2020: GA 668863, SyBill-AA “Systems Biology of Alcohol Addiction: Modeling and validating disease state networks in human and animal brains for understanding pathophysiolgy, predicting outcomes and improving therapy”, 1/1/2016 - 31/12/2019, Principal Investigator

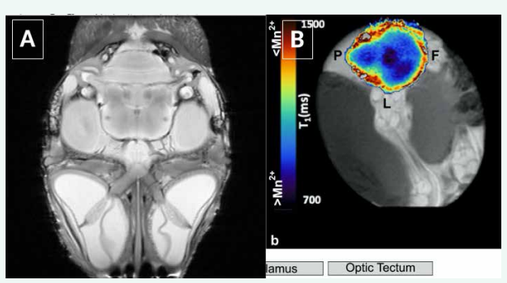
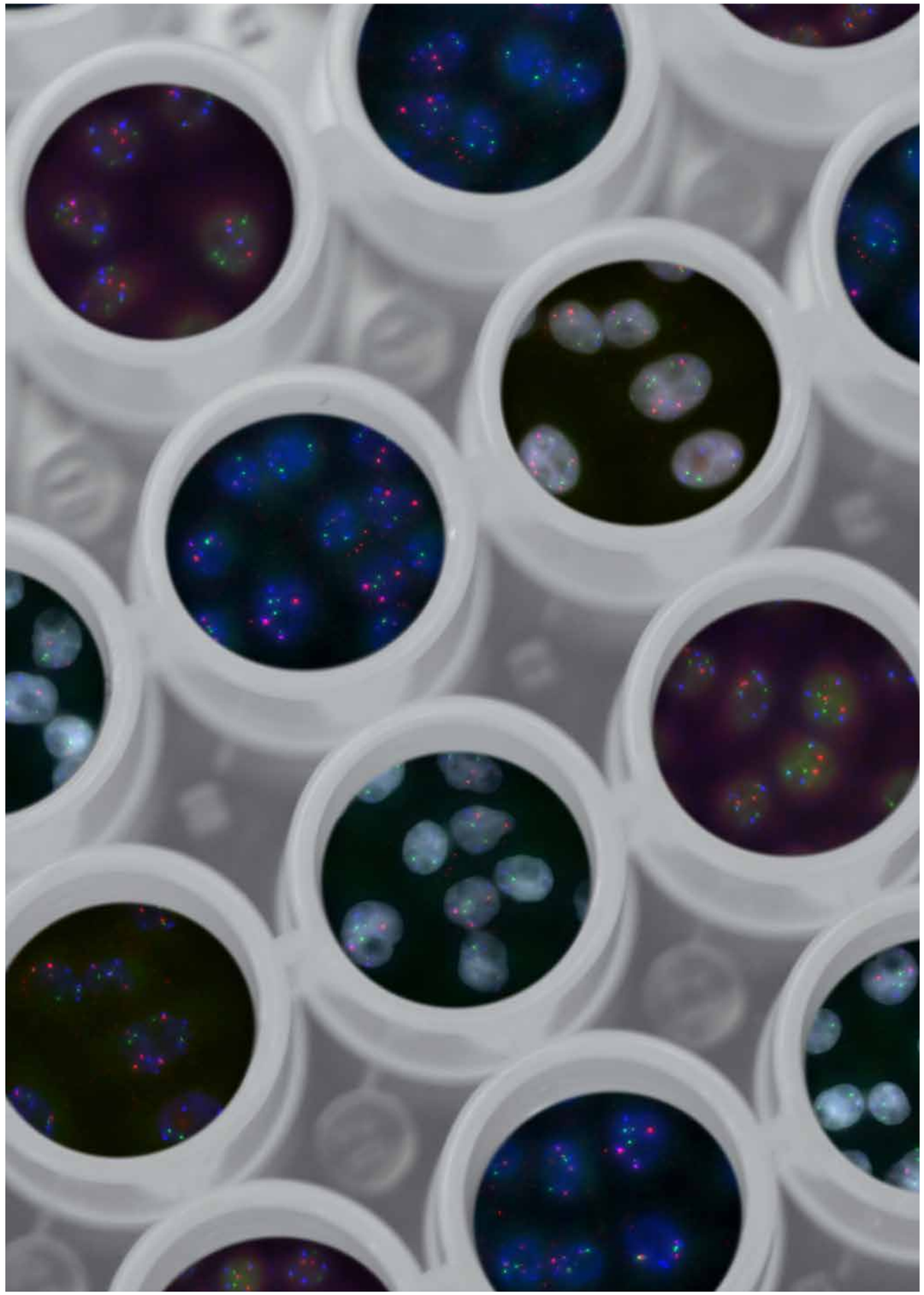


Figure 4.
A) Anatomy of the visual system in the chick’s brain; the optic nerves and chiasm are clearly visible, showing almost complete decussation of the nerve fibers. (B) Brain activity induced by light stimulation mapped in ovo by Manganese Enhanced Magnetic Resonance Imaging. Light stimulation through the egg-shell results in lateralization of the visual system already at an embryonic stage of development. Adapted from Lorenzi et al., 2023.

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=7004669829

- → “Manganese Enhanced Magnetic Resonance Imaging reveals light-induced brain asymmetry in embryo” E. Lorenzi, S. Tambalo, G. Vallortigara, and A. Bifone eLife 12, e86116 (2023) https://doi.org/10.7554/eLife.86116
- → “Amplifying the effects of Contrast Agents on Magnetic Resonance Images using a Deep Learning method trained on synthetic data”A. Fringuello Mingo et al., Investigative Radiology (2023) doi.org/10.1097/RLI.0000000000000998
- → “Increased network centrality of the anterior insula in early abstinence from alcohol”C. Bordier, G. Weil, P. Bach, G. Scuppa, C. Nicolini, G. Forcellini, U. Pérez-Ramirez, D. Moratal, S. Canals, S. Hoffmann, D. Hermann, S. Vollstädt-Klein, F. Kiefer, P. Kirsch, W.H. Sommer, and A. Bifone Addiction Biology e13096, doi.org/10.1111/adb.13096, 27(1) e13096 (2022) (featured on the cover) https://doi.org/10.1111/adb.13096
- → “Anterior insula stimulation suppresses appetitive behavior while inducing forebrain activation in alcohol-preferring rats” M. Haaranen et al., Translational Psychiatry 10:150, 1-11 (2020) https://doi.org/10.1038/s41398-020-0833-7
- → “Aberrant insular cortex connectivity in abstinent alcohol-dependent rats is reversed by dopamine D3 receptor blockade” G. Scuppa, S. Tambalo, S. Pfarr, W.H. Sommer, and A. Bifone Addiction Biology 25(3), e12744 (2020) https://doi.org/10.1111/adb.12744
- → “Fast and sensitive detection of paramagnetic species using coupled charge and spin dynamics in strongly fluorescent nanodiamonds” F. Gorrini, R. Giri, C.E. Avalos, S. Tambalo, P. Marzola, A. Miotello, and A. Bifone ACS Applied Materials and Interfaces 11:27, 24412-24422 (2019) https://doi.org/10.1021/acsami.9b05779
- → “phMRI, neurochemical and behavioral responses to psychostimulants distinguishing genetically selected alcohol-preferring from genetically heterogenous rats” A. Bifone, A. Gozzi, A. Cippitelli, A. Matzeu, E. Domi, H. Li, G. Scuppa, N. Cannella, M. Ubaldi, F. Weiss, R. Ciccocioppo Addiction Biology 24(5):981-993 (2019) https://doi.org/10.1111/adb.12671
- → Disrupted modular organization of primary sensory brain areas in schizophrenia” C. Bordier, C. Nicolini, G. Forcellini and A. Bifone NeuroImage: Clinical 18, 682-693 (2018) doi.org/10.1016/j.nicl.2018.02.035
- → “Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior” Y. Zhan et al., Nature Neuroscience 17(3), 400-4006 (2014) https://doi.org/10.1038/nn.3641
- → Distributed BOLD and CBV-weighted resting-state networks in the mouse brain F Sforazzini, AJ Schwarz, A Galbusera, A Bifone, A Gozzi Neuroimage 87, 403-415 (2014) https://doi.org/10.1016/j.neuroimage.2013.09.050



MARA BRANCACCIO

CHAPERON PROTEINS STRESS RESPONSE

BIOGRAPHICAL SKETCH

2023-present	Professor of Cell Biology in the Department of Molecular Biotechnology and Health Sciences, University of Torino
2006-2023	Associate Professor of Cell Biology in the Department of Molecular Biotechnology and Health Sciences, University of Torino
2002-2006	Assistant Professor at the Department of Genetics, Biology and Biochemistry, Faculty of Medicine, University of Torino
2000-2002	Research fellow at the Department of Genetics, Biology and Biochemistry Faculty of Medicine, University of Torino
2000	PhD in “Human Biology: molecular and cellular basis”
1996-1997	Harvard Medical School, Massachusetts General Hospital (Boston), USA

MAIN GROUP MEMBERS

Matteo Sorge *Post-doc*
Pietro Poggio *phD student*
Francesca Zuppini *phD student*
Davide Acquarone *phD student*
Lucia Renzullo *phD student*
Francesca Tornatore *MD student*

My research interests are focused on chaperones, a class of proteins involved in the recovery from stress conditions. As a visiting scientist at the Harvard University, I discovered the protein Melusin as a new interactor for the beta1 integrin in the myocardium (1). Further investigations revealed that Melusin is a muscle specific chaperone protein with the remarkable ability to protect the myocardium against pressure overload, myocardial infarction and ischemia reperfusion injury (2,3). My research journey continued with the cloning and characterization of Melusin paralog in vertebrates, which I named Morgana (4). The subsequent research revealed that the expression of Morgana is essential for the development of both drosophila and mouse (4). Intriguingly, we also found that alteration in Morgana expression, both through haploinsufficiency (5,6) and overexpression, are implicated in the onset and progression of tumors (7,8,9,10).

RESEARCH ACTIVITY

The heat shock response (HSR) is an ancient and universal mechanism for preserving homeostasis and enhancing cell viability during stressful circumstances. This mechanism has played a vital role in the evolution and adaptation of organisms.

My primary research interests are focused on chaperone proteins, pivotal players in the HSR, upregulated in response to various types of stress and responsible for facing the emergency imposed by changes in environmental conditions. Chaperone proteins are also often essential for routine cellular maintenance tasks, acting as machineries for protein folding and the formation of supramolecular complexes.

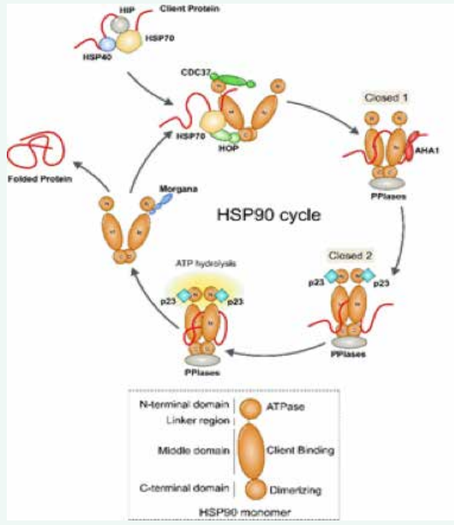


Figure 1.
The intracellular HSP90 cycle. The figure highlights the role of co-chaperones that participate to the HSP90 intracellular machinery and that have been also found to be part of extracellular HSP90 complexes.

Chaperone proteins in cancer growth and progressio

Cancer cells experience various stresses in the tumor environment and they depend on a network of chaperone proteins to ensure their survival and to facilitate their adaptive responses. Chaperone inhibitors can be effective in causing damage and death to cancer cells, but they may also harm normal cells, as chaperones also serve essential housekeeping functions.

In addition to upregulate chaperone proteins, cancer cells release them in the extracellular milieu. Among these chaperones, Hsp90 is secreted by numerous cancer cells and human tumors. In the extracellular milieu its binds to various co-chaperones, forming complexes and machineries with specific tasks. These complexes often play a role in supporting tumor progression by aiding the chaperoning of extracellular client proteins and by enhancing cell survival and motility through interactions with surface receptors. However, it is important to note that extracellular HSP90 (eHSP90) also promotes anti-tumor immune response. By targeting specific co-chaperones, it becomes possible to separate the pro-tumor activity of eHSP90 from its ability to trigger anti-cancer immunity. In line with this approach, we generated an antibody that specifically targets the eHSP90 co-chaperone Morgana. This antibody is capable of disrupting the formation of complexes that induce cancer cell migration. In preclinical trials, treatment with this antibody has demonstrated the ability to inhibit metastasis and reduce tumor growth by promoting anti-cancer immunity (Figure 2).

Chaperone proteins in heart function

The rhythmic contraction of the heart is a result of the efficient performance of sarcomere structures, which are intricate assemblies of numerous proteins held together by non-covalent bonds. Chaperone proteins are responsible for ensuring the quality control and turnover of sarcomere proteins, thereby preserving their efficiency and enabling adaptation. Among these, Melusin stands out as a unique chaperone, expressed selectively in skeletal and cardiac muscles and acting as a mechanical stretch sensor. Melusin exerts its protective function through its interaction with the cytoplasmic region of beta 1 integrin, subsequently activating intracellular signaling pathways

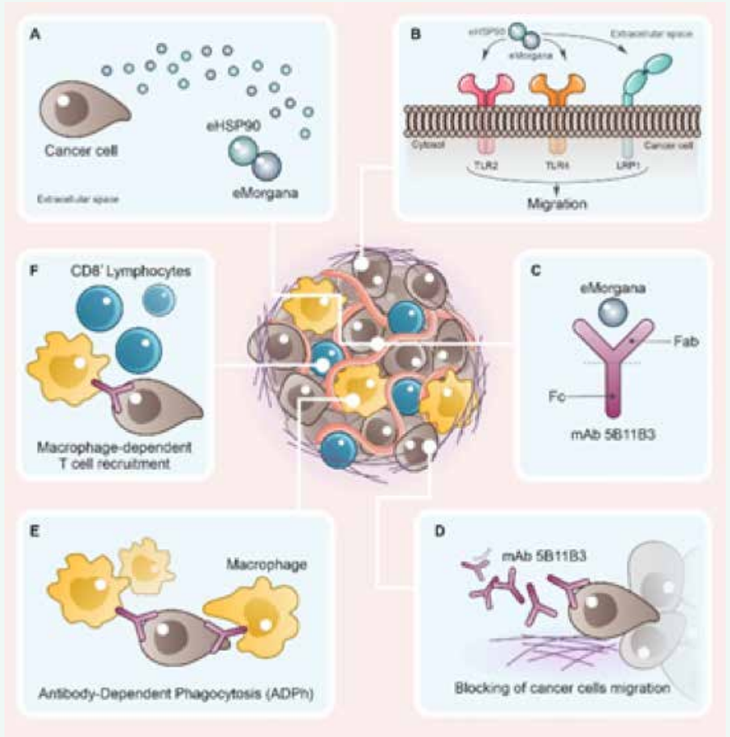


Figure 2.
The extracellular chaperone Morgana promotes cancer cell migration and targeting Morgana with a mAb inhibits tumor growth by inducing antibody-dependent phagocytosis and macrophage-dependent recruitment of CD8+ T lymphocytes. (Seclì L, et al., Cancer Res. 2021 Sep 15;81(18):4794-4807. doi: 10.1158/0008-5472.CAN-20-3150. PMID: 34193441.)

such as MAPK and PI3K/AKT, thus leading to cardiomyocyte hypertrophy and enhanced cell survival. Indeed, Melusin overexpression in cardiomyocytes has shown promising results in improving myocardial function under various stress conditions, including pressure overload, myocardial infarction, and ischemia/reperfusion injury. Understanding the underlying molecular mechanisms and evaluating the potential of Melusin-based gene therapy approaches holds promise for the treatment of cardiomyopathy.

Skeletal muscle

Skeletal muscle, the largest organ in our body, consists of thousands of muscle fibers that come together to form muscles. The efficiency of muscle activity, similar to the heart, relies on the continuous and meticulous maintenance of sarcomeres. Muscle homeostasis depends on a delicate balance between protein synthesis, assembly and degradation. Various factors, such as physical activity, nutrient availability, aging, and stressful situations like inflammatory diseases or cancer, influence these processes. When trophic pathways are activated through exercises or growth factors, protein synthesis surpasses degradation, resulting in muscle hypertrophy, the increase in muscle size. On the other hand, muscle atrophy occurs when the protein degradation machinery disassembles sarcomeres in response to physical inactivity or specific signals. Muscle atrophy leads to a reduction in muscle mass and strength. Melusin has a relevant role in protecting muscle tissue from atrophy. Our research aims to explore the molecular mechanisms and to elucidate its role in regulating trophic and atrophic pathways.

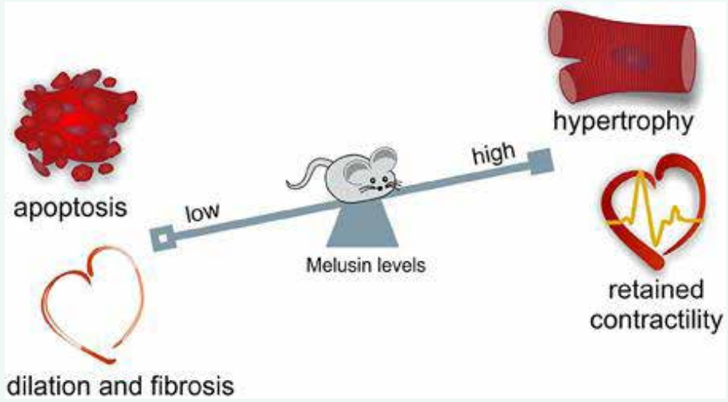


Figure 3.
Melusin protects the heart from “maladaptive remodeling” during mechanical stress.

Stress into the wild

Alpine ecosystems are particularly vulnerable to the effects of climate change due to their unique and fragile nature. Rising temperatures, altered precipitation patterns, and changing snow cover dynamics profoundly impact the flora and fauna of these regions. Alpine organisms face the challenge of either adapting to the changing conditions or seeking alternative habitats to survive. Heat shock proteins play a crucial role in cellular stress responses and are known to be highly responsive to environmental changes. By monitoring the expression levels of HSPs, we can gain insights into the stress levels experienced by alpine organisms. We are setting methods to track the changes in HSP expression over time and perform longitudinal studies.

FUTURE RESEARCH PLANS

Our current research focuses on the characterization of extracellular chaperone complexes and of their roles in cancer growth and progression to design in innovative treatments. We are also actively involved in the identification of the indispensable house-keeping role of Morgana in mammals. Moreover, we are engaged in studying chaperone function in myocardial resilience and in skeletal muscle atrophy. Through a collaboration with Prof. Alessandro Bertero’s group, we will utilize 3D-engineered tissues generated from human induced pluripotent stem cell (iPSCs) derived cardiomyocytes and skeletal muscle cells as innovative tools.

FUNDING ID (PAST 5 YERS)

- → 2021-2026: “Targeting extracellular HSP90 complexes to fight cancer progression”, AIRC IG 2020 24930, PI, 607,000,
- → 2018-2022: Digital Technology For Lung Cancer Treatment (DEFLeCT), Piedmont Region, Head of research unit, €70.000.
- → 2017-2019: “Linking cardiac metabolism to inflammation”, Compagnia di San Paolo and University of Turin, PI, €70,800.
- → 2016-2020: “Characterization of adaptive or maladaptive influences of innate immune system on cardiac hypertrophic remodeling in response to pressure overload”, Ministry of University and Research Head PRIN 2015, head of research unit, €50,000.

SELECTED PUBLICATIONS

- Brancaccio M, Guazzone S, Menini N, Sibona E, Hirsch E, De Andrea M, Rocchi M, Altruda F, Tarone G, Silengo L. Melusin is a new muscle-specific interactor for beta(1) integrin cytoplasmic domain. J Biol Chem. 1999 Oct 8;274(41):29282-8. doi: 10.1074/jbc.274.41.29282. PMID: 10506186
- Brancaccio M, Fratta L, Notte A, Hirsch E, Poulet R, Guazzone S, De Acetis M, Vecchione C, Marino G, Altruda F, Silengo L, Tarone G, Lembo G. Melusin, a muscle-specific integrin beta1-interacting protein, is required to prevent cardiac failure in response to chronic pressure overload. Nat Med. 2003 Jan;9(1):68-75. doi: 10.1038/nm805. Epub 2002 Dec 23. PMID: 1249695
- Tarone G, Brancaccio M. The muscle-specific chaperone protein melusin is a potent cardioprotective agent. Basic Res Cardiol. 2015 Mar;110(2):10. doi: 10.1007/s00395-015-0466-9. Epub 2015 Feb 5. PMID: 25653116.
- Ferretti R, Palumbo V, Di Savino A, Velasco S, Sbroggiò M, Sportoletti P, Micale L, Turco E, Silengo L, Palumbo G, Hirsch E, Teruya-Feldstein J, Bonaccorsi S, Pandolfi PP, Gatti M, Tarone G, Brancaccio M. Morgana/chp-1, a ROCK inhibitor involved in centrosome duplication and tumorigenesis. Dev Cell. 2010 Mar 16;18(3):486-95. doi: 10.1016/j.devcel.2009.12.020. PMID: 20230755.
- Di Savino A, Panuzzo C, Rocca S, Familiari U, Piazza R, Crivellaro S, Carrà G, Ferretti R, Fusella F, Giugliano E, Camporeale A, Franco I, Miniscalco B, Cutrin JC, Turco E, Silengo L, Hirsch E, Rege-Cambrin G, Gambacorti-Passerini C, Pandolfi PP, Papotti M, Saglio G, Tarone G, Morotti A, Brancaccio M. Morgana acts as an oncosuppressor in chronic myeloid leukemia. Blood. 2015 Apr 2;125(14):2245-53. doi: 10.1182/blood-2014-05-575001. Epub 2015 Feb 12. PMID: 25678499.
- Morotti A, Rocca S, Carrà G, Saglio G, Brancaccio M. Modeling myeloproliferative neoplasms: From mutations to mouse models and back again. Blood Rev. 2017 May;31(3):139-150. doi: 10.1016/j.blre.2016.11.004. Epub 2016 Nov 24. PMID: 27899218.
- Fusella F, Ferretti R, Recupero D, Rocca S, Di Savino A, Tornillo G, Silengo L, Turco E, Cabodi S, Provero P, Pandolfi PP, Sapino A, Tarone G, Brancaccio M. Morgana acts as a proto-oncogene through inhibition of a ROCK-PTEN pathway. J Pathol. 2014 Oct;234(2):152-63. doi: 10.1002/path.4341. Epub 2014 Aug 6. PMID: 24615293.
- Fusella F, Seclì L, Busso E, Krepelova A, Moiso E, Rocca S, Conti L, Annaratone L, Rubinetto C, Mello-Grand M, Singh V, Chiorino G, Silengo L, Altruda F, Turco E, Morotti A, Oliviero S, Castellano I, Cavallo F, Provero P, Tarone G, Brancaccio M. The IKK/NF-κB signaling pathway requires Morgana to drive breast cancer metastasis. Nat Commun. 2017 Nov 21;8(1):1636. doi: 10.1038/s41467-017-01829-1. PMID: 29158506; PMCID: PMC5696377.
- Fusella F, Seclì L, Cannata C, Brancaccio M. The one thousand and one chaperones of the NF-κB pathway. Cell Mol Life Sci. 2020 Jun;77(12):2275-2288. doi: 10.1007/s00018-019-03402-z. Epub 2019 Dec 6. PMID: 31811308.
- Seclì L, Avalle L, Poggio P, Fragale G, Cannata C, Conti L, Iannucci A, Carrà G, Rubinetto C, Miniscalco B, Hirsch E, Poli V, Morotti A, De Andrea M, Turco E, Cavallo F, Fusella F, Brancaccio M. Targeting the Extracellular HSP90 Co-Chaperone Morgana Inhibits Cancer Cell Migration and Promotes Anticancer Immunity. Cancer Res. 2021 Sep 15;81(18):4794-4807. doi: 10.1158/0008-5472.CAN-20-3150. Epub 2021 Jun 30. PMID: 34193441.

BENEDETTO BRUNO

Transplant Biology - Laboratory of Clinical and Translational Research in Hematology

BIOGRAPHICAL SKETCH

2022 to date	Director, Division of Hematology and Cell Therapy Unit, Univ. of Torino, AOU Città della Salute e della Scienza di Torino, Professor of Hematology, Dpt. of Molecular Biotechnology and Health Sciences, School of Medicine, University of Torino, Torino, Italy
2021-2022	Myeloma Transplant and Immune and Cell Therapy (Program Coordinator) – NYU Grossman School of Medicine - New York, NY
2020-2022	Professor of Medicine (Clinical) - NYU Grossman School of Medicine New York, NY
2014-2020	Associate Professor of Hematology, University of Torino
2013-2020	Program Head, Bone Marrow Transplantation Unit (CIC-231- To01), Azienda Ospedaliera Universitaria Città della Salute e della Scienza, and School of Medicine, University of Torino, Italy
1999–2014	Assistant Professor of Hematology, School of Medicine, University of Torino, Torino, Italy
1995-1999	Visiting Physician at The Fred Hutchinson Cancer Research Center, Seattle, WA

Transplant Biology Group MAIN GROUP MEMBERS

Alessia MELIS *student*
Alessia CARGNINO *student*
Giuseppe LIA *Research Associate*
Luisa GIACCONE *Associate Professor*

RESEARCH ACTIVITY

In addition to a robust clinical activity in the field of hematological malignancies, the group has focused on translational projects with particular emphasis on the role of the thymus in post-transplant immune-reconstitution (Figure 1), on the role of biomarkers such as extracellular vesicles (EVs) in the pathogenesis and early diagnosis of acute and chronic “graft versus host disease” (Figure 2), and, more recently, on the role of the endothelial damage in the complications following the infusion of chimeric antigen receptor T (CAR T) cells (Fig. 3).

Briefly, thymus-dependent recovery of peripheral T cells ensures long term T cell-mediated protection and tolerance. We measured the TCR gene rearrangements in T cells. This technique measures the extrachromosomal DNA excision circles that are generated in the thymus only in naïve T cells following TCR gene rearrangement [i.e. joined T cell receptor excision circles (sjTRECs)]. These stable DNA circles do not replicate during mitosis, but they are diluted within each cellular division and can persist in mature T cells, thus providing an excellent measure of thymic functions. Our preliminary data demonstrated a progressive increase in absolute numbers of all T cell subsets and of sjTRECs from the 3rd month up to 2 years post-Haploidentical-HCT. The increased sjTREC values provide evidence of an active thymic function despite age-dependent involution that substantially contributes to T cell IR after Haplo-HSCT. Moreover, we observed that both CD4+ and CD8+ T cell levels and sjTRECs levels are lower in recipients after 2 years from transplant compared to healthy donors. Of note, chronic GVHD and older age are significantly correlated with thymic activity.

EVs are natural carrier of several bioactive molecules, such as lipids, proteins, and nucleic acids (like DNA and miRNA), and their quantity relies on patient status. EVs are physiologically present in body fluids, including blood and urine from where they can be easily extracted without invasive procedures, making them very attractive targets for diagnostic applications. The content inside each EV reflects the cellular origin and it can be quantified. EVs are shed by cells under both normal and pathological conditions. They carry nucleic acids and proteins from

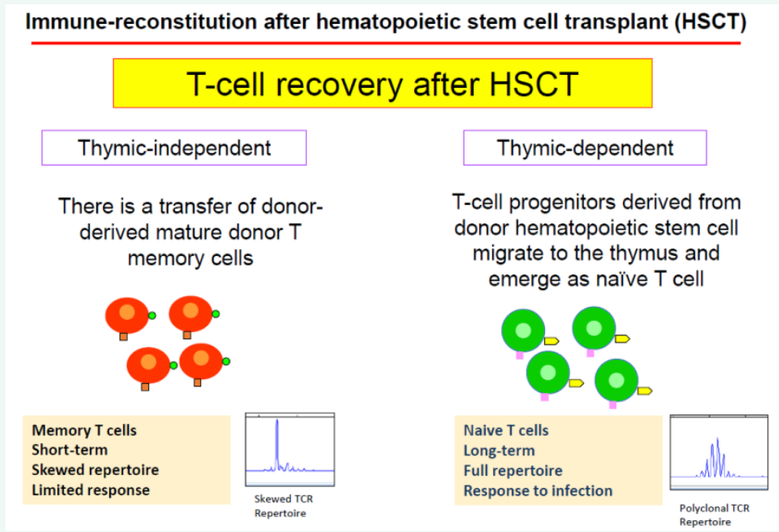


Figure 1.
Immune reconstitution after HSCT. Thymic-independent and thymic-dependent immune reconstitutions are key factors for clinical outcomes. Quantification of different immune cell subsets, by flow cytometry, and quantification of thymic output (sjTRECs) by real-time PCR (Taqman) are employed to monitor the thymic activity.

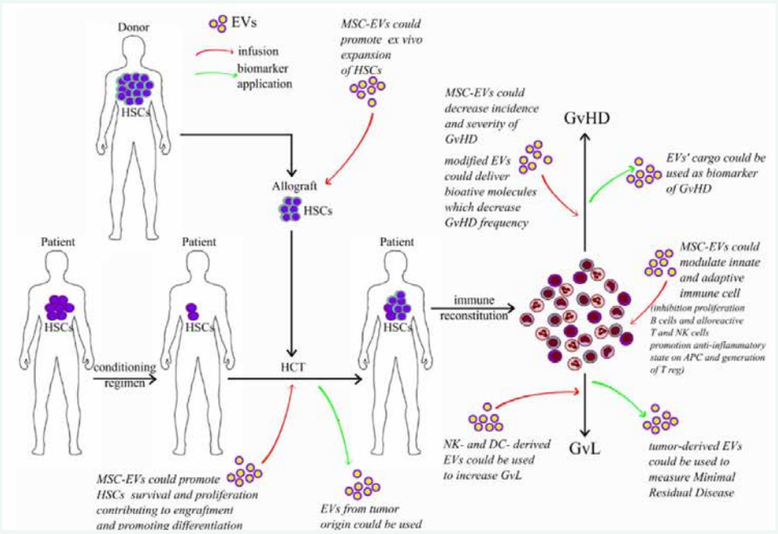


Figure 2.
(adapted form Lia et al) Potential role of extracellular vesicles (EVs) in hematopoietic stem cell transplant. Red arrows illustrate potential effects of infused EVs; green arrows of potential use of EVs as biomarkers. Abbreviations: HSC, hematopoietic stem cells; HCT, hematopoietic cell transplantation; EVs, extracellular vesicles; MSCs, mesenchymal stromal cells; GvL, graft-versus-leukemia; GvHD, graft-versus-host disease.

their host cells that are indicative of pathophysiological conditions, and, for these reasons, they are considered crucial for the understanding of pathophysiological processes. Over the past few years, numerous studies have demonstrated that exosomes contain nucleic acids and proteins implicated in cancer as well as inflammatory conditions. We previously showed that the immune-profile of serum extra cellular vesicles, including their content, and the degree of endothelial dysfunction provide important insights in the pathophysiology and severity of post-transplant life-threatening complications (i.e. graft-vs.-host disease, venous-occlusive disease, transplant-associated microangiopathy).

Novel cell therapies such as chimeric antigen receptor (CAR) – T cells and T cell receptor (TCR)

T cells have recently changed the treatment landscape of hematological malignancies with high rates of remission, and potentially reaching a cure, in particular in relapsed/refractory lymphoproliferative diseases and multiple myeloma. However, these immunotherapies are associated with significant toxicities that not only limit their administration but have also become an area of intensive research with the goal of improving clinical outcomes by reducing untoward side effects. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are life-threatening complications that follow CAR T infusions. While CAR T cell expansion and their persistence after infusion appear associated with disease response, inflammation and a supraphysiological response appear pivotal processes in the development of both CRS and ICANS. Mechanisms of endothelial activation and dysfunction have also emerged as important factors associated with CAR T cell toxicity. A significant consequence of endothelial activation, reported in a number of inflammatory processes, is represented by a dysfunctional switch from an antithrombotic to a prothrombotic endothelial phenotype, sustained by the triggering of fibrin formation and platelet adhesion and aggregation. Overall our aim is to investigate the dynamic changes of endothelial activation in association with the immune-profile of EVs in peripheral blood from baseline to given time-points after the infusion of CAR T cells in patients with relapsed/refractory lymphoproliferative diseases. Importantly, laboratory findings will be correlated with clinical outcomes to design potential pre-emptive strategies to prevent CAR T cells toxicity and expand patient eligibility criteria.

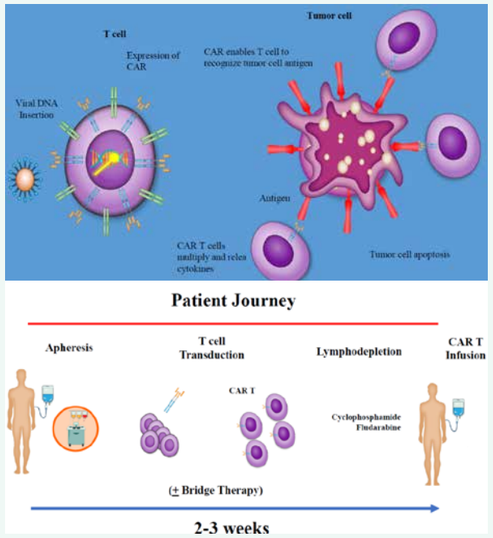


Figure 3.
Current chimeric antigen receptor (CAR) T-cell therapies have been a major breakthrough in immunotherapy. In particular, CD19-directed CAR T- cells have dramatically changed the treatment of relapse/refractory B-cell origin hematological malignancies. CAR T-cells are primarily derived from autologous T-cells, collected by leukopheresis, and genetically engineered to express a CAR, specific for a given antigen expressed on cancer cells, followed by ex vivo CAR T cell expansion and re-infusion into the patient. CARs are fusion proteins of a selected single-chain fragment variable from a specific monoclonal antibody and one or more T-cell receptor intracellular signaling domains. This genetic T-cell modification can be obtained by viral-based gene transfer or non-viral technologies such as DNA-based transposons, CRISPR/Cas9 technology or direct transfer of in vitro transcribed-mRNA by electroporation. In the clinical setting, treatment protocols (either for FDA/EMA approved products or for clinical trials) incorporate a short, non-intense lymphodepleting regimen prior to CAR T-cell infusion with the aim to increase efficacy. Overall, this "patient journey" from collection to infusion usually requires between two and four weeks.

FUTURE RESEARCH PLANS

We plan to compare the immune-reconstitution, and in particular the role of the thymus, in different settings of hematopoietic stem cell transplantation and novel cell therapies. We will continue to investigate the role of EVs as biomarkers of post-transplant complications of endothelial origin and infections. Some projects will be carried out with national and international Research Institutes.

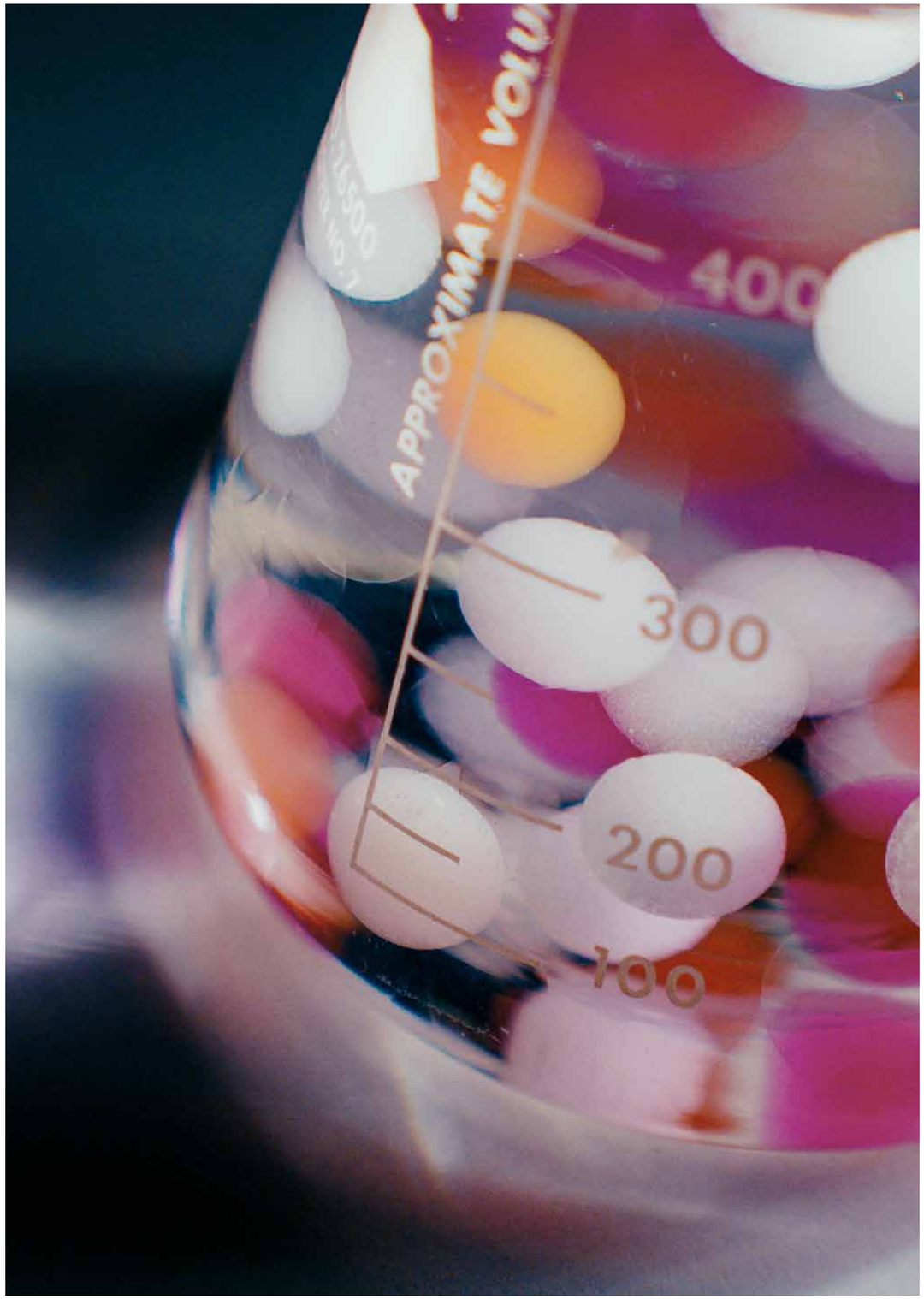
FUNDING ID (PAST 5 YERS)

M.I.U.R. (Ministry of University and Scientific Research) Research Grants (2018-2023):

- → Role of Plasmatic Extracellular vesicles as biomarkers of inflammatory and endothelial complications after hematopoietic stem cell transplantation.
- → Plasmatic Extracellular Vesicles in Cytomegalovirus reactivation and acute Graft Versus Host Disease after Haploidentical Stem Cell Transplantation
- → Plasmatic Extracellular Vesicles as potential biomarkers of viral infection and endothelial complications after allogeneic stem cell transplantation
- → Extracellular vesicles as potenzial biomarkers of chronic GVHD"
- → Integrating Next Generation Sequencing in minimal residual disease and prognosis stratification of acute myeloid leukemia and high risk myelosplastic syndromes patients. (JANSSEN/CILAG)
- → Efficacy of the biosimilar filgrastim Nivestim on the mobilization of hematopoietic CD34+ cells and on the kinetics of engraftment after high dose chemotherapy and mobilized peripheral hematopoietic cell support. (HOSPIRA ITALIA SRL)
- → Bando PRIN 2022 PNRR Endothelial cell dysfunction and extracellular vesicles as biomarkers of clinical outcomes (toxicity and disease responses) after chimeric antigen receptor (CAR) T-cell therapy for lymphoproliferative diseases

SELECTED PUBLICATIONS

- Bruno B, et al. Purified canine CD34+Lin- marrow cells transduced with retroviral vectors give rise to long-term multi-lineage hematopoiesis. Biol Blood Marrow Tr. 2001;7:543-51. doi: 10.1016/s1083-8791(01)70020-1
- Bruno B, et al. A comparison of allografting with autografting for newly diagnosed myeloma. New Engl J Med. 2007; 356:1110-20. doi: 10.1056/NEJMoa065464
- Bruno B, et al. Non-myeloablative allografting for newly diagnosed multiple myeloma: the experience of the Gruppo Italiano Trapianti di Midollo. Blood. 2009;113:3375-82. doi: 10.1182/blood-2008-07-167379
- Giaccone L, …, Bruno B. Long-term follow-up of a comparison of nonmyeloablative allografting with autografting for newly diagnosed myeloma. Blood. 2011;117:6721-7. doi: 10.1182/blood-2011-03-339945
- Lia G, …, Bruno B. Extracellular vesicles as potential biomarkers of acute graft-vs-host disease. Leukemia. 2018;32:765-773. doi: 10.1038/leu.2017.277
- Mariotti J, … Bruno B. Impact of donor age and kinship on clinical outcomes after T-cell- replete haploidentical transplantation with PT-Cy. Blood Adv. 2020;4:3900-3912. doi: 10.1182/bloodadvances.2020001620
- Bruno B, et al. European Myeloma Network Perspective on CAR T-Cell Therapies for Multiple Myeloma. Haematologica, 2021;106:2054-2065. doi: 10.3324/haematol.2020.276402
- Lia G, … Bruno B. Extracellular Vesicles as Biomarkers of acute Graft-vs.-host Disease after Haploidentical Stem Cell Transplantation and Post-transplant Cyclophosphamide. Front in Immunol ;12:816231. doi: 10.3389/fimmu.2021.816231
- Boccalatte F, …, Bruno B. Advances and hurdles of CAR T cell immune therapy in solid tumors. Cancers (Basel). 2022 Oct 18;14(20):5108. doi: 10.3390/cancers14205108
- Rejeski K, …, Bruno B, … Yakoub-Agha I. Immune Effector Cell-Associated Hematotoxicity (ICAHT): EHA/EBMT Consensus Grading and Best Practice Recommendations. Blood. 2023. doi: 10.1182/blood.2023020578.



ALFREDO BRUSCO

Medical Genetics and Rare Diseases

BIOGRAPHICAL SKETCH

2023 to date	Full Professor in Medical Genetics, University of Turin
2016-2023	Associate Professor in Medical Genetics, University of Turin
2000-2016	Assistant Professor in Medical Genetics, University of Turin
2000 to date	Head biologist Città della Salute e della Scienza University Hospital, Turin
1998 to date	Group leader of the Medical Genetics and Rare Diseases laboratory

TRANSPLANT BIOLOGY GROUP

Lisa Pavinato *post-doc*
Slavica Trajkova *post-doc*
Simona Cardaropoli *Research assistant*
Verdiana Pullano *PhD student*
Silvia Carestiato *PhD student*
Chiara Giovenino *Trainee in Medical genetics*
Serena Rizzo *Trainee in Medical genetics*
Chiara Leso *fellowship student*

RESEARCH ACTIVITY

Neurodevelopmental disorders (NDDs) are a broad spectrum of conditions that include autism spectrum disorders (ASD), intellectual disability, epilepsy, and ADHD. NDDs have a strong genetic component, but our knowledge about the molecular mechanisms involved in their onset is still limited.

Our research is focused on studying Mendelian forms of NDDs at several levels (Figure 1).

We are interested in improving the yield of genetic tests by providing implemented pipelines of exome sequencing (ES) analysis. The interpretation of genetic variants is often hindered by the lack of functional tests, which allow us to sustain the pathogenicity of variants of uncertain significance. We are thus fascinated by studying complex genetic mechanisms causing genetic diseases, such as mobile element insertions, complex splicing anomalies, uncommon imprinting pathogenic variants, and TADopaties causing enhancer adoption. We are also particularly interested in the role of non-random X chromosome inactivation (XCI) in NDDs, which can disclose X-linked forms. Genetic NDDs frequently present with overlapping clinical features and inconclusive or ambiguous genetic findings, which can confound accurate diagnosis and clinical management. An increasing number of genetic syndromes have been shown to have unique genomic DNA methylation patterns (called “episignatures”). We are working to define novel episignatures and apply these methods in diagnostics.

The number of NDD-associated genes is still limited; for instance, more than 1,000 genes are expected to be related to ASDs, but only 150 are presently known. One major aim of our laboratory is to define novel NDD genes by exploiting a large cohort of NDD cases collected and sequenced in the last years (NeuroWES project). This work has already allowed us to find more than 10 novel genes, among which CAPRIN1, whose haploinsufficiency causes NDD. The work done on this gene exemplifies our interest in the study of NDDs. We generated CAPRIN1+/– human induced pluripotent stem cells (hiPSCs) via CRISPR–Cas9 mutagenesis and differentiated them into neuronal progenitor cells and cortical neurons. CAPRIN1 loss caused reduced neuronal processes, overall disruption of the neuronal organization, and increased neuronal degeneration. We also observed an alteration of mRNA translation in CAPRIN1+/– neurons, compatible with its suggested function as a translational inhibitor. As suggested above, our work is also interested in finding new diagnostic tools, and for CAPRIN1 patients we are also working on the generation of a specific methylation profile (Figure 2).

Understanding gene function in neuronal models is one of our main targets. We are presently working on several other candidate and known NDD genes/regions, such as the duplication 15q11.2, one of the most common genetic causes of ASD. In this case, we are exploiting

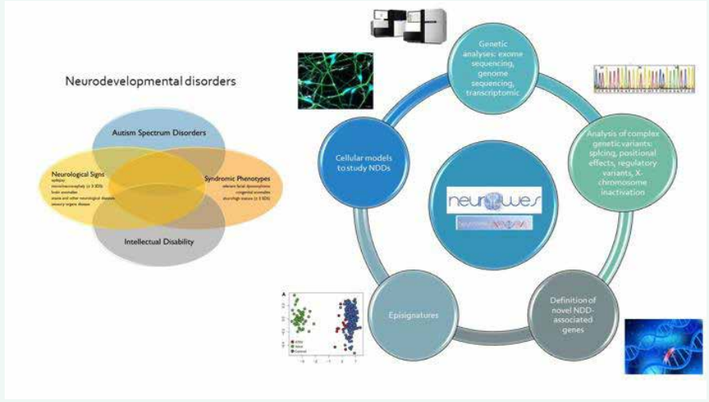


Figure 1.
Schematized summary of the research activity in Brusco’s laboratory

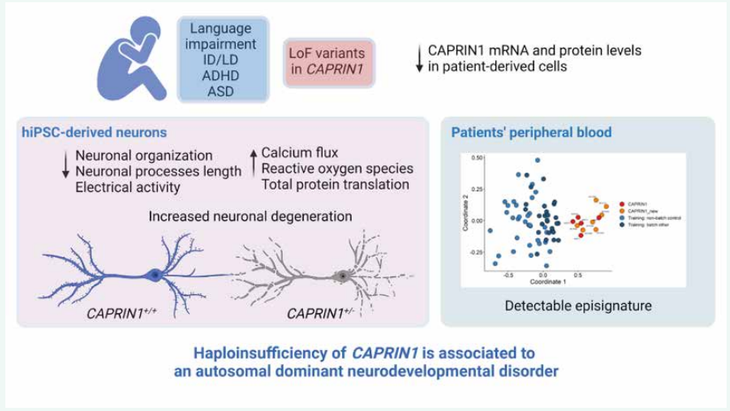


Figure 2.
CAPRIN1 as exemplifying gene associated with NDD, recently described by our group.

Micro Electrode Arrays to test for drugs potentially effective on epilepsy. Finally, we are particularly interested in modifier genes, which can be a target of disease-specific therapies. In this topic, we are studying TANGO2-autosomal recessive disease, where we have identified phenotypically discordant siblings with loss of function variants in the gene. The study of neuron-induced hiPSCs by morphological, functional, and transcriptome analysis is shedding light on the role of compensatory genetic mechanisms.

As an innovative method to study NDDs in vitro we are using Dental Pulp Stem Cells (DPSC), pluripotent mesenchymal stem cells that can be differentiated into neurons. These cells can be easily collected from deciduous teeth and used in vitro in functional experiments.

FUTURE RESEARCH PLANS

We plan to use short and long-read genome sequencing and transcriptomics to search for causative variants in NDD cases that are negative at exome analysis. We will use in vitro neuronal and organoid models to study the pathogenic mechanisms of novel NDD genes. We will also develop novel episignature profiles that may help with diagnosis in NDDs and neurologic disorders.

FUNDING ID (PAST 5 YEARS)

- Coordinator of the multicentric project: “Multiomic strategies to implement the diagnostic workflow of rare diseases”. Ministero della Salute, PNRR-MR1-2022-12376067. 2023-2025. 240000 €.
- Co-Coordinator TANGO2 foundation project: “Understanding TANGO2 pathogenic mechanisms in hiPSCs-derived neurons”. June 2022 - December 2023. 22000 €.
- Partner PRIN 2020: “Unveiling the hidden side of NEUrodevelopmental DIsorder Genetics (NEUDIG): a multidisciplinary pathway to new molecular diagnoses by integrating genomic, transcriptomic, and functional analyses”. 2021-2024. 143590 €.
- Coordinator Genetica e Farmacogenomica, Progetto di Eccellenza Dipartimento Scienze Mediche, Università di Torino 2018-2022. 150000 €.
- Partner PRIN Project 2017: “Multidisciplinary approach to study protocadherin 19: from neuronal function to the “cellular interference” pathogenic mechanism”. 20172C9HLW. 2019-2022. 153.137 €
- Principal investigator of a Telethon multicenter project GGP14225: “Translating molecular pathology into a therapeutic strategy in SCA38, a newly identified form of spinocerebellar ataxia”. 2015-2019. 143.000 €.

SELECTED PUBLICATIONS

- Melo, U.S., Jatzlau, J., Prada-Medina, et al. Enhancer hijacking at the ARHGAP36 locus is associated with connective tissue to bone transformation. (2023) Nature Communications, 14 (1), DOI: 10.1038/s41467-023-37585-8
- Pavinato, L., Stanic, J., Barzasi, M., … and Brusco, A. Missense variants in RPH3A cause defects in excitatory synaptic function and are associated with a clinically variable neurodevelopmental disorder. (2023) Genet Med. Nov;25(11):100922, DOI: 10.1016/j.gim.2023.100922.
- Pavinato, L., Delle Vedove, A., Carli, D., … and Brusco, A. CAPRIN1 haploinsufficiency causes a neurodevelopmental disorder with language impairment, ADHD and ASD (2023) Brain, 146 (2), pp. 534-548. DOI: 10.1093/brain/awac278
- Ferrero, E., Di Gregorio, E., Ferrero, … and Brusco, A. Spinocerebellar ataxia 38: structure–function analysis shows ELOVL5 G230V is proteotoxic, conformationally altered and a mutational hotspot (2023) (2023) Hum Genet. Aug;142(8):1055-1076. DOI: 10.1007/s00439-023-02572-y
- Giovenino, C., Trajkova, S., Pavinato, L., … and Brusco, A. Skewed X-chromosome inactivation in unsolved neurodevelopmental disease cases can guide re-evaluation For X-linked genes (2023) (2023) Eur J Hum Genet. Nov;31(11):1228-1236. DOI: 10.1038/s41431-023-01324-w
- Pavinato, L., Villamor-Payà, M., Sanchiz-Calvo, … and Brusco, A. Functional analysis of TLK2 variants and their proximal interactomes implicates impaired kinase activity and chromatin maintenance defects in their pathogenesis (2022) Journal of Medical Genetics, 59 (2), pp. 170-179. DOI: 10.1136/jmedgenet-2020-107281
- Fu, J.M., Satterstrom, F.K., Peng, M., et al., The Autism Sequencing Consortium (ASC), Broad Institute Center for Common Disease Genomics (Broad-CCDG), iPSYCH-BROAD Consortium. Rare coding variation provides insight into the genetic architecture and phenotypic context of autism (2022) Nat Genet. Sep;54(9):1320-1331, DOI: 10.1038/s41588-022-01104-0
- Satterstrom, F.K., Kosmicki, J.A., Wang, J., et al., Autism Sequencing Consortium, iPSYCH-Broad Consortium. Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism (2020) Cell, 180 (3), pp. 568-584.e23. DOI: 10.1016/j.cell.2019.12.036
- Giorgio, E., Lorenzati, M., Di Val Cervo, … and Brusco, A. Allele-specific silencing as treatment for gene duplication disorders: Proof-of-principle in autosomal dominant leukodystrophy (2019) Brain, 142 (7), pp. 1905-1920. DOI: 10.1093/brain/awz139
- Giorgio, E., Robyr, D., Spielmann, … and Brusco, A. A large genomic deletion leads to enhancer adoption by the lamin B1 gene: A second path to autosomal dominant adult-onset demyelinating leukodystrophy (ADLD), (2014) Human Molecular Genetics, 24 (11), pp. 3143-3154. DOI: 10.1093/hmg/ddv065

BENEDETTA BUSSOLATI

Extracellular Vesicles in regenerative medicine

BIOGRAPHICAL SKETCH

2022-present	Full Professor of Laboratory Medicine, Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy
2006-2021	Associate Professor of Nephrology, Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy
2001-2006	Assistant Professor of Pharmacology, Department of Clinical Sciences, University of Torino, Italy
1999-2001	post-doctoral fellow, Laboratory of Renal and Vascular Pathophysiology, University of Torino, Italy
1998-1999	research fellow, Laboratory of Vascular and Reproductive Physiopathology, University of Birmingham, UK
1995-1998	PhD in Nephrology, University of Parma, Italy
1988-1994	MD in Medicine and Surgery, University of Torino, Italy

TRANSPLANT BIOLOGY GROUP

Alessia Brossa *RTDA Roberta Verta-PhD*
Adele Tanzi *PhD*
Tunahan Ergünay *PhD*
Alessia Dalmasso *PhD*
Marta Fornaro *PhD*
Michela Arena *PhD*

RESEARCH ACTIVITY

Our group has a strong background on renal pathophysiology, on angiogenesis and on the mechanisms of renal damage and progression. In addition, we have extensive experience in studies of stem cell biology and regenerative medicine that include characterization of various stem cell types and derived bioproducts and their potential use for tissue regeneration.

In particular, we are investigating investigate the role of extracellular vesicles in regenerative medicine as delivery systems of therapeutic cargo, such as RNA species and as diagnostic tool.

A platform for single-EV characterization with dedicated instruments is presentin the lab.

Moreover, we are currently studying other cell derived bioproducts, such as mitochondria, for regenerative medicine, with a particular focus on graft protection during reperfusion.

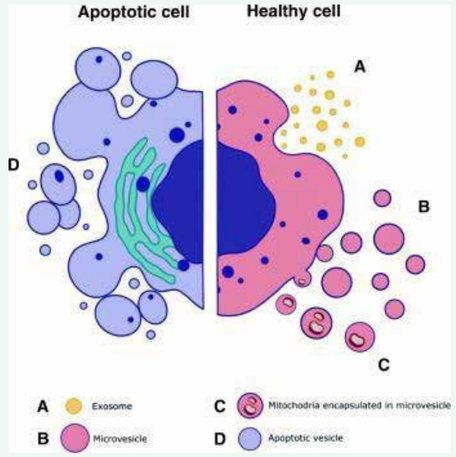


Figure 1.
Representation of stem cell bioproducts for regenerative medicine

EVs as diagnostic tools

We are interested in the characterization of urinary EVs in order to identify disease biomarkers, as well as markers of renal regeneration reserve.

We characterized EVs in amniotic fluid, which is mainly derived from fetal urine, and identified their alteration in pre-eclamptic patients (Fig.2). We also extensively characterized urinary EVs These vesicles are of interest as mediators of glomerular-tubular and intersegment tubular crosstalk and are involved in the amplification of kidney damage and inflammation. The molecular profile of extracellular vesicles reflects the type and pathophysiological status of the originating cell so could potentially be exploited for diagnostic and prognostic purposes.

In recent studies, we showed that extracellular vesicles present in urine may represent useful markers of renal regenerative ability of interest to assess the risk of chronic kidney disease progression in transplanted patients (Fig.3).

EVs as therapeutic tools

Extracellular vesicles from different stem cell types might be used for regeneration of renal tissue after injury. We showed that urinary EVs can contain Klotho, a single-pass transmembrane hormon identified as an anti-aging factor, expressed in the kidney where it exherts a regenerative effect (Fig.5). Our results demonstrated the novel potential use of urinary EVs with factor Klotho to contrast the acute kidney injury

Extracellular vesicles, especially from autologous sources, are also attractive candidates for drug delivery and various engineering strategies are being investigated to modify their cargo and increase their efficacy (Fig. 6).

Finally, we recently demonstrated the regenerative effect of mitochondrial transplant in a model of ex vivo kidney reperfusion, resembling cardiac death donation.

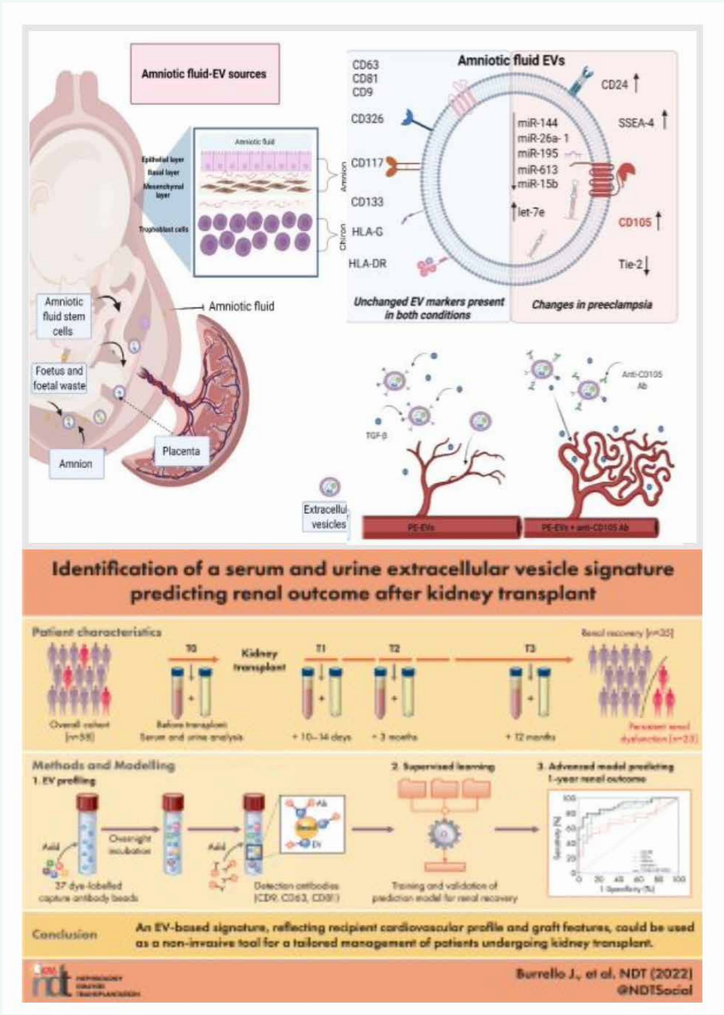


Figure 2.
Illustration of the multiple possible sources of term amniotic fluid-derived EVs and the main changes in preeclampsia- derived-EVs. Their antiangiogenic properties are supported by the specific upregulation of CD105 surface. (Gebara et al. JEV 2022)

Figure 3.

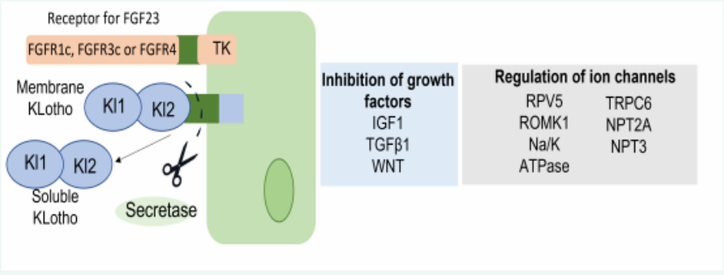


Figure 5.
Main functions of the Klotho protein

FUTURE RESEARCH

We aim to extend our results on urinary EVs and to identify an early signature of renaldamage progression, to be transplated into the clinic.

We also aim to exploit EVs and mitochondria for organ regeneration, with a main focus on graft function

FUNDING ID (PAST 5 YEARS)

- 2023-2026 PNRR National Center for the development of RNA-based therapies. Spoke 8.1 - Translational development of smart delivery platforms. PI of the 8.1.4 – Biological vesicles and platforms
- 2020-2025. NIH R01 Grant No. R01DK121037 Extracellular vesicles derived from amniotic fluid stem cells normalize glomerular function during progressive kidney disease, Co-PI.
- 2018-2023 European H2020 project RenalToolBox H2020-MSCA-ITN-2018
- 2016-2022 Unicyte EG AV Pre-clinical development of stem cell-derived EVs for treatment of Renal Carcinomas
- 2017-2021 European H2020 project iPLACENTA: H2020-MSCA-ITN-2017
- 2015-2020 Grant of the Italian Association for Cancer Research (AIRC), IG2015 169173

SELECTED PUBLICATIONS

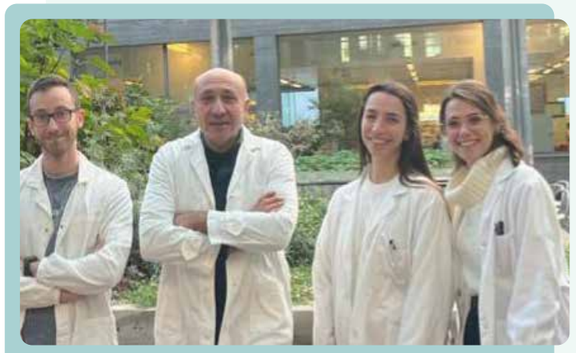
- Rossi A, Asthana A, Riganti C, Sedrakyan S, Byers LN, Robertson J, Senger RS, Montali F, Grange C, Dalmasso A, Porporato PE, Palles C, Thornton ME, Da Sacco S, Perin L, Ahn B, McCully J, Orlando G, Bussolati B. Mitochondria Transplantation Mitigates Damage in an In Vitro Model of Renal Tubular Injury and in an Ex Vivo Model of DCD Renal Transplantation. Ann Surg. 2023 Jul 14. doi: 10.1097/SLA.0000000000006005.
- Burrello J, et al. Identification of a serum and urine extracellular vesicle signature predicting renal outcome after kidney transplant. Nephrol Dial Transplant. 2023 28;38(3):764-777. doi: 10.1093/ndt/gfac259.
- Grange C, Bussolati B. Extracellular vesicles in kidney disease. Nat Rev Nephrol. 2022;18:499-513. 10.1038/s41581-022-00586-9
- Gebara N, et al. Single extracellular vesicle analysis in human amniotic fluid shows evidence of phenotype alterations in preeclampsia. J Extracell Vesicles. 2022;11(5):e12217. doi: 10.1002/jev2.12217.
- Börger V, et al. International Society for Extracellular Vesicles and International Society for Cell and Gene Therapy statement on extracellular vesicles from mesenchymal stromal cells and other cells: considerations for potential therapeutic agents to suppress coronavirus disease-19. Cytotherapy. 2020; 22(9):482-485. doi: 10.1016/j.jcyt.2020.05.002.
- Bellucci L, et al. Mesenchymal Stromal Cell-Derived Extracellular Vesicles Pass through the Filtration Barrier and Protect Podocytes in a 3D Glomerular Model under Continuous Perfusion. Tissue Eng Regen Med. 2021;18(4):549-560. doi: 10.1007/s13770-021-00374-9.
- .Erdbrügger U, et al. Urinary extracellular vesicles: A position paper by the Urine Task Force of the International Society for Extracellular Vesicles. J Extracell Vesicles. 2021;10(7):e12093. doi: 10.1002/jev2.12093.
- Iampietro C, et al. Bussolati B. Molecular and functional characterization of urine-derived podocytes from patients with Alport syndrome. J Pathol. 2020; 252(1):88-100. doi: 10.1002/path.5496.
- Grange C, et al. Urinary Extracellular Vesicles Carrying Klotho Improve the Recovery of Renal Function in an Acute Tubular Injury Model. Mol Ther. 2020; 28(2):490-502. doi: 10.1016/j.ymthe.2019.11.013
- Lopatina T, et al. Extracellular vesicles from human liver stem cells inhibit tumor angiogenesis. Int J Cancer.2019;144(2):322-333.5. 6. doi: 10.1002/ijc.31796

ENZO CALAUTTI

Transplant Biology

BIOGRAPHICAL SKETCH

2018 to present	Full Professor of Laboratory Medicine, Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy
2008-2018	Assistant Professor of Cell Biology (Department of Molecular Biotechnology and Health Sciences), University of Turin, Italy
2006-2011	Assistant Telethon Scientist, Dulbecco Telethon Institute, Molecular Biotechnology Center, University of Turin, Italy
2004-2006	Group leader, Epithelial Stem Cell Research Center, Fondazione Banca degli Occhi del Veneto, Venezia, Italy
1996-2004	Instructor in Dermatology/ Assistant in Cellular Biology, Harvard Medical School and Massachusetts General Hospital, Boston, MA, USA
1996	PhD degree in Cell Biology, University of Turin/Harvard Medical School
1992-1996	Research Fellow in Dermatology, Cutaneous Biology Research Center, Dermatology Department, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
1991-1992	Research Fellow, Department of Pathology, School of Medicine, Yale University, New Haven, CT, USA.
1989	MD degree



TRANSPLANT BIOLOGY GROUP

Luca Ponzone *PhD student*
Eleonora Marina *Student*
Sara Ferrua *Student*

RESEARCH ACTIVITY

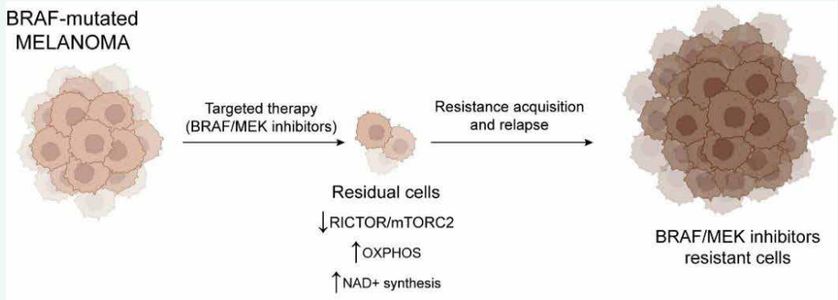


Figure 1.
A novel tumor-suppressive role of mTORC2 in melanoma therapeutic resistance

Adaptation to stressful conditions is key for the evolution and maintenance of organisms but also allows tumor cells to escape anti-cancer therapeutic interventions. The main research activity of my laboratory is aimed at understanding how normal- and cancer (stem) cells cope with unfavorable conditions by activating stress-protective programs. The identification of such adaptive signaling mechanisms is crucial for the design of therapeutic strategies aimed either at improving tissue regeneration and healthy aging or at developing more effective oncological therapies. Our laboratory is presently focused on the identification of adaptive mechanisms at the basis of cancer therapeutic resistance. For instance, nearly 50% of cutaneous melanomas carry activating mutations of the BRAF oncogene, and the combination of BRAF- and MEK-inhibitors (BRAF/MEKi) is frequently used in their clinical management. This therapeutic approach often induces a rapid regression of tumors at advanced stages of disease, but has the major drawback of the nearly inevitable acquisition of therapeutic resistance. This can be driven by multiple adaptive and/or genetic mechanisms of tumor cells that often converge on the upregulation of mitochondrial bioenergetics and NAD+ biosynthetic pathways. Building on the knowledge gained in Rictor-depleted epidermal cells that exhibit a high stress tolerance that depends on a glycolysis-to-OXPHOS metabolic switch (Tassone B. et al. 2017), we hypothesized that mTORC2 depletion may also protect BRAFV600E metastatic melanoma (MM) cells from therapeutic stress induced by targeted therapy, by promoting similar changes in mitochondrial bioenergetics. Bioinformatic analysis of the TCGA database revealed that in the context of cutaneous MM, tumors expressing low RICTOR levels are associated with a poorer patients' prognosis as compared to high-RICTOR expressing counterparts. Gene-set enrichment analysis (GSEA) of Low-RICTOR MM specimens also evidenced a prominent molecular signature suggestive of activation of ETC-based mitochondrial OXPHOS. We found that mTORC2-deficient BRAFV600E MM cells obtained through RICTOR knockdown are intrinsically tolerant to BRAF/MEKi, and anticipate the acquisition of BRAFi resistance after prolonged drug exposure, indicating that mTORC2 counteracts the acquisition of targeted therapy resistance in MM. Proteomic analysis of RICTOR-deficient cells has revealed increased expression and/or differential post-translational modification of proteins involved in mitochondrial OXPHOS and NAD metabolism. Consistently, RICTOR-deficient cells show enhanced mitochondrial respiratory potential and increased expression of nicotinamide phosphoribosyltransferase (NAMPT) protein, the rate-limiting enzyme of the NAD+ salvage pathway. Notably, pharmacological inhibition of either NAMPT or the ETC in RICTOR-deficient cells is sufficient to restore sensitivity to BRAFi. We also found that in drug naïve BRAFV600E MM cells, the endogenous RICTOR protein is downregulated in the few proliferating cells that survive a prolonged BRAFi exposure. Because these cells represent the seeds of BRAFi-resistant cell populations, the downregulation of endogenous RICTOR may provide

an early adaptation mechanism that allows MM cells to escape BRAF inhibition. This adaptive resistance mechanism may set the basis for the occurrence of further genetic and/or epigenetic alterations that permanently stabilize BRAF/MEKi-resistance in tumors. Overall, our findings unveil a novel tumor suppressive function for RICTOR/mTORC2, whose inactivation promotes the acquisition of MM therapeutic resistance. Thus, measurement of intra-tumor RICTOR levels may have a prognostic value, and help to predict the responsiveness of BRAFV600E MM to targeted therapy. Moreover, the NAMPT-ETC axis may represent a specific therapeutic vulnerability of low-RICTOR MM, with potential therapeutic implications.

Like in melanoma, also in epithelial cancers the lethality of tumors largely depends on the ability of cancer cells to evade therapeutic interventions thanks to adaptive stress-protective programs that foster the maintenance of “persister” cell populations reigniting tumor growth and metastasis. This includes changes in the expression of genes that protect cells from oxidative damage. Increasing evidence indicates that persister cancer cells can be sensitized to ferroptosis, a non-apoptotic form of cell death characterized by iron-dependent lipid peroxidation, being “addicted” to anti-ferroptotic mechanisms for their survival. Importantly, being ferroptosis an immunogenic form of cell death, tumor cells engaging anti-ferroptotic mechanisms also acquire immune evasive properties. As a consequence, the induction of ferroptosis may enhance the responses of tumors to immunotherapies.

Our recent evidence point to the whole p53 family of transcription factors (p53, p63 and p73) as major transcriptional regulators of redox genes whose expression can dictate the sensitivity or resistance of cells to ferroptotic stimuli. One prominent gene in this network is SLC7A11, encoding for the functional subunit of the cystine/glutamate antiporter system xc- involved in glutathione biosynthesis. SLC7A11 is highly expressed in tumors and persister cancer cells, and its activity plays a key role in the protection of cells from ferroptosis, thus representing a therapeutic target in ferroptosis-based anti-cancer strategies. Whereas p53 inhibits SLC7A11 expression, our recent data indicate that in squamous cell carcinoma models, ΔNp63 (and to a lesser extent p73), may exert an even broader control of the glutathione metabolic pathway, including a direct positive regulation of SLC7A11 expression. Thus, epithelial cancers overexpressing p63 and/or p73 may be protected from cancer therapy, at least in part, by engaging anti-ferroptotic transcriptional programs. We are presently investigating the transcriptome changes of epithelial cells derived from breast cancers and squamous cell carcinomas, in response to oxidative stress as a function of p53 family members expression.

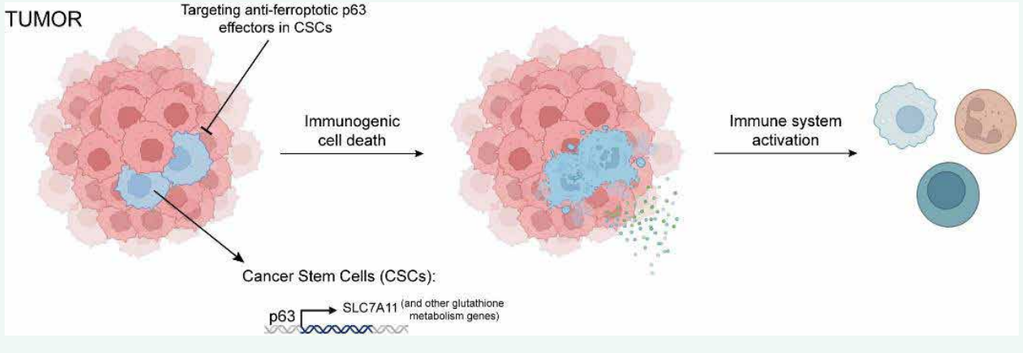


Figure 2.
Role of p63/p73 in the resistance of epithelial tumors to ferroptosis

FUTURE RESEARCH PLANS

We aim at elucidating in MM the interplay of mTORC2 signaling, NAD biosynthetic pathways and mitochondrial ETC activation. Another interesting venue of research that we plan to pursue is based on our unpublished data indicating that RICTOR-deficient melanoma cells activate genes involved in the cross-talk between the tumor and the immune system, with potential impacts on the response of RICTOR-deficient tumors to Immune Checkpoint Inhibitors.

In the context of the regulation of epithelial tumor anti-ferroptotic mechanisms, we will address how the transcriptional changes induced by p63/73 modulation impact on epithelial cancer cell survival and resistance to oxidative stress, immunogenicity, and response to immunotherapies, including cancer vaccination against SLC7A11 and immune checkpoint blockade. These studies will be carried out in tight collaboration with the teams of Profs. Federica Cavallo (UniTO), and Caterina Missero (UniNA, Ceinge).

FUNDING ID

- 2023-2025: PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022, MUR.
- 2020-2022: Liberal contribution, Banca d’Italia.

SELECTED PUBLICATIONS

- Ruiu R, Cossu C, Iacoviello A, Conti L, Bolli E, Ponzone L, Magri J, Rumandla A, Calautti E, Cavallo F. Cystine/glutamate antiporter xCT deficiency reduces metastasis without impairing immune system function in breast cancer mouse models. J Exp Clin Cancer Res. 2023 Sep 29;42(1):254 PMID: 37770957
- Indini A, Fiorilla I, Ponzone L, Calautti E, Audrito V. NAD/NAMPT and mTOR Pathways in Melanoma: Drivers of Drug Resistance and Prospective Therapeutic Targets. Int J Mol Sci. 2022 Sep 1;23(17):9985. PMID: 36077374
- Centonze G, Centonze S, Ponzone L, Calautti E. ROCK ‘n TOR: An Outlook on Keratinocyte Stem Cell Expansion in Regenerative Medicine via Protein Kinase Inhibition. Cells. 2022 Mar 27;11(7):1130. PMID: 35406693
- Pergolizzi B, Panuzzo C, Ali MS, Lo Iacono M, Levra Levron C, Ponzone L, Prelli M, Cilloni D, Calautti E, Bozzaro S, Bracco E. Two conserved glycine residues in mammalian and Dictyostelium Rictor are required for mTORC2 activity and integrity. J Cell Sci. 2019 Nov 14;132(22):jcs236505. PMID: 31653780
- Calautti E, Avalle L, Poli V. Psoriasis: A STAT3-Centric View. Int J Mol Sci. 2018 Jan 6;19(1):171. PMID: 29316631
- Del Pilar Camacho Leal M, Costamagna A, Tassone B, Saoncella S, Simoni M, Natalini D, Dadone A, Sciortino M, Turco E, Defilippi P, Calautti E, Cabodi S. Conditional ablation of p130Cas/BCAR1 adaptor protein impairs epidermal homeostasis by altering cell adhesion and differentiation. Cell Commun Signal. 2018 Nov 26;16(1):90. PMID: 30477510
- Tassone B, Saoncella S, Neri F, Ala U, Brusa D, Magnuson MA, Provero P, Oliviero S, Riganti C, Calautti E. (2017). “Rictor/mTORC2 deficiency enhances keratinocyte stress tolerance via mitohormesis”. Cell Death Differ. Apr;24(4):731-746. doi: 10.1038/cdd.2017.8. Epub 2017 Feb 17
- Raimo M, Orso F, Grassi E, Cimino D, Penna E, De Pittà C, Stadler MB, Primo L, Calautti E, Quaglino P, Provero P, Taverna D. miR-146a Exerts Differential Effects on Melanoma Growth and Metastatization. Mol Cancer Res. 2016 Jun;14(6):548-62. PMID: 27311960
- Saoncella S, Tassone B, Deklic E, Avolio F, Jon C, Tornillo G, De Luca E, Di Iorio E, Piva R, Cabodi S, Turco E, Pandolfi PP, Calautti E. Nuclear Akt2 opposes limbal keratinocyte stem cell self-renewal by repressing a FOXO-mTORC1 signaling pathway. Stem Cells. 2014 Mar;32(3):754-69. PMID: 24123662

RAFFAELE A CALOGERO

BGcore lab

MAIN GROUP MEMBERS

Maddalena Arigoni *Research Technician*
Luca Alessandri *Researcher type A*
Maria Luisa Ratto *PhD Student*
Sofia Fasciolo, Sebastian Bucatariu, Jacopo Di Mauro, Agata D'Onofrio *B.Sc Students*

BIOGRAPHICAL SKETCH

Raffaele Calogero serves as the Principal Investigator (PI) of the Bioinformatics and Genomics core lab (BGcore) at the Molecular Biotechnology Center in Torino. He became Associate Professor of Molecular Biology at the University of Naples (Italy) in 1992, and he established the B&Gu lab at the University of Turin in 1998. From 2000 to 2010, his research endeavors were primarily focused on transcriptomics data analysis and mining, with a specific emphasis on microarray technolog and then on Next Generation Sequencing (NGS) data analysis. He co-founded the Reproducible Bioinformatics Project, a community of scientists dedicated to advancing the development of reproducible bioinformatics workflows in genomics research. Presently, his primary research focus revolves around the development of single-cell data analysis workflows and the establishment of long-read protocols for single-cell and spatial transcriptomics. Between 2002 and 2010, Prof. Calogero collaborated with Affymetrix to organize training courses for biologists on microarray data analysis. Since 2010, he has been actively involved in bioinformatics training at the European Molecular Biology Laboratory (EMBL) in Heidelberg. Additionally, he conducts courses on NGS data analysis in collaboration with Illumina in Italy and DUKE-NUS in Singapore. He is the President of the Bioinformatics Italian Society (BITS), Representative for University of Torino at the General Assembly of Elixir Italian Node, Co-coordinator of Elixir IT single cell Omics community, Member of the management committee of Elixir IT node, Member of the scientific committee of CINI InfoLife lab.

RESEARCH ACTIVITY

BGcore specializes in transcriptomics, offering targeted services such as miRNA/total RNA sequencing from circulating microvesicles, as well as single-cell spatial transcriptomics services using cutting-edge technologies like Visium and 10XGenomics. BGcore expertise also offers comprehensive data analysis support and conducts hands-on courses dedicated to data analysis to empower researchers in effectively interpreting their results.

Our primary research focus revolves around single-cell Omics, with a specific emphasis on the development of a novel autoencoder known as the sparsely connected autoencoder, exceptionally suitable for extracting valuable biological information from single-cell Omics data [Alessandrì et al. NPJ Syst Biol Appl. 2021, Alessandrì et al. Int J Mol Sci. 2021, Alessandrì et al. Methods Mol Biol. 2023]. Furthermore, we are actively investigating the utilization of advanced computational architectures, such as the INTEL neuromorphic computing infrastructure, to create a more efficient implementation of sparsely connected autoencoders.

When researchers in computational biology and related scientific disciplines face the task of analyzing their data, they often confront the challenge of selecting the most suitable computational methods. To address this challenge and offer valuable recommendations, benchmarking studies are conducted.

Within the framework of the Single Cell Community implementation study, our primary focus is on offering three benchmark experiments (BE1-3). These benchmark experiments aim to tackle the extraction of valuable biological insights from "controlled" cancer heterogeneity.

BE1: We are performing a 10XGenomics scRNAseq experiment including the following elements:

PC9 (EGFR Del19, activating mutation [Simonetti et al. (2010)]

A549 (KRAS p.G12S, growth and proliferation [Yoon et al. (2010)]

NCI-H596 (MET Del14 , enhanced protection from apoptosis and cellular migration [Cerqua et al. (2022)]

NCI-H1395 (BRAF p.G469A, gain of function, resistant to all tested MEK +/− BRAF inhibitors [Negrao et al. (2020)]

DV90 (ERBB2 p.V842I, increases kinase activity, [Boese et al. (2013)

HCC78 (SLC34A2-ROS1 Fusion, ROS1 inhibitors have antiproliferative effect [Davies et al. (2012)]. EML4-ALK Fusion-A549 Isogenic Cell. White cells from donor buffy coat (PBMC)

PBMC were isolated from healthy donor. Before single cell generation, PBMC were marked with Biolegend TotalSeq™-B human universal cocktail v1.0. 10XGenomics analysis was performed using CellPlex 10XGenomics technology, allowing samples labelling.

The experiment was done using CellPlex technology from 10XGenomics allowing multiplexing samples into a single channel and therefore removing unwanted batch effects. Immunological cell types from PBMC will be annotated thanks to the help of an experienced immunologist.

The count tables from the entire BE1 experiment will be made available through an R Shiny app, allowing users to construct datasets encompassing different cell lines at varying ratios.

Actual state of the project: 10XGenomics libraries on going.

Expected data availability: September 2023

BE2: The cell lines from for BE1 will be used to generate surrogate tumor-tissues for spatial transcriptomics, by embedding in matrigel pools of the 7 cell lines at different ratios. Surrogate tissues will be analysed using Visium for FFPE samples and Curio Bioscience spatial platform for OTC fresh frozen samples.

Actual state of the project: Expecting results from BE1.

Expected data availability: December 2023

BE3: The cell lines for BE1 will be used to generate a combined scRNAseq and scATACseq experiment using 10X genomics technology for multi-omics.

Actual state of the project: Expecting results from BE1.

Expected data availability: July 2024

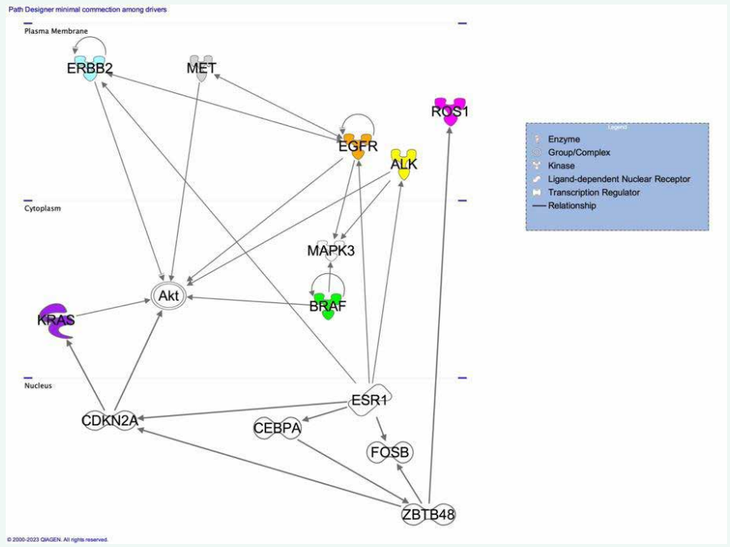


Figure 1.
The figure describes the driver genes associated to each cell line. Only a minimal part of the connections has been shown to easy readability of the image. Full list of the interactions depicted by IPA are available at figshare [10.6084/m9.figshar

FUTURE RESEARCH PLANS

At the heart of our mission lies the goal of pioneering innovative computational methods capable of unearthing hitherto undiscovered targetable genes in the realm of lung cancer. Leveraging the power and potential of single-cell omics data, we aim to revolutionize the landscape of cancer research by identifying elusive genetic targets that hold promise for therapeutic interventions. By harnessing cutting-edge algorithms and data analysis techniques, our endeavor seeks to unlock invaluable insights that could pave the way for novel treatment strategies and ultimately improve patient outcomes in the fight against lung cancer..

FUNDING ID (PAST 5 YERS)

- PNRR HPC Spoke 8, In Silico Medicine and Omics Data (2022-2025) EUR 744,000
- Retrieving, handling and analyzing publicly available TCRseq data (2021) EUR 120,000
- EPIGEN Bioinformatics work package (2012-2018) EUR 200,000

SELECTED PUBLICATIONS

- Chancellor A, Alan Simmons R, Khanolkar RC, Nosi V, Beshirova A, Berloffa G, Colombo R, Karuppiah V, Pentier JM, Tubb V, Ghadbane H, Suckling RJ, Page K, Crean RM, Vacchini A, De Gregorio C, Schaefer V, Constantin D, Gligoris T, Lloyd A, Hock M, Srikannathasan V, Robinson RA, Besra GS, van der Kamp MW, Mori L, Calogero R, Cole DK, De Libero G, Lepore M. Promiscuous recognition of MR1 drives self-reactive mucosal-associated invariant T cell responses. J Exp Med. 2023 Sep 4;220(9):e20221939. doi: 10.1084/jem.20221939.
- Mastini C, Campisi M, Patrucco E, Mura G, Ferreira A, Costa C, Ambrogio C, Germena G, Martinengo C, Peola S, Mota I, Vissio E, Molinaro L, Arigoni M, Olivero M, Calogero R, Prokoph N, Tabbò F, Shoji B, Brugieres L, Geoerger B, Turner SD, Cuesta-Mateos C, D'Aliberti D, Mologni L, Piazza R, Gambacorti-Passerini C, Inghirami GG, Chiono V, Kamm RD, Hirsch E, Koch R, Weinstock DM, Aster JC, Voena C, Chiarle R. Targeting CCR7-PI3Kγ overcomes resistance to tyrosine kinase inhibitors in ALK-rearranged lymphoma. Sci Transl Med. 2023 Jun 28;15(702):eabo3826. doi: 10.1126/scitranslmed.abo3826.
- Pernice S, Sirovich R, Grassi E, Viviani M, Ferri M, Sassi F, Alessandrì L, Tortarolo D, Calogero RA, Trusolino L, Bertotti A, Beccuti M, Olivero M, Cordero F. CONNECTOR, fitting and clustering of longitudinal data to reveal a new risk stratification system. Bioinformatics. 2023 May 4;39(5):btad201. doi: 10.1093/bioinformatics/btad201.
- Avesani S, Viesi E, Alessandrì L, Motterle G, Bonnici V, Beccuti M, Calogero R, Giugno R. Stardust: improving spatial transcriptomics data analysis through space-aware modularity optimization-based clustering. Gigascience. 2022 Aug 10;11:giac075. doi: 10.1093/gigascience/giac075.
- Alessandri L, Cordero F, Beccuti M, Licheri N, Arigoni M, Olivero M, Di Renzo MF, Sapino A, Calogero R. Sparsely-connected autoencoder (SCA) for single cell RNAseq data mining. NPJ Syst Biol Appl. 2021 Jan 5;7(1):1. doi: 10.1038/s41540-020-00162-6.
- Christodoulou C, Spencer JA, Yeh SA, Turcotte R, Kokkaliaris KD, Panero R, Ramos A, Guo G, Seyedhassantehrani N, Esipova TV, Vinogradov SA, Rudzinskas S, Zhang Y, Perkins AS, Orkin SH, Calogero RA, Schroeder T, Lin CP, Camargo FD. Live-animal imaging of native haematopoietic stem and progenitor cells. Nature. 2020 Feb;578(7794):278-283. doi: 10.1038/s41586-020-1971-z.
- Alessandrì L, Cordero F, Beccuti M, Arigoni M, Olivero M, Romano G, Rabellino S, Licheri N, De Libero G, Pace L, Calogero RA. rCASC: reproducible classification analysis of single-cell sequencing data. Gigascience. 2019 Sep 1;8(9):giz105. doi: 10.1093/gigascience/giz105.
- Kulkarni N, Alessandrì L, Panero R, Arigoni M, Olivero M, Ferrero G, Cordero F, Beccuti M, Calogero RA. Reproducible bioinformatics project: a community for reproducible bioinformatics analysis pipelines. BMC Bioinformatics. 2018 Oct 15;19(Suppl 10):349. doi: 10.1186/s12859-018-2296-x.
- Beccuti M, Cordero F, Arigoni M, Panero R, Amparore EG, Donatelli S, Calogero RA. SeqBox: RNAseq/ChIPseq reproducible analysis on a consumer game computer. Bioinformatics. 2018 Mar 1;34(5):871-872. doi: 10.1093/bioinformatics/btx674.
- Rodriguez-Fraticelli AE, Wolock SL, Weinreb CS, Panero R, Patel SH, Jankovic M, Sun J, Calogero RA, Klein AM, Camargo FD. Clonal analysis of lineage fate in native haematopoiesis. Nature. 2018 Jan 11;553(7687):212-216. doi: 10.1038/nature25168.

CANCER METABOLISM AND CACHEXIA (CaMeCa)

PORPORATO AND MENGA GROUP MEMBERS:

Elisabeth Wyart *Post-Doc*
Erica Mina *PhD Student*
Rita Vacca *Research Fellow*
Ivan Zaggia *Research Fellow*
Roberta Basile *Research Fellow*

RESEARCH ACTIVITY

Tumors are highly energy demanding to support survival and growth in a harsh environment. Hence, they promote nutrient mobilization from the body to support their increased metabolic demand. Ultimately promoting systemic wasting and cachexia. In particular, we are working on the metabolic competitions occurring between skeletal muscle and growing tumor with the aim of breaking the vicious circle which leads to skeletal muscle wasting and tumor growth. Mitochondrial metabolism plays a crucial role in cancer aggressiveness and immune escape, offering a fascinating perspective on the intricate relationship between cancer cells and the immune system. Understanding this connection can provide valuable insights for developing innovative cancer therapies. Cancer cells often outcompete immune cells for essential nutrients, such as glucose and amino acids, within the tumor microenvironment. By hijacking these resources, cancer cells can maintain their energy-intensive metabolism, while immune cells become metabolically compromised and less effective in their anti-tumor activities. Cancer cells can manipulate mitochondrial metabolism to produce metabolites and signaling molecules that suppress the immune response. For example, cancer cells may release lactate, which not only serves as an energy source but also acidifies the tumor microenvironment, impairing the function of immune cells and promoting their exhaustion. Moreover, the fusion and fission of mitochondria in cancer cells can impact the expression of immune checkpoint molecules, such as PD-L1, which inhibit the activity of immune cells like T cells. Understanding the interplay between mitochondrial metabolism and cancer aggressiveness, as well as immune evasion, has significant therapeutic implications. Alteration in metabolism eventually will lead to cachexia. Cachexia is a wasting syndrome characterized by devastating skeletal muscle atrophy that dramatically increases mortality in various diseases, most notably in cancer patients with a penetrance of up to 80%. Knowledge regarding the mechanism of cancer-induced cachexia remains very scarce, making cachexia an unmet medical need.

The Cancer Metabolism and Cachexia (CaMeCa) Lab is focused on the definition of metabolic dependencies occurring in cancer cell, immune landscape and in the host.

To this aim, we are working on multiple fronts to address various aspect of this metabolic interactions:

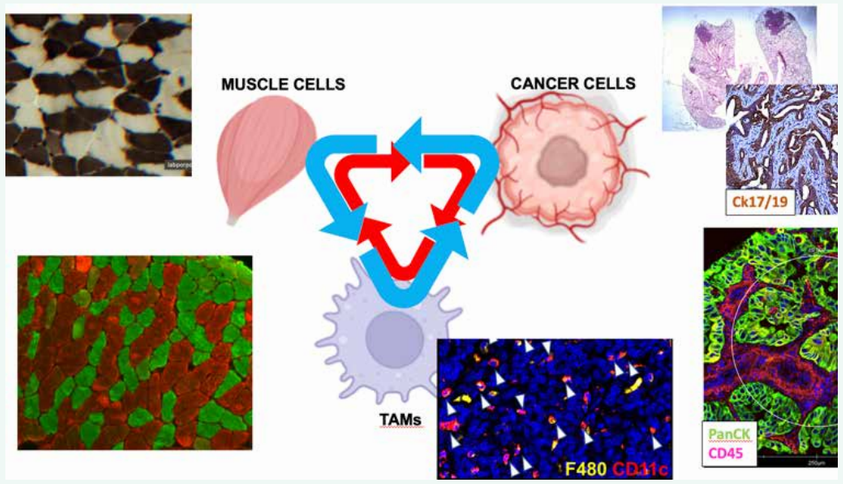


Figure 1.

The role of altered iron metabolism in regulating skeletal muscle function during tumor growth

We discovered strong alterations of iron metabolism in the skeletal muscle of both cancer patients and tumor-bearing mice, characterized by decreased iron availability in mitochondria. We found that modulation of iron levels directly influences myotube size in vitro and muscle mass in otherwise healthy mice. Furthermore, iron supplementation was sufficient to preserve both muscle function and mass, prolong survival in tumor-bearing mice, and even rescues strength in human subjects within an unexpectedly short time frame. Importantly, iron supplementation refuels mitochondrial oxidative metabolism and energy production. Overall, our findings provide new mechanistic insights in cancer-induced skeletal muscle wasting, and support targeting iron metabolism as a potential therapeutic option for muscle wasting diseases.

The impact of mitochondrial function and aminoacid metabolism in regulating cancer growth and immunoregulation

Targeting mitochondrial metabolism in cancer cells represents a promising avenue for treatment. Strategies include inhibiting glycolysis, targeting mitochondrial enzymes, or modulating mitochondrial dynamics to sensitize cancer cells to immune attack. Additionally, combining metabolic therapies with immunotherapies has shown promise in clinical trials. By disrupting cancer cell metabolism and simultaneously bolstering the immune response, these combination therapies have the potential to enhance treatment outcomes and overcome resistance mechanisms.

FUTURE RESEARCH PLANS:

To this aim, we are working on multiple fronts to address various aspect of this metabolic interactions: i) to study the mitochondrial metabolism of alternative amino acids as central player in the complex web of cancer aggressiveness and immune escape. Expanding our knowledge of these processes will provide opportunities for innovative cancer treatments that will disrupt cancer cell energetics and restore the immune system's ability to recognize and eliminate malignant cells. This intersection of metabolism and immunology holds great promise in the ongoing battle against cancer; ii) to understand the impact of altered metabolic activities in skeletal muscle on the development of tumor and its aggressiveness.

FUNDING ID:

- Bando POC-TOINPROVE
- Bando POC-LIFFT on the development of proprietary patent Novel BMP modulators and use thereof.
- Bando MFAG-AIRC My First AIRC Grant. "Targeting crosstalk between muscle and cancer" (2019-2024),
- Fondo Rita Levi-Montalcini 2014 "Understanding and targeting cancer-related muscle atrophy" (2016-2019)
- Bando Finalizzata Giovani Ricercatori (2023-26): "NF-Kb positively regulates Fatty Acid Oxidation in cancer cells, which hinders CD8+ T lymphocytes and promotes tumor progression". PI: Alessio Menga. GR-2021-12374957
- Bando MFAG-AIRC My First AIRC Grant (2021-26): "Dissecting mitochondrial lysine and tryptophan metabolism to target metabolic symbiosis in lung adenocarcinoma". PI: Alessio Menga MFAG, Rif. 25908

SELECTED PUBLICATIONS

- Wyart E, Hsu M, Sartori R, Mina E, Rausch V, Pierobon ES, Mezzanotte M, Pezzini C, Bindels LB, Penna F, Lauria A, Filigheddu N, Hirsch E, Martini M, Roetto A, Geninatti-Crich S, Prenen H, Mazzone M, Sandri M, Menga A, and Porporato PE. Iron supplementation is sufficient to rescue cancer-induced muscle wasting and function. EMBO Reports, e53746 (2022)
- Wyart E, Reano S, Longo D, Ghigo A, Riganti C, Porporato PE. Metabolic characterization of a new model of PDAC-induced cancer cachexia, Oxid Med Cell Longev. 2018 Feb 26;
- Payen VL, Hsu MY, Rädecke KS, Wyart E, Vazeille T, Bouzin C, Porporato PE*, Sonveaux P. Monocarboxylate Transporter MCT1 Promotes Tumor Metastasis Independently of Its Activity as a Lactate Transporter. Cancer Res 2017;77(20):5591-5601. doi: 10.1158/0008-5472.CAN-17-0764. *co-last
- Porporato PE, Filigheddu N, Pedro JMB, Kroemer G, Galluzzi L. Mitochondrial metabolism and cancer. Cell Res. 2018 Mar;28(3):265-280.
- Brisson L, Bański P, Sboarina M, Dethier C, Danhier P, Fontenille MJ, Van Hée VF, Vazeille T, Tardy M, Falces J, Bouzin C, Porporato PE, Frédérick R, Michiels C, Copetti T, Sonveaux P. Lactate Dehydrogenase B Controls Lysosome Activity and Autophagy in Cancer. Cancer Cell 2016
- Porporato PE, Filigheddu N, Reano S, Ferrara M, Angelino E, Gnocchi V, Prodam F, Ronchi G, Fagoone S, Chianale F, Chianale F, Baldanzi G, Sinigaglia F, Surico N, Perroteau I, Smith R, Sun Y, Geuna S, Graziani A. Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. J Clin Invest. 2013 Jan 2;123(2):611-22.
- Porporato PE, Payen VL, De Saedeleer CJ, Préat V, Thissen JP, Feron O, Sonveaux P. Lactate stimulates angiogenesis and accelerates the healing of superficial and ischemic wounds in mice. Angiogenesis. 2012 Jun 3;15(4):581-92.
- Menga A, Favia M, Spera I, Vegliante MC, Gissi R, De Grassi A, Laera L, Campanella A, Gerbino A, Carrà G, Canton M, Loizzi V, Pierri CL, Cormio G, Mazzone M, Castegna A. N-acetylaspartate release by glutaminolytic ovarian cancer cells sustains protumoral macrophages. EMBO Rep. 2021 Jul 14:e51981. doi: 10.15252/embr.202051981. Epub ahead of print. PMID:34260142. 2-s2.0-85109871059
- Menga A, Serra M, Todisco S, et al. Glufosinate constrains synchronous and metachronous metastasis by promoting anti-tumor macrophages [published online ahead of print, 2020 Sep 4]. EMBO Mol Med. 2020; e11210. doi:10.15252/emmm.201911210. 2-s2.0-85090134974
- Palmieri EM, Menga A, Martín-Pérez R, Quinto A, Riera-Domingo C, De Tullio G, Hooper DC, Lamers WH, Ghesquière B, McVicar DW, Guarini A, Mazzone M & Castegna A (2017) Pharmacologic or Genetic Targeting of Glutamine Synthetase Skews Macrophages toward an M1-like Phenotype and Inhibits Tumor Metastasis. Cell Rep. 20, 1654–1666. 2-s2.0-85027849350

PAOLO E. PORPORATO

CANCER METABOLISM AND CACHEXIA (CaMeCa) GROUP

BIOGRAPHICAL SKETCH

2020-to date	Associate Professor in Experimental Biology at the University of Turin, Torino, Italy
2016-2019	Assistant Professor in Experimental Biology at the University of Turin, Torino, Italy.
2015	Visiting professor in Biochemistry at the University of Piemonte Orientale (UPO), Novara, Italy
2013-2016	post-doctoral research scientist (Chargé de recherche), Unit of Pharmacology & Therapeutics, Research
2018-2014	Post doctoral fellow at the Dept. of Molecular Biotechnologies and Health Sciences and Molecular Imaging Center (www.cim.unito.it) of the University of Torino

See the group resarch at page 68

EDUCATION AND TRAINING

- M.Sc. in Medical Biotechnologies, University of Turin, 2004, with a focus on Iron Metabolism.
- Ph.D. in Molecular Medicine, University of Piemonte Orientale “A. Avogadro”, 2009, specializing in Muscle Mass Homeostasis.
- Postdoctoral training in Cancer Metabolism and Angiogenesis, University of Louvain Medical School, 2010.

HONORS

- Eliezer Rachmilewitz Prize Recipient,at BioIron EMBL (2019) “Role of Iron metabolism in cancer cachexia”
- Prix d’Alvarenga, de Piauhy (2015) “Role of mitochondrial superoxide targeting on tumor signaling and metastasis prevention”. Royal Belgian Academy of Medicine
- Best scientific poster at the Biowin day 2012, Louvain LN, Belgium 28 Nov 2012.
- Aegean Conference Trainee Award Tumor microenvironment and cellular stress conference, Chania Greece, 4-9 Oct, 2012

COMMISSIONS OF TRUST AND MEMBERSHIPS

- Selected reviewer for PNAS, Oncogene, Science Signalling, Science Advances, Oncogenesis, Oncotarget, American Journal of Physiology, Trends in Cancer, Journal of Translational Medicine, Frontiers in Oncology, IJMS, Cancer Res
- Grant Reviewer: Health Research Council (NZ) 2021, Swiss National Science Foundation-SNSF (CH)2020, MRC-University of Cambridge 2020,2019; National Fellowship Committee for Graduate Women in Science 2019; Research Foundation Flanders – FWO 2018, Luxembourg National Research Fund

ALESSIO MENGA

CANCER METABOLISM AND CACHEXIA (CaMeCa) GROUP

BIOGRAPHICAL SKETCH

2/2022 - to date	Researcher PON (RTDa-BIO/13)- junior lecturer at Molecular Biotechnology Center, University of Turin. Mentor: Prof. Paolo Porporato
6/2019 to 1/2022	Postdoctoral Researcher at Molecular Biotechnology Center, University of Turin. PI: Prof. Paolo E Porporato
1/2019 to 5/2019	FEBS and ViB Postdoctoral Fellow, at Laboratory of Tumor Inflammation and Angiogenesis, VIB Center for Cancer Biology, Department of Oncology, University of Leuven, Belgium. PI: Prof. Massimiliano Mazzone.
2016-2018	PhD in Pharmaceutical and Biomolecular Science at the Department of Molecular Biotechnologies & Health Sciences, University of Torino
2008-2010	unior Researcher at the I.R.C.C.S. Oncologic Hospital “Giovanni Paolo II” Bari, Italy. PI: Prof. Alessandra Castegna

See the group resarch at page 68

EDUCATION AND TRAINING

- Master Degree in Pharmacy, University of Bari, 12/11/2009
- Ph.D. in Biochemical and Pharmacological Sciences, University of Bari, 25/03/2013

HONORS

- FEBS Fellowship Short-Term (2 Months) at Laboratory of Tumor Inflammation and Angiogenesis, VIB Center for Cancer Biology, Department of Oncology, University of Leuven, Leuven, Belgium. PI: Prof. Massimiliano Mazzone, PhD
- EMBO Fellowship Short-Term (3 Months) at Laboratory of Tumor Inflammation and Angiogenesis, VIB Center for Cancer Biology, Department of Oncology, University of Leuven, Leuven, Belgium. PI: Prof. Massimiliano Mazzone, PhD
- TRANSMIT best poster award: “Pharmacological Targeting of Glutamine Synthetase Skews Macrophages Toward An Inflammatory Phenotype and Inhibits Metastasis”: Alessio M, Erika Mariana P, Massimiliano M & Alessandra C. Course in Cancer Metabolism in Bertinoro on November 29-30, 2018.
- EACR best poster award: “Overexpression of the mitochondrial S-Adenosylmethionine carrier in cervical cancer cells leads to rewiring of the methyl metabolism and sensitivity to cisplatin”. Alessio M, Erika Mariana P, Antonia C, Vito I, Alessandra C. Bertinoro 19-21 October 2017 ISCaM2017 - 4th Annual Meeting - Cancer Metabolism
- OECI Meeting Bursary award: OECI Meeting Bursary to attend the 8th Edition of the “Molecular Pathology Approach to Cancer” training course, Amsterdam 4-6 June 2018.

GIULIA CARON

CASSMedChem Lab

BIOGRAPHICAL SKETCH

Since 2013	Associate Professor, Dept. Molecular Biotechnology and Health Sciences, University of Turin, (I).
1999-2012	Assistant Professor, Pharmacy Faculty, University of Turin (I).
1997-1998	Postdoctoral Fellow, University of Lausanne (CH)
1994-1997	PhD in Pharmaceutical Sciences, University of Lausanne (CH)
1994	Master's Degree in Pharmacy, University of Turin (I)
1992	Master's Degree in Medicinal Chemistry and Pharmaceutical Technology, University of Turin I()

The CASSMedChem members

Giuseppe Ermondi *Associate Professor*
Maura Vallaro *Technician*
Matteo Rossi Sebastiano *Research fellow*
Diego Jimenez Garcia *PhD student*
Giulia Apprato *PhD student*

RESEARCH ACTIVITY

Scenario: drug discovery in the beyond-Rule-of-5 (bRo5) chemical space

The increasing understanding of biological systems is providing a range of new difficult-to-drug targets with small-molecule approaches. Other types of molecules, or modalities, are therefore required in modern drug discovery and Targeted Protein Degradation (TPD) and macrocycles (MCs) are two of the most promising.

At the forefront of the TPD field is the small molecule bifunctional degrader (a general term commonly referred to as PROteolysis Targeting Chimeras [PROTACs]). A PROTAC (Fig. 1A) consists of a ligand (warhead) for a target protein of interest (POI), covalently bound via a linker to an E3 ligase complex ligand. TPD technology functions by initiating the formation of a ternary complex between the POI, the PROTAC (Fig. 1B) and the E3 ligase, resulting in polyubiquitination of the POI and subsequent degradation by the 26S proteasome machinery. It is essential for the PROTAC to establish a stable ternary complex, whose stability affects the degradation potential.

In practice, by harnessing the cell's own disposal system to degrade proteins of choice, PROTACs can eliminate otherwise undruggable disease-causing proteins. PROTACs technology exhibits several advantages in a drug discovery context. For instance, it does not need to have a high affinity with the target to degrade. Moreover, because of the catalytic mechanism, a low dose is required, and thus off-target toxicity is reduced. PROTACs also limit drug resistance since they do not cause overexpression of target proteins and mutations of the target rarely affect the outcome. Overall, the potential of TPD as therapeutics is enormous and the number of heterobifunctional degraders in advanced development is rapidly growing. Although many disease areas stand to benefit from this modality, the greatest impact is for degraders against oncology targets, for instance, two PROTACs targeting the AR and ER are already in Phase 2 clinical trials, whereas other candidates targeting oncoproteins are in Phase1.

Macrocycles are defined as organic molecules which contain a ring of at least 12 heavy atoms. The benefit of MCs as drugs is that they can provide functional diversity and stereochemical complexity in a semirigid, preorganized structure. As compared to ring-opened analogues, this can allow MCs to bind with higher affinity and selectivity to targets.

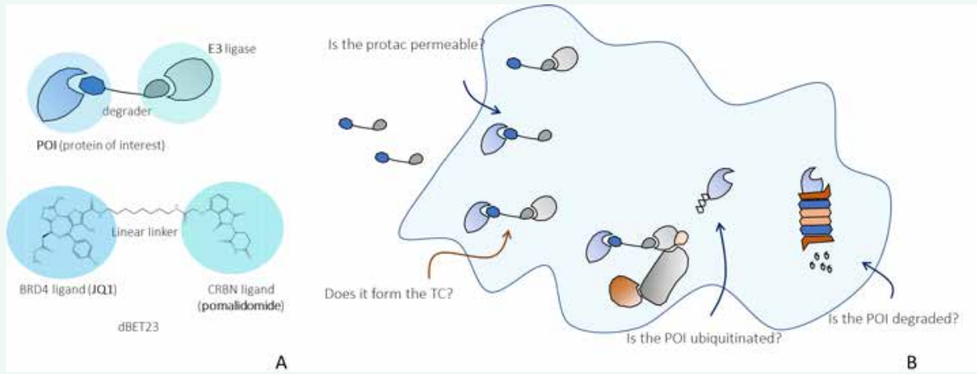


Figure 1.
PROTAC A) general structure and a representative compound and B) mechanism of action

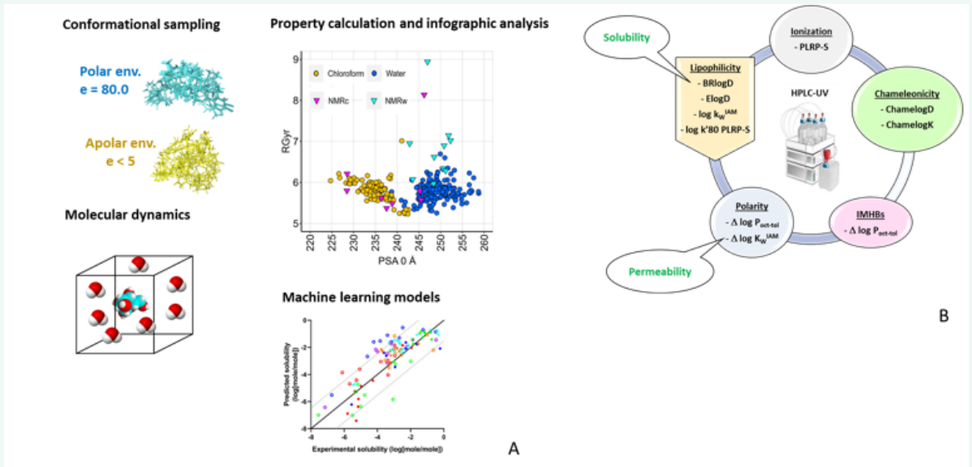


Figure 2.
Strategies applied by the CASSMedChem team A) computational and B) experimental

PROTAC targets are intracellular proteins, and their mode of action requires them to be cell permeable. Moreover, PROTAC and MC oral administration offers convenience for patients and improves compliance. Notably, most PROTACs in clinical trials and some commercial macrocyclic drugs are oral available, supporting that obtaining oral bRo5 drugs is a reachable goal. However, achieving oral bioavailability includes efforts to adjust absorption and thus the optimization of physicochemical properties, such as lipophilicity and polarity and in vitro ADME properties like solubility and permeability.

Aim: ranking bRo5 drug candidates for their oral bioavailability potential

Literature supports that there is a need for structural, physicochemical and in vitro ADME descriptors impacting the bRo5 drug metabolism and pharmacokinetic (DMPK) profiles. The main aim of the CASSMedChem team is therefore to design and produce innovative high quality structural, physicochemical and in vitro ADME data and build guidelines to discover oral bioavailable MCs and PROTACs, i.e. molecules with a future as oral drugs.

Methods

A battery of computational and experimental techniques (Fig. 2) are used and combined to reach the goal.

The computational part (Fig. 2A) first includes the production of conformer ensembles in polar and nonpolar environments, mimicking the molecular behavior in water and membrane core, respectively. Conformational sampling and molecular dynamic techniques are applied in this phase. On the resulting conformer ensembles, we calculate Polar Surface Area (3D PSA) to quantify polarity, Radius of gyration (Rgyr) to characterize molecular size and shape and Intramolecular Hydrogen Bonds (IMHBs) formation. Finally, the data matrix is analysed with infographic and machine learning tools to obtain models that predict physicochemical and in vitro ADME properties of MCs and PROTACs. All the models are then combined to obtain a ranking tool for oral bioavailability and identify structural features responsible for it.

The experimental part (Fig. 2B) includes the physicochemical and in vitro ADME characterization of MCs and PROTACs with a pool of chromatographic descriptors previously reported by the CASSMedChem team and suitable for the bRo5 chemical space. Remarkably, we recently set-up a chameleonicity descriptor (Chamelogk) that quantifies the molecular skills to adapt to the environment. Chamelogk is therefore a unique and specific tool for bRo5 compounds. Experimental data are used to validate models obtained with computational strategies and as an input to generate more models.

FUTURE RESEARCH PLANS

Collaboration with pharma and biotech companies will allow to apply the obtained guidelines on commercial derivatives on large proprietary datasets. Finally, the optimised strategies will be used to obtain a few potential oral bioavailable PROTAC drug candidates targeting POIs related to anticancer activity in collaboration with other teams of our department.

FUNDING ID (PAST 5 YERS)

- Research contracts with R&D departments of leading international pharma/biotechnological companies (Amgen, Boheringer Ingelheim, Chiesi Farmaceutici, Kymera, Pfizer)

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=7005430971

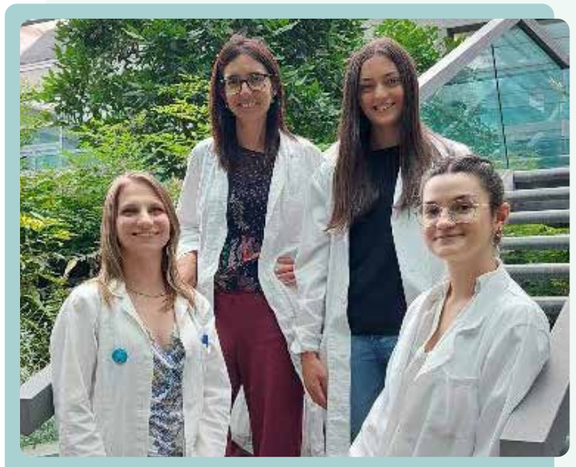
- Apprato G., Ermondi G. and Caron G. The Quest for Oral PROTAC drugs: Evaluating the Weaknesses of the Screening Pipeline ACS Med. Chem. Lett., 14, 7, 879 (2023).
- García Jiménez, D., … and Caron, G. Chamelogk: a chromatographic chameleonicity quantifier to design orally bioavailable beyond-Rule-of-5 drugs J. Med. Chem. 66, 15, 10681 (2023).
- Apprato, G., … and Caron, G. In Silico Tools to Extract the Drug Design Information Content of Degradation Data: The Case of PROTACs Targeting the Androgen Receptor Molecules, 28 (3), 1206 (2023).
- García Jiménez, D., … and Caron, G. Designing Soluble PROTACs: Strategies and Preliminary Guidelines J. Med, Chem., 65 (19), 12639-12649 (2022).
- Rossi Sebastiano, M., … and Caron, G.. Refinement of Computational Access to Molecular Physicochemical Properties: From Ro5 to bRo5 J. Med. Chem. 65 (18), 12068 (2022).
- Ermondi, G., … and Caron, G. Rational Control of Molecular Properties Is Mandatory to Exploit the Potential of PROTACs as Oral Drugs ACS Med. Chem. Lett., 12 (7), 1056 (2021).
- Caron, G., et al. Steering New Drug Discovery Campaigns: Permeability, Solubility, and Physicochemical Properties in the bRo5 Chemical Space ACS Med. Chem. Lett., 12 (1), 13-23 (2021).
- Poongavanam, V.… and Caron, G., Kihlberg, J. Predicting the Permeability of Macrocycles from Conformational Sampling – Limitations of Molecular Flexibility J. Pharm. Sci., 110 (1), 301 (2021).
- Poongavanam, V., … Caron, G., Kihlberg, J. Predicting the Permeability of Macrocycles from Conformational Sampling – Limitations of Molecular Flexibility J. Pharm. Sci., 110 (1), 301, (2021).
- Ermondi, G., Vallaro, M., Caron, G. Degraders early developability assessment: face-to-face with molecular properties DDT, 25 (9), 1585, (2020).

DEBORAH CHIABRANDO

Neuro-Metabolism Research Lab

BIOGRAPHICAL SKETCH

Since 2021	RTDB, Dept. of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy
2020-2021	RTDA Dept. of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy
2011-2020	PostDoc Fellow at the Molecular Biotechnology Center, University of Turin, Turin, Italy
2007-2010	PhD Student at the Department of Genetics, Biology and Biochemistry and Molecular Biotechnology Center, University of Turin, Turin, Italy
2019	International Master in Peripheral Nervous System Disorder. University of Milan, Milan, Italy
2018	National Scientific Abilitation in Applied Biology (BIO/13)
2006	Master's degree in Molecular Biotechnology, Faculty of Biotechnology, University of Turin, Turin, Italy
2004	Degree in Molecular Biotechnology, Faculty of Biotechnology, University of Turin, Turin, Italy



The CASSMedChem members

Francesca Bertino – *Postdoc*
Elisa Quarta *PhD student*
Diletta Diletta Isabella Zanin *PhD student*
Livia Metani *Undergraduate student*

RESEARCH ACTIVITY

Mitochondria serve as the central hub for cellular metabolism, orchestrating energy production, nutrient utilization, and signaling pathways that are vital for maintaining proper cellular function and homeostasis. During neurodevelopment, mitochondria play a crucial role in regulating the proliferation, differentiation, migration, and survival of neuronal progenitors. In adulthood, maintaining proper metabolic control is essential to support the long-term survival of neurons, particularly those in the peripheral nervous system or the retina, which have high energy requirements.

We are interested in rare neurodegenerative disorders linked to mitochondrial metabolism. Our current research focuses on a group of autosomal recessive disorders caused by FLVCR1 mutations. FLVCR1-related diseases include Posterior Column Ataxia and Retinitis Pigmentosa (PCARP), non-syndromic Retinitis Pigmentosa (RP) and Hereditary Sensory and Autonomic Neuropathies (HSAN).

These disorders are characterized by the progressive degeneration of sensory neurons responsible for proprioception and nociception as well as degeneration of photoreceptors. The loss of these specific neuronal subtypes leads to sensory ataxia, pain insensitivity and vision loss. FLVCR1 has been implicated in the regulation of heme metabolism, choline uptake and overall energetic metab-

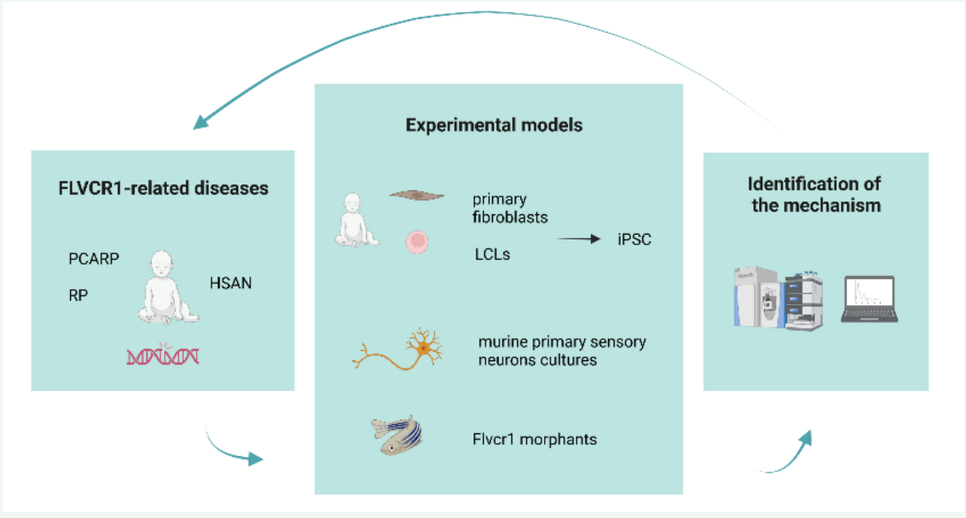


Figure 1.
Elucidating the mechanisms underlying FLVCR1-related disorders. FLVCR1-disorders encompass a group of genetic diseases due to mutations in the heme exporter FLVCR1. FLVCR1-related disorders include Posterior Column Ataxia and Retinitis Pigmentosa (RP) and Hereditary Sensory and Autonomic Neuropathies (HSAN). These disorders are characterized by the progressive degeneration of sensory neurons responsible for proprioception and nociception as well as degeneration of photoreceptors. The loss of these specific neuronal subtypes leads to sensory ataxia, pain insensitivity and vision loss. To study the molecular mechanisms underlying these disorders we developed several experimental models: (i) patients derived fibroblasts and lymphoblastoid cells (LCLs); (ii) patients derived induced pluripotent stem cells (iPSCs) that will be differentiated into specific neuronal types; (iii) murine and zebrafish models of the disease; (iv) murine primary sensory neurons. We plan to use a combination of genetic, metabolic, and proteomic approaches to elucidate the underlying molecular mechanisms and to identify therapeutic targets.

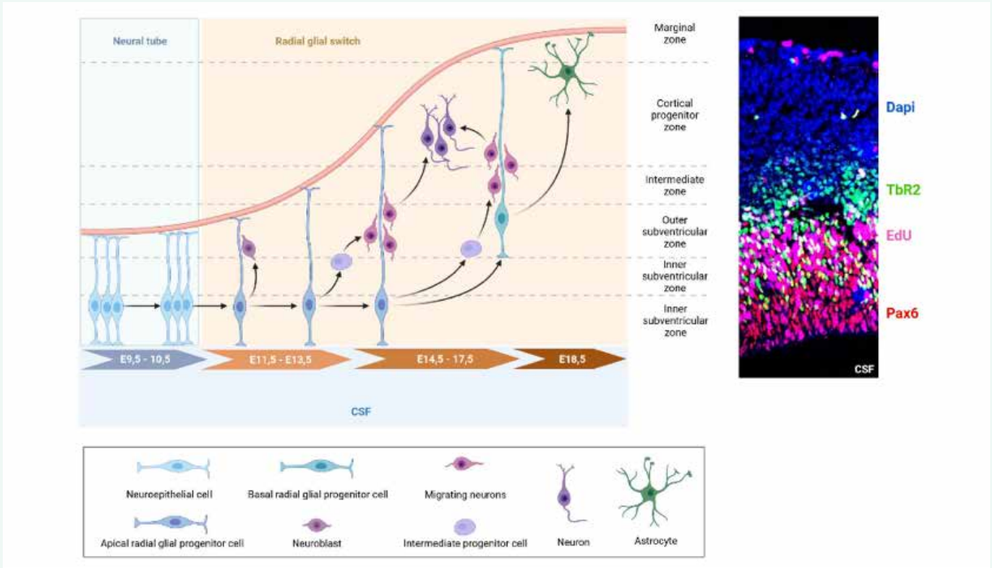


Figure 2.
Spatiotemporal development of the cerebral cortex in mice. The cartoon illustrates the key steps in mouse cortical neurogenesis. On the right, a representative immunofluorescence performed on a E14,5 murine cortex section is shown. (Pax6=marker of neuronal progenitors; EdU=staining for proliferating cells; TbR2= marker of intermediate progenitors; Dapi=nuclei staining).

olism but the pathogenetic mechanisms underlying the diseases are still unclear. Our research aims to elucidate the role of FLVCR1 in the nervous system and to uncover the molecular mechanisms leading to sensory neurons and photoreceptors failure in FLVCR1-related diseases.

To this end, we have developed several experimental models: (i) patients derived fibroblasts and lymphoblastoid cells (LCLs); (ii) patients derived induced pluripotent stem cells (iPSCs) that will be differentiated into specific neuronal types; (iii) murine and zebrafish models (iv) murine primary sensory neurons. We plan to use a combination of genetic, metabolic, and proteomic approaches to elucidate the underlying molecular mechanisms and to identify therapeutic targets (Fig.1). Our long-term goal is to discover a therapeutic entry point to effectively treat rare neurological disorders.

FUTURE RESEARCH PLANS

Our future research aims are: (1) to develop novel experimental models of FLVCR1-related disease; (2) to unravel the molecular mechanisms responsible for the degeneration of sensory neurons and photoreceptors in FLVCR1-related diseases; (3) to elucidate the role of FLVCR1 during the development of the nervous system (Fig. 2), with a particular focus on the pathogenesis of congenital hydrocephalus; (4) to get mechanistic insights into heme trafficking, by focusing on the role of different isoforms of the FLVCR1 gene.

FUNDING ID (PAST 5 YERS)

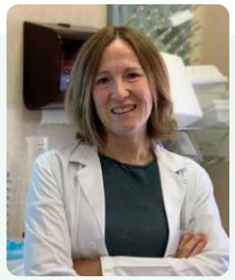
- 2023-2025 Principal Investigator (PI) in the PRIN project entitled “Unlocking the structure and function of the heme transporter FLVCR1”. Grant number: 2022PX3SR3
- 2023-2025 Principal Investigator (PI) in the “Fondazione Telethon ETS” project, entitled “Posterior Column Ataxia and Retinitis Pigmentosa: new pathogenetic insights from the study of mitochondria-associated membranes”. Grant number: GMR22T1076
- 2021-2025 Co-PI in the project entitled “Defective heme transport in the development of congenital hydrocephalus”, supported by the National Institute of Health (NIH). Grant number: R01 NS123168-01

SELECTED PUBLICATIONS:

- Fiorito V, Allocco AL, Petrillo S, Gazzano E, Torretta S, Marchi S, Destefanis F, Pacelli C, Audrito V, Provero P, Medico E, Chiabrando D, Porporato PE, Cancelliere C, Bardelli A, Trusolino L, Capitanio N, Deaglio S, Altruda F, Pinton P, Cardaci S, Riganti C, Tolosano E. The heme synthesis-export system regulates the tricarboxylic acid cycle flux and oxidative phosphorylation. Cell Reports 2021, doi: 10.1016/j.celrep.2021.109252.
- Chiabrando D, Fiorito V, Petrillo S, Bertino F, Tolosano E. HEME: a neglected player in nociception? Neuroscience and Biobehavioural Reviews 2021, doi: 10.1016/j.neubiorev.2021.01.011.
- Bertino F, Firestone K, Bellacchio E, Jackson KE, Asamoah A, Hersh J, Fiorito V, Destefanis F, Gonser R, Tucker ME, Altruda F, Tolosano E and Chiabrando D. HEME AND SENSORY NEUROPATHY: INSIGHTS FROM NOVEL MUTATIONS IN THE HEME EXPORTER FLVCR1. PAIN, 2019, doi: 10.1097/j.pain.0000000000001675
- Fiorito V*, Chiabrando D* and Tolosano E. MITOCHONDRIAL TARGETING IN NEURODEGENERATION: A HEME PERSPECTIVE. Pharmaceuticals, 2018, doi: 10.3389/fnins.2018.00712
- Petrillo S, Chiabrando D, Genova T, Fiorito V, Ingoglia G, Vinchi F, Mussano F, Carossa S, Silengo L, Altruda F, Merlo GR, Munaron L and Tolosano E. HEME ACCUMULATION IN ENDOTHELIAL CELLS IMPAIRS ANGIOGENESIS BY TRIGGERING PARAPTOSIS. Cell Death and Differentiation, 2018, doi: 10.1038/s41418-017-0001-7
- Castori M, Morlino S, Ungelenk M, Pareyson D, Salsano E, Grammatico P, Tolosano E, Kurth I and Chiabrando D. POSTERIOR COLUMN ATAXIA WITH RETINITIS PIGMENTOSA COEXISTING WITH SENSORY-AUTONOMIC NEUROPATHY AND LEUKEMIA DUE TO A RECURRENT FLVCR1 MUTATION. Am Journal Medical Genetics B, 2017, doi: 10.1002/ajmg.b.32570
- Chiabrando D, Castori M, di Rocco M, Ungelenk M, Gießelmann S, Di Capua M, Madeo A, Grammatico P, Bartsch S, Hübner CA, Altruda F, Silengo L, Tolosano E, Ingo Kurth. MUTATIONS IN THE HEME EXPORTER FLVCR1 CAUSE SENSORY NEURODEGENERATION WITH LOSS OF PAIN PERCEPTION. PLOS Genetics, 2016, doi: 10.1371/journal.pgen.1006461
- Chiabrando D, Vinchi F, Fiorito V, Mercurio S and Tolosano E. HEME IN PATHOPHYSIOLOGY: A MATTER OF SCAVENGING, METABOLISM AND TRAFFICKING ACROSS CELL MEMBRANES. Frontiers in Pharmacology, 2014, doi: 10.3389/fphar.2014.00061
- Vinchi F, Ingoglia G, Chiabrando D, Mercurio S, Turco E, Silengo L, Altruda F and Tolosano E. HEME EXPORTER FLVCR1A REGULATES HEME SYNTHESIS AND DEGRADATION AND CONTROLS ACTIVITY OF CYTOCHROMES P450. Gastroenterology, 2014, doi: 10.1053/j.gastro.2014.01.053
- Chiabrando D, Marro S, Mercurio S, Giorgi C, Petrillo S, Vinchi F, Fiorito V, Fagoonee S, Camporeale A, Turco E, Merlo GR, Silengo L, Altruda F, Pinton P and Tolosano E. THE MITOCHONDRIAL HEME EXPORTER FLVCR1B MEDIATES ERYTHROID DIFFERENTIATION. Journal of Clinical Investigation, 2012. doi: 10.1172/JCI62422

MARTA COSCIA

Laboratory of Translational Hematology

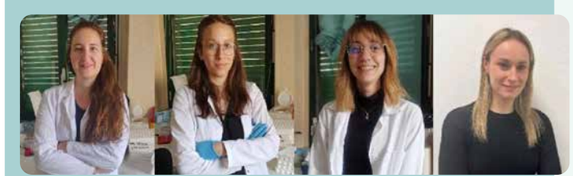


BIOGRAPHICAL SKETCH

2020-2023	Director of the Postgradute School of Hematology, University of Torino
2019	Associate Professor of Hematology, Department of Molecular Biotechnology and Health Sciences, University of Torino
2015	Head of the clinical research group on chronic lymphoproliferative diseases, Division of Hematology, Città della Salute e della Scienza University, Hospital of Torino - Via Genova, 10126, Torino
2015	Head of the Laboratory of Translational Hematology, Department of Molecular Biotechnology and Health Sciences, University of Torino
2009	PhD in Immunology and Cell Biology, University of Torino
2007	Staff physician, Division of Hematology, Città della Salute e della Scienza University Hospital of Torino - Via Genova, 3 10126, Torino
2007–2019	Assistant Professor, Department of molecular biotechnology and health sciences, University of Torino
2003–2004	Visiting Scientist hired by S.A.I.C. Inc., Experimental transplantation and Immunology Branch, National Cancer Institute, National Institute of Health, Bethesda, MA, U.S.A.
2002–2003	Visiting scientists at National Cancer Institute (NCI/NIH), Bethesda, MD, U.S.A.
1998–2002	Post-doctoral fellow, Post-graduate School of Hematology, Department of Medicine and Experimental Oncology, University of Torino

CLINICAL TEAM

Candida Vitale *MD PhD - Tenure track researcher*
Francesca Perutelli *MD - Hematology fellow*
Maria Chiara Montalbano *MD - Hematology fellow*
Velleda Zorzetto, *MS Clinical Study Coordinator*



LABORATORY TEAM

Valentina Griggio *PhD - Postdoctoral fellow*
Rebecca Jones *MS - PhD student*
Giorgia Mancin *MS - Research fellow*
Giacomo Ortone *medical school student (MD/PhD program)*
Giulia Bondielli *MS - Clinical Study Coordinator*

RESEARCH ACTIVITY

The research activity of the Laboratory of Translational Hematology is focused on:

- The evaluation of immune dysfunctions occurring in patients with lymphoproliferative diseases, with a special interest in patients with chronic lymphocytic leukemia (CLL).(Fig.1)
- The impact of conventional and novel targeted therapies on the immune system of patients with lymphoproliferative diseases and CLL. (Fig. 2)
- The study of intrinsic, microenvironment-related or immune-mediated mechanisms favouring tumor progression and inducing resistance to apoptosis and to drug-induced cell death in CLL cells.
- Evaluation of vaccine response in patients affected by lymphoproliferative diseases and impact of targeted treatment on vaccine response (Fig3).
- Development of novel strategies of adoptive immunotherapy, with the aim of improving anti-tumor efficacy and reducing side effects in lymphoproliferative diseases. Study of the impact of targeted drugs on the generation and anti-tumor potency of CAR-T cells in CLL

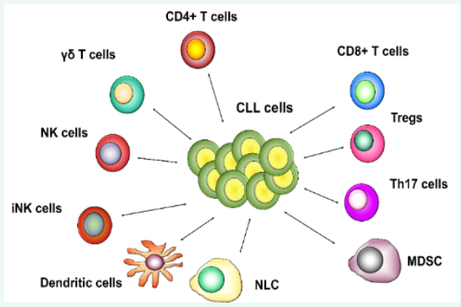


Figure 1.
CLL is characterized by a wide range of tumor-induced alterations, which progressively accumulate during disease evolution. Reviewed in Griggio V et al. Front Immunol.2021

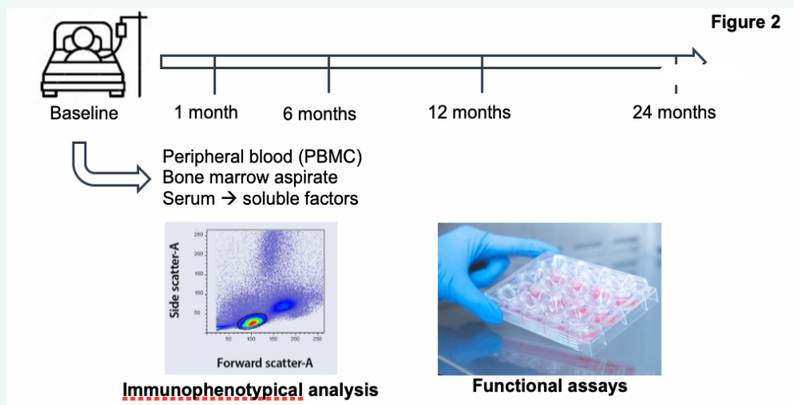


Figure 2.
Schematic representation of immunophenotypcal and functional assays on patients affected by CLL and treated with target therapies

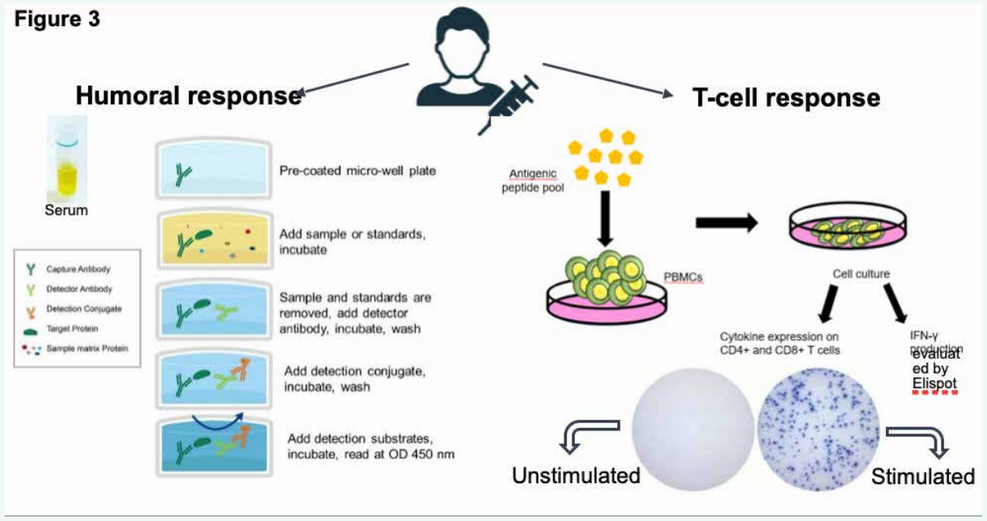


Figure 3.
Humoral and T cell response evaluation in patients affected by lymphoproliferative diseases

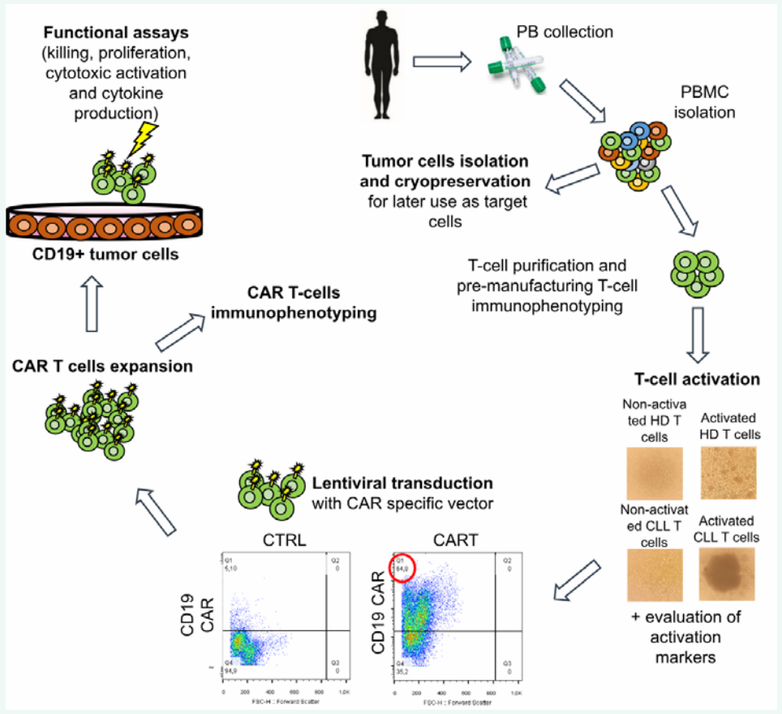


Figure 4.
Anti-CD19 CAR-T cell production workflow applied in our laboratory

FUTURE RESEARCH PLANS

- To extend currently ongoing projects:
  1. Immune reconstitution in patients undergoing treatment with new targeted agents;
  2. Impact of targeted drugs treatment on CAR T-cell generation and functionality and on the metabolic profile of pre-manufacturing T cells and post-manufacturing CAR T cells;
- Optimization of the CAR T generation process in CLL To evaluate the impact of incorporation of immune cell modifiers in the CAR T-cell manufacturing process.
- To study the polymorphisms of transmembrane transporters and metabolic enzymes in primary cells from patients with CLL treated with target therapies and to evaluate the impact of polymorphisms on clinical parameters, response to therapy and drug resistance.

FUNDINGS

Projects funded from competitive calls:

- Fondazione GIMEMA Onlus: “Impact of treatment with targeted therapies on the generation of effective CAR T CELLS in patients with chronic lymphocytic leukemia (2020 – present). Role: Principal Investigator.
- PRIN call 2022, “Overcoming T cell impairment in CLL to bust CAR-T cell function”. Role: Head of research unit

Research contract with Foundations and Private Companies:

- Research contract with Abbvie S.r.l.: “Immunomodulatory effects of venetoclax treatment in patients with chronic lymphocytic leukemia”, Role: Principal Investigator (2021 – present).
- Research contract with Karyopharm Therapeutics Inc., a Delaware corporation: “In vivo evaluation of the activity of Selinexor (KPT-330) in combination with bendamustine or idelalisib in chronic lymphocytic leukemia”, Role: Principal Investigator (2018 – present).
- Research contract with Janssen Research & Development, LLC: “Immunomodulatory effects of single-agent ibrutinib treatment in chronic lymphocytic leukemia patients”, Role: Principal Investigator (2016 – present).

PUBBLICATIONS

- Salvetti C et al. Front Oncol. 2022. doi: 10.3389/fonc.2022.917115.
- Vitale C et al. Cancers (Basel). 2021. doi: 10.3390/cancers13122883.
- Vitale C et al. Salvetti Blood 2021. doi: 10.1182/blood.2020008201.
- Griggio V et al. Haematologica. 2020. doi: 10.3324/haematol.2019.217430.
- Griggio V et al. Oncotarget. 2017; 8:3274-3288.
- Coscia M et al. Blood. 2012; 120:3271-9.
- Coscia M et al. Leukemia. 2011; 25:828-37.
- Coscia M et al. J Cell Mol Med. 2010; 14:2803-15.
- Coscia M et al. Leukemia. 2004; 18:139-45

SILVIA DEAGLIO

Laboratory of Functional Genomics

BIOGRAPHICAL SKETCH

I obtained my MD degree (1998), Board Certification in Oncology (2002) and PhD in Genetics (2006) from the University of Turin. I then trained at the Transplant Immunology Unit of the Beth Israel Deaconess Medical Center of Harvard University, where we identified molecular components and genetic regulation of the suppressive machinery of regulatory T cells. In 2006 I established my own laboratory at the University of Turin as Assistant (2005) and then Associate (2011) Professor. From 2010 to 2019 I was principal investigator (PI) of the Immunogenetics Unit of the Human Genetics Foundation (now Italian Institute for Genomic Medicine) in Turin, Italy. During that time, my lab contributed to the identification and functional characterization of recurrently mutated genes in CLL. From 2014 to 2016, I was visiting associate professor at Weill Cornell Medical College, to set up patient-derived xenograft models of CLL and Richter syndrome. Since September 2016 I joined the Immunogenetics and Transplant Biology Service of the Città della Salute e della Scienza University Hospital of Torino, with the aim of setting up a diagnostic platform to provide genetic diagnosis and counseling to patients in need of organ transplant with a suspected underlying monogenic disease. Starting July 1, 2022 I am Full Professor of Genetics and starting November 2023 I am Director of the Service.

The CASSMedChem members

Valeria Bracciamà *assegnista di ricerca*
Amelia Fascì *assegnista di ricerca*
Matilde Micillo *PhD student*
Martina Migliorero *PhD student*
Monica Sorbini *PhD student*
Nahal Nabelsi *PhD student*
Angelo Corso Faini *MD in training (specializzando in genetica medica)*
Claudia Saglia *Biotechnologist in training (specializzando in genetica medica)*
Caterina Scolari *Biotechnologist in training (specializzando in genetica medica)*

RESEARCH ACTIVITY

The research activity of the lab revolves around 3 major research areas.

Molecular pathogenesis of Chronic Lymphocytic Leukemia.

Chronic lymphocytic leukemia (CLL) is a fascinating disease for at least four different reasons. The first is that the tumor and the host co-exist for several years, even without therapy. Several studies, including some from our group, have uncovered many and complex interactions undergoing between the host microenvironment and the tumor, leading to disruption of the host defenses (see references1-4).

The second is that while several recurrently mutated genes have been identified, none of them is predominant or pathognomonic, leaving the question of leukemogenesis still unanswered. The third is that in a small and still unpredictable percentage of patients the disease evolves into an aggressive lymphoma, a condition known as Richter's syndrome, with a dire prognosis. We have established unique patient-derived xenograft models of this conditions that have been extensively used to model therapy (see references 5-7). The fourth and final reason is that today CLL is effectively treated with small molecules that inhibit oncogenic pathways or that drive apoptosis, without chemoimmunotherapy.

Currently, there are two main CLL – RS projects ongoing in the lab:

The first one is dedicated to the study of the connections between the B cell receptor (BCR), which is the driving force in the disease and NOTCH1, which is the most common single gene abnormality in the disease. The hypothesis behind this project is that oncogenic signaling in CLL and RS cells, as driven through BCR and NOTCH1 acts directly through the up-regulation of the metabolic capabilities of the cancer cell. Hence, the combination of drugs that target oncogenic pathways with drugs that inhibit central metabolism of cancer cells may show synergistic effects. (see Fig. 1). Reference scientists: Amelia Fascì and Nahal Nabelsi

The second one is a collaboration with a novel pharma company dedicated to understanding the role of WNT/planar cell polarity (WNT/PCP) signaling pathway in CLL. The choice of this pathway stems from very encouraging results obtained using antibodies targeting ROR1, which is one of the receptors. This pathway is important in regulating homing to the leukemic niche and hence in generating therapy resistance. For this reason, we have embarked in a project to study the other ligands of this family, namely ROR2, RYK and PTK7. Reference scientist: Matilde Micillo

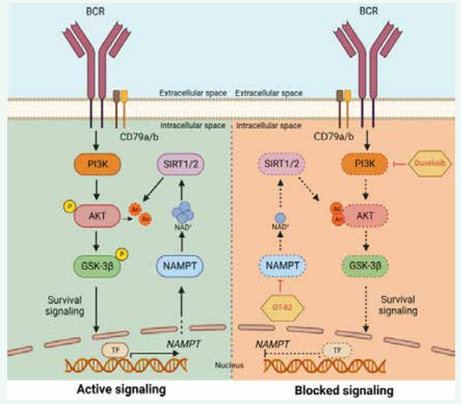


Figure 1.
Molecular circuits involving the B cell receptor and NAMPT in CLL and RS cells

Identification of novel markers of organ rejection.

We have exploited the concept of liquid biopsy, extensively used to monitor tumor relapse, in the field of transplantation, to monitor rejection. To do so, we set-up an assay to measure donor-derived cell free DNA (dd-cfDNA, see Figure 2).

Research showed that dd-cfDNA values correlate with the state of the transplanted organ and can therefore be predictive of the occurrence of rejection episodes. The lab has devised an innovative monitoring system based on HLA-DRB1 and HLA-DQB1 mismatches between donor and recipient and a strictly quantitative droplet digital PCR assay. Preliminary results obtained in cohorts of heart and lung recipients are encouraging (see references 8,9). Reference Scientist: Monica Sorbini

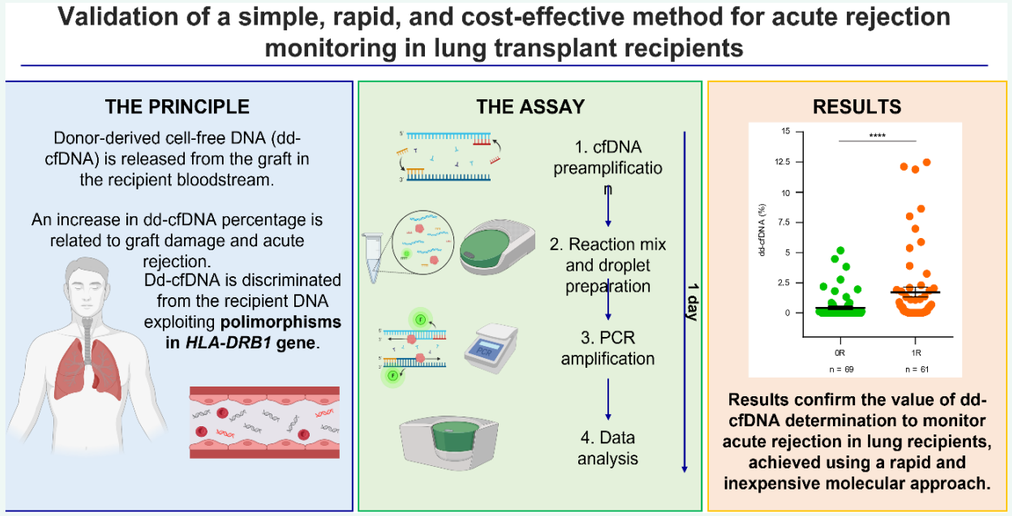


Figure 2.
Schematic representation of the droplet digital PCR-based assay to monitor donor-derived cell-free DNA using HLA-DRB1 mismatches in heart and lung recipients.

Functional characterization of novel mutations in patients with monogenic conditions

My clinical activity provides diagnosis and genetic counseling to patients who have received a solid organ transplant or are at risk of receiving one. This activity was started thanks to an Excellence Grant awarded to the Department of Medical Sciences in late 2018 and is now an integral part of the diagnostic offer of the Service of Immunogenetics and Transplant Biology of the Città della Salute e della Scienza Hospital.

In many instances, the impact of genetic damage on the phenotype remains unknown, particularly in the presence of previously unreported mutations or of diseases with unclear pathogenetic mechanisms. Part of the lab activities are being dedicated to design easy and ready to use genome editing approaches to generate experimental models to better understand the impact of specific mutations on the phenotype or genotype-phenotype correlations (see reference 10).

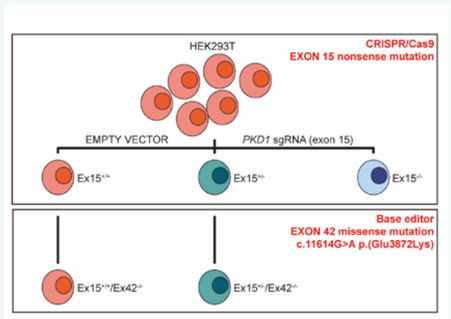


Figure 3.
Schematic representation of the approaches selected to study the impact of PKD1 mutations targeting different domains of the coded protein

FUTURE RESEARCH PLANS

Research area 1: Identification of actionable pathways in CLL and RS.

We plan to focus on epigenetic modifications activated in CLL and RS cells through master signaling pathways, including the BCR and NOTCH1.

Research area 2: Development of an assay to study graft versus host disease

We plan to implement an assay based on methylation profiles of cfDNA to determine tissue of origin, to be used in the context of allogeneic bone marrow transplantation to monitor graft versus host disease.

Research area 3: Genotype-phenotype correlations in patients with monogenic conditions

We aim to devise rapid and easy to use experimental models we aim to be able to recreate in vitro the most interesting and novel mutations we see in patients.

Title	Funding agency	Duration	Role
A ménage à trois involving the B cell receptor, NOTCH1 and NAMPT: therapeutic implications for CLL and RS patients	AIRC	2020-2024	PI
Leukemic cell and microenvironment interactions as the culprit of chronicity in CLL	PNRR project	2023-2025	PI of operative unit
Understanding the role of the WNT/PCP pathway in CLL and RS	Solve Therapeutics	2023-2025	PI
Exploring the molecular landscape of pediatric idiopathic nephrotic syndrome-associated glomerular damage and proteinuria	Miur – PRIN project	2023-2025	PI of operative unit
Cell free DNA profiling as a tool to monitor clinically-relevant events in allogeneic hematopoietic stem cell transplantation	Miur – PRIN project	2024-2026	PI

10 SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=6701775996

- Audrito V,.., Deaglio S: Extracellular nicotinamide phosphoribosyltransferase (NAMPT) promotes M2 macrophage polarization in chronic lymphocytic leukemia. Blood 125:111-23, 2015
- Bologna C,.., Deaglio S: SLAMF1 regulation of chemotaxis and autophagy determines CLL patient response. J Clin Invest 126:181-94, 2016
- Arruga F,.., Deaglio S: Bidirectional linkage between the B-cell receptor and NOTCH1 in chronic lymphocytic leukemia and in Richter's syndrome: therapeutic implications. Leukemia 34:462-477, 2020
- Arruga F, …, Deaglio S: The immunomodulatory molecule TIGIT is expressed by chronic lymphocytic leukemia cells and contributes to anergy. Haematologica, 2023
- Vaisitti T,.., Deaglio S: Novel Richter Syndrome Xenograft Models to Study Genetic Architecture, Biology, and Therapy Responses. Cancer Res 78:3413-3420, 2018
- Vaisitti T,., Deaglio S: ROR1 targeting with the antibody-drug conjugate VLS-101 is effective in Richter syndrome patient-derived xenograft mouse models. Blood 137:3365-3377, 2021
- Vaisitti T,.., Deaglio S: Anti-CD37 alpha-amanitin-conjugated antibodies as potential therapeutic weapons for Richter syndrome. Blood 140:1565-1569, 2022
- Sorbini M,..,Deaglio S: HLA-DRB1 mismatch-based identification of donor-derived cell free DNA (dd-cfDNA) as a marker of rejection in heart transplant recipients: A single-institution pilot study. J Heart Lung Transplant 40:794-804, 2021
- Sorbini M,.., Deaglio S: Validation of a Simple, Rapid, and Cost-Effective Method for Acute Rejection Monitoring in Lung Transplant Recipients. Transpl Int 35:10546, 2022
- Migliorero M, Deaglio S: A novel COLEC10 mutation in a child with 3MC syndrome. Eur J Med Genet 64:104374, 2021

PAOLA DEFILIPPI

Signaling platforms and translational strategies

BIOGRAPHICAL SKETCH

1980	MSC in Biological Science, University of Torino, Italy
1982-1986	PhD, Biochemistry, Université Libre de Bruxelles (Belgium)
1987-1992	Post-doctoral fellow, University of Torino, Italy
1992-1997	Research Assistant, University of Torino, Italy
1998-2000	Associate professor, University of Torino, Italy
Since 2000	Full Professor Applied Biology, University of Torino, Italy

LAB MEMBERS:

Giuseppe Ermondi *Associate Professor*
Alessandro Morellato, Vincenzo Salemme *Post doc*
Giorgia Centonze, Mario De Gregorio, Francesca Nigrelli *PhD student*
Beatrice Bersia, Olga Teresa Bianciotto, Arianna Colombino, Matteo Fragomeni, Zoe Lesti, Matteo Poncina, Lucrezia Rosgen, Andrea Scavuzzo, Noemi Zuccaro *Undergraduate students*

Honours:

2001-2003 Secretary of the Italian ABCD Society (Associazione Biologia Cellulare e del Differenziamento)

2001-2003 Board of Director FISV (Federazione Italiana Scienze della Vita)

2013-2015 Board of the AIBG (Associazione Italiana Biologia e Genetica)

since 2012: member AACR (American Association Cancer Research)

since 2018: member EACR (European Association Cancer Research)

2016-2018: Member of the 05F1, BIO/13 ASN panel Italian Ministry of University

RESEARCH ACTIVITY

Molecular and functional characterization of signaling platforms and translational strategies

Cancer cell Biology. Since the early ’90, my group has been involved in studying integrin signaling with special interest in the pathophysiology of integrin- and growth factor- dependent control of cell growth and migration. Over the years, we generated cell and animal models to deeply investigate at the genetic and functional level two adaptor proteins, the p130Cas (encoded by the BCAR1 gene) and the p140Cap (encoded by the SRCIN1 gene) that build up very upstream molecular platforms in integrin/RTK-dependent signals, cell motility and cell invasion. This is particularly relevant in cancer cells, regulating cancer progression (Cabodi et al., Cancer Res 2006 PMID:16651418; Di Stefano et al., EMBO J 2007 PMID:17525734; Damiano et al., Oncogene 2010 PMID:20453886; Cabodi et al., Nat Rev Cancer 2010, PMID:21102636; Grasso et al., Nat Commun 2017 PMID 28300085). The above findings provide evidence for a role of p130Cas as a positive regulator of both proliferation and survival in breast cancer. In contrast, p140Cap, behaves as an oncosuppressor which opposes and interferes with breast (Centonze et al., Front Cell Dev Biol. 2021 PMID: 34708040; Salemme et al., Cell Mol Life Sci 2021 PMID: 33079227) and neuroblastoma cancer features (Grasso, Cangelosi et al., Cell Death and Diff 2019 PMID:31488891). We recently dissected intra-tumor heterogeneity as a current urgent need to better define breast cancer biology and to develop therapeutic strategies targeting the microenvironment as helpful tools for combined and personalized treatment (Salemme et al., Front Oncol. 2021 PMID: 3377775; Salemme et al., Front Oncol. 2023 PMID: 37265795). We presented evidence that, by its ability to interact with and inhibit β-Catenin in the breast cancer stem cell compartment, p140Cap can orchestrate an anti-tumor immune response, influencing the composition of the TME immune infiltrate to prevent the establishment of a tumor conducive immune environment (Salemme et al., Nat Commun 2023 PMID: 37169737). It is our goal to mechanistically address the relevance of the WNT/β-Catenin pathway in BC, specifically on the TIC compartment, to depict the molecular interplay between p140Cap and BC heterogeneity, and the impact of p140Cap on drug responsiveness. We will offer a comprehensive picture of new opportunities for therapeutic interventions aimed at providing an effective response to counteract tumor progression.

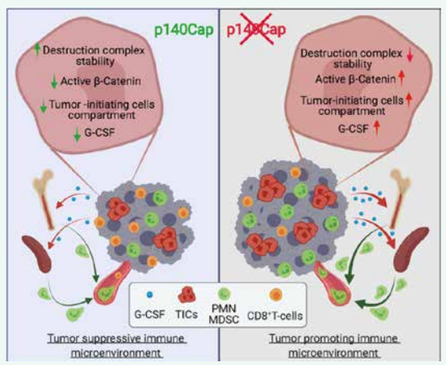


Figure 1.

p140Cap family proteins and synaptic plasticity

We have shown that p140Cap is highly enriched in synaptic structures, where it is an active member of dynamic synaptic network involved in memory consolidation, LTP and LTD that are known to be altered in neurological and psychiatric disorders (Jaworski et al., Neuron 2009 PMID:19146815; Nat Comm 2013 PMID:23868368; Repetto et al., J Neurosc 2014 PMID:24453341; Alfieri et al., Front Mol Neurosc 2017 PMID 28713243; Russo et al., Cer Cortex 2017 PMID:29161354). p140Cap directly binds the GluN2A subunit of NMDAR and modulates the GluN2A-associated molecular network. (Angelini, Morellato et al, JNS, 2022, PMID: 35953295).

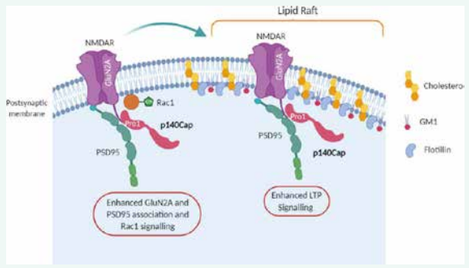


Figure 2.

We have generated iPSC-derived neurons (iN) where we will study the role of p140Cap in human synaptic signaling, mainly focusing on molecular pathways related to the glutamatergic synaptic activity. Several single-point mutations of p140Cap have been identified in the GeneMatcher platform. These variations give rise to missense variants of p140Cap in subjects presenting neurological disorders such as epilepsy, autism spectrum disorders, and intellectual disorders. Thus, we will use p140Cap-/- iN to analyze how the mutants affect the molecular signaling and electrophysiological activity of iN.

FUNDING ID, PAST 5 YEARS

- → Piedmont Region F.E.S.R. 2014-2020 DEFLeCT: Digital tEchnology For Lung Cancer treatment co-PI, 70,000 €.
- → MUR PRIN 2015 “Biological modifiers of HER2 in cancer: from genomic landscape and mechanistic signaling to therapeutic efficacy of HER2 inhibitors”. PI and Project co-ordinator, 369,000 €.
- → AIRC ID20107 2018-2022“Functional events and molecular mechanisms controlled by p140Cap in curbing the aggressiveness of ERBB2 breast cancers” PI, 664,000 €.
- → AIRC ID27353 2023-2027 “Functional role of the adaptor protein p140cap in breast cancer: molecular mechanisms and tumor sensitivity to therapy”, PI, 825,000 €.
- → Health Ministry Finalized Research 2021 “Capturing tumor heterogeneity exploiting a living biobank of ER-positive Breast Cancer Patient-Derived Organoids (PDO)and Xenografts (PDX) to personalize cancer treatment and overcome treatment resistance” Co-applicant, 67.500 €.
- → MUR PRIN 2022 “Protein-protein interactions as regulators of molecular complexes involved in synaptic plasticity: the paradigm of the adaptor protein p140Cap” PI and Project coordinator 189.409 €.
- → MUR PRIN PNRR 2022 “Mevalonate pathway and cholesterol modulation by p140Ca and UBIAD1 in breast cancer” PI 232.000 €.
- → PNRR M4C2-Investimento 1.4-CN00000041 “Finanziato dall’Unione Europea NextGenerationEU” 60.000 €.

10 SELECTED PUBLICATIONS

https://scholar.google.com/citations?hl=it&user=uv9dveoAAAAJ&view_op=list_works&sortby=pubdate

- → Salemme V, et al. p140Cap inhibits β-Catenin in the breast cancer stem cell compartment instructing a protective anti-tumor immune response. Nat Commun. 2023 May 11;14(1):2350. PMID: 37169737.
- → Costamagna A, Natalini D et al. Docking Protein p130Cas Regulates Acinar to Ductal Metaplasia During Pancreatic Adenocarcinoma Development and Pancreatitis. Gastroenterology. 2022 Apr;162(4):1242-1255.e11. PMID: 34922945.
- → Angelini C, Morellato A, et al. p140Cap regulates the composition and localization of the NMDAR complex in synaptic lipid rafts. J Neurosci. 2022 Aug 10;42(38):7183–200. PMID: 35953295.
- → Grasso S, Cangelosi D, Chapelle J, et al. The SRCIN1/p140Cap adaptor protein negatively regulates the aggressiveness of neuroblastoma. Cell Death Differ. 2020 Feb;27(2):790-807.
- → Grasso S, et al. The scaffold protein p140Cap limits ERBB2-mediated breast cancer progression interfering with Rac GTPase-controlled circuitries. Nat Commun. 2017 Mar 16;8:14797. doi: 10.1038/ncomms14797.
- → Repetto D, Camera P, et al. p140Cap regulates memory and synaptic plasticity through Src-mediated and citron-N-mediated actin reorganization. J Neurosci. 2014 Jan 22;34(4):1542-53. PMID: 24453341.
- → Morello V, et al. β1 integrin controls EGFR signaling and tumorigenic properties of lung cancer cells. Oncogene. 2011 Sep 29;30(39):4087-96. PMID: 21478906.
- → Cabodi S, et al. Integrin signalling adaptors: not only figurants in the cancer story. Nat Rev Cancer. 2010. PMID: 21102636.
- → Di Stefano P, et al. p140Cap protein suppresses tumour cell properties, regulating Csk and Src kinase activity. EMBO J. 2007 Jun 20;26(12):2843-55 PMID: 17525734.
- → Moro L, et al. Integrins induce activation of EGF receptor: role in MAP kinase induction and adhesion-dependent cell survival. EMBO J. 1998 Nov 16;17(22):6622-32. PMID: 9822606.

DANIELA DELLI CASTELLI

Molecular and metabolic imaging

BIOGRAPHICAL SKETCH

2000	Chemistry degree
2000-2003	PhD
2004-2011	post doctoral fellows
2011-2019	Research fellow
from 2019	Associate

LAB MEMBERS:

Claudia Quattrociocchi *postgraduate fellow*
Giulia Vassallo *PhD*

RESEARCH ACTIVITY

Molecular Imaging is a cutting-edge medical technique that enables the visualization and analysis of biological processes at molecular and cellular levels within living organisms. The main imaging technologies that are used to this purpose, at clinical or preclinical level, are: positron emission tomography (PET), single-photon emission computed tomography (SPECT), optical Imaging (OI), computed tomography (CT) and magnetic resonance imaging (MRI). Molecular Imaging approach holds immense promise in various fields, including oncology, neurology, cardiology, and immunology, as it provides insights into disease progression, early detection, and treatment response assessment. The combination of advanced imaging technologies with targeted molecular probes empowers scientists to unravel the intricacies of complex biological processes, leading to enhanced diagnostic accuracy and to the development of personalized therapeutic strategies. The research activity of Prof. Delli Castelli and her team belong to this area of research. In particular, in the last years, they have contributed to the advancement of research in the field of contrast agents for Magnetic Resonance Imaging for molecular targeting and metabolic imaging applications. Within the array of magnetic resonance imaging contrast agents, this group has specialized in advancing a relatively emerging category referred to as Chemical Exchange Saturation Transfer (CEST) contrast agents. The distinctiveness of these contrast agents, as opposed to conventional gadolinium-based ones, lies in their capability to simultaneously visualize multiple entities within a single image. Such implementation cannot be achieved in MRI using conventional gadolinium-based agents since the response these molecules induce in the system is undistinguishable from one molecule to another. Another distinct characteristic of this category of contrast agents, as opposed to conventional ones, is that CEST contrast can be easily modulated through tissue microenvironment parameters. Consequently, these molecules have proven to be excellent reporters of temperature, pH, redox potential, and catalytic activity. Despite these significant potentials, CEST contrast agents suffer from low sensitivity, a characteristic that the scientific community involved in their development has constantly addressed. One of the primary contribution of this research team regarding the sensitivity issue has focused on the development of nanosystems. These nanosystems, called LipoCEST have led to a remarkable increase in sensitivity by several orders of magnitude. This advancement has shifted the detection thresholds from millimolar concentrations to nanomolar concentrations, which are much more aligned with the purposes of molecular imaging. Due to the exceptional versatility of LipoCEST, these nanovesicles can be readily customized with molecular targeting vectors. Furthermore, these systems have been engineered to align themselves in a magnetic field accordingly with the sign of the magnetic susceptibility of their membrane thus altering the chemical shift (DLIPO) of the mobile protons connected to these systems. This innovation facilitates the establishment of a library for multifaceted visualization. Regarding applications in metabolic imaging, the contribution of this research team has predominantly revolved around the advancement of pH-responsive probes, particularly involving paramagnetic molecules (ParaCEST agents). In parallel with the research in the CEST field, this team has recently exploited their expertise in nanosystems to contribute to in vitro diagnostic test development. The reporting systems for ligand/antiligand assays based on pH variations that was designed by this team have shown a strong competitive edge, particularly in cost effectiveness, when compared to the ELISA counterparts.

FUTURE RESEARCH PLANS

At the moment, this research team is focusing its attention on the development of heteronuclear CEST agents. Utilizing heteronuclei could lead to a reduction in sensitivity threshold; however, it might come at the cost of compromising spatial resolution. This represents a groundbreaking initiative that has not been attempted before, paving the way for an entirely novel field of research. The goal is to optimize the parameters of heteronuclear CEST to expand the boundaries of diagnostic potential in CEST-MRI. The primary focus of our efforts will be directed towards the development of probes and pulse sequences, all aimed at successfully attaining this particular objective.

FUNDING (PAST 5 YEARS)

- 2019 Horizon 2020 project “GLINT: GlucoCEST Imaging in Neoplastic Tumours”
- 2021-FISR2020 Fondo integrativo speciale per la ricerca istituito dal Ministero dell’Istruzione dell’Università e della Ricerca “Test Covid-19 basato su turbidimetria”
- 2023 Proof of Concept (PoC) – TOINPROVE/2023 “Composizione di liposomi e metodo di dosaggio basato sull’uso degli stessi”

SELECTED PUBLICATIONS

- Vassallo, Giulia, Garello, Francesca, Aime, Silvio, Terreno, Enzo, Delli Castelli, Daniela* (2022). 31P ParaCEST: 31P MRI-CEST Imaging Based on the Formation of a Ternary Adduct between Inorganic Phosphate and Eu-DO3A. INORGANIC CHEMISTRY, vol. 61, p. 19663-19667, ISSN: 0020-1669, doi: 10.1021/acs.inorgchem.2c03329
- Tripepi, Martina, Bennardi, Paolo O., Ferrauto, Giuseppe, Aime, Silvio, Delli Castelli, Daniela* (2021). LipHosomes: Reporters for Ligand/Anti-Ligand Assays Based On pH Readout. ANALYSIS & SENSING, vol. 1, p. 48-53, ISSN: 2629-2742, doi: 10.1002/anse.202000001
- Ferrauto G., Tripepi M., Di Gregorio E., Bitonto V., Aime S., Delli Castelli D.* (2021). Detection of U-87 Tumor Cells by RGD-Functionalized/Gd-Containing Giant Unilamellar Vesicles in Magnetization Transfer Contrast Magnetic Resonance Images. INVESTIGATIVE RADIOLOGY, vol. 56, p. 301-312, ISSN: 0020-9996, doi: 10.1097/RLI.0000000000007425 2020
- Tripepi M., Ferrauto G., Bennardi P. O., Aime S., Delli Castelli D.* (2020). Multilamellar LipoCEST Agents Obtained from Osmotic Shrinkage of Paramagnetically Loaded Giant Unilamellar Vescicles (GUVs). ANGEWANDTE CHEMIE. INTERNATIONAL EDITION, vol. 59, p. 2279-2283, ISSN: 1433- 7851, doi: 10.1002/anie.201912327
- Ferrauto, Giuseppe, Beauprez, Frederik, Di Gregorio, Enza, Carrera, Carla, Aime, Silvio, Terreno, Enzo, Delli Castelli, Daniela* (2019). Development and characterization of lanthanide-HPDO3A-C16-based micelles as CEST-MRI contrast agents. DALTON TRANSACTIONS, vol. 48, p. 5343-5351-5351, ISSN: 1477-9226, doi: 10.1039/c8dt04621b 6 2019
- Dastrù, Walter, Menchise, Valeria, Ferrauto, Giuseppe, Fabretto, Serena, Carrera, Carla, Terreno, Enzo, Aime, Silvio, Delli Castelli, Daniela * (2018). Modulation of the Prototropic Exchange Rate in pH-Responsive Yb-HPDO3A Derivatives as ParaCEST Agents. CHEMISTRYSELECT, vol. 3, p. 6035-6041, ISSN: 2365- 6549, doi: 10.1002/slct.201800283
- Delli Castelli Daniela, Tei Lorenzo, Carniato Fabio, Aime Silvio, Botta Mauro (2018). [Yb(AAZTA)(H2O)]−: an unconventional ParaCEST MRI probe.. CHEMICAL COMMUNICATIONS, p. 2004-2007, ISSN: 1364-548X, doi: 10.1039/c8cc00193f
- Delli Castelli, Daniela, Ferrauto, Giuseppe, Di Gregorio, Enza, Terreno, Enzo, Aime, Silvio (2015). Sensitive MRI detection of internalized T1 contrast agents using magnetization transfer contrast. NMR IN BIOMEDICINE, vol. 28, p. 1663-1670, ISSN: 0952-3480, doi: 10.1002/nbm.3423
- Garello, Francesca, Stefania, Rachele, Aime, Silvio, Terreno, Enzo, Delli Castelli, Daniela* (2014). Successful entrapping of liposomes in glucan particles: An innovative micron-sized carrier to deliver water-soluble molecules. MOLECULAR PHARMACEUTICS, vol. 11, p. 3760-3765, ISSN: 1543-8384, doi: 10.1021/mp500374f
- Delli Castelli, Daniela, Ferrauto, Giuseppe, Cutrin, Juan Carlos, Terreno, Enzo, AIME, Silvio (2014). In Vivo Maps of Extracellular pH in Murine Melanoma by CEST–MRI. MAGNETIC RESONANCE IN MEDICINE, vol. 71, p. 326-332, ISSN: 0740-3194, doi: 10.1002/mrm.24664

GIACOMO DONATI

Chromatin and stem cell adaptation in wound healing and skin cancer

BIOGRAPHICAL SKETCH

2019 to date	Associate Professor of Genetics, Department of Life Sciences and Systems Biology, University of Turin, Italy.
2017 to date	Group leader, (www.donatilab.org) Molecular Biotechnology Center, University of Turin
2016-2019	RTDb, Department of Life Sciences and Systems Biology, University of Turin
2012-2016	Senior Research Associate, Centre for Stem Cells and Regenerative Medicine, King's College London, Fiona Watt Laboratory
2009-2012	APost-doc, Cancer Research UK Cambridge Research Institute, Fiona Watt Laboratory

LAB MEMBERS:

Carlotta Duval *PhD student*
Chiara Levra Levron *PhD student*
Gabriele Piacenti *PhD student*
Luca Elettrico *PhD student*
Alessandro Croce *Research fellow*
Dilay Yılmaz *Research fellow*
Osamu Ansai *MD Dermatologist, PhD, Postdoc*

RESEARCH ACTIVITY

In numerous adult epithelia, specific pools of stem cells continually renew multiple cell types throughout life. While maintaining their lineage identity, stem cells and their differentiated progeny tightly crosstalk to ensure proper tissue homeostasis. The skin is a physical, chemical and immunological barrier that protect us from the outside environment. Following an injury, distinct cell lineages at various stages of differentiation collaborate to restore skin integrity through the wound healing process, in which they acquire an unexpected plasticity. Indeed, when epithelial cells are recruited to repair an injury, they progressively lose their initial identity and are reprogrammed to acquire the lineage of the repaired epithelial niche. Recent studies highlight the existence of shared molecular mechanisms in wound healing and skin cancer, suggesting possible detrimental consequences of cell plasticity. Moreover, the precise transcriptional and chromatin factors that govern this process remain unidentified, and the connection between plasticity and cancer is still incompletely understood.

In the lab (www.donatilab.org), we integrate state-of-the-art molecular and cellular biology techniques in vitro and in vivo to decipher the regulatory mechanisms of cell plasticity in wound healing and its consequences on cell fate, such as cancer. The first approach that we set up in our lab employs in vitro genetic screenings, such as shRNA pooled screenings. The technology allows to target, in an unbiased way, thousands of transcriptional and chromatin factors. Pooled lentiviral screenings (with shRNAs or CRISPR-Cas9) are powerful tools to elucidate phenotype drivers, gene-gene interactions and determine critical nodes in biological pathways and processes. The combination of functional genomics in primary cell cultures with histological and 'omics approaches in in vivo models represents a powerful strategy for the study of a complex process as cell plasticity. Therefore, in the lab we use in vivo lineage tracing of different epidermal lineages in longitudinal experiments such as at different phases of wound healing. Lineage tracing allows to follow the progeny of a selected stem cell population; it represents an essential tool for studying stem cell properties in adult mammalian tissues and it is particularly helpful to study cell fate after plasticity acquisition and its long-term consequences. Our research employs a combination of in vitro and in vivo approaches, complemented by high-throughput methods that analyze the transcriptional and epigenetic profiles of cells, both at the bulk and single-cell levels. In particular, to study the transcriptome we employ single cell or bulk RNA-sequencing and a more recent technique, named Spatial Transcriptomic, that add the spatial resolution component to the transcript quantification. Additionally, chromatin profile and protein-DNA binding are assessed through chromatin immunoprecipitation coupled with sequencing (Chip-seq)

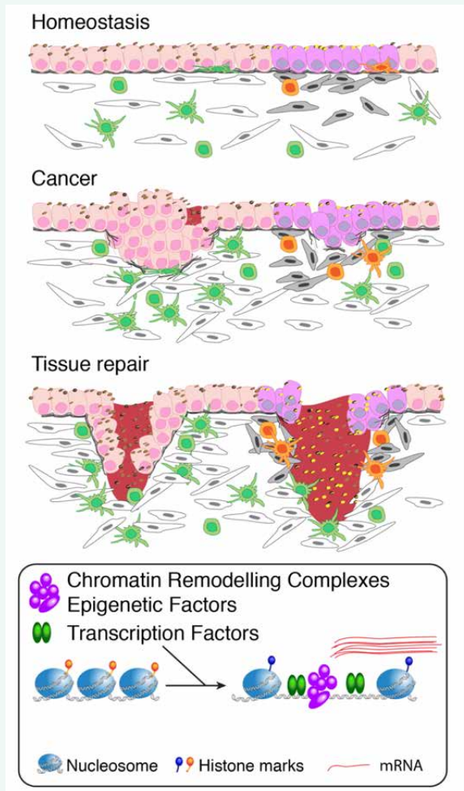


Figure 1.
Overview of Donati's Lab cellular and molecular research

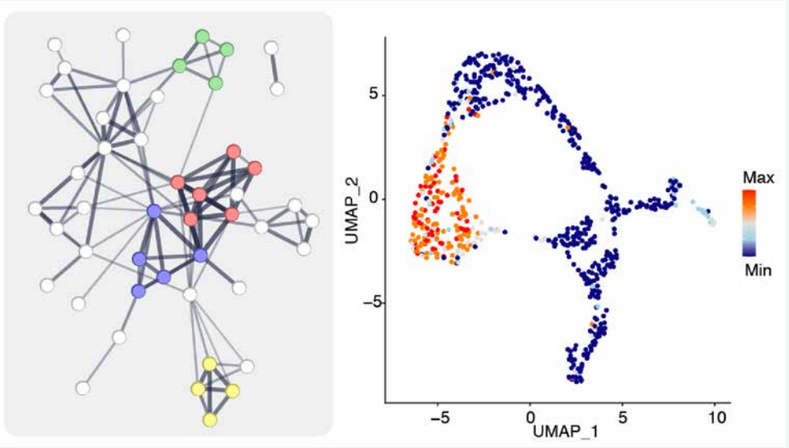


Figure 2.
PPI Network analysis of genes' hits from a pooled genetic screening highlighting known epigenetic complexes (left). UMAP representation of single cell RNAseq of epidermal cells colored by the expression of a cluster marker (right).

and chromatin accessibility assay (ATAC-seq). Finally, the lab comprises a team of computational biologists that enable the interpretation and the integration of the data coming from high-throughput techniques. To validate our finding in human skin diseases, we have access to fresh and stored skin samples from our network of national and international of collaborators including several dermatologists.

Recent findings. We recently identified and functionally characterized a so-called distal memory, a cell adaptation of specific epidermal stem cells. Cellular adaptation refers to the capacity of cells to respond to diverse stimuli and adverse environmental changes. While adaptive programs have been widely described in adaptive and innate immune cells, it has recently emerged that epithelial cells in vivo can also acquire a memory. We established a two consecutive skin injuries in vivo model, combining lineage tracing with single cell omics to understand the spatial extent of wound memory and the full spectrum of the adaptive responses of multiple epithelial stem cells in skin. We demonstrated that away from injured site, after a first injury a specific epithelial stem cell population gives rise to long term wound-memory progenitors residing in their own niche of origin. Notably these progenitors have not taken part in the first wound healing but become pre-activated through priming. Mechanistically, we demonstrated that a transcriptional de-repression, linked to the reduction of a H2AK119 ubiquitination (a chromatin marker of transcriptional repression), is functional for memory onset. We also analyzed the the consequences of distal memory. We found that not only this wound memory exacerbated tumorigenesis, but which onset occurs earlier within these primed cells and follow their spatial distribution. Therefore, we showed that sub-organ scale adaptation of an injury relies on spatially organized and memory-dedicated progenitors, characterized by a chromatin actionable cell state, that establishes an epigenetic field cancerization and predisposes to tumour onset.

FUTURE RESEARCH PLANS

We recently performed multiple shRNA pooled screens to identify key regulatory networks of transcription and chromatin factors controlling cell plasticity and adaptation in epithelia.

Through our research, we aim to investigate the impact of these factors on various cellular phenotypes and elucidate their downstream targets in homeostatic, regenerative and neoplastic conditions of epithelial cells.

Beside our fundamental regenerative and cancer biological questions, our data will have the potential to identify new molecular markers of early and late field cancerization establishment in ageing skin, as well as to identify new therapeutic targets of the early stage of epithelial carcinogenesis.

FUNDING ID (PAST 5 YERS):

- My First AIRC Grant (Id. 21640) by AIRC;
- Single-Cell Analysis of Inflammation (Id. DAF2020-217532) by Chan Zuckerberg Initiative

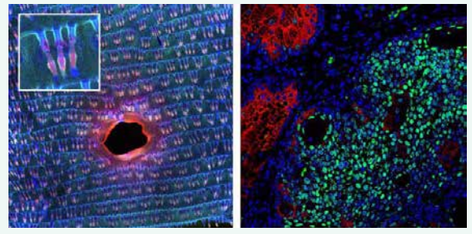


Figure 3.
My First AIRC Grant (Id. 21640) by AIRC;
Single-Cell Analysis of Inflammation (Id. DAF2020-217532) by Chan Zuckerberg Initiative

SELECTED PUBLICATIONS

- Tissue memory relies on stem cell priming in distal undamaged areas. Levra Levron C*, Watanabe M*, Proserpio V*, Piacenti G, Lauria A, Kaltenbach S, Tamburrini A, Nohara T, Anselmi F, Duval C, Elettrico L, Donna D, Conti L, Baev D, Natsuga K, Hagai T, Oliviero S, Donati G. Nat Cell Biol. 2023 May;25(5):740-753. doi: 10.1038/s41556-023-01120-0. (*Co-first authors)
- DNMT3B supports meso-endoderm differentiation from mouse embryonic stem cells. Lauria A, Meng G, Proserpio V, Rapelli S, Maldotti M, Polignano IL, Anselmi F, Incarnato D, Krepelova A, Donna D, Levra Levron C, Donati G, Neri F, Oliviero S. Nat Commun. 2023 Jan 23;14(1):367. doi: 10.1038/s41467-023-35938-x.
- Molecular and spatial design of early skin development. Jacob T, Annusver K, Czarnewski P, Dalessandri P, Kastriti ME, Levra Levron C, Mikkola ML, Rendl M, Lichtenberger BM, Donati G, Björklund A, Kasper M. (accepted in Dev Cell)
- Van Hove L, Lecomte K, Roels J, Vandamme N, Vikkula HK, Hoorens I, Ongenae K, Hochepied T, Donati G, Saeys Y, Quist SR, Watt FM, van Loo G, Hoste. Fibrotic enzymes modulate wound-induced skin tumorigenesis. EMBO Rep. 2021 May 5;22(5):e51573. doi: 10.15252/embr.202051573.
- Oulès B, Philippeos C, Segal J, Tihy M, Vietri Rudan M, Cujba AM, Grange PA, Quist S, Natsuga K, Deschamps L, Dupin N, Donati G & Watt FM. Contribution of GATA6 to homeostasis of the human upper pilosebaceous unit and acne pathogenesis. Nature Comm 2020. https://doi.org/10.1038/s41467-020-18784-z
- Oulès B, Rognoni E, Hoste E, Goss G, Fiehler R, Natsuga K, Quist S, Mentink R, Donati G*, Watt FM*. Mutant Lef1 controls Gata6 in sebaceous gland development and cancer. EMBO J 2019 (* Co-last authors)
- Hagai T, Chen X, Miragaia RJ, Rostom R, Gomes T, Kunowska N, Henriksson J, Park JE, Proserpio V, Donati G, Bossini-Castillo L, Vieira Braga FA, Naamati G, Fletcher J, Stephenson E, Vegh P, Trynka G, Kondova I, Dennis M, Haniffa M, Nourmohammad A, Lässig M, Teichmann SA. A Gene expression variability across cells and species shapes innate immunity. Nature 2018 doi: 10.1038/s41586-018-0657-2.
- Donati G*, Rognoni E, Hoste E, Liakathali K, Kar G, Kayikci M, Russel R, Mulder K, Teichmann SA, Watt FM*. Wounding induces dedifferentiation of epidermal Gata6+ cells and acquisition of stem cell properties. doi: 10.1038/ncb3532. (* Co-corresponding authors).
- Donati G and Watt FM. Stem cell heterogeneity and plasticity in epithelia. Cell Stem Cell 2015 doi: 10.1016/j.stem.2015.04.014.
- Nardini M*, Gnesutta N*, Donati G*, Gatta R, Forni C, Fossati A, Vonrhein C, Moras D, Romier C, Bolognesi M, Mantovani R. Sequence-Specific Transcription Factor NF-Y Displays Histone-like DNA Binding and H2B- like Ubiquitination. Cell 2013 doi: 10.1016/j.cell.2012.11.047. (*Co-first authors)

GIUSEPPE ERMONDI

CASSMedChem Lab

BIOGRAPHICAL SKETCH

Since 2013	Associate Professor, Dept. Molecular Biotechnology and Health Sciences, University of Turin, ITALY
2001-2012	Associate Professor, Pharmacy Faculty, University of Turin, ITALY
1993-2011	Researcher, Pharmacy Faculty, University of Eastern Piedmont, ITALY
1997	Postdoctoral Fellow, Institut de Chimie Thèrapeutique, Ecole de Pharmacie in Lausanne, SWITZERLAND
1991-1994	PhD in Chemistry, Dept. Chemistry, University of Turin, ITALY

LAB MEMBERS:

Giulia Caron *Associate Professor*
Maura Vallaro *Technician*
Matteo Rossi Sebastiano *Research fellow*
Diego Jimenez Garcia *PhD student*
Giulia Apprato *PhD student*

RESEARCH ACTIVITY

Scenario: Rare Diseases (RDs)

The portal for rare diseases and orphan drugs (https://www.orpha.net/) defines RDs as diseases that affects a small number of people compared to the general population. In Europe, a disease is rare if there is an incidence of 1 case per 2000 people.

There are more than 6,000 different rare diseases in the EU, so while one rare disease may affect only a handful of patients, another may affect up to 250,000. Globally, there are up to 36 million people in the EU who are living with a RD. Around 80% of rare diseases are of genetic origin and of those 70% already start in childhood (EU Fact sheet https://health.ec.europa.eu/system/files/2023-05/ncd_2023_rare-diseases_factsheet_en.pdf)

Despite the big efforts done by EU action on rare diseases to improve the diagnosis, care, and treatment of patients, most RDs still lack specific treatments. Research on the treatment of RDs has focused on various modalities, including the use of antibodies, gene therapy, stem cell therapy and enzyme replacement therapy. However, the use of small molecules and drug repositioning remains a promising area of research, Figure 1.

Aim: developing a screening method to prioritize mutated proteins that are potentially druggable

One of the main limitations in finding cures for RDs is undoubtedly the low level of commercial investment. In our view, the development of rapid and cost-effective methods to assess the feasibility of RD drug development could be an important driver.

Our mission in RDs research is to support clinical geneticists in identifying specific mutations that result in the production of a mutated protein, which can serve as a target for small molecules capable of effectively restoring its function To this end, it is necessary to assess the druggability of protein products derived from mutant DNA by means of a structure-based method.

Methods: in silico pipeline

We are developing a filtering process to select the most promising mutated proteins to be targeted with small molecules, which will then become potential candidates for a drug discovery program, Figure 2.

First, a panel of mutations arising from clinical data and related to the protein involved in a given RD is collected. The panel can be retrieved from the literature but is mainly provided by clinical geneticists collaborating with our group.

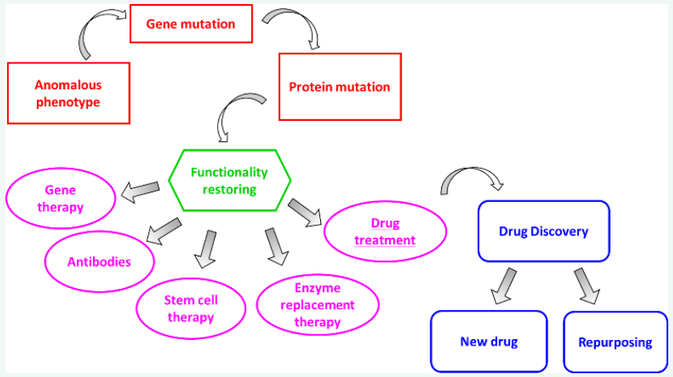


Figure 1.
Typical pipeline followed in RD studies

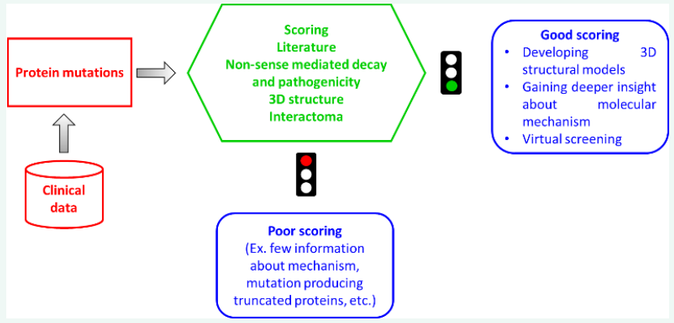


Figure 2.
Filtering pipeline to prioritize potential druggable mutate proteins

Then the protein is filtered on the basis of four criteria:

- → available information in the literature
- → mRNA nonsense-mediated decay and pathogenicity scores
- → protein WT 3D structure availability
- → protein interactome features.

The four criteria contribute to define a score that allows to discard mutations that are poorly characterized or that probably produce instable or truncated proteins.

The mutated proteins with the highest scores are then modelled using experimental structures, when possible, otherwise using homology models obtained in house or downloaded by AlphaFold. Protein complexes arising from the interactome analysis are also modelled.

The following step includes the investigation of the protein structure in terms of stability, flexibility, surface properties and molecular interactions in order to characterize the potential pathogenic mechanism introduced by each mutation. Docking tools are finally used to find small molecules potentially able to bind to the proteins and restore the functionality of the mutated protein.

The method was successfully applied to mutations in the gene ALS2 which are responsible for rare motor neuron diseases, such as infantile onset ascending hereditary spastic paralysis (IAHSP) and juvenile primary lateral sclerosis (JPLS) and involved in some cases of amyotrophic lateral sclerosis (ALS). There are only ~50 reported cases of IAHSP, but it is estimated that ~150 children have this disease worldwide, for which no specific treatment is available.

Alsin is a protein essential for the development and maintenance of motor neurons through the endosomal/endocytic pathway, Figure 3A shows Alsin domains with the main known interacting proteins. To carry out its function, WT Alsin forms dimers by interaction of VPS9 domains and then tetramerizes; R1611W mutation responsible for IAHSP acts by destabilising the tetramer, Figure 3B.

We collected a panel of mutations of ALS2 suggested by several sources: the non-profit IAHSP patient organisation Help Olly (https://helpolly.it), the Paediatric neuropsychiatry unit specialising in rare genetic neurological disorders of IRCCS Stella Maris, and the literature. Among the mutations with a favourable score calculated with the procedure depicted above, Figure 2, we focused on R1611W. This was also the first mutation reported to our laboratory. R1611W occurs in the core of VPS9 and blocks the initial formation of the dimer, Figure 2 B. The domains of the Alsin structure involved in dimer formation and their potential reciprocal interactions were modelled for the WT and the W1611R mutation. These models suggested the possibility of searching for a small molecule that could bind to W1611R in the region affected by the mutation and mask the arginine charge. In principle, this would restore the hydrophobicity of the protein in VPS9, allowing the formation of the dimer.

To this aim, we looked for a binding pocket near R1611W, Figure 3C, and then the pocket was submitted to a structure-based virtual screening of 9815 approved molecules, retrieved from ZINC (v.v. 15) and Drug Bank (www.drugbank.com).

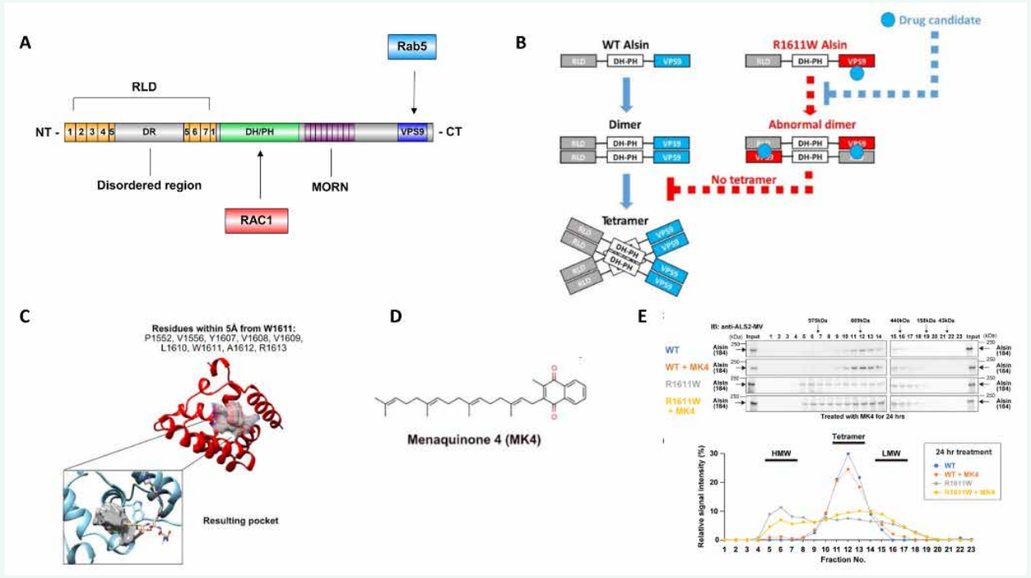


Figure 3.
Figure 3. Discover of MK4, more details can be found in Rossi Sebastiano, M. et al. Molecules 2022, 27, 7063. https://doi.org/10.3390/molecules27207063

The initial screening outputs were filtered with blood–brain barrier (BBB) permeability in silico models and after some refinement runs the virtual screening identified Menaquinone 4 (MK4, Figure 3D) as the most promising candidate.

MK4 was approved for the treatment of osteoporosis in Japan and commercialized as supplements in Europe and USA. To confirm the in-silico predictions, we performed biochemical analyses in the presence/absence of MK4. Biochemical analysis suggested that in the presence of MK4, the mutated Alsin can tetramerise again and thus its function is restored, Figure 4E. MK4 is now administered to an IHASP patient under compassionate use.

FUTURE RESEARCH PLANS

- → Translate the prioritisation process into a simple form that can be used for rapid analysis of mutation panels provided by clinical geneticists.
- → Refinement of the procedure by increasing the number of cases treated.
- → Implementation of experimental methods for the identification of biomarkers which can be used to test the efficacy of compounds identified in virtual screening.

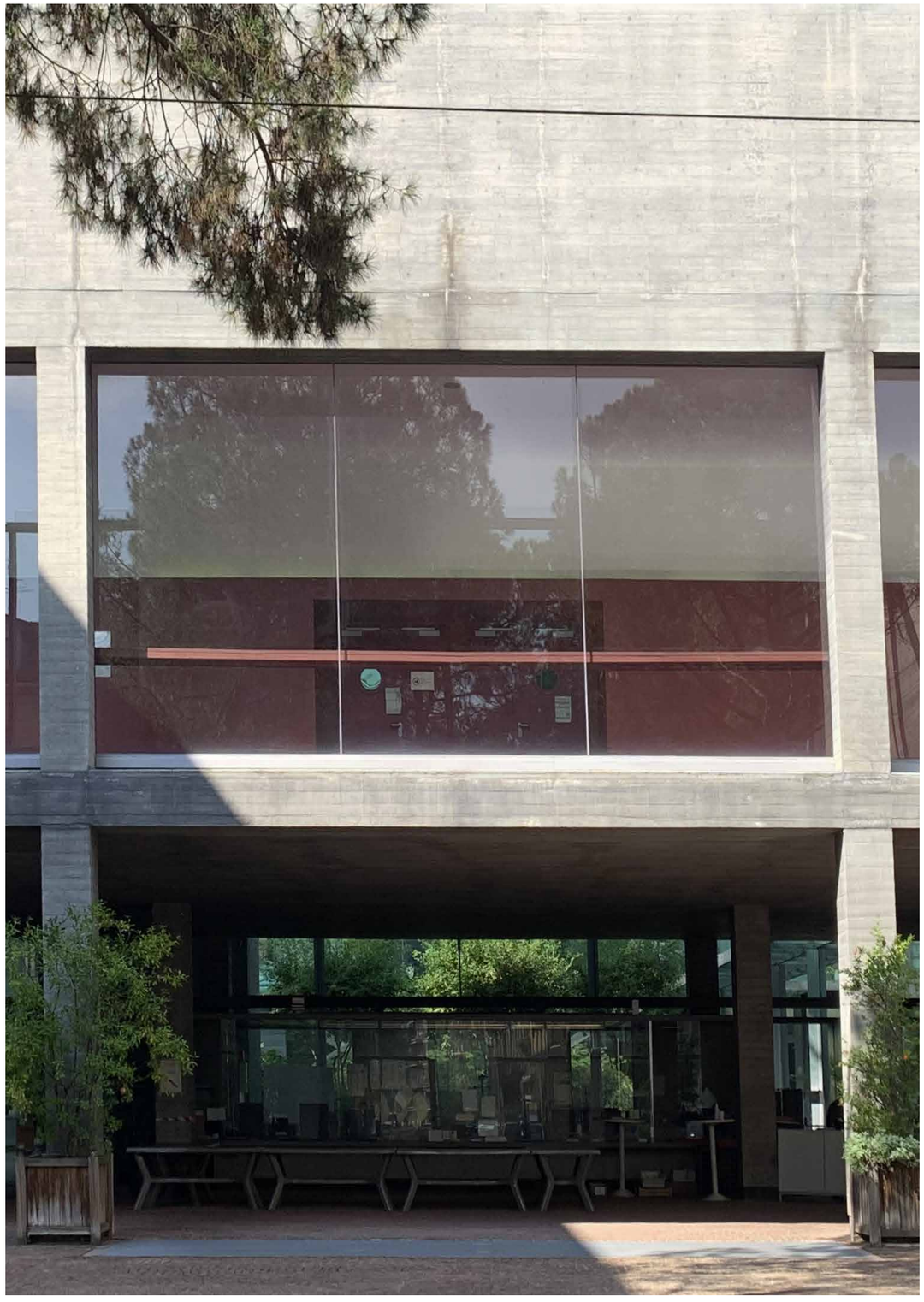
FUNDING

- → Investigating common pathogenic mechanisms of rare genetic hereditary spastic paraplegia – Prot. 20224YX5ZX - PRIN 2022

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=6602743907

- Hiatt, S.M., … S., Sebastiano, ..., Ermondi, G., et al. Deleterious, protein-altering variants in the transcriptional coregulator ZMYM3 in 27 individuals with a neurodevelopmental delay phenotype, Am. J. Hum. Genet., 110 (2), 215-227 (2023) DOI: 10.1016/j.ajhg.2022.12.007
- Rossi Sebastiano, M., Ermondi, G., Sato, K., Otomo, A., Hadano, S., Caron, G. Personalized Treatment for Infantile Ascending Hereditary Spastic Paralysis Based on In Silico Strategies
- Molecules, 27 (20), 7063 (2022) DOI: 10.3390/molecules27207063
- Rossi Sebastiano, M., Ermondi, G., Hadano, S., Caron, G. AI-based protein structure databases have the potential to accelerate rare diseases research: AlphaFoldDB and the case of IAHSP/Alsin, DDT, 27 (6), 1652-1660, (2022) DOI: 10.1016/j.drudis.2021.12.018
- Carrà, G., Ermondi, G., Riganti, C., Righi, L., Caron, G., Menga, A., Capelletto, E., Maffeo, B., Lingua, M.F., Fusella, F., Volante, M., Taulli, R., Guerrasio, A., Novello, S., Brancaccio, M., Piazza, R., Morotti, A. IκBα targeting promotes oxidative stress-dependent cell death
- J. Exp. Clin. Cancer Res., 40 (1), 136 (2021) DOI: 10.1186/s13046-021-01921-x
- Costamagna, A., Rossi Sebastiano, M., Natalini, D., Simoni, M., Valabrega, G., Defilippi, P., Visentin, S., Ermondi, G., Turco, E., Caron, G., Cabodi, S. Modeling ErbB2-p130Cas interaction to design new potential anticancer agents. Sci. Rep., 9, 3089-3104, (2019). DOI: 10.1038/s41598-019-39510-w

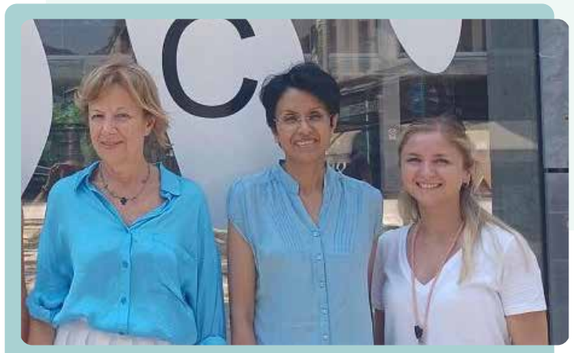


SHARMILA FAGOONEE

Biomarkers and post-transcriptional regulation in human pathologies

BIOGRAPHICAL SKETCH

2014-to date	Researcher at Institute of Biostructure and Bioimaging (IBB-CNR), Molecular Biotechnology Center “Guido Tarone”, Turin, Italy
2021-ongoing	co-coordinator for the internship activities, member of the scientific committee and lecturer of the Master program in Stem cells in regenerative medicine and Cell Factory management (STEM-MED), University of Turin, Italy
2011-2013	Research fellowship at Institute of Biostructure and Bioimaging (IBB-CNR), Molecular Biotechnology Center “Guido Tarone”, Turin, Italy
2005-2011	Post-doctoral fellow, University of Turin, Italy



LAB MEMBERS:

Fiorella Altruda *Professor Emeritus, University of Turin*
Giorgia Ammirata *Ph.D. student*
Carola Ronco *M.Sc. student*

EDUCATION

- 2012-2013-M.Sc. in Biotechnology, University of Turin, Italy
- 2005-2006- Master in Bioinformatics, first level, University of Turin, Italy
- 2000-2004- Ph.D. in Human Cell and Molecular Biology, University of Turin, Italy
- 1997-1999- M.Sc. in Cellular Biology and Physiology: Molecular Biology and Genetics, Université Victor Segalen, Bordeaux II, France

RESEARCH ACTIVITY

Extracellular vesicles-based research

Search for clinically applicable biomarkers for human diseases.

Liver fibrosis, the prime event which is often reversible if identified early, remains asymptomatic in its early stage, and remains undiagnosed until complications occur. Cholestatic diseases, resulting from impaired bile flow from the liver to the duodenum, account for 10% of all liver diseases and exert a significant burden on healthcare systems. Accumulation of bile compounds during cholestasis leads to non-specific cellular damage, initiating a cascade of inflammatory and fibrogenic events in the liver. There is urgent need for reliable biomarkers in the clinic to intervene early with therapy. The evolution and resolution of liver fibrosis are influenced by the underlying cause, and general biomarkers may not be sufficient to detect fibrotic development in all liver diseases. There is increasingly strong evidence demonstrating that the molecular cargo of extracellular vesicles (EVs), which represents the phenotypic state of donor cells, is a critical determinant of EV action in the progression of fibrosis in various liver diseases.

Our study was designed to lay the foundation for translational research in humans by identifying potential biomolecules indicative of cholestasis-induced liver fibrosis in mouse models. Whole transcriptome and small RNA sequencing analyses of circulating EVs revealed enrichment of RNA species, such as hepatic mRNAs, like Albumin and Haptoglobin and microRNAs like miR192-5p, miR194-5p, miR22-3p, and miR29a-3p, in cholestatic mice versus controls. This panel of mRNAs and miRNAs contained in circulating EVs indicates hepatic damage and fibrosis in mice, hence offering promising biomarkers for human severe cholestasis-induced liver fibrosis.

EV-based therapy

The secretome of stem cells, especially extracellular vesicles (EVs), have recently attracted much attention due to their small size, biomolecule cargo, and low immunogenicity compared to the parent cells in novel, cell-free therapeutic strategies for human diseases. We assessed the potential of EVs derived from bone marrow mesenchymal stem cells (BMSC-EVs), applied in a bio-adhesive and lubricant material that enables prolonged effects in vivo and reduces the frequency of daily applications, in promoting corneal repair. Our study showed that BMSC-EV treatment mitigated corneal damage while controlling inflammation and neo-angiogenesis, crucial for repairing the avascularized cornea. Importantly, we observed no side effects despite the non-autologous nature of the EVs, as they were not internalized in the non-damaged corneas, indicating that as the damage resolves, these EVs no longer penetrate the corneal epithelium. Thus, BMSC-EVs are promising as a new cell-free approach for intervening on burn wounds in the avascularized region of the eye and deserve further investigation.

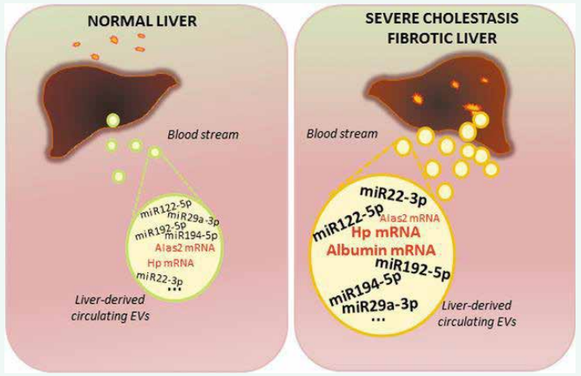


Figure 1.
Schematic representation of biomarkers-containing EVs release from the fibrotic liver. Under normal conditions, physiological quantities of EVs containing liver-derived biomolecules are released. Upon cholestasis, the damage that occurs in the liver induces massive release of EVs that are enriched with bioactive molecules such as miRNAs and mRNAs, which provide a molecular fingerprint of the underlying pathological events in the liver and can thus be used as biomarkers (taken from Antioxid Redox Signal. 2022 Mar;36(7-9):480-504).

Post-transcriptional regulation of gene expression in human diseases.

RNA binding proteins are well recognized as critical regulators of tumorigenic processes through their capacity to modulate RNA biogenesis, including alternative splicing, RNA stability and mRNA translation. We have previously identified a new role for the RNA-binding protein Epithelial Splicing Regulatory Protein 1, ESRP1, as a physiological regulator of the finely-tuned balance between self-renewal and commitment to a restricted developmental fate. In particular, ESRP1 exerted its action by modulating the polysomal loading of some core pluripotency factors, such as Oct4 and Sox2, thus controlling their expression and the responsiveness of Embryonic Stem (ES) cells to differentiative stimuli. In fact, ESRP1-silenced ES cells showed enhanced self-renewal and defective differentiation capacity.

This initial study sparked further research in the field of colorectal cancer (CRC), considering that certain embryogenesis-related pathways are reactivated during tumorigenesis. ESRP1 is mostly known for its tumor suppressive role through the control of alternative splicing events. However, our work regarding the role of ESRP1 in CRC indicated, for the first time, that ESRP1 played a role in anchorage-independent growth of CRC cells, hence acting as pro-oncogene. ESRP1 also promoted the ability of CRC cells to generate macrometastases in mice livers. High ESRP1 expression may thus stimulate growth of cancer epithelial cells in the colon as well as at distant sites, and promote colorectal cancer progression.

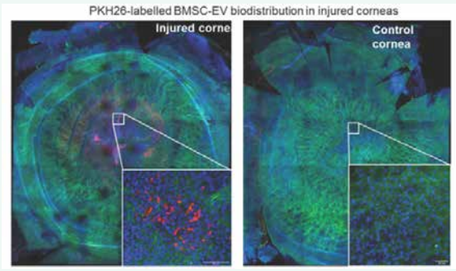


Figure 2.
MSC-EV treatment of murine corneas. Confocal microscopy images of corneal whole mounts, reconstructed using the mosaic function of Leica Sp5 confocal microscope, following treatment of the injured or uninjured control corneas with PKH26-labelled (Red) MSC-EVs. F-actin and nuclei were stained with phalloidin-FITC (green) and DAPI (blue), respectively (Cells. 2022 Dec; 11(23): 3892)..

FUTURE RESEARCH PLANS

- Clarify, using a transcriptomic approach, the relationship between serum Vitamin D levels, genomic response to Vitamin D treatment (analysis of serum EVs) and SARS-CoV-2 positivity during the follow-up of IBD patients recently affected by Covid-19, with the aim of offering personalized therapy based on Vit.D.
- Dissect the molecular mechanisms through which ESRPs participate in liver tumorigenesis and develop molecules to modulate the expression of these proteins in vivo.
- Characterize the molecular events involved in epithelial-mesenchymal transition that occur in pediatric idiopathic nephrotic syndrome, using dynamic three-layer millifluidic glomerulus.

FUNDING ID (PAST 5 YEARS)

- 2023: PRIN MUR; Exploring the molecular landscape of pediatric idiopathic nephrotic syndrome-associated glomerular damage and proteinuria.
- 2020-ongoing: PI, ARGO project, Ricerca di biomarcatori genomici per la risposta alla vitamina D nei pazienti con Covid-19 e IBD
- 2017-2020: PRIN MIUR, Regenerative potential of extracellular vesicles-derived from mesenchymal stem cells on epithelial wound healing

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=6603221574

- Saccu G et al, and Fagoonee S. Cells. 2022 Dec 2;11(23):3892. doi: 10.3390/cells11233892. Bone Marrow Mesenchymal Stromal/Stem Cell-Derived Extracellular Vesicles Promote Corneal Wound Repair by Regulating Inflammation and Angiogenesis.
- Rosso C, et al, and Bugianesi E*, Fagoonee S*. Expression of SARS-Cov-2 Entry Factors in Patients with Chronic Hepatitis. Viruses. 2022 Oct 29;14(11):2397. doi: 10.3390/v14112397.
- Fuentes-Vélez S*, Fagoonee S*, et al., Electrical Impedance-Based Characterization of Hepatic Tissue with Early-Stage Fibrosis. Biosensors (Basel). 2022 Feb 13;12(2):116. doi: 10.3390/bios12020116.
- Fagoonee S, et al. Circulating Extracellular Vesicles Contain Liver-Derived RNA Species as Indicators of Severe Cholestasis-Induced Early Liver Fibrosis in Mice. Antioxid Redox Signal. 2022 Mar;36(7-9):480-504. doi: 10.1089/ars.2021.0023.
- Manco M, Ala U, Cantarella D, Tolosano E, Medico E, Altruda F, Fagoonee S. The RNA-Binding Protein ESRP1 Modulates the Expression of RAC1b in Colorectal Cancer Cells. Cancers (Basel). 2021 Aug 13;13(16):4092. doi: 10.3390/cancers13164092.
- Fuentes-Vélez S*, Fagoonee S*, et al. Impedance-based drug-resistance characterization of colon cancer cells through real-time cell culture monitoring. 2020. doi:10.1016/j.talanta.2020.121441
- Ala U, Manco M, Mandili G, Tolosano E, Novelli F, Provero P, Altruda F, Fagoonee S. Proteomics-Based Evidence for a Pro-Oncogenic Role of ESRP1 in Human Colorectal Cancer Cells. Int J Mol Sci. 2020 Jan 16;21(2). doi: 10.3390/ijms21020575.
- Famulari ES, et al, and Fagoonee S*, Altruda F*. Human liver stem cells express UGT1A1 and improve phenotype of immunocompromised Crigler Najjar syndrome type I mice. Sci Rep. 2020 Jan 21;10(1):887. doi: 10.1038/s41598-020-57820-2.
- Fagoonee S, et al. Oncotarget. The RNA-binding protein ESRP1 promotes human colorectal cancer progression. 2017 Feb 7;8(6):10007-10024. doi: 10.18632/oncotarget.14318.
- Fagoonee S, et al. The RNA binding protein ESRP1 fine-tunes the expression of pluripotency-related factors in mouse embryonic stem cells. PLoS One. 2013;8:e72300. doi: 10.1371/journal.pone.0072300.

ADA FUNARO

Immunogenetics

BIOGRAPHICAL SKETCH

Since 2018	Head of the Laboratory of Immunogenetics, Department of Medical Sciences University of Torino, Torino, Italy
Since 2005	Associate Professor of Medical Genetics, University of Torino Medical School, Italy

LAB MEMBERS:

Enza Ferrero *Assistant professor*
Erica Ortolan *Research technician*
Yuliya Yakymiv *Post-doc*
Sara Marchisio *PhD student*
Rebecca Maria Casu, Letizia Vassoney, Elena Giuliano *Undergraduate students*

RESEARCH ACTIVITY

We study the interaction between tumor cells and microenvironment and in particular, the molecular mechanisms driving tumor immunosuppression.

Among the pathological alterations that give tumor cells invasive potential, disruption of the inflammatory response and the purinergic signaling are emerging as important components of cancer progression. Nucleotide/nucleoside receptor-mediated cell communication is orchestrated by ectonucleotidases, which efficiently hydrolyze ATP, ADP, and AMP to adenosine (ADO). ATP can act as danger signaling whereas adenosine, acts as a negative feedback mechanism to limit inflammation. Extracellular adenosine (eADO) signaling has emerged as an important regulator of immune responses, including anti-tumor immunity. eADO mediates T-cell immunosuppression through the activation of specific receptors, among which the adenosine receptor 2A (A2AR) is the most important. eADO is generated through two pathways. In the canonical pathway, extracellular ATP is hydrolyzed to ADP and AMP by CD39, then AMP is converted into eADO by CD73. The non-classical eADO production is mediated by CD38/CD157 which converts NAD+ into ADPR; then converted by CD203a to AMP, which is hydrolyzed by CD73 to eADO (Figure 1). The ectonucleotidases involved in the two adenosinergic pathways are expressed by selected cell populations both in the tumor microenvironment (TME) and in the malignant cells.

We study the interaction between tumor cells and microenvironment and in particular, the molecular mechanisms driving tumor immunosuppression.

Among the pathological alterations that give tumor cells invasive potential, disruption of the inflammatory response and the purinergic signaling are emerging as important components of cancer progression. Nucleotide/nucleoside receptor-mediated cell communication is orchestrated by ectonucleotidases, which efficiently hydrolyze ATP, ADP, and AMP to adenosine (ADO). ATP can act as danger signaling whereas adenosine, acts as a negative feedback mechanism to limit inflammation. Extracellular adenosine (eADO) signaling has emerged as an important regulator of immune responses, including anti-tumor immunity. eADO mediates T-cell immunosuppression through the activation of specific receptors, among which the adenosine receptor 2A (A2AR) is the most important. eADO is generated through two pathways. In the canonical pathway, extracellular ATP is hydrolyzed to ADP and AMP by CD39, then AMP is converted into eADO by CD73. The non-classical eADO production is mediated by CD38/CD157 which converts NAD+ into ADPR; then converted by CD203a to AMP, which is hydrolyzed by CD73 to eADO (Figure 1). The ectonucleotidases involved in the two adenosinergic pathways are expressed by selected cell populations both in the tumor microenvironment (TME) and in the malignant cells.

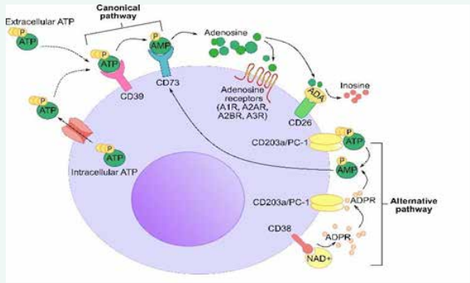


Figure 1.
Schematic representation of the Canonical and alternative adenosinergic pathways

T-cell immunosuppression through the activation of specific receptors, among which the adenosine receptor 2A (A2AR) is the most important. eADO is generated through two pathways. In the canonical pathway, extracellular ATP is hydrolyzed to ADP and AMP by CD39, then AMP is converted into eADO by CD73. The non-classical eADO production is mediated by CD38/CD157 which converts NAD+ into ADPR; then converted by CD203a to AMP, which is hydrolyzed by CD73 to eADO (Figure 1). The ectonucleotidases involved in the two adenosinergic pathways are expressed by selected cell populations both in the tumor microenvironment (TME) and in the malignant cells.

The role of (ecto)enzymes involved in the coordinated control of ADO metabolism and signaling, and how these distinct, but interrelated molecules interact during tumor progression is poorly understood.

We recently reported aberrant expression of CD39 and/or CD73 in the circulating CD4+ T cells from patients with Sézary syndrome (SS) an aggressive, leukemic form of cutaneous T cell lymphoma. We demonstrated that the altered expression of CD39 or CD73 in lymphocytes circulating in the blood and infiltrating the skin in SS patients is an intrinsic feature of each patient since diagnosis, persisting during the progression of the disease, regardless of the therapy administered, with the exception of patients being treated with Mogamulizumab (anti-C-C chemokine receptor 4 monoclonal antibody). CD39 or CD73 have important functional implications.

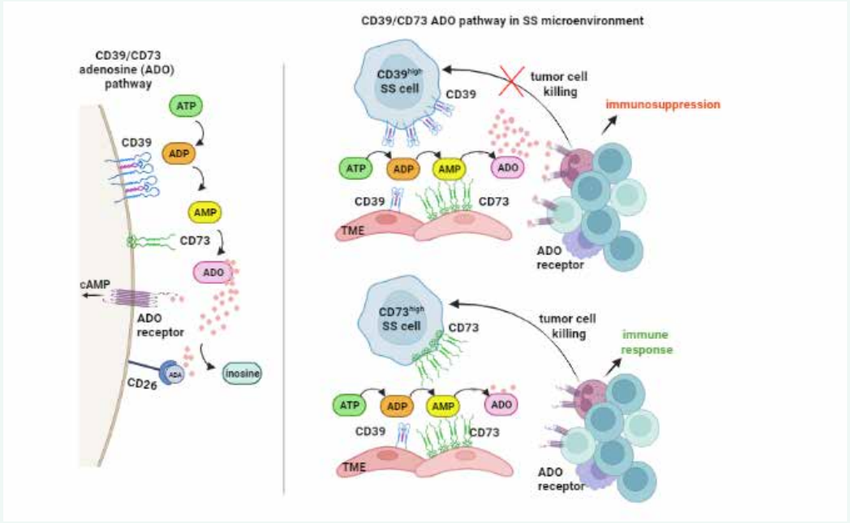


Figure 2. Ado-mediated immunosuppression in SS.

Indeed, in vivo, we found significantly higher levels of AMP in plasma from CD39+ patients compared to both CD73+ patients and healthy subjects. In keeping with this finding, ex vivo experiments demonstrated that both CD39 and CD73 are enzymatically active and that the CD39/CD73/adenosine axis implemented in the TME remarkably contributes to immunosuppression. In vitro, in the CD39+ patients, the CD39/CD73/adenosine axis promotes immunosuppression. These findings support the view that high CD39 expression in SS cells, combined with loss of CD26 (a docking site for adenosine deaminase that inactivates adenosine preventing its binding to A2AR on immune cells) and with abundant expression of CD73 in the TME (Figure 2) drive immunosuppression. Overall our data suggest that directly inhibiting the adenosinergic pathways has the potential to unleash an immune-mediated anti-tumor response via two mechanisms: i) increasing the availability of immunostimulatory eATP released by damaged and/or dying cells, and ii) reducing the generation and accumulation of suppressive ADO within the TME.

Through better understanding of various immune modulating pathways, superior combination therapies may be developed, and prolonged remissions realized, eventually improving the quality of life for patients. SS cells are usually resistant to chemotherapy and, although clinical responses to a wide variety of biologic agents are seen in 30% to 40% of patients, complete and durable responses are rare. This therapeutic challenge is strengthened by recent studies showing marked genomic heterogeneity in SS affecting a variety of signaling pathways, which suggests that the identification of appropriate small-molecule inhibitors or biological drugs for targeted therapy will also be challenging. Nonetheless, analysis of patient samples over time is an urgent need to identify subgroups of SS patients who may benefit from specific targeted therapies.

FUTURE RESEARCH PLANS

- to define subgroups of patients in which altered CD39/CD73 or CD38/CD203a/CD73 expression in the tumor cells and the TME provides the optimal milieu to sustain high concentrations of adenosine causing immunosuppression;
- to elucidate the molecular mechanisms underpinning this circuit
- to test ex vivo the ability of specific inhibitors to counteract immunosuppression in order to identify patients who are most likely to benefit from eADO-targeting agents;
- to establish whether the adenosine pathway is involved in the SS aggressive behavior and resistance to Moganulizumab treatment.

FUNDING ID (PAST 5 YERS)

- 2015-2019: Menarini Research (Roma, Italy). Functional effects of MEN1112 in leukemic blasts and in the bone marrow microenvironment in acute myeloid leukemia. (PI)
- 2020-2022: RiLo, MUR – New molecular markers for the design of targeted therapies in patients with acute myeloid leukemia (PI)
- 2021: Cutaneous Lymphoma Foundation Research Awards Program - Mechanistic insights into the CD39/CD73 adenosinergic immunosuppressive axis in patients with Sézary Syndrome: association with disease course and treatment response (Co-PI).
- 2022-24: RiLo, MUR- CD39/CD73 expression and adenosine metabolism in the cross-talk between Sézary Syndrome cells and blood-skin microenvironment (PI).

SELECTED PUBLICATIONS

- Yakymiv Y, Marchisio S, Ortolan E, Bracci C, Senetta R, Rumore MR, Tampieri C, Fia M, Ribero S, Funaro A, Quaglino P. CD39/CD73 dysregulation and adenosine metabolism contribute to T-cell immunosuppression in patients with Sézary syndrome. Blood. 2023 Jan 5;141(1):111-116. doi: 10.1182/blood.2022017259
- Funaro A, Nakagawa T, Ishihara K. Revisiting immunological roles for bone marrow stromal cell antigen-1; an entero-neuro-immune regulator. Front. Immunol. Jun 27;14:1239546. https://doi.org/10.3389/fimmu.2023.1239546
- Quaglino P, Novelli M, Fava P, Ortolan E, Astrua C, Tonella L, Tomasini CF, Senetta R, Ribero S, Ponti R, Fierro MT, Funaro A. Dis Markers. 2022 Feb 24;2022:342441 10.1155/2022/3424413
- Yakymiv Y, Augeri S, Bracci C, Marchisio S, Aydin S, D'Ardia S, Massaia M, Ferrero E, Ortolan E, Funaro A. CD157 signaling promotes survival of acute myeloid leukemia cells and modulates sensitivity to cytarabine through regulation of anti-apoptotic Mcl-1. Sci Rep. 2021 Oct 27;11(1):21230. doi: 10.1038/s41598-021-00733-5
- Yakymiv Y, Augeri S, Fissolo G, Peola S, Bracci C, Binaschi M, Bellarosa D, Pellacani A, Ferrero E, Ortolan E, Funaro A. CD157: From Myeloid Cell Differentiation Marker to Therapeutic Target in Acute Myeloid Leukemia. Cells. 2019 Dec 5;8(12):1580. doi: 10.3390/cells8121580
- Augeri S, Capano S, Morone S, Fissolo G, Giacomino A, Peola S, Drace Z, Rapa I, Novello S, Volante M, Righi L, Ferrero E, Ortolan E, Funaro A. Soluble CD157 in pleural effusions: a complementary tool for the diagnosis of malignant mesothelioma. Oncotarget. 2018 Apr 27;9(32):22785-22801. doi: 10.18632/oncotarget.25237
- Ferrero E, Lo Buono N, Morone S, Parrotta R, Mancini C, Brusco A, Giacomino A, Augeri S, Rosal-Vela A, García-Rodríguez S, Zubiaur M, Sancho J, Fiorio Pla A, Funaro A. Human canonical CD157/Bst1 is an alternatively spliced isoform masking a previously unidentified primate-specific exon included in a novel transcript. Sci Rep. 2017 Nov 21;7(1):15923. doi: 10.1038/s41598-017-16184-w
- Svegliati S, Amico D, Spadoni T, Fischetti C, Finke D, Moroncini G, Paolini C, Tonnini C, Grieco A, Rovinelli M, Funaro A, Gabrielli A. Agonistic Anti-PDGF Receptor Autoantibodies from Patients with Systemic Sclerosis Impact Human Pulmonary Artery Smooth Muscle Cells Function In Vitro. Front Immunol. 2017 Feb 8;8:75. 10.3389/fimmu.2017.00075
- Moroncini G, Grieco A, Nacci G, Paolini C, Tonnini C, Pozniak KN, Cuccioloni M, Mozzicafreddo M, Svegliati S, Angeletti M, Kazlauskas A, Avvedimento EV, Funaro A, Gabrielli A. Epitope Specificity Determines Pathogenicity and Detectability of Anti-Platelet-Derived Growth Factor Receptor α Autoantibodies in Systemic Sclerosis. Arthritis Rheumatol. 2015 Jul;67(7):1891-903. doi: 10.1002/art.39125
- Ortolan E, Giacomino A, Martinetto F, Morone S, Lo Buono N, Ferrero E, Scagliotti G, Novello S, Orecchia S, Ruffini E, Rapa I, Righi L, Volante M, Funaro A. CD157 enhances malignant pleural mesothelioma aggressiveness and predicts poor clinical outcome. Oncotarget. 2014 Aug 15;5(15):6191-205. doi: 10.18632/oncotarget.2186

ALESSANDRA GHIGO

PI3K signaling in cardiovascular and pulmonary diseases

BIOGRAPHICAL SKETCH

Since April 2020	Associate Professor of Applied Biology, Dept. of Molecular Biotechnology and Health Sciences, University of Torino, Italy
Since 2023	Member, Study Group in Basic and Translational Science of the Council of Cardio-Oncology of the European Society of Cardiology (ESC)
Since 2022	Member, Heart Failure Association (HFA) Basic and Translational Research Committee
Since 2021	Member, Editorial Board of Circulation Research
2020-2025	Member, Council of the Italian Society for Cardiovascular Research (SIRC)
Since 2017	Co-founder, Shareholder and Scientific Advisor, Kither Biotech
2017-2019	Member, Board of Directors of Kither Biotech
2015-2019	Member, Council of the Int.Society for Heart Research–Eur. Section (ISHR-ES)
2014-2019	Assistant Professor of Applied Biology, Dept. of Molecular Biotechnology and Health Sciences, University of Torino, Italy
2012-2014	Post-doctoral Fellow, Dept. of Molecular Biotechnology and Health Sciences, University of Torino, Italy
2008-2009	Visiting Fellow, Paris-Sud XI University, Chatenay-Malabry, France

LAB MEMBERS:

Alessandra Murabito *Post-doctoral fellow*
Angela Della Sala *PhD student*
Sophie Cnudde *PhD student*
Marco Mergiotti *PhD student*
Giulia Guerra *PhD student*

RESEARCH ACTIVITY

The PI3K signaling pathway regulates diverse cellular functions, including survival, proliferation and metabolism, and deregulation of this axis has been implicated in a variety of pathological conditions, ranging from cancer to cardiovascular disease. Our research is focused on understanding the role of the PI3K signal transduction in two different disease contexts: 1) cardiac disease, with a main focus on the cardiomyopathy induced by anti-cancer agents (Cardio-Oncology arm), and 2) chronic respiratory disease, with a major emphasis on cystic fibrosis lung pathology (Airway disease arm) (Fig. 1).

Figure 1.
Overview of the main research topics of Ghigo's lab.

Cardiotoxicity is a major drawback of commonly employed anti-cancer agents, often requiring the use of lower and less effective doses and, in the worst-case scenario, treatment discontinuation. Among the most cardiotoxic therapies are anthracyclines, like doxorubicin (DOX), which remain a cornerstone in the treatment of several solid and hematological cancers. Although clinical evaluation can identify patients at risk of anthracycline-induced cardiotoxicity (AIC), there is currently no effective means for preventing this complication, primarily because of an incomplete understanding of the underlying molecular mechanisms.

We believe that the early molecular alterations that occur in myocardial cells in response to DOX are still reversible and tractable, and their identification is key to the design of effective means for preventing the evolution of the disease towards an irreversible and intractable state.

Our projects aim at pinpointing the early molecular signs of AIC by exploiting in vitro and in vivo murine and zebrafish models (Fig. 2), and by combining molecular and biochemical characterization with in vivo functional phenotyping and single cell RNA sequencing approaches.

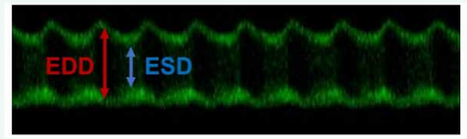


Figure 2.
Cardiac contractility evaluation in Myl7-EGFP zebrafish exposed to doxorubicin (DOX).

The second line of Ghigo's lab research is dedicated to the study of the role of the PI3K signaling axis in the pathogenesis of airway diseases, with a special attention to the lung pathology of patients with cystic fibrosis (CF). CF, the most common among rare lethal genetic diseases, is caused by mutations in the gene encoding the Cystic Fibrosis Conductance Regulator (CFTR), a chloride channel activated by cyclic AMP, expressed on the apical surface of epithelial cells, and favoring mucus hydration. In CF patients, CFTR dysfunction results in abnormally dehydrated mucus, which clogs the airways and fuels recurrent infections and inflammation, eventually culminating in respiratory failure. The standard of care for CF patients includes the so-called CFTR modulators which, however, reinstate the function of the mutant channel only up to 60% of the wild-type protein. Furthermore, CF patients treated with CFTR modulators display a residual disease characterized by lung inflammation and infections.

We showed that class I PI3Kγ is a key negative regulator of cAMP-mediated activation of the CFTR channel. We demonstrated that PI3Kγ functions as an A-kinase anchoring protein (AKAP) that tethers protein kinase A (PKA) in close proximity to its targets, cAMP phosphodiesterases (PDEs), favoring their phosphorylation and activation,

and ultimately ensuring cAMP degradation. Accordingly, we designed a cell-permeable PI3Kγ mimetic peptide (PI3Kγ MP) that, by displacing the PI3Kγ-anchored pool of PKA, inhibits PDE4 and safely increases cAMP in the lungs upon inhalation. We showed that, in virtue of its ability to increase cAMP, the PI3Kγ MP can ensure three independent therapeutic effects in CF, namely airway smooth muscle relaxation, reduced pulmonary inflammation (Fig. 3) and maximization of the efficacy of CFTR modulators.

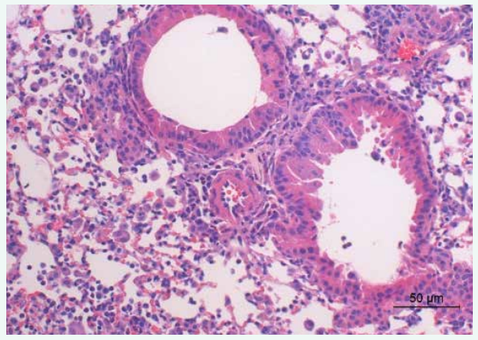


Figure 3.
Evaluation of the anti-inflammatory effect of the PI3K mimetic peptide in a murine model of inflammatory lung disease.

FUTURE RESEARCH PLANS

Cardio-Oncology arm. We plan to 1) dissect the molecular mechanisms underlying the PI3K-dependent cardiac metabolic rewiring that occurs in AIC and 2) identify and characterize new potential regulators of AIC through single cell RNA sequencing of murine and human hearts exposed to DOX.

Airway disease arm: We plan to demonstrate that PI3Kγ can be targeted, either alone or as an-add on to the standard of care, to treat 1) the lung pathology of different subsets of CF patients, including those carrying rare mutations as well as 2) non-genetic CFTR-related pulmonary disorders, like chronic obstructive pulmonary disease (COPD).

FUNDING ID (PAST 5 YEARS)

COST ACTION CA22169, PRIN 2022 20223YPL49, PRIN PNRR 2022 P2022ZB72T, Ricerca Sanitaria Finalizzata GR-2021-12371950, H2020-MSCA-ITN-2020, Telethon GGP20079, Bando Roche per la Ricerca 2019, Novel Disease Mechanisms for AstraZeneca Preclinical Compounds Award

SELECTED PUBLICATIONS

All publications: https://www.scopus.com/authid/detail.uri?authorId=24067048900

- Ghigo, A., Murabito, A., Sala, V., Pisano, A.R., Bertolini, S., Gianotti, A., Caci, E., Premchandar, A., Pirozzi, F., Ren, K., Della Sala, A., Richter, W., de Poel, E., Matthey, M., Montresor, A., Caldrer, S., Cardone, R.A., Civiletti, F., Costamagna, A., Quinney, N., Butnarasu, C., Visentin, S., Ramel, D., Laffargue, M., Tocchetti, C.G., Levi, R., Conti, M., Lu, X., Melotti, P., Sorio, C., De Rose, V., Facchinetti, F., Fanelli, V., Wenzel, D., Fleischmann, B.K., Mall, M.A., Beekman, J., Laudanna, C., Gentzsch, M., Lukacs, G.L., Pedemonte, N., and Hirsch, E. A PI3Kγ mimetic peptide triggers CFTR gating, bronchodilation and reduced inflammation in obstructive airway diseases. Sci Transl Med. 2022 Mar 30;14(638):eabl6328. doi: 10.1126/scitranslmed.abl6328. Epub 2022 Mar 30.
- Gulluni F, Prever L, Li H, Krafcikova P, Corrado I, Lo WT, Margaria JP, Chen A, De Santis MC, Cnudde SJ, Fogerty J, Yuan A, Massarotti A, Sarijalo NT, Vadas O, Williams RL, Thelen M, Powell DR, Schueler M, Wiesener MS, Balla T, Baris HN, Tiosano D, McDermott BM Jr, Perkins BD, Ghigo A, Martini M, Haucke V, Boura E, Merlo GR, Buchner DA, Hirsch E. PI(3,4)P2-mediated cytokinetic abscission prevents early senescence and cataract formation. Science. 2021 Dec 10;374(6573):eabk0410. doi: 10.1126/science.abk0410. Epub 2021 Dec 10.
- Li M, Sala V, De Santis MC, Cimino J, Cappello P, Pianca N, Di Bona A, Margaria JP, Martini M, Lazzarini E, Pirozzi F, Rossi L, Franco I, Bornbaum J, Heger J, Rohrbach S, Perino A, Tocchetti CG, Lima BHF, Teixeira MM, Porporato PE, Schulz R, Angelini A, Sandri M, Ameri P, Sciarretta S, Lima-Júnior RCP, Mongillo M, Zaglia T, Morello F, Novelli F, Hirsch E, Ghigo A. Phosphoinositide 3-Kinase Gamma Inhibition Protects from Anthracycline Cardiotoxicity and Reduces Tumor Growth. Circulation. 2018 Aug 14;138(7):696-711. doi: 10.1161/CIRCULATIONAHA.117.030352.
- Ghigo A., Perino A., Mehel H., Zahradníková A. Jr, Morello F., Leroy J., Nikolaev V.O., Damilano F., Cimino J., De Luca E., Richter W., Westenbroek R., Catterall W.A., Zhang J., Yan C., Conti M., Gomez A.M., Vandecasteele G., Hirsch E., Fischmeister R. Phosphoinositide 3-kinase γ protects against catecholamine-induced ventricular arrhythmia through protein kinase A-mediated regulation of distinct phosphodiesterases. Circulation. 2012 Oct 23;126(17):2073-83. doi: 10.1161/CIRCULATIONAHA.112.114074. Epub 2012 Sep 24.

ELIANA GIANOLIO

Paramagnetic MRI contrast agents

BIOGRAPHICAL SKETCH

Since July 2021	Associate Professor of Inorganic Chemistry at the Dep. Of Molecular Biotechnologies and Health Science of the University of Torino
Since May 2020	Joint Research Unit (JRU) Manager for the Euro-BioImaging Multi-Modal Molecular Imaging (MMMI) Italian Node infrastructure (www.mmmi.unito.it)
2010 to date	Member of the European Society for Molecular Imaging (ESMI), the European Society of Magnetic Resonance in Medicine and Biology (ESMRMB), the Italian Group of Discussion on Magnetic Resonance (GIDRM) and of the European Gadolinium Retention Evaluation consortium (e-GREC)
2005-2021	Permanent position as research technician (EP4) at the Dept. of Molecular Biotechnologies and Health Sciences and Molecular Imaging Center (www.cim.unito.it) of the University of Torino
2000-2005	Post doctoral fellow at the Dept. of Chemistry IFM of the University of Torino
1997-2000	PhD in Biochemical Science, University of Torino
1996	Master's degree in Chemistry

LAB MEMBERS:

Lorenzo Palagi *assegnista di ricerca*
Serena Rizzuti *dottoranda*
Ferdeze Hasallari *borsista di ricerca*

RESEARCH ACTIVITY

Our research activity is in the field of design and testing of imaging agents for in vitro and in vivo applications, with a special focus on the characterization and application of paramagnetic contrast agents as contrast agents (CA) in Magnetic Resonance Imaging (MRI). This class of substances is mainly represented by Gadolinium(III)-based contrast agents (GBCAs).

Along the years, our research work has dealt with the elucidation of the relationships between solution structure/ dynamics of GBCAs and the determinants of their proton relaxation enhancement with the aim of generating imaging reporters endowed with high sensitivity and specificity. To this scope, our research is always active, at the preclinical level, in the synthesis, characterization and validation of new Gd complexes endowed with high relaxivity (high efficiency), while maintaining a high thermodynamic and kinetic stability. In Figure 1, some examples are reported of new Gd-complexes studied by our group whose relaxometric efficiency is far higher than that of GBCAs used today in the clinical settings.

In recent years some concerns raised on the use of GBCAs because it has been acknowledged that a portion of the injected gadolinium can remain in the body for extended periods even in subjects with normal renal functions. In this context, our research has been focused on the comprehension, on preclinical animal models, of the mechanisms behind Gd(III) retention and the identification of the organs where the accumulation mostly occurs. Efforts were also put into the elucidation of the chemical form (free or chelated) of retained Gd (Figure 2).

FUTURE RESEARCH PLANS

Currently, we are interested in the study of multicomponent peptide-based hydrogels and nanogels matrices for the incorporation/conjugation of Fe(III) complexes as a novel generation of MRI CAs characterized by lower toxicity and enhanced sensitivity.

In another project, we aim to develop novel MRI molecular probes targeted to specific collagen types. Three new peptide sequences specifically targeting either type-I or type-III collagen will be functionalized with Gd(III)-based

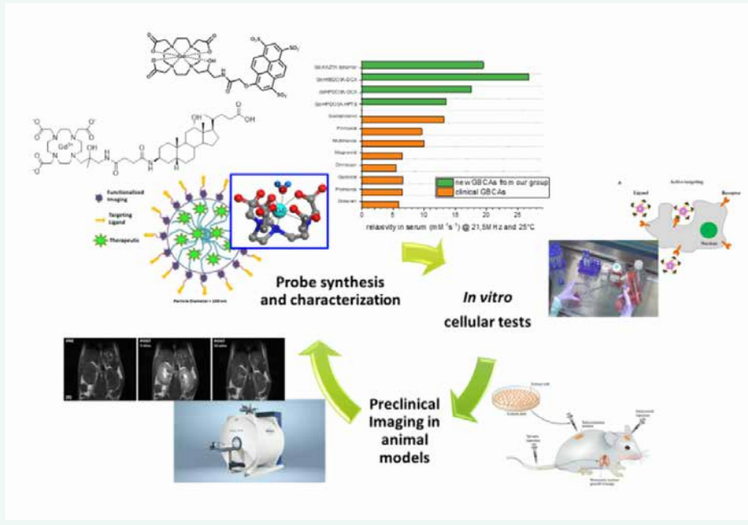


Figure 1.
Design, characterization and validation of paramagnetic magnetic resonance imaging (MRI) contrast agents (CAs)
Another interesting field of action is related to the obtainment of a number of MRI CAs whose relaxation enhancement properties are responsive to a specific parameter of the biological microenvironment where they distribute, i.e. pH, temperature, enzymatic activity, metabolites' concentration, oxygenation.

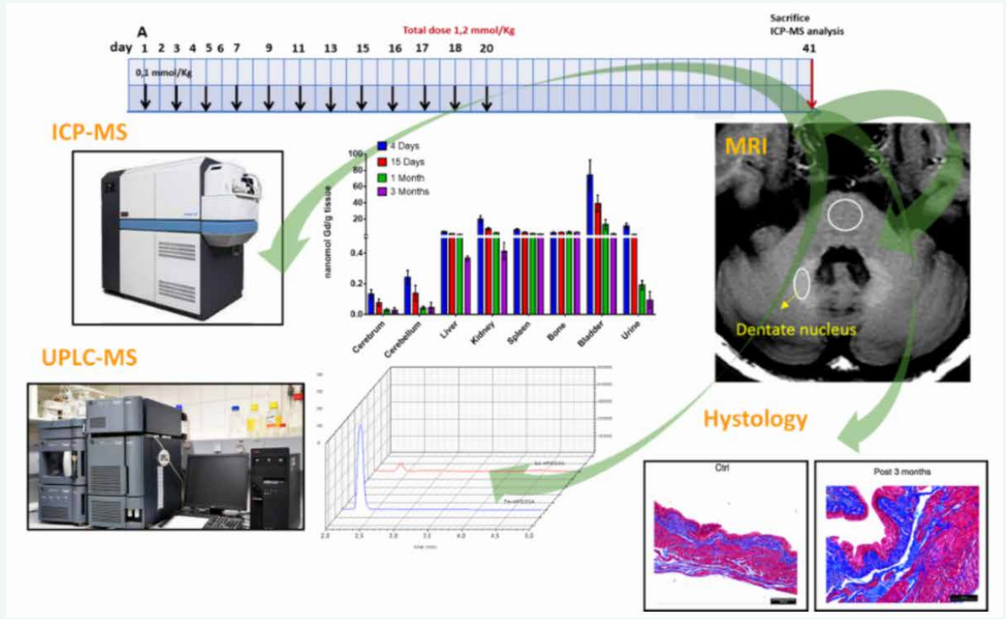


Figure 2.

Preclinical investigation of gadolinium retention in the body of mice upon multiple administrations of gadolinium-based contrast agents (GBCAs).
On the other hand, these discoveries have sparked renewed interest in seeking alternatives to gadolinium(III) for MR contrast imaging. From our side, we are interested in the study of Fe(III) complexes bearing ligands that are represented by clinically approved iron sequestering agents with the expectation of a facilitated clinical translation as MRI CAs. For some applications, with the aim of increasing both the efficacy (in terms of signal enhancement) and the vehicolation efficiency, our Gd- and Fe-based imaging probes are embedded into nano-sized carriers (from endogenous systems to liposomes, micelles, hydrogels, nanogels,etc.) for diagnostic and “theranostic” applications.

complexes. By detecting and quantifying the two different types of collagens, we hypothesise that it is possible to stage the tumour and predict its progression.

FUNDING ID (PAST 5 YEARS)

- European Project H2020-PHC-2015RIA n° 668119 IDentIFY (Improving Diagnosis by Fast Field-Cycling MRI), 2016-2019, Participant
- Progetto di Ateneo –Research for the Territory-finaced by “Compagnia di San Paolo” entitled “ Gd-retention in the brain after Contrast Enhanced MRI” 2016-2019, WP leader
- Regional Project (Piemonte), F.E.S.R. 2014/2020 entitled “Sviluppo preclinico e clinico di Gadoplus, un mezzo di contrasto per risonanza magnetica dotato di elevata relassività” 2017-2020, WP leader
- European Project H2020 FET OPEN Primogaia (Prepolarized MRI at Earth Field), 2019-2023, Participant
- European Project H2020 FET OPEN Nectar (NEutron Capture-enhanced Treatment of neurotoxic Amyloid aggRegates), 2021-2024, Participant

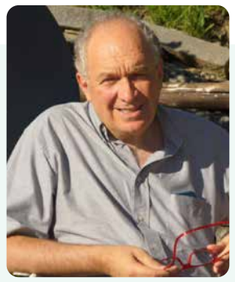
SELECTED PUBLICATIONS

Link Scopus: https://www.scopus.com/authid/detail.uri?authorId=6603359686

- Romano, F., Di Gregorio, E., Riccardi, G., Furlan, C., Cavallini, N., Savorani, F., Di Porzio, A., De Tito, S., Randazzo, A., Gianolio, E.*, Iaccarino, N. Comparison of the biological effects of gadodiamide (Omniscan) and gadoteridol (ProHance) by means of multi-organ and plasma metabolomics (2023) Analyst, 148 (11), pp. 2415-2424.DOI: 10.1039/d3an00353a
- Furlan, C., Montarolo, F., Di Gregorio, E., Parolisi, R., Atlante, S., Buffo, A., Bertolotto, A., Aime, S., Gianolio, E.*Analysis of the Gadolinium retention in the Experimental Autoimmune Encephalomyelitis (EAE) murine model of Multiple Sclerosis (2021) Journal of Trace Elements in Medicine and Biology, 68, art. no. 126831 DOI: 10.1016/j.jtemb.2021.126831
- Palagi, L., Di Gregorio, E., Costanzo, D., Stefania, R., Cavallotti, C., Capozza, M., Aime, S., Gianolio, E.* Fe(deferasirox)2: An Iron(III)-Based Magnetic Resonance Imaging T1Contrast Agent Endowed with Remarkable Molecular and Functional Characteristics (2021) Journal of the American Chemical Society, 143 (35), pp. 14178-14188. DOI: 10.1021/jacs.1c04963
- Tear, L.R., Carrera, C., Dhakan, C.B., Cavallari, E., Travagin, F., Calcagno, C., Aime, S., Gianolio, E.* An albumin-binding Gd-HPDO3A contrast agent for improved intravascular retention (2021) Inorganic Chemistry Frontiers, 8 (17), pp. 4014-4025. DOI: 10.1039/d1qi00128k
- Di Gregorio, E., Lattuada, L., Maiocchi, A., Aime, S., Ferrauto, G., Gianolio, E.* Supramolecular adducts between macrocyclic Gd(iii) complexes and polyaromatic systems: a route to enhance the relaxivity through the formation of hydrophobic interactions (2021) Chemical Science, 12 (4), pp. 1368-1377. DOI: 10.1039/d0sc03504a
- Kock, F.V.C., Forgács, A., Guidolin, N., Stefania, R., Vágner, A., Gianolio, E.*, Aime, S., Baranyai, Z. [Gd(AAZTA)]− Derivatives with n-Alkyl Acid Side Chains Show Improved Properties for Their Application as MRI Contrast Agents (2021) Chemistry - A European Journal, 27 (5), pp. 1849-1859. DOI: 10.1002/chem.202004479
- Tear, L.R., Carrera, C., Gianolio, E.*, Aime, S. Towards an Improved Design of MRI Contrast Agents: Synthesis and Relaxometric Characterisation of Gd-HPDO3A Analogues (2020) Chemistry - A European Journal, 26 (27), pp. 6056-6063. DOI: 10.1002/chem.202000479
- Di Gregorio, E., Furlan, C., Atlante, S., Stefania, R., Gianolio, E.*, Aime, S. Gadolinium retention in erythrocytes and leukocytes from human and murine blood upon treatment with gadolinium-based contrast agents for magnetic resonance imaging (2020) Investigative Radiology, 55 (1), pp. 30-37. DOI: 10.1097/RLI.0000000000000608
- Di Gregorio, E., Ferrauto, G., Furlan, C., Lanzardo, S., Nuzzi, R., Gianolio, E.*, Aime, S. The Issue of Gadolinium Retained in Tissues: Insights on the Role of Metal Complex Stability by Comparing Metal Uptake in Murine Tissues Upon the Concomitant Administration of Lanthanum-A nd Gadolinium-Diethylentriamminopentaacetate (2018) Investigative Radiology, 53 (3), pp. 167-172. DOI: 10.1097/RLI.0000000000000423
- Gianolio, E., Bardini, P., Arena, F., Stefania, R., Gregorio, E.D., Iani, R., Aime, S. Gadolinium retention in the rat brain: Assessment of the amounts of insoluble gadolinium-containing species and intact gadolinium complexes after repeated administration of gadolinium-based contrast agents (2017) Radiology, 285 (3), pp. 839-849. DOI: 10.1148/radiol.2017162857

ANDREA GRAZIANI

Tumor-Host signaling interactions Lab



BIOGRAPHICAL SKETCH

1983-86 Ph.D. fellow of the School of Pharmacology at the Mario Negri Institute Pharmacological Research (Lab of Enzymology, head Dr. Mario Salmona), Milano

1986-90 post-doctoral fellow at the Dept. of Cellular and Molecular Physiology (Lab of Lipid Signaling, head prof. Lewis C. Cantley), Tufts University Medical School, Boston, USA)

1991-95 Research Associate, Dept. of Biomedicine and Oncology (Lab of Oncogene Signaling, head Prof. Paolo Comoglio), University of Torino Medical School, Torino

1996-99 Assistant Professor of Biochemistry, Dept. of Genetics, Biology and Biochemistry, University of Torino Medical School

2000-06 Associate Professor of Biochemistry, Dept. of Medical Sciences, University of Piemonte Orientale Medical School, Novara

2006-14 Full professor of Biochemistry and of Molecular Biology, Dept. of Translational Medicine University of Piemonte Orientale Medical School, Novara

2014-18 Full professor of Biochemistry, Medical School University Vita-Salute San Raffaele, Milan, and Head of the Lipid Signaling unit at the Division of Experimental Oncology, Ospedale San Raffaele, Milano

2019 Full Professor of Molecular Biology, Dept. of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino Medical School.

LAB MEMBERS:

Elia Angelino *RTDA (UniUPO)*
Valeria Malacarne *RTDA (UniUPO)*
Giulia Rossino *Assegnista di Ricerca (UniUPO)*
Suvham Barua *Ph.D. student*
Lorenza Bodo *Ph.D. student*
Sabrina Mula *Ph.D. student*
Raluca Minea *Ph.D. student*
Alessia Labate, Carolina Sciavolino, Camilla Racca, Alessia Meschi, Beatrice D'Anna *Master and undergraduate students*

RESEARCH ACTIVITY

The current research projects in our lab are deeply rooted in my long-time interest in investigating the regulation of the cell surface receptor signaling mechanisms mediating the communication between cancer cells and their environment. The two current major areas of interests are to investigate 1) defective TCR signaling in both tumor-infiltrating T cells and aging T cells, and 2) the molecular mechanisms mediating skeletal muscle atrophy in cancer cachexia and ageing-associated sarcopenia.

1) defective TCR signaling in both tumor-infiltrating T cells and aging T cells

Through the years, our lab pioneered the role of Diacylglcyerol kinase alpha (DGKA) in oncogene signaling, by showing that its activation is required for tumoriegenesis and invasion by regulating atypical PKC, Rho-family small GTPases and integrin recycling (Cutrupi, EMBO J, 2000; Baldanzi, Oncogene, 2004; Chianale, Mol.Biol.Cell, 2007, Baldanzi, Oncogene, 2008; Chianale, PNAS; 2010; Rainero, J.Cell.Biol. 2012). By virtual screening for DGKA inhibitors, in collaboration with pharmaceutical chemists, we identified a novel DGKA inhibitor (Velnati, Eur. J Med. Chem 2018; Velnati et al. 2019).

In addition, we showed the negative regulation of DGKA activity upon T cells antigen stimulation, and its deregulation in immunoproliferative syndromes and leukemia (Bachiocchi, Blood, 2005; Baldanzi, J. Immunol. 2011). Indeed, we reported that DGKA targeting in vitro rescues defective TCR signaling strength and the pathology in in vitro and in vivo models of X-linked immunoproliferative syndrome (XLP) (Ruffo, Malacarne et al. Sci Transl Med, 2016).

Though the years we have developed interested in investigating the regulation of TCR and Chimeric T cell Antigen Receptor (CAR) expression at the cell surface (Greco, Malacarne, et al. Sci Transl Med, 2021). We are currently investigating the hypothesis that both DGKA and SAP, whose LOF mutations cause XLP, reciprocally regulate TCR and Chimeric T cell Antigen Receptor (CAR) signaling strength by controlling their trafficking to and from the T cell surface. We are also investigating the role of DGKA deregulation in driving defective TCR signaling and exhaustion in tumor-infiltrating T cells and in immune-aging.

2) the molecular mechanisms mediating skeletal muscle atrophy in cancer cachexia and ageing-associated sarcopenia.

Upon our pioneering work reporting the first evidence that Ghrelin, an orexigenic hormone, acts as a survival factor in the myocardium by activating mitophagy through binding to a novel receptor yet to be identified (Baldanzi, J.Cell.Biol. 2002; Ruozi, Nature Commun. 2015), we then showed that in the skeletal muscle is part of a stress-induced adaptive response, which counteracts muscle wasting and sarcopenia, enhances Insulin sensitivity, and triggers muscle regeneration by activating satellite cells (Filigheddu, Mol.Biol.Cell. 2007, Porporato, J. Clin. Invest. 2013, Gortan-Capellari, Diabetes 2015, Reano, Angelino, Stem Cells, 2017, Angelino, Endocrine, 2019, Agosti, Aging, 2020). However, as Ghrelin does not prevent muscle wasting in cancer cachexia, we are currently investigating the role of tumor-induced impairment of cAMP signaling and CREB1-dependent transcriptome reprogramming driving mitochondrial dysfunction in cancer cachexia. Indeed, through in vitro and in vivo models, we have identified the molecular mechanisms linking tumor-released factors to defective cAMP signaling and mitochondrial dysfunction, and we are currently verifying these findings in human skeletal muscle biopsies. We are also planning to investigate the significance of these finding in aging associated sarcopenia.

FUNDING ID (PAST 5 YERS)

- 2022-2026: AIRC (ID 25702) Diacylglcyerol kinase alpha as mediator of tumor-induced immune escape.
- 2023-2025: Ricerca Finalizzata Ministero della Salute (RF-2021-12373598-) Uncover and overcome senescence and dysfunction of genetically engineered T lymphocytes for cancer immunotherapy
- 2023-2025: PNRR MUR MC4C2 PE8 “Age-it: Ageing individuals in an ageing society”) “Molecular mechanisms coupling DNA damage to T cell senescence and dysfunction”
- 2023-2025: MUR PRIN 2022 (coordinator naz.) Role of mRNA splicing regulation in cancer cachexia
- 2018-2021, Telethon: SAP-mediated DGKα inhibition triggers a novel cell fate switch in antigen-activated T cells: implications for XLP1 therapy
- 2018-2021, Fondazione CARIPLO, Role of unacylated ghrelin and autophagy in counteracting aging-associated frailty
- 2017-2020, MIUR – PRIN 2016 (coordinatore naz.): Diacylglcyerol kinase alpha regulates self-renewal and tumorigenesis of glioblastoma cancer stem cells
- 2016-2019, AIRC: Role of tumor-induced PI3-kinase-gamma in promoting skeletal muscle ghrelin resistance in cancer cachexia
- 2018-2019, AFM-Telethon (France), Acylated and Unacylated Ghrelin, inflammation, and muscle wasting: the unexpected role of novel and old ghrelin receptors.

SELECTED PUBLICATIONS (10)

- Velnati S et al (2023) Wiskott-Aldrich syndrome protein interacts and inhibits diacylglycerol kinase alpha promoting IL-2 induction. Front. Immunol. 14, 1043603, DOI: 10.3389/fimmu.2023.1043603
- Greco B, Malacarne V. et al. (2022) Disrupting N-glycan expression on tumor cells boosts chimeric antigen receptor T cell efficacy against solid malignancies. Sci Transl Med 14, eabg3072 DOI: : 10.1126/scitranslmed.abg3072
- Agosti E et al (2020) Both ghrelin deletion and unacylated ghrelin overexpression preserve muscles in aging mice. Aging 12, 13939–57 DOI: 10.18632/aging.103802
- Velnati S et al (2018). Identification of a novel DGKα inhibitor for XLP-1 therapy by virtual screening European Journal of Medicinal Chemistry 164(Chem. Rev. 111 2011), 378-390. DOI: 10.1016/j.ejmech.2018.12.061
- Reano S, Angelino E et al. (2017). Unacylated Ghrelin Enhances Satellite Cell Function and Relieves the Dystrophic Phenotype in Duchenne Muscular Dystrophy mdx Model STEM CELLS 35(7), 1733-46. DOI: 10.1002/stem.2632
- Ruffo E, Malacarne, V et al. (2016). Inhibition of diacylglycerol kinase α restores restimulation-induced cell death and reduces immunopathology in XLP-1 Science Translational Medicine 8(321), 321ra7-321ra7. DOI: 10.1126/scitranslmed.aad1565
- Cappellari G et al. (2016). Unacylated Ghrelin Reduces Skeletal Muscle Reactive Oxygen Species Generation and Inflammation and Prevents High-Fat Diet–Induced Hyperglycemia and Whole-Body Insulin Resistance in Rodents, Diabetes 65(4), 874-86 DOI: 10.2337/db15-1019
- Ruozi, G et al. (2015). AAV-mediated in vivo functional selection of tissue-protective factors against ischaemia Nature Communications 6, 7388. DOI: 10.1038/ncomms8388
- Porporato, P et al. (2013) Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice Journal of Clinical Investigation 123, 611-22. DOI: 10.1172/jci39920
- Rainero E et al. (2012). Diacylglycerol kinase α controls RCP-dependent integrin trafficking to promote invasive migration The Journal of Cell Biology 196(2), 277-295. DOI: 10.1083/jcb.201109112

FEDERICO GULLUNI

Targeting aneuploidy in breast cancer

BIOGRAPHICAL SKETCH

2017-2019	ostdoctoral fellow, AIRC-FIRC, University of Turin, Dept. of Molecular Biotechnology and Health Sciences, Italy
2019-2021	Postdoctoral fellow, Pezcoller-SIC, University of Turin, Dept. of Molecular Biotechnology and Health Sciences, Italy
2021-present	Assistant professor, University of Turin, Medical Faculty, Dept. of Molecular Biotechnology and Health Sciences, Italy

GROUP MEMBERS:

Ping Zhang *PhD student (co-tutor)*
Roberta Rubino *PhD student (co-tutor)*
Emanuele Fantastico *Postgraduate fellow*

RESEARCH ACTIVITY

Aneuploidy, characterized by an abnormal number of chromosomes, is a well-established characteristic linked to cancer development. Unlike normal cells, which usually maintain a stable and predictable number of chromosomes (euploid), cancer cells frequently exhibit an erratic count (aneuploid). The objective of our research is to comprehend the significance of aneuploidy in cancer and to determine whether it presents vulnerabilities that can be targeted for treatment. Specifically, recent studies suggest that certain drugs, by making cancer cells even more aneuploid than they already are, might push them past a point of no return, causing catastrophic errors during cell division and ultimately leading to cell death (Figure 1).

Thinking of it like fixing a malfunctioning assembly line in a factory, the introduction of changes that make the process even more chaotic may reach a breaking point, causing the entire system to fail. In cancer cells, this failure is known as mitotic catastrophe (Figure 2).

In our research focused on breast cancer, we explore the deficiency of phosphoinositide kinases, including PI3K-C2α. When these kinases are dysfunctional, they disrupt the normal arrangement and segregation of chromosomes during cell division, resulting in genomic instability. Our particular focus lies on two phosphoinositides (PIs) generated during this process: PI(3,4)P2 and its precursor PI(4)P, both playing a crucial role in concluding the cell division process. Essentially, our research suggests that disturbing the production of specific phosphoinositides during mitosis can prompt a rapid merging of cells in the initial phases of cell division. This results in an unusual increase in chromosome count, inducing chromosomal instability (CIN) and rendering cancer cells more susceptible to drugs targeting the cell division apparatus (Figure 2).

Moreover, disrupting the early stages of cell division in cancer cells, thus inducing CIN, may potentially trigger

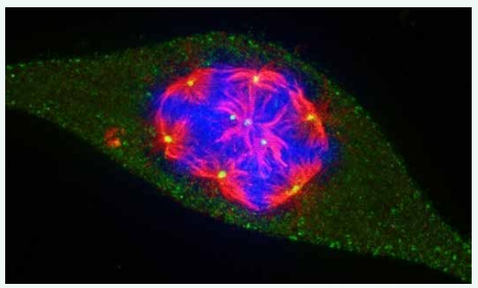


Figure 1.
Immunofluorescence analysis was conducted on HeLa cells undergoing mitotic cell division, wherein α-tubulin was utilized as a marker for the spindle, ɣ-tubulin in green was employed to delineate centrosomes, and DAPI in blue was applied for DNA staining.

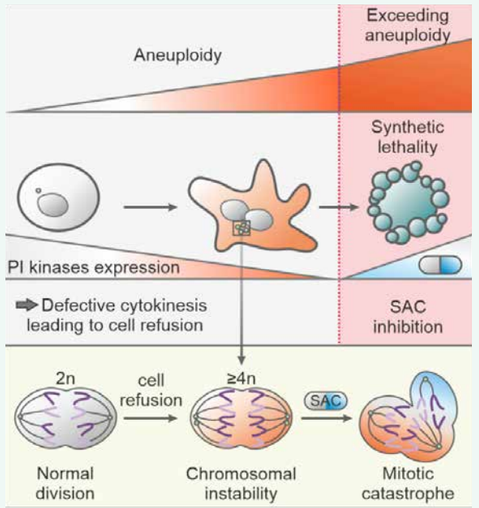


Figure 2.
Illustrates that the knockdown or inhibition of PI kinases results in tetraploidy and chromosomal instability (CIN). Disrupting the spindle assembly checkpoint (SAC) can induce mitotic catastrophe, emphasizing a mechanism of synthetic lethality in breast cancer cells.

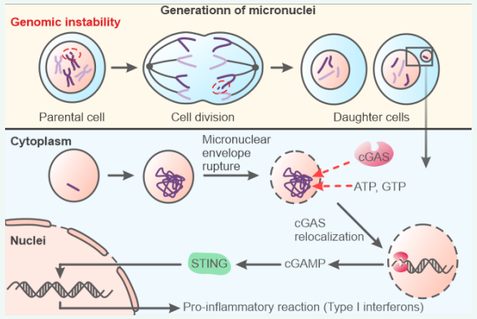


Figure 3.
Depicts a schematic representation of the activation of the cGAS-STING pathway and the ensuing pro-inflammatory reaction as a consequence of chromosomal instability (CIN).

the expression of neoantigens derived from genomic instability itself. These neoantigens can activate the body's natural defense against cancer – the immune response. Concurrently, CIN can activate signaling pathways that modulate the immune response, including the cGAS-STING pathway. Therefore, we are adopting a dual strategy: 1) utilizing drugs to enhance aneuploidy in tumors, combined with drugs targeting chromosome segregation, to induce a catastrophic failure in cell division; and 2) identifying cancer vulnerabilities resulting from the activation of cGAS-STING-dependent responses to chromosomal instability (CIN) and the subsequent immune modulation associated with cancer progression (Figure 3). Through this dual-strategy approach, our goal is to enhance the efficacy of treatments in suppressing the growth of breast cancer cells.

FUTURE RESEARCH PLANS

Our upcoming research aims to unravel the intricate processes that lead to aneuploidy during the early stages of cell division in breast cancer. Our main objective is to investigate the molecular mechanisms orchestrating this phenomenon, employing genetic modifications and pharmacological interventions. The focus will be on manipulating key proteins, particularly phosphoinositide kinases and phosphatases, crucial in mitotic cell division. Concurrently, our future plans involve exploring the potential connection of these events to immune system alterations, specifically through the activation of the cGAS-STING pathway. Furthermore, we seek to determine whether heightened aneuploidy renders breast cancer cells more susceptible to specific inhibitors targeting essential cell division checkpoints, such as the spindle assembly checkpoint (SAC) or cyclin-dependent kinases (CDKs). Essentially, our main goal is to unravel the complex coordination of cellular processes contributing to aneuploidy induction in breast cancer, with the ultimate objective of paving the way for potential breakthroughs in treatment strategies.

FUNDING ID (PAST 5 YERS)

- → 2023 – 2027 MFAG. Targeting aneuploidy for breast cancer therapeutics.
- → 2023 – 2025 PRIN 2022. Characterization of extracellular vesicle biogenesis and content in Hepatocellular Carcinoma: circulating biomarkers of tumorigenesis and progression.
- → 2020 – 2021 PEZCOLLER FOUNDATION-SIC. Defining The Role of PI3K-C2α as a New Prognostic Marker in Breast Cancer Progression.
- → 2017 – 2019 AIRC/FIRC FELLOWSHIP FOR ITALY. Study of the role of PtdIns(3,4)P2 and PI3K-C2α in breast cancer.

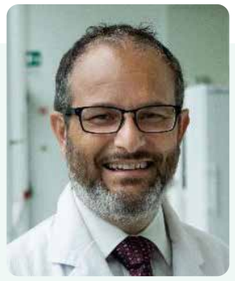
SELECTED PUBLICATIONS

Link Scopus profile: https://www.scopus.com/authid/detail.uri?authorId=55778214900

- → Phosphoinositide Conversion Inactivates R-RAS and Drives Metastases in Breast Cancer. Li H, Prever L, Hsu MY, Lo WT, Margaria JP, De Santis MC, Zanini C, Forni M, Novelli F, Pece S, Di Fiore PP, Porporato PE, Martini M, Belabed H, Nazare M, Haucke V, Gulluni F*, Hirsch E*. Adv Sci (Weinh). 2022 Mar;9(9):e2103249. doi: 10.1002/advs.202103249.
- → PI(3,4)P2-mediated cytokinetic abscission prevents early senescence and cataract formation. Gulluni F, Prever L, Li H, Krafcikova P, Corrado I, Lo WT, Margaria JP, Chen A, De Santis MC, Cnudde SJ, Fogerty J, Yuan A, Massarotti A, Sarijalo NT, Vadas O, Williams RL, Thelen M, Powell DR, Schueler M, Wiesener MS, Balla T, Baris HN, Tiosano D, McDermott BM Jr, Perkins BD, Ghigo A, Martini M, Haucke V, Boura E, Merlo GR, Buchner DA, Hirsch E. Science. 2021 Dec 10;374(6573):eabk0410. doi: 10.1126/science.abk0410.
- → Targeting PI3K/AKT/mTOR Signaling Pathway in Breast Cancer. Li H, Prever L, Hirsch E, Gulluni F. Cancers (Basel). 2021 Jul 14;13(14). doi: 10.3390/cancers13143517.
- → Mutations in PIK3C2A cause syndromic short stature, skeletal abnormalities, and cataracts associated with ciliary dysfunction. Tiosano D*, Baris HN*, Chen A*, Hitzert MM*, Schueler M*, Gulluni F*, Wiesener A, Bergua A, Mory A, Copeland B, Gleeson JG, Rump P, van Meer H, Sival DA, Haucke V, Kriwinsky J, Knaup KX, Reis A, Hauer NN, Hirsch E, Roepman R, Pfundt R, Thiel CT, Wiesener MS, Aslanyan MG, Buchner DA. PLoS Genet. 2019 Apr;15(4):e1008088. doi: 10.1371/journal.pgen.1008088.
- → Class II PI3K Functions in Cell Biology and Disease. Gulluni F, De Santis MC, Margaria JP, Martini M, Hirsch E. Trends Cell Biol. 2019 Apr;29(4):339-359. doi: 10.1016/j.tcb.2019.01.001.
- → Cytokinetic Abscission: Phosphoinositides and ESCRTs Direct the Final Cut. Gulluni F, Martini M, Hirsch E. J Cell Biochem. 2017 Nov;118(11):3561-3568. doi: 10.1002/jcb.26066.
- → Autoregulation of Class II Alpha PI3K Activity by Its Lipid-Binding PX-C2 Domain Module. Wang H, Lo WT, Vujičić Žagar A, Gulluni F, Lehmann M, Scapozza L, Haucke V, Vadas O. Mol Cell. 2018 Jul 19;71(2):343-351.e4. doi: 10.1016/j.molcel.2018.06.042.
- → Mitotic Spindle Assembly and Genomic Stability in Breast Cancer Require PI3K-C2α Scaffolding Function. Gulluni F, Martini M, De Santis MC, Campa CC, Ghigo A, Margaria JP, Ciraolo E, Franco I, Ala U, Annaratone L, Disalvatore D, Bertalot G, Viale G, Noatynska A, Compagno M, Sigismund S, Montemurro F, Thelen M, Fan F, Meraldi P, Marchiò C, Pece S, Sapino A, Chiarle R, Di Fiore PP, Hirsch E. Cancer Cell. 2017 Oct 9;32(4):444-459.e7. doi: 10.1016/j.ccell.2017.09.002.
- → PI3K class II α controls spatially restricted endosomal PtdIns3P and Rab11 activation to promote primary cilium function. Franco I*, Gulluni F*, Campa CC*, Costa C*, Margaria JP, Ciraolo E, Martini M, Monteyne D, De Luca E, Germena G, Posor Y, Maffucci T, Marengo S, Haucke V, Falasca M, Perez-Morga D, Boletta A, Merlo GR, Hirsch E. Dev Cell. 2014 Mar 31;28(6):647-58. doi: 10.1016/j.devcel.2014.01.022.
- → Spatiotemporal control of endocytosis by phosphatidylinositol-3,4-bisphosphate. Posor Y, Eichhorn-Gruenig M, Puchkov D, Schöneberg J, Ullrich A, Lampe A, Müller R, Zarbakhsh S, Gulluni F, Hirsch E, Krauss M, Schultz C, Schmoranzer J, Noé F, Haucke V. Nature. 2013 Jul 11;499(7457):233-7. doi: 10.1038/nature12360.

EMILIO HIRSCH

PI3K signaling



BIOGRAPHICAL SKETCH

1995-2000	Assistant Professor, University of Turin, Medical Faculty
2000-2005	Associate Professor, University of Turin, Medical Faculty
2005-present	Full Professor of Biology, University of Turin, Medical Faculty, Dept. of Molecular Biotechnology and Health Sciences, Italy
2020-present	Scientific Director of Molinette Research Foundation
2021-present	Director of the Mol.Biotech. Center, University of Torino, Italy

GROUP MEMBERS:

Lorenzo Prever *PhD student*
Ping Zhang *PhD student*
Roberta Rubino *PhD student*

RESEARCH ACTIVITY

Phosphoinositide 3-kinases (PI3Ks) constitute a family of lipid kinases crucial for converting phosphatidylinositol lipids into phosphoinositide second messengers. Within this PI3K family, class II PI3Ks emerge as a distinct subgroup actively involved in various cellular signaling pathways. More specifically, class II PI3Ks play a key role in generating two essential second messengers: phosphatidylinositol 3-phosphate [PtdIns(3)P] and phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P2]. These second messengers exert regulatory control over a diverse array of cellular processes, including cell growth, survival, metabolism, and intracellular trafficking.

Class II PI3Ks can be further classified into three subtypes: PI3K-C2α, PI3K-C2β, and PI3K-C2ɣ. Distinguishing themselves from class I PI3Ks, these class II variants exhibit unique structural characteristics and regulatory mechanisms. Notably, class II PI3Ks feature a distinct domain architecture, comprising a C-terminal phosphoinositide-binding PX (phox homology) domain and an N-terminal unstructured region involved in protein-protein interactions. These structural distinctions play a pivotal role in shaping the functional diversity of class II PI3Ks and determining their specific involvement in cellular processes (see Figure 1).

While the exact functions of class II PI3Ks are still being investigated, extensive research, including studies conducted in our laboratory, has yielded significant insights into their participation in a wide range of cellular processes. Through our investigations, we have elucidated the specific roles of class II PI3K isoforms in various contexts. For instance, our research indicates that PI3K-C2α is not only crucial for mouse embryo development but also plays a vital role in clathrin-dependent endocytosis and vesicle trafficking. We observed that the disruption of PI3K-C2α results in impaired embryonic development and disturbed intracellular transport, underscoring its

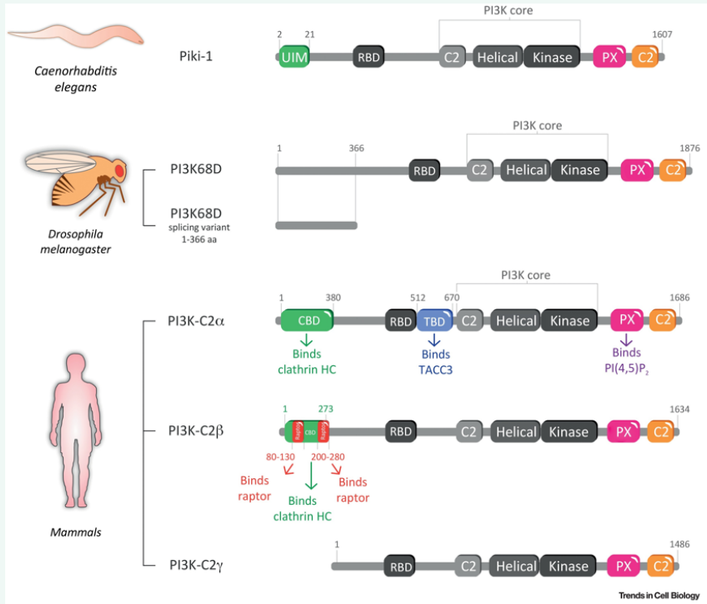


Figure 1. Schematic Representation of Class II Phosphoinositide 3-Kinases (PI3Ks) in Worms (piki-1), Fly (PI3K68D), and Mammals (PI3K-C2α, PI3K-C2β, and PI3K-C2γ) and Relative Domains. CBD, Clathrin-binding domain; RBD, Ras-binding domain; TBD, TACC3-binding domain; UIM, ubiquitin interacting motif.

Figure 1.

significance in these processes (Figure 2-3). Additionally, our investigations have uncovered that PI3K-C2α is implicated in the activation of Rab11, a small GTPase that governs intracellular transport and recycling (Figure 3). These findings provide insights into the regulatory mechanisms mediated by PI3K-C2α in membrane trafficking.

Furthermore, our investigations have revealed the significance of PI3K-C2α in the control of cell division, particularly in the context of breast cancer tumorigenesis. We have observed that altered expression or activity of PI3K-C2α impacts cell proliferation and division, suggesting its involvement in the regulation of these processes in cancer cells.

Similarly, our studies have concentrated on the role of PI3K-C2β in mitosis progression, specifically in prostate cancer. Through our research, we have clarified the importance of PI3K-C2β in modulating mitotic events and ensuring proper cell division in prostate cancer cells. These findings shed light on the specific functions of PI3K-C2β in the context of cancer biology and provide a potential target for therapeutic interventions.

More recently, our investigations have revealed an unexpected role of PI3K-C2β-mediated lipid signaling in the regulation of mTORC1-dependent neuronal excitability. We discovered that PI3K-C2β activity influences neuronal excitability in both mice and humans through its impact on mTORC1 signaling. This discovery highlights the involvement of class II PI3Ks in neuronal function and unveils a novel link between lipid signaling and neuronal excitability.

Additionally, our research has clarified the subcellular localization of PI3K-C2γ on early endosomes, offering insights into its compartmentalization within the cell. We have established that PI3K-C2γ is instrumental in regulating Akt2 activation and glycogen storage in the liver. Our studies indicate that the disruption of PI3K-C2γ expression or activity results in the dysregulation of Akt2 signaling, thereby impeding glycogen synthesis and storage in hepatocytes.

Collectively, these findings emphasize the varied functions of class II PI3K isoforms and their substantial implications in cellular processes and disease contexts. Ongoing research, including investigations conducted in our laboratory, persists in enhancing our comprehension of the exact mechanisms and signaling pathways governed by class II PI3Ks. This progress lays the groundwork for potential therapeutic interventions and improved clinical outcomes.

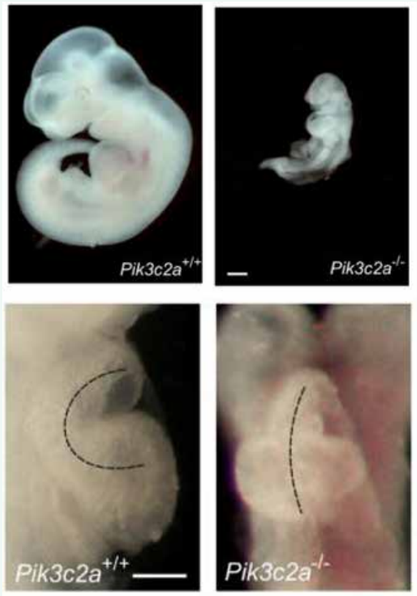


Figure 2. *Upper panels*. E10.5 *Pik3c2a*$^{-/-}$ embryos fail to complete axial rotation and turning. Bar = 500 μm. *Lower panels*. Defective cardiac looping in *Pik3c2a*-null embryos at E10.5.

Figure 2.

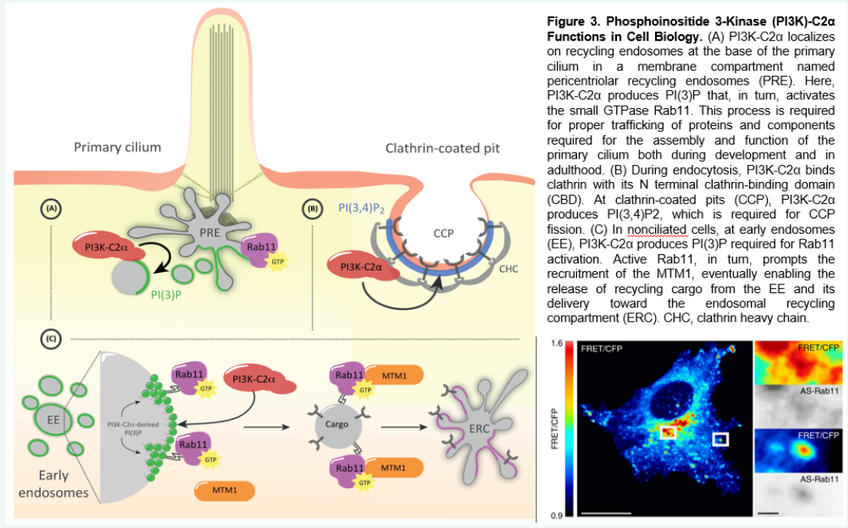


Figure 3. Phosphoinositide 3-Kinase (PI3K)-C2α Functions in Cell Biology. (A) PI3K-C2α localizes on recycling endosomes at the base of the primary cilium in a membrane compartment named pericentriolar recycling endosomes (PRE). Here, PI3K-C2α produces PI(3)P that, in turn, activates the small GTPase Rab11. This process is required for proper trafficking of proteins and components required for the assembly and function of the primary cilium both during development and in adulthood. (B) During endocytosis, PI3K-C2α binds clathrin with its N terminal clathrin-binding domain (CBD). At clathrin-coated pits (CCP), PI3K-C2α produces PI(3,4)P2, which is required for CCP fission. (C) In nonciliated cells, at early endosomes (EE), PI3K-C2α produces PI(3)P required for Rab11 activation. Active Rab11, in turn, prompts the recruitment of the MTM1, eventually enabling the release of recycling cargo from the EE and its delivery toward the endosomal recycling compartment (ERC). CHC, clathrin heavy chain.

Figure 3.

FUTURE RESEARCH PLANS

Our recent study highlighted the significance of PI(3,4)P2, produced by PI3K-C2α at the midbody during cytokinesis, in ensuring proper cell division at the end of mitosis. The localized production of PI(3,4)P2 plays a critical role in the positioning and activation of the Endosomal Sorting Complex Required for Transport (ESCRT) proteins. These proteins organize essential complexes crucial for cytokinesis, and any dysfunction in ESCRT function during this process can lead to cell refusion and aneuploidy, characterized by an abnormal number of chromosomes.

In our examination of breast cancer patients, we observed frequent downregulation of various components of the ESCRT-II/III machinery. This downregulation is associated with increased aneuploidy, suggesting a connection between dysfunctional ESCRT-dependent mechanisms and cell refusion, leading to the development of micronuclei and the leakage of DNA into the cytoplasm. Additionally, we propose that the loss of nuclear membrane repair might further contribute to the presence of cytoplasmic DNA. These processes potentially converge on the activation of the cGAS-STING pathway, involving cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING). Ultimately, this pathway can promote immune modulation, epithelial-mesenchymal transition (EMT), and cancer progression.

In our ongoing projects, we are focused on exploring the interplay between phosphoinositides and ESCRTs in the progression of breast cancer. We aim to address these hypotheses to gain a deeper understanding of the molecular mechanisms underlying cytokinesis and its implications in cancer development.

FUNDING ID (PAST 5 YERS)

LEDUCQ TRANSATLANTIC NETWORKS OF EXCELLENCE: Targeting approaches for prevention and treatment of anthracycline-induced cardiotoxicity, GA no. 19CVD02, 2020-2024

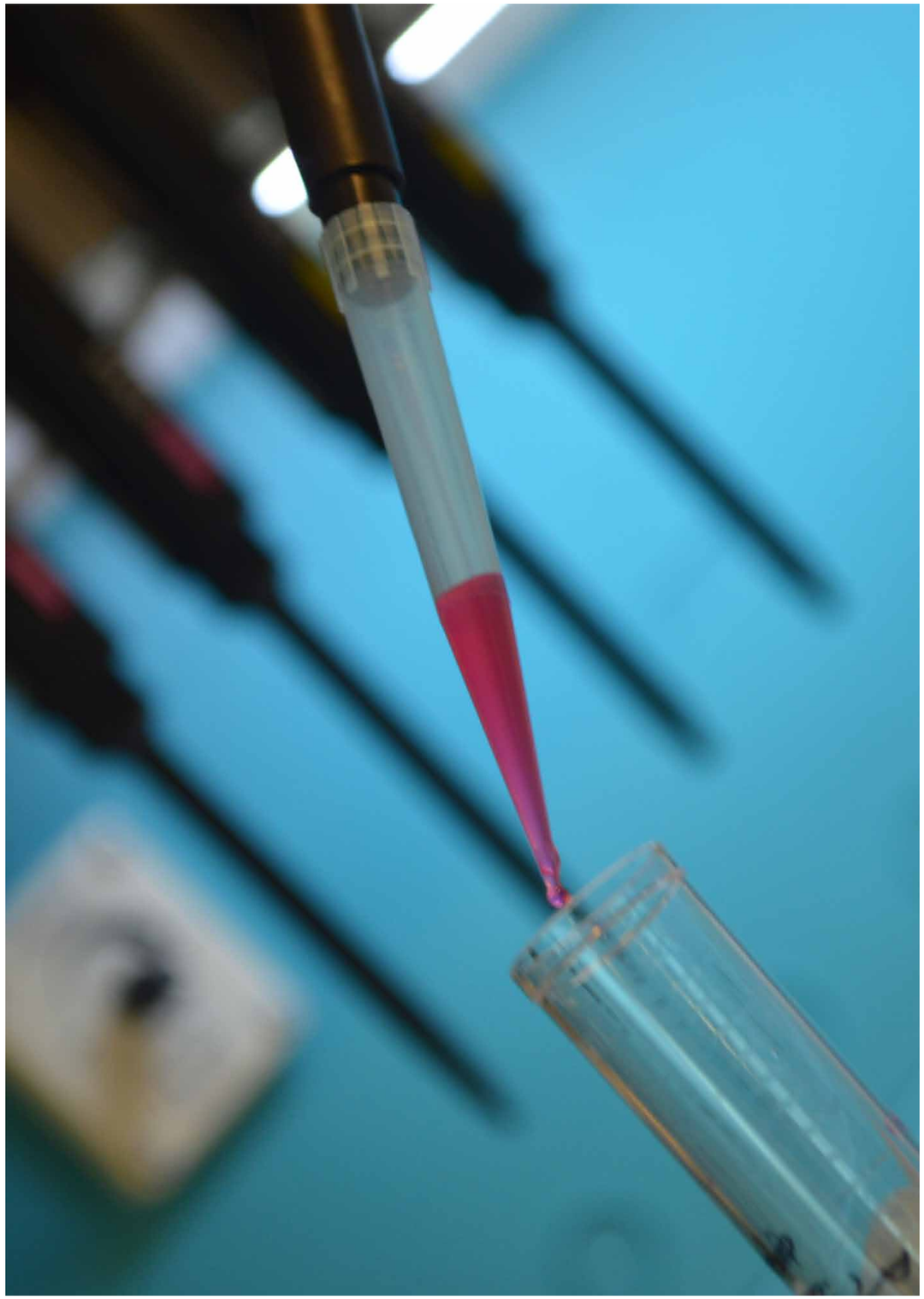
AIRC: Understanding the mechanism of action of PI3K-C2α in breast cancer progression, IG 21875, 2019-2024

MIUR PRIN: Cellular mechanisms of breast cancer stem cell-driven aggressiveness, GA no. 20177E9EPY, 2018-2022

SELECTED PUBLICATIONS

Link Scopus profile: https://www.scopus.com/authid/detail.uri?authorId=7201435266

- A PI3Kγ mimetic peptide triggers CFTR gating, bronchodilation, and reduced inflammation in obstructive airway diseases. Ghigo A, et al., and Hirsch E. Sci Transl Med. 2022 Mar 30;14(638):eabl6328. doi: 10.1126/scitranslmed.abl6328. Epub 2022 Mar 30.
- Phosphoinositide Conversion Inactivates R-RAS and Drives Metastases in Breast Cancer. Li H, et al, and Hirsch E. Adv Sci (Weinh). 2022 Mar;9(9):e2103249. doi: 10.1002/advs.202103249.
- PI(3,4)P2-mediated cytokinetic abscission prevents early senescence and cataract formation.
- Gulluni F, et al., and Hirsch E. Science. 2021 Dec 10;374(6573):eabk0410. doi: 10.1126/science.abk0410. Epub 2021 Dec 10.
- Inhalation of the prodrug PI3K inhibitor CL27c improves lung function in asthma and fibrosis. Campa CC, et al., and Hirsch E. Nat Commun. 2018 Dec 12;9(1):5232. doi: 10.1038/s41467-018-07698-6.
- Rab11 activity and PtdIns(3)P turnover removes recycling cargo from endosomes.
- Campa CC, et al., and Hirsch E. Nat Chem Biol. 2018 Aug;14(8):801-810. doi: 10.1038/s41589-018-0086-4. Epub 2018 Jun 18.
- Phosphoinositide 3-Kinase Gamma Inhibition Protects From Anthracycline Cardiotoxicity and Reduces Tumor Growth. Li M, et al., and Ghigo A. Circulation. 2018 Aug 14;138(7):696-711. doi: 10.1161/CIRCULATIONAHA.117.030352.
- Mitotic Spindle Assembly and Genomic Stability in Breast Cancer Require PI3K-C2α Scaffolding Function. Gulluni F, et al., and Hirsch E. Cancer Cell. 2017 Oct 9;32(4):444-459.e7. doi: 10.1016/j.ccell.2017.09.002.
- PI3K class II α controls spatially restricted endosomal PtdIns3P and Rab11 activation to promote primary cilium function. Franco I, et al., and Hirsch E. Dev Cell. 2014 Mar 31;28(6):647-58. doi: 10.1016/j.devcel.2014.01.022.
- PI3Kgamma modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. Patrucco E, et al., and Hirsch E. Cell. 2004 Aug 6;118(3):375-87. doi: 10.1016/j.cell.2004.07.017.
- Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. Hirsch E, et al., and Wymann MP. Science. 2000 Feb 11;287(5455):1049-53. doi: 10.1126/science.287.5455.1049.



DARIO LIVIO LONGO

IMAGING TUMOR MICROENVIRONMENT

BIOGRAPHICAL SKETCH

2020-current	Head of the Research Unit of Torino, (IBB), CNR
2019-current	Adjunct Lecturer in Cancer Imaging, Dpt. of Molecular Biotechnology and Health Sciences, University of Torino, Italy)
2018-current	First Researcher, IBB, CNR
2017-2018	Assistant Professor (RTDA), Dpt. of Molecular Biotechnology and Health Sciences, University of Torino, Italy
2014-2017	First Researcher, IBG, CNR
2012-2014	Preclinical MRI scientist, Molecular Imaging Center, University of Torino, Italy
2007-2012	Post-doctoral fellow, Department of Chemistry, University of Torino, Italy

GROUP MEMBERS:

Antonella Carella *post-doctoral fellow*
Alessia Corrado *PhD student*
Elisa Pirotta *PhD student*
Elena Botto *post-graduate fellow*
Feriel Romdhane *post-doctoral fellow*
Francesco Gammaraccio *post-graduate fellow*
Kranthi Thei Kandula *post-graduate fellow*
Elisabetta Spinazzola *post-graduate fellow*

RESEARCH ACTIVITY

The scientific activity is directed towards the development and characterization of contrast agents for Magnetic Resonance Imaging (MRI) based on i) paramagnetic coordination complexes of Gd (III) and / or other lanthanides as responsive probes or with high-relaxivity, ii) molecules of natural origin (natural compounds, proteins, polymers) as biocompatible and safe contrast agents and iii) iodinated compounds used in computed tomography (CT) applications as innovative contrast agents for CEST (Chemical Exchange Saturation Transfer) imaging at the preclinical level. These new contrast agents are exploited for developing new procedures for the evaluation of the tumor microenvironment (vascularization, acidosis) and to quantify the therapeutic response to drugs in several murine pathological models.

A first line of research has concerned the development of molecules as pH responsive agents, to be able to quantify pH in vivo using magnetic resonance imaging. The candidate contributed to advance the understanding of tumor acidosis, a key aspect of tumor metabolism, through the development and validation of pH-responsive contrast agents and of new methods for the measurement of the tissue pH, for image acquisition and to improve its quantification. For the first time it was possible to measure the acidification of the tumor microenvironment in vivo with high spatial resolution, accuracy and on the whole tumor allowing to demonstrate in vivo the association between acidity of the tumor environment and aggressiveness and providing a new non-invasive method to evaluate the efficacy of innovative anticancer drugs acting on tumor metabolism (Figure 1).

A second line of research concerned the use and development of new methods for measuring pH at the level of the kidneys, promoting their use to evaluate renal function and to evaluate damage and recovery. The kidney is a highly complex organ consisting of well-defined structures that function in a deeply coordinate fashion to allow for fine regulation of pH homeostasis. Although the principal role of the kidney is the maintenance of acid–base balance, current imaging approaches are unable to assess this important parameter and clinical biomarkers are not robust enough in evaluating the severity of kidney damage. Therefore, our lab is developing novel non-invasive imaging approaches to assess the acid–base homeostasis in vivo and to monitor pH evolution following kidney injury (Figure 2).

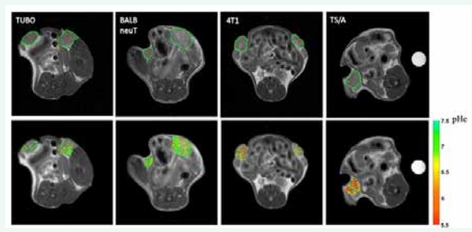


Figure 1.
Extracellular tumor pH maps showing increased tumor acidosis in murine breast tumors with higher metastatic potential (adapted from Anemone A. et al; British Journal of Cancer 2021).

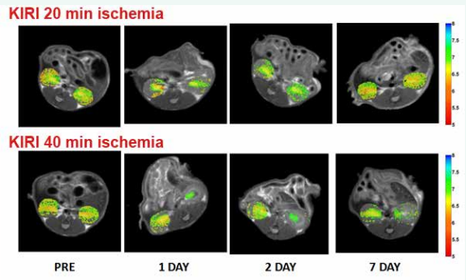


Figure 2.
Imaging renal pH for evaluating renal functionality and recovery in the kidney ischemic and reperfusion injury (KIRI) model upon 20 or 40 minutes of ischemia (adapted from Longo D.L. et al.; NMR in Biomedicine 2017)

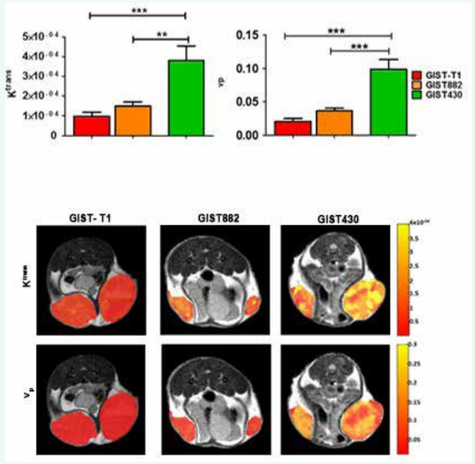


Figure 3.
Assessing differences in tumor vascular permeability (Ktrans) and in vessek density (vp) by DCE-MRI (dynamic contrast-enhanced magnetic resonance imaging) method with a novel blood-pool Gadolinium-based contrast agent to distinguish between Imatinib-sensitive and resistant gastrointestinal stromal tumors (adapted from Consolino L. et al; Gastric Cancer 2017)

A third line of research concerns the development of new molecules, both synthetic and natural, such as new contrast agents for MRI with the aim of improving the efficiency of the contrast or as alternatives to gadolinium complexes to solve the problems of accumulation following repeated injections of the same gadolinium-based agents. On this issue, the results have led to fundamental contributions in the development of contrast agents based on natural compounds with a high safety index, such as pharmaceutical excipients, sugars and glucose derivatives, or manganese complexes or iodinated agents (currently used as a contrast medium for computed tomography) or coordination complexes with paramagnetic ions other than gadolinium or nanosystems demonstrating their use as alternatives to gadolinium which is currently used in clinical investigations by MRI.

A fourth line of research involved the development of macromolecular compounds such as gadolinium-based contrast agents to characterize tumor vasculature and to evaluate response to antiangiogenic drugs or immunotherapies, combined with the development of low-field (1 Tesla) MRI scanners where these contrast agents, following the interaction with macromolecules, have a higher contrast efficiency. Within this vein I have developed new computational methods to predict the interaction with albumin and to characterize the contrast efficiency which led to the development of probes with an efficiency of superior contrast to that of gadolinium-based contrast agents commonly used in the clinic for the development of protocols for DCE-MRI having as application the study of vascularization and permeability/perfusion changes following the angiogenic switch, or to study therapies that target angiogenesis on different tumor models (Figure 3).

FUTURE RESEARCH PLANS

We plan to continue with the development and validation of new molecules as pH-responsive contrast agents for MRI and we will expand the fields of use to further validate this method as a non-invasive biomarker to predict the aggressiveness of solid tumors and to evaluate the therapeutic efficacy of anticancer drugs and of novel immunotherapies.

FUNDING ID (PAST 5 YERS)

- 2018-2023, “Multidimensional MRI mapping of tumor acidosis: tracking spatial heterogeneity and temporal evolution”, 495K €, Project Coordinator and PI, AIRC – MFAG 2017 / #20153
- 2022-2025 “A European-wide foundation to accelerate Data-driven Cancer Research (EOSC4Cancer)”, 132K €, Local unit PI, HORIZON-INFRA-2021-EOSC-01/ #101058427
- 2017-2019, “Imaging extracellular pH as a new MRI diagnostic tool (PHeMRI)”, 70K €,
- Co-PI, Progetti di Ateneo - CSP 2016 / CSTO165925

SELECTED PUBLICATIONS

Link Scopus profile: https://www.scopus.com/authid/detail.uri?authorId=8564616500

- Irrera P, Consolino L, Roberto M, Capozza M, Dhakan C, Carella A, Corrado A, Villano D, Anemone A, Navarro-Tableros V, Bracesco M, Dastrù W, Aime S, Longo DL. In Vivo MRI-CEST Tumor pH Imaging Detects Resistance to Proton Pump Inhibitors in Human Prostate Cancer Murine Models. Cancers (Basel). 2022 Oct 7;14(19):4916. doi: 10.3390/cancers14194916. PMID: 36230838;
- Leone L, Anemone A, Carella A, Botto E, Longo DL, Tei L. A Neutral and Stable Macrocyclic Mn(II) Complex for MRI Tumor Visualization. ChemMedChem. 2022 Dec 16;17(24):e202200508. doi: 10.1002/cmdc.202200508. PMID: 36198652;
- Anemone A, Consolino L, Conti L, Irrera P, Hsu MY, Villano D, Dastrù W, Porporato PE, Cavallo F, Longo DL. Tumour acidosis evaluated in vivo by MRI-CEST pH imaging reveals breast cancer metastatic potential. Br J Cancer. 2021 Jan;124(1):207-216. doi: 10.1038/s41416-020-01173-0. PMID: 33257841;
- Irrera P, Consolino L, Cutrin JC, Zöllner FG, Longo DL. Dual assessment of kidney perfusion and pH by exploiting a dynamic CEST-MRI approach in an acute kidney ischemia-reperfusion injury murine model. NMR Biomed. 2020 Jun;33(6):e4287. doi: 10.1002/nbm.4287. PMID: 32153058.
- Anemone A, Consolino L, Conti L, Reineri F, Cavallo F, Aime S, Longo DL. In vivo evaluation of tumour acidosis for assessing the early metabolic response and onset of resistance to dichloroacetate by using magnetic resonance pH imaging. Int J Oncol. 2017 Aug;51(2):498-506. doi: 10.3892/ijo.2017.4029. PMID: 28714513.
- Anemone A, Consolino L, Longo DL. MRI-CEST assessment of tumour perfusion using X-ray iodinated agents: comparison with a conventional Gd-based agent. Eur Radiol. 2017 May;27(5):2170-2179. doi: 10.1007/s00330-016-4552-7. PMID: 27572810.
- Longo DL, Arena F, Consolino L, Minazzi P, Geninatti-Crich S, Giovenzana GB, Aime S. Gd-AAZTA-MADEC, an improved blood pool agent for DCE-MRI studies on mice on 1 T scanners. Biomaterials. 2016 Jan;75:47-57. doi: 10.1016/j.biomaterials.2015.10.012. PMID: 26480471.
- Longo DL, Michelotti F, Consolino L, Bardini P, Digilio G, Xiao G, Sun PZ, Aime S. In Vitro and In Vivo Assessment of Nonionic Iodinated Radiographic Molecules as Chemical Exchange Saturation Transfer Magnetic Resonance Imaging Tumor Perfusion Agents. Invest Radiol. 2016 Mar;51(3):155-62. doi: 10.1097/RLI.0000000000000217. PMID: 26460826.
- Longo DL, Dastrù W, Consolino L, Espak M, Arigoni M, Cavallo F, Aime S. Cluster analysis of quantitative parametric maps from DCE-MRI: application in evaluating heterogeneity of tumor response to antiangiogenic treatment. Magn Reson Imaging. 2015 Jul;33(6):725-36. doi: 10.1016/j.mri.2015.03.005. PMID: 25839393.
- Longo DL, Bartoli A, Consolino L, Bardini P, Arena F, Schwaiger M, Aime S. In Vivo Imaging of Tumor Metabolism and Acidosis by Combining PET and MRI-CEST pH Imaging. Cancer Res. 2016 Nov 15;76(22):6463-6470. doi: 10.1158/0008-5472.CAN-16-0825. PMID: 27651313.

MARINA MARCHISIO CONTE

DELTA, Digital Education for Learning and Teaching Advances

BIOGRAPHICAL SKETCH

Since Dec 2019	Full Professor, Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy
Since Oct 2022	President of the Interdepartmental University School of Strategic Sciences (SUISS), University of Turin, Italy
Since Nov 2022	Delegate of the Rector for the development and promotion of Digital Education strategies, University of Turin, Italy
Since Oct 2020	Deputy Director for Education of the Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy
Jul 2020-Sep 2020	Member of the Digital Education Expert Group for the Digital Education Action Plan 2021-27, European Commission
2012-2014	Preclinical MRI scientist, Molecular Imaging Center, University of Torino, Italy
Since May 2023	Secretary of the Council of the School of Medicine
2005-2019	Associate Professor, University of Turin, Italy
1999-2005	Senior researcher, University of Turin, Italy

GROUP MEMBERS:

Fabio Roman, Matteo Sacchet, Daniela Salusso, Cecilia Fissore, Alice Barana *post-doctoral fellow*
Giulia Boetti, Valeria Fradiante *Phd Students*
Francesca Casasso, Marta Pulvirenti *Postgraduate Fellows*
Francesco Floris *adm. technician and Phd Student*
Sergio Rabellino *Res. technician, ICT Services*

RESEARCH ACTIVITY

The main research lines include: i)Integrated Digital Learning Environments and Learning Analytics in Open Educational Practices ii) Adaptive, inclusive and data driven digital methodologies for learning and teaching scientific disciplines, iii) Computational Mathematical Models for Biotechnologies, iv) Biostatistical Methods applied in Biotechnology and Medicine.

In today's fast-paced world, education is evolving at a rapid rate, driven by advancements in technology. Digital tools have become an integral part of modern education, transforming teaching methodologies and empowering educators to create engaging and interactive learning experiences for their students. A key element of Digital Education is the Digital Learning Environment (DLE), which can be defined as a learning ecosystem in which teaching, learning and the development of competence are fostered. It is composed of a human component, a technological component, and the interrelationships between the two. The DLEs we mainly focus on are composed by a Moodle platform (developed by the Department of Computer Science - ICT Services of the University of Turin) integrated with an Advanced Computing Environment (ACE), an Automatic Assessment System (AAS) and a web conference system. The research group study the interactions occurring among the components of a DLE during learning activities in online, blended, hybrid, and classroom-based modalities (Fig. 1). In a human-centered approach, at the center of the DLE, there is the learning community, composed of students, teachers, and peers: they can interact with the DLE through its functions receiving and sending information. The blue dotted arrows represent the interactions between the community and the digital systems that occur through human actions, such as reading, receiving, inserting, providing, digiting. The continuous double-ended orange arrows represent the interactions among students, teachers, and peers, which in classroom-based settings can be verbal while in online settings can be mediated by the technology.

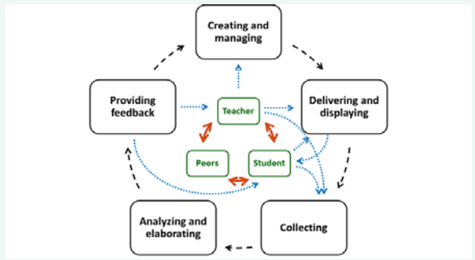


Figure 1.
Modelling interactions in a Digital Learning Environment

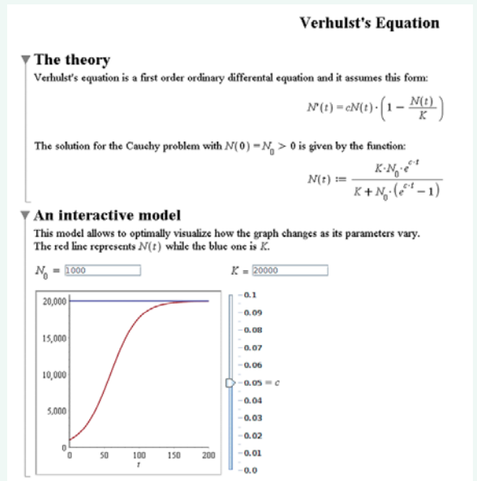


Figure 2.
Example of computational mathematical models with an ACE

The research group develops innovative teaching and learning methodologies enhanced by technologies, such as: automatic formative assessment, problem posing and solving, adaptive teaching and learning, gamification, data driven learning, collaborative learning, learning by doing and so on. For example, the research group has developed and tested a model to design automatic formative assessment activities in a DLE, with immediate and interactive feedback. Students are guided step by step in solving tasks, individually or in groups, having multiple attempts to answer.

We use an Advanced Computing Environment to model various phenomena in biology and medicine, such as population growth and the spread of viruses (Fig. 3). An ACE is a system that can perform numerical and symbolic computations, build graphical representations in 2 and 3 dimensions, and create mathematical simulations through interactive components. Mathematical modelling is the process of translation between the real world (including nature, society, everyday life and other scientific disciplines) and Mathematics in both directions. One of the aims of STEM education is to encourage students to develop models: representations that can describe and explain the phenomena of reality and predict the course of experiments, mechanisms and processes. Modelling is a valid approach to many different problems and therefore a common ground between the different STEM disciplines.

Thanks to technology, Educational Data, which is continuously updated and growing, has now become Big Data and, to make the most of it, we use Learning Analytics (LA) to analyse it, interpret it, and derive enough information to make decisions. LA can be defined as: "the measurement, collection, analysis and reporting of data about learners and their contexts, for purposes of understanding and optimizing learning and the environments in which it occurs". LA can inform not only teachers and researchers, but also students about their achievements, thus letting them keep control of their learning path.

FUTURE RESEARCH PLANS

Our future research intends to harness new technologies, such as AI, Deep Learning, Augmented Reality and Large Language Models to innovate STEM learning and teaching, fostering the teaching methodologies mentioned above. For instance, a DLE integrated with AI tools can give real-time information on student learning. This can enable teachers to adapt their teaching according to students' needs, and students to have personalized learning. AI tools offer new ways of teaching to better engage students, and Large Language Models have opened new horizons in digital education and educational research. Moreover, we plan to use these technologies also in order to enhance the development of computational models and biostatistical methods, thanks to the tools' capabilities in assisting these processes.

FUNDING ID (PAST 5 YEARS)

- Progetto europeo di ricerca "MGS" (MILITARY GENDER STUDIES) Erasmus+ KA2. Grant 2020-1-PT01-KA203-078544
- Progetto europeo di ricerca "DIGICODE" DIGItal COmpetences for improving security and Defence Education Programme. Erasmus+ KA2. 2020-1-PL01-KA226-096192.
- Progetto europeo di ricerca "Interdisciplinary Education and Training on Hybrid Warfare" (HYBRID). Erasmus+ KA2. KA220-HED-B9458C8A.
- Progetto europeo di ricerca "Developing Competences and Innovative Designs for International Virtual and Blended Modalities" (INVITE). Erasmus+ KA2. 2021-1-DK01-KA220-HED-000031145.
- Progetto europeo di ricerca "Time-Spatial-Linguistic Teaching and Learning Travel Machine platform for Connecting UNITA - CONNECT- UNITA". Erasmus+ KA2. KA220-HED-E60423AD.
- Progetto europeo di ricerca DIMAS - DIGITAL MATHEMATICS APPLIED IN DEFENSE AND SECURITY EDUCATION, Erasmus+ KA2. 2023-1-BG01-KA220-HED-000156664

SELECTED PUBLICATIONS

Link Scopus profile: https://www.scopus.com/authid/detail.uri?authorId=57225427057&origin=recordPage

- Barana, A., Boetti, G., Marchisio, M., Perrotta, A., & Sacchet, M. (2023). Investigating the Knowledge Co-Construction Process in Homogeneous Ability Groups during Computational Lab Activities in Financial Mathematics. Sustainability, 15(18), 13466. https://doi.org/10.3390/su151813466
- Fissore, C., Floris, F., Marchisio, M., & Rabellino, S. (2023). Learning analytics to monitor and predict student learning processes in problem solving activities during an online training. IEEE 47th Annual Computers, Software, and Applications Conference (COMPSAC), Torino, Italy, 2023, (pp. 481-489), doi: 10.1109/COMPSAC57700.2023.00070.
- Barana, A., Boetti, G., & Marchisio, M. (2022). Self-Assessment in the Development of Mathematical Problem-Solving Skills. Education Sciences, 12(2), 81. doi: 10.3390/educsci12020081
- Marchisio, M., Remogna, S., Roman, F., & Sacchet, M. (2022). Teaching mathematics to non-mathematics majors through problem solving and new technologies. Education Sciences, 12(1), 34. doi: 10.3390/educsci12010034
- Corino, E., Fissore, C., & Marchisio, M. (2022). Data Driven Learning activities within a Digital Learning Environment to study the specialized language of Mathematics. In 2022 IEEE 46th Annual Computers, Software, and Applications Conference (COMPSAC) (pp. 167-176). IEEE. doi: 10.1109/COMPSAC54236.2022.00032
- Barana, A., & Marchisio, M. (2021). A Model for the Analysis of the Interactions in a Digital Learning Environment During Mathematical Activities. In International Conference on Computer Supported Education (pp. 429-448). Cham: Springer International Publishing. doi: 10.1007/978-3-031-14756-2_21
- Barana, A., Marchisio, M., & Sacchet, M. (2021). Interactive feedback for learning mathematics in a digital learning environment. Education Sciences, 11(6), 279. doi: 10.3390/educsci11060279
- Barana, A., Fissore, C., & Marchisio, M. (2020). Automatic Formative Assessment Strategies for the Adaptive Teaching of Mathematics. In International Conference on Computer Supported Education (pp. 341-365). Cham: Springer International Publishing. doi: 10.1007/978-3-030-86439-2_18
- Barana, A., Conte, A., Fissore, C., Marchisio, M., & Rabellino, S. (2019). Learning analytics to improve formative assessment strategies. JE-LKS. Journal of e-learning and knowledge society, 15(3), 75-88. doi: 10.20368/1971-8829/1135057
- Marchisio, M., Barana, A., Fioravera, M., Rabellino, S., & Conte, A. (2018). A model of formative automatic assessment and interactive feedback for STEM. In 2018 IEEE 42nd Annual Computer Software and Applications Conference (COMPSAC) (Vol. 1, pp. 1016-1025). IEEE. doi: 10.1109/COMPSAC.2018.00178

MIRIAM MARTINI

Cancer Cell Signaling

BIOGRAPHICAL SKETCH

2021-present	Associate Professor, Univ. of Torino, Dept. of Molecular Biotechnology and Health Sciences
2018 to date	Group leader, Molecular Biotechnology Center, Torino
2018-2021	RTDB (Assistant Professor), Univ. of Torino, Dept. of Molecular Biotechnology and Health Sciences
2016-2017	Postdoctoral Fellow (Fondazione Umberto Veronesi)
2011-2015	Postdoctoral fellow, Molecular Biotechnology Center, Italy
2008-2011	Postdoctoral fellow, IRCCS Candiolo (TO), Italy
2009	PhD in Cell Science and Technology, University of Torino

GROUP MEMBERS:

Maria Chiara De Santis *senior postdoc*
Andrea Costamagna *postdoc*
Damiano Abbo *PhD student*
Noemi Ghiglione *PhD student*
Anastasia Bushunova *PhD student*

RESEARCH ACTIVITY

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal form of cancer and is projected to become the second leading cause of cancer-related deaths worldwide by 2030. Despite advancements in diagnostic and treatment methods, the prognosis and early intervention for PDAC remain unsatisfactory. A comprehensive understanding of the molecular mechanisms governing the early stages of PDAC development is essential to devise improved prognostic and early intervention approaches.

Our laboratory focuses on pancreatic cancer biology, and we use a combination of approaches, including biochemistry, cell and molecular biology, and 3D cell culture models to define new signaling crosstalk mechanisms (Figure 1).

Studies using mouse models have indicated that PDAC originates from acinar cells in response to a mutation known as KrasG12D, a process termed acinar to ductal metaplasia (ADM). However, the specific mechanism by which mutant Kras induces ADM and contributes to PDAC development has not been fully elucidated. We have recently identified a connection between KrasG12D, ADM, and PDAC development through the involvement of the adaptor protein p130Cas. We demonstrated that p130Cas acts downstream of Kras, inducing the PI3K-AKT signaling pathway, thereby supporting its role in the early stages of PDAC development (Figure 2). We are currently studying more in depth this molecular mechanism in ductal-derived PDAC and its interaction with various subpopulations within the tumor microenvironment, as this knowledge could be pivotal in devising targeted therapeutic approaches for PDAC.

Resistance to chemotherapies represents a critical challenge for pancreatic cancer patients, thus novel therapeutic approaches for PDAC are desperately needed. A phenomenon that contributes to the dismal prognosis and chemoresistance is a unique characteristic of pancreatic cancer called desmoplasia, which results in reduced level of oxygen and nutrients. Deregulation of critical signaling pathways endows pancreatic cancer cell with specific features that support their survival in a harsh microenvironment. However, within this adversity, pancreatic cancer cells display remarkable adaptability through the deregulation of critical signaling pathways that confer specific traits supporting their survival. One

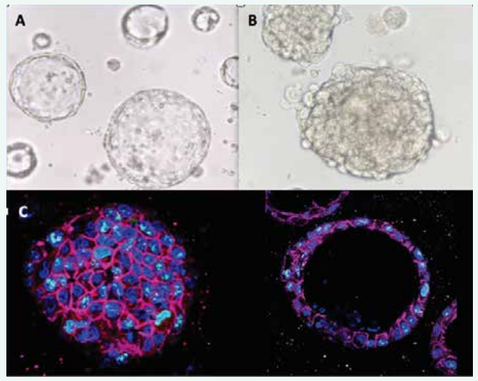


Figure 1.
Images of pancreatic cancer organoids: bright-field (A-B) and confocal immunofluorescence images (C).

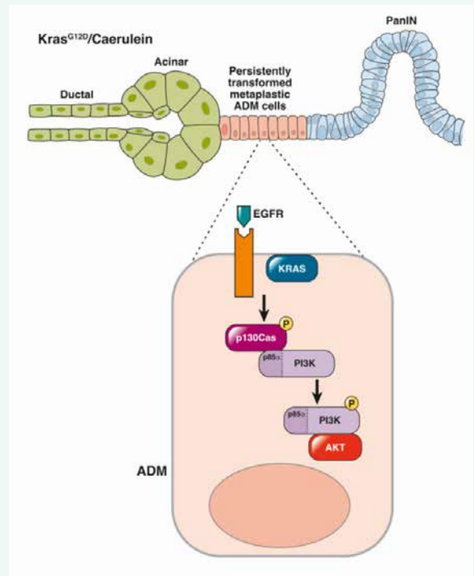


Figure 2.
Schematic representation of the molecular mechanism of the p130-driven ADM process. From Costamagna et al, Gastroenterology, 2021.

such pathway that has garnered considerable attention in the context of pancreatic cancer is the mTOR signaling pathway. The hyperactivation of the mTORC1 pathway plays a pivotal role in the pathogenesis of PDAC, as it is essential for sustaining malignant growth and orchestrating a rewiring of cellular metabolism to meet the demands of rapid proliferation. The activation of mTORC1 is under the influence of various inputs, and amino acids play a particularly crucial role in driving its full activation. Among the recent discoveries in this area, we recently reported that PI(3,4)P2, a product of class II PI3K signaling, functions as a local repressor of mTORC1 signaling in response to fluctuations in glutamine levels (Figure 3). This newly uncovered interaction between PI(3,4)P2 and mTORC1 sheds light on the intricate regulatory mechanisms that enable pancreatic cancer cells to sense and respond to changes in nutrient availability. The tight control of mTORC1 activity through PI(3,4)P2 not only highlights the adaptability of cancer cells in harsh conditions but also presents a potential avenue for therapeutic intervention. Understanding these specific molecular interactions could pave the way for targeted therapies aimed at disrupting the survival strategies employed by pancreatic cancer cells and sensitizing them to existing treatments.

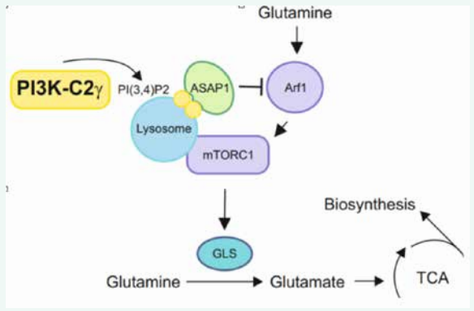


Figure 3.
Schematic representation of the proposed working model. Adapted from De Santis,

FUTURE RESEARCH PLANS

Our research endeavors hold the promise of revolutionizing our understanding of pancreatic cancer biology, paving the way for novel and innovative treatment approaches to combat this formidable and life-threatening disease. Our primary focus lies in delving deep into the intricacies of the disease, aiming to shed new and illuminating light on its underlying mechanisms and vulnerabilities.

Specifically, we are committed to exploring alternative treatment strategies that can effectively challenge and overcome the resilience of pancreatic cancer. One of the key aspects we will investigate is the identification of new regulators within signaling pathways that play a crucial role in supporting the survival and growth of pancreatic cancer cells, especially during conditions of extreme nutrient deprivation, which is commonly observed in this particular type of cancer.

By deciphering the intricate signaling mechanisms that enable pancreatic cancer cells to thrive under harsh nutrient-deprived conditions, we aim to uncover potential therapeutic targets that can be harnessed to disrupt and debilitate the cancer cells, ultimately hindering their growth and progression. Our ultimate goal is to contribute to the validation of groundbreaking therapeutic strategies that hold the promise of significantly improving the prognosis and overall outcomes for patients with pancreatic cancer.

As it stands, pancreatic cancer is notoriously known for having one of the most unfavorable prognoses among all human cancers, with an alarmingly low 5-year survival rate of only 1%. However, we firmly believe that with our rigorous and cutting-edge research efforts, we can tip the scales in favor of the patients. By identifying and validating effective therapeutic approaches, we aspire to bring hope and a brighter future for those affected by this devastating disease.

The potential impact of our research extends far beyond the realms of pancreatic cancer. The insights gained from our investigations may have broader implications for cancer biology as a whole, offering valuable lessons and innovative strategies that could be adapted and applied to other challenging cancers as well.

In conclusion, through our dedicated pursuit of knowledge, our research aims to open up new avenues for understanding, diagnosing, and treating pancreatic cancer. By pushing the boundaries of scientific exploration, we aspire to contribute to the arsenal of weapons in the fight against this relentless disease, ultimately bringing renewed hope to patients, their families, and the medical community at large.

FUNDING ID (PAST 5 YERS)

2020 Worldwide Cancer Research Grant, 2023: AIRC Individual Grant.

SELECTED PUBLICATIONS

Link to Scopus profile: https://www.scopus.com/authid/detail.uri?authorId=21743032500

- De Santis MC, et al., and Martini M. Lysosomal lipid switch sensitises to nutrient deprivation and mTOR targeting in pancreatic cancer. Gut, 2022. doi: 10.1136/gutjnl-2021-325117.
- Gozzelino L, et al., and Martini M*, Haucke V*, Hirsch E*, Pippucci T*. Defective lipid signaling caused by mTOR-activating ultra-rare variants in PIK3C2B underlies focal epilepsy. Brain. 2022. doi: 10.1093/brain/awac082.
- Costamagna A, et al., and Martini M. Docking protein p130Cas regulates acinar to ductal metaplasia during pancreatic adenocarcinoma development and pancreatitis. Gastroenterology, 2021. doi: 10.1053/j.gastro.2021.12.242.
- Gulluni F*, Martini M*,#, De Santis MC*, et al., and Hirsch E#. Mitotic spindle assembly and genomic stability in breast cancer require PI3K-C2A scaffolding function. CANCER CELL, 2017. doi: 10.1016/j.ccell.2014.11.007.
- Costa C, Ebi H, Martini M, et al, and Engelman JA. Measurement of PIP3 levels reveals an unexpected role for p110β in early adaptive responses to p110α-specific inhibitors in luminal breast cancer. CANCER CELL, 2015. doi: 10.1016/j.ccell.2014.11.007.
- Martini M, et al., and Bardelli A(2013). Mixed lineage kinase MLK4 is activated in colorectal cancers where it synergistically cooperates with activated RAS signaling in driving tumorigenesis. CANCER RESEARCH, 2013. doi: 10.1158/0008-5472.CAN-12-3074
- Bottos A*, Martini M*, et al., and Bardelli A. Targeting oncogenic serine/threonine-protein kinase BRAF in cancer cells inhibits angiogenesis and abrogates hypoxia. PNAS, 2012. doi: 10.1073/pnas.1105026109
- Martini M, Vecchione L, Siena S, Tejpar, Bardelli A. Targeted therapies: how personal should we go?. NATURE REVIEWS. CLINICAL ONCOLOGY, 2011. doi: 10.1038/nrclinonc.2011.164
- Sartore-Bianchi A*, Martini M*, Molinari F*, et al, and Bardelli A. PIK3CA Mutations in Colorectal Cancer Are Associated with Clinical Resistance to EGFR-Targeted Monoclonal Antibodies. CANCER RESEARCH, 2009. doi: 10.1158/0008-5472.CAN-08-2466.
- Di Nicolantonio F*, Martini M*, Molinari F*, et al., and Bardelli A. Wild-Type BRAF Is Required for Response to Panitumumab or Cetuximab in Metastatic Colorectal Cancer. JOURNAL OF CLINICAL ONCOLOGY, 2008. doi: 10.1200/JCO.2008.18.0786

MASSIMO MASSAIA

Blood Tumor Immunology Lab

BIOGRAPHICAL SKETCH

Massimo Massaia holds the position of Associate Professor of Hematology and Head of the Laboratory of Blood Tumor Immunology at the University of Turin, Italy. He is Director of the Division of Hematology at Azienda Ospedaliera Santa Croce & Carle in Cuneo, Italy, after being Acting Director of SCDU Hematology and Cell Therapy at Azienda Ospedaliera Ordine Mauriziano in Turin, Italy.

He studied medicine and graduated with honors as M.D. from the University of Turin, Italy. Then, he completed his post-graduate degree in Hematology at the University of Roma and in Internal Medicine at the University of Turin. During this period, he did his post-doctoral research at the Royal Free Hospital in London, UK. He has been a Visiting Investigator, Visiting Scientist, and Visiting Professor at the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, USA, at Oklahoma Medical Research Foundation, Oklahoma City, USA, at Frederick Cancer Research and Development Center (FCRDC), Frederick, USA, at Stanford University, Stanford, USA, at Leiden University, The Netherlands, and at MD Anderson Cancer Center, Houston.His research activity has been driven for more than 30 years by an unfailing interest in immune surveillance and immunotherapy in multiple myeloma (MM) and chronic lymphocytic leukemia (CLL). He has pioneered idiotype vaccination in MM, showing the feasibility, safety, and efficacy of active specific immunotherapy in hematological malignancies. His contributions to these topics have gained him recognition through the publication of over 160 manuscripts in peer-reviewed journals and the receipt of numerous grants and awards. He has a track record of experience in more than 30 phase I/II, phase II, and phase III clinical studies in Multiple Myeloma (MM), Chronic Lymphocytic Leukemia (CLL), non-Hodgkin lymphoma (NHL), tumor vaccination, and adoptive immunotherapy.

He is a member of the Italian Society of Hematology (SIE), the Fondazione Italians Linfomi (FIL), the German CLL Study Group (GCLLSG), the American Hematology Association (ASH), and European Hematology Association (EHA)

GROUP MEMBERS

Claudia Giannotta *Postdoctoral fellow*
Federica Autino *PhD student*
Ignazio Ezio Tripoli *Clinical Research Coordinator*
Alessia Castellino *MD and PhD student*
Serena Pignola *Postgraduate fellow*

RESEARCH ACTIVITY

The Laboratory of Blood Tumor immunology is focused on the discovery of immune mechanisms involved in the pathogenesis of lymphoproliferative disorders [i.e., Multiple Myeloma (MM), Chronic Lymphocytic Leukemia (CLL)] and other hematological malignancies. The final goal is to identify actionable targets of immune interventions to improve the clinical outcome of patients with hematological malignancies.

In the last years, the immune system has emerged as major player in tumor progression and as an actionable target for therapeutic interventions. The interactions between tumor cells and immune cells occur in the tumor microenvironment (TME) which is the battlefield where the balance tip is progressively shifted from effective to ineffective antitumor control. MM is a paradigm disease in which progression is fueled by intrinsic alterations of myeloma cells and TME tumor-host interactions. Disease evolution from monoclonal gammopathy of undetermined significance (MGUS) to smoldering myeloma (SMM), and symptomatic disease is characterized by a progressive increase of myeloma cells in the bone marrow (BM) paralleled by immunological and metabolic changes in the TME (Figure 1).

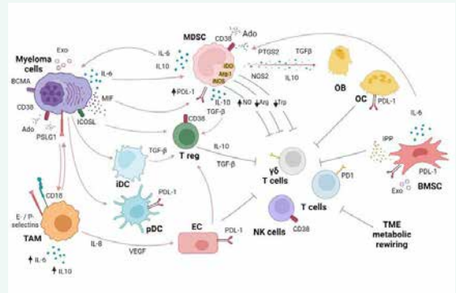


Figure 1.
Immune suppressive network operating in the bone marrow(BM) of multiple myeloma (MM) patients.

The discovery of immune checkpoints (ICPs) and other mechanisms of immune suppression and/or immune escape have paved the way to the development of more effective immune-based interventions, from passive immunotherapy with monoclonal antibodies (mAb) such as CD38-targeted therapy to bispecific antibodies (bAbs), and Chimeric Antigen Receptor (CAR)-T cells. However, clinical results remain suboptimal and many patients relapse after an initial response indicating that this is another critical setting to decode tumor-intrinsic and tumor-extrinsic mechanisms playing a role in tumor cell regrowth.

presentation which is highly relevant for their use as allogeneic ('off-the-shelf') cell therapies. Two main strategies have been developed to manipulate Vγ9Vδ2 T-cell activation which include the in vivo administration of phosphoantigens (pAgs) or aminobisphosphonates (NBP) or the adoptive transfer of ex vivo-expanded Vγ9Vδ2 T cells, but results have been short of clinical expectations. Our laboratory has dedicated a great effort in understanding the mechanisms of pAg-induced activation of Vg9Vd2 T cells in healthy individuals and patients with MM and CLL with the final aim to recover the peculiar antitumor activity of these cells. In healthy individuals, we have identified the

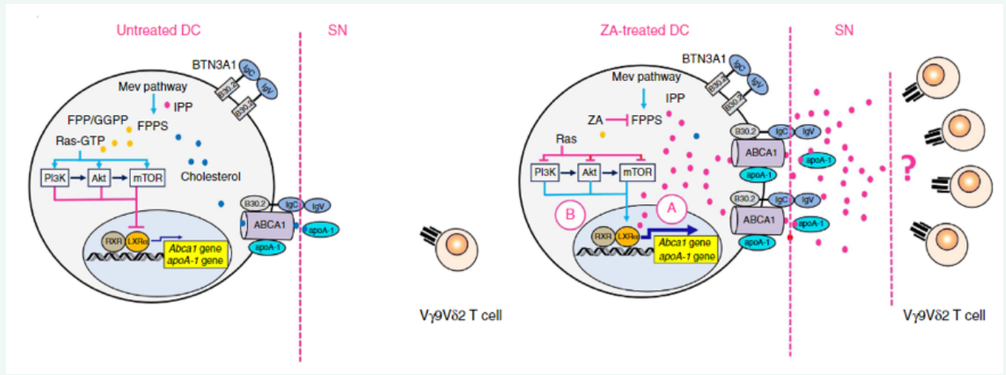


Figure 2.
ATP-binding cassette transporter A1 (ABCA1) mediates extracellular IPP release from dendritic cells (DC) in cooperation with apolipoprotein A-I (apoA-I) and butyrophilin-3A (Castella et al., Nat Commun. 2017).

In the last years, the Laboratory of Blood Tumor Immunology (LBTI) has focused on Vγ9Vδ2 T cells which are a peculiar subset of immune effector cells halfway between innate and adaptive immunity. Vg9Vd2 T cells are excellent candidates for immune interventions in lymphoproliferative disorders because they exploit multiple mechanisms to target tumor cells, including the capacity to sense metabolic alterations [intracellular phosphoantigen (pAg) accumulation], and to recognize stress-induced self-ligands. Vg9Vd2 T cells combine activation mechanisms of conventional T cells and NK cells, and act independently of MHC class I-mediated antigen cell surface proteins involved in pAg release and activation of bystander Vg9Vd2 T cells (Figure 2).

In MM and CLL, we have shown that Vg9Vd2 T cells are highly dysfunctional combining phenotypic and TCR-associated alterations consistent with chronic exhaustion and immune senescence hampering their antitumor activity (Figure 3). Interestingly, Vg9Vd2 T-cell dysfunctions are correlated with the disease status and we have shown that different approaches are needed to rescue their antitumor activity in patients at diagnosis, in remission, or in relapse.

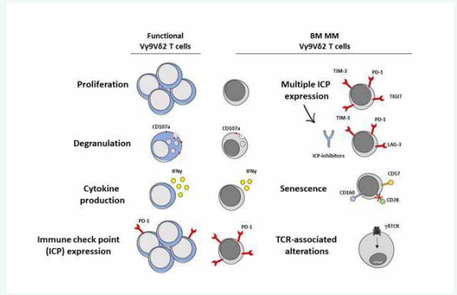


Figure 3.
The immune competence of bone marrow (BM)-resident Vγ9Vδ2 T cells is deeply compromised in multiple myeloma (MM) patients.

FUTURE RESEARCH PLANS

Future plans are to improve the knowledge of tumor-intrinsic and tumor-extrinsic mechanisms impairing the immune competence of Vg9Vd2 T cells and to use this knowledge to develop more effective immune interventions using Vg9Vd2 T cells as single agents or in association with other drugs like monoclonal antibodies, and bispecific antibodies. We are currently investigating the interactions with anti-CD38 mAbs and the possibility to develop allogeneic "off-the-shelf" CAR-Vg9Vd2 T cells that are more resistant to the immune suppressive TME and able to exert antitumor activity even under hypoxic or acidic conditions. To this end, we are investigating the metabolic asset of resting and activated Vg9Vd2 T cells from healthy individuals and MM/CLL patients to reinforce their capacity to face the energetic challenge occurring in the TME of MM patients.

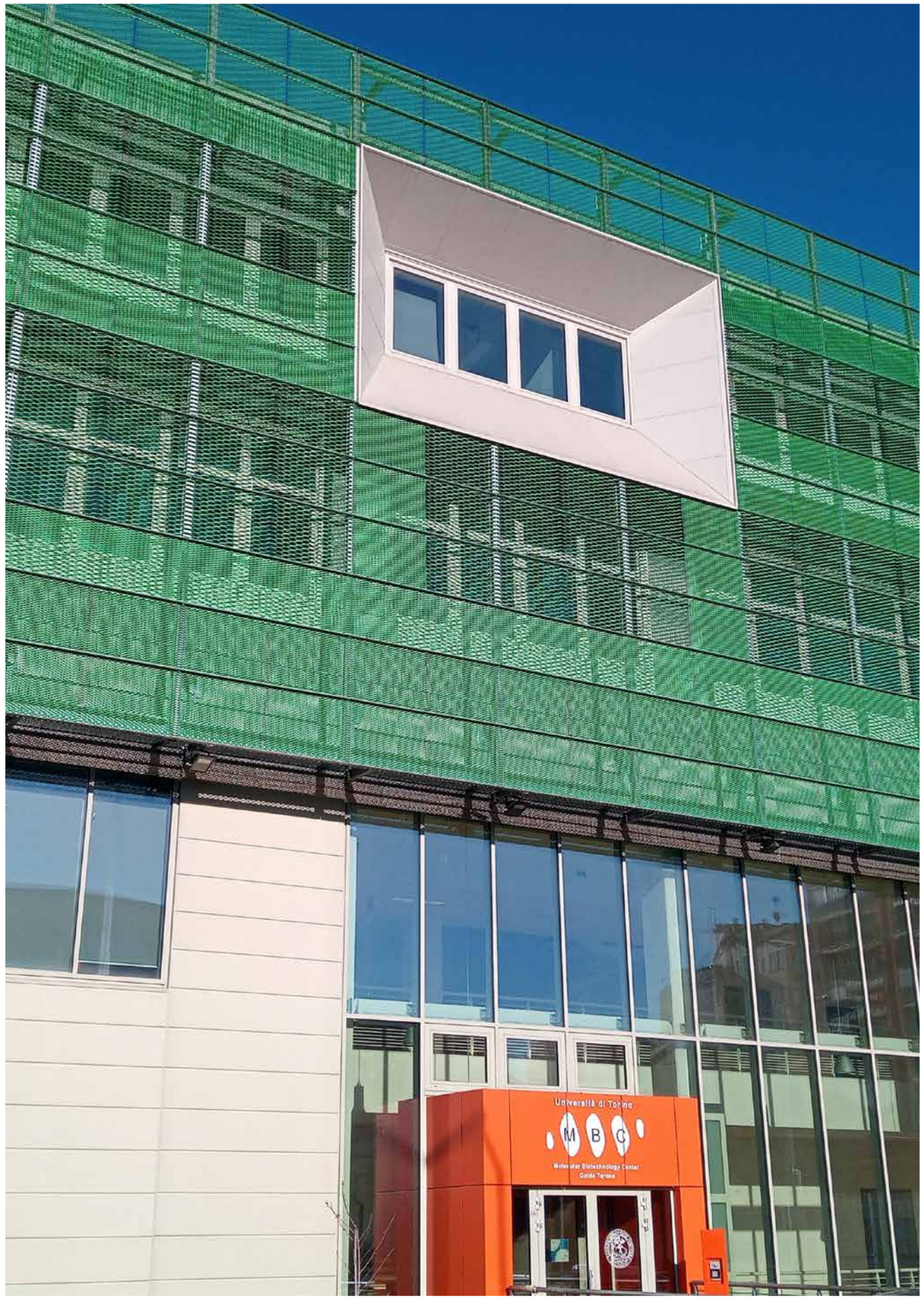
FUNDING ID (PAST 5 YERS)

- → 2019-2024 AIRC: "Immune checkpoint neutralization to recover antitumor Vγ9Vδ2 T-cell activity and reinforce anti-CD38 therapy in myeloma (PI);
- → 2021-2023 Fondazione Cassa di Risparmio di Cuneo (CRC): Development and activation of a phase I clinical unit in Hematology (PI);
- → 2022-2024 Sanofi - Research to Care Onco-hematology Edition, Sanofi: Immunomodulatory effects of anti-CD38 monoclonal antibodies on immune cells in myeloma patients (PI)

SELECTED PUBLICATIONS

Link Scopus profile: https://www.scopus.com/authid/detail.uri?authorId=7005447404

- Giannotta C, Autino F, Massaia M. Vɣ9Vδ2 T-cell immunotherapy in blood cancers: ready for prime time? Front Immunol. 2023 Apr 18;14:1167443. doi: 10.3389/fimmu.2023.1167443. PMID: 37143664; PMCID: PMC10153673.
- Giannotta C, Autino F, Massaia M. The immune suppressive tumor microenvironment in multiple myeloma: The contribution of myeloid-derived suppressor cells. Front Immunol. 2023 Jan 16;13:1102471. doi: 10.3389/fimmu.2022.1102471. PMID: 36726975; PMCID: PMC9885853
- Giannotta C, et al, and Massaia M. Immune dysfunctions affecting bone marrow Vɣ9Vδ2 T cells in multiple myeloma: Role of immune checkpoints and disease status. Front Immunol. 2022 Dec 20;13:1073227. doi: 10.3389/fimmu.2022.1073227. PMID: 36605214; PMCID: PMC9808386.
- Castella B, Riganti C, Massaia M. Metabolic approaches to rescue antitumor Vɣ9Vδ2 T-cell functions in myeloma. Front Biosci (Landmark Ed). 2020 Jan 1;25(1):69-105. doi: 10.2741/4795. PMID: 31585878.
- Castella B, Melaccio A, Foglietta M, Riganti C, Massaia M. Vɣ9Vδ2 T Cells as Strategic Weapons to Improve the Potency of Immune Checkpoint Blockade and Immune Interventions in Human Myeloma. Front Oncol. 2018 Nov 6;8:508. doi: 10.3389/fonc.2018.00508. PMID: 30460198; PMCID: PMC6232124.
- Castella B, Foglietta M, Riganti C, Massaia M. Vɣ9Vδ2 T Cells in the Bone Marrow of Myeloma Patients: A Paradigm of microenvironment-Induced Immune Suppression. Front Immunol. 2018 Jun 25;9:1492. doi: 10.3389/fimmu.2018.01492. eCollection 2018. Review PubMed PMID: 30013559; PubMed Central PMCID: PMC6036291.
- Riganti C, Castella B, Massaia M. ABCA1, apoA-I, and BTN3A1: A Legitimate Ménage à Trois in Dendritic Cells. Front Immunol. 2018 Jun 8;9:1246. doi: 10.3389/fimmu.2018.01246. PMID: 29937767; PMCID: PMC6002486.
- Castella B, et al., and Massaia M. The ATP-binding cassette transporter A1 regulates phosphoantigen release and Vɣ9Vδ2 T cell activation by dendritic cells. Nat Commun. 2017 Jun 5;8:15663. doi: 10.1038/ncomms15663. PMID: 28580927; PMCID: PMC5465356.
- Castella B, et al., and Massaia M. Anergic bone marrow Vɣ9Vδ2 T cells as early and long-lasting markers of PD-1-targetable microenvironment-induced immune suppression in human myeloma. Oncoimmunology. 2015 May 26;4(11):e1047580. doi: 10.1080/2162402X.2015.1047580. PMID: 26451323; PMCID: PMC4589055.
- Foglietta M, et al., and Massaia M. The bone marrow of myeloma patients is steadily inhabited by a normal-sized pool of functional regulatory T cells irrespective of the disease status. Haematologica. 2014 Oct;99(10):1605-10. doi: 10.3324/haematol.2014.105866. Epub 2014 Jun 27. PMID: 24972771; PMCID: PMC4181257.



CLAUDIO MEDANA

Unit of Mass Spectrometry

BIOGRAPHICAL SKETCH

Massimo Massaia holds the position of Associate Professor of Hematology and Head of the Laboratory of Blood Tumor Immunology at the University of Turin, Italy. He is Director of the Division of Hematology at Azienda Ospedaliera Santa Croce & Carle in Cuneo, Italy, after being Acting Director of SCDU Hematology and Cell Therapy at Azienda Ospedaliera Ordine Mauriziano in Turin, Italy.

He studied medicine and graduated with honors as M.D. from the University of Turin, Italy. Then, he completed his post-graduate degree in Hematology at the University of Roma and in Internal Medicine at the University of Turin. During this period, he did his post-doctoral research at the Royal Free Hospital in London, UK. He has been a Visiting Investigator, Visiting Scientist, and Visiting Professor at the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, USA, at Oklahoma Medical Research Foundation, Oklahoma City, USA, at Frederick Cancer Research and Development Center (FCRDC), Frederick, USA, at Stanford University, Stanford, USA, at Leiden University, The Netherlands, and at MD Anderson Cancer Center, Houston.His research

GROUP MEMBERS:

Federica Dal Bello *associate professor*
Riccardo Aigotti *senior technician (ep)*
Enrica Mecarelli *junior technician*
Valentina Schiavo *research fellow*
Alberto Asteggiano, Sandra Vietti, Alex Affricano *PhD students*
5 *Graduate* ***and 3*** *undergraduate students* ***belong to the group.***

RESEARCH ACTIVITY

The group research activity is focused on the development of analytical mass spectrometry (MS) methods for the identification, structure determination and quantification of bioactive molecules.

Group professors serve as mentor and tutor of Master degrees in Biotechnology, Chemistry, and Biology and PhD degree in Chemical and Materials Sciences. Group members have developed collaborations and agreements with companies, public control, forensic and research organizations. The main third mission activity of the laboratory is related to the quality control of the mineral waters of the springs of central-northwestern Italy.

The laboratory instrumentation involves ten hyphenated mass spectrometers: GC (gas chromatography)-MS, HPLC (liquid chromatography)-MS and ICP (inductively coupled plasma)-MS. The research projects deal with development and application of new analytical methods both in the field of small molecules (metabolomics, lipidomics, enviromics) and macromolecules (proteomics).

The most recent research topics are:

Characterization of mineral water quality official quality control of physical-chemical parameters: inorganic anions and cations by ion chromatography and trace elements by ICP-MS; evaluation of volatile organic pollutants by purge&trap-GC-MS and stirring bar sorptive extraction (SBSE)-GC-MS; study of food contact materials: release of contaminants via LC-MS (fig. 1).

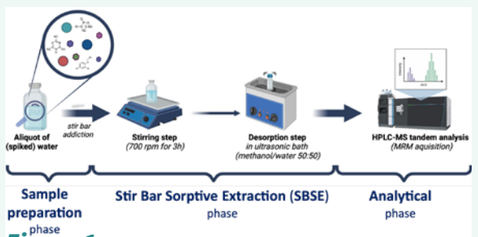


Figure 1.
preanalytical/analytical workflow of a SBSE-LC-MS/MS determination of water pollutants

Quantitation of bioactive peptides and proteins

Development and validation of targeted nanoLC-HRMS methods to detect neuropeptides (GnRH, AMH) in human plasma and nervous tissues of laboratory animals; identification and quantitation of proteins in biological/archaeological samples.

Characterization of plant secondary metabolites

Development and validation of targeted GC and HPLC-MS methods to quantify plant bioactive principles in vegetal extracts; Plant metabolomics to identify putative biomarkers of exposure to pathogens.

Targeted and untargeted metabolomics

molecular determination of biochemicals: endogenous toxins formed by interaction of sugars and proteins; enzymatic products of drugs and lipid molecules; food bioactive components quantitation.

Assessment of the transformation of bioactive molecules by HPLC coupled to high resolution multistage mass spectrometry

to study drug metabolism, environmental fate of drugs

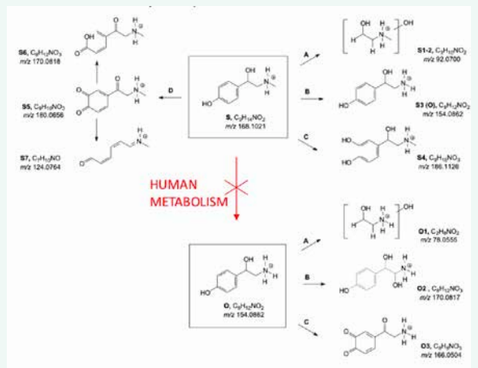


Figure 2.
Structure determination based on high resolving power MS untargeted molecular annotation: towards identification of synephrine metabolism and degradation products.

and toxics compounds. - Studies about MS fragmentation pathways and application to structural determination of synthetic intermediates or unknown molecules. These studies are centered on molecular identification of metabolites or degradants formed in vitro, in vivo or in lab simulation based on photocatalytical experiments (Fig. 2).

FUTURE RESEARCH PLANS

We are planning to complete and extend our research projects dealing about: i) drinking water quality characterization (by developing and applying new sensitive methods aimed to identify and quantify emerging pollutants and contaminants); ii) protein and peptide identification and quantitation (by developing and applying new protocols for allergens and biomarkers account); iii) studies on pathology conditions by the use of metabolomics (analytical and bioinformatics) protocols in order to characterize extracellular vesicles, microbiome and bacterial/human biomarkers.

FUNDING ID (PAST 5 YEARS)

- PRIN 2020 20204YRYS5 (Impact of microplastics on reproduction and development)
- H2020-MSCA-RISE-2020 101007578 (SusWater)
- HORIZON-MSCA-2022-DN-01 101119555 (In2aquas)
- Contract agreements with Fiat Research Center, Sanofi, IDI and multiple mineral water bottling companies
- FSE Piemonte Regional financing of apprentice PhD 2530726 (RP/2020/607) and 2544191 (RP/2021/959)

SELECTED PUBLICATIONS

Link Scopus profile: https://www.scopus.com/authid/detail.uri?authorId=6602482783

- E. Mecarelli, R. Aigotti, A. Asteggiano, P. Giacobini, M. Chasles, Y. Tillet, F. Dal Bello, C. Medana. Quantitation of endogenous GnRH by validated nanoHPLC-HRMS method: a pilot study on ewes plasma. Analytical and Bioanalytical Chemistry 414, 7623-7634 (2022). doi: 10.1007/s00216-022-04293-z
- F. Dal Bello, E. Mecarelli, R. Aigotti, E. Davoli, P. Calza, C. Medana. Development and application of high resolution mass spectrometry analytical method to study and identify the photoinduced transformation products of environmental pollutants. Journal of Environmental Management 308, 114573 (2022). doi: 10.1016/j.jenvman.2022.114573
- P. Calza, B. Guarino, F. Dal Bello, A. Dioni, M. Bergero, C. Medana. Integrated approach for the analysis of neonicotinoids in fruits and food matrices. Food Chemistry 372, 131153 (2022). doi: 10.1016/j.foodchem.2021.131153
- Asteggiano, A. Occhipinti, A. Capuzzo, E. Mecarelli, R. Aigotti, C. Medana. Quali-Quantitative characterization of volatile and non-volatile compounds in Protium heptaphyllum (Aubl.) Marchand resin by GC-MS validated method, GC-FID and HPLC-HRMS2. Molecules 26, 1447 (2021). doi: 10.3390/molecules26051447
- F. Dal Bello, C. Lamberti, M. Giribaldi, C. Garino, M. Locatelli, D. Gastaldi, C. Medana, L. Cavallarin, M. Arlorio, M. G. Giuffrida. Multi-target detection of egg-white and pig gelatin fining agents in Nebbiolo-based aged red wine by means of nanoHPLC-HRMS. Food Chemistry 345, 128822 (2021). doi: 10.1016/j.foodchem.2020.128822
- F. Dal Bello, M. Zorzi, R. Aigotti, D. Medica, V. Fanelli, V. Cantaluppi, E. Amante, V.T. Orlandi, C. Medana. Targeted and untargeted quantification of quorum sensing signaling molecules in bacterial cultures and biological samples via HPLC-TQ MS techniques. Analytical and Bioanalytical Chemistry 413, 853-864 (2021). doi: 10.1007/s00216-020-03040-6
- F. Dal Bello, C. Medana, M. Zorzi, B. Kuck, D. Fabbri, P. Calza. LC-MS analytical determination of gabapentin transformation products by heterogeneous photocatalysis and environmental evaluation. Rapid Communications in Mass Spectrometry 34, e8925 (2020). doi: 10.1002/rcm.8925
- F. Dal Bello, R. Aigotti, M. Zorzi, C. Medana. Multi-Analyte MS Based Investigation in Relation to the Illicit Treatment of Fish Products with Hydrogen Peroxide. Toxics 8, 2 (2020). doi: 10.3390/toxics8010002
- V. Santoro, C. Baiocchi, F. Dal Bello, D. Gastaldi, R. Aigotti, M. Zorzi, A. Pellegrino, E. Forte, F. Romaniello, M. Magni, M. Fontana, M. Somenzi, C. Medana. Formation of by-products during chemical interesterification of lipids. Detection and characterization of dialkyl ketones by nonaqueous reversed-phase LC-HRMS and GC-MS. Journal of Chromatography A 1581-1582, 63-70 (2018). doi: 10.1016/j.chroma.2018.11.001.
- F. Dal Bello, V. Santoro, V Scarpino, C. Martano, R. Aigotti, A. Chiappa, E. Davoli and C. Medana. Antineoplastic drugs determination by HPLC/HRMSn to monitor occupational exposure. Drug Testing and Analysis 8, 730-737 (2016). doi:10.1002/dta.1827

VALERIA MENCHISE

Nanosystems for Molecular Imaging

BIOGRAPHICAL SKETCH

January 2000	PhD, Structural studies on thioredoxins, Universities of Naples "Federico II" (Italy) and of Nancy 1 "Henri Poincarè" (France)
Since 2001	Researcher at IBB, CNR
2003-2006	Cooperative research program between Public and Private Res. institutions at Bioindustry Park del Canavese SpA. Colleretto Giacosa
2007 to date	Researcher in Torino, IBB, CNR (Institute of Biostructures and Bioimaging, National Research Council of Italy).
	IBB-CNR unit at the Molecular Biotechnology Centre (MBC) of the University of Turin.

GROUP MEMBERS:

Carla Carrera *Postdoctoral fellow*
Claudia Quattrociocchi *Postgraduate fellow*

RESEARCH ACTIVITY

The main research line is focused on the development of multimodal diagnostic procedures relying on the use of structure based designed nanosystems, properly planned to quantify the expression of molecular targets, overexpressed in tumoral and/or viral pathologies:

-During the COVID-19 emergency, we developed a project for a diagnostic test based on a turbidimetric method. The overall idea involves the development of a simple and low-cost test capable of providing rapid diagnostic results for a large number of subjects. The objective is to design liposomal systems that display peptides on their surface, which effectively interact with the RBD domain of the Spike protein. Subsequently, the formation of a liposome/virus-like network based on the peptide/RBD interaction was confirmed, resulting in the turbidity of the solution (Figure 1). This turbidity measurement serves as an indication of the presence of the virus in the sample under examination. Peptides targeting the RBD domain were designed, synthesized, and incorporated into the membrane of appropriately functionalized liposomes.

Furthermore, the nanomolar affinity of the isolated peptides and the liposomes conjugated with the Spike RBD domain was confirmed using BLI. By utilizing extracellular vesicles (EVs) that expose a portion of the Spike protein, the necessary conditions for the formation of an aggregate based on liposome/RBD recognition were determined using DLS.

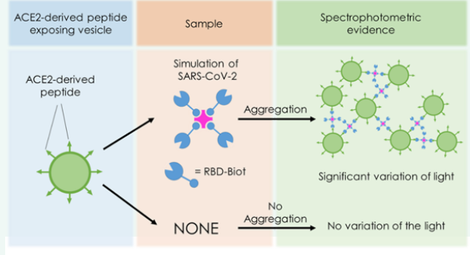


Figure1. Turbidimetric test: liposomes exposing the ACE-derived peptide bind the RBD on the virus-like vescicles, forming a network that affects the turbidity of the sampe.

Figure 1.

-We are developing a multi-parametric in vivo diagnostic approach (MRI/OI) to evaluate the correlation between the expression level of hCA IX, hCA XII, and pH deregulation in a xenografted breast cancer murine model. Despite significant advances in therapy, breast cancer remains the second leading cause of cancer death in women. This indicates the need for a better understanding of this complex disease. In recent years, the study of H+ dynamics in cancer has led to a new paradigm known as the pH-centric anticancer paradigm. This metabolic reprogramming involves the intracellular alkalization of cancer cells and, consequently, extracellular and intratumoral microenvironmental acidosis. Among many others, two key players in pH regulation of cancer cells are the transmembrane isoforms of carbonic anhydrases, hCA IX and hCA XII. These proteins are known to be overexpressed in many common tumors and play a critical role in hypoxia-associated tumor acidosis. The extracellular part of CAIX contains a catalytic CA domain and a region with high sequence identity to the keratan sulfate attachment domain of a large proteoglycan called aggrecan, named the PG domain (Figure 2). Unlike hCA IX, hCA XII does not contain the PG domain. hCA IX and hCA XII have been shown to contribute to the growth and survival of tumor cells, and their expression is correlated with metastasis and resistance to therapies. Recent studies have demonstrated that although hCA IX and hCA XII have similar catalytic activities, they exhibit distinct and non-overlapping expression patterns. High expression of hCA XII is associated with better survival statistics, while hCA IX expression is associated with a more aggressive tumor phenotype and poor prognosis. However, most of this information has

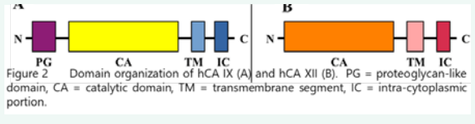


Figure 2 Domain organization of hCA IX (A) and hCA XII (B). PG = proteoglycan-like domain, CA = catalytic domain, TM = transmembrane segment, IC = intra-cytoplasmic portion.

Figure 2.

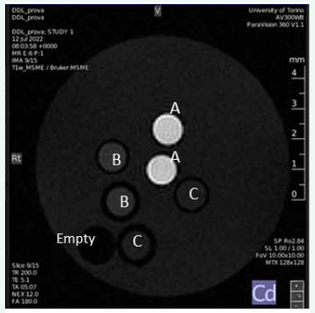


Figure 3. Representative MRI T1W image of cell pellets 8 hours after incubation with liposome with specific peptide (A), peptide scramble (B) and without any peptide (C).

Figure 3.

been obtained through ex-vivo indirect measurements, such as immunohistochemical analyses of tumor biopsies. Therefore, we are developing an in vivo molecular imaging approach using agents that selectively target hCA IX and hCA XII, which are overexpressed at sites of hypoxia. So far, there have been no Magnetic Resonance Imaging (MRI) studies on tumor imaging that exploit CAIX as a target. MRI is a technique that provides superior soft tissue contrast and excellent spatial resolution. However, the inherent sensitivity of MRI is relatively low, and the ability to visualize a molecular marker in vivo depends on the target concentration and/or the efficacy and amount of contrast agent accumulated at the site. To enhance the concentration of the contrast agent delivered to the target, the most common strategy involves the use of paramagnetic liposomes. These liposomes can transport a large amount of contrast agents per vesicle, amplifying the effect of the molecular recognition event. Their structure allows for the encapsulation of active components in the aqueous core and the incorporation of multifunctionalized phospholipids in the membrane, making them suitable for theranostics and image-guided drug delivery, including MRI imaging.

We are developing a Gd-based MRI nanoprobe that carries a CAIX PG domain targeting vector on the surface and a quenched T1-contrast agent in the interior space. This allows for the specific delivery of a cargo of MRI probes to the receptor site and the tracking of its intracellular fate. As a recognition peptide for the target, we modified some sequences developed from the crystallographic structure between the PG of CAIX and one of its antibodies. The nano-system thus prepared resulted in specific in vitro MRI-labeling of breast cancer cells, up to 80 times more effective than that obtained using a scramble sequence. Interestingly, this huge specificity emerges in the MRI signal only 8 h after the end of incubation, when the probe was likely entirely internalized and has released the paramagnetic molecule into the cells (Figure 3). This could be ascribed to the process of quenching, consisting in silencing the probe relaxing ability on bulk water signal as long as it is entrapped in the inner core of a nanovesicle at high concentration. These T1-quenched nanosystems are designed to recover the signal enhancement ability following the release of vesicle contents in response to biological events. The strategy of our MRI procedure is based on incapsulating a high concentration of contrast agent in the liposome that is quite silent in the condition of our MRI experiment, producing a high MRI signal only after the release of the contrast agent (de-quenching). In collaboration with Prof. Delli Castelli's group at MBC, we have successfully translated the procedure in vivo using three groups of murine models with breast cancer: one group treated with the liposomes functionalized with the specific peptide, another group treated with a scramble peptide, and a control group without any peptide treatment. Consistent with previous experiments, the group treated with the specific peptide-functionalized liposomes showed a significant increase in signal enhancement. The peak of T1 signal enhancement in the tumor was observed at 8 hours after injection, indicating the internalization of liposomes into cells through PG-CAIX binding and subsequent release of the contrast media. The developed probe in this study has the ability to generate a high signal enhancement in response to the interaction between liposomes and CAIX, as well as the degradation of the vesicles. This enables sensitive detection that can be monitored in vivo through MRI.

FUTURE RESEARCH PLANS

In the context of developing theranostic procedures targeting CAIX and CAXII for breast cancer, my focus will be on designing targeting liposomes that can deliver alkalizing agents, such as bicarbonate, directly into the tumor microenvironment characterized by strong pH deregulation. These liposomes will be guided to cancer cells by incorporating molecules that can recognize the target proteins, and they will release their content upon the application of low-frequency ultrasound at the target site. The binding of this ligand to the enzyme and the controlled release of the drug can help regulate the pH environment and influence the action of the enzyme itself.

FUNDING ID (PAST 5 YERS)

PRIN 2015-2019: Regenerative potential of extracellular vescicles derived from mesenchimal stem cells on epithelial wound healing

2021- FISR2020IP_02416: Turbidity based Covid-19 test- TURBO

SELECTED PUBLICATIONS

Link Scopus profile: https://www.scopus.com/authid/detail.uri?authorId=6602387450

→ V. Menchise, et al, and S. Aime. In Vivo Labeling of B16 Melanoma Tumor Xenograft with a Thiol-Reactive Gadolinium Based MRI Contrast Agent Molecular Pharmaceutics (2011) 1750-1756 DOI: 10.1021/mp2001044.

→ E. Terreno, C. Boffa, V. Menchise, et al, and Aime, S. (2011) Gadolinium-doped LipoCEST agents: a potential novel class of dual 1H-MRI probes. Chem. Comm. (2011) 47, 4667-4669.DOI: 10.1039/c1cc10172b

→ C. V. Gringeri, V. Menchise, et al., and S. Aime Novel Gd(III)-based probes for MR molecular imaging of Matrix Metalloproteinases. Contrast Media Mol. Imaging 2011, (DOI: 10.1002/cmmi.478)

→ C. Fiorillo, et al., V. Menchise, R. Biancheri, F. M. Santorelli, C. Bruno RPV4 mutations in children with congenital distal spinal muscular atrophy. NEUROGENETICS (2012) 13, 195-203 (DOI: 10.1007/s10048-012-0328-7)

→ V. Catanzaro, C. V. Gringeri, V. Menchise, et al, and G. Digilio*, S. Aime* A R2p/R1p ratiometric procedure to assess Matrix Metalloproteinase-2 activity by Magnetic Resonance Imaging. Angew. Chem. Int. (2013), 52, 3926–3930. (DOI: 10.1002/anie.201209286)

→ C. Chirizzi, W. Dastrù, D. Delli Castelli, V. Menchise, S. Aime and E. Terreno. Glucan Particles Loaded with Fluorinated Emulsions: a Sensitivity Improvement for the Visualization of Phagocytic Cells by 19F-MRI. Current Molecular Imaging (2015) 4, 29-34. (DOI: 10.2174/2211555204666150702160805)

→ E. Calce, G. Digilio, V. Menchise, M. Saviano, S. De Luca. Chemoselective Glycosylation of Peptides through S-Alkylation Reaction. Chemistry (2018) 24, 6231-6238 (DOI:10.1002/chem.201800265)

→ W. Dastrù, V. Menchise, G. Ferrauto, S. Fabretto, C. Carrera, E. Terreno, S. Aime, D. Delli Castelli. Modulation of the Prototropic Exchange Rate in pH-Responsive Yb-HPDO3A Derivatives as ParaCest Agents. ChemistrySelect (2018) 3, 1-8. (DOI: 10.1002/slct201800283)

→ G. Saccu, V. Menchise, et al., and S. Fagonee. Regenerative Approaches and Future Trends for the Treatment of Corneal Burn Injuries. J. Clin. Med. 2021, 10, 317; (DOI: https://doi.org/10.3390/jcm10020317)

→ .G. Saccu, V. Menchise, et al., and S. Fagoonee. Bone Marrow Mesenchymal Stromal/Stem Cell-Derived Extracellular Vesicles Promote Corneal Wound Repair by Regulating Inflammation and Angiogenesis. Cells. 2022 Dec 2;11(23):3892. doi: 10.3390/cells11233892.

GIORGIO ROBERTO MERLO

Genetics and Development laboratory

BIOGRAPHICAL SKETCH

Oct 2022-current	Director of the Master Course II level “Stem Cells Regeneration and Cell Factory” at Univ. of Torino
Nov 2010-current	Associate Professor, Dept Molecular Biotechnology, Univ. of Torino
May 2000-Dec 2009	Assistant Telethon Scientist, Career Project
Dec 1998-Feb 1999	Visiting Scientist at the Weizmann Institute of Science, Rehovot, Israel
Jan 1996-April 2000	Research Associate at the Advanced Biotechnology Center CBA-IST, Genova, Italy.
Sept 1995-Jan 1996	Guest Scientist at Ciba LTD, Basel, Switzerland.
Jan 1993-Aug 1995	Research Contract at the Friedrich Miescher Institute, Basel, Switzerland.
Jan 1992-Jan 1993	Recipient of a European Community Bursary, Senior Scientist at the Friedrich Miescher Institute, Basel, Switzerland.
Mar 1989-Dec 1991	Visiting Associate at the NIH, in the Laboratory of Tumor Immunology and Biology.
Jan 1986-Mar 1989	Visiting Fellow at the NIH, in the Laboratory of Tumor Immunology and Biology.

GROUP MEMBERS:

Carla Liaci *Dottoranda*
Simona Rando *Borsista*
Lucia Prandi *Studentessa Magistrale*
Sara Palermo *Studentessa Magistrale*
Lorenzo Licari *Studente Magistrale*
Emma Leonetti *Studente Magistrale*
Giovanni Catapano *Studente Magistrale*
Daniela Micheletto *Studentessa Magistrale*

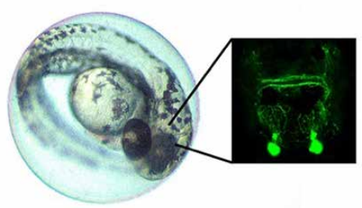
RESEARCH ACTIVITY

Development of the Olfactory and GnRH System

The development of the olfactory neurons is associated to the genesis and migration of the GnRH neuroendocrine neurons, a complex process that is specifically impaired in the Kallmann Syndrome. The molecules involved in guidance and connectivity of olfactory axons are not well known. We are investigating the role of specific disease genes and microRNAs for olfactory/GnRH development, combining transcription-profiling, analysis of conserved co-expression and specific animal models. Zebrafish strains with fluorescent neurons, such as GnRH3::GFP, turn out useful to image the effect of exposure to endocrine-interfering compounds or other contaminants on the development of hypothalamic neurons.

Development and Maturation of Inhibitory Neurons

Using Neural Stem cells in vitro and specific mutant mouse strains, the team aims to clarify a molecular signature for GABA+ differentiation, and to comprehend the role of Dlx genes in this process. Current results indicate that alterations of the migration, differentiation and neuritogenesis of inhibitory neurons affect cognitive and memory functions of the hippocampus and may contribute to the risk of epilepsy.

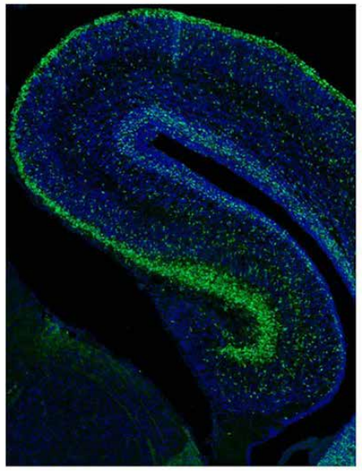


On the left: pre-hatching zebrafish embryo – 48 hpf
On the right: GnRH3-GFP+ neurons in the olfactory area

Figure 1.

The RacGTPase and Models of Intellectual Disability

The small GTPases RhoA, Rac1 and cdc42 have been implicated in genetically transmitted Intellectual Disability (ID) conditions, either directly or upon mutation of one of their several regulators in developing neurons. In the case of Rac1 it appears that most genetic mutations causing ID result in a hypoactive Rac1. Based on our previous work on the protein ArhGAP15, a negative regulator of Rac1, we can hypothesize that interfering with the protein::protein interaction between ArhGAP15 and Rac1 should result in hyperactivation of Rac1. This applied to the condition of hypoactive Rac1 causing ID might restore normal activity and the development of normal neuronal circuitry. We are currently setting up cellular models to prove this hypothesis.



Radially and tangentially migrating inhibitory neurons in the mouse embryonic hippocampus, labeled with the GAD67-GFP transgene.

Figure 2.

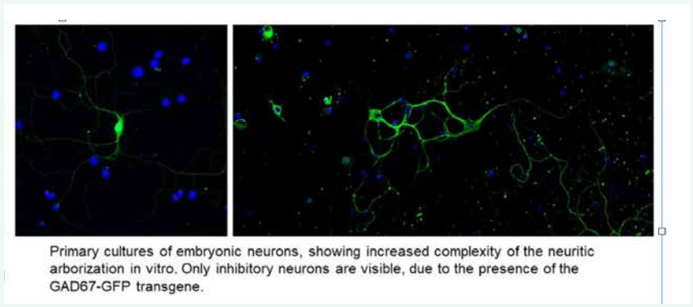


Figure 3.

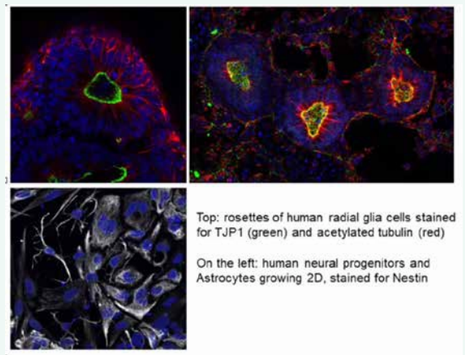


Figure 4.

FUTURE RESEARCH PLANS

Recently the team is implementing a program for the use of human pluripotent stem cells to explore the effects of pathogenic mutations on human brain development. In particular, the experimental work focuses on two selected disease-genes, TRIO and ArhGEF6, that encode for proteins that regulate the dynamic of actin cytoskeleton, whose mutations cause hereditary Microcephaly and ID. Human iPSc have been mutagenized and are currently being analysed for neurogenesis, kinetic and orientation of cell division, neuronal differentiation, morphogenesis and neuritogenesis, 3D organization, network formation.

A new project, in collaboration with Dept. of Life Science, deals with the impact of microplastics (MPs) and Endocrine Disrupting Chemicals (EDC) on living organisms. It has been recently found that they can distribute systemically within an organism and they are associated with disease risk. At present there is no evidence of a direct action of MPs, there is growing evidence of the potential of MPs to be vehicles of environmental contaminants, including EDC. We aim to evaluate the impact of MP and EDC on health, in particular on reproduction and metabolism. Studies are in progress on zebrafish embryos and on mammalian cells (adipocytes, hepatocytes, neurons) exposed to environmentally relevant doses.

FUNDING ID (PAST 5 YEARS)

- Eit Food EU program, 2019. Consumers and Environmental Safety: Food Packaging and Kitchenware
- Coordinator, E. 65.000
- Telethon Foundation, Research Grant 2021 GGP20039. Rac GTPase in Intellectual Disability: preclinical opportunities from interfering with a Rac1 protein::protein interaction. Principal Investigator E. 185,000
- MUR PRIN 2022. Neural cytoskeleton and Rho GTPases in human models of Intellectual Disability and Microcephaly. Principal Investigator, 199.000.

SELECTED PUBLICATIONS

- Garaffo G., Conte D., Provero P., Tomaiuolo D., Luo Z., Pinciroli P, Peano C., D'Atri I., Gitton Y., Etzion E., Gothilf Y., Gays D., Santoro M.M., Merlo G.R. (2015) The Dlx5 and Foxg1 transcription factors, linked via miRNA-9 and -200, are required for the development of the olfactory and GnRH system. Mol. Cell. Neurosci. 68: 103-119. doi: 10.1016/j.mcn.2015.04.007 PubMed PMID: 25937343; PubMed Central PMCID: PMC4604252
- Conte D, Garaffo G, Lo Iacono N, Mantero S, Piccolo S, Cordenonsi D, Perez-Morga D, Orecchia V, Poli V and Merlo GR (2015) The Apical Ectodermal Ridge of Dlx-5;Dlx6-/- ectrodactylous limbs shows altered Wnt5a expression and planar-cell polarity pathway, rescued by exogenous Wnt5a ligand. Hum Mol Genet. 25(4): 740-754. doi: 10.1093/hmg/ddv514. PubMed PMID: 26685160; PubMed Central PMCID: PMC4743692
- Zamboni V, Armentano M, Berto G, Ciraolo E, Ghigo A, Garzotto D, Umbach A, DiCunto F, Parmigiani E, Boido M, Vercelli A, El-Assawi N, Mauro A, Priano L, Ponzoni L, Murru L, Passafaro M, Hirsch E, Merlo GR. (2018) Hyperactivity of Rac1-GTPase pathway impairs neuritogenesis by altering actin dynamics. Scientific Report, 8: 7254. doi: 10.1038/s41598-018-25354-3. PMID 29740022
- Grassi E, Santoro R, Umbach A, Grosso A, Oliviero S, Neri F, Conti L, Ala U, Provero P, DiCunto F, Merlo GR (2019) Choice of alternative polyadenylation sites, mediated by the RNA-binding protein Elavl3, plays a role in differentiation of inhibitory neuronal progenitors. Front. Cell. Neurosci. 12:518. doi: 10.3389/fncel.2018.00518
- Zuccarini G, D'Atri I, Cottone E, Mackie K., Shainer I, Gothilf Y, Provero P, Bovolin P and Merlo GR (2019) Interference with the cannabinoid receptor CB1R results in miswiring of GnRH+ and AgRP1+ axons in zebrafish embryos. Int. J. Molecular Sci, special issue 21(1) pii:E168. doi:10.3390/ijms21010168. PMID: 31881740
- Messina A., Pulli K., Santini S., Acierno J., Känsäkoski J., Cassatella D., Xu C., Casoni F., Malone S.A., Ternier G., Conte D., Sidis Y., Tommiska J., Vaaralahti K., Dwyer A., Gothilf Y., Merlo G.R., Santoni F., Niederländer N.J., Giacobini P., Raivio T., Pitteloud N. (2020) Neuron-derived neurotrophic factor (NDNF) is mutated in patients with Congenital Hypogonadotropic Hypogonadism, Am. J. Hum Genet, 106(1): 58-70. doi: 10.1016/j.ajhg.2019.12.003. PMID: 31883645
- Liaci C., Camera M., Rando S., Caslini G., Contino S., Romano V. and Merlo GR (2021) Neuronal cytoskeleton in intellectual disability: from systems biology and modeling to therapeutic opportunities. Int. J. Molecular Sci. 22(11) 6167. doi: 10.3390/ijms22116167 PMID: 34200511
- Camera M, Russo I, Zamboni V, Ammoni A, Rando S, Morellato A, Cimino I, Angelini C, Giacobini P, Oleari R, Amoruso F, Cariboni A, Franceschini I, Turco E, Defilippi P and Merlo GR (2022) p140Cap controls female fertility in mice acting via glutamatergic afference on hypothalamic GnRH neurons. Front. Neuroscience 16: 744693. doi: 10.3389/fnins.2022.744693. eCollection 2022 PMID: 35237119
- Liaci C., Prandi L., Brusco A., Pavinato L., Maldotti M., Molineris I., Oliviero S. and Merlo G.R. (2022) The emerging role of non-coding lncRNAs in Intellectual Disability and related neurodevelopmental disorders. Int. J. Mol. Sci., 23: 6118. doi: 10.3390/ijms23116118 PMID: 35682796
- Liaci C., Camera M., Zamboni V., Sarò G., Ammoni A., Parmigiani E., Ponzoni L., Hidisoglu E., Chiantia G., Marcantoni M., Giustetto M., Tomagra G., Carabelli V., Torelli F., Yanagawa Y., Obata K., Hirsch E., Merlo G.R. (2022) Loss of ARHGAP15 affects the directional control of migrating interneurons in the embryonic cortex and increases susceptibility to epilepsy. Front Cell Develop Biol 10:875468. Pag 1-20 doi: 10.3389/fcell.2022.875468

MOLECULAR AND TRANSLATIONAL ONCOLOGY

CHIARLE AND VOENA LABORATORY MEMBERS:

Alessandro Gasparetto *MD PhD student in Biomedical Sciences and Oncology*
Andrea Macioce *Postgraduate Fellow*
Martina Maggiore *Postgraduate Fellow*
Marta Rubin *PhD student in Molecular Medicine*
Mariapia Russo *PhD student in Biomedical Sciences and Oncology*

CHIARLE AND VOENA LABORATORY MEMBERS:

Dominik-Laurentius Candea *PhD student in Molecular Medicine, MSCA – DN*
Maria Vittoria Di Marco *PhD student in Molecular Medicine*
Giulia Mura *Postgraduate Fellow*

RESEARCH ACTIVITY

Several hematological and solid tumors display genetic alterations in the gene encoding Anaplastic Lymphoma Kinase (ALK) (FIGURE 1). ALK rearrangements define a distinct molecular subset of non-Hodgkin T cell lymphoma, namely Anaplastic Large Cell Lymphoma (ALCL), and non-small cell lung cancer (ALK+ NSCLC), whereas ALK activating single point mutations characterize neuroblastoma (NB). ALK chromosomal rearrangements involve different partner genes and result in fusion genes that encode for new oncogenic chimeric proteins, such as NPM-ALK in ALCL and EML4-ALK in NSCLC.

ALK fusion proteins are constitutively active and contribute to tumorigenesis and maintenance of neoplastic phenotype. ALK-rearranged tumors are totally dependent on ALK activity, making ALK an attractive target for therapy. Different ALK tyrosine kinase inhibitors (TKIs), crizotinib, ceritinib, brigatinib, alectinib and lorlatinib, have been developed and FDA approved for treatment of ALK+ NSCLC in first and second-line treatment. Crizotinib has been recently approved for refractory/relapsed ALK+ ALCL and ALK TKIs are currently evaluated in several clinical trials for different ALK-driven tumors. Overall, ALK TKIs have revolutionized the therapeutic opportunities of ALK-driven tumors. Nonetheless, the development of resistance limits their long-term clinical impact. We have extensively studied the role of ALK in lymphoma and in lung cancer using in vitro and in vivo models. Our lab aims at discovering the key mechanisms of ALK-mediated transformation and resistance to therapy while developing therapeutic approaches to completely eradicate ALK-positive cancers.

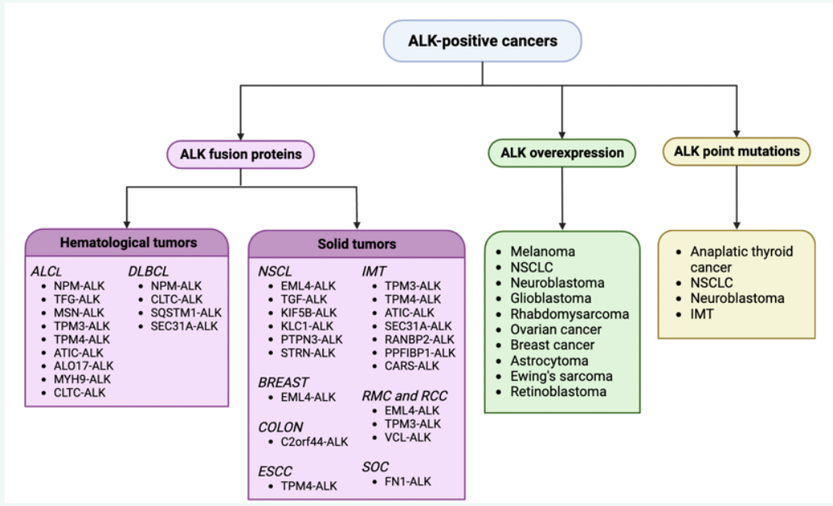


Figure 1.
ALK positive tumor

ROBERTO CHIARLE

TEAM LEADER: IMMUNOTHERAPIES FOR ALK-DRIVEN CANCERS

BIOGRAPHICAL SKETCH

Throughout clinical work and research, Dr. Chiarle has gained deep experience and expertise both as a clinical pathologist as well as in the areas of Haematopathology, Oncology, Immunology, and Molecular Biology. He received his M.D. degree from the University of Torino, Italy, and then refined his clinical skills as a Visiting Fellow in the Department of Pathology, New York University Medical Center. In Italy, his academic ranks eventually progressed up to the promotion to Professor of Pathology at the University of Torino Medical School in 2014. He started a new laboratory in 2012 at the Department of Pathology at Children's Hospital and Harvard Medical School, Boston, USA. In 2020, he was appointed Professor of Pathology at Harvard Medical School.

As a clinician, he is currently an attending hematopathologist at the at Boston Children's Hospital and Director of Hematopathology at the European Institute of Oncology (Milan, Italy). In research, Dr. Chiarle has being leading his own groups in the University of Torino since 2001 and in Boston since 2012. He won several national and international awards, including an award from the Italian National Academy of Science, two prestigious European Research Council (ERC) grants, an AICR-UK award, several NIH grants and grants from the LUNGevity foundation, V Foundation, the Ellison Foundation, the Bridge Project and others.

LAB MEMBERS:

Alessandro Gasparetto *MD PhD student in Biomedical Sciences and Oncology*
Andrea Macioce *Postgraduate Fellow*
Martina Maggiore *Postgraduate Fellow*
Marta Rubin *PhD student in Molecular Medicine*
Mariapia Russo *PhD student in Biomedical Sciences and Oncology*

See the group resarch at page 170

RESEARCH ACTIVITY

One major research interest has been to study the mechanisms and pathways of tumor formation activated by the ALK oncogene, as well as the development of innovative therapies for AK-positive cancers, such as an ALK-specific cancer immunotherapy. Chiarle's group has developed mouse models for both ALK-rearranged lymphoma and lung carcinoma to study the molecular pathogenesis of ALK-driven tumors. Recently, the group has developed an ALK vaccine that instructs the immune system to recognize and eliminate ALK-positive lymphoma and lung cancer cells. Additional areas of interest involve the study of the biological mechanisms of chromosomal translocation formation that initiate tumor formation.

ALK-TKIs have produced impressive results in ALK-driven cancer. Unfortunately, such therapies have setbacks associated with the development of TKI resistance. Acquired resistance is commonly mediated by secondary mutations in the ALK kinase domain, bypass track activation, and other mechanisms. In this scenario, tumors are almost never fully eradicated by ALK inhibitors, and most patients eventually relapse being left without other valuable therapeutic options.

We are currently implementing innovative ALK-specific immunotherapies (FIGURE 2) because the ALK protein has several features suitable for a tumor onco-antigen for cancer immunotherapy. ALK is expressed almost exclusively by tumor cells and is naturally immunogenic in humans. Patients with ALK-rearranged lymphoma and lung cancer spontaneously develop a natural immune response against ALK. Importantly, ALK is a potent driver oncogene required for tumor survival and growth, which minimizes the chances of escape of ALK negative tumor cells. We developed an ALK vaccine that generates strong and specific immune responses against tumors cells that express ALK as an oncogenic driver in a pre-clinical setting. We are now translating our results into the clinic. We are designing a

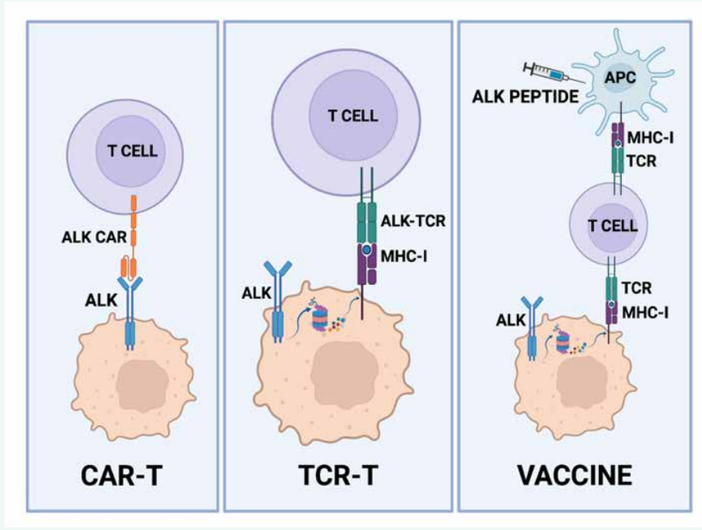


Figure 1.
ALK-specific immunotherapies

Phase I clinical trial to combine ALK TKIs with an ALK vaccine based on the human ALK immunogenic peptides that we recently discovered and published.

ALK vaccine is not effective in neuroblastoma (NB), therefore we are also developing ALK-specific chimeric antigen receptor (CAR) T cells to target tumors that express ALK on their surface, such as neuroblastoma. We have already generated and validated ALK-specific CAR T cells in pre-clinical models of neuroblastoma. We tested in vitro and in vivo the anti-tumor activity of hALK.CAR-Ts. We have cell lines or PDX models of NB to establish a biomarker that predicts therapeutic response and test the combination with ALK TKIs. We are implementing a Phase I clinical trial to treat neuroblastoma with a combination of hALK.CAR-Ts and ALK TKIs.

Lastly, we are generating T cell receptor (TCR)-engineered T cells (TCR-T) against ALK instead of a vaccine. TCRs are naturally developed for sensitive antigen detection and can recognize epitopes at far lower concentrations than required for CAR-T activation. In addition, TCR-T cells can be exploited to attack antigens not expressed on the cell membrane, as EML4-ALK in ALK+ NSCLC. The generation of such cells will be a powerful tool used to eradicate ALK positive lung cancer cells and form the foundation of a TCR-T cell based clinical trial for patients with TKI-resistant ALK positive NSCLC.

FUTURE RESEARCH PLANS

We plan

- to enhance ALK.CAR-Ts efficacy by combination with lorlatinib and by generation of dual ALK/GD2.CAR-Ts or ALK.CAR-Ts expressing IL15;
- to enhance the expansion and in vivo persistence of ALK CAR-T by amph-CAR-T ligands;
- to implement a phase I clinical trial in patients with ALK+ NSCLC to test the ALK vaccine in combination with ALK inhibitors and immuncheckpoint inhibitors (ICI);
- to implement a Phase I clinical trial to treat neuroblastoma with a combination of hALK.CAR-Ts and ALK TKIs;
- to develop TCR-T cells for the treatment of ALK+ cancers.

FUNDS (5 PAST YEARS)

- 2023-2027: Associazione Italiana Ricerca sul Cancro (AIRC) – “Development of CAR T cells to target oncogenic ALK for neuroblastoma therapy”
- 2023-2025: 101072735 –HORIZON – MSCA – 2021 – DN – “FANTOM - Future of ALCL: Novel Therapies, Origins, Biomarkers and Mechanisms of Resistance”

SELECTED PUBLICATIONS

- Stat3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. Nature Medicine 11:623, 2005. DOI: 10.1038/nm1249
- he Anaplastic Lymphoma Kinase in the pathogenesis of cancer. Nature Reviews Cancer 8: 11, 2008. DOI: 10.1038/nrc2291
- The anaplastic lymphoma kinase is an effective oncoantigen for lymphoma vaccination. Nature Medicine 14: 676, 2008. DOI: 10.1038/nm1769
- Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells. Cell 147:107, 2011. DOI: 10.1016/j.cell.2011.07.049
- Simple and rapid in vivo generation of chromosomal rearrangements using CRISPR/Cas9 technology. Cell Reports 9:1219, 2014. DOI: 10.1016/j.celrep.2014.10.051
- Phosphatidylinositol 3-kinase d blockade increases genomic instability in B cells. Nature 542:489, 2017. DOI: 10.1038/nature21406
- Wiskott-Aldrich Syndrome protein (WASP) is a tumor suppressor in T cell lymphoma. Nature Medicine 25:130, 2019. DOI: 10.1038/s41591-018-0262-9
- Tyrosine phosphatases regulate resistance to ALK inhibitors in ALK+ anaplastic large cell lymphoma. Blood 139:717, 2022. DOI: 10.1182/blood.2020008136
- CCR7-PI3Kg signaling supports resistance to tyrosine kinase inhibitors in ALK-rearranged lymphoma. Science Translational Medicine 15, 2023. DOI: 10.1126/scitranslmed.abo3826
- ALK peptide vaccination restores the immunogenicity of ALK-rearranged non-small cell lung cancer. Nature Cancer 4:1016, 2023. DOI: 10.1038/s43018-023-00591-2.

CLAUDIA VOENA

TEAM LEADER: Mechanisms of ALK-mediated transformation and drug resistance to therapy in ALK-driven tumors

BIOGRAPHICAL SKETCH

Claudia Voena is currently an Associate Professor in Laboratory Medicine at the University of Torino in the Department of Molecular Biotechnology and Health Sciences. She is a recipient of a Biology degree and a PhD in Experimental Hematology. She is also board certified in Clinical Pathology. During her PhD she developed new approaches for the detection of minimal residual disease in hematological malignancies under the guidance of Prof. Paolo Corradini at the University of Torino and then, moved to the Istituto San Raffaele in Milano, to keep studying minimal residual disease and set up a lab of molecular diagnostics for hematological malignancies. As a senior scientist, Claudia worked with Prof. Giorgio Inghirami and Prof. Roberto Chiarle at the University of Torino and at the Boston Children's Hospital, Boston, USA. During this time, she investigated mechanisms of lymphomagenesis, mainly related to the ALK oncogene, and became an expert of ALK driven tumors. She is a member of the European Association for Cancer Research (EACR) and Società Italiana Ricerca Traslazionale e Professioni Sanitarie (SIRTEPS). She is also part of the research network ERIA (European Research Initiative on ALK-related malignancies).

LAB MEMBERS:

Dominik-Laurentius Candea *PhD student in Molecular Medicine, MSCA – DN*
Maria Vittoria Di Marco *PhD student in Molecular Medicine*
Giulia Mura *Postgraduate Fellow*

See the group resarch at page 170

RESEARCH ACTIVITY

By exploiting different in vitro (2D and 3D cell lines, and patient-derived organoids- PDO), and in vivo models (patient-derived xenografts -PDX and mouse models for ALCL and NSCLC), our research aims at defining relevant mechanisms of transformation in ALK-positive tumors to find new therapeutic vulnerabilities and to validate innovative treatments (FIGURE 3). Proper cytoskeletal regulation is fundamental in lymphoid cells for almost any aspect of T-cell biology, and small GTPases (Ras and Rho-family GTPases) are among the major players in this regulation. We are investigating the role of the RHO GTPases in lymphomagenesis, specifically in anaplastic large cell lymphomas (ALK+ and ALK- ALCL). Using in vitro and in vivo models we will study how RHOA impacts the biology of lymphoma cells. RHO GTPases are key biological regulators of both ALK+ and ALK- ALCL therefore they can represent novel targetable vulnerabilities that can be exploited in the treatment of ALCL alone or in combination with other existing treatments. In addition, our findings can potentially highlight therapeutic targets for other type of T cell lymphoma.

Targeted therapy has changed the clinical outcome of ALK+ patients. However, the development of resistance is inevitable. There is still limited understanding on how acquired resistance develops and undermines the effects of ALK TKIs. In addition, it is now clear that the heterogeneity of cancer cells is associated with resistance and that the presence of sub-population of drug-tolerant cells (called persister cells) can play a major role in resistance. We have recently demonstrated that CCL19/21-CCR7-PI3Kg axis drives resistance to ALK tyrosine kinase inhibitors (TKIs) and promotes survival of ALK+ ALCL persister cells in the perivascular niche during TKI treatment. We are then working on the hypothesis that the concomitant blockade of PI3Kg and/or the CCR7 receptor during ALK TKI treatment would contribute to reduce primary resistance as well as the survival of residual lymphoma cells. We will use

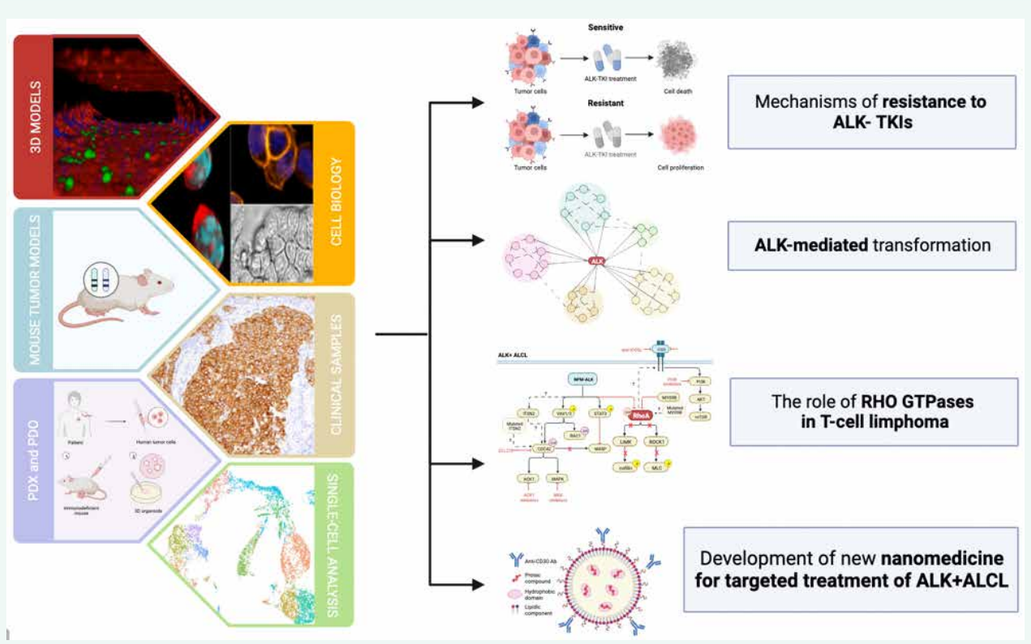


Figure 3.
Overview of research on ALK+ tumors

3D microvessel cell culture system that mimic the lymphoma perivascular niche and sub cutis or intravenous grafts of ALK+ ALCL cell lines and ALK+ ALCL patient derived xenografts (ALCL-PDX).

In ALK+ lung cancer, there is unmet medical need for ALK+ NSCLC patients with co-occurring TP53 mutations, which account for about 25% of ALK-positive NSCLC patients, as these patients suffer from unfavorable outcome treated either with ALK-inhibitors or conventional chemotherapy. Recently, the selective inhibitors of nuclear export (SINEs) represent a promising therapy in single or in combination with standard therapies. We previously demonstrated that treatment of ALK+ ALCL cells with SINE together with the ALK-inhibitor crizotinib exerts a pronounced synergistic activity on cell viability and survival of lymphoma cells. Based on these data, we are now investigating the efficacy of SINEs as single agents and in combination with ALK-inhibitors in ALK TKI-sensitive and resistant ALK+ lung cancer cell lines and in ALK TKI-resistant patient-derived organoids (PDOs).

In addition, we are also looking for novel mechanisms of resistance to ALK TKI treatment in ALK-driven tumors. To discover these mechanisms, we will use high throughput screening techniques such as whole genome sequencing (WES), RNA-sequencing, sc-RNA sequencing and proteomics and we will develop murine models along with more innovative 3D models to study the biology of these mechanisms.

FUTURE RESEARCH PLANS

We plan

- to identify and characterize new mechanisms of drug resistance in ALK-driven tumors;
- to characterize the ALK+ lymphoma niche that contributes to tumor persistence through sc-RNA sequencing and spatial transcriptomics in collaboration with the University of Milano-Bicocca and the emerging CyTOF technology;
- to define new druggable vulnerabilities in ALK+ lymphoma that can be exploited for other T-cell lymphoma;
- to develop new nanomedicines for targeted treatment of persistent lymphoma cells in ALK+ tumors in collaboration with the Politecnico of Torino.

FUNDS (5 PAST YEARS)

- 2023-2025: Fondazione CRT – “Inibizione dell’export nucleare dipendente da Exportina 1 (XPO-1) come nuova strategia terapeutica per i tumori del polmone”
- 2023-2025: 101072735 –HORIZON – MSCA – 2021 – DN – “FANTOM - Future of ALCL: Novel Therapies, Origins, Biomarkers and Mechanisms of Resistance”
- 2020-2024: Associazione Italiana Ricerca sul Cancro (AIRC) - “Characterization of cytoskeleton regulators as novel oncogenic mechanisms in anaplastic large cell lymphoma”

SELECTED PUBLICATIONS

- The tyrosine phosphatase Shp2 interacts with NPM-ALK and regulates anaplastic lymphoma cell growth and migration. Cancer Research 67: 4278, 2007. DOI: 10.1158/0008-5472.CAN-06-4350
- The Anaplastic Lymphoma Kinase in the pathogenesis of cancer. Nature Reviews Cancer 8: 11, 2008. DOI: 10.1038/nrc2291
- The enzymatic activity of 5-aminoimidazole-4-carboxamide ribonucleotide transformylase/inosine 5’-monophosphate cyclohydrolase (ATIC) is enhanced by NPM-ALK: new insights in ALK-mediated pathogenesis and the treatment of ALCL. Blood 113: 2776, 2009. DOI: 10.1182/blood-2008-06-161018
- The EGFR family members sustain the neoplastic phenotype of ALK+ lung adenocarcinoma via EGR1. Oncogenesis 2: e43, 2013. DOI: 10.1038/oncsis.2013.7
- Efficacy of an ALK cancer vaccine against ALK-rearranged lung tumors. Cancer Immunology Research 3: 1333, 2015. DOI: 10.1158/2326-6066.CIR-15-0089
- Excess of NPM-ALK oncogenic signaling promotes cellular apoptosis and drug dependency. Oncogene 35: 3854, 2016. DOI: 10.1038/onc.2015.456
- ALK oncogene regulates epithelial-mesenchymal transition (EMT) in ALK-rearranged Non-Small Cell Lung Carcinoma through repression of the epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2). Oncotarget 7: 33316, 2016. DOI: 10.18632/oncotarget.8955
- Wiskott-Aldrich Syndrome protein (WASP) is a tumor suppressor in T cell lymphoma. Nature Medicine 25:130, 2019. DOI: 10.1038/s41591-018-0262-9
- Regulation of ALK activity by CD45 phosphatase in anaplastic large cell lymphoma. Frontiers Oncology 12:1085672, 2023. DOI: 10.3389/fonc.2022.1085672
- CCR7-PI3Kg signaling supports resistance to tyrosine kinase inhibitors in ALK-rearranged lymphoma. Science Translational Medicine 15, 2023. DOI: 10.1126/scitranslmed.abo3826

MOLECULAR IMAGING AND NANOBIOTECHNOLOGIES

FERRAUTO AND DI GREGORIO GROUP MEMBERS:

Dr. Alessandro Amaolo *PhD Student in "Pharmaceutical and Biomolecular Sciences", University of Torino*

Dr. Chiara Pa pi *PhD Student in "Innovation in the diagnosis, prevention and therapy of infections at epidemic-pandemic risk", University of Siena*

Dr. Chiara Romiti *PhD "Student in Innovation in the diagnosis, prevention and therapy of infections at epidemic-pandemic risk", University of Siena*

Dr. Angelo Scarciglia *PhD Student in "Legal and Strategic Studies for innovation in defense and security", Centro Alti Studi per la Difesa (CASA), University of Roma*

RESEARCH ACTIVITY

Our research deals with the merge of molecular imaging and nanobiotechnologies, at the cross between biotechnological and chemical sciences. We focus on the 3 research activities:

- Design and development of innovative and "smart" nano systems for:
  - in vivo diagnosis (imaging) and targeted therapy.
  - in vitro assays for quantification of bioanalytes.
  - detection and decontamination from chemical and biological weapons of mass destruction (CB-NRe).
- In vivo imaging of oncological, neurological (multiple sclerosis) and infectious diseases in preclinical murine models by Magnetic Resonance Imaging (MRI), Optical Imaging (OI) and Photoacoustic Imaging (PAI):
  - analysis of tumor microenvironment (pH, vascularization, hypoxia…).
  - analysis of water exchange across cells' membranes.
  - design of innovative metal-based imaging contrast agents.
- Design of new strategies for reducing environmental impact of Gd-based MRI contrast agents. Synthesis and the characterization of nano-/micro-systems are under investigation for the decontamination from heavy metals and water contaminants. The three main research fields have strong interconnections. In fact, nanosystems are also used as imaging agents and in vivo imaging is used for monitoring nanoystems' distribution in vivo (Fig1).

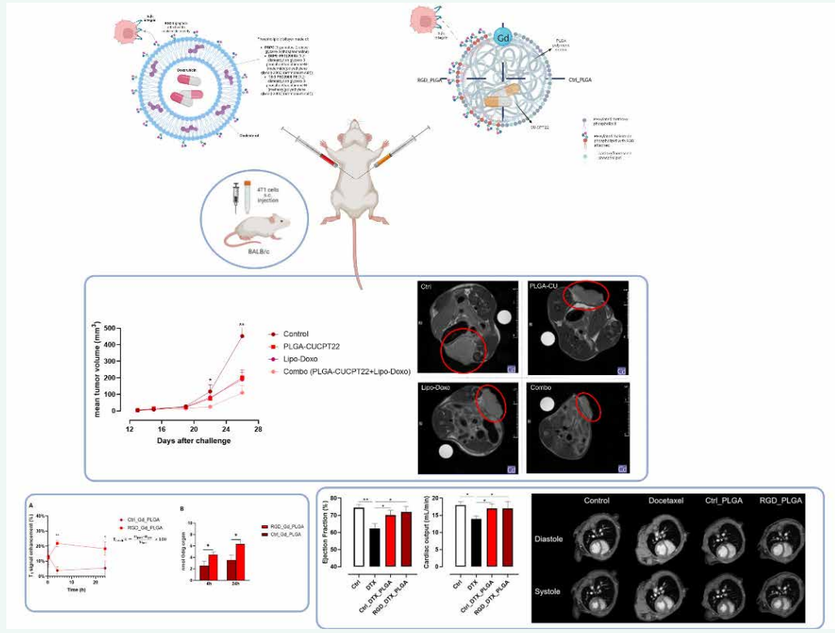


Figure 1. Combined targeted nanosystem therapy and application in triple negative breast cancer murine models reveals an improved in the therapeutic efficacy and reduced side

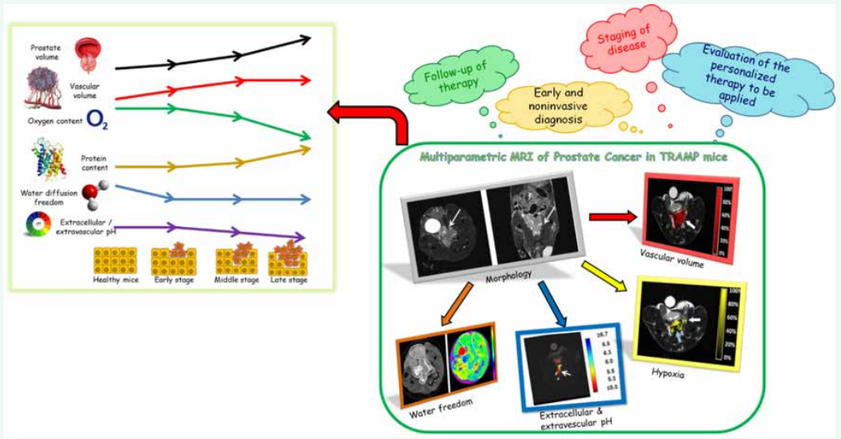


Figure 2.
Multiparametric MRI of Prostate cancer in TRAMP mice reveals specific phenotypes of the cancer.

A significant application of imaging relies on the possibility to have multiparametric MR imaging, i.e. the simultaneous quantification of different hallmarks of the disease. In particular, we focused on the assessment of i) tumor size, ii) protein content, iii) water freedom degree, iv) extracellular / extravascular pH, v) vascular volume and vi) hypoxia (Fig.2).

These last two parameters were assessed upon in vivo injection of ex vivo RBCs labelled with proper Gd-complexes. The Gd-labelled- RBCs can provide quantitative maps of vascular volume (Ferrauto G, et al. S. Biomaterials. 2015) and of hypoxia (Di Gregorio E, et al. ACS Nano. 2015). These Gd-RBCs appeared to be stable and biocompatible, suitable for in vivo applications.

As far as concern the measurement of the extracellular / extravascular pH, several attempts have been carried out by our group, obtaining especially results using YbH-PDO3A as pH responsive probe, translable for preclinical applications. With this MRI probe, we were able to measure pH of melanoma and glioblastoma in murine models (Delli Castelli D., Ferrauto G. et al. Magn Reson Med. 2014; Ferrauto G, et al. NMR Biomed. 2018).

Another topic of great interest is the quantitative analysis of water exchange across cells' membranes, e.g. across cancer cells. Recently, we deposited a patent and published a paper (Di Gregorio E. et al. Angewandte Chemie In. Ed.) reporting an innovative in vivo imaging approach to quantify water cycling across the membrane through transporters (Fig.3). It can be considered a hallmark of cellular metabolism, of high diagnostic relevance in the characterization of tumors and other diseases. The method relies on the response of intracellular proton exchanging molecules to the presence of extracellular Gd-based contrast agents (GBCAs). The effect is detected at the MRI-CEST (Magnetic Resonance Imaging - Chemical Exchange Saturation Transfer) signal of intracellular proton exchanging molecules. The method has been tested on RBC and on orthotopic murine models of breast cancer with different degree of malignancy (4T1, TS/A and 168FARN) and it allows obtaining high resolution and quantitative maps of membrane permeability. Water membrane permeability was correlated to the cells' aggressiveness. Moreover, it can act as an early reporter to monitor therapeutic treatments.

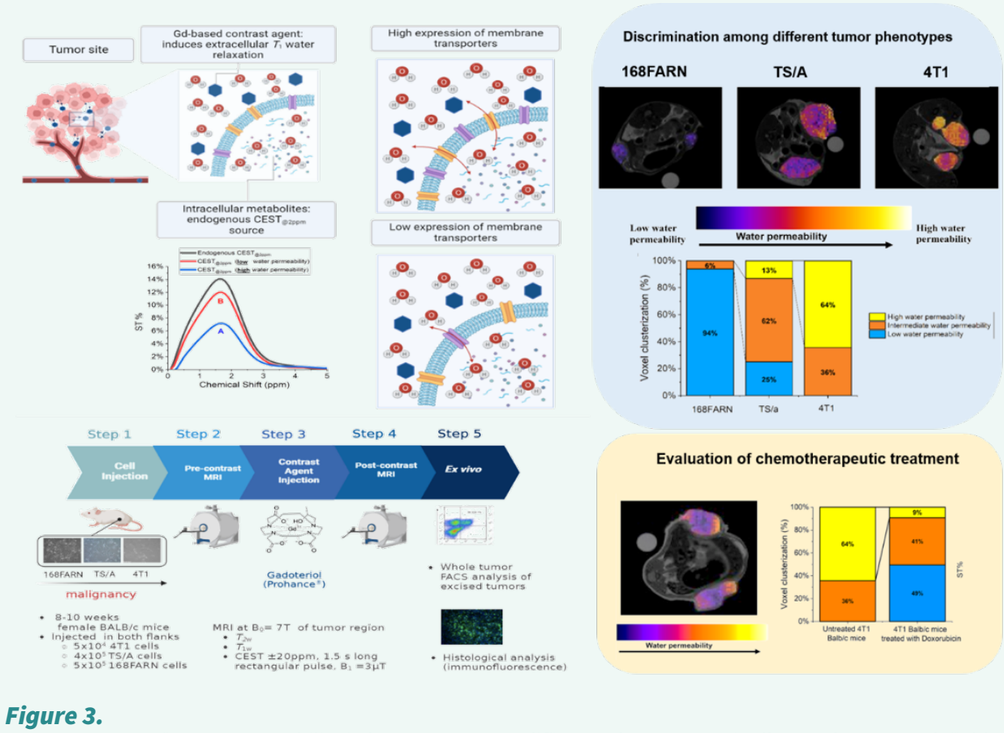


Figure 3.
CEST-MRI maps of water permeability in breast cancers at different phenotypes. Finally, another research field relies on "In vivo imaging of oncological, neurological (multiple sclerosis) and infectious diseases in preclinical murine models by Magnetic Resonance Imaging (MRI), Optical Imaging (OI) and Photoacoustic Imaging (PAI)". In vivo imaging techniques have revolutionized the ability to study and understand the complexities of oncological, neurological, and infectious diseases in preclinical murine models. These techniques provide insights into the tumor microenvironment, water exchange across cell membranes, and the development of innovative metal-based imaging contrast agents, ultimately advancing our knowledge and potential treatment strategies for these diseases.

FUTURE RESEARCH PLANS

As far as concerns the molecular imaging projects, we will continue focusing on triple negative breast cancer (TNBC) and pancreatic adenocarcinoma (PDAC) murine models, since they represent two of the most aggressive cancers for which the design of innovative early diagnosis and targeted therapy approaches is challenging. For these models we will look for new targeted systems for the early diagnosis and nanomedicine-based therapy and the study of water exchange rate across cell membranes.

In addition, our research will be also focused on the study of innovative MRI approaches for quantification of myelin in murine models of multiple sclerosis.Moreover, we will continue working on the design of nanosystems for detection and decontamination of CBRNe weapons of mass destruction. Finally, we will start application of molecular imaging and nanosystems to models of infectious diseases, with the aim of developing new tools for diagnosis and characterization of infectious diseases (both in vitro assays and in vivo imaging) and for targeted therapy.

FUNDING ID (PAST 5 YERS)

- PRIN PNRR 2022 - P2022R2YW3- entitled “myREPAIR: a new method to induce myelin repair in multiple sclerosis” – P.I. of the local unit: Ferrauto
- PoC Nodes- SPOKE 2 – Green Technologies e Industria Sostenibile MUR – M4C2 1.5 of PNRR with grant agreement no. ECS00000036 entitled” REDiRECt-Gd “ REDucing and RECovering the Gadolinium from Gd-based contrast agents for Magnetic resonance Imaging” – P.I.: Ferrauto
- Grant for Internationalization - GFI - Programmazione Triennale 21-23 - II tornata Macroarea 2 - Ambito 2 Ricerca traslazionale e applicata entitled “Strategies for recovery and recycling of Rare Earth Gadolinium from Gd-based contrast agents for Magnetic Resonance Imaging”. P.I.: Ferrauto
- Grant for Internationalization - GFI - Programmazione Triennale 21-23 - II tornata Macroarea 2 - Ambito 2 Ricerca traslazionale e applicata entitled “New methods for tumor phenotyping based on high and low field MRI”. P.I.: Di Gregorio
- PNRR M4C2-Investimento 1.4-CN00000041 finanziato dall’Unione Europea-NextGenerationEU. P.I.: Ferrauto

SELECTED PUBLICATIONS

Scopus IDs: Ferrauto, Giuseppe - Di Gregorio, Enza

- Di Gregorio E, Boccalon M, Furlan C, Gianolio E, Bényei A, Aime S, Baranyai Z, Ferrauto G. Inorganic Chemistry Frontiers. DOI:10.1039/D2QI00596D. https://www.scopus.com/record/display.uri?eid=2-s2.0-85131731219&origin=resultslist&sort=plf-f.
- Di Gregorio, E., Lattuada, L., Maiocchi, A., Aime, S., Ferrauto, G*., Gianolio, E. Chemical Science, 12 (4), pp. 1368-1377. DOI: 10.1039/d0sc03504a. https://www.scopus.com/record/display.uri?eid=2-s2.0-85100517986&origin=resultslist&sort=plf-f.
- Di Gregorio E, Ferrauto G, Gianolio E, Lanzardo S, Carrera C, Fedeli F, Aime S. ACS Nano. 2015 Aug 25;9(8):8239-48. DOI:10.1021/acsnano.5b02604. https://www.scopus.com/record/display.uri?eid=2-s2.0-84940121373&origin=resultslist&sort=plf-f.
- Ferrauto G, Di Gregorio E, Dastrù W, Lanzardo S, Aime S. Biomaterials. 2015. Jul;58:82-92. DOI:10.1016/j.biomaterials.2015.04.026. https://www.scopus.com/record/display.uri?eid=2-s2.0-84929259744&origin=resultslist&sort=plf-f.
- Di Gregorio E, Romiti C., Di Lorenzo A., Cavallo F., Ferrauto G*, Conti L. Cancers, 2023, 15(1), 8. DOI:10.3390/cancers15010008. https://www.scopus.com/record/display.uri?eid=2-s2.0-85146191487&origin=resultslist&sort=plf-f.
- Scarciglia A. Di Gregorio E., Aime S., Ferrauto G. Molecules 2022, 27, 2490. DOI: 10.3390/molecules27082490. https://www.scopus.com/record/display.uri?eid=2-s2.0-85128801748&origin=resultslist&sort=plf-f.
- Di Gregorio E, Arena F, Gainolio E, Ferrauto G*, Aime S. Magn. Res. Med, 88(1), pp. 357–364,2022. DOI: 10.1002/mrm.29190. https://www.scopus.com/record/display.uri?eid=2-s2.0-85126037039&origin=resultslist&sort=plf-f.
- Ferrauto G*, Tripepi M., Di Gregorio E., Bitonto V., Aime S., Delli Castelli D. Invest. Radiol. 2021 May 1;56(5):301-312. DOI:10.1097/RLI.0000000000000742. https://www.scopus.com/record/display.uri?eid=2-s2.0-85103992774&origin=resultslist&sort=plf-f.
- Ferrauto G*, Di Gregorio E, Lanzardo S, Ciolli L, Iezzi M, Aime S. Sci Rep. 2018 Jul 12;8(1):10567. DOI: 10.1038/s41598-018-28926-5. https://www.scopus.com/record/display.uri?eid=2-s2.0-85049902910&origin=resultslist&sort=plf-f.
- Di Gregorio E., Ferrauto G, Lanzardo S, Gianolio E, Aime S. Use of FCC-NMRD relaxometry for early detection and characterization of ex-vivo murine breast cancer. Sci Rep. 2019 Mar 15;9(1):4624. DOI:10.1038/s41598-019-41154-9. https://www.scopus.com/record/display.uri?eid=2-s2.0-85062978130&origin=resultslist&sort=plf-f

GIUSEPPE FERRAUTO

MOLECULAR IMAGING AND NANOBIOTECHNOLOGIES GROUP

BIOGRAPHICAL SKETCH

2019	Assistant Professor of General and Inorganic Chemistry at the Dept. of Molecular Biotechnologies and Health Sciences of the University of Torino; Associate Professor since 2022
2019-2021	Master Degree in Forensic Chemistry and Doping Control, University of Torino
2021	Master in Eu projects design and coordination, Europa Business School
2018-2019	Research technician at University of Torino Department of Molecular Biotechnologies and Health Sciences- University of Torino (IT)
2018-2014	Post doctoral fellow at the Dept. of Molecular Biotechnologies and Health Sciences and Molecular Imaging Center (www.cim.unito.it) of the University of Torino
2011-2013	PhD in Pharmaceutical and Biomolecular Science at the Department of Molecular Biotechnologies & Health Sciences, University of Torino
2008-2010	European Master in Molecular Imaging (EMMI)- European training on molecular imaging techniques. Paris Sud University- University of Torino (IT)
2008-2010	Master's degree in Molecular Biotechnologies, University of Torino

See the group resarch at page 180

ENZA DI GREGORIO

MOLECULAR IMAGING AND NANOBIOTECHNOLOGIES GROUP

BIOGRAPHICAL SKETCH

2022 -today	Assistant Professor of "General and Inorganic Chemistry", SSD Chim/03 at the Dep. of Molecular Biotechnologies and Health Science of the University of Torino
2014-2022	Post doctoral fellow at the Dept. of Molecular Biotechnologies and Health Sciences and Molecular Imaging Center (www.cim.unito.it) of the University of Torino
2012 to date	Member of the European Society for Molecular Imaging (ESMI), the European Society of Magnetic Resonance in Medicine and Biology (ESMRMB), the Italian Group of Discussion on Magnetic Resonance (GIDRM), the Italian Chemical Society (SCI) and the International Photoacoustic Standardization Consortium (IPSC)
2011-2013	PhD in Pharmaceutical and Biomolecular Science at the Department of Molecular Biotechnologies & Health Sciences, University of Torino
2008-2010	European Master in Molecular Imaging (EMMI)- European training on molecular imaging techniques. Paris Sud University- University of Torino (IT)
2008-2010	Master's degree in Molecular Biotechnologies, University of Torino

See the group resarch at page 180

ALESSANDRO MOROTTI

Cancer and thrombosis laboratory

BIOGRAPHICAL SKETCH

I received my MD degree at the University of Piemonte Orientale, my residency and PhD at the University of Turin, and master in thrombosis and hemostasis at the University of Firenze.

clinical resarch

lab research

GROUP MEMBERS:

Giovanna Carrà *PhD postdoctoral fellow*
Maria Vittoria Manno *student*
Arianna Nebbia Colomba *student*
Sofia Camerlo *MD*
Giorgio Rosati *MD*

RESEARCH EXPERIENCES

I started my first research experiences in 1998 with the supervision of Prof. Carola Ponzetto; next I Joined the Hematological Division directed by Prof. Giuseppe Saglio at the AUO San Luigi - Orbassano, where I mostly studied signaling pathways in myeloproliferative neoplasms. After my residency, I worked as a research fellow in Prof. Pier Paolo Pandolfi laboratory at the Memorial Sloan Kettering Cancer center in New York and then at the BIDMC of Boston. Clinical experiences: I develop my clinical expertise at that the Division of Hematology of San Luigi Hospital, under the supervision of Prof. Giuseppe Saglio. I am currently working as associate Professor at the same division, where I am mostly focusing on cancer associated thrombosis and hematological malignancies.

Background

Cancer Associated Thrombosis (CAT) is the second most frequent cause of death in patients with cancer, second only to disease progression. Conversely, cancer is the most frequent cause of death in patients with venous thromboembolism. Cancer and thrombosis are indeed intimately connected. Beside individual risk factors, CAT incidence varies based on tumor type, with cancers of the pancreas, lung, stomach and lymphomas at the highest risk of thrombosis. Various mechanisms have been associated with increased risk of thrombosis, including the expression of pro-coagulant factors from the tumor and the recruitment of inflammatory cells by the tumor itself. However, a more general and targetable mechanisms is still missing. Cancer is also the most frequent cause of death in patients with venous thromboembolism, therefore it is tempting assuming that thrombosis could favor cancer progression as well. Overall, deciphering the crosstalk between coagulation pathways and cancer is of essential clinical importance, with potentially relevant implications in thrombosis prevention and inhibition of cancer progression.

Research aims

our lab aims to address the link between aberrantly activated signal transduction pathways and the hemostatic process in cancers of different origin. We intend to determine:

- → how cancer affects microenvironment including endothelial cells and immune system recruitment.
- → how cancer, in particular lung cancer and myeloproliferative neoplasms, triggers clot system and platelets, therefore leading to thrombosis.

Previous discoveries

We start our investigations to assess the contribution of various pathways in the development of both myeloproliferative neoplasms and lung cancers. We were interested in both types of cancers due to the observation that similar pathways were able to develop both myeloproliferative neoplasia and lung cancer (as we published in Nat Genet. 2010 Mar;42(3):216-23.). We spent most of our energies in the assessment of the role of NFkB/IkBalpha in such cancer models. Very recently, we demonstrated that this pair is playing an essential role in the modulation of cancer cell metabolism, with potentially relevant implications in the modification of the tumoral microenvironment, involving endothelial cell activation and immune system recruitment. Parallel to these investigations, we were also interested in the determination of how aberrant pathways can lead to thrombosis. In particular, we recently demonstrated that various gene mutations (DNMT3A and TET2 point mutations) are associated with increased thrombosis manifestations in a particular group of patients. We are therefore trying to link aberrant pathways to thrombosis development.

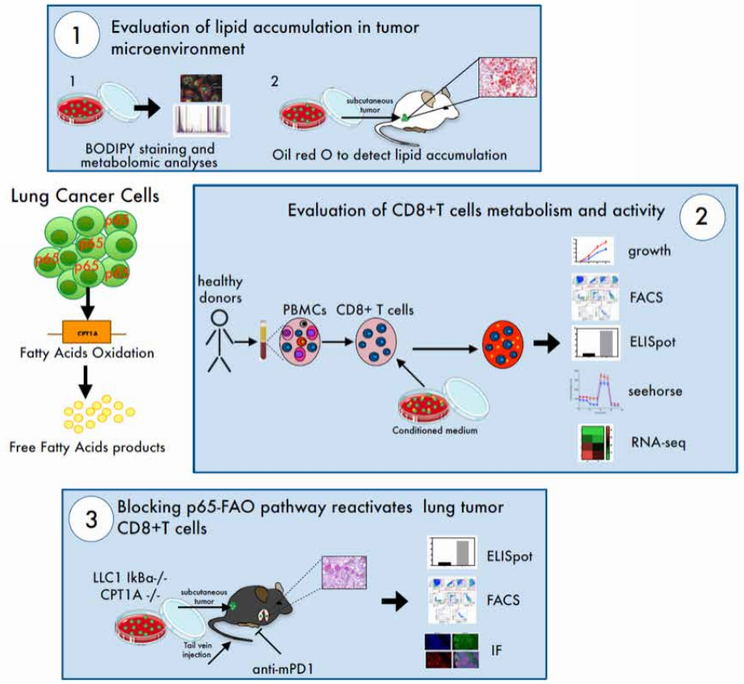


Figure 1.

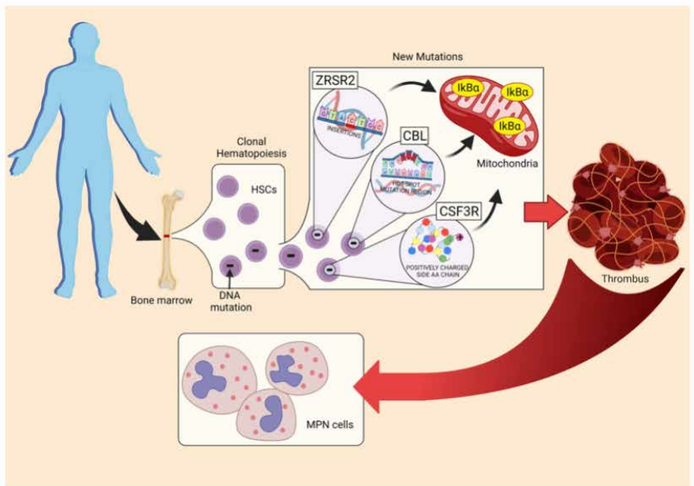


Figure 2.

FUTURE PLAN

Current research is focusing to assess whether aberrant activation of NF-KB/IkB-alpha is able to affect tumor microenvironment, favoring the activation of the endothelial cells of tumoral vessels. Activated endothelial cells are indeed able to promote both neo-angiogenesis and favoring cancer associated thrombosis. Such investigations are designed to identify mechanisms of CAT development and to define new therapeutic strategies to prevent thrombosis. Finally, the contribution of thrombosis, as a potential source of growth factor derived from the clots, in tumorigenesis are also investigated.

FUNDING ID

- Ricerca finalizzata Ministero della salute to Alessandro Morotti: “Definition of the role of IkB-alpha in the pathogenesis and progression of Chronic Myeloid Leukemia”. 304.109 euros
- Ricerca finalizzata Ministero della salute to Giovanna Carrà: “GR-20212374957 denominato “NF-kB positively regulates Fatty Acid Oxidation in cancer cells, which hinders CD8+ T lymphocytes and promotes tumor progression

SELECTED PUBLICATIONS

Scopus ID Alessandro Morotti: 6507221286

- Carrà G, Giugliano E, Camerlo S, Rosati G, Branca E, Maffeo B, Russo I, Piazza R, Cilloni D, Morotti A. Clonal hematopoiesis by DNMT3A mutations as a common finding in idiopathic splanchnic vein thrombosis. Haematologica. 2023 May 1;108(5):1447-1449
- Carrà G, Ermondi G, Riganti C, Righi L, Caron G, Menga A, Capelletto E, Maffeo B, Lingua MF, Fusella F, Volante M, Taulli R, Guerrasio A, Novello S, Brancaccio M, Piazza R, Morotti A. “IκBα targeting promotes oxidative stress-dependent cell death”. J Exp Clin Cancer Res. 2021 Apr 16;40(1):136.
- Barale C, Senkeev R, Napoli F, De Gobbi M, Guerrasio A, Morotti A, Russo I. “Transferrin saturation inversely correlates with platelet function”. Thromb Haemost. 2019 May;119(5):766-778
- Di Savino A, Panuzzo C, Rocca S, Familiari U, Piazza R, Crivellaro S, Carrà G, Ferretti R, Fusella F, Giugliano E, Camporeale A, Franco I, Miniscalco B, Cutrin JC, Turco E, Silengo L, Hirsch E, Rege-Cambrin G, Gambacorti-Passerini C, Pandolfi PP, Papotti M, Saglio G, Tarone G, Morotti A, Brancaccio M. (CO-LAST AUTHOR) “Morgana acts as an oncosuppressor in chronic myeloid leukemia”. Blood. 2015 Apr2;125(14):2245-53.
- Morotti A, Panuzzo C, Crivellaro S, Pergolizzi B, Familiari U, Berger AH, Saglio G, Pandolfi PP. “BCR-ABL disrupts PTEN nuclear cytoplasmic shuttling throught phosphorylation dependent activation of HAUSP”. Leukemia. 2014 Jun;28(6):1326-33.
- Berger AH, Niki M, Morotti A, Taylor BS, Socci ND, Viale A, Brennan C, Szoke J, Motoi N, Rothman PB, Teruya-Feldstein J, Gerald WL, Ladanyi M, and Pier Paolo Pandolfi. “Identification of DOK family genes as lung tumor suppressors”. Nature Genetics. 2010 Mar;42(3):216-23.
- Ito K, Bernardi R, Morotti A, Matsuoka S, Saglio G, Ikeda Y, Rosenblatt J, Avigan DE, Teruya-Feldstein J and Pandolfi PP. “PML targeting eradicates quiescent leukaemia-initiating cells”. Nature. 2008 Jun 19;453(7198):1072-8.
- Morotti A, Parvis G, Cilloni D, Familiari U, Pautasso M, Bosa M, Messa F, Arruga F, Defilippi, Catalano R, Rosso V, Carturan S, Bracco E, Guerrasio A and Saglio G. “CD7/CD56 positive Acute Myeloid Leukemias are characterized by constitutive phosphorylation of the NF-kB subunit p65 at Ser536”. Leukemia. 2007 Jun;21(6):1305-6.
- Morotti A, Cilloni D, Messa F, Arruga F, Defilippi I, Carturan S, Catalano R, Rosso V, Chiarenza A, Pilatrino C, Guerrasio A, Taulli R, Bracco E, Pautasso M, Baraban D, Gottardi E and Saglio G. “Valproate enhances Imatinib-induced growth arrest and apoptosis in Chronic Myeloid Leukemia Cells”. Cancer.2006;106:1188-1196.
- Morotti A, Mila S, Ponzetto C: “K252a inhibits the oncogenic properties of Met, the HGF receptor”. Oncogene, 2002 (21): 4885-4893.

FRANCESCO NERI

Epigenetics of aging and cancer

BIOGRAPHICAL SKETCH

2020-to date	Associate Professor, University of Torino
2021-to date	Visiting Scientist at the Leibniz Institute on Aging – FLI, Jena (DE)
2020-2022	Member of the Board of the German Stem Cell Network (GSCN)
2017-2020	GSCN 2017 Young Investigator Award
2016-2021	Group leader at the FLI, Jena (DE)
2016	Sofja Kovalevskaja Award
2015-2016	EMBO STF, Radboud University Medical Centre, Nijmegen (Netherlands
2014	Nicolo Copernico Award
2011-2015	Post-doc Fellow, Epigenetics unit, Human Genetics Foundation, Torino
2007-2011	PhD in Biotechnology, University of Siena, Italy

GROUP MEMBERS:

Carla Carrera *Postdoctoral fellow*
Nadia Ducano *postdoctoral fellow*
Anna Krepelova *TA*
Ilenia Caracciolo *predoctoral fellowship*
Alberto Minetti *PhD student*

RESEARCH ACTIVITY

Transcriptional and epigenetic alterations during aging and cancer

Aging is associated with defective organ maintenance and increased tissue dysfunction as well as with a higher risk for the development of pathological conditions, including cancer. Colorectal cancer is one of the most frequent and lethal neoplasms and its incidence exponentially increases with age. The focus of the Neri lab is the functional characterization of transcriptome and epigenome alterations that occur during adult stem cell aging in the intestinal system. The main aim is to characterize transcriptional and epigenetic alterations of stem cells during aging (focusing on DNA methylation changes together with principal histone modifications). The group employs genome-wide and single-cell technology to dissect alterations of the transcriptional and epigenetic landscape of the stem cells of the mouse small intestine and colon. Functional experiments are carried out by utilizing in vitro systems (intestinal organoids) and in vivo mouse models. In addition, the group has developed novel tools to identify dormant stem cells in intestine in vivo, to characterize in vitro organoid systems and to analyze DNA methylation in rare cells.

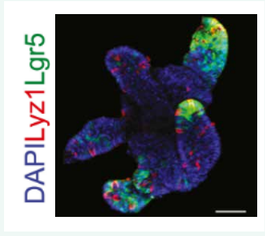


Figure 1.
Intestinal organoids represent a novel tool for in vitro studies of biological processes and drug screening since all the cell types of a specific organ are present in this 3D culture system. The figure shows an organoid of small intestine isolated from an old mouse. DAPI stains the cell nuclei, Lyz1 is a marker of the epithelial Paneth cells, Lgr5 is a marker of the intestinal stem cells.

Epigenetic drift and epigenetic clocks

Several studies have demonstrated that intestinal stem cells represent the cells-of-origin of intestinal cancer and that clonal dominance of mutant stem cells appears frequently during aging. Emerging evidence indicates that genetic and epigenetic factors impact on the functionality and homeostasis of adult stem cells during aging, thereby favoring the selective advantage of dominant clones and the onset of cancer. Among these factors, DNA methylation (a stable and heritable epigenetic modification) has been associated with aging-induced diseases and cancer development. Recent discovery that DNA methylation can be actively removed by the TET proteins (ten-eleven-translocation) has revealed the importance of this epigenetic modification in several biological models. Epigenetic drift refers to the gradual and stochastic changes that occur in the epigenome over time. Epigenetic drift can occur naturally as a consequence of aging, as well as in response to environmental exposures and lifestyle choices. These changes can accumulate over the lifespan of an individual, leading to alterations in gene expression patterns, driving clonal selection and potentially contributing to age-related diseases and conditions.

Our group identified an intestine-specific DNA methylation drift that is associated with colon cancer. We are currently studying the origin of this epigenetic drift and its consequences in cancer development.

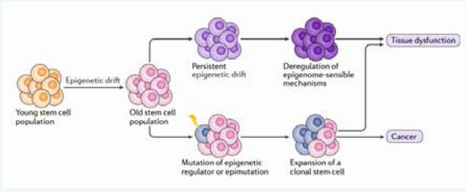


Figure 2.
During lifetime, adult stem cells accumulate epigenetic alterations that lead to the appearance and expansion of mutant stem cell clones. These cells lead to tissue dysfunction and cancer. Figure adapted from Ermolaeva, Neri, et al., Nature Reviews MCB, 2018.

Deciphering and counteracting Inflammaging.

Inflammaging refers to a state of chronic low-grade inflammation that occurs with aging. We recently found that inflammaging is driven by upregulation of innate immune receptors and systemic interferon gamma (IFN gamma) signaling. Importantly, we found that inflammaging can be ameliorated by dietary restriction (DR) interventions in a tissue-specific manner. Moreover, DR ameliorates aging-induced alterations of chromatin accessibility and RNA transcription of the inflammaging gene network while failing to rescue those alterations on the rest of the genome. Our results present a comprehensive understanding of the molecular network regulating inflammation in aging and DR and provide anti-inflammaging therapeutic targets.

In addition, we have further demonstrated that the intestinal epithelium shows this pro-inflammatory phenotype during aging and that it is lost following long in-vitro culturing suggesting that is driven by external factors (e.g. the gut microbiome) and that can be reverted (opening therapeutics opportunity). Moreover, we found that treating mice with an antibody anti-IFNg can revert the intestinal inflammaging phenotype by rescuing the number of Lgr5+ intestinal stem cells (ISCs) and of Muc2+ Goblet cells (epithelial cells of the intestine responsible for the anti-microbial response) as well as the number of pro-inflammatory Cytotoxic Ccl5+ T-cells resident in the intestinal lamina propria to a young-like state. Importantly, pre-treatment with anti-IFNg antibody is able to improve the gut regeneration after treatment with the chemotherapeutic Fluorouracil (5FU) that induces intestinal damage as demonstrated by analysis of the mice body weight and histochemistry. Overall, our data indicate that inhibition of IFNg can have beneficial effects in elderly with intestinal pathologies and/or that undergo chemotherapy. Indeed, for some cancers (e.g. colon cancer), the chemotherapeutic drug cocktail has very severe side effects and, very often, drugs are combined according to the patient's health status.

Figure 3.
The multi-tissue gene network regulating inflammaging. This network is characterized by chromatin opening and upregulation in the transcription of innate immune system receptors and by activation of interferon signaling through interferon regulatory factors, inflammatory cytokines, and Stat1-mediated transcription.

FUTURE RESEARCH PLANS

Our group aims to understand the aging-associated alterations in the somatic stem cells that lead to tissue dysfunctions and cancer, especially in the intestinal system. The final goal is to identify molecular mechanisms that can be targets of preventive or curative therapeutic approaches. We plan to define and functionally characterize intestinal epigenetic drifts and their contribution to aging-associated diseases including colon cancer. We want to assess their relevance to the development of intestine-specific epigenetic clocks.

In addition, we started to test non-invasive approaches like treatment with natural compounds, nutrient supplements, pre- and pro-biotics, specific dietary regimens in the amelioration of the aging-associated intestinal dysfunctions and cancer, gut dysbiosis, and neuroinflammation.

FUNDING ID

- 2016-21 Sofja Kovalevskaja Starting grant from von Humboldt Foundation (1.6M€)
- 2017-19 Fritz Thyssen Foundation grant (180K€)
- 2018-21 RTG1715 from DFG (1 PhD student position for 3 years)
- 2018-21 SAW-DRFZ grant from Senate Competition Committee of the Leibniz (20K€)
- 2019-22 DFG grant NE 2144/5-1 (258K€) and DFG grant NE 2144/6 (9.5K€)
- 2021-26 AIRC-MFAG (497K€)
- 2023-25 Fondazione Molinette (200k€)

SELECTED PUBLICATIONS

- Omrani, O., Krepelova, A., ... and Neri, F. (2023). IFNgamma-Stat1 axis drives aging-associated loss of intestinal tissue homeostasis and regeneration. Nat Commun, 14, 6109. doi.org/10.1038/s41467-023-41683-y
- Lu, J., ... and Neri, F. (2022). Establishment and evaluation of module-based immune-associated gene signature to predict overall survival in patients of colon adenocarcinoma. J Biomed Sci 29, 81. 10.1186/s12929-022-00867-2.
- Rasa, S.M.M., Annunziata, F., ... and Neri, F. (2022). Inflammaging is driven by upregulation of innate immune receptors and systemic interferon signaling and is ameliorated by dietary restriction. Cell Reports 39, 111017. 10.1016/j.celrep.2022.111017.
- Annunziata, F., ... and Neri, F. (2022). Paneth Cells drive Intestinal Stem Cell Competition and Clonality in Aging and Calorie Restriction. Eur J Cell Biol, 151282. 10.1016/j.ejcb.2022.151282.
- Freter, R., ... and Neri, F. (2021). Establishment of a fluorescent reporter of RNA-polymerase II activity to identify dormant cells. Nat Commun 12, 3318–16. 10.1038/s41467-021-23580-4.
- Lu, J., ... and Neri, F. (2021). Characterization of an in vitro 3D intestinal organoid model by using massive RNAseq-based transcriptome profiling. Scientific Reports -uk 11, 16668. 10.1038/s41598-021-96321-8.
- Ermolaeva, M., Neri, F., Ori, A., and Rudolph, K.L. (2018). Cellular and epigenetic drivers of stem cell ageing. Nature reviews. Molecular cell biology 19, 594–610. 10.1038/s41580-018-0020-3.
- Schwoerer, S., ... Neri, F., et al. (2016). Epigenetic stress responses induce muscle stem cell aging by Hoxa9 developmental signals. Nature 540, 428–432. 10.1038/nature20603.
- Neri, F., et al. (2017). Intragenic DNA methylation prevents spurious transcription initiation. Nature 543, 72–77. 10.1038/nature21373.
- 1Neri, F., et al. (2013). Dnmt3L antagonizes DNA methylation at bivalent promoters and favors DNA methylation at gene bodies in ESCs. Cell 155, 121–134. 10.1016/j.cell.2013.08.056.

SALVATORE OLIVIERO

Epigenetics and embryonic stem cell development

BIOGRAPHICAL SKETCH

From 2000	Full Professor in Molecular Biology, from 2013 at University of Torino – Italy
2007–2010	Associate Professor, Dept Molecular Biotechnology, Univ. of Torino
2003–2013	Director of the Doctorate School in Biotechnology, Santa Chiara, University of Siena – Italy
2007-2010	Scientific secretary of the Italian Federation of Life Sciences, FISV
2005-2007	Visiting Professor Albert Einstein College of Medicine, Bronx - USA
1993-2000	Associated professor in Molecular Biology, University of Siena – Italy
1988–1992	Post-doctoral fellow Dept Biochemistry, Harvard University, Boston – USA
1988	PhD Human Genetics. University of Torino, Italy (1983 – 1985) and EMBL Heidelberg, Germany (1985 – 1988)
2011-2016	Member of the Scientific Council of the Human Frontier of Science Program (HSFP). From 2014 Chair of the Scientific Council
From 2018	EMBO member
From 2023	President of Italian Society of Biophysics and Molecular Biology (SIBBM)

GROUP MEMBERS:

Carla Liaci *Dottoranda*
Andrea Lauria, Livia Caizzi *Researchers*
Isabelle Polignano, Hassan Dastsooz, Fang Yang, Annalaura Tamburrini *Postdoctoral fellow*
Mirko Giuseppe Scrivano, Claudia Vaccari, Chiara Cicconetti, Jacopo Pinto *PhD students*
Francesca Anselmi, Daniela Donna *Technicians*
Alessandro Salamone, Marco Gaspari *Fellows*

RESEARCH ACTIVITY

Our research group combines molecular, cellular, and global genomic approaches to investigate the network of regulations that take place in response to environmental or developmental signals and that are deregulated in developmental disorders or in cancer. We study the regulation and function of epigenetic modifications to understand their role in the control of cell identity, the regulation of the correct transitions during the process of embryonic development, and they alteration in tumorigenesis. DNAme is introduced by the de novo DNMT3A and DNMT3B DNA methyltransferases together with DNMT3L, while DNMT1 together with UHRF1 are mainly involved in the propagation of the DNAme on the genome during DNA replication. The exit from pluripotency is characterized by the transition from the pre- to the post-implantation epiblast of the embryo. To analyse the gene expression patterns of the EBs at the resolution of individual cells, we collected samples at 3 and 9 days during EBs differentiation from WT and mutant cells and profiled their gene expression by single-cell RNA sequencing (scRNAseq) (Figure1). During this transition genome-wide DNAme is established by the de novo DNMT3A and DNMT3B DNA methyltransferases which are strongly upregulated to establish the DNAme essential for cell fate specification during development and transcription integrity. By whole genome DNAme coupled with gene expression and cell phenotype analysis we demonstrated a specific role of DNMT3B, but not DNMT3A, in the meso-endoderm (ME) lineage specification and identified DNMT3B genomic targets that prime EpiLCs toward ME.

Thus, DNMT3B-dependent methylation at the epiblast stage is essential for the priming of the meso-endodermal lineages and provides functional characterization of the de novo DNMTs during EpiLCs lineage determination. To differentiate into meso-endoderm, EpiLCs should re-

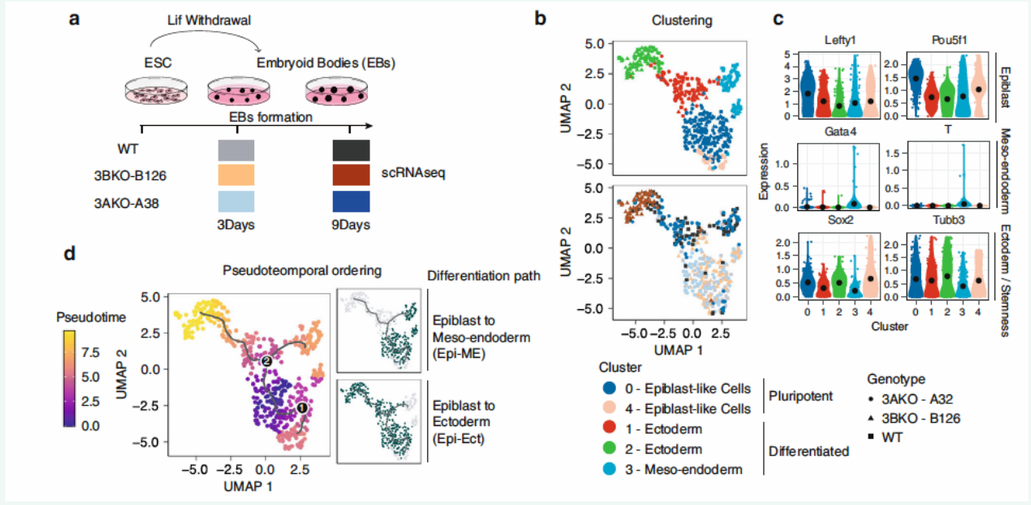


Figure 1.
scRNA-seq profiling of Dnmt3a−/− and Dnmt3b−/− differentiating EBs. a Overview of the experimental design and visualisation of collected time points. b UMAP embedding of 487 WT, 3AKO and 3BKO single cell transcriptomes. Cells are coloured by cluster (top panel) and genotype/time of cells' collection (bottom panel). 3AKO = lightblue/darkblue, 3BKO= light orange (3Days)/ dark orange (9Days), WT= light grey (3Days)/dark grey (9Days). c Gene expression levels distribution of representative epiblast (Lefty1, Pou5f1), meso-endoderm (Gata4, T) and ectoderm markers (Sox2, Tubb3) in the five identified cell clusters.

press the chromatin of a number of ectoderm enhancers that at this stage are open and demethylated and should be decommissioned in the cells primed to be able to differentiate into meso-endoderm. DNMT3B-dependent DNAme establishes the meso-endodermal epigenetic landscape by repressing the expression of key TFs that would otherwise induce the default differentiation into neuroectoderm.

Recently, it has become clear that nuclear long non-coding RNAs (lncRNAs) might be involved in gene transcription by interacting with nuclear factors and epigenetic modifiers. Whole-genome screens have shown that a large fraction of lncRNAs play a role in development.

We recently found that histone acetyltransferase p300/CBP, a general transcriptional coactivator that introduces the H3K27ac modification on enhancers of active genes, interacts with over a hundred of lncRNAs. We found that lncSmad7, a lncRNA specifically expressed in embryonic stem cells, is required to maintain maintains ESC self-renewal (Figure 2). lncSmad7 also contains predicted RNA-DNA Hoogsteen forming base pairing. By Chromatin Isolation by RNA precipitation followed by sequencing (ChIRPseq) together with CRISPR/Cas9 mutagenesis of the target sites we demonstrated that lncSmad7 binds and recruits p300 to enhancers to trigger enhancer acetylation and transcriptional activation of its target genes. Thus, these results unveil a new mechanism by which p300 is recruited to the genome.

FUTURE RESEARCH PLANS

Projects in progress in the laboratory address the study of epigenetic modifications that determine embryonic stem cells fate in particular to neural development of human embryonic stem cell (hESCs) differentiation into 3D brain organoids. In particular we identified a number of lncRNAs and nuclear factors that are specifically expressed along the neural differentiation pathways or expressed in terminally differentiated neurons. By the use of CRISPR/Cas technology we will dissect their role in neural differentiation. We will analyse the network of gene regulation s and epigenetic landscape along neural differentiation.

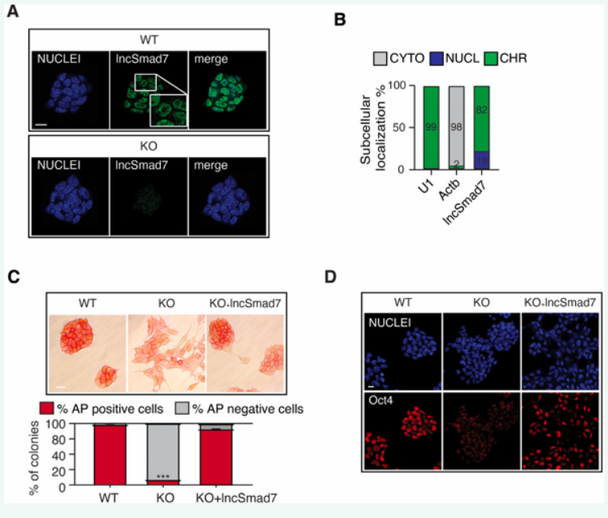


Figure 2.
The lncRNA lincSmad7 in required for embryonic stem cell self-renewal. (A) RNA fluorescent in situ hybridization (RNA-FISH) of lncSmad7 in ESCs. (B) Expression analysis of lncSmad7 in subcellular fractionation of ESCs by RT-qPCR. (C) Alkaline phosphatase (AP) staining of WT, lncSmad7 KO cells and rescued ESC colonies. (D) Immunostaining for the pluripotency marker Oct4 in WT, lncSmad7 KO ESCs and rescued ESCs.

FUNDING ID (PAST 5 YERS)

- Telethon GGP19201A (2018-2023)
- PRIN 2022: 2022RA8E3T; 2020: 2020HEFHY2; 2017: 2017P352Z4
- AIRC: IG2017 ID 20240; IG 2022 ID 27155

SELECTED PUBLICATIONS

- Neri F, Rapelli S, Krepelova A, Incarnato D, Parlato C, Basile G, Maldotti M, Anselmi F, Oliviero S. (2017) Intragenic DNA methylation prevents spurious transcription initiation. Nature 543: 72-77. DOI: 10.1038/nature21373 codice SCOPUS: 2-s2.0-85014546354
- Incarnato D, Morandi E, Simon LM, Oliviero S. (2018) RNA Framework: an all-in-one toolkit for the analysis of RNA structures and post-transcriptional modifications. Nucleic Acids Res. 46: e97. DOI: 10.1093/nar/gky486 codice SCOPUS: 2-s2.0-85066849476
- Simon LM, Morandi E, Luganini A, Gribaudo G, Martinez-Sobrido L, Turner DH, Oliviero S*, Incarnato D*. (2019) In vivo analysis of influenza A mRNA secondary structures identifies critical regulatory motifs. Nucleic Acids Res. 47: 7003-7017.DOI: 10.1093/nar/gkz318 codice SCOPUS: 2-s2.0-85070182306
- Lauria A, Peirone S, Giudice MD, Priante F, Rajan P, Caselle M, Oliviero S, Cereda M.U (2020) Identification of altered biological processes in heterogeneous RNA-sequencing data by discretization of expression profiles. Nucleic Acids Res. 48:1730-1747. DOI: 10.1093/nar/gkz1208 codice SCOPUS: 2-s2.0-85081097473
- Di Timoteo G, Dattilo D, Centrón-Broco A, Colantoni A, Guarnacci M, Rossi F, Incarnato D, Oliviero S, Fatica A, Morlando M, Bozzoni I. (2020) Modulation of circRNA Metabolism by m 6 A Modification. Cell Report 31:107641. DOI: 10.1016/j.celrep.2020.107641 codice SCOPUS: 2-s2.0-85084373798
- Zorzan I, Pellegrini M, Arboit M, Incarnato D, Maldotti M, Forcato M, Tagliazucchi GM, Carbognin E, Montagner M, Oliviero S*, G. Martello* (2020) The transcriptional regulator ZNF398 mediates pluripotency and epithelial character downstream of TGF-beta in human PSCs. Nature Commun. 11: 2364. DOI: 10.1038/s41467-020-16205-9 codice SCOPUS: 2-s2.0-85084455816
- Betto RM, Diamante L, Perrera V, Audano M, Rapelli S, Lauria A, Incarnato D, Arboit M, Pedretti S, Rigoni G, Guerineau V, Touboul D, Stirparo GG, Lohoff T, Boroviak T, Grumati P, Soriano ME, Nichols J, Mitro N, Oliviero S*, G. Martello* (2021) Metabolic control of DNA methylation in naive pluripotent cells. Nature Genetics 53 :215-229.DOI: 10.1038/s41588-020-00770-2 codice SCOPUS: 2-s2.0-85100264695
- Morandi E, Manfredonia I, Simon LM, Anselmi F, van Hemert MJ, Oliviero S*, Incarnato D*. (2021) Genome-scale deconvolution of RNA structure ensembles. Nature Methods, 18: 249-252 DOI: 10.1038/s41592-021-01075-w codice SCOPUS: 2-s2.0-85101203392
- Maldotti M, Lauria A, Anselmi F, Molineris I, Tamburrini A, Meng G, Polignano IL, Scrivano MG, Campestre F, Simon LM, Rapelli S, Morandi E, Incarnato D, Oliviero S. (2022) The acetyltransferase p300 is recruited in trans to multiple enhancer sites by lncSmad7. Nucleic Acids Res. 50: 2587-2602. DOI: 10.1093/nar/gkac083 codice SCOPUS: 2-s2.0-85127541928
- Lauria A, Meng G, Proserpio V, Rapelli S, Maldotti M, Polignano IL, Anselmi F, Incarnato D, Krepelova A, Donna D, Levra Levron C, Donati G, Molineris I, Neri F, Oliviero S. (2023) DNMT3B supports meso-endoderm differentiation from mouse embryonic stem cells. Nat Commun. 14(1):367. DOI: 10.1038/s41467-023-35938-x codice SCOPUS: 2-s2.0-85146771241

ONCOIMMUNOLOGY LAB

CAVALLO, CONTI AND QUAGLINO GROUP MEMBERS:

Federica Cavallo, Laura Conti, Elena Quaglino *Principal Investigators*
Irene Fiore Merighi *Technician*
Federica Riccardo *Research Fellow*
Giuseppina Barutello, Elisabetta Bolli, Roberto Ruiu, Lidia Tarone *Senior Post-doctoral Fellows*
Zubyeah Arshad, Chiara Cossu, Antonino Di Lorenzo, Antonella Iacoviello, Giulia Peppino *Ph.D. Students*

RESEARCH ACTIVITY

The OncoImmunology Lab, led by Prof. Federica Cavallo, Prof. Laura Conti and Prof. Elena Quaglino, has dedicated the last decade to developing and testing immunotherapy for cancer in translational pre-clinical settings. Their focus includes the study of Human epidermal growth factor receptor 2 (HER2) as a primary target antigen in a subset of breast cancers. Using pre-clinical models, such as transgenic mice carrying an activated form of HER2, or mice challenged with HER2+ cancer cells, the Lab has demonstrated the efficacy of anti-HER2 DNA vaccines (patents US8389494B2 and US8207141B2) and virus-based vaccines in preventing and controlling mammary tumor progression.

The current research lines of the OncoImmunology Lab focus on targeting antigen expressed by cancer-stem-cells (CSC). Indeed, being CSC refractory to most current anti-cancer therapies, they are responsible for the development of recurrences and metastasis. The Lab has identified several promising CSC antigens (Quaglino, E, Cavallo, F, Conti, L. Cancer stem cell antigens as targets for new combined anti-cancer therapies. Int J Biochem Cell Biol. 2020; Doi: 10.1016/j.biocel.2020.105861) employing a high-throughput screening based on Next-Generation Sequencing (Quaglino, E, Conti, L, Cavallo, F. Breast cancer stem cell antigens as targets for immunotherapy. Semin Immunol. 2020. Doi: 10.1016/j.smim.2020.101386) and is exploring various vaccination platforms, including oncolytic viruses, virus-like particles, and primarily DNA-based vaccines, to target these CSC antigens.

Other research areas include understanding the role of immunosurveillance in controlling mammary tumor initiation, growth, and response to vaccination, applying maternal immunization to prevent cancer, and using combined therapies to revert the immunosuppressive tumor microenvironment.

The collaborative efforts of the three Principal Investigators have led to significant milestones in tumor immunology and immunotherapy. Each Investigator leads a research program examining the functional role of one or more identified CSC antigens in cancer and the anti-tumor efficacy of their targeting in vivo.

The OncoImmunology Lab's achievements have also been made possible thanks to international, national, and "in-house" – at the Molecular Biotechnology Center "Guido Tarone" - collaborations, and partnerships with companies, such as Galena Biopharma Inc (San Ramon, US), ImmunoGenesis (Houston, US), Indena srl (Milan, Italy), OSIVAX (Lyon, France), Vaxxas Inc (Cambridge, US) and YGION Biomedical GmbH (Vienna, Austria).

Overall, the OncoImmunology Lab aims to understand the role of tumor-associated antigens in cancer initiation, growth, metastasis, and resistance to conventional therapies. Its goal is to develop innovative immunotherapies that activate the patients' immune system to safely eradicate cancer, preventing recurrence and metastases.

FEDERICA CAVALLO

ONCOIMMUNOLOGY LAB - Scopus Author ID: 34568031100; ORCID 0000-0003-4571-1060

BIOGRAPHICAL SKETCH

2016 to present	Full Professor of Immunology (General Pathology), University of Turin, Italy
2006-2016	Associate Professor of Immunology (General Pathology), University of Turin, Italy
2001-2006	Assistant Professor of Immunology (General Pathology), University of Turin, Italy
1998-2000	Post-doctoral Fellow, Centre of Immunogenetics and Experimental Oncology, National Research Council (CNR), Turin, Italy
1994-1998	Post-doctoral Fellow, Dept of Clinical and Biological Sciences, University of Turin, Italy
1990-1994	Ph. D in Tumor Immunology, University of Turin, Italy
1989	Master's degree in Biological Sciences, University of Turin, Italy

See the group resarch at page 180

Roberto Ruiu *Senior Post-doctoral Fellow*
Lidia Tarone *Post-doctoral Fellow*
Antonella Iacoviello *Ph.D. Students*
Irene Fiore Merighi *Technician*

See the group resarch at page 200

RESEARCH ACTIVITY

My team's current research primarily focuses on the targeting of two antigens: the cystine/glutamate antiporter xCT and the other is the Chondroitin Sulfate Proteoglycan (CSPG)4.

Cystine/glutamate antiporter xCT is vital for shielding cancer cells from oxidative stress. It is expressed by various tumor types, but it is also expressed by immune cells, influencing proliferation and effector function. To gain insight into the role of xCT, we generated xCTnull BALB/c mice for investigating its impact on the immune system. Additionally, HER2-transgenic mice were made xCTnull to study xCT in a mammary cancer-prone model. Mammary cancer cells derived from BALB-neuT/xCTnull mice and xCTKO cells were used to assess xCT's contribution to malignant properties in vitro and in vivo. Our findings reveal that xCT disruption doesn't prevent tumor initiation and growth but sensitizes cancer cells to oxidative stress, reducing cancer-cell metastasization to the lungs. This is accompanied by altered immune-cell recruitment in the pre-metastatic niche. This is accompanied by altered immune-cell recruitment in the pre-metastatic niche. Systemic xCT depletion in host mice doesn't impact tumor growth, metastasis, or hinder proper humoral and cellular immune responses in vivo.

We've developed various vaccination platforms (DNA-based, VLP-based, and Oncolytic-virus-based) to target xCT. These platforms effectively induce an immune response against xCT, mitigating the malignant features of mammary cancer cells expressing it. Overall, these results indicate that xCT isn't essential for proper immune system function, supporting the safety of xCT targeting in oncology. Nevertheless, it plays a crucial role in processes vital to the metastatic seeding of mammary cancer cells, broadening the scope of xCT-targeting approaches (Figure 1).

Another promising target for immunotherapeutic interventions that we are studying is CSPG4. It has a limited expression in normal tissues and substantial overexpression in various tumor histotypes, where it regulates crucial cancer-related processes. Our research delves into DNA-based vaccination strategies targeting CSPG4 for treating challenging CSPG4-expressing tumors. While extensively characterized in malignant melanoma, our group has recently unveiled CSPG4 up-regulation and

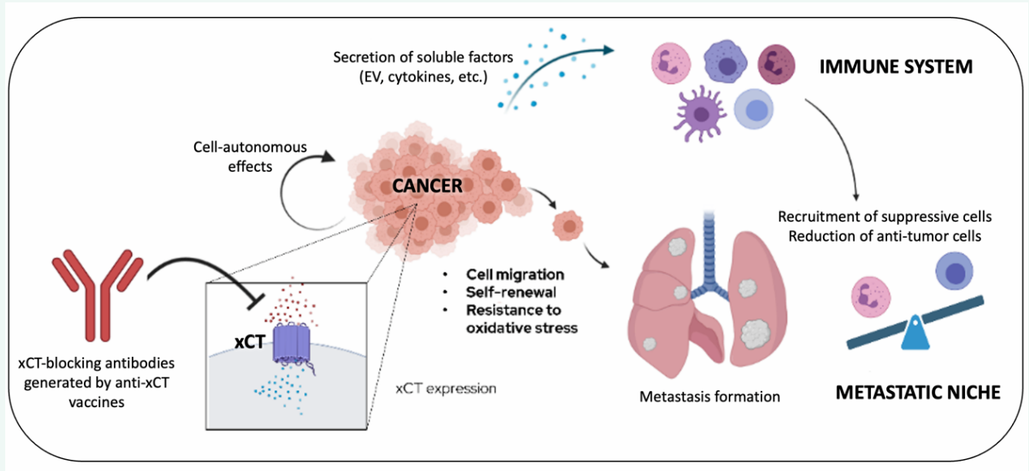


Figure 1.
The cystine/glutamate antiporter xCT plays a crucial role in promoting breast cancer metastasis through both cell-autonomous and non-cell-autonomous mechanisms.

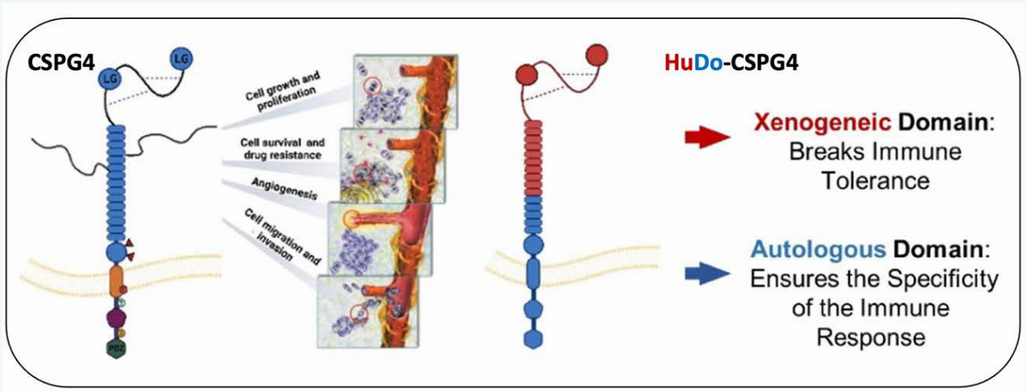


Figure 2.
Structure of the chondroitin sulfate proteoglycan (CSPG)4 protein and scheme representing our human/dog chimeric CSPG4 (HuDo-CSPG4) vaccine.

pivotal oncogenic role in osteosarcoma. However, being a self-antigen, CSPG4 is tolerated by the patient's immune system, posing a significant obstacle to effective vaccine development. To overcome this challenge, our team has innovatively explored the concept of a chimeric CSPG4 vaccine, blending human and canine CSPG4 sequences. This approach aims to disrupt immune tolerance, triggering a robust anti-CSPG4 immune response capable of eliminating tumor cells and preventing recurrence and metastasis (Figure 2).

In recent years, my research group has been actively involved in evaluating the safety, immunogenicity, and clinical potential of this chimeric anti-CSPG4 vaccine. We conducted studies using pre-clinical mouse models of melanoma and osteosarcoma. Additionally, we explored its efficacy in client-owned dogs affected by spontaneous CSPG4+ melanoma and osteosarcoma, in collaboration with the Veterinary Teaching Hospital of the University of Turin. Interestingly, this canine model is considered highly predictive of the response to immunotherapy in humans.

FUTURE RESEARCH PLANS

We aim to advance the development of innovative combinatorial therapies targeting breast cancer, melanoma, and osteosarcoma to enhance patient outcomes. Our focus will be on investigating how xCT regulates ferroptosis, extracellular vesicle (EV) release, immunosuppression, and the formation of pre-metastatic niches. This knowledge will guide the development of combinatorial approaches involving xCT and immune checkpoint blockade (ICB), along with the establishment of an EV signature to predict ICB response in breast cancer. Concurrently, our research will delve into the role of CSPG4 in conventional therapy resistance. We plan to test anti-CSPG4 vaccination in conjunction with BRAF/MEK inhibitors and ICB for melanoma treatment. Additionally, we will explore its combination with chemotherapy and 'non-conventional' ICB for the management of osteosarcoma.

FUNDING ID (PAST 5 YERS)

- 2023-2025: PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022, MUR.
- 2023-2024: Proof of Concept (PoC) – TOINPROVE/2023, University of Turin.
- 2022-2023: Research agreement, YGION Biomedical, Vienna, Austria.
- 2022-2023: Research agreement, ImmunoGenesis, Huston, Texas, US.
- 2022: Liberal contribution, Banca d'Italia.
- 2021-2022: PoC Instrument grant, University of Turin and Compagnia di San Paolo.
- 2019-2023: Investigator Grant, Fondazione AIRC per la Ricerca (AIRC IG 2018; ID 21468).
- 2019-2020: Research agreement, Indena S.p.A., Milano, Italy.

SELECTED PUBLICATIONS

- Ruiu R, Cossu C, Iacoviello A, Conti L, Bolli E, Ponzone L, Magri J, Rumandla A, Calautti E, Cavallo F. Cystine/glutamate antiporter xCT deficiency reduces metastasis without impairing immune system function in breast cancer mouse models. J Exp Clin Cancer Res. 2023. doi: 10.1186/s13046-023-02830-x.
- Tarone L, Giacobino D, Camerino M, Maniscalco L, Iussich S, Parisi L, Giovannini G, Dentini A, Bolli E, Quaglino E, Merighi IF, Morello E, Buracco P, Riccardo F, Cavallo F. A chimeric human/dog-DNA vaccine against CSPG4 induces immunity with therapeutic potential in comparative preclinical models of osteosarcoma. Mol Ther. 2023. Doi: 10.1016/j.ymthe.2023.06.004. Epub ahead of print.
- Riccardo F, Tarone L, Camerino M, Giacobino D, Iussich S, Barutello G, Arigoni M, Conti L, Bolli E, Quaglino E, Merighi IF, Morello E, Dentini A, Ferrone S, Buracco P, Cavallo F. Antigen mimicry as an effective strategy to induce CSPG4-targeted immunity in dogs with oral melanoma: a veterinary trial. J Immunother Cancer. 2022. Doi: 10.1136/jitc-2021-004007.
- Rolih V, Caldeira J, Bolli E, Salameh A, Conti L, Barutello G, Riccardo F, Magri J, Lamolinara A, Parra K, Valenzuela P, Francia G, Iezzi M, Pericle F, Cavallo F. Development of a VLP-Based Vaccine Displaying an xCT Extracellular Domain for the Treatment of Metastatic Breast Cancer. Cancers (Basel). 2020. Doi: 10.3390/cancers12061492.
- Riccardo F, Tarone L, Iussich S, Giacobino D, Arigoni M, Sammartano F, Morello E, Martano M, Gattino F, Maria R, Ferrone S, Buracco P, Cavallo F. Identification of CSPG4 as a promising target for translational combinatorial approaches in osteosarcoma. Ther Adv Med Oncol. 2019. Doi: 10.1177/1758835919855491.
- Donofrio G, Tebaldi G, Lanzardo S, Ruiu R, Bolli E, Ballatore A, Rolih V, Macchi F, Conti L, Cavallo F. Bovine herpesvirus 4-based vector delivering the full length xCT DNA efficiently protects mice from mammary cancer metastases by targeting cancer stem cells. Oncoimmunology. 2018. Doi: 10.1080/2162402X.2018.1494108.
- Bolli E, O'Rourke JP, Conti L, Lanzardo S, Rolih V, Christen JM, Barutello G, Forni M, Pericle F, Cavallo F. A Virus-Like-Particle immunotherapy targeting Epitope-Specific anti-xCT expressed on cancer stem cell inhibits the progression of metastatic cancer in vivo. Oncoimmunology. 2017. Doi: 10.1080/2162402X.2017.1408746.
- Tallerico R, Conti L, Lanzardo S, Sottile R, Garofalo C, Wagner AK, Johansson MH, Cristiani CM, Kärre K, Carbone E, Cavallo F. NK cells control breast cancer and related cancer stem cell hematological spread. Oncoimmunology. 2017. Doi: 10.1080/2162402X.2017.1284718.
- Lanzardo S, Conti L, Rooke R, Ruiu R, Accart N, Bolli E, Arigoni M, Macagno M, Barrera G, Pizzimenti S, Aurisicchio L, Calogero RA, Cavallo F. Immunotargeting of Antigen xCT Attenuates Stem-like Cell Behavior and Metastatic Progression in Breast Cancer. Cancer Res. 2016. Doi: 10.1158/0008-5472.CAN-15-1208.
- Riccardo F, Iussich S, Maniscalco L, Lorda Mayayo S, La Rosa G, Arigoni M, De Maria R, Gattino F, Lanzardo S, Lardone E, Martano M, Morello E, Prestigio S, Fiore A, Quaglino E, Zabarino S, Ferrone S, Buracco P, Cavallo F. CSPG4-specific immunity and survival prolongation in dogs with oral malignant melanoma immunized with human CSPG4 DNA. Clin Cancer Res. 2014. Doi: 10.1158/1078-0432.CCR-13-3042.

LAURA CONTI

ONCOIMMUNOLOGY LAB - Scopus Author ID: 16041417500; ORCID 0000-0003-1780-098X

BIOGRAPHICAL SKETCH

2023 to present	Associate Professor of Immunology (General Pathology), University of Turin, Italy.
2019-2022	Assistant Professor of Immunology (General Pathology), University of Turin, Italy.
November 2018	Visiting Scientist at AgilVax Inc., Houston, Texas, US
2018-2019	Post-doctoral Fellow, University of Turin, Italy.
2014-2017	Post-doctoral Fellow, Fondazione Umberto Veronesi, University of Turin, Italy
June 2013	Visiting Scientist at Transgene SA, Illkirch Graffenstaden Cedex, France
2009-2014	Post-doctoral Fellow, University of Turin, Italy
2007-2009	Post-doctoral Fellow, Bracco Imaging SpA, Colleretto Giacosa, Turin, Italy
2003-2007	Ph.D. in Immunology and Cell Biology, University of Turin, Italy
1998-2003	Master's degree in Medical Biotechnology, University of Turin, Italy

GROUP MEMBERS:

Elisabetta Bolli *Senior Postdoctoral Fellow*
Antonino Di Lorenzo and Chiara Cossu *Ph.D. Students*
Irene Fiore Merighi *Technician*

See the group resarch at page 200

RESEARCH ACTIVITY

Breast cancer (BC) is the leading cause of cancer death in women. Despite early diagnosis and advancements in therapy, more than 30% of patients relapse, and 80% of BC deaths occur in patients who progressed to metastatic BC because of the development of primary or secondary resistance to therapy. Resistance to chemotherapy and immunotherapy can be attributed to several mechanisms, many of which rely on the complex crosstalk between cancer cells, immune cells, the tumor microenvironment and the microbiota. Cancer cells shape the tumor microenvironment in a pro-tumoral manner through several mechanisms, including by acquiring the expression and activation of molecules typically expressed by the innate immune system, such as Pattern-Recognition Receptors (PRRs). Physiologically, PRRs expressed by immune cells sense infections as well as tissue damage by binding to Pathogen-Associated Molecular Patterns (PAMPs) and Damage-Associated Molecular Patterns (DAMPs), respectively, and activate an inflammatory response. The expression of PRRs by tumor cells may promote tumor progression and resistance to therapies. By exploiting the pipeline developed in the OncoImmunology Lab to identify breast cancer-stem-cell (CSC) oncoantigens, we have discovered that BC cells express the PRR Toll-like Receptor (TLR)2 and demonstrated that TLR2 is activated by DAMPs released by cancer cells, either actively or following chemotherapy-induced cell death. By generating TLR2KO and TLR2WT ErbB2-transgenic mice, which spontaneously develop mammary cancer, and using several other BC models, we showed that TLR2 activation promotes CSC self-renewal through the activation of the MyD88/NF-kB pathway, fostering BC cell survival, metastasis and drug resistance. Moreover, TLR2 activation on immune cells induces T-regulatory-cell expansion and promotes immunosuppression. High expression of TLR2 is associated with poor prognoses and inferior responses to chemotherapy in BC patients, and we have demonstrated that the deletion or pharmacologic inhibition of TLR2 (using monoclonal antibodies or the CU-CPT-22 inhibitor) sensitizes BC cells to chemotherapy (Figure 1). Since TLR2 also detects PAMPs, we are currently studying whether TLR2 functions as a bridge between BC cells and the local microbiota, and thus mediates the detrimental effects that dysbiosis may exert on tumor response to chemotherapy.

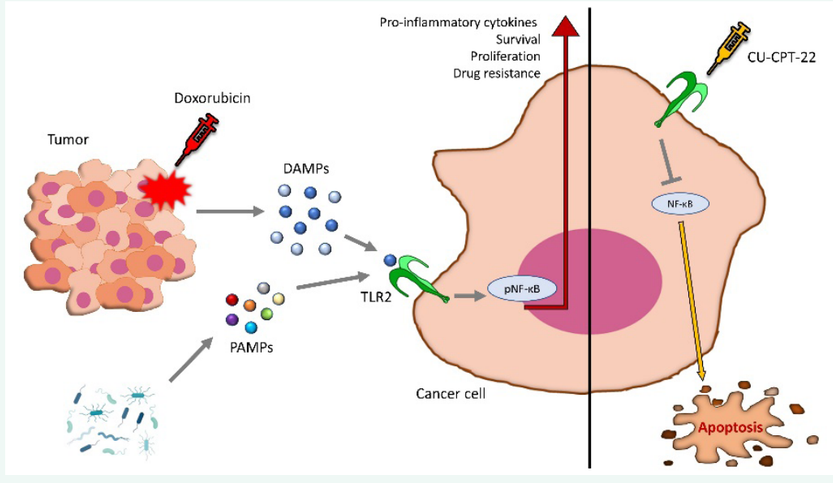


Figure 1.
TLR2 inhibition reinstates chemosensitivity in breast

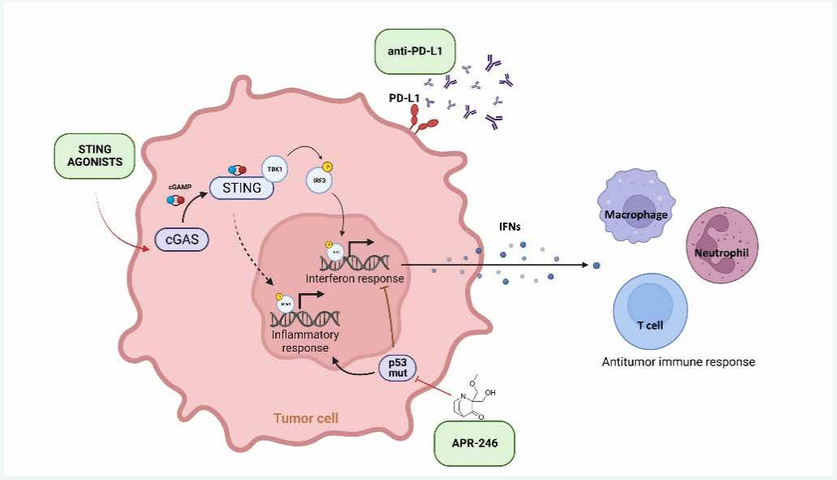


Figure 2.
Combinatorial treatment with STING agonists, ICB and mutant-p53 targeted drugs (APR-246) to target BC cells.

A second line of research focuses on another PRR expressed by cancer cells; the stimulator of interferon genes (STING). Normally, the cyclic GMP-AMP synthase (cGAS)-STING pathway is activated by cytosolic double stranded DNA, released after tissue damage or viral infection, leading to the activation of the type I interferon (IFN) response, which recruits T lymphocytes. This may be a promising strategy to foster T cell tumor infiltration and turn cold tumors hot, possibly rendering BC responsive to immune checkpoint blockade (ICB) therapy. However, high levels of chromosomal instability and DNA damage lead to the altered activation of the cGAS-STING pathway in many BC, with the induction of a NF-kB-dependent inflammatory progression and resistance to therapy. Altered STING signaling in BC is associated with high rates of TP53 mutation, which blocks the canonical cGAS-STING pathway, promoting the production of pro-tumoral cytokines via the activation of NF-kB (Figure 2). We are currently studying the regulation of the cGAS-STING pathway in BC and developing combined therapies that can restore the activation of the STING-dependent IFN response, using STING agonists combined with re-activators of mutant P53 (such as APR-246) or other drugs, in combination with ICB.

FUTURE RESEARCH PLANS

We aim to further characterize the role of the TLR2 and STING pathways in the crosstalk between BC cells and the tumor microenvironment, including the tumor microbiota. In particular, to dissect the tumor-cell-intrinsic and immune-cell-mediated effects of these pathways. Moreover, we are characterizing the interaction between TLR2 and other CSC oncoantigens, such as the cystine-glutamate antiporter xCT, and developing theranostic nanoparticles to target tumor cells and for the intratumoral delivery of chemotherapy and TLR2 inhibitors. These nanosystems will be tested in combination with immunotherapies, including xCT-targeting vaccines developed by the OncoImmunology Lab. In parallel, we will evaluate new strategies for the reactivation of the canonical STING pathway (Figure 3).

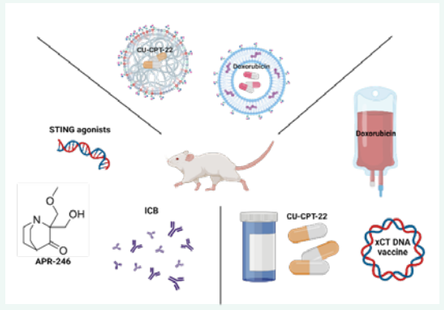


Figure 3.
Development of new combinatorial therapies for BC.

Moreover, we are endeavoring to understand whether alterations in the tumor microbiota are responsible for TLR2-mediated chemoresistance and tumor progression by identifying the bacterial species involved in pro-tumoral and anti-tumoral effects during treatment, and to identify possible correlations between the composition of the mammary microbiota, tumor stemness and the clinical response to chemotherapy. This information will be helpful for patient stratification, while the design of innovative approaches for microbiota manipulation would ameliorate the prognosis of patients who do not currently respond to chemotherapy.

FUNDING ID (PAST 5 YERS)

- 2022-2027: Investigator Grant, Fondazione AIRC per la Ricerca (AIRC IG 2021; ID 25766).
- 2022-2024: Fondazione CRT, RF 2021.1774.
- 2016-2019: Ministero della Salute, bando progetti di ricerca giovani ricercatori, rf-2013-02354892.

SELECTED PUBLICATIONS

- Park SC*, Conti L*, Franceschi V, Oh BK, Yang MS, Ham G, Di Lorenzo A, Bolli E, Cavallo F, Kim B, Donofrio G. Assessment of BoHV-4-based vector vaccine intranasally administered in a hamster challenge model of lung disease. Frontiers in Immunology. 2023. *Equal contribution. Doi: 10.3389/fimmu.2023.1197649.
- Di Gregorio E, Romiti C, Di Lorenzo A, Cavallo F, Ferrauto G, Conti L. RGD_PLGA Nanoparticles with Docetaxel: A Route for Improving Drug Efficiency and Reducing Toxicity in Breast Cancer Treatment. Cancers (Basel). 2022;15(1):8. Doi: 10.3390/cancers15010008.
- Di Lorenzo A, Bolli E, Ruiu R, Ferrauto G, Di Gregorio E, Avalle L, Savino A, Poggio P, Merighi IF, Riccardo F, Brancaccio M, Quaglino E, Cavallo F, Conti L. Toll-like receptor 2 promotes breast cancer progression and resistance to chemotherapy. Oncoimmunology. 2022;2086752. Doi: 10.1080/2162402X.2022.2086752.
- Ruiu R, Di Lorenzo A, Cavallo F, Conti L. Are Cancer Stem Cells a suitable target for breast cancer immunotherapy? Frontiers in Oncology. 2022; Vol. 12. Doi: 10.3389/fonc.2022.877384.
- Di Lorenzo A, Bolli E, Tarone L, Cavallo F, Conti L. Toll-Like Receptor 2 at the Crossroad between Cancer Cells, the Immune System, and the Microbiota. Int. J. Mol. Sci. 2020; 21(24), 9418. Doi: 10.3390/ijms21249418.
- Salemme V, Centonze G, Cavallo F, Defilippi P, Conti L. The crosstalk between tumor cells and the immune microenvironment in breast cancer: implications for immunotherapy. Frontiers in Oncology. 2021; 11:610303. Doi: 10.3389/fonc.2021.610303.
- Conti L, Franceschi V, Macchi F, Riccardo F, Ruiu R, Russo L; Quaglino E, Donofrio G, Cavallo F. Immunotargeting of the xCT cystine/glutamate antiporter potentiates the efficacy of Her2-targeted immunotherapies in breast cancer. Cancer Immunol Res. 2020; 8(8), 1039-1053. Doi: 10.1158/2326-6066.CIR-20-0082.
- Ruiu R, Rolih V, Bolli E, Barutello G, Riccardo F, Quaglino E, Merighi IF, Pericle F, Donofrio G, Cavallo F, Conti L. Fighting breast cancer stem cells through the immune-targeting of the xCT cystine-glutamate antiporter. Cancer Immunol Immunother. 2019; 68(1):131-141. Doi: 10.1158/2326-6066.CIR-20-0082.
- Donofrio G, Tebaldi G, Lanzardo S, Ruiu R, Bolli E, Ballatore A, Rolih V, Macchi F, Conti L*, Cavallo F*. Bovine herpesvirus 4-based vector delivering the full length xCT DNA efficiently protects mice from mammary cancer metastases by targeting cancer stem cells. Oncoimmunology. 2018; 7(12):e1494108. *Equal contribution. Doi: 10.1080/2162402X.2018.1494108.
- Conti L, Lanzardo S, Arigoni M, Antonazzo R, Radaelli E, Cantarella D, Calogero RA, Cavallo F. The non-inflammatory role of High mobility group box 1/Toll-like Receptor 2 axis in the self-renewal of mammary cancer stem cells. FASEB Journal. 2013; 27:4731-44. Doi: 10.1096/fj.13-230201.

ELENA QUAGLINO

ONCOIMMUNOLOGY LAB - Scopus Author ID: 6603501602; ORCID 0000-0002-8151-9124

BIOGRAPHICAL SKETCH

2015 to present	ssociate Professor of Immunology (General Pathology), University of Turin, Italy.
2011-2015	Assistant Professor of Immunology (General Pathology), University of Turin, Italy.
June 2009	Visiting Scientist at Karmanos Cancer Institute, Wayne State University, Detroit, USA.
2009-2011	Post-doctoral Fellow, “Indena Spa”, Milano, Italy.
2003-2008	Post-doctoral Fellow, University of Turin, Italy.
1999-2002	Ph.D. in Immunology, University of Turin, Italy.
1997	Degree in Biological Sciences, University of Turin, Italy.

GROUP MEMBERS:

Giuseppina Barutello *Senior Post-doctoral Fellow*
Zubyeah Arshad and Giulia Peppino *Ph.D. Students*
Irene Fiore Merighi *Technician*

See the group resarch at page 200

RESEARCH ACTIVITY

Teneurin-4 (TENM4) is one of the oncoantigens that has been identified, via the pipeline setup by the Onco-Immunology Lab, as being overexpressed by triple negative breast cancer (TNBC) stem cells. TENM4 is a glycosylated type-II transmembrane protein belonging to the Teneurins family, which includes four highly conserved members that are involved in cell-cell and cell-extracellular matrix interactions and play a pivotal role in neural and cellular differentiation during embryonic development. TENM4 possesses a short N-terminal intracellular (IC), a transmembrane and a large extracellular (EC) domain. There is evidence to indicate that TENM4 can form both homo- and heterodimers on the same cell and between different, adjacent cells. Data suggests that the TENM4 EC portion can be cleaved from the plasma membrane and released. The IC domain has a predicted cleavage site with a canonical nuclear localization sequence, indicating that this protein plays a role both as a receptor and a transcriptional regulator (Figure 1). However, few data on TENM4 protein-protein interactions are available.

While the involvement of TENM4 in embryonic and neuronal development has been extensively documented, its function in cancer biology is understudied and an object of controversy. Indeed, only two papers on the role of TENM4 in solid tumors exist at this time: Graumann and colleagues demonstrated that the down-regulation of TENM4 in ovarian cancer cells induces an increased proliferation rate and decreased sensitivity to cisplatin; on the other hand, my research group has recently demonstrated that TENM4 has a protumoral role in TNBC. An in-silico analysis of publicly available data sets showed a significant decrease in both relapse-free and overall survival in TNBC patients bearing high, rather than low, TENM4-expressing tumors. Using RNA interference-based silencing and CRISPR/Cas9 technology, we have generated TENM4-deficient and TENM4 knock out (TENM4 KO) murine TNBC cells, showing a significant reduction in tumorsphere-forming ability, clonogenicity, migration and invasion, compared to their TENM4 wild type (TENM4 WT) counterpart.

No significant differences in the tumor growth rate of TENM4-deficient were observed, compared to TENM4 WT cells. However, TENM4 was demonstrated, for the first time, to have a significant role in in-vivo TNBC-derived lung metastatization. Thanks to the collaboration with Prof. Castellano (Department of Medical Science, Pathology Unit, University of Turin), we found that patients bearing TENM4-high expressing TNBC do recur and/or metastasize at a significantly higher frequency than those with TENM4-low expressing TNBC (Figure 2).

Since several mechanisms are involved in TNBC progression, including tumor-cell features and changes in the tumor microenvironment and distant organs, which become permissive environments for the outgrowth of disseminating tumor cells, our research group is now focused on: i) studying which genes can be affected by TENM4 expression; ii) identifying TENM4 molecular interactors (transcriptomic and interactomic analyses); iii) characterizing TENM4’s involvement in affecting the TNBC microenvironment and the lung pre-metastatic niche; and, iv) exploring TENM4’s role in chemoresistance via endoplasmic reticulum (ER) stress alteration. The elu-

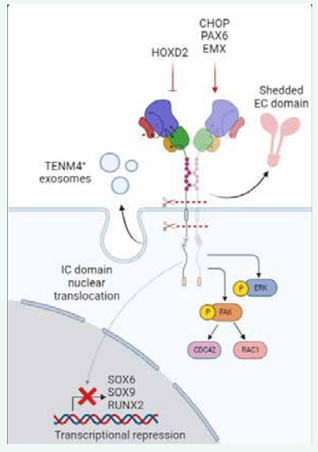


Figure 1. TENM4 structure and its molecular interactions

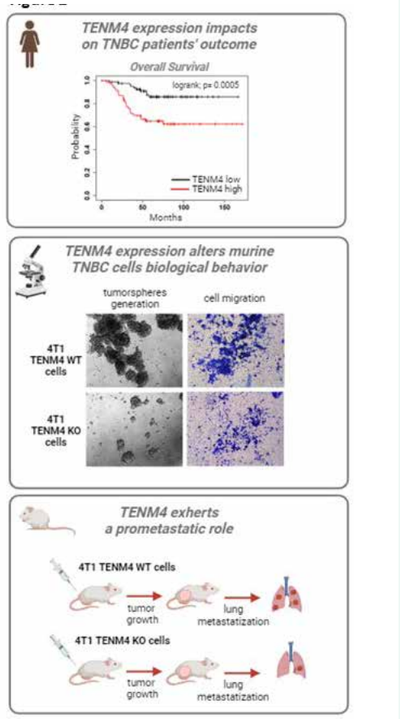


Figure 2.
TENM4 role in TNBC biology.

cidation of the mechanisms involving TENM4 in TNBC lung metastatization may validate this protein as a therapeutically relevant target and lead to the identification of novel molecular vulnerabilities to be exploited in the clinical management of TNBC patients.

My team is also focused on the evaluation of the anti-tumor efficacy of oncolytic virotherapy against non-small cell lung cancer (NSCLC). Many features, including its safety, its cytopathogenic effects against tumor cells from different histotypes, and the lack of pre-existing neutralizing antibodies in humans, make the bovine herpesvirus 4 (BoHV-4) a good candidate for introduction into the clinic. Preliminary results exploiting a preclinical model of NSCLC show that BoHV-4 blocks the proliferation of NSCLC tumor cells in vitro and, induce the regression of established NSCLC when injected intratumorally. Beside the direct anti-tumor effect, the cancer cell killing induced by the oncolytic activity of the BoHV-4 infection is effective in stimulating the host's immune system, leading to the induction of a systemic anti-cancer immune response that can counteract the growth of second tumor challenge (Figure 3).

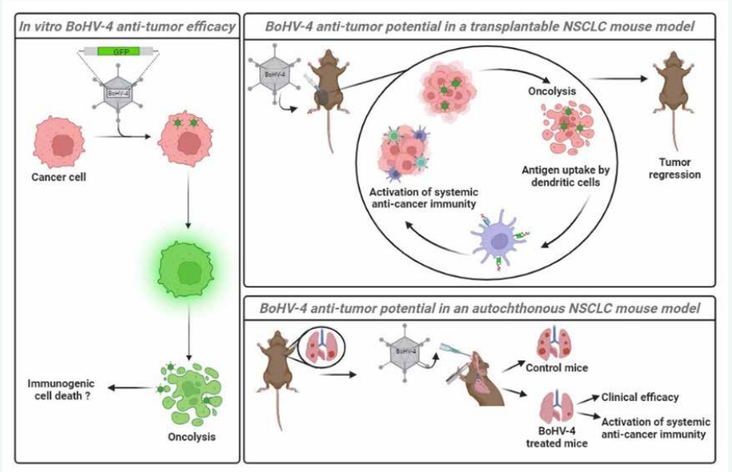


Figure 3.
BoHV-4-based oncolytic virotherapy.

FUTURE RESEARCH PLANS

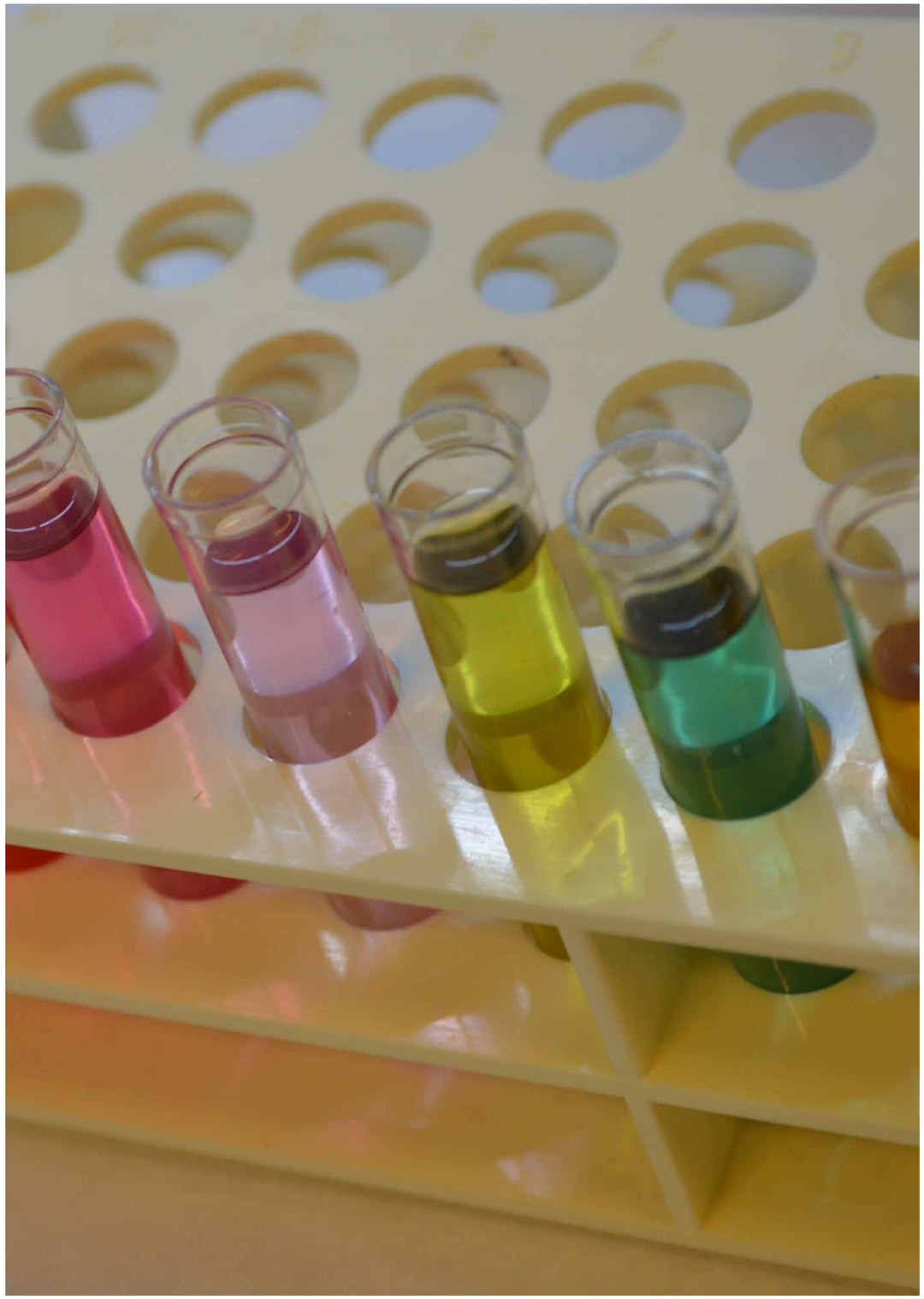
Two DNA vaccines coding for TENM4 have been developed and their safety, immunogenicity and anti-tumor effectiveness will be tested. If a role for TENM4 in ER stress, whether protective or harmful, is identified, we will also investigate the efficacy of TENM4 targeting via DNA vaccination, both alone and in combination with drugs that can maximize or decrease cell ER-stress levels. The possible involvement of TENM4 in the progression of other solid tumors and the effects of its immune targeting will also be unraveled. On the other hand, the safety of the intratracheal instillation of BoHV-4, its efficacy in hampering the growth of NSCLC developed by K-RasG12D transgenic mice, and the ability of BoHV-4-infected tumor cells to act as an in situ vaccine will be investigated in depth. The effectiveness of combinatorial strategies using BoHV-4 and immune checkpoint inhibitors administration in the NSCLC preclinical model will lay the groundwork for the development of a novel weapon for the treatment of NSCLC, which will be providentially then extendable to the treatment of lung metastases derived from other malignant cancers, thus strongly influencing the human clinical setting.

FUNDING ID (PAST 5 YERS)

→ 2017-2021: Investigator Grant, Fondazione AIRC per la Ricerca, (AIRC IG 2017; ID 20505)
→ 2023-2024: Research agreement, OSIVAX, Paris, France

SELECTED PUBLICATIONS

- Macagno M, Bandini S, Bolli E, Bello A, Riccardo F, Barutello G, Merighi IF, Forni G, Lamolinara A, Del Pizzo F, Iezzi M, Cavallo F, Conti L, Quaglino E. Role of ADCC, CDC, and CDCC in Vaccine-Mediated Protection against Her2 Mammary Carcinogenesis. Biomedicines. 2022. Doi: 0.3390/biomedicines10020230.
- Ruiu, R, Barutello, G, Arigoni, M, Riccardo, F, Conti, L, Peppino, G, Annaratone, L, Marchiò, C, Mengozzi, G, Calogero, RA, Cavallo, F, Quaglino, E. Identification of TENM4 as a Novel Cancer Stem Cell-Associated Molecule and Potential Target in Triple Negative Breast Cancer. Cancers (Basel). 2021. Doi: 10.3390/ijms22052321.
- Peppino, G, Ruiu, R, Arigoni, M, Riccardo, F, Iacoviello, A, Barutello, G, Quaglino, E. Teneurins: Role in Cancer and Potential Role as Diagnostic Biomarkers and Targets for Therapy. Int J Mol Sci. 2021. Doi: 10.3390/ijms22052321.
- Riccardo, F, Barutello, G, Petito, A, Tarone, L, Conti, L, Arigoni, M, Musiu, C, Izzo, S, Volante, M, Longo, DL, Merighi, IF, Papotti, M, Cavallo, F, Quaglino, E. Immunization against ROS1 by DNA electroporation impairs K-ras-driven lung adenocarcinomas. Vaccines. 2020. Doi: 10.3390/vaccines8020166.
- Jacca, S, Rolih, V, Quaglino, E, Franceschi, V, Tebaldi, G, Bolli, E, Rosamilia, A, Ottonello, S, Cavallo, F, Donofrio, G. Bovine herpesvirus 4-based vector delivering a hybrid rat/human HER-2 oncoantigen efficiently protects mice from autochthonous Her-2+ mammary cancer. Oncoimmunology. 2015. Doi: 10.1080/2162402X.2015.1082705.
- Riccardo, F, Arigoni, M, Buson, G, Zago, E, Iezzi, M, Longo, D, Carrara, M, Fiore, A, Nuzzo, S, Bicciato, S, Nanni, P, Landuzzi, L, Cavallo, F, Calogero, R, Quaglino, E. Characterization of a genetic mouse model of lung cancer: a promise to identify Non-Small Cell Lung Cancer therapeutic targets and biomarkers. BMC Genomics. 2014. Doi: 10.1186/1471-2164-15-S3-S1.
- Arigoni, M, Barutello, G, Riccardo, F, Ercole, E, Cantarella, D, Orso, F, Conti, L, Lanzardo, S, Taverna, D, Merighi, I, Calogero, RA, Cavallo F, Quaglino E. miR-135b coordinates progression of ErbB2-driven mammary carcinomas through suppression of MID1 and MTCH2. Am J Pathol. 2013. Doi: 10.1016/j.ajpath.2013.02.046.
- Quaglino E, Mastini C, Amici A, Marchini C, Iezzi M, Lanzardo S, De Giovanni C, Montani M, Lollini PL, Masucci G, Forni G, Cavallo F. A better immune reaction to Erbb-2 tumors is elicited in mice by DNA vaccines encoding rat/human chimeric proteins. Cancer Res. 2010. Doi: 10.1158/0008-5472.CAN-09-2548.
- Quaglino E, Iezzi M, Mastini C, Amici A, Pericle F, Di Carlo E, Pupa SM, De Giovanni C, Spadaro M, Curcio C, Lollini PL, Musiani P, Forni G, Cavallo F. Electroporated DNA vaccine clears away multifocal mammary carcinomas in her-2/neu transgenic mice. Cancer Res. 2004. Doi: 10.1158/0008-5472.can-03-2962.
- Quaglino E, Rolla S, Iezzi M, Spadaro M, Musiani P, De Giovanni C, Lollini PL, Lanzardo S, Forni G, Sanges R, Crispi S, De Luca P, Calogero R, Cavallo F. Concordant morphologic and gene expression data show that a vaccine halts HER-2/neu preneoplastic lesions. J Clin Invest. 2004. Doi: 10.1172/JCI19850.



ROBERTO PIVA

The FUNctional Genomics LAB

BIOGRAPHICAL SKETCH

2020-to date	Full Professor, Dept. Molecular Biotechnology and Health Sciences, University of Torino, Italy.
2015-to date	Executive Biologist, Division of Medical Genetics, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Italy; program: molecular diagnosis of hereditary cancers
2007-2020	Associate Professor, Dept. Molecular Biotechnology and Health Sciences, University of Torino, Italy
2007-2010	Adjunct Assistant Professor, Dept. Pathology, New York University School of Medicine, USA
2005-2007	Assistant Professor, Center of Experimental Research and Medical Science (CeRMS), University of Torino, Italy
2003-2004	Senior Scientist, Charterhouse Therapeutics Ltd, Roma, Italy
1999-2002	Research Scientist, Dept. Pathology, New York University School of Medicine, USA

GROUP MEMBERS:

Elisabetta Mereu *Postdoctoral fellow*

Mariangela Porro, Michela Cumerlato, María Labrador, Nariman Gharari *PhD students*

Monica Maccagno, Beatrice Luciano, Alessia Malisan *Graduate student*

RESEARCH ACTIVITY

Our laboratory is focused in the comprehensive study of Multiple Myeloma (MM) and other hematological malignancies (B and T cell lymphomas). We apply integrated functional screenings with the overarching goal of uncovering co-essential genes and pathways that can serve as novel therapeutic targets. Our objective is to enhance the effectiveness of current pharmacological strategies and prevent the emergence of drug resistance (Figure 1).

A key focus of our investigations lies in proteasome inhibitors (PIs), a class of drugs commonly employed in clinical settings for treating MM patients. Despite their therapeutic efficacy, the development of resistance poses a significant challenge and complicates disease management. To address this issue, we have conducted a series of genetic screenings utilizing techniques such as shRNA and CRISPRa, alongside pharmacological screenings. These efforts are aimed at identifying new targets that can synergistically interact with the proteasome inhibitor Carfilzomib (CFZ) (Figure 2).

We meticulously validate the therapeutic potential of these combination treatments in a diverse array of MM cell lines, encompassing both those sensitive and resistant to PIs. To mimic the tumor microenvironment, we assess the efficacy of these combinations in MM cells co-cultured with bone marrow stromal cells in 2D and 3D culturing systems. Additionally, we extend our investigations to primary cells from MM patients. The most promising combinations are then chosen for rigorous in-vivo assessments using zebrafish and mouse MM xenograft models. To gain comprehensive insights into the molecular mechanisms driving therapy response of the new drug combinations, we harness multi-omics approaches that will enable us to unravel the intricate interplay between genetic, epigenetic, and proteomic factors.

Recognizing the challenges posed by low bioavailability and high toxicity associated with certain therapeutic agents, we are actively engaged in the refinement of drug delivery systems. Our approach involves encapsulating drugs within functionalized biomimetic lipid nanoparticles, designed for targeted delivery to tumor cells (Figure 3). Furthermore, we are keen on exploring the potential of these combinations in in a broader range of hematological malignancies and solid tumors.

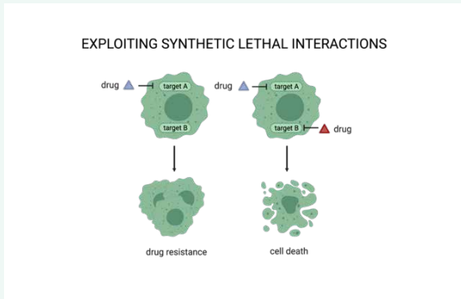


Figure 1.
Co-essential gene pathways in drug resistance

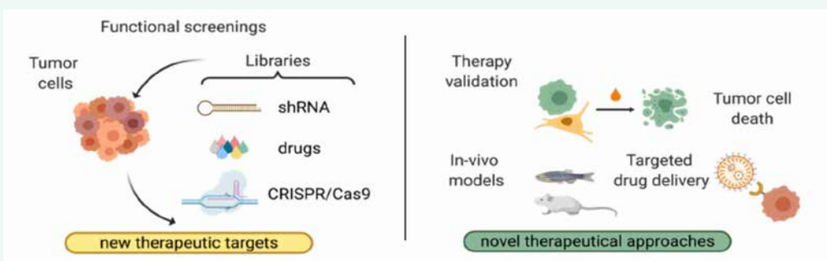


Figure 2.
Overview of lab research

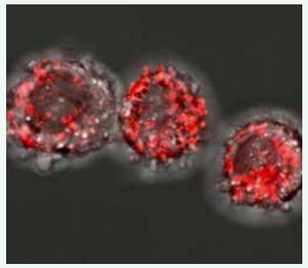


Figure 3. Internalized nanoparticles in MM cells

FUTURE PLANS

Being aware of the complex genetic and molecular scenario of MM disease, we believe that there is an urgent need to explore novel cancer vulnerabilities and design new personalized therapeutic strategies. To this end, our functional workflow will be expanded to novel classes of molecules approved for the treatment of MM such as T-cell engagers bispecific antibodies and chimeric antigen receptor (CAR) therapies. To more efficiently define the therapeutic efficacy of combination protocols, preclinical trials will be executed using patient-derived xenografts (PDX).

A pivotal element of our future endeavors involves the integration of patient-derived xenografts (PDX) into our preclinical trial strategy. This approach, leveraging the transplantation of patient tumor samples into animal models, holds immense promise in replicating the complexities of human MM within an in vivo context. By employing PDX models, we aim to bridge the gap between bench and bedside, providing a more accurate representation of the disease microenvironment and therapeutic responses. This step is critical in evaluating the clinical relevance and efficacy of combination protocols, enabling us to make informed decisions on therapeutic approaches with the highest potential for success.

In parallel, our laboratory remains committed to fostering collaborations with clinical researchers, pharmaceutical companies, and academic institutions. These partnerships are pivotal in translating our discoveries from the bench to the clinic, ensuring that our findings directly impact patient care and therapeutic decision-making.

FUNDING ID (PAST 5 YEARS)

- 2019-2021: CRT Foundation: Richieste Ordinarie 2018 (PI)
- 2019-2024: AIRC, Investigator Grant (PI)
- 2020-2021: Compagnia di San Paolo: POC Instrument 2020 (Co-PI)
- 2022:2025: PRIN 2020; (Co-PI)
- 2023-2024: CRT Foundation: Richieste Ordinarie 2022 (PI)
- 2023-2024: Compagnia di San Paolo: POC Instrument 2022 (Co-PI)

SELECTED PUBLICATIONS

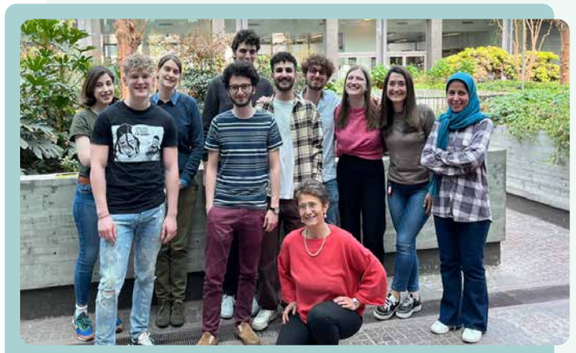
- Bandini C, Mereu E, Paradzik T, Labrador M, Maccagno M, Cumerlato M, Oreglia F, Prever L, Manicardi V, Taiana E, Ronchetti D, D'Agostino M, Gay F, Larocca A, Merlo G, Hirsch E, Ciarrocchi A, Inghirami G, Neri A, Piva R. Lysin (K)-Specific Demethylase 1 Inhibition Enhances Proteasome Inhibitor Response and Overcomes Drug Resistance in Multiple Myeloma. Experimental Hematology and Oncology, 12: 71 (2023). https://doi.org/10.1186/s40164-023-00434-x
- Mereu E, Abbo D, Paradzik T, Cumerlato M, Bandini C, Labrador M, Maccagno M, Ronchetti D, Manicardi V, Neri A, Piva R. Euchromatic Histone Lysine Methyltransferase 2 Inhibition Enhances Carfilzomib Sensitivity and Overcomes Drug Resistance in Multiple Myeloma Cell Lines. Cancers 15, 2199 (2023). https://doi.org/10.3390/cancers15082199
- Taiana E, Bandini C, Favasuli VK, Ronchetti D, Silvestris I, Puccio N, Todoerti K, Erratico S, Giannandrea D, Bolli N, Amodio N, Ciarrocchi A, Chiaramonte R, Torrente Y, Piva R, Neri A. Activation of lncRNA NEAT1 leads to survival advantage of multiple myeloma cells by supporting a positive regulatory loop with DNA repair proteins. Haematologica, 108: 219-233 (2022). https://doi.org/10.3324/haematol.2022.281167
- Cauda V, Xu TT, Nunes I, Mereu E, Villata S, Bergaggio E, Labrador M, Limongi T, Susa F, Chiodoni A, Cumerlato M, Rosso G, Stefania R, Piva R. Biomimetic mesoporous vectors enabling the efficient inhibition of wild-type isocitrate dehydrogenase in multiple myeloma cells. Microporous and Mesoporous Materials, 325, 111320 (2021). https://doi.org/10.1016/j.micromeso.2021.111320
- Sindi H, Russomanno G, Satta S, Abdul-Salam V, Beom Jo K, Chaudhry B, Ainscough A, Szulcek R, Bogaard H, Morgan C, Pullamsetti S, Alzaydi M, Rhodes C, Piva R, Eichstaedt C, Grünig E, Wilkins M, Wojciak-Stothard B. Krüppel-like factor 2-induced microRNAs: implications for treatment of pulmonary hypertension. Nat Commun, 11(1):1185 (2020). https://doi.org/10.1038/s41467-020-14966-x
- Menotti M, Ambrogio C, Cheong TC, Pighi C, Mota I, Cassel SH, Compagno M, Wang Q, Dall'Olio R, Minero V, Poggio T, Sharma G, Patrucco E, Mastini C, Choudhari R, Pich A, Zamò A, Piva R, Giliani S, Mologni L, Collings CK, Kadoch C, Gambacorti-Passerini C, Notarangelo LD, Anton IM, Voena C, Chiarle R. Wiskott–Aldrich syndrome protein (WASP) is a tumor suppressor in T cell lymphoma. Nature Med, 25(1):130-140 (2019). https://doi:10.1038/s41591-018-0262-9

VALERIA POLI

Gene expression control in tumor biology and autoimmunity

BIOGRAPHICAL SKETCH

1984	MSC in Biological Science, University of Torino, Italy
1984-1988	PhD, Human biology, University of Torino, Italy
1988-1990	Post-doctoral fellow, European Molecular Biology Laboratories (EMBL), Heidelberg, Germany
1990-1992	Post-doctoral fellow, College of Physicians and Surgeons of Columbia University
1992-1997	Principal Investigator, Istituto di Ricerche di Biologia Molecolare (IRBM), Rome, Italy
1997-2001	Principal Investigator, Wellcome Trust Senior Research Fellow, Department of Biochemistry, University of Dundee, Dundee, UK
2001-2005	Associate Professor in Molecular Biology, University of Turin, Italy
Since 2005	Full Professor
1998	Elected member of EMBO
2014	Elected member of Academia Europaea
2016-2022	President of SIBBM (Italian Society of Biophysics and Molecular Biology)
2012-2015	Member of the BIO/11 ASN panel
2010-2017	Member of the LS4 advanced ERC grants reviewing panel
2021-2022	Coordinator of the Panel of Evaluation Experts for Life Sciences, BIO sector (GEV05) for the National Research Quality Evaluation (VQR) 2015-2019



LAB MEMBERS:

Somayeh Mirzagahaei, Luana Bataglia *Post-doctoral fellows*
Daniele Viavattene *PhD student*
Andrea Lobascio *master student*
Andrea Marchetti, Gregorio Pep *Undergraduate students*

RESEARCH ACTIVITY

Gene expression control in tumor biology and autoimmunity

Controlled production of cytokines and growth factors, leading to the activation of specific signalling pathways and to the modulation of gene expression, is essential for life, while aberrant cytokines/growth factors expression, activity or signalling is at the basis of many pathological conditions including chronic inflammation, auto-immunity and oncogenesis. The laboratory has long focused on the axis between the pro-inflammatory cytokine IL-6 and the oncogenic transcription factor STAT3 at the crossroads between inflammation, auto-immunity and cancer, aiming at characterizing STAT3 involvement in both physiological and pathological conditions and making use of both in vitro approaches and genetically modified mouse model (GEMMs).

Tumor biology:We have demonstrated that constitutively active STAT3 can cooperate with oncogenes such as the HER2/Neu to confer aggressive features to breast tumors and support metastasis formation (Barbieri et al., 2010, Barbieri et al., 2010). We have then shown that aberrantly active STAT3 can regulate energy metabolism and mitochondrial activity and can act as a first hit in tumor transformation (Demaria et al., 2010, Demaria et al, 2012)

More recently, we have demonstrated that STAT3 is essential in mediating the pro-tumorigenic activities of breast Cancer Associated Fibroblasts (CAFs). We identified a STAT3-dependent signature that is conserved in humans, and validated several STAT3-dependent genes encoding for secreted proteins responsible of CAF activities (Fig. 1, Avalle et al, 2022).

Ongoing research:

- Genome wide CRISPR Cas screening to identify breast cancer genes responsible of increased drug resistance in response to CAFs.
- Identification of regulatory networks and novel therapeutic targets in breast cancer. We have generated breast cancer gene co-expression networks and identified several Transcription Factor hubs as crucial regulators of breast cancer aggressiveness features . We are now functionally validating our findings and and assessing inhibitory strategies (Savino et al., 2021, 2020, 2019).
- STAT3 anti-apoptotic roles at the ER: We have demonstrated that STAT3 can localize to the ER, where its Serine-phosphorylated form triggers degradation of the calcium channel IP3R3, decreasing calcium release towards the mitochondria and enhancing resistance to apoptosis of basal-like breast cancer cells (Avalle et al., 2019). We are now characterizing the mechanisms mediating STAT3 Serine-phosphorylation at the ER to identify potential novel targets.
- Disentangling the relationships with tumor microenvironment in prostate cancer to better model and target tumor progression. This is a collaborative effort,

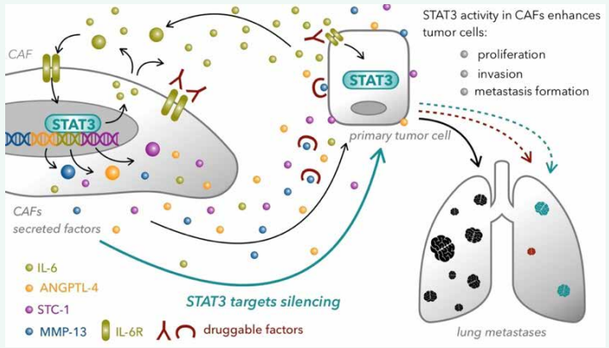


Figure 1.
The scheme depicts how IL-6 secreted by primary tumor cells activates CAFs, triggering STAT3-dependent induction of a set of genes encoding for secreted proteins, responsible for supporting CAF pro-tumorigenic actions (Avalle et al, 2022.)

based on the analysis of CAFs and tumor cells/organoids derived from high risk patient samples.

→ Heart auto-immunity. We have shown that STAT3 is both sufficient and necessary to trigger the onset of immune-mediated myocarditis, activating a feed-forward loop involving enhanced IL-6 signalling and complement factors production in the liver (Camporeale et al., 2013), and demonstrated that liver-specific STAT3 or C3 siRNA silencing can both prevent and cure immune-mediated myocarditis (Avalle et al., 2020). Current research aims at repurposing already approved inhibitors of STAT3 activation such as JAK kinase inhibitors, and assessing the value of dosing complement activation to predict disease progression in a cohort of patients.

FUNDING ID, PAST 5 YEARS

→ 2014-2023, Truus and Gerrit van Riemsdijk Foundation, Vaduz, Liechtenstein. Role of Stat3 in tumorigenesis (Donation). PI, 150,000 €

→ 2014-2020, Piedmont Region F.E.S.R. DEFLeCT: Digital tEchnology For Lung Cancer Treatment, co-PI, 70,000 €.

→ 2019-2021, MUR PRIN 2017 , “Prostate cancer: disentangling the relationships within the tumour microenvironment to better model and target tumour progression”. PI and Project coordinator, 216,000 €.

→ 2021-2025, AIRC IG 24851, “Exploiting network analysis to unravel breast cancer molecular features and identify novel targets”, PI, 684,000 €.

→ 2021-2024, Health Ministry Finalized Research, “Biopsy-proven myocarditis: genetic background, predictors of dismal prognosis and of response to immunosuppressive therapy and preclinical evaluation of innovative immunomodulatory therapies”. Co-applicant, 90,000 €.

→ 2023-2025, MUR PRIN 2022 , DEsiRE-Pre-clinical development of siRNA-based non-targeted and targeted approaches to interfere with STAT3 activity and CAF-tumor cross-talk in breast cancer. PI and Project coordinator, 203,329 €.

SELECTED PUBLICATIONS

All my research products (Link Scopus)

→ Savino A, De Marzo N, Provero P, Poli V. Meta-Analysis of Microdissected Breast Tumors Reveals Genes Regulated in the Stroma but Hidden in Bulk Analysis. Cancers 2021, 13(13):3371. doi: 10.3390/cancers13133371.

→ Avalle L, Raggi L, Monteleone E, Savino A, Viavattene D, Statello L, Camperi A, Aversano Stabile S, Salemme V, De Marzo N, Marino F, Guglielmi C, Lobascio A1, Zanini C, Forni M, Incarnato D, Defilippi P, Oliviero S, Poli V. STAT3 induces breast cancer growth via ANGPTL4, MMP13 and STC1 secretion by Cancer Associated Fibroblasts, 2022 Oncogene, Online ahead of print, doi: 10.1038/s41388-021-02172-y

→ Avalle L., Marino F., Camporeale A., Guglielmi C., Viavattene D., Bandini S., Conti L., Cimino J., Forni M., Zanini C., Ghigo A., Bogorad R.L., Cavallo F., Provero P., Koteliansky V. and Poli V. Liver-specific siRNA-mediated Stat3 or C3 knock-down improves the outcome of experimental autoimmune myocarditis, Mol Ther Methods Clin Dev. 2020, 18:62-78. https://doi.org/10.1016/j.omtm.2020.05.023

→ D’Alise AM, Leoni G, Cotugno G, Troise F, Langone F, Fichera I, De Lucia M, Avalle L, Vitale R, Leuzzi A, Bignone V, Di Matteo E, Tucci FG, Poli V, Lahm A, Catanese MT, Folgori A, Colloca S, Nicosia A, Scarselli E. Adenoviral vaccine targeting multiple neoantigens as strategy to eradicate large tumors combined with checkpoint blockade. Nat Commun. 2019, 10:2688. doi: 10.1038/s41467-019-10594-2.

→ Avalle L, Camporeale A, Morciano G, Caroccia N, Ghetti E, Orecchia V, Viavattene D, Giorgi C, Pinton P, Poli V. STAT3 localizes to the ER, acting as a gatekeeper for ER-mitochondrion Ca2+ fluxes and apoptotic responses. (2018) Cell Death Differ. 2019, 26(5):932-942. doi: 10.1038/s41418-018-0171-y.

→ Avalle L, Incarnato D, Savino A, Gai M, Marino F, Pensa S, Barbieri I, Stadler MB, Provero P, Oliviero S, Poli V. MicroRNAs-143 and -145 induce epithelial to mesenchymal transition and modulate the expression of junction proteins. (2017) Cell Death Differ., 24(10):1750-1760. doi:10.1038/cdd.2017.103. doi: 10.1038/cdd.2017.103. PMID: 28644441.

→ Laklai H, Miroshnikova YA, Pickup MW, Collisson E, Kim GE, Barrett AS, Hill RC, Lakins JN, Schlaepfer DD, Mouw JK, LeBleu VS, Novitskiy SV, Johansen JS, Poli V, Kalluri R, Iacobuzio-Donahue CA, Wood LD, Hebrok M, Hansen K, Moses HL and Weaver VW. Genotype tunes PDAC tension to induce matricellular-fibrosis and tumor aggression. (2016) Nature Medicine 22, 497–505, doi:10.1038/nm.4082.

→ Camporeale A, Marino F, Papageorgiou A, Carai P, Fornero S, Fletcher S, Page BDG, Gunning P, Forni M, Chiarle R, Morello M, Jensen O, Levi R, Heymans S, Poli V. STAT3 activity is necessary and sufficient for the development of immune-mediated myocarditis in mice and promotes progression to dilated cardiomyopathy. (2013) EMBO Mol. Medicine 5: 572–590, DOI: 10.1002/emmm.201201876.

→ Demaria M, Misale S, Giorgi C, Miano V, Camporeale A, Campisi J, Pinton P and Poli V. STAT3 can serve as a hit in the process of malignant transformation of primary cells. 2012, Cell Death and Differentiation 19: 1390-1397.

→ Kreuzaler PA, Staniszewska AD, Li W, Omidvar N, Kedjouar B, Turkson J, Poli V, Flavell RA, Clarkson RWE, and Watson CJ. Stat3 controls lysosomal mediated cell death in vivo. (2011) Nature Cell Biology 13:303-309.

VALENTINA PROSERPIO

Women's Health Genomics Lab

BIOGRAPHICAL SKETCH

Since 2021	Researcher (RTDb) at the Department of Life Sciences and Systems Biology of the University of Turin
2016-2021	Post-Doctoral Fellow at the University of Turin, Department of Life Sciences and Systems Biology and Italian Institute for Genomic Medicine (IIGM), Prof. Oliviero's lab
2013-2016	Post-doctoral fellow in Sarah Teichmann's lab at EMBL-European Bioinformatics Institute, Hinxton, UK
2010-2013	MRC Career Development Fellow at MRC Laboratory of Molecular Biology, Cambridge, UK
2010	Visiting Scientist in Fiona Watt Lab, Cambridge Research Institute, Cancer Research UK
2009	Short term visiting in the Laboratory of Muscle Stem Cells and Gene Regulation, NIAMS, NIH, Bethesda USA
2006-2010	PhD In Biomolecular Sciences and Biotechnology at the University of Milan
2004-2006	MSC at the University of Milan, Biology applied to Medical Sciences
2001-2004	BSC at the University of Milan, Biology

GROUP MEMBERS:

Alessia Albano *Postgraduate Fellow*
Federica Loperfido *Master Student*

RESEARCH ACTIVITY

Since my Doctorate fellowship at the University of Milan, my focus has revolved around investigating molecular changes that can transform one cell type into another. Specifically, I have been fascinated by the transcriptional regulation of gene expression by transcription factors, which play a crucial role in controlling cell differentiation and plasticity in both health and disease.

In 2022, after becoming a researcher, I made the decision to apply my knowledge in the biomolecular field to study neglected diseases that affect women. Thus, I embarked on a project aimed at unraveling the molecular mechanisms underlying vulvodynia. Now, you might be wondering, what on earth is vulvodynia? Well, according to recent definition, vulvodynia refers to vulvar pain without an identifiable origin that persists for more than 3 months. Despite affecting 1 out of 7 women, as revealed by recent studies, vulvodynia remains largely unknown to the general public.

Vulvodynia can significantly impair a woman's sexual life and, depending on its severity, it can also impact everyday activities such as urination, sitting, wearing tight clothing, engaging in sports, and sometimes even walking. The diagnosis of vulvodynia is considered a "diagnosis of exclusion," meaning it is made when all other possible diseases have been ruled out. Despite being poorly acknowledged, vulvodynia is a frequently occurring women's health condition that is often overlooked and challenging to diagnose due to the lack of specific markers. To address this gap, we are conducting transcriptomic analyses on both patients and healthy controls in order to identify biomarkers specific to vulvodynia.

The findings of our project will not only assist clinicians in making accurate diagnoses, but also have the potential to guide therapy direction, helping to prevent the progression and chronicity of the disease. In 2022, in order to secure funding for this research project, we collaborated with the "Comitato Vulvodinia e Neuropatia del Pudendo," of which I am a member, to organize a crowdfunding campaign called "doppiaVproject." This campaign successfully raised over 20,000 euros. Furthermore, we have recently received funding from the Ministry of Research (MUR) to further pursue our objectives.

Figure 1.

FUTURE RESEARCH PLANS:

In addition to our ongoing research, we are leveraging our extensive network of collaborators to initiate a project focusing on Extramammary Paget Disease in women. This particular form of epithelial cancer primarily affects the vulvar region and its surrounding areas, and its characterization in women is relatively limited. Unfortunately, it is frequently misdiagnosed, leading to surgical interventions that can be extensive and mutilating. Our objective is to support clinicians in stratifying patients, enabling the implementation of personalized therapeutic strategies and minimizing the need for surgical interventions as a last resort.

FUNDING ID

- PRIN2022 - Project code: 2022CLTAYH
- Project title: Vestibulodynia at high resolution: omics approach to improve diagnosis
- Raccolta fondi Ideaginger - Studiamo insieme la Vulvodinia
- Bando Ricerca Finalizzata 2019 - Targeting alternative splicing neo-junctions as a novel source of neo-antigens in pediatric and adult tumors
- EASI-genomics, Third Call for Proposals for Transnational Access Projects at EASI-Genomics

SELECTED PUBLICATIONS

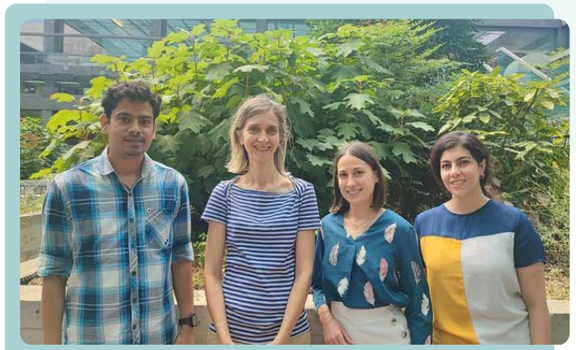
- Lauria, Andrea, et al. “DNMT3B Supports Meso-Endoderm Differentiation from Mouse Embryonic Stem Cells.” Nature Communications, vol. 14, no. 1, Jan. 2023, p. 367, https://doi.org/10.1038/s41467-023-35938-x.
- Levra Levron, Chiara, et al. “Tissue Memory Relies on Stem Cell Priming in Distal Undamaged Areas.” Nature Cell Biology, vol. 25, no. 5, May 2023, pp. 740–53, https://doi.org/10.1038/s41556-023-01120-0.
- Mahata, Bidesh, et al. “Single-Cell RNA Sequencing Reveals T Helper Cells Synthesizing Steroids de Novo to Contribute to Immune Homeostasis.” Cell Reports, vol. 7, no. 4, May 2014, pp. 1130–42, https://doi.org/10.1016/j.celrep.2014.04.011.
- Mirzadeh Azad, Fatemeh, et al. “Long Noncoding RNAs in Human Stemness and Differentiation.” Trends in Cell Biology, vol. 31, no. 7, July 2021, pp. 542–55, https://doi.org/10.1016/j.tcb.2021.02.002.
- Proserpio, Valentina, Andrea Piccolo, et al. “Single-Cell Analysis of CD4+ T-Cell Differentiation Reveals Three Major Cell States and Progressive Acceleration of Proliferation.” Genome Biology, vol. 17, May 2016, p. 103, https://doi.org/10.1186/s13059-016-0957-5.
- Proserpio, Valentina. Single Cell Methods: Sequencing and Proteomics. 2019, https://books.google.com/books/about/Single_Cell_Methods.html?hl=&id=V-jMkzAEACAAJ.
- Proserpio, Valentina, Carlotta Duval, et al. “Single-Cell Sequencing for Everybody.” Methods in Molecular Biology , vol. 2421, 2022, pp. 217–29, https://doi.org/10.1007/978-1-0716-1944-5_15.
- Proserpio, Valentina, Raffaella Fittipaldi, et al. “The Methyltransferase SMYD3 Mediates the Recruitment of Transcriptional Cofactors at the Myostatin and c-Met Genes and Regulates Skeletal Muscle Atrophy.” Genes & Development, vol. 27, no. 11, June 2013, pp. 1299–312, https://doi.org/10.1101/gad.217240.113.
- Proserpio, Valentina, and Tapio Lönnberg. “Single-Cell Technologies Are Revolutionizing the Approach to Rare Cells.” Immunology and Cell Biology, vol. 94, no. 3, Mar. 2016, pp. 225–29, https://doi.org/10.1038/icb.2015.106.
- .Proserpio, Valentina, and Bidesh Mahata. “Single-Cell Technologies to Study the Immune System.” Immunology, vol. 147, no. 2, Feb. 2016, pp. 133–40, https://doi.org/10.1111/imm.12553.
- (Proserpio, Duval, et al.; Proserpio, Piccolo, et al.; Proserpio and Lönnberg; Proserpio; Levra Levron et al.; Lauria et al.; Mirzadeh Azad et al.; Proserpio, Fittipaldi, et al.; Proserpio and Mahata; Mahata et al.)

FRANCESCA REINERI

MR Hyperpolarization for diagnostics

BIOGRAPHICAL SKETCH

March 2022 to date	Associate Professor of General and Inorganic Chemistry, Dept. Molecular Biotechnology and Health Sciences, Univ. of Torino
2012-2022	Assistant Professor, Dept. Molecular Biotechnology and Health Sciences, Univ. of Torino
2007-2012	Assistant Professor, Dept. Chemistry, Univ. of Torino
2005-2007	Research Fellow, Dept. of Chemistry, Univ. of Torino
2003-2005	Postdoctoral Fellow, Dept. Chemistry, University of Torino



LAB MEMBERS:

Ginevra Di Matteo *PhD Student*
Sumit Mishra *Postdoctoral fellow*
Bhahreh Saadat *Deghan Master Student*

RESEARCH ACTIVITY

MRI is a powerful diagnostic tool to obtain information about the health state of tissues and to detect the onset of pathologies non-invasively. Due to its intrinsic low sensitivity, the wealth of chemical information that can be obtained from MR remains widely unexploited. Hyperpolarization increases the intensity of the MR signals of specific molecules by orders of magnitude, making them clearly detectable by MRS/MRI and allowing the investigation of metabolic processes in-vivo, in real time.

A hyperpolarization procedure, named PHIP-SAH (ParaHydrogen Induced Polarziation -Side Arm Hydrogenation) (figure 1) has been invented in our laboratory, to generate hyperpolarized metabolites in an easy to handle and affordable way. Among them, pyruvate (usually obtained by the expensive and technically demanding d-DNP technique) is the most widely used for the study of metabolism and its alterations in different pathologies. A pilot study has been carried out in-vivo using this hyperpolarized probe, that demonstrated its applicability of PHIP polarized pyruvate for metabolic investigations (figure 2).

The up-regulated conversion of pyruvate into lactate, even in the presence of oxygen (i.e. the Warburg effect), is a common feature of cancer tissues. HP-pyruvate, obtained using parahydrogen, has also been used to investigate these metabolic changes in cancer cell lines characterized by different aggressiveness (figure 3).

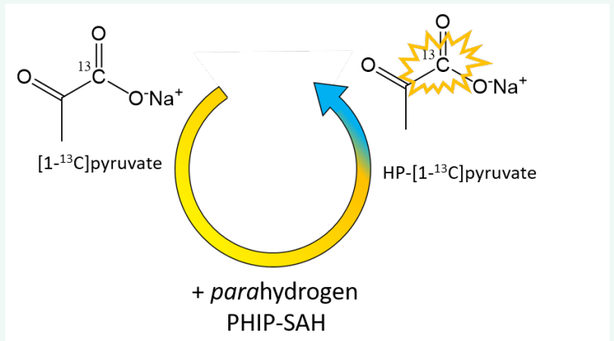


Figure 1.
PHIP-SAH (ParaHydrogen Induced Polarization – Side Arm Hydrogenation) hyperpolarization of pyruvate is obtained through a sequence of steps (hydrogenation using para-enriched hydrogen of an unsaturated derivative, spin-order transfer form parahydrogen to 13C, hydrolysis of the hydrogenated ester). The aqueous solution of this metabolite can be used for metabolic investigations in-vivo and in-cells.

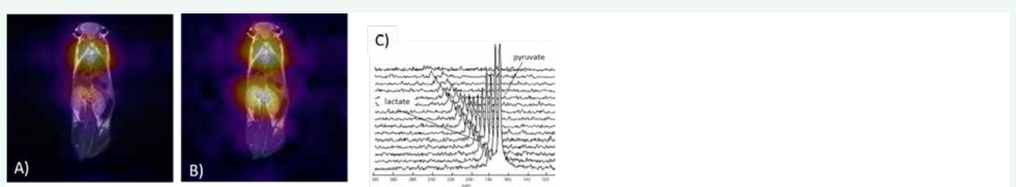


Figure 2.
^{13}C-CSI (chemical shift images) of HP-pyruvate (A) and lactate (B), overlapped with anatomical images (grey scale) of a healthy mouse. 13C images have been acquired following to the injection of parahydrogen HP-pyruvate, lactate derives from the metabolic transformation of pyruvate in-vivo. Both images have been acquired within about 2 minutes after the injection. C) Stacked plot of 13C-MR spectra acquired in-vivo, using small flip angles, after the injection of HP-pyruvate. The time delay among spectra is 2s, the build-up of the lactate signal is clearly visible.

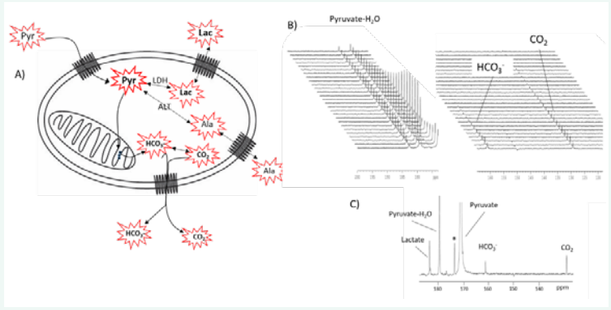


Figure 3.
A) pyruvate is at the crossing of several metabolic pathways: it is transformed into lactate by LDH, into alanine by ALT an can enter the TCA cycle following to decarboxylation by PDH. All these pathways can be observed by means of HP-pyruvate, as reported in the stacked plot of 13C-NMR spectra (B) acquired following to the perfusion of PHIP polarized pyruvate through cancer cells suspended in their growth medium. C) 13C-NMR spectrum from the stacked plot: the signals of HP lactate and carbonate are clearly visible (the peak marked with the asterisk is an impurity).

FUTURE RESEARCH PLANS

For the future we plan to: 1) optimize the hyperpolarization procedure, using different precursors of the target molecules and different methods for spin order transfer, in order to increase the hyperpolarization level on the final product; 2) use the hyperpolarized substrates (in particular HP-pyruvate) for the investigation of metabolic changes in different pathologies and following to treatment; 3) explore different biological substrates suitable for hyperpolarization using parahydrogen.

FUNDING ID (PAST 5 YERS)

- Since October 2019 to September 2023. Partner in the H2020 FETOpen Project Alternatives to Gd (www.alternativestogd.eu). Project n. 848149
- Since February 2018 to December 2021 . Partner in the Marie-Curie ITN H2020-MSCA-ITN-2017, n. 766402, Zero and Ultra-Low Field Nuclear Magnetic Resonance (ZULF)
- Since 01 May 2017 to 30 April 2020. Athenaeum call 2016 (Compagnia San Paolo), Research for the territory, projet n. CSTO164550, Hyperpolarized metabolites for MRI of cancer
- Since January 2016 to December 2018. AIRC- Cariplo foundation TRIDEO 2015, “Metabolic Imaging with ParaHydrogen Polarized tracers for cancer detection and treatment monitoring”

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=6506369230

- ParaHydrogen Induced Polarization of 13C carboxylate resonance in Acetate and pyruvate” Reineri F., Boi T., Aime S., Nat. Commun. 2015, 6:5858. doi: 10.1038/ncomms6858
- ^{13}C-MR Hyperpolarization of Lactate using ParaHydrogen and metabolic transformation in vitro. CAVALLARI, ELEONORA, CARRERA, CARLA, AIME, Silvio, REINERI, Francesca (2016). CHEMISTRY-A EUROPEAN JOURNAL, p. 1-5, ISSN: 0947-6539, doi: 10.1002/chem.201605329.
- The ^{13}C hyperpolarized pyruvate generated by ParaHydrogen detects the response of the heart to altered metabolism in real time, Eleonora Cavallari, Carla Carrera, Matteo Sorge, Gisèle Bonne, Antoine Muchir, Silvio Aime, Francesca Reineri Scientific Reports, 2018, 8:8366 DOI:10.1038/s41598-018-26583-2.
- “Metabolic Studies of Tumor Cells Using [1-13C] Pyruvate Hyperpolarized by Means of PHIP-Side Arm Hydrogenation” Eleonora Cavallari, Carla Carrera, Silvio Aime, Francesca Reineri ChemPhysChem 2019, 20, 318–325. Doi: 10.1002/cphc.201800652
- Real-Time Nuclear Magnetic Resonance Detection of Fumarase Activity Using Parahydrogen-Hyperpolarized [1-13C]Fumarate. James Eills,, Eleonora Cavallari, Carla Carrera, Dmitry Budker, Silvio Aime and Francesca Reineri J. Am. Chem. Soc. 2019, 141, 20209−20214. Doi 10.1021/jacs.9b10094
- In-vitro NMR Studies of Prostate Tumor Cell Metabolism by Means of Hyperpolarized [1-13C]Pyruvate Obtained Using the PHIP-SAH. Eleonora Cavallari , Carla Carrera, Ginevra Di Matteo, Oksana Bondar , Silvio Aime and Francesca Reineri Method Front. Oncol. April 2020 | Volume 10 | Article 497. doi: 10.3389/fonc.2020.00497
- Singlet-contrast Magnetic Resonance Imaging: Unlocking Hyperpolarization with Metabolism” Eills J., Cavallari E., Kircher R., Di Matteo G., Carrera C., Dagys L., Levitt M.H., Ivanov K.L., Aime S., Reineri F., Münnemann K., Budker D., Buntkowsky G., Knecht S. (2021) Angewandte Chemie - International Edition, 60 (12), pp. 6791 - 6798, Cited 16 times. DOI: 10.1002/anie.202014933
- ParaHydrogen polarized ethyl-[1-13C]pyruvate in water, a key substrate for fostering the PHIP-SAH approach to metabolic imaging. C. Carrera, E. Cavallari, G. Digilio, S. Aime F. Reineri; Chem Phys Chem 2021, 22(11), pp.1042-1048. Doi: 10.1002/cphc.202100062
- Rapid hyperpolarization and purification of the metabolite fumarate in aqueous solution” Knecht S., Blanchard J.W., Barskiy D., Cavallari E., Dagys L., van Dyke E., Tsukanov M., Bliemel B., Münnemann K., Aime S., Reineri F., Levitt M.H., Buntkowsky G., Pines A., Blümler P., Budker D., Eills J. (2021) Proceedings of the National Academy of Sciences of the United States of America, 118 (13), art. no. e2025383118, DOI: 10.1073/pnas.2025383118
- Effect of the hydrogenation solvent in the PHIP-SAH hyperpolarization of [1-13C]pyruvate” Bondar O., Cavallari E., Carrera C., Aime S., Reineri F. (2022) Catalysis Today, 397-399, pp. 94 - 102, DOI: 10.1016/j.cattod.2021.11.030

CHIARA RIGANTI

Biochemical Pharmacology of Cancer

BIOGRAPHICAL SKETCH

2021-present	full professor of Biochemistry, Dept. of Oncology, University of Torino
2016-2021	associate professor of Biochemistry, Dept. of Oncology, University of Torino
2011 and 2013	visiting professor at Dept. of Molecular Genetics, The Weizmann Institute of Science (Prof. Menachem Rubinstein), Rehovot, Israel
2010	visiting scientist at Institut Cochin (Prof. Pierre-Olivier Couraud), Universitè Renè Descartes, Paris, France
2006-2016	assistant professor of Biochemistry, Dept. of Genetics, Biology and Biochemistry/Dept. of Oncology, University of Torino
2003-2006	post-degree medical Specialization in Clinical Biochemistry (70/70 cum laude and honors), University of Torino
1996-2002	degree in Medicine and Surgery (110/110 cum laude and honors) at University of Torino

GROUP MEMBERS:

Joanna Kopecka *Assistant Professor*
Iris Chiara Salaroglio *post-doc researcher*
Costanzo Costamagna *Lab technician*
Muhlis Akman *PhD student*
Giulia Antonello *PhD student*
Sabrina Digiovanni *PhD student*
Simona Fontana *PhD student*
Martina Godel *PhD student*

RESEARCH ACTIVITY

Main research line: New predictive biomarkers and therapeutic targets of tumor chemo-immuno-resistance.

Achieving a good efficacy of chemotherapy and an effective activation of the host immune system against the tumor are two major challenges still unresolved in patients with disseminated and metastatic tumors. The main limitation to the efficacy of chemotherapy is multidrug resistance (MDR), a multiple cross-resistance towards different anticancer drugs.

Besides killing MDR cells, the successful tumor eradication depends on the ability of chemotherapy to kill tumor cells in a way detectable by the immune system, i.e. inducing an immunogenic cell death. We aim to:

- → investigate the molecular and metabolic basis of chemo-immunoresistant phenotype to identify new predictive markers of sensitivity/resistance;
- → validate newly synthesized or repurposed compounds, with the dual property of inducing immunogenic cell death, bypassing chemo- and immuno-resistance.

Subline research a: Dissecting the endoplasmic reticulum-mitochondria network to overcome chemo-immuno-resistance in cancer cells

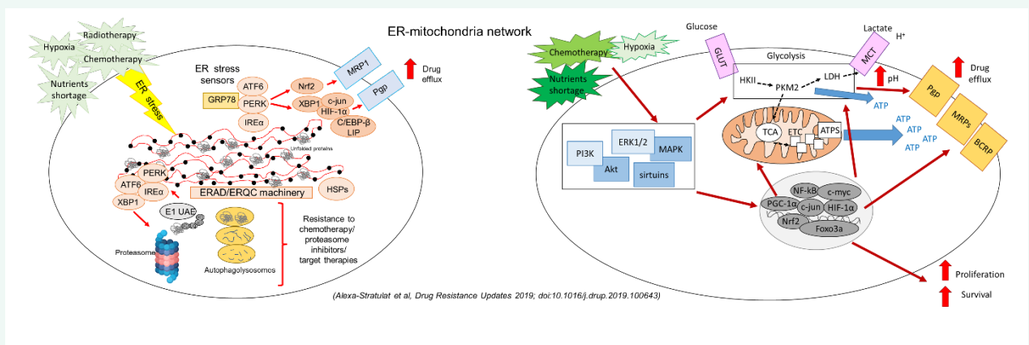
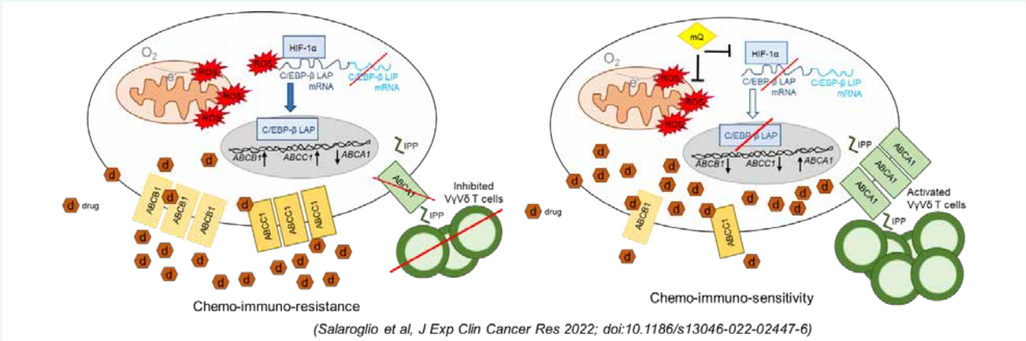


Figure 1.

Research subline b: Disrupting the bad metabolic liaisons determining chemo-immuno-resistance



(Salaroglio et al, J Exp Clin Cancer Res 2022; doi:10.1186/s13046-022-02447-6)

Figure 2.

FUTURE RESEARCH PLANS

A key factor determining chemo- and immuno-resistance in non-small cell lung cancer is the ER stress-sensitive transcription factor CCAAT/Enhancer Binding Protein-β (C/EBP-β) that has two splicing isoforms: LAP and LIP that produce opposite effects and opposite metabolic rewiring via ncRNA-circuitries in murine embryonic fibroblasts. No data exists in cancer. We are investigating a series of competing endogenous RNAs (ceRNAs), predicted to control the splicing of C/EBP-β, to find new chemo-immunosenistizer strategy.

FUNDING ID (PAST 5 YERS)

- 2019-2022: Project title: STRATAGEM – New diagnostic and therapeutic tools against multidrug resistant tumours. Source: Horizon 2020.
- 2019-2023:Project title: Tipping the balance between ABCB1/ABCC1 and ABCA1: a new approach to reverse chemo-immuno-resistance in solid tumors. Source: AIRC, Italian Association for Cancer Research; Investigator Grant 2018 program.
- 2021-2023: Project title: A biological passport to rationalize the use of the immunotherapy in oncological patients in Piemonte. Source: CRT Foundation.
- 2022-2023: Project title: PANDORA – A Pan-European Educational Platform on Multidrug Resistant Tumours and Personalised Cancer Treatment. Source: Horizon Europe
- 2022-2023: Project title: Real-time monitoring of drug response in non-small cell lung cancer patient avatars. Source: San Paolo Foundation, Torino, Italy, EX-POST 2021 program
- 2023-2025: Project title: Inducing BRCAness in pancreatic cancer by modulating a glycolytic branch. Source: PRIN 2022 (MUR)

SELECTED PUBLICATIONS

Riganti Scopus ID

- Anobile DP, Salaroglio IC, Tabbò F, La Vecchia S, Akman M, Napoli F, Bungaro M, Benso F, Aldieri E, Bironzo P, Kopecka J, Passiglia F, Righi L, Novello S, Scagliotti GV, Riganti C. Autocrine 17-β-estradiol/estrogen receptor-α loop determines the response to immune-checkpoint inhibitors in non-small cell lung cancer. Clin Cancer Res. 2023 Jun 7:CCR-22-3949. doi: 10.1158/1078-0432.CCR-22-3949
- Salaroglio IC, Belisario DC, Akman M, La Vecchia S, Godel M, Anobile DP, Ortone G, Digiovanni S, Fontana S, Costamagna C, Rubinstein M, Kopecka J, Riganti C. Mitochondrial ROS drive resistance to chemotherapy and immune-killing in hypoxic non-small cell lung cancer. J Exp Clin Cancer Res 2022, 41:243; doi: 10.1186/s13046-022-02447-6
- Kopecka J, Salaroglio IC, Perez-Ruiz E, Sarmento-Ribeiro AB, Saponara S, De Las Rivas J, Riganti C. Hypoxia as a driver of resistance to immunotherapy. Drug Resist Updat 2021, 18: 100787. doi: 10.1016/j.drup.2021.100787.
- Salaroglio IC, Kopecka J, Napoli F, Pradotto M, Maletta F, Costardi L, Gagliasso M, Milosevic V, Ananthanarayanan P, Bironzo P, Tabbò F, Cartia CF, Passone E, Comunanza V, Ardissone F, Ruffini E, Bussolino F, Righi L, Novello S, Di Maio M, Papotti M, Scagliotti GV, Riganti C. Potential diagnostic and prognostic role of micro-environment in malignant pleural mesothelioma. J Thor Oncol 2019, 14(8):1458-1471; doi: 10.1016/j.jtho.2019.03.029
- Alexa-Stratulat T, Pešić M, Čipak Gašparović A, Trougakos IP, Riganti C.. What sustains the multidrug resistance phenotype beyond ABC efflux transporters? Looking beyond the tip of the iceberg. Drug Resist Updat 2019, 46:100643. doi: 10.1016/j.drup.2019.100643
- Castella B, Kopecka J, Sciancalepore P, Mandili G, Foglietta M, Mitro N, Caruso D, Novelli F, Riganti C*, Massaia M*. Mechanisms of phosphoantigen release and Vγ9Vδ2 T-cell activation by dendritic cells. Nat Commun 2017, 8:15663 * co-last authors. doi: 10.1038/ncomms15663
- Salaroglio IC, Panada E, Moiso E, Buondonno I, Provero P, Rubinstein M, Kopecka J, Riganti C. PERK induces resistance to cell death elicited by endoplasmic reticulum stress and chemotherapy. Mol Cancer 16:e91, 2017; doi: 10.1186/s12943-017-0657-0
- Riganti C, Kopecka J, Panada E, Barak S, Rubinstein M. The Role of C/EBP-β LIP in Multidrug Resistance. J Natl Cancer Inst 2015, 107(5): djv046; doi: 10.1093/jnci/djv046
- Gelsomino G, Corsetto PA, Campia I, Montorfano G, Kopecka J, Castella B, Gazzano E, Ghigo D, Rizzo AM, Riganti C. Omega 3 fatty acids chemosensitize multidrug resistant colon cancer cells by down-regulating cholesterol synthesis and altering detergent resistant membranes composition. Mol Cancer 2013; 12 (1):e137; doi: 10.1186/1476-4598-12-137
- Riganti C, Salaroglio IC, Caldera V, Campia I, Kopecka J, Mellai M, Annovazzi L, Bosia A, Ghigo D, Schiffer D. Temozolomide down-regulates P-glycoprotein expression in glioblastoma stem cells by interfering with the Wnt3a/GSK3/β-catenin pathway. Neuro-Oncol 2013; 15(11):1502-17; doi: 10.1093/neuonc/not104

GIUSEPPE SAGLIO

Translational Hematology Laboratory

BIOGRAPHICAL SKETCH

2020-present	Emeritus Professor of Hematology and Internal Medicine at the University of Turin
1990-2020	Full Professor in Internal Medicine – Univ. of Perugia (90/91), Univ. of Turin in Novara (91/98), Univ. of Turin in Turin (98-20)
1983-1990	Assistant Professor in Internal Medicine –University of Turin
1980-1983	Residency in Hematology at the University of Milan
1975-1980	Residency in Internal Medicine at the University of Turin
1975	Degree in Medicine at the University of Turin

Coordinator of the PhD programme in Medicine and Experimental Therapy of the Univ. of Turin

Director of the Department of Clinical and Biological Sciences of the same University.

He has published more than 600 peer-reviewed articles in the fields of molecular pathogenesis of haematological diseases, molecularly targeted therapy and molecular characterization of haematological malignancies.
In 2017, Prof Saglio was nominated Knight of the Italian Republic for scientific merits and for his long lasting service for the Italian University.

GROUP MEMBERS:

Paola Circosta *PhD, Post doc*
Marco Lucio Lolli *Associate Professor in Medicinal Chemistry*
Donatella Boschi *PhD, Associate Professor in Medicinal Chemistry*
Stefano Sainas *PhD, Assistant Professor in Medicinal Chemistry*
Marta Giorgis *PhD, Assistant Professor in Medicinal Chemistry*
Agnese Chiara Pippione *Assistant Professor in Medicinal Chemistry*
Valentina Gaidano *MD, PhD*
Alessandro Cignetti *MD, PhD*
Carmen Fava *MD, PhD*

RESEARCH ACTIVITY

In Acute Myeloid Leukemia (AML), leukemic cells lose their ability to differentiate into adult white blood cells, remaining immature and dysfunctional; they accumulate in the bone marrow, affecting the production of normal blood cells. Major advances have been made in the last years in the understanding of the genomic and epigenetic landscape of AML: it turned out that AML is an extremely heterogeneous disease, both biologically and clinically. As we learned from APL, differentiating therapy can force leukemic stem cells out of quiescence, significantly increasing the possibility of cure; moreover, despite existing side effects, it is generally better tolerated than chemotherapy, and could be offered to the elderlies. Efforts in dissecting the molecular complexity of acute myeloid leukemia (AML) have been finally crowned by the recent approval of several targeted therapies. However, compounds like FLT3 and IDH1/2 inhibitors do not address all AML subtypes nor every subclone in a single patient, potentially leading to clonal escape. A promising approach is the so-called synthetic lethality, i.e., a combination of treatments that results lethal to cells. Differentiation therapy could be an essential ingredient in this combination, forcing immature cells to mature and increasing their exposure to other drugs. Indeed, this strategy has already brought acute promyelocytic leukemia (APL) to a cure rate >90%. Recently, Dihydroorotate Dehydrogenase (DHODH) inhibitors were found to induce myeloid differentiation in AML models. DHODH is an enzyme located in the inner mitochondrial membrane, which catalyses the oxidation of dihydroorotate to orotate and is a fundamental enzyme in the de novo pyrimidine biosynthesis (figure1). Pyrimidines are crucial for the proliferation of living entities, as tumor cells. The current research focus is the characterization of new DHODH inhibitors and their preclinical study for the treatment of AML patients. This project starts from a fruitful collaboration, dedicated to the design and the biologically profiling of human DHODH inhibitors, that bond since 2017 the research group of the PI and of Medicinal Chemists at Department of Drug Science and Technology (DSTF) at the University of Turin. A novel class of DHODH inhibitors, with a strong differentiating and pro-apoptotic activity in vitro has been developed. We hypothesize that a therapy based on DHODH inhibitors could cause apoptosis and differentiation of leukemic cells and promote their sensitization to further drugs, leading to synthetic lethality. Moreover, we think that the evaluation of the molecular and metabolic profile of leukemic cells treated with DHODH inhibitors, and the correlation with response, will allow to identify specific predictive biomarkers.

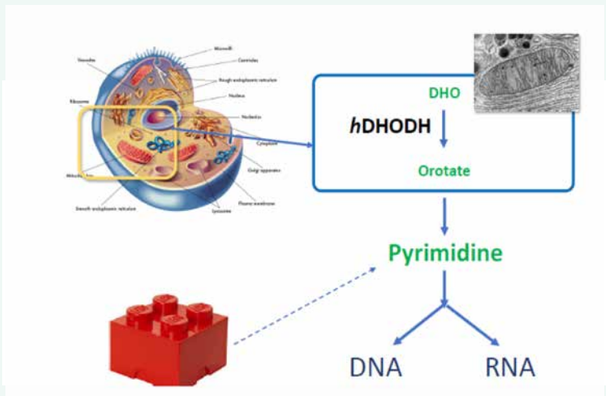


Figure 1.
hDHODH as drug target in AML

The main goals of the laboratory are therefore:

- Understanding how DHODH inhibitors induce differentiation and apoptosis, investigating molecular and metabolic pathways influenced by these drugs.
- Development of new, pharmacokinetically optimized, DHODH inhibitors with strong differentiating and pro-apoptotic activity on AML models.
- Identification of drugs that, associated with DHODH inhibitors, could lead to synthetic lethality.
- Identification of biomarkers predictive for response to DHODH inhibitors.
- Prognostic stratification of patients according to metabolomic profiles

The team is able to cover all the skills necessary for the design of a preclinical candidate effective in hematological field (Fig. 2).

In particular:

- Medicinal Chemistry. Development and optimization of new DHODH inhibitors
- Biochemistry. Assaying the candidate on AML models both in vitro and in vivo.
- Clinic: This part of the project will be focused on the recruitment of patients to test new drugs on primary cells and to correlate in vitro response with clinical features. We collaborate with University Division of Hematology and Cell Therapy, Mauriziano Hospital, University of Turin.

Thanks to excellent research experience in the field of medicinal chemistry, biological and clinical knowledge in 2020, the Spin Off of UniTo Drug Discovery and Clinic s.r.l. was funded.

Drug Discovery & Clinic

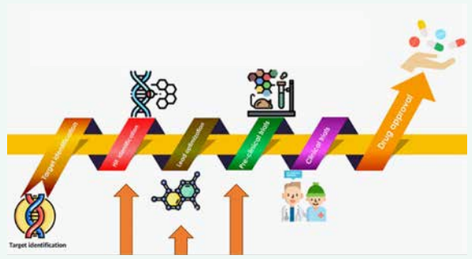


Figure 2.
Drug discovery and development pipeline

FUTURE RESEARCH

AML is still characterized by a disappointing 5-year 25 % overall survival. Moreover, many patients are elderly and could not tolerate chemotherapy or transplantation. Therefore, there is an urgent need for new therapeutic approaches that: i) minimize the risk of relapse, and ii) can be tolerated by unfit patients. The comprehensive approach could lead, in the next 5 years, to the creation of a new differentiating and pro-apoptotic drug ready to be tested in clinical trials; the drug will probably work in combination with other therapeutics, and will be offered to “biologically selected” patients. Our objective is to discover new biomarkers that can predict response to DHODHi enabling clinicians in recruiting responsive patients.

FUNDING ID (PAST 5 YEARS)

- 2019-2024: Dihydroorotate Dehydrogenase Inhibition to Induce Apoptosis and Differentiation in Myeloid Leukemias (DIORAMA)”- Italian Association for Cancer Research (AIRC) – Investigator Grant 2019 Project IG 2019 ID 23344

SELECTED PUBLICATIONS

- Sainas S et al., Targeting Acute Myelogenous Leukemia Using Potent Human Dihydroorotate Dehydrogenase Inhibitors Based on the 2-Hydroxypyrazolo[1,5-a]pyridine Scaffold: SAR of the Aryloxyaryl Moiety. .J Med Chem. 2022 Oct 13;65(19):12701-12724. doi:10.1021/acs.jmedchem.2c00496.
- Mughal TI et al., Perspective: Pivotal translational hematology and therapeutic insights in chronic myeloid hematopoietic stem cell malignancies. Hematol Oncol. 2022 Oct;40(4):491-504. doi: 10.1002/hon.2987.
- Houshmand M, et al, and Saglio G. Dihydroorotate dehydrogenase inhibition reveals metabolic vulnerability in chronic myeloid leukemia. Cell Death Dis. 2022 Jun 30;13(6):576. doi: 10.1038/s41419-022-05028-9.
- Houshmand M, et al., and Saglio G, Circosta P. Shedding Light on Targeting Chronic Myeloid Leukemia Stem Cells. J Clin Med. 2021 Dec 11;10(24):5805. doi: 10.3390/jcm10245805.
- Sainas S, et al., Targeting Acute Myelogenous Leukemia Using Potent Human Dihydroorotate Dehydrogenase Inhibitors Based on the 2-Hydroxypyrazolo[1,5-a]pyridine Scaffold: SAR of the Biphenyl Moiety. J Med Chem. 2021 May 13;64(9):5404-5428. doi: 10.1021/acs.jmedchem.0c01549.
- Houshmand M, et al., and Saglio G. Targeting Chronic Myeloid Leukemia Stem/Progenitor Cells Using Venetoclax-Loaded Immunoliposome. Cancers 2021, 13(6):1311. doi: 10.3390/cancers13061311.
- Gaidano V, et al., and Saglio G, Circosta P. The Synergism between DHODH Inhibitors and Dipyridamole Leads to Metabolic Lethality in Acute Myeloid Leukemia. Cancers 2021, Feb 28;13(5):1003. doi: 10.3390/cancers13051003.
- Saglio G, Fava C, Gale RP. Precision tyrosine kinase inhibitor dosing in chronic myeloid leukemia?
- Haematologica. 2019 May;104(5):862-864. doi: 10.3324/haematol.2018.214445.PMID: 31040230
- Houshmand M, et al, and Saglio G, Gale RP. Chronic myeloid leukemia stem cells.Leukemia. 2019 Jul;33(7):1543-1556. doi:10.1038/s41375-019-0490-0.
- Mughal TI, et al, and Saglio G, Van Etten RA. Emerging translational science discoveries, clonal approaches, and treatment trends in chronic myeloproliferative neoplasms. Hematol Oncol. 2019 Aug;37(3):240-252. doi: 10.1002/hon.2622
- Sainas S, et al., and Saglio G, Lolli ML. Targeting Myeloid Differentiation Using Potent 2-Hydroxypyrazolo[1,5- a]pyridine Scaffold-Based Human Dihydroorotate Dehydrogenase Inhibitors.
- J Med Chem. 2018 Jul 26;61(14):6034-6055. doi: 10.1021/acs.jmedchem.8b00373.

RICCARDO TAULLI

Molecular Biochemistry & Translational Oncology Laboratory

BIOGRAPHICAL SKETCH

2018-present	Associate Professor, University of Torino
2008-2018	Assistant Professor of Biochemistry, University of Torino
2015-present	Group Leader, Department of Oncology, University of Torino
2011-2013	Marie Curie Fellow, BIDMC, Harvard Medical School, Boston and Torino

GROUP MEMBERS:

Paola Circosta *PhD, Post doc*
Francesca Picca, Gouji Toyokawa *Post-doctoral fellows*
Jiahao Tao, Hafiz Muhammad Waqas *PhD students*
Eleonora D'Amore, Virginia Botta, Margherita Lambertini *Students*

RESEARCH ACTIVITY

Since the early stages of my scientific career, I have contributed to define the role of the HGF/MET axis in tumorigenesis. The expertise gained in gene silencing/expression allowed me to generate vector cassettes for the conditional expression of long and short RNAs, including microRNAs, lncRNAs and, more recently, circRNAs. By the employment of these genetic tools, I defined the biological significance of several microRNAs involved in c-Met regulation as well as in the control of epigenetic programs disrupted in cancer. During my training in the US, I acquired novel skills in RNA biology, in generating Genetically Engineered Mouse Models (GEMMs) and in deconstructing complex cancer phenotypes using genomic analyses and innovative pre-clinical platforms. In 2014, I moved back to Turin and thanks to an AIRC-Start-Up Grant, I established my independent research group.

The research carried out in my lab is mainly focused on coding and non-coding RNA regulatory circuits involved in tumor development and evolution, with the long-term aim to develop novel therapeutic strategies for cancer treatment. As the Head of the Laboratory of Molecular Biochemistry and Translational Oncology at the Department of Oncology, I have carried out deep ncRNA profiling approaches to identify novel actionable targets in cancer treatment (Oncogene 2015, Cell Cycle 2015; Cancer Res 2016). Concomitantly, we have generated a versatile GEMM for conditional and tissue-specific expression of HGF. This model revealed a critical role of the tumor microenvironment, exemplified by HGF, in the maintenance of stem cell quiescence as well as in the selection and expansion of rare MET-amplified subclones as a mechanism of acquired resistance (Elife, 2016). Moreover, we have recently demonstrated in primary co-cultures that specific epigenetic drug treatments exert a dual benefit by inhibiting cancer cell proliferation and playing an instructive role on the autologous immune system (Onco-immunology 2018).

Our current effort is focused on understanding the key molecular determinants of lung cancer adaptation and evolution under molecular targeted therapy pressure.

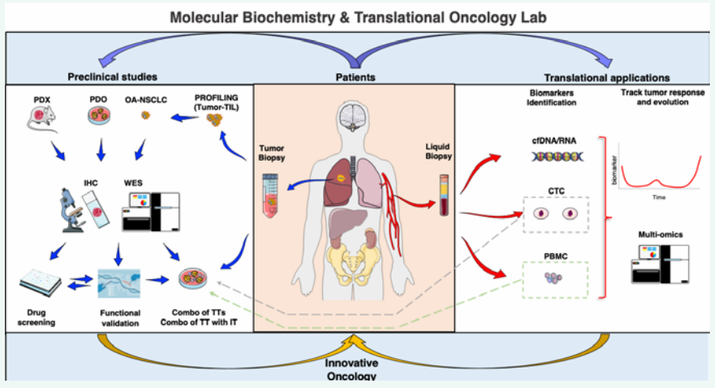


Figure 1.
The translational platform

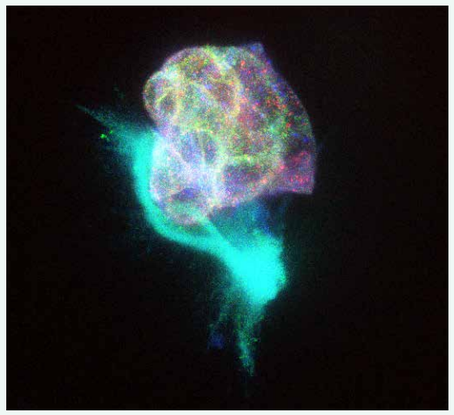


Figure 2.
Immunofluorescence analysis of a lung cancer organoid

FUTURE PLANS

We are developing a platform of tumor-derived organoids from advanced NSCLC to define the complex genetic and transcriptional interplay underlying lung cancer adaptation, persistence and evolution under molecular therapy pressure. By exploiting genomic profiling, microfluidic technologies, pharmacogenetic screening and functional validation analyses we aimed at identifying novel predictive biomarkers of tumor response and key molecular determinants involved in lung cancer plasticity and evolution. This approach will shed light on novel targetable therapeutic avenues for intercept and abrogate therapy evasion.

FUNDING ID

AIRC Start-Up Grant-15405 and AIRC-IG-25978 to R.T.; Italian Ministry of University and Research (RILO to R.T.); University of Turin, Progetti Ateneo, Compagnia di San Paolo Excellent Young PI (to R.T.). Grant for Internationalization to F.B.; National Foundation for Cancer Research Grant to F.B., PRIN 2022 to F.B.

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=8351211100

- → Bersani F, Picca F, Morena D, Righi L, Napoli F, Russo M, Oddo D, Rospo G, Negrino C, Castella B, Volante M, Listì A, Zambelli V, Benso F, Tabbò F, Bironzo P, Monteleone E, Poli V, Pietrantonio F, Di Nicolantonio F, Bardelli A, Ponzetto C, Novello S, Scagliotti GV, Taulli R. Exploring circular MET RNA as a potential biomarker in tumors exhibiting high MET activity. J Exp Clin Cancer Res. 2023;42:120.
- → Toyokawa G, Bersani F, Bironzo P, Picca F, Tabbò F, Haratake N, Takenaka T, Seto T, Yoshizumi T, Novello S, Scagliotti GV, Taulli R. Tumor plasticity and therapeutic resistance in oncogene-addicted non-small cell lung cancer: from preclinical observations to clinical implications. Crit Rev Oncol Hematol. 2023 Apr;184:103966.
- → Riganti C, Lingua ML, Salaroglio IC, Falcomatà C, Righi L, Morena D, Picca F, Oddo D, Kopecka J, Pradotto M, Libener R, Orecchia S, Bironzo P, Comunanza V, Bussolino F, Novello S, Scagliotti GV, Di Nicolantonio F, Taulli R. Bromodomain inhibition exerts its therapeutic potential in malignant pleural mesothelioma by promoting immunogenic cell death and changing the tumor immune-environment. Oncoimmunology. 2017DOI:10.1080/2162402X.2017.1398874.
- → Bersani F, Lingua MF, Morena D, Foglizzo V, Miretti S, Lanzetti L, Carrà G, Morotti A, Ala U, Provero P, Chiarle R, Singer S, Ladanyi M, Tuschl T, Ponzetto C, Taulli R. Deep Sequencing Reveals a Novel miR-22 Regulatory Network with Therapeutic Potential in Rhabdomyosarcoma. Cancer Res. 2016;76:6095-6106.
- → Morena D, Maestro N, Bersani F, Forni PE, Lingua MF, Foglizzo V, Šćepanović P, Miretti S, Morotti A, Shern JF, Khan J, Ala U, Provero P, Sala V, Crepaldi T, Gasparini P, Casanova M, Ferrari A, Sozzi G, Chiarle R, Ponzetto C, Taulli R. Hepatocyte Growth Factor-mediated satellite cells niche perturbation promotes development of distinct sarcoma subtypes. Elife. 2016 doi: 10.7554/eLife.12116.
- → Guarnerio J, Riccardi L, Taulli R, Maeda T, Wang G, Hobbs RM, Song MS, Sportoletti P, Bernardi R, Bronson RT, Castillo-Martin M, Cordon-Cardo C, Lunardi A, Pandolfi PP. A genetic platform to model sarcomagenesis from primary adult mesenchymal stem cells. Cancer Discov. 2015;5:396-409.
- → Lunardi A, Varmeh S, Chen M, Taulli R, Guarnerio J, Ala U, Seitzer N, Ishikawa T, Carver BS, Hobbs RM, Quarantotti V, Ng C, Berger AH, Nardella C, Poliseno L, Montironi R, Castillo-Martin M, Cordon-Cardo C, Signoretti S, Pandolfi PP. Suppression of CHK1 by ETS Family Members Promotes DNA Damage Response Bypass and Tumorigenesis. Cancer Discov. 2015;5:550-63.
- → Taulli R, Foglizzo V, Morena D, Coda D, Ala U, Bersani F, Maestro N, Ponzetto C. Failure to downregulate the BAF53a subunit of the SWI/SNF chromatin remodeling complex contributes to the differentiation block in rhabdomyosarcoma. Oncogene 33:2354-62, 2014
- → Kats LM, Reschke M, Taulli R, Pozdnyakova O, Burgess K, Bhargava P, Straley K, Karnik R, Meissner A, Small D, Su SM, Yen K, Zhang J, Pandolfi PP. Proto-oncogenic role of mutant IDH2 in leukemia initiation and maintenance. Cell Stem Cell. 2014;14:329-41
- → Taulli R, Bersani F, Foglizzo V, Linari A, Vigna E, Ladanyi M, Tuschl T, Ponzetto C. The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xeno-transplanted mice by promoting myogenic differentiation. J Clin Invest. 119:2366-78, 2009.

DANIELA TAVERNA

MicroRNAs in oncogenesis

BIOGRAPHICAL SKETCH

2019-Present	Full Professor of Molecular Biology, Dept. of Molecular Biotechnology and Health Sciences, University of Torino, Italy
2010-2019	Associate Professor of Molecular Biology, Dept. of Molecular Biotechnology and Health Sciences, University of Torino, Italy
2002-2010	Assistant Professor of Molecular Biology, Dept. of Oncology and later of Molecular Biotechnology and Health Sciences, University of Torino, Italy
1994-2002	Post-doctoral Fellow, Associate, Instructor Massachussets Institute of Technology (MIT), Cambridge, MA, USA
1993	Ph.D. in Molecular and Cellular Biology, University of Basel and Friedrich Miescher Institute, Basel, Switzerland
1988	Laurea/Master in Biological Sciences (Biomedical), summa cum laude and honours, University of Torino, Italy

GROUP MEMBERS:

Francesca Orso *RTDB/UPO, collaborator*
Sabrina Rizzolio *RTDA*
Lorena Quirico *Post-Doc*
Martina Coco *PhD student*
Sara Cozzubbo *PhD student*
Andrea Caiola *Technician*
Alessia Gecchele *MS student*
Alessia Marchese *MS student*

RESEARCH ACTIVITY

Solid cancer are usually fatal to patients when cells can detach from primary tumors and form metastases in distant organs, which are responsible for about 90% of deaths. Based on Global Cancer Statistics, in 2018, 18.1 million cancer cases were diagnosed around the world and 9.5 million people died because of cancer. It has also been anticipated that by 2040, the number of new cases will rise to 29.5 million with the consequent death of 16.4 million patients. Tumor dissemination depends on the capability of cancer cells to detach from the primary tumor mass, travel in the blood circulation and finally seed and form metastasis in distant organs. The tumor microenvironment, formed by Cancer Associated Fibroblasts (CAFs), Mesenchymal Stem Cells (MSCs), Myeloid-derived Suppressor Cells (MDSCs), Tumor Associated Macrophages (TAMs), Endothelial cells, Lymphocytes and Extracellular Matrix Proteins (ECMs), plays an essential role in malignancy. Thus, when investigating malignancy, it is essential focus on the microenvironment and on the cross-talk between tumor and stroma cells. Altered expression of protein-coding genes and of various RNAs, such as microRNAs (miRs), in tumor and stroma cells is linked to metastasis formation since it is responsible for biological and metabolic alterations. Because of the lack of efficacious therapies against tumor spreading, it is necessary to invest in new strategies to hit tumor and stroma cells to revert metabolic and adhesion/migration alterations.

Malignant Melanoma (MM) is one of the most invasive, therapy-resistant and metastatic tumors, with a recent dramatic increased incidence. Similarly, malignant Breast Cancer (BC), in particular Triple Negative (TN) BC, is highly aggressive and often fatal because of frequent dissemination in distant organs since specific therapies are still missing. Our laboratory aims to understand the role and molecular mechanism of specific miRs in MM and BC progression and to develop new tumor/stroma specific therapeutic strategies.

By comparing malignant versus non-malignant conditions, we could identify miR-214 and miR-148b as respectively upregulated and downregulated in MM and aggressive BC and, thanks to in vitro and in vivo function-

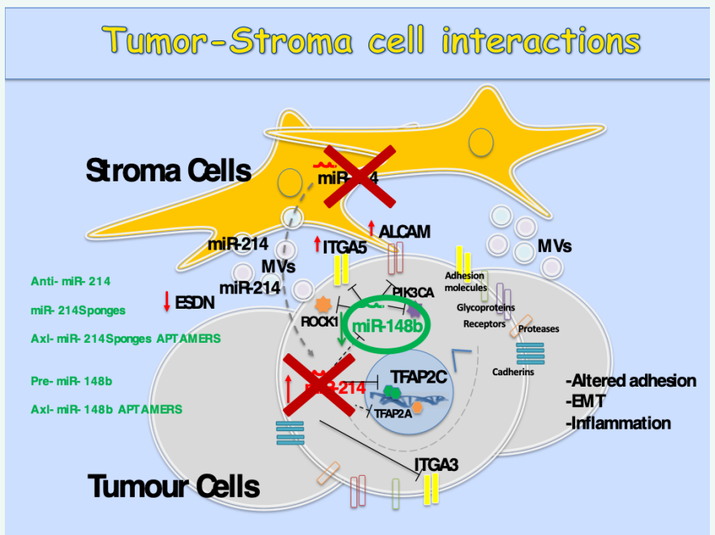


Figure 1.
miR-214 is highly present in cancer and stroma cells in malignant tumors. Chimeric aptamers can be used to specifically hit miR-214 in the different cells.

al studies, we evidenced the pro-metastatic function of miR-214 as well as the protective, anti-metastatic role of miR-148b. No major interventions on tumor growth were observed. Mechanistically, we evidenced that miR-214 could downregulate miR-148b and that, partially, the two small RNAs acted on the same axis. We know that, together, they can coordinate a metastatic molecular pathway which includes adhesion molecules (i.e. ALCAM, ITGA5) and transcription factors (TFAP2C) involved in the control of cell movement, invasion, extravasation and metastasis formation. We recently observed that high levels of miR-214 are present not only in tumor cells but also in the stroma counterparts, in particular in CAFs and that stroma miR-214 is highly relevant for cancer cell dissemination. See Fig. 1. We know that tumor cells stimulate miR-214 expression in stroma cells via the activation of IL-6/STAT3 pathway.

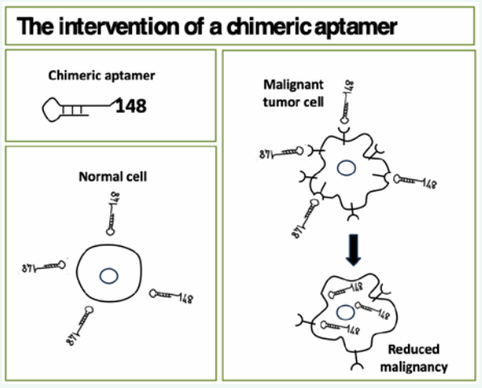


Figure 2.
Chimeric aptamers get internalized only in cells expressing the specific receptors, which are able to target.

From a therapeutic point of view, we first attempted to block miR-214 by systemically delivering anti-miR-214 to mice carrying tumors and observed relevant inhibition of metastasis formation. Then, we generated tools for the specific delivery of miR-148b or of miR-214 inhibitors (sponges). Precisely, we designed conjugates made of an aptamer (a short oligonucleotide able to recognize specific targets on the cell membrane), which recognizes axl, a tyrosine kinase receptor highly expressed on MM and aggressive BC cells but not on the normal counterparts, linked to miR-148b or miR-214sponge as in Fig. 2. We delivered axl-miR-148b (to upregulate miR-148b levels) or axl-miR-214sponge (to decrease miR-214 levels) to mice with tumors and obtained promising pre-clinical results suggesting that both conjugates can impair MM and TNBC dissemination, giving hope for a transfer to the clinics. We patented the conjugates and aim to analyze their relevance in clinical trials. These conjugates received the FIRST Intellectual Property Award (IPA) in Life Sciences and Healthcare, by the Italian Ministero dello Sviluppo Economico (MISE), in 2022.

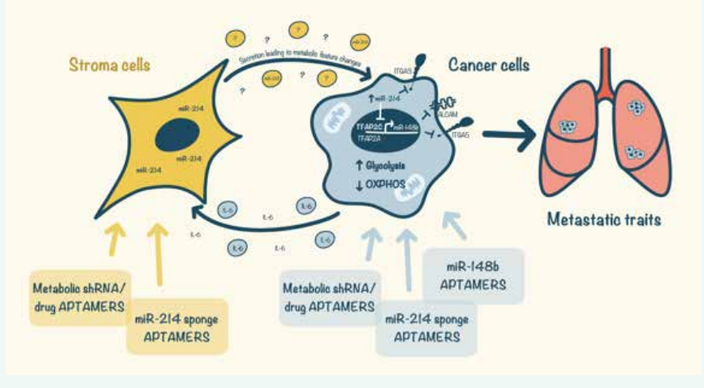


Figure 3.
miR-214, miR-148b or metabolic players as targets for therapy via chimeric aptamers for tumor or stroma cells.

FUTURE RESEARCH PLANS

Metabolic alterations play a major role in cancer progression and miRs are highly involved in metabolism. We have evidences that miR-214 and miR-148b are able to induce metabolic switches. Thus, we aim at identifying the molecular players associated with metabolism, which are controlled by these small RNAs and to generate therapeutic tools against them to use in combination with instruments able to hit miR-214 or increase miR-148b levels.

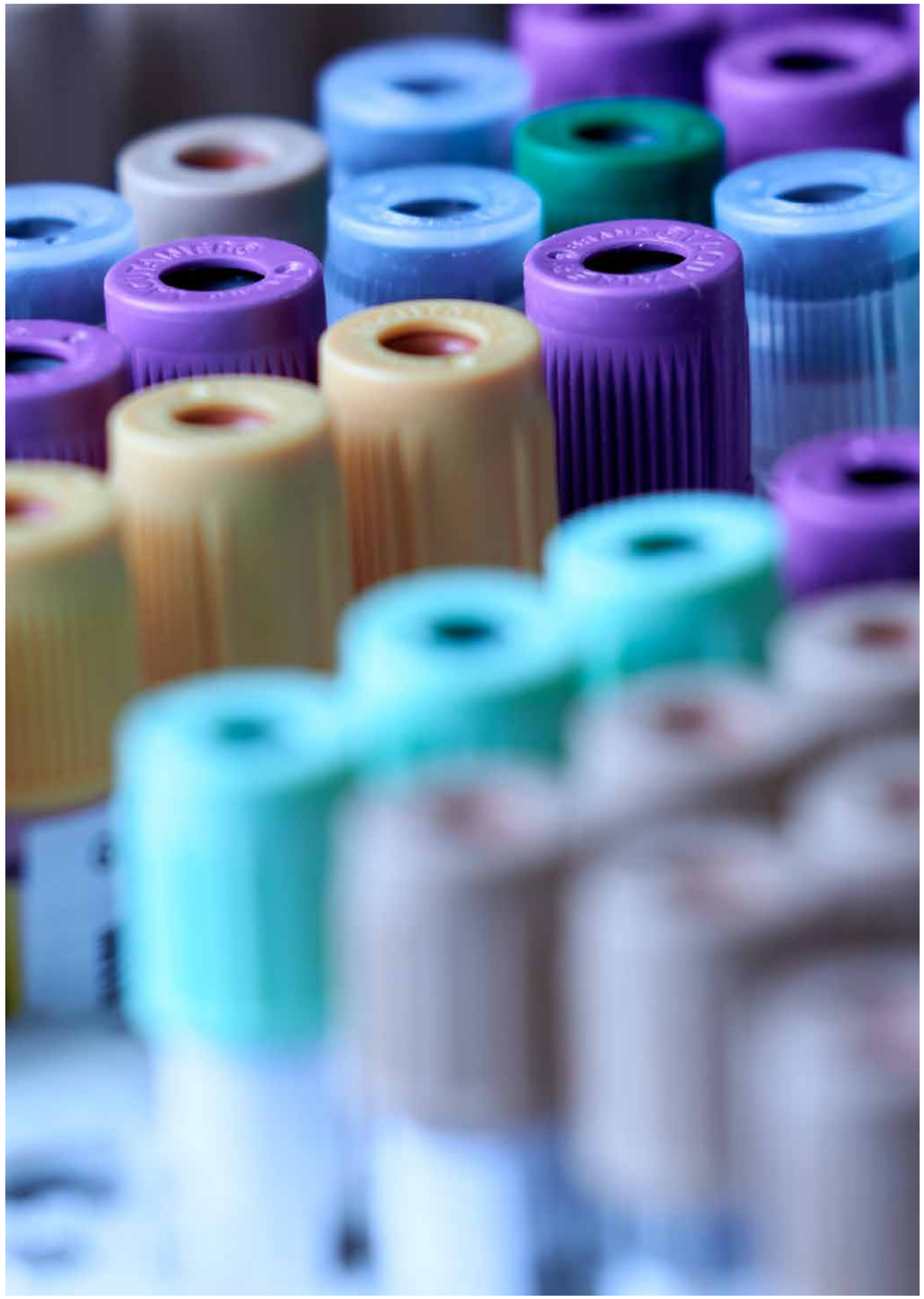
FUNDING ID (PAST 5 YEARS, MAJOR GRANTS)

- Fondazione CRT RF. 2018.1311 Bersagli terapeutici nella cura del melanoma, PI, 25,000 €
- Compagnia di San Paolo 2016/2018 - New therapeutic targets and biomarkers in melanoma - PI, 70,800 €
- Compagnia di San Paolo 2019/2021 - TArgeted Delivery of RNA Therapeutics to Cancer Cells, PI, 45,000 €
- Regione Piemonte 2014/2020 DEFLeCT - Digital tEchnology For Lung Cancer Treatment, co-PI, 70,000 €
- AIRC IG 2017 ID 20258-5 years, Role of miR-214 and miR-148b in tumor/stromal cell interactions and as therapeutic targets in cancer progression, PI, 587,000 €
- AIRC IG 2022 ID 272054-5 years, miR-214 and miR-148-driven metabolic alterations in tumor progression and chimeric aptamer-based targeted therapy, PI, 739, 000 €
- FISR 2021 Covid, Blood-based non-coding RNA Biomarkers to identify COVID-19, co-PI, 25,000 €
- Ricerca Sanitaria Finalizzata 2016/2023, Identification of new biomarkers for Oligoarticular Juvenile Idiopathic Arthritis by exosomal miRNA assessment in blood and synovial fluid, co-PI, 80,000 €.
- Intellectual Property Award 2021, Chimeric complex and its therap. use in cancer met. treatm., PI, 10,000 €
- PoC Instrument – Comp. di San Paolo 2019/2021, Complesso chimerico e suoi usi terapeutici, PI, 50,000 €
- PoC TOINPROVE, 2023/2024, CHIMERE (Apt CHImerico per il tratt. dei MElan REsist BRAFi), PI 50,000 €
- PNRR M4C2 Iniziativa 1.4 - Centri Nazionali (CN), RNA Therapy, 2022-2025, PI, 60, 000 €
- PRIN MUR 2022, 2023-2025, Influence of stromal miR-425-5p and miR-214 in Triple Negative Breast Cancer Progression and aptamer-based targeting intervention, co-PI, 122,600 €

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=6701587316

- Penna E., Orso F, Cimino D,….Taverna D. miR-214 coordinates melanoma progression by upregulating ALCAM through TFAP2 and miR-148b downmodulation. Cancer Res. 2013 Jul 1;73(13):4098-111. doi: 10.1158/0008-5472. CAN-12-3686. IF 12.701
- Cuiffo BG, Campagne A, ...Taverna D, Karnoub AE. MSC-regulated microRNAs converge on the transcription factor FOXP2 and promote breast cancer metastasis. Cell Stem Cell. 2014 Dec 4;15(6):762-74. doi: 10.1016/j.stem.2014.10.001. IF 24.633
- Wicki A, …Taverna D,…Xue G. Acquired Resistance to Clinical Cancer Therapy: A Twist in Physiological Signaling. Physiol Rev. 2016 Jul;96(3):805-29. doi: 10.1152/physrev.00024.2015. Review. IF 37.312
- Orso F, Quirico L,… Taverna D. miR-214 and miR-148b targeting inhibits dissemination of melanoma and breast cancer. Cancer Res. 2016 Jun 21, 1322.2015. doi: 10.1158/0008-5472, CAN-15-1322.IF 12.701.
- Comunanza V,….Taverna D, Bussolino F. VEGF blockade enhances the antitumor effect of BRAFV600E inhibition. EMBO Mol Med. 201 Feb;9(2):219-237. doi: 10.15252/emmm.201505774. IF 12.137
- Bellazzo A,…Taverna D,…Collavin L. Cell-autonomous and cell non-autonomous downregulation of tumor suppressor DAB2IP by microRNA-149-3p promotes aggressiveness of cancer cells. Cell Death Differ. 2018 Jul;25(7):1224 1238. doi: 10.1038/s41418-018-0088-5. Epub 2018 Mar 22. IF 15.828
- Dettori D,…Taverna D. Therapeutic Silencing of miR-214 Inhibits Tumor Progression in Multiple Mouse Models. Mol Ther. 2018 Aug 1;26(8):2008-2018. doi: 10.1016/j.ymthe.2018.05.020. IF 11.454
- Orso F, Quirico L, Dettori D, Coppo R, Virga F, Ferreira LC, Paoletti C, Baruffaldi D, Penna E, Taverna D. Role of miRNAs in tumor and endothelial cell interactions during tumor progression. Semin Cancer Biol. 2020 Feb;60:214-224. doi: 10.1016/j.semcancer.2019.07.024. Epub 2019 Aug 3. IF 15.707
- Virga F, ….Taverna D*, Mazzone M*. Macrophage miR-210 induction and metabolic reprogramming in response to pathogen interaction boost life-threatening inflammation. Sci Adv. 2021 May 7;7(19):eabf0466. doi: 10.1126/sciadv.abf0466. IF 14.136. * co-last authors.
- Orso F, …Taverna D. Stroma-derived miR-214 coordinates tumor dissemination. J Exp Clin Cancer Res. 2023 Jan 13;42(1):20. doi: 10.1186/s13046-022-02553-5. IF 12.658.



ENZO TERRENO

Imaging Lab

BIOGRAPHICAL SKETCH

2020 to date Coordinator of the Italian Node Multi-Modal Molecular Imaging (MMMI) within the European Research Infrastructure Consortium (ERIC) EuroBioImaging

2018 to date Full Professor of General and Inorganic Chemistry at the Department of Molecular Biotechnology and Health Sciences of the University of Torino

2007-2017 Associate Professor of General and Inorganic Chemistry at the Department of Chemistry (until 2012) and Molecular Biotechnology and Health Sciences of the University of Torino

1999-2007 Researcher Fellow of General and Inorganic Chemistry at the Department of Chemistry of the University of Torino

1997-1999 Manager of the NMR Laboratory at the Department of Chemistry IFM of the University of Torino

GROUP MEMBERS:

Francesca Garello *Research Fellow*
Gianluca Destro *Research Fellow*
Martina Capozza *Research Technician*
Carla Carrera *Research Technician*
Diana Costanzo *PhD student*
Rebecca Rizzo *PhD student*
Miriam Roberto *PhD student*
Alberto Mangia *PhD student*

RESEARCH ACTIVITY

The group is strongly committed to the design, in vitro chemical/biological characterization, and in vivo preclinical validation of imaging probes for various in vivo imaging technologies, primarily MRI, Optical, Nuclear Imaging, CT, and multimodal hybrid technologies. These probes are intended for both diagnostic and theranostic applications within the broad field of Molecular Imaging.

In addition to working with small molecules, the team has developed expertise in the preparation of various types of nanoparticles, including phospholipid- and polymer-based micelles, liposomes, nanoemulsions, and inorganic nanoparticles.

As the design of a Molecular Imaging probe typically involves the conjugation of an imaging reporter (dependent on the imaging technology of interest) with a targeting vector, the group has acquired extensive experience in synthesizing different types of vectors. These vectors encompass organic moieties, peptides, proteins, antibodies, and their derivatives.

The main areas of expertise, categorized according to the specific imaging technology, are as follows:

MRI

the team possesses extensive experience in the development and testing of MRI probes. Over the years, we have synthesized and thoroughly characterized metal-based probes acting as T1/T2 or CEST agents. Some of these probes have also been incorporated into nano- or micro-systems to enhance contrast, create multimodal systems (for instance, by decorating particles with fluorescent moieties), and achieve selective delivery through the attachment of targeting vectors, such as peptides or antibodies. This approach allows us to detect pathological conditions and monitor disease progression. More recently, we have explored the development of theranostic MRI probes. Theranostic probes have the potential to enable both diagnosis and therapy simultaneously. An example of a theranostic protocol developed by our group involves the use of ultrasound to locally stimulate the release of both the chemotherapeutic drug doxorubicin and an MRI contrast agent from liposomes, in a more specific and efficient manner than the commonly used therapeutic protocols (see Figure 1). Furthermore, our

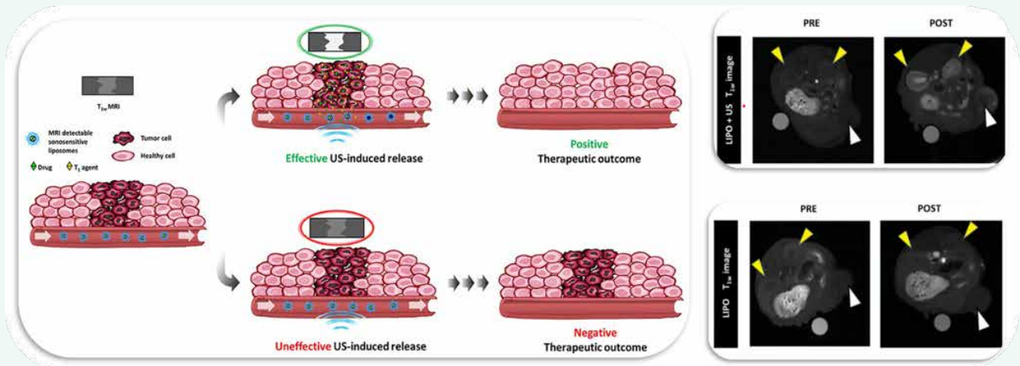


Figure 1.
Schematic representation of the theranostic protocol involving ultrasound stimulation for the release of doxorubicin and the Gd-based contrast agent, aimed at enhancing tumor treatment efficiency and predicting patient outcomes

group has also directed its focus towards more biocompatible (non-metallic) probes. This includes the synthesis of highly efficient 19F-based compounds, the development and testing of 19F-loaded nanosystems, which are ideal for imaging inflammatory pathologies, and the use of Chemical Shift Imaging Protocols, which enable the visualization of nanosystems containing highly protonated components without the need for additional metallic contrast agents.

Optical imaging

the team has experience creating targeted fluorescent probes with NIR emission and testing them in cell lines and in tumour bearing mice. These molecules can be used in fluorescence-guided surgery (FGS), which is an excellent option to allow a high spatial resolution discrimination between tumour and healthy tissues. In this area, we also possess expertise in dual-imaging agents and theragnostic, here, we produce new fluorescent probes for Guided Surgery and PhotoDynamic Therapy. The imaging probe is composed by: i) a targeting vector to cancer cells, and ii) a dye that can be detected by NIRF imaging and produce ROS for photodynamic therapy.

Nuclear Imaging

In this context, the group is taking its first steps. The main research lines are related to the development of new chemical reactions with potential translatability to radiolabelling with different radionuclides. On the other hand, the design of new chemical probes bearing fluorine-18 and iodine-123 and radiometals to study their behaviour towards cancer is ongoing.

CT

Whereas CT imaging of hard tissues such as bones and cartilages are very sensitive to X-rays, imaging of soft structures (fatty tissues or neoplastic formations) required contrast agents to improve the image performance. Bismuth-based small complex has been recently synthetized and tested in healthy mice showing excellent renal enhancement.

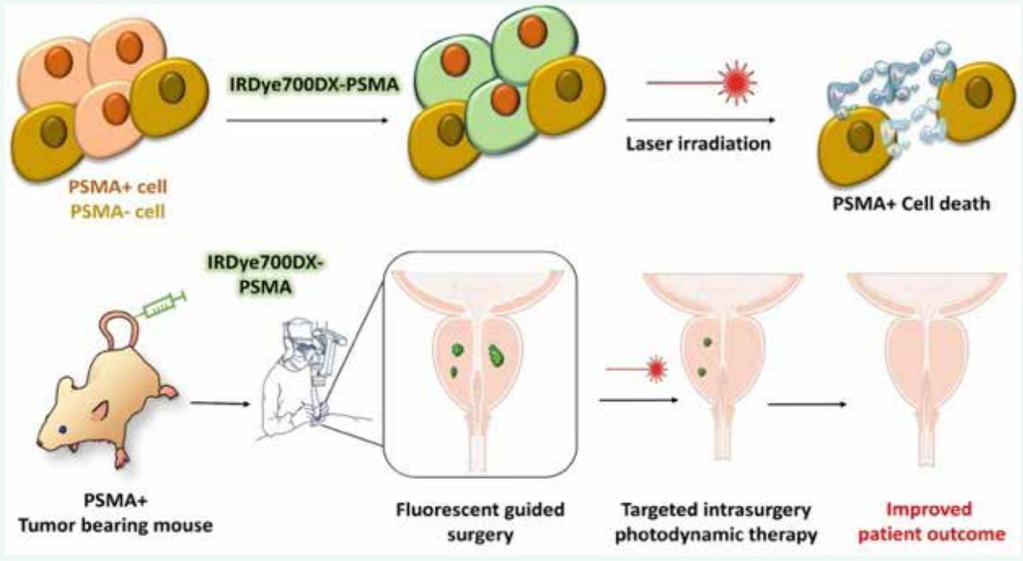


Figure 2.
Combined FGS and intra surgery PDT approach for prostate cancer treatment (ref 8).

FUTURE RESEARCH PLANS

Our future research will be dedicated to advancing diagnostic and theranostic tools and exploring novel avenues in various domains like: i) to broaden the application of ultrasound technology to trigger the release of nanocarrier content and facilitating cell sonoporation for drug activation in diverse cancer models, ii) to develop environmentally friendly (non-metallic) probes for Magnetic Resonance Imaging (MRI) (e.g., synthesis of innovative 19F-based probes), iii) to refine and optimize new protocols for Chemical Shift Imaging (CSI) of various types of free-label nanosystems.

In the realm of nuclear medicine, we aim to implement our novel radiolabeling strategies for creating new vectors and deepen our understanding of theranostics.

For our optical imaging initiatives, we have plans to enhance our library of compounds, with a focus on targeting a broader range of tumour types. Furthermore, we will employ ultrasound-guided techniques to facilitate the release of photosensitizers, thus enhancing the uptake and efficacy of photodynamic agents.

By pursuing these multifaceted research objectives, we aspire to contribute to the advancement of diagnostic and therapeutic approaches across various medical disciplines.

FUNDING ID (PAST 5 YEARS)

- FOE MUR: ERIC Eurobioimaging - Coordination of the Multi Modal Molecular Imaging Italian Node
- COST Action CA15209 (”European Network on NMR Relaxometry”)
- AIRC IG 2018 - n. 22041 “Boosting the efficacy of liposomal doxorubicin against ovarian cancer by local ultrasound stimulation under MRI guidance”
- PRIN 2018: “Rationally designed nanogels embedding paramagnetic ions as MRI probes”
- PNRR-4.2-3.1: Project “SEE LIFE - StrEngthEning the ItaLIan InFrastructure of Euro-bioimaging”
- PNC-E3: Project “INNOVA - Italian network of excellence for advanced diagnosis”
- PRIN 2022: Project “Design of paramagnetic metal complexes for improved MRI-guided drug-release applications”

SELECTED PUBLICATIONS

- Terreno E, Uggeri F, Aime S. Image guided therapy: the advent of theranostic agents. J Control Release. 2012 Jul 20;161(2):328-37. doi: 10.1016/j.jconrel.2012.05.028.
- Filippi M, Martinelli J, Mulas G, Ferraretto M, Teirlinck E, Botta M, Tei L, Terreno E. Dendrimersomes: a new vesicular nano-platform for MR-molecular imaging applications. Chem Commun (Camb). 2014 Apr 4;50(26):3453-6. doi: 10.1039/c3cc49584a. Epub 2014 Feb 19. PMID: 24553970.
- Pagoto A, Stefania R, Garello F, Arena F, Digilio G, Aime S, Terreno E. Paramagnetic Phospholipid-Based Micelles Targeting VCAM-1 Receptors for MRI Visualization of Inflammation. Bioconjug Chem. 2016 Aug 17;27(8):1921-30. doi: 10.1021/acs.bioconjchem.6b00308.
- Rizzitelli S, Giustetto P, Faletto D, Delli Castelli D, Aime S, Terreno E. The release of Doxorubicin from liposomes monitored by MRI and triggered by a combination of US stimuli led to a complete tumor regression in a breast cancer mouse model. J Control Release. 2016 May 28;230:57-63. doi: 10.1016/j.jconrel.2016.03.040.
- Capozza M, Blasi F, Valbusa G, Oliva P, Cabella C, Buonsanti F, Cordaro A, Pizzuto L, Maiocchi A, Poggi L. Photoacoustic imaging of integrin-overexpressing tumors using a novel ICG-based contrast agent in mice. Photoacoustics 11, 36-45 (2018). https://doi.org/10.1016/j.pacs.2018.07.007
- Filippi M, Garello F, Pasquino C, Arena F, Giustetto P, Antico F, Terreno E. Indocyanine green labeling for optical and photoacoustic imaging of mesenchymal stem cells after in vivo transplantation. J Biophotonics. 2019 May;12(5):e201800035. doi: 10.1002/jbio.201800035.
- Garello F, Boido M, Miglietti M, Bitonto V, Zenzola M, Filippi M, Arena F, Consolino L, Ghibaudi M, Terreno E. Imaging of Inflammation in Spinal Cord Injury: Novel Insights on the Usage of PFC-Based Contrast Agents. Biomedicines. 2021 Apr 3;9(4):379. doi: 10.3390/biomedicines9040379.
- Capozza M, Stefania R, Dinatale V, Bitonto V, Conti L, Grange C, Skovronova R, Terreno E. A Novel PS-MA-Targeted Probe for NIRF-Guided Surgery and Photodynamic Therapy: Synthesis and Preclinical Validation. Int J Mol Sci 23 (2022). https://doi.org/10.3390/ijms232112878
- Capozza M, Anemone A, Dhakan C, Della Peruta M, Bracesco M, Zullino S, Villano D, Terreno E, Longo D, Aime S.. GlucoCEST MRI for the Evaluation Response to Chemotherapeutic and Metabolic Treatments in a Murine Triple-Negative Breast Cancer: A Comparison with[(18)F]F-FDG-PET. Mol Imaging Biol 24, 126-134 (2022). https://doi.org/10.1007/s11307-021-01637-6
- Rizzo, R., Capozza, M., Carrera, C. & Terreno, E. Bi-HP-DO3A as a novel contrast agent for X-ray computed tomography. Sci Rep 13, 16747 (2023). https://doi.org/10.1038/s41598-023-43031-y

EMANUELA TOLOSANO

Heme-Iron Biology Unit

BIOGRAPHICAL SKETCH

1988	Graduated in Biology, University of Torino, Italy
1994	PhD in Endocrinological and Metabolical Sciences, University of Milano, Italy
1994-2000	Postdoctoral fellow, Dept. of Genetics, Biology and Biochemistry, Univ. of Torino, Italy
2001-2005	Assistant Professor, University of Torino, School of Medicine, Italy
2001 to date	Group leader, Molecular Biotechnology Center “Guido Tarone”, Torino, Italy
2005-2017	Associate Professor Biology, University of Torino, School of Medicine, Italy
2017 to date	Full Professor Biology, University of Torino, School of Medicine, Italy

GROUP MEMBERS:

Veronica Fiorito *Senior Post-doc*
Sara Petrillo *Senior Post-doc*
Anna Lucia Allocco *Dottoranda*
Francesco De Giorgio *Dottorando*

RESEARCH ACTIVITY

Heme, an iron-containing porphyrin, serves as the prosthetic group of proteins involved in various biological processes, such as oxygen transport and storage, cell respiration, drug metabolism, and gene expression control. Its role in regulating oxidative metabolism is crucial, as heme influences oxygen availability, electron transport chain complexes activity, and participates in tricarboxylic acid (TCA) cycle cataplerosis. Consequently, it is not surprising that disruptions in heme metabolism occur in numerous pathological conditions, including genetic diseases, hematological disorders, and cancer.

Maintaining cellular heme homeostasis relies on the coordinated expression and activity of enzymes and transporters involved in heme synthesis, acquisition from external sources, degradation, and transport within subcellular compartments and across the plasma membrane.

Our long-term research objective is to elucidate the mechanisms that regulate cellular heme homeostasis. By comprehending how heme homeostasis is upheld in healthy individuals and disrupted in pathological conditions, we aim to develop innovative therapeutic approaches.

Our research is primarily focused on characterizing the functional interplay between ALAS1, the initial and rate-limiting enzyme in the heme biosynthetic pathway, and FLVCR1, a heme transporter responsible for transporting heme out of mitochondria and across the plasma membrane. Through gene silencing techniques, genetic manipulation, and pharmacological interventions, we have established various cellular and animal models that either inhibit or enhance the heme synthesis-export system (Figure 1).

These models have enabled us to (i) identify biological processes that depend on a functional heme synthesis-export system (as exemplified in Figure 2), (ii) define the heme-controlled mechanisms underlying these processes, and (iii) design strategies to restore heme homeostasis and regain a healthy state or (iv) exploit heme dependencies to selectively target pathological cells such as cancer cells.

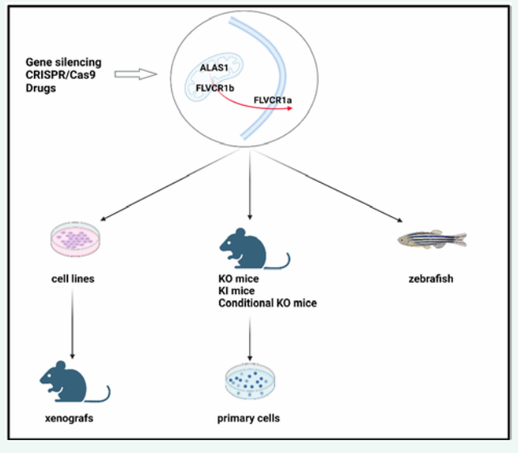


Figure 1.
Cellular and animal models available in the lab. We have developed cancer cell lines with modified heme synthesis-export systems, enabling us to study both inhibitory and enhancing effects. Additionally, we have created mouse models with knockout, knockin, or conditional alleles that perturb the heme system. Furthermore, we have zebrafish models with impaired heme synthesis. These models provide valuable tools for studying the underlying mechanisms. Cancer cell lines can be utilized to generate xenografts in mice, while primary cell cultures derived from the mouse models allow for detailed investigations into the molecular aspects of heme regulation. (Created by Biorender).

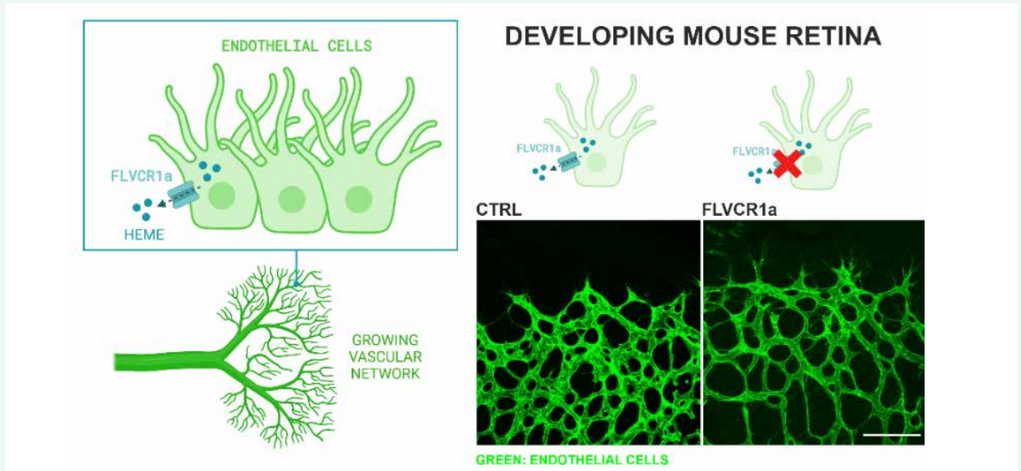


Figure 2.
Defective angiogenesis in endothelial specific Flvcr1a-null mice. The retinas were isolated from endothelial specific Flvcr1a-null pups and controls at P8 and stained with an antibody against the endothelial marker CD31. Scale bar: 100 µm. Deletion of Flvcr1a in endothelial cells results in impaired heme metabolism that cannot sustain the proper development of the vascular network.

FUTURE RESEARCH PLANS

We plan to utilize models that exhibit a malfunctioning heme synthesis-export system to accomplish the following objectives: (i) assess the influence of heme metabolism on the advancement and progression of tumors and leverage this system as a potential target for therapeutic interventions; (ii) gain a deeper understanding of the dependency of endothelial cells on a functional heme synthesis-export system to support both physiological and pathological angiogenesis; (iii) evaluate the potential impact of impaired heme synthesis-export system on liver and skeletal muscle, leading to alterations in glucolipid metabolism and potentially influencing susceptibility to the development of metabolic syndrome.

FUNDING ID (PAST 5 YERS)

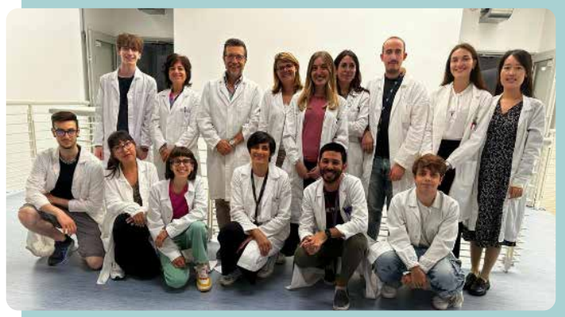
- Title of contract: Diagnosi e terapia del cancro al colon-retto: un approccio innovativo basato sul metabolismo del ferro-eme, 2018-2021, Fondazione CRT
- Title of contract: EV-ER - Vescicole extracellulari ed insufficienza d'organo, 2018-2022, Regione Piemonte - PIATTAFORMA TECNOLOGICA "SALUTE E BENESSERE"
- Title of contract: Exploiting heme-driven metabolic rewiring in tumor cell and tumor endothelial cell to control lung cancer progression, 2021-2026, AIRC
- Title: Defective heme transport in the development of congenital hydrocephalus, 2021-2026, NIH, 1R01NS123168-01.
- Title: Disentangling genetic, epigenetic and hormonal regulation of Fe/heme metabolism in the gender-specific nature of NAFLD (DEFENDER), 2023-2025, MIUR Italian Ministry of University and Research
- Title: Unsolved challenges in metabolic syndrome: the role of heme metabolism in liver and muscle gluco-lipid homeostasis, 2023-2025, MIUR Italian Ministry of University and Research

SELECTED PUBLICATIONS

Scopus ID

- Petrillo S. et al, "Endothelial cells require functional FLVCR1a during developmental and adult angiogenesis", Angiogenesis doi: 10.1007/s10456-023-09865-w (2023) https://doi.org/10.1007/s10456-023-09865-w
- Allocco, A.L. et al.,"Inhibition of Heme Export and/or Heme Synthesis Potentiates Metformin Anti-Proliferative Effect on Cancer Cell Lines". Cancers 2022, 14, 1230 (2022). https://doi.org/10.3390/cancers14051230
- Petrillo S et al.,"Endothelial Heme Dynamics Drive Cancer Cell Metabolism by Shaping the Tumor Microenvironment", Biomedicines 9(11):1557 (2021). https://doi.org/10.3390/biomedicines9111557
- Fiorito V et al., "The heme synthesis-export system regulates the tricarboxylic acid cycle flux and oxidative phosphorylation", Cell Reports 35(11):109252. https://doi.org/10.1016/j.celrep.2021.109252
- Petrillo S et al.., "Heme accumulation in endothelial cells impairs angiogenesis by triggering paraptosis", Cell Death & Differentiation 25:573–588 (2018). https://doi.org/10.1038/s41418-017-0001-7
- Chiabrando D et al.,"MUTATIONS IN THE HEME EXPORTER FLVCR1 CAUSE SENSORY NEURODEGENERATION WITH LOSS OF PAIN PERCEPTION', PLOS Genetics 12(12):e1006461 (2016). https://doi.org/10.1371/journal.pgen.1006461
- Vinchi F et al.,, "Hemopexin therapy reverts heme-induced pro-inflammatory phenotypic switching of macrophages in a mouse model of sickle cell disease", Blood 127(4): 473-486 (2016) https://doi.org/10.1182/blood-2015-08-663245
- Fiorito V et al., "Crucial role of Flvcr1a in the maintenance of intestinal heme homeostasis", Antioxidants & Redox Signaling 23(18): 1410-1423 (2015). https://doi.org/10.1089/ars.2014.6216
- Vinchi F et al., "Heme Exporter FLVCR1a Regulates Heme Synthesis and Degradation and Controls Activity of Cytochromes P450", Gastroenterology 146: 1325–1338 (2014). https://doi.org/10.1053/j.gastro.2014.01.053
- Vinchi F et al, "Hemopexin Therapy Improves Cardiovascular Function by Preventing Heme-Induced Endothelial Toxicity in Mouse Models of Hemolytic Diseases", Circulation 127(12):1317-29 (2013). https://doi.org/10.1161/circulationaha.112.130179

TUMOR IMMUNOLOGY LAB



NOVELLI, CAPPELLO AND CURCIO GROUP MEMBERS:

Dr Silvia Brugiapaglia, *PhD student*
Dr Ermes Candiello *PhD post-doc*
Dr Tullia Carradori *Manager of EnoApa biobank*
Ms Annamaria Cena *Lab manager*
Dr Giorgia Guadagnin *Research fellow*
Dr Florian Jazai *PhD student*
Dr Alessandro Scagliotti *PhD student*
Dr Ferdinando Spagnolo *PhD student*
Dr Giorgia Tiberi *PhD student*
Dr Paula Ariadna Diaz Villegas *Research fellow*
Dr Jianjian Zhang *PhD student*

NOVELLI, CAPPELLO AND CURCIO GROUP STUDENTS:

Mr Simone Basile
Mrs Elisa D'Angelo
Mr Ivan Depetro
Mrs Giulia Dessì
Mrs Erika Ostorero
Ms Davide Verrengia
Mrs Ylenia Vitolo

→ Follow at @ItalianPCC & i-pcc.org

Piazza Nizza 44 –
10126 Torino (TO)
0116706336
0116334463
immunotum@unito.it

INTRO

The Tumor Immunology Lab aims at the identification of novel therapeutic strategies for the treatment of a very dismal cancer such as the pancreatic ductal adenocarcinoma (PDAC). Dr Novelli and Dr Cappello have been studying the immune response in PDAC patients for twenty years, and Dr Curcio joined the group as an expert in anti-tumor vaccine eight years ago.

Our goal, indeed, is the development of immunotherapeutic strategies based on the use of a DNA vaccine that codes for molecules selected among those that when expressed by the cancer cells induced a strong antibody and cellular reaction in PDAC patients. For this each PI is focused on different aspects:

- → i) identification of molecular targets to promote an antitumor immune response (Dr Novelli);
- → ii) development of DNA vaccines, to set up and implement clinical protocols for pancreatic cancer immunotherapy (Dr Curcio);
- → iii) characterization of tumor microenvironment before or after different immunotherapy (Dr Cappello).

RESEARCH ACTIVITY

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in Western countries, and it is projected to become the second leading cause of cancer death by 2030. The overall 5-year survival rate is about 10% and the poor prognosis is attributed to failure in an early diagnosis, when the tumor would be surgically resectable, its propensity to disseminate, and its resistance to chemo, radio and immunotherapies. For these reasons, innovative and effective therapies that can be rapidly translated to clinical practice represent an urgent medical need.

Through a SERological Proteome Analysis we have identified a dozen antigens expressed by PDAC cancer cells and recognized by autoantibodies present in the sera of PDAC patients, but not in the sera of patients with other tumors, or affected by pancreatitis, or healthy donors. One of these antigens, alpha-enolase (ENO1), is recognized by more than 60% of PDAC patients, which also displayed T cells able to specifically being activated by ENO1. We used genetically engineered mice, that devel-

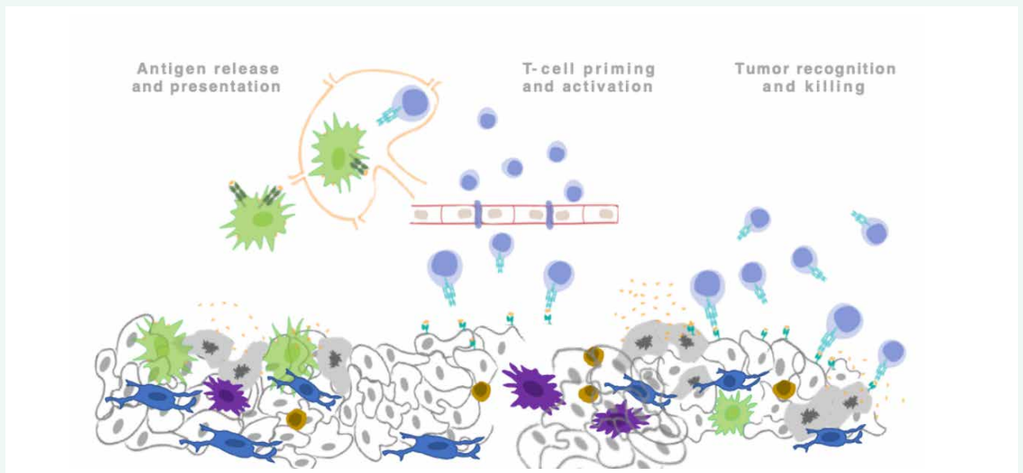


Figure 0.
Mucciolo G et al., Cancer Drug Resistance, May 2020;3 PMID: 35582441

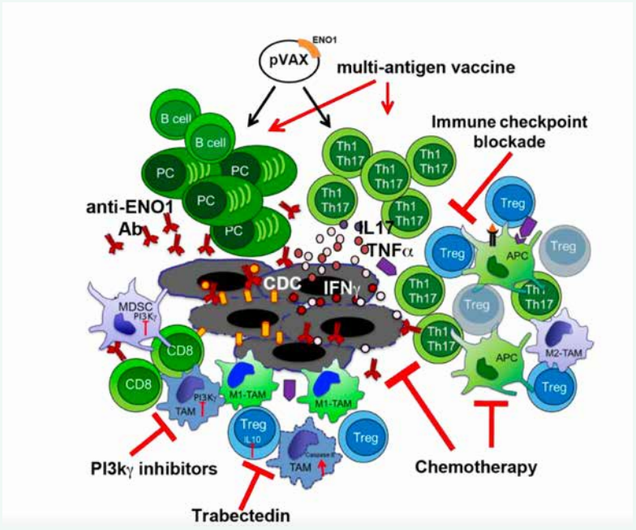


Figure 1.
ENO1 DNA vaccine and potential combo-partners. Cartoon shows the multiple effects of potential combinatory treatments (red arrows) with ENO1 DNA vaccination (black arrows) on the anti-tumor immune response. (DOI: 10.3390/cancers10020051)

op autochthonous lethal pancreatic carcinomas with different kinetics, to evaluate the protective effect of a DNA vaccine expressing human full-length ENO1. ENO1 DNA vaccine elicited an integrated humoral and cellular immune response that significantly extends mouse survival (Cappello et al., Gastroenterology 2013; DOI: 10.1053/j.gastro.2013.01.020). Unfortunately, after few months the immune pressure on tumors decline as consequence of the increase in myeloid suppressor and T regulatory cells. To overcome this problem, different strategies are ongoing: we are combining ENO1 vaccine with other antigens such as Ezrin, or with chemotherapy, or inhibitors of specific suppressive pathways (Figure 1).

Notably, combining ENO1 DNA vaccine with chemotherapy treatment significantly enhanced antitumor responses and efficacy to counteract tumor progression compared the vaccine or the chemotherapy alone. Indeed, the robust integrated cellular and humoral immune response led to a reduction of pancreatic tumor lesions (Mandili et al., JITC 2020 DOI: 10.1136/jitc-2020-001071, Figure 2).

In the recent years, the global scientific interest focused on the characterization of the crosstalk between immune, stromal and cancer cells. We have revealed how a cytokine, messenger between immune and non-immune cells, is profoundly changing the transcription and secre-

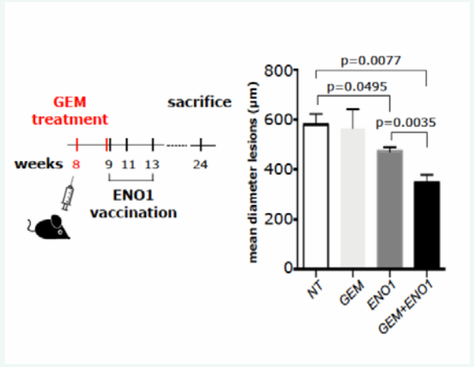


Figure 2.
Effect of combination of GEM and ENO1 vaccination in KC mice. On the left, schematic representation of mice treatment schedule. On the right, evaluation of the mean of tumor lesion diameter in treated mice sacrificed at 24 weeks of age. (DOI: 10.1136/jitc-2020-001071)

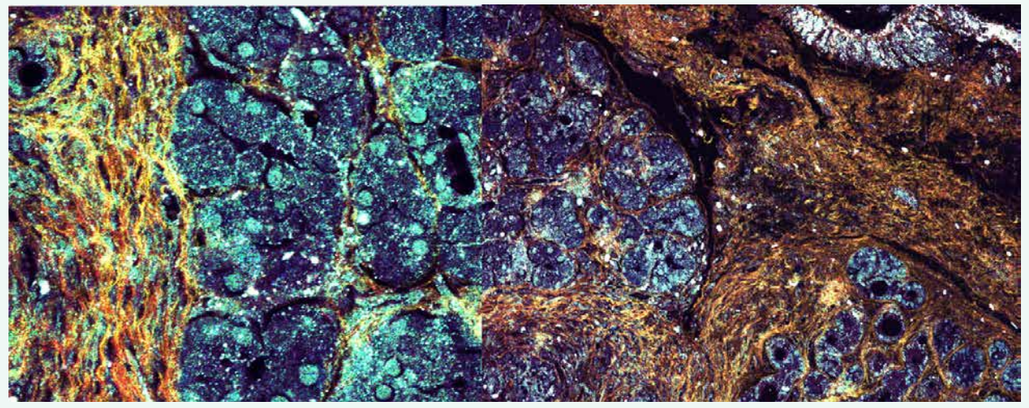


Figure 3.
Pancreatic tumor microenvironment visualized in second harmonic generation 2photon microscopy.

tome profile of cancer-associated fibroblast. This has the consequence of re-shape also the immune cell infiltrate, and globally the tumor microenvironment (Figure 3), and lead to a major pressure on cancer cells (Mucciolo et al., PNAS 2021; DOI: 10.1073/pnas.2020395118). Our interest is now open to other potential candidate targets that directly affect both immune anti-tumor response and tumor behavior. These results will pave the way to novel combination with conventional and immunotherapy to treat pancreatic cancer.

In the last years, our group has been also worked on the amelioration of ENO1 DNA vaccine and has designed a second generation of vaccine, called ENO3PEP which is matter of an international patent. From the lab will also stem NexTher, a startup aimed at the development of novel immunotherapies for the cure of pancreatic cancer.

SELECTED PUBBLICATIONS

- Srivastava AK, Guadagnin G, Cappello P and Novelli F. (2022) Post-Translational Modifications in Tumor-Associated Antigens as a Platform for Novel Immuno-Oncology Therapies. Cancers 2023, 15, 138. DOI:org/10.3390/cancers1501013
- Curcio C, Brugiapaglia S, Bulfamante S, Follia L, Cappello P, Novelli F. (2021) The Glycolytic Pathway as a Target for Novel Onco-Immunology Therapies in Pancreatic Cancer. Molecules 2021 Mar 15;26(6):1642. DOI: 10.3390/molecules26061642
- Mucciolo G, Curcio C, Roux C, Li WY, Capello M, Curto R, Chiarle R, Giordano D, Satolli MA, Lawlor R, Scarpa A, Lukac P, Stakheev D, Provero P, Vannucci L, Mak TW, Novelli F, Cappello P. (2021) IL17A critically shapes the transcriptional program of fibroblasts in pancreatic cancer and switches on their protumorigenic functions. Proc Natl Acad Sci U S A, 9;118:e2020395118, 2021; DOI: 10.1073/pnas.2020395118
- Mandili G, Curcio C, Bulfamante S, Follia L, Ferrero G, Mazza E, Principe M, Cordero F, Satolli MA, Spadi R, Evangelista A, Giordano D, Viet D, Cappello P, Novelli F. (2020) In pancreatic cancer, chemotherapy increases antitumor responses to tumor-associated antigens and potentiates DNA vaccination. J Immunother Cancer, 8:e001071, 2020; DOI: 10.1136/jitc-2020-001071
- Borgoni S, Iannello A, Cutrupi S, Allavena P, D'Incalci M, Novelli F and Cappello P. (2018) Depletion of tumor-associated macrophages switches the epigenetic profile of pancreatic cancer infiltrating T cells and restores their anti-tumor phenotype. OncoImmunology; Nov 13;7(2):e1393596. DOI: 10.1080/2162402X.2017.1393596
- Principe M, Borgoni S, Cascione M, Chattaragada MS, Ferri-Borgogno S, Capello M, Bulfamante S, Chapelle J, Di Modugno F, Defilippi P, Nisticò P, Cappello P, Riganti C, Leporatti S, Novelli F. (2017) Alpha-enolase (ENO1) controls alpha v/beta 3 integrin expression and regulates pancreatic cancer adhesion, invasion, and metastasis. J Hematol Oncol. 13;10(1):16. DOI: 10.1186/s13045-016-0385-8.
- Kaneda MM, Cappello P, Nguyen AV, Ralainirina N, Hardamon CR, Foubert P, Schmid MC, Sun P, Mose E, Bouvet M, Lowy AM, Valasek MA, Sasik R, Novelli F, Hirsch E, Varner JA. (2016) Macrophage PI3Kγ drives pancreatic ductal adenocarcinoma progression. Cancer Discov. 6(8):870-85. DOI: 10.1158/2159-8290
- Cappello P, Tonoli E, Curto R, Giordano D, Giovarelli M and Novelli F. (2016) Anti-α-enolase antibody limits the invasion of myeloid-derived suppressor cells and attenuates their restraining effector T cell response. OncoImmunology 21;5(5):e1112940. doi:10.1080/2162402X.2015.1112940
- Cappello P, Rolla S, Chiarle R, Principe M, Cavallo F, Perconti G, Feo S, Giovarelli M, Novelli F. (2013) Vaccination with ENO1 DNA prolongs survival of genetically engineered mice with pancreatic cancer. Gastroenterology. 144(5):1098-106. DOI:
- Tomaino B, Cappello P, Capello M, Fredolini C, Sperduti I, Migliorini P, Salacone P, Novarino A, Giacobino A, Ciuffreda L, Alessio M, Nisticò P, Scarpa A, Pederzoli P, Zhou W, Petricoin E, Liotta L, Giovarelli M, Milella M, Novelli F. (2011) Circulating autoantibodies to phosphorylated alpha-enolase are a hallmark of pancreatic cancer. J Proteome Res. 10(1); 105-112. DOI:

LINK SCOPUS ALLE PUBBLICAZIONI COMPLETE:

- https://www-scopus-com.bibliopass.unito.it/authid/detail.uri?authorId=7004313816
- https://www-scopus-com.bibliopass.unito.it/authid/detail.uri?authorId=7004378482
- https://www.scopus.com/authid/detail.uri?authorId=7006091781

FRANCESCO NOVELLI

TUMOR IMMUNOLOGY LAB - GROUP

BIOGRAPHICAL SKETCH

1986-1988	Tecnhogenetics s.p.a. Laboratories , S. Mauro T.se; Responsible of monoclonal antibody production;
1993-1994	Visiting scientist at Wistar Institute, Phyladelphia
1995	Visiting scientist at Cell Growth Regulation's Laboratory, King's College London
1996-2002	Assistant Professor, University of Turin, Dept. of Clinical and Biol. Sciences, Lab of Immunol, Orbassano, Italy
2001- 2002	Visiting Professor, Laboratory of Human Genetics of Infectious Diseases, Enfants Malades Medical School, Paris, France
2002-today	Director of Centro Ricerche in Medicina Sperimentale, Laboratory of Tumor Immunology Città della Salute e della Scienza di Torino
2006-2016	Associate Professor of Immunology (MED/04), Medical School, University of Torino, Università di Torino
2010-today	Responsible of the Cellular Immunology Unit of the Immunology Transplantation Department of the Azienda Ospedaliera-Universitaria Città della Salute e della Scienza di Torino
2014-today	Shareholder of NatiMab Therapeutics srl, Colleretto Giacosa (Torino)
2015-today	Member of Molecular Biotechnology Centre, University of Turin
2016-today	Full Professor of Immunology (MED/04), University of Turin, Dept. of Molecular Biotechnology and Healthy Science
2017-today	Chair of PhD Program in Molecular Medicine, University of Turin
2018-today	Director of the Department of Molecular Biotechnology and Health Sciences, University of Turin

See the group resarch at page 260

EDUCATION

- Master Degree, Biological Sciences, University of Turin, Turin, 1984
- NA (research training), Microbiology, University of Turin, Turin, 1988-1993
- NA (research training), Cytokine/Interferon signaling, Research Unit Hoffmann La Roche, Basel, Switzerland, 1988-1989
- PhD, Physiopathology, University of L'Aquila, L'Aquila, 1993

AWARDS AND HONORS

- 1988: Winner of National Exam for ENI-Sclavo fellowship in cancer research;
- 1990: Winner of National Exam for AIDS research fellowship by Istituto Superiore di Sanità;
- 2004: Winner of Author Prize-Cytokine & Growth Factor Reviews (Elsevier) Most Down-loaded review;
- 2013: Winner of Author Prize FEBS letter, Most Down-loaded review;
- 2014: National Habilitation as Full professor of General Pathology(MED/04).

PATENTS

- Ribovax Biotechnologies, SA (CH), Bioline Diagnostici srl (IT). Novelli F, Tomaino B, Cappello P. Novel antigens and antibodies associated to pancreatic ductal adenocarcinoma. WO/2008/037792A1;
- NatiMab Therapeutics srl, F. Novelli, B.Tomaino, P. Cappello. A HUMAN Alpha-enolase derived-monophosphorylated peptide useful for diagnosis and treatment of pancreatic adenocarcinoma and for generate antibodies to the above mentioned mophosphorylarted peptide and their uses; WO2011030302;
- NatiMab Therapeutics srl, F. Novelli, M. Capello, P. Cappello. A method and a kit for the in vitro diagnosis of pancreatic ductal adenocarcinoma or for determining the predisposition to pancreatic ductal adenocarcinoma WO 2013186748 A1.
- University of Turin, Italy; F. Novelli, P. Cappello, C. Curcio, S. Brugiapaglia. A DNA vaccine for use in the therapeutic and /or prophylactic treatment of tumor diseases; PCT E141583.

MOST RECENT GRANTS OBTAINED

- 2018: Italian Association for Cancer Research (AIRC)(IG 19931): "Combination of chemotherapy and immunotherapy as new paradigm to cure pancreatic cancer " Euro 523000 (five years)
- 2022: Piano Nazionale Di Ripresa E Resilienza (PNRR) missione 6 (PNRR-POC-2022-12375658): "A next generation DNA vaccine coding for immunodominant sequences of alpha-enolase to cure pancreatic cancer", 950000 (two years)
- 2023: AIRC (IG 27020): "Toward a novel precision chemo-immune therapy to cure pancreatic cancer" Euro 589270 (five years)
- 2023: PRIN 2022ELLA9L: "Combination of IDO-P3Kgd axis inhibition and tumor associated antigens DNA vaccination to cure pancreatic cancer" Euro 245236 (two years)

PAOLA CAPPELLO

TUMOR IMMUNOLOGY LAB - GROUP

BIOGRAPHICAL SKETCH

2001-2006	Post-Doctoral Research Scientist, Dept of Clinical and Biological Sciences, University of Turin;
2007-2011	Post-Doctoral Fellowship, Dept of Medicine and Experimental Oncology, University of Turin
2010-2011	Post-Doctoral Research Scientist, Campbell Family Institute for Breast Cancer Research, University Health Network, Toronto (ON, Canada)
2012-today	Over this time she has served as an Advisory Committee member for 18 Graduate Students, as 1st opponent on 3 PhD dissertations, University of Turin, Dept. Molecular Biotechnology and Healthy Sciences, Italy
2011-2019	Assistant Professor, Dept of Molecular Biotechnology and Health Sciences, University of Turin
2015-today	Member of Molecular Biotechnology Centre, University of Turin
2017	External reviewer of two PhD theses for the admission to the defence: University of Verona and University of Milan, PhD Program in Experimental Medicine and Medical Biotechnologies
2018-present	Member of the Department Board, Dept. Molecular Biotechnology and Health Sciences, University of Turin, Italy
2019-today	Associate Professor, Dept of Molecular Biotechnology and Health Sciences, University of Turin
2020-present	Deputy Director of the PhD program in Molecular Medicine at the University of Turin
mobility 2010-2011	Dr Cappello spent 16 months at the University Health Network, lab of Prof Tak W. Mak Toronto (Canada) at the Campbell Family Institute for Breast Cancer Research as post-doctoral scientist research working on the kinome and its implication in breast cancer metastatization. She went back in the University Health Network, lab of Prof Tak W. Mak Toronto (Canada) several times for weeks, as visiting professor to perform and discuss experimental results in collaboration.

See the group resarch at page 260

EDUCATION

- Master's degree, Biological Sciences, University of Turin, Italy, 1996
- Phd, Immunology, University of Turin, Italy, 2001

AWARDS AND HONORS

- 1997: Awarded of one of 2 fellowships for Research in Neuroscience, Cavalieri Ottolenghi Foundation, Italy;
- 1999: European Cytokine Society Student Award (1st Prize);
- 2004: "Honorary fellow of Immunology";
- 2007-2009: Awarded of one of the 40 FIRC-Fellowships, Associazione Italiana per la Ricerca sul Cancro (AIRC), Italy;
- 2016: National Habilitation as Associate professor of General Pathology(MED/04);
- 2021: Awarded of the prize entitled "Talent Women" 2nd Edition from the Cenacolo della Scienza e della Cultura, Locri, Italy;
- 2022: Awarded of the prize entitled "Amelia Earhart 2022: donne che volano nella medicina" from Interclub Zonta, Cuneo, Italy;
- 2022: National Habilitation as Full professor of General Pathology(MED/04).

PATENTS

- Ribovax Biotechnologies, SA (CH), Bioline Diagnostici srl (IT). Novelli F, Tomaino B, Cappello P. Novel antigens and antibodies associated to pancreatic ductal adenocarcinoma. WO/2008/037792A1;
- NatiMab Therapeutics srl, F. Novelli, B.Tomaino, P. Cappello. A HUMAN Alpha-enolase derived-monophosphorylated peptide useful for diagnosis and treatment of pancreatic adenocarcinoma and for generate antibodies to the above mentioned mophosphorylarted peptide and their uses; WO2011030302;
- NatiMab Therapeutics srl, F. Novelli, M. Capello, P. Cappello. A method and a kit for the in vitro diagnosis of pancreatic ductal adenocarcinoma or for determining the predisposition to pancreatic ductal adenocarcinoma WO 2013186748 A1.
- University of Turin, Italy; F. Novelli, P. Cappello, C. Curcio, S. Brugiapaglia. A DNA vaccine for use in the therapeutic and /or prophylactic treatment of tumor diseases; PCT E141583.

MOST RECENT GRANTS OBTAINED

- 2020: CRT Foundation (RF= 2020.0719): "Characterization of a new immunological target for the development of inhibitors to be used in the treatment of pancreatic cancer" Euro 35000 (two years)
- 2022: Italian Association for Cancer Research (AIRC)(IG 26341): "Harnessing IL17 family to trigger pancreatic cancer microenvironment and improve immunotherapy" Euro 503000 (five years)
- 2022: Fondazione Compagnia di San Paolo (ID 116390), Italy: "ENO3PEP: il vaccino di nuova generazione per la cura del tumore del pancreas, a un passo dalla clinica" Euro 50000 (six months)
- 2023: MAECI Italy-Singapore in collaboration with Singapore Immunology Network: "Development of a three-dimensional in vitro pancreatic tumor model to study the microenvironment and design more effective immunotherapies", 300000 (three years)
- 2023: Progetti di Rilevante Interesse Nazionale (PRIN), Ministero dell'Università e della Ricerca, co-PI: "Unraveling novel microenvironment-induced mechanisms in the pathogenesis of pancreatic intraductal papillary mucinous neoplasms" Euro 59845 (two years)

CLAUDIA CURCIO

TUMOR IMMUNOLOGY LAB - GROUP

BIOGRAPHICAL SKETCH

1999-2007	Internship and fellowship, Immunology Lab (prof. Guido Forni), Department of Clinical and Biology Sciences, University of Turin, Italy
2007-2016	Research Fellow and PhD Student, Vision Sciences Lab (prof. Leonardo Mastropasqua), Department of Medicine and Aging Sciences, University of Chieti-Pescara, Italy
2016-2019	Research Fellow, Tumor Immunology Lab (prof. Francesco Novelli), Department of Biotechnology and Health Sciences, University of Turin, Italy
2019-today	Assistant professor, Tumor Immunology Lab, Department of Biotechnology and Health Sciences, University of Turin, Italy
2021-today	Member of the Committee of the PhD program in “Scienze strategiche e giuridiche dell’innovazione per la difesa e la sicurezza”, University of Turin-CASD (Centro Alti Studi per la Difesa)
2021-today	Member of the Department Board, Dept. Molecular Biotechnology and Health Sciences, University of Turin, Italy
2023-today	Member of the Committee of the PhD program in “Molecular Medicine”, University of Turin

See the group resarch at page 260

EDUCATION

- Master’s degree, Biology, University of Turin, Italy, 2001
- Specialty’s degree, Clinical Pathology, University of Turin, Italy, 2006
- PhD program, Basic and applied medical sciences, University of Chieti-Pescara, 2015

AWARDS AND HONORS

- 2003: “Travel grant for best poster” Summer School in Cancer Immunology and Immunotherapy, Ionian Village, Greece;
- 2006: “SIICA award for best abstract” at IV Pontignano Workshop on Molecular basis and therapeutic implication of angiogenesis, Italy;
- 2007: “Pezcoller award for best abstract” at 19° Pezcoller symposium “Hypothesis driven clinical investigation in cancer”.

PATENTS

- -University of Turin, Italy; E. Hirsch, E. Ciraolo, C. Curcio, G. Forni. Regulation of expression of PI3Kbeta protein in tumors; WO/2008/099280.
- -University of Turin, Italy; F. Novelli, P. Cappello, C. Curcio, S. Brugiapaglia. A DNA vaccine for use in the therapeutic and /or prophylactic treatment of tumor diseases; PCT E141583.

MOST RECENT GRANTS OBTAINED

- 2022:University of Turin: “Combination of PI3Kgamma inhibition, chemotherapy and DNA vaccination to cure pancreatic cancer”, Euro 16.231 (1 year)
- 2023:Progetti di Rilevante Interesse Nazionale (PRIN), Ministero dell’Università e della Ricerca: “A supplementary diet as therapeutic vaccine for pancreayic cancer”, Euro 248.024 (2 years)

TIZIANA VAISITTI

Functional Genomics Lab – Vaisitti unit

BIOGRAPHICAL SKETCH

2021 to date	Associate Professor of Medical Genetics, Dept. of Medical Sciences, University of Torino, Italy
2017-2021	Assistant Professor of Medical Genetics, Dept. of Medical Sciences, University of Torino, Italy
2020 to date	Group Leader, Dept. of Medical Sciences and Molecular Biotechnology Center, University of Torino, Italy
2014-2016	Visiting Fellow, Dept. of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, USA
2009-2014	Senior Post-Doc, Dept. of Genetics, Biology and Biochemistry, University of Torino, Italy
2009-2009	Visiting Fellow, Dept. of Medical Biochemistry and Immunology, School of Medicine, Cardiff University, UK
2007-2007	Visiting Fellow, The Feinstein Institute for Medical Research, North Shore-Long Island Jewish, Manhasset, NY, USA

GROUP MEMBERS:

Lorenzo Brandimarte *PhD Student*
Giulia Omezzolli *Post-graduate Fellow*
Francesco Edoardo Vallone *Post-graduate Fellow*

RESEARCH ACTIVITY

The Research Unit is focused on two distinct topics: Richter's syndrome, a hematological cancer, and monogenic diseases leading to organ failure. Even though they appear as distinct fields of interest, there is a common denominator: the use of "omics" and functional approaches to understand pathogenetic mechanisms underlying the diseases.

Richter's syndrome (RS) is a high-grade lymphoma occurring in 10-12% of patients diagnosed with chronic lymphocytic leukemia, the most frequent leukemia in Western countries. RS is a clinical need since the current chemotherapeutic regimes result in no or limited responses. Our research is focused on the understanding of mechanisms characterizing RS cells biology and contributing to disease aggressiveness, with the final goal of identifying potential targets to be translated into the clinics.

To this, we are exploiting both primary cells and patient-derived xenograft (RS-PDX) models, that recapitulate the human disease allowing for functional analyses and genetic manipulation. Moreover, PDXs are useful tools to preclinically validate the impact of selective drugs. Primary RS cells and PDXs have been characterized from the genetic and transcriptomic points of view. The profiling of these cells and models allowed us to i) obtain a fingerprint of RS, ii) identify critical biological features (e.g., cellular metabolism, RNA translation) and iii) pinpoint molecular pathways that are detrimentally activated (e.g., apoptosis) or molecules that are aberrantly expressed (e.g., CD37, ROR1). We are currently validating and molecularly characterize these pathways to understand their pathogenic role and whether they can be exploited in a translational perspective (Figure 1).

In line with this view, recently, we have shown that RS cells are characterized by the expression of selective receptors whose expression is restricted to cancer cells, making them good candidates for targeting approaches. By exploiting RS-PDXs, we proved the ex-vivo and in vivo efficacy of antibody-drug conjugates (ADCs) targeting these receptors (Figure 2).

The second main topic of investigation of the Research Unit is the identification, by sequencing approaches, of variants relevant for the diagnosis of rare genetic diseases responsible for organ failure and their functional validation to better understand their clinical impact and meaning.

Approximately 80% of all rare diseases have a genetic background and most of them are monogenic conditions.

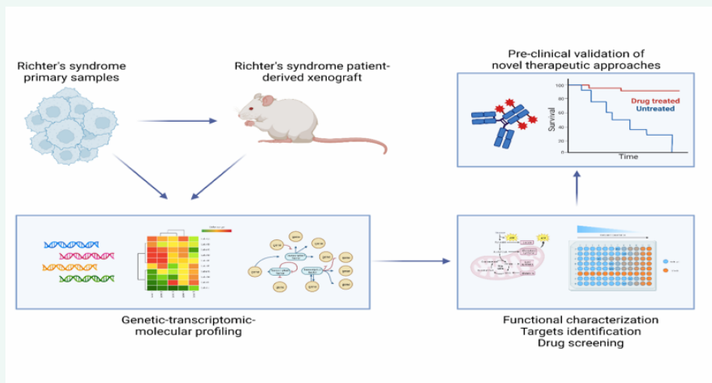


Figure 1.
Overview of Vaisitti's lab research on Richter's syndrome

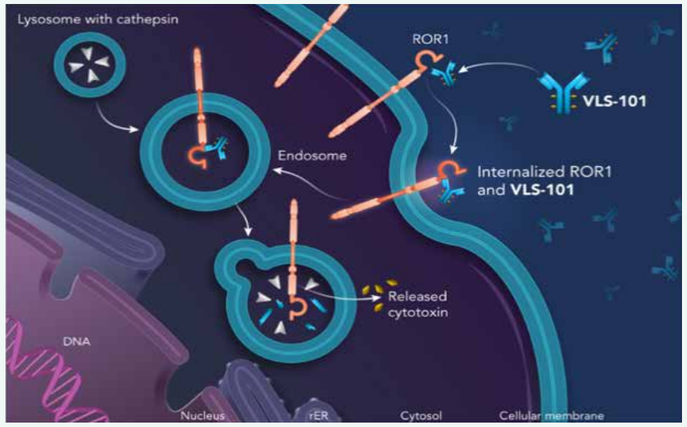


Figure 2.
Richter's syndrome cells express selective receptors that can be targeted using antibody drug conjugates (ADCs) limiting the toxic effect to cancer cells.

Early diagnosis and specific treatments can improve patients' quality of life. Transplantation is among the therapeutic options and represents the definitive treatment for end-stage organ failure, both in children and adults. A significant number of patients in transplant waiting lists is affected by rare monogenic diseases, while approximately 20% of them are undiagnosed.

We are applying a clinical exome sequencing based approach to uncover genetic variants that lead to organ failure, thus ultimately requiring organ transplantation. This approach allows the identification of causative genetic variants in a significant proportion of patients. However, one of the main drawbacks of applying sequencing technology in the diagnostic field, is the identification of the so called "variants of unknown significance - VUS", meaning that their role in contributing to the disease remains to be defined. This classification poses questions on the biological meaning of the identified variants and whether they may be involved in the pathogenesis of the disease. To address this point, it is crucial to reproduce these variants using gene editing approaches (e.g., CRISPR/Cas9) to generate ad hoc cellular models that allow for functional validation of the identified variants and ultimately to understand their role in the disease (Figure 3). This approach is currently used to validate VUS variants identified in patients with kidney diseases (e.g., polycystic kidney disease, hypocalciuric hypercalcemia). This part of the research is performed in a joint effort with the group of Prof. Deaglio.

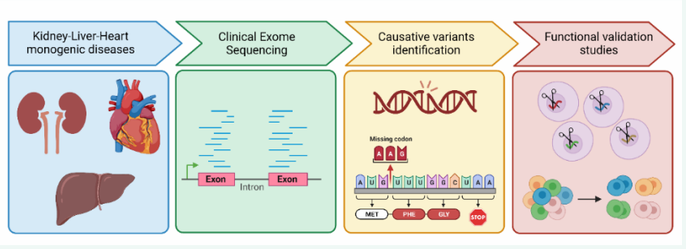


Figure 3.
Schematic workflow from the identification of genetic variants responsible for rare monogenic diseases leading to organ failure to their functional validation to understand their pathogenic role.

FUTURE RESEARCH PLANS:

Regarding the first topic, the available DNA and RNA sequencing data are a treasure trove of information and may open for novel paths of investigation. Specifically, we plan to more deeply characterize those pathways that emerged as the most differentially expressed between the CLL and RS phases and that contribute to the aberrant behavior of these neoplastic cells (e.g., DNA repair mechanisms, migration, apoptosis). Molecular characterization is a necessary step to explore novel therapeutic opportunities.

Regarding the second topic, we plan to investigate the genetic basis of the clinical heterogeneity typical of some monogenic diseases by re-analyzing genetic data and looking for variants, besides the causative ones, in genes that may act as disease modifiers and characterize their role.

FUNDING ID (PAST 5 YERS)

- Italian Ministry of Health, Young Investigator Grant "Analysis of the in vitro and in vivo role of ET-1/ETAR and CD38/CD31 axes in chronic lymphocytic leukemia: prognostic, functional and therapeutic implications"
- Italian Ministry of Health, Young Investigator Grant "Highlighting the tumorigenic role of long non-coding RNA in patients with Anaplastic Large cell Lymphoma".
- Italian Association for Cancer Research (AIRC) – My First AIRC Grant "Probing Richter's syndrome by multiple "omics" approaches to find its Achille's heel".
- Fondazione CRT - Erogazioni Ordinarie 2021 "Analysis of the therapeutic response of Richter's syndrome cells: targeting novel molecular players".
- Fondazione Ricerca Molinette – Ricerca Scientifica d'Eccellenza "DeRiMe – Deciphering Richter's Syndrome Metabolism to identify novel therapeutic opportunities".
- Progetti di Rilevanza Nazionale – PRIN 20229LATWH "Cell homing and apoptosis resistance in Richter's syndrome: 2 features opening translational opportunities".

SELECTED PUBLICATIONS

SCOPUS ID Link: https://www.scopus.com/authid/detail.uri?authorId=14066641800

- Vaisitti T, Gaudino F, Ouk S, Moscvin M, Vitale N, Serra S, Arruga F, Zakrzewski JL, Liou HC, Allan JN, Furman RR, Deaglio S. “Targeting metabolism and survival in chronic lymphocytic leukemia and Richter syndrome cells by a novel NF-κB inhibitor.” Haematologica. 2017 Nov;102(11):1878-1889. doi: 10.3324/haematol.2017.173419
- Vaisitti T, Braggio E, Allan JN, Arruga F, Serra S, Zamò A, Tam W, Chadburn A, Furman RR, Deaglio S. “Novel Richter Syndrome Xenograft Models to Study Genetic Architecture, Biology, and Therapy Responses.” Cancer Res. 2018 Jul 1;78(13):3413-3420. doi: 10.1158/0008-5472.CAN-17-4004
- Vaisitti T, Arruga F, Vitale N, Lee TT, Ko M, Chadburn A, Braggio E, Di Napoli A, Iannello A, Allan JN, Miller LL, Lannutti BJ, Furman RR, Jessen KA, Deaglio S. “ROR1 targeting with the antibody-drug conjugate VLS-101 is effective in Richter syndrome patient-derived xenograft mouse models.” Blood. 2021 Jun 17;137(24):3365-3377. doi: 10.1182/blood.2020008404.
- Iannello A, Vitale N, Coma S, Arruga F, Chadburn A, Di Napoli A, Laudanna C, Allan JN, Furman RR, Pachter JA, Deaglio S, Vaisitti T. “Synergistic efficacy of the dual PI3K-δ/γ inhibitor duvelisib with the Bcl-2 inhibitor venetoclax in Richter syndrome PDX models.” Blood. 2021 Jun 17;137(24):3378-3389. doi: 10.1182/blood.2020010187.
- Chakraborty S, Martines C, Porro F, Fortunati I, Bonato A, Dimishkovska M, Piazza S, Yadav BS, Innocenti I, Fazio R, Vaisitti T, Deaglio S, Zamò A, Dimovski AJ, Laurenti L, Efremov DG. “B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation.” Blood. 2021 Sep 23;138(12):1053-1066. doi: 10.1182/blood.2020008276.
- Vaisitti T, Vitale N, Micillo M, Brandimarte L, Iannello A, Papotti MG, Jaksic O, Lopez G, Di Napoli A, Cutrin JC, Orlik C, Kulke M, Pahl A, Deaglio S. “Anti-CD37 α-amanitin-conjugated antibodies as potential therapeutic weapons for Richter syndrome.” Blood. 2022 Sep 29;140(13):1565-1569. doi: 10.1182/blood.2022016211.
- Martines C, Chakraborty S, Vujovikj M, Gobessi S, Vaisitti T, Deaglio S, Laurenti L, Dimovski AJ, Efremov DG. “Macrophage- and BCR-derived but not TLR-derived signals support the growth of CLL and Richter syndrome murine models in vivo.” Blood. 2022 Dec 1;140(22):2335-2347. doi: 10.1182/blood.2022016272.
- Vaisitti T, Sorbini M, Callegari M, Kalantari S, Bracciamà V, Arruga F, Vanzino SB, Rendine S, Togliatto G, Giachino D, Pelle A, Cocchi E, Benvenuta C, Baldovino S, Rollino C, Fenoglio R, Sciascia S, Tamagnone M, Vitale C, Calabrese G, Biancone L, Bussolino S, Savoldi S, Borzumati M, Cantaluppi V, Chiappero F, Ungari S, Peruzzi L, Roccatello D, Amoroso A, Deaglio S. “Clinical exome sequencing is a powerful tool in the diagnostic flow of monogenic kidney diseases: an Italian experience.” J Nephrol. 2021 Oct;34(5):1767-1781. doi: 10.1007/s40620-020-00898-8.
- Vaisitti T, Peritore D, Magistroni P, Ricci A, Lombardini L, Gringeri E, Catalano S, Spada M, Sciveres M, Di Giorgio A, Limongelli G, Varrenti M, Gerosa G, Terzi A, Pace Napoleone C, Amodeo A, Ragni L, Dello Strologo L, Benetti E, Fontana I, Testa S, Peruzzi L, Mitrotti A, Abbate S, Comai G, Gotti E, Schiavon M, Boffini M, De Angelis D, Bertani A, Pinelli D, Torre M, Poggi C, Deaglio S, Cardillo M, Amoroso A. “The frequency of rare and monogenic diseases in pediatric organ transplant recipients in Italy.” Orphanet J Rare Dis. 2021 Sep 4;16(1):374. doi: 10.1186/s13023-021-02013-x.
- Vaisitti T, Bracciamà V, Faini AC, Brach Del Prever GM, Callegari M, Kalantari S, Mioli F, Romeo CM, Luca M, Camilla R, Mattozzi F, Gianoglio B, Peruzzi L, Amoroso A, Deaglio S. “The role of genetic testing in the diagnostic workflow of pediatric patients with kidney diseases: the experience of a single institution.” Hum Genomics. 2023 Feb 13;17(1):10. doi: 10.1186/s40246-023-00456-w.

SONJA VISENTIN

CASSMEDCHEM- MucLab

BIOGRAPHICAL SKETCH

Prof. Sonia Visentin is an associate professor in the scientific disciplinary sector Chim/08 at the Department of Molecular Biotechnology and Health Sciences, University of Turin.

1994 University of Torino M.D.IN PHARMACY Title: Studies of potential NO-donors

1995-1998 University of Torino, Department of Drug Science and Technology; PhD IN MEDICINAL CHEMISTRY Title: Synthesis, structure- activity relationship of NO-donors/Ca2+-channel antagonist hybrids

1998-1999 University of Torino, Department of Drug Science and Technology; POST-DOCTORAL FELLOWSHIP Title: Studies on calcium channel modulators

1998 University of Torino, Department of Drug Science and Technology; SCHOLARSHIP ASSIGNED BY PRIMITIVE IPERTROPHIC CARDIOMYOPATHY CONSORTIUM C.A.I.P

Eng. Lorenzo Sardelli

Dr. Olga Valentina Garbero

Dr. Enrica Frasca

GROUP MEMBERS:

Lorenzo Sardelli
Olga Valentina
Enrica Frasca

RESEARCH ACTIVITY

Whatever we do in life, it is not possible to exist and function without the world and the things it offers. This is true also for our body: lungs need oxygen and muscles need energy to move. In other words, resources need to get from the outside to the inside. Hence, our body cannot be a closed system and parts of our “inside” are unavoidably in touch with parts of the “outside”. Surprisingly, the very first interface between our body tissues and the overwhelming numbers and types of external stimuli is very often a misunderstood and disqualified actor. Despite its fundamental role, this gatekeeper was named, in our opinion, by one of the most unpleasant, disturbing and underestimated names of biology: mucus.

Our research is centred on the remarkable and yet not fully understood properties of mucus. Employing a highly interdisciplinary approach, we consider mucus not merely as a passive element of human mucosal tissues, but rather as a truly active agent that, through its unique compositional, chemical, mechanical, and biological characteristics, performs essential functions crucial for maintaining our state of health. For these reasons, our primary focus is on the main and key type of proteins forming mucus: the mucins.

Mucins are a family of high molecular weight, heavily glycosylated proteins that confer the protective and lubrication properties of mucus in various tissues and fluids throughout the body, including the respiratory, gastrointestinal, and reproductive tracts.

Several diseases are associated with alterations in mucins production, secretion, or function. For example, it is well-known that mucins are overproduced in the lungs of cystic fibrosis (CF) patients, leading to impaired mucins clearance and increased susceptibility to respiratory infections. With our interdisciplinary approach among chemistry, material science and industry, we developed Bac3Gel, a new and robust Mucins-based technological tool to challenge CF infections (Figure 1). In the case of Bac3Gel, we used mucins as components of an in vitro model exhibiting the key characteristics of the mucus environment, where we observed that the low permeability limit the amount of drug that encounters bacteria, thus affecting its efficacy.

Since the evaluation of efficacy and bioavailability of potential therapeutic molecules depends heavily on permeability assessments, it is an essential step to include mucins in available permeability assay, thus maximising the probability of clinical success. For this reason, we constantly optimise and adapt our mucins-based technology to be integrated within standard and newly-developed tools for rapid-permeability profiling. With our collaborations in industry, we provided insights into the

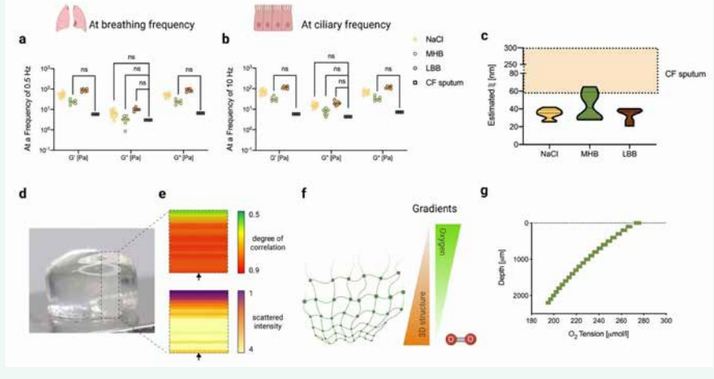


Figure 1.
Bac3Gel: mucins-containing hydrogel with gradients of mechanical (A, B), structural (C, D and E) and physical (F) properties that are bioinspired from the mucus of cystic fibrosis patients.

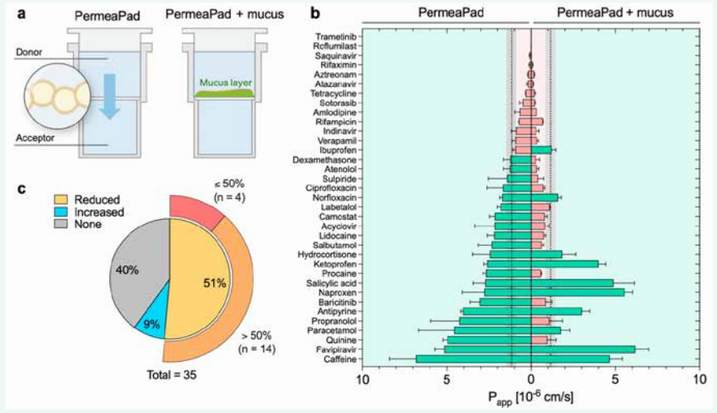


Figure 2.
The integrated system of mucins-containing Bac3Gel hydrogel with PermeaPad 96-well plate (A) expose the impact of mucus on the apparent permeability of the investigated compounds (B), showing the potent overall filtering power of mucus compared to control group (C).

permeability mechanisms of an heterogeneous dataset of drugs in commercial products such as Permeapad, unveiling the physicochemical properties ruling the permeation flux.

In our fundings, mucins and mucus showed in vitro their pivotal role as the primary active filter of our body, with the capability of governing the diffusion of drugs from “outside” to the “inside” (Figure 2). Despite the remarkable technological advancements and our understanding of human diseases, the successful translation of these advances into therapeutic solutions remains a significant challenge. We aimed at revolutionising the current drug delivery strategies by drawing inspiration from nature itself and harnessing the potential of our body’s primary defence mechanism, mucins, as carriers for therapeutic agents.

The implementation of our innovative approach culminated in the development of an entirely new category of nanocarriers: NanoMug (Figure 3). The NanoMug nanocarriers represent a new breakthrough in nanotechnology, as they are made of the distinctive components of mucus, the mucins, conferring remarkable glycosylated bioinspired-branches to the nanocarriers and providing mucoadhesive properties in a unique and stand-alone system. These features enable a high affinity of mucosomes for a wide range of molecules, as well as natural compatibility with both prokaryotic and eukaryotic cells. This makes our approach a suitable nanosystem for tailored drug delivery, but also lays the groundwork for exploring diverse applications simultaneously, including pioneering fields such as gene therapy and cutting-edge diagnostics.

FUTURE RESEARCH PLANS

Embracing the advances provided by Bac3Gel as a bioinspired environment and NanoMug as a new category of nanocarriers, our natural future research perspective relies on applying our technologies in the frontiers of pharmaceutical sciences, each one considered as a stand-alone tool and in combination. We plan to expand the formulations of Bac3Gel to model both healthy and pathological environments, including cultures of healthy and pathological strains of bacteria or microbiota populations. We also aim at using NanoMugs to craft cutting-edge high-throughput screening tools, as well as to advance the delivery of drugs for cancer treatment and to implement new strategies of immunomodulation.

By exploiting the synergy between Bac3Gel and NanoMug, we pursue to stand at the threshold where science and innovation hold the key to unlock mysteries of biology, in the reimaging of drug delivery and pharmaceutical science.

FUNDING ID (PAST 5 YEARS)

- 2020 Project title: Nanoparticelle per combattere la resistenza batterica, Progetti di Ricerca di Ateneo PoC Instrument - Compagnia di San Paolo 2019/20212019.2260
- 2021 Project title Mucus4COVID: un prototipo di modello in vitro per determinare il ruolo del muco polmonare nell’infezione di Sars-Cov2, la sua trasmissione e lo sviluppo di terapie efficaci per bloccare la (Mucus4Covid), progressione della malattia FISR 2020 Covid
- 2022 Project title: Smart NIR dye-based wound dressings to fight bacteria Grant for Internationalization - GFI - Programmazione Triennale 21-23
- 2023 Project title: New generation of substrates to harness the full power of microorganisms, Bac3Gel LDA, EIC accelerator 2023
- 2023 Project title: Epithelial Exchange Surfaces – From organizing principles to novel culture models of the gatekeepers of the body (SurfEx) HORIZON-TMA-MSCA-DN
- 2023 Project title: Multitasking mucosomes bioinspired from mucin immunomodulatory and antimicrobial activity: the turning point of the host-pathogen interaction? (MITIGATE), TRAPEZIO - Linea 1 - Paving the way to research excellence and talent attraction
- 2023 Project title: Multitasking mucosomes: empowering the immune response of the host while treatment respiratory pathologies (MUMMI), PRIN 2022
- 2023 Project title: Nature-inspired prophylactic lubricants against HIV-1 and HSV-2 (NatProLub), EIC Pathfinder 2023

SELECTED PUBLICATIONS

Scopus ID: https://www.scopus.com/authid/detail.uri?authorId=7004184798

- Peneda Pacheco, et al., Heterogeneity Governs 3D-Cultures of Clinically Relevant Microbial Communities (2023) Advanced Functional Materials DOI: 10.1002/adfm.202306116
- Butnarasu, C., Pontremoli, C., Moran Plata, M.J., Barbero, N., Visentin, S. Squaraine Dyes as Fluorescent Turn-on Probes for Mucins: A Step Toward Selectivity (2023) Photochemistry and Photobiology, 99 (2), pp. 562-569. DOI: 10.1111/php.13722
- Butnarasu, C., Garbero, O.V., Petrini, P., Visai, L., Visentin, S. Permeability Assessment of a High-Throughput Mucosal Platform (2023) Pharmaceutics, 15 (2), art. no. 380 DOI: 10.3390/pharmaceutics15020380
- Butnarasu, C., Petrini, P., Bracotti, F., Visai, L., Guagliano, G., Fiorio Pla, A., Sansone, E., Petrillo, S., Visentin, S. Mucosomes: Intrinsically Mucoadhesive Glycosylated Mucin Nanoparticles as Multi-Drug Delivery Platform (2022) Advanced Healthcare Materials, 11 (15), art. no. 2200340, DOI: 10.1002/adhm.202200340
- Kretschmer, M., et al., T., Yan, H. Synthetic Mucin Gels with Self-Healing Properties Augment Lubricity and Inhibit HIV-1 and HSV-2 Transmission (2022) Advanced Science, 9 (32), art. no.2203898 DOI: 10.1002/advs.202203898
- Sardelli, L., Vangosa, F.B., Merli, M., Ziccarelli, A., Visentin, S., Visai, L., Petrini, P. Bioinspired in vitro intestinal mucus model for 3D-dynamic culture of bacteria (2022) Biomaterials Advances, 139, art. no. 213022. DOI: 10.1016/j.bioadv.2022.213022
- Ghigo, A., et al., A PI3Kγ mimetic peptide triggers CFTR gating, bronchodilation, and reduced inflammation in obstructive airway diseases (2022) Science Translational Medicine, 14 (638), art. no. abl6328. DOI: 10.1126/scitranslmed.abl6328
- Butnarasu, C., Caron, G., Pacheco, D.P., Petrini, P., Visentin, S. Cystic Fibrosis Mucus Model to Design More Efficient Drug Therapies (2022) Molecular Pharmaceutics, 19 (2), pp. 520-531. DOI: 10.1021/acs.molpharmaceut.1c00644
- Guillaume, O., Butnarasu, C., Visentin, S., Reimhult, E. Interplay between biofilm microenvironment and pathogenicity of Pseudomonas aeruginosa in cystic fibrosis lung chronic infection (2022) Biofilm, 4, art. no. 100089. DOI: 10.1016/j.bioflm.2022.100089
- Peneda Pacheco, D., Suárez Vargas, N., Visentin, S., Petrini, P. From tissue engineering to engineering tissues: The role and application of: The vitro models (2021) Biomaterials Science, 9 (1), DOI: 10.1039/d0bm01097a
- Butnarasu, C., Barbero, N., Barolo, C., Visentin, S. Squaraine dyes as fluorescent turn-on sensors for the detection of porcine gastric mucin: A spectroscopic and kinetic study(2020) Journal of Photochemistry and Photobiology B: Biology, 205, art. no. 111838. DOI: 10.1016/j.jphotobiol.2020.111838

Università di Torino

MBC

Molecular Biotechnology Center
Guido Tarone

Dipartimento di Biotecnologie Molecolari e Scienze per la Salute

UNIVERSITÀ DI TORINO

2023 - 2027 DIPARTIMENTO DI ECCELLENZA
Ministero dell'Università e della Ricerca

Dipartimento di Oncologia

UNIVERSITÀ DI TORINO

2023 - 2027 DIPARTIMENTO DI ECCELLENZA
Ministero dell'Università e della Ricerca

Dipartimento di Neuroscienze "Rita Levi Montalcini"

UNIVERSITÀ DI TORINO

2023 - 2027 DIPARTIMENTO DI ECCELLENZA
Ministero dell'Università e della Ricerca

DBios

Università di Torino

Molecular Biotechnology Center
Guido Tarone