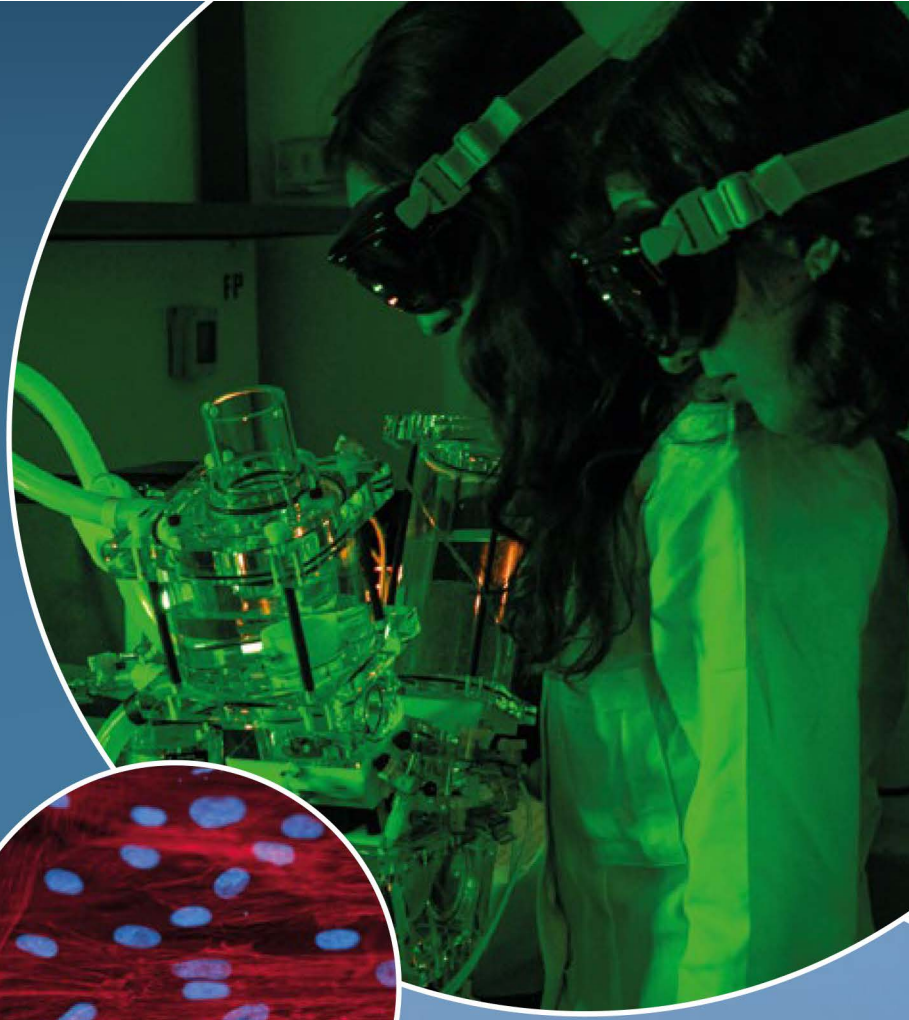
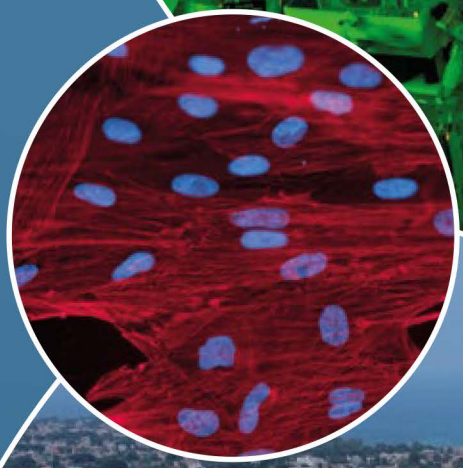
Fondazione Ri.MED

ANNUAL REPORT 2022





Fondazione Ri.MED

FOUNDING PARTNERS

Presidenza del Consiglio dei Ministri

Regione Siciliana

Consiglio Nazionale delle Ricerche

University of Pittsburgh

UPMC LIFE CHANGING MEDICINE

PARTNER

UPMC IRCCS ISMETT Palermo

Paolo Aquilanti

PRESIDENT AND CEO

Ri.MED has closed successfully 2022, moving forward important steps towards the realization of its missions. I am proud that we have achieved positive results both in terms of research, training and scientific dissemination, as well as in terms of construction of our Biomedical Research and Biotechnology Center, also thanks to the support of our founding partners: the Presidency of the Italian Council of Ministers, the Region of Sicily and the Italian National Research Center (CNR), the University of Pittsburgh (UP) and the University of Pittsburgh Medical Center (UPMC).

Ri.MED keeps on growing: new professionals have been appointed in order to strengthen our research teams, specific technology platforms and projects: during 2022, Ri.MED has recruited 14 new resources and hosted 25 fellows and 31 PhD students. Nurturing new talents and training highly qualified resources is one of our missions, which also contributes to increasing local competitiveness.

The pandemic highly impacted the BRBC project timeline and 2022's challenge has been the war in Ukraine, which is still ongoing and is causing a shortage of building materials all over Europe. Despite those unpredictable events, a good amount of resilience, combined with the passion of all the people involved in the project, are bringing the center to life, which will be completed by 2025. Ri.MED has been increasingly committed in 2022 to Carini, the community that will host the BRBC, also in terms of public engagement: with the event "Carini, scienza in cantiere", the Foundation disseminated scientific knowledge while at the same time involving and informing people on the construction of the research center. Photo exhibitions, film programs, meetings with school groups and workshops were offered from April to June at the Castle of Carini, involving all the schools of the area and hundreds of citizens.

Our research network is becoming more and more competitive: sixteen agreements were signed in 2022 and today Ri.MED boasts a total 45 agreements for the development of research activities and technological innovation with European and U.S. institutions. In 2022, four new research funds have been granted to the Ri.MED Foundation and there has also been a strong effort to submit new grant requests. Researchers have also created strategic partnerships with SMEs and start-up businesses for the development of new products, whose tangible outcomes are the more than 30 patents. At the same time, technology platforms and research areas have been consolidated, with the continuous support of the administrative office.

Our 2022 International Symposium on "Inflammation and Aging" was a successful event - the first one in person after the pandemic - highlighting also the strength of the Ri.MED-ISMETT cluster, with the underlying contribution of UPMC and UP. That is the reason why our annual Research Retreat has become, starting from this year, a "joint cluster retreat".

I am grateful to all the people at Ri.MED who, with passion and knowledge, contribute every day to the development of our vision.

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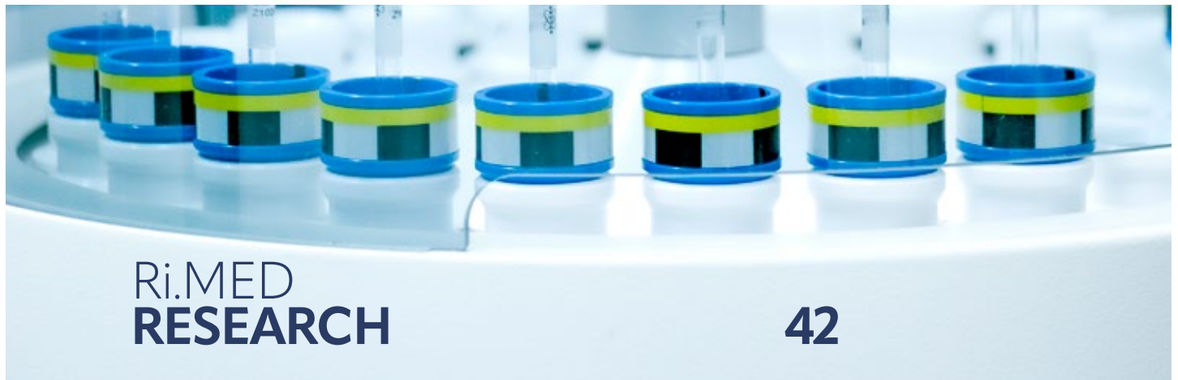


Ri.MED OVERVIEW

UP TO 31.12.2022

Networking

5 Agreements for labs management

45 Active scientific collaborations and technology transfer agreements

16 Scientific collaborations and technology transfer agreements signed in 2022

Training & employment

69 Employees in 2022

63%

37%

41 Scholarships

18 University of Pittsburgh

Post- Doc Fellowships c/o University of Pittsburgh

36 Ph.D. Fellowships

40 Internships

Intellectual property

About **530** Scientific publications

28 Patent

Fundings for Research

22,730,150€

Awarded through national and international GRANTS

8,000,000€

Sicilian Region operational contributions for Ri.MED-ISMETT cluster

Scientific knowledge dissemination

40 Ri.MED scientific meetings

11 Ri.MED internal meetings

10 Ri.MED institutional events

37 Participations in scientific events or local development activities

Building the BRBC

17,070 sq m of laboratories

€ 220,000,000 € Value of the investment

600 Planned occupancy opportunities

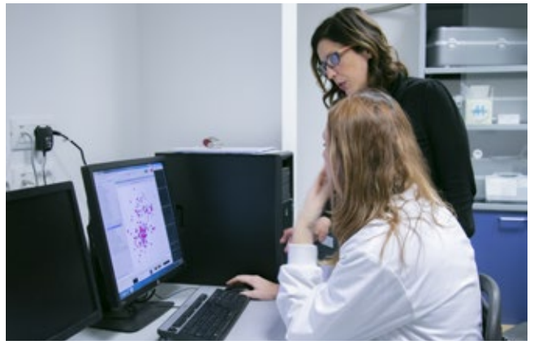
HUMAN RESOURCES

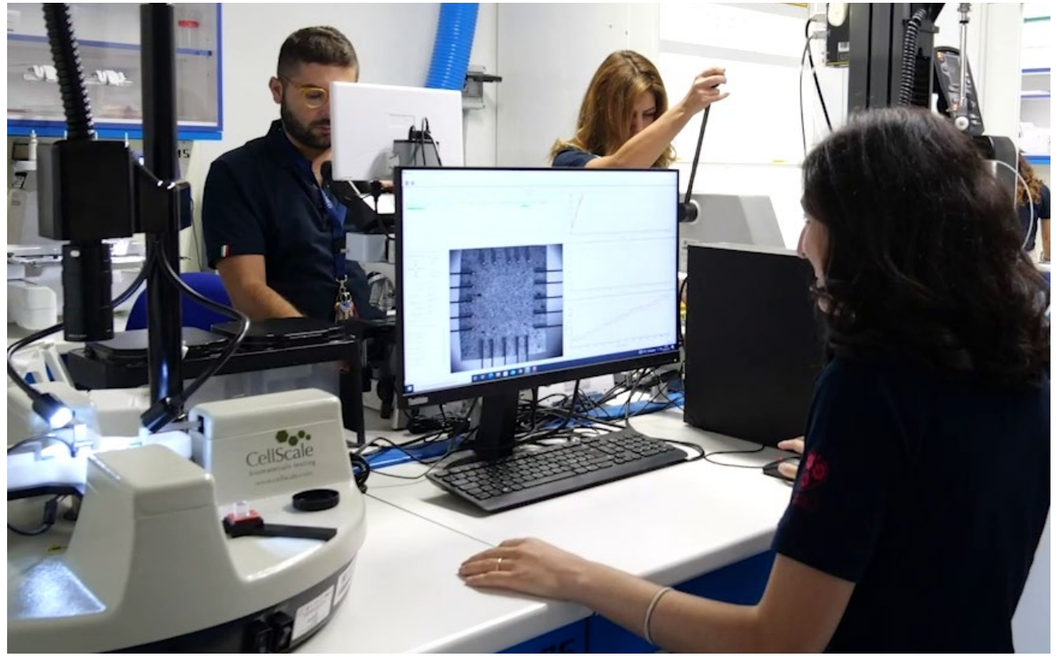
NURTURING NEW TALENTS

Ri.MED is particularly focused on training highly-qualified staff, recognizing their key role in accomplishing successful scientific results, and also to guarantee the competitiveness and growth of the territory.
Some of these training programs were implemented in partnership with the University of Pittsburgh, which has already hosted 18 postdocs under the Ri.MED Fellowship program. Additional training was activated using EU funds (ERC grant "Biomitral"), ministerial funds ("4Frailty"), and regional calls ("Obind" and "Prometeo").

In 2022, there was an increase in the number of PhD and internship applications at Ri.MED, as a result of the growing interest of young researchers in the Foundation's activities. In 2022, Ri.MED hosted 25 internships (4 from Northern Italy universities) and 31 PhD offering over 50 young professionals to develop new skills.

The fellowships and research grants were awarded to junior and senior researchers wishing to spend part of their career at Ri.MED. The Foundation has become a center of attraction of high scientific value for young promising researchers.





HUMAN RESOURCES

GROWING TOGETHER

As of December 2022, the Ri.MED Foundation has a staff of over 100 employees and fellows, of which 86 are researchers. Eighty-nine percent of researchers are Sicilians, and about 20% of them decided to return to Sicily thanks to the professional opportunities offered by Ri.MED.

The remaining 11% of researchers arrived from other parts of the world, a further demonstration of the international reputation of the Foundation. Since January 2022, Ri.MED is in fact hosting researchers from non-EU countries after its inclusion in the list of authorized research institutions issued by the Italian Ministry of University and Research (MUR).

While this has increased the Foundation's international reputation, it has always been the strategy of Ri.MED, also thanks to the partnership with the University of Pittsburgh and UPMC, to offer researchers rotations in top scientific institutions, in line with the EU recommendations on mobility of scientific research as a means of developing research networks and consolidating the role of European research at a global level.

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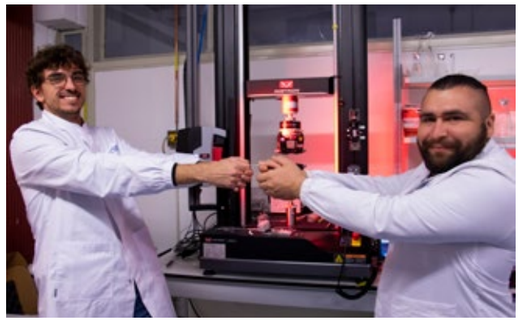
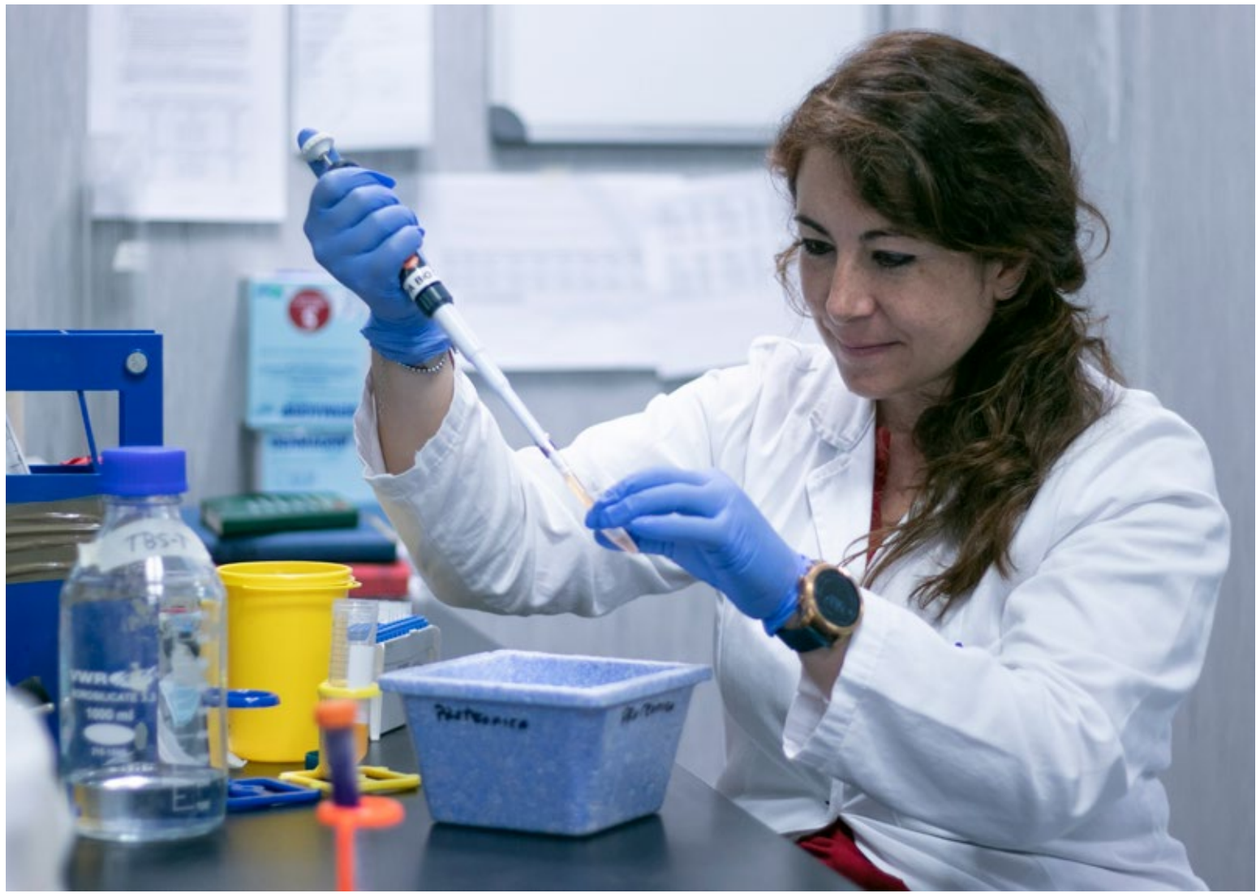
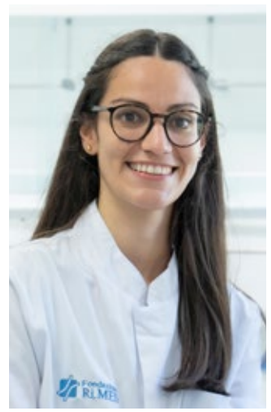
In view of the forthcoming opening of the Foundation's new research center in Carini, near Palermo, and thanks to new research funds, in the next coming years Ri.MED's research teams will continue to grow and the Foundation will become an international host institution.

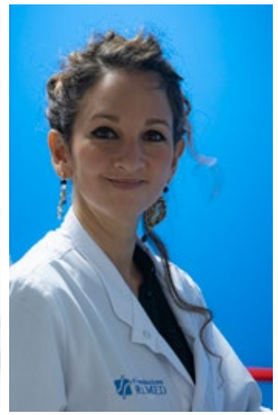


HUMAN RESOURCES

WOMAN IN SCIENCE & GENDER EQUALITY PLAN







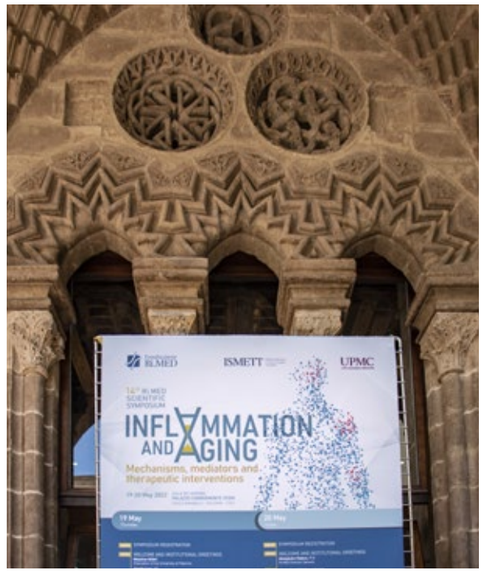
In December 2022, the Ri.MED Board of Directors approved the Gender Equality Plan (GEP), an important tool to ensure recognition and respect of gender equality at all levels.
The aim is to increase opportunities and enhance skills of employees of any gender and orientation, in line with the European Commission's recommendations. The GEP, and its indicators and tasks, are designed as a flexible tool that can easily be updated and that includes measurable goals.
Even before the introduction of this tool, however, the Ri.MED Foundation demonstrated the absence of gender bias, selecting over the years a team of researchers composed by 62% of women. This is particularly relevant when analyzed against numbers from European universities where the so-called STEM (Science, Technology, Engineering and Mathematics) studies are still the frontier of the gender equality challenge. In contrast, Ri.MED is an example of valorization of women in science. Brilliant and skilled women managing important medical and scientific research projects, contribute decisively to the achievement of Ri.MED's outcomes.

DISSEMINATION OF SCIENTIFIC KNOWLEDGE

EVENTS

Right from its very inception, Ri.MED Foundation has focused on scientific dissemination, sharing research outcomes, organizing meetings, keynote lectures, and workshops attracting vast audiences and networking with international partners.

In post-pandemic 2022, the Ri.MED Annual Scientific Symposium was held in Palermo on May 18-19. The chairman of the event entitled "Inflammation and Aging: Mechanisms, mediators and therapeutic interventions" was Toren Finkel, MD, PhD, Director, Aging Institute at UPMC and the University of Pittsburgh.



The symposium featured studies focusing on the development of innovative diagnostic and therapeutic solutions to slow down or even reverse the genesis of the chronic inflammatory state, allowing to treat a wide range of age-related diseases. International speakers included Ri.MED researchers Chiara Cipollina, PhD who with Dr. Alessandro Bertani, Chief of the Division of Thoracic Surgery and Lung Transplantation at ISMETT, presented a study on innate immune responses in age-related lung diseases, and Roberto Di Gesù with a presentation on new frontiers in osteoarthritis as an inflammatory age-related disease.

In July, Ri.MED participated in Innovabiomed, a network for biomedical innovation in Verona with over 50 companies and organizations and hundreds of visitors attending.

Our researchers presented various projects including new generation tissue-engineered and transcatheter heart valves, advanced solutions to support diagnostics and therapeutic planning, electrochemical immunosensors to quantify biomarkers of oxidative stress and inflammation, ATMPs, and customized solutions for the treatment of organ failures.

The Ri.MED speakers were Antonio D'Amore and Maria Giovanna Francipane, Principal Investigator in Regenerative Medicine.
Caterina Alfano brought to Sicily the EMBO circuit organizing the workshop "When prediction meet experiments: The future of structure determination" that took place in Palermo in September.

The workshop was also a chance for Ri.MED researchers to present their scientific activity, often as invited speakers. Year 2022 was an important time in terms of scientific publications, with over fifty articles in peer reviewed journals with relevant impact factors.

The role of the Foundation's press office was also instrumental to share the main research results to a wider public of non-experts, thus contributing to the dissemination of scientific knowledge.

DISSEMINATION OF SCIENTIFIC KNOWLEDGE

PUBLIC ENGAGEMENT

Involving and inspiring a heterogeneous public is one of our priorities, offering a public engagement program for people of all ages, in collaboration with the main local institutions. We are present in the community with activities that facilitate and promote knowledge, from science and health to investments and employment opportunities, legality, and meritocracy.

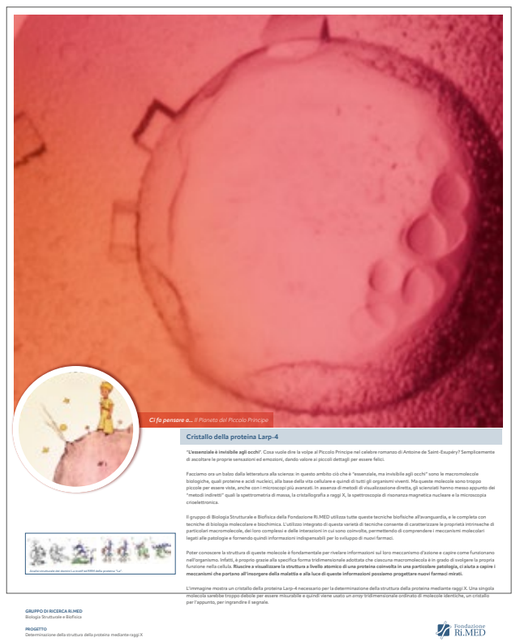
Ri.MED Foundation's was increasingly committed in 2022 in Carini, the community that will host the Biomedical Research and Biotechnology Center (BRBC). With the event **"CARINI, SCIENZA IN CANTIERE"**, Ri.MED Foundation disseminated scientific knowledge while at the same time involving and informing people on the construction of the BRBC. Photo exhibitions, film programs and workshops were offered from April to June at the La Grua-Talamanca Castle in Carini:

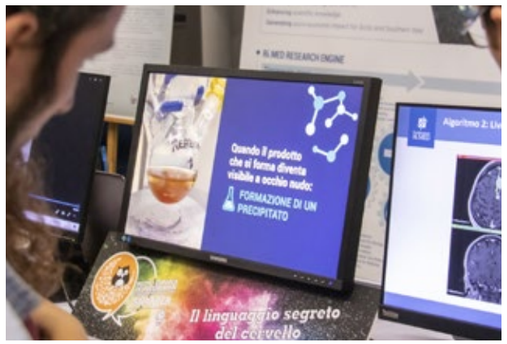
BRBC Photo exhibition: a selection of photographs of the construction site, the project, and the workers. Inaugurated on April 7 in the presence of the president of the Ri.MED Foundation Paolo Aquilanti and of the Mayor of Carini Giovi Monteleone, the exhibition curated by Nadia Consiglio illustrates the space, projects, materials, surrounding environment and workforce at the BRBC construction site.

Exhibition "Enchanted Science": an exhibition of scientific images proposing a striking combination of nature, art and research". Art is a form of language that interprets the world and creates enchantment. Sometimes rational science can steal the stage from art or merge with nature. These beautiful images were illustrated to all visitors by the Ri.MED researchers.

Science Movies film review: a selection of biographical and fantasy movies inspired by scientific characters and themes.

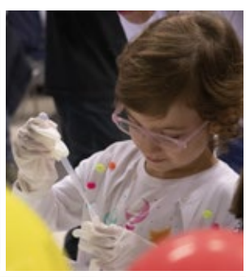
Bartender's Chemistry workshop: Ri.MED researcher Ester Badami, Principal Investigator in Experimental Immunology conducted the workshop "The Bartender's Chemistry" during which aspiring bartenders/scientists were able to discover the secrets of molecular mixology, a new and fun way to approach science.





Ri.MED took part in the event **"SHARPER - THE EUROPEAN RESEARCHERS' NIGHT"** with the initiative "The Secret Language of the Brain", an event that enjoyed a vast popularity among young and older visitors. Our researchers revealed the secrets of the human brain: exploring the molecules that regulate normal neuronal processes and those of synthetic origin used in healthcare. Lab gloves and glasses were available to visitors wishing to try small chemical experiments.

A bioinformatics and AI approach was also proposed to discover the so-called "brain grammar", from regulating DNA activities of each individual neuron, to morphological and functional imaging mapping the neuronal networks of the brain. Audiences were entertained with perceptual games designed to discover that "you don't see it with your eyes, but with your brain".



DISSEMINATION OF SCIENTIFIC KNOWLEDGE

SCHOOLS

The Foundation was particularly active in 2022 carrying out educational projects in local schools. Ri.MED entered various agreements with local high schools to activate pathways for transversal skills and guidance. With theoretical and hands-on knowledge, the aim of these programs was to complement the student's curriculum and encourage their choice in their future study or work. Our researchers were asked to conduct sessions for various groups of students. In particular, researchers of our Molecular Informatics and Advanced Data Analysis teams were invited by two high schools in Palermo, while the Structural Biology and Biophysics team held a course at the Liceo Scientifico in Carini.

Collaborations with other schools included the "Research Friday" laboratories of applied sciences at the STEM high school of the Gonzaga Institute in Palermo, and the two-year course at ITS Academy A. Volta of Palermo, the first industrial technical institute in Sicily specialized in "new technologies of life," aimed at training technicians in the use of diagnostic and biomedical devices.

Ri.MED devoted a special attention to Carini students: in addition to continuing the collaboration with Mursia Institute started in 2017, a new series of events named "School of Science" was launched in 2022, involving over 500 students.

Our researchers introduced students to Ri.MED's research areas, case studies of ongoing projects, acting as ambassadors of the "true life of the researcher". Students were also offered a tour of the exhibitions that Ri.MED organized at the La Grua-Talamanca Castle in Carini.

The goal of Ri.MED is to gradually increase its presence in the territory developing new educational programs with schools, and involving the local community.

DISSEMINATION OF SCIENTIFIC KNOWLEDGE

PRESS

la Repubblica PALERMO — 17-GIU-2022

L'intervista a Roberto Di Gesù della fondazione Rimed

“Io, ricercatore premiato negli Usa agli studenti do un consiglio viaggiate tanto e non arrendetevi”

Grazie a me chi ha l'osteoartrite potrà ricominciare più facilmente a camminare

LA SICILIA — Malattie legate all'invecchiamento, esperti a confronto a Palermo

abba news — notizie senza confine — Antonio D'Amore: attrattore di cervelli

LA SICILIA — Fondazione Ri.Med, la squadra della ricerca composta al 62% da donne

GIORNALE DI SICILIA

«Key innovator»

Cartilagine, Ue promuove i test del Rimed

Fabio Geraci

Il centro Rimed di Carini è stato selezionato come «key innovator», per la realizzazione della piattaforma di test meccanici per l'ingegneria della cartilagine, uno strumento innovativo per la lotta all'osteoartrite e alle patologie muscolo-scheletriche sviluppato dai ricercatori Riccardo Gottardi e Roberto Di Gesù. Gli esperti indipendenti

GIORNALE DI SICILIA — a cura di Italpress

Biologia molecolare

Ri.MED porta a Palermo scienziati da 28 Paesi

Favorire confronto e sinergie tra scienziati sperimentali e computazionali per una maggior integrazione dell'Intelligenza Artificiale nella pratica canonica di determinazione della struttura delle proteine. E' questo l'obiettivo del workshop "When prediction meet experiments: The future of structure determination", il convegno patrocinato dalla European Molecular Biology Organization (EMBO) in collaborazione con Fondazione Ri.MED, che si è svolto a Palermo, a Palazzo Branciforte.

GIORNALE DI SICILIA — 04-OTT-2022

Ricerca contro il tumore al seno Accordo tra Ri-Med e Gemelli

Verrà favorito lo scambio delle informazioni scientifiche

Sanità24 — Tumore seno, accordo di collaborazione scientifica tra Fondazione Ri.MED e Fondazione Policlinico Universitario Agostino Gemelli Irccs

CROTONESE — Sanità, ricerca scientifica: accordo tra la fondazione siciliana Ri.Med ed il Marrelli Health

QUOTIDIANO DI SICILIA — A Palermo un workshop sull'intelligenza artificiale che riunisce scienziati da 28 Paesi

Sicilia — 22-APR-2022 — Il RiMed "innovatore chiave" per gli esperti della commissione europea

GIORNALE DI SICILIA — Fondazione Rimed, le iniziative al Castello

Due mostre a Carini Una finestra aperta sul centro di ricerca

Corrado Lo Piccolo

Come nasce il polo di ricerca Rimed, gli scatti dal cantiere in mostra a Carini

Zootecnico Palermo, nasce Centro sperimentazione pre-clinica

GIORNALE DI SICILIA — Rimed, leader in ingegneria dei tessuti

Ricerca e invenzioni, premio internazionale ad Antonio D'Amore

Scienza e traguardi Sta sviluppando una tecnologia che annulla la dipendenza dalle terapie anticoagulanti

A lui il Nai senior member È pure professore dell'ateneo di Pittsburgh

«Prevenire» l'artrosi con i Big data

GIORNALE DI SICILIA

Carini, Terna libera un'area per il nuovo centro Rimed

Da interrare le linee elettriche legate a una cabina primaria La Fondazione: così si riducono gli impatti sul territorio

NETWORKING

PARTNERSHIP & COLLABORATIONS

The aim of collaborations is to integrate complementary skills with joint translational research projects, increasing critical mass and potential for success.

Creating networks that generate competitive research financing is crucial. Ri.MED pays great attention to the ongoing development of its network of scientific collaborations and scientific agreements with centers and institutions operating in its areas of interest: there are currently 45 agreements in place for the development of technological innovation, promotion of research activities, and sharing laboratories and resources with European and U.S. institutions.

Sixteen agreements were signed in 2022.
Ri.MED has five ongoing agreements for lab hosting: the management of the Regenerative Medicine and Immunology laboratories at ISMETT of strategic importance for integrating basic and clinical research, the Structural Biology and Biophysics labs at ATeN Center, the Bioengineering and Medical Devices lab at the University of Palermo, and the High Throughput Screening lab at CNR.

Ri.MED Foundation attended the BIO International Convention organized by the Italian Trade & Investment Agency, the governmental agency supporting business development of Italian companies abroad and promoting the attraction of foreign investments in Italy.

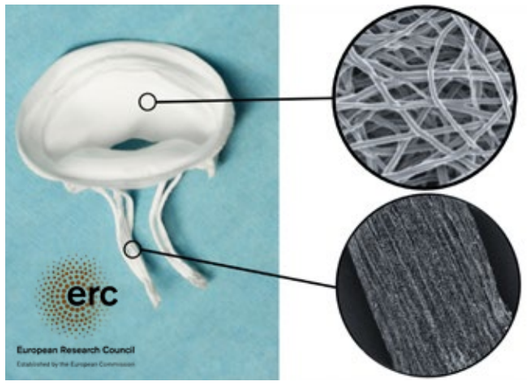
GRANTS

RESEARCH FOUNDING

To create new knowledge for contributing to the scientific progress in the biomedical and biotechnological area, Ri.MED Foundation participates in competitive scientific grants funded by public and private financing bodies, at the regional, national, and international level.

To achieve this goal, the Grant Area provides technical support to all Ri.MED researchers, from the identification of the most suitable grants, up to the project management and coordination once projects are approved for funding. In 2022, in addition to the widely known prestigious competitive grants, the new opportunities offered by the National Recovery and Resilience Plan (NRRP), lead Ri.MED Foundation to the submission of more than 40 project proposals.

At this regard, it is worth to mention the Ri.MED participation in the Italian Fund for scientific excellence (FISA - Ministry of Research), and the opportunities offered by the most challenging Italian academic grants (PRIN), financially integrated by the NRRP, allowing the strengthening of the scientific collaboration with Universities. While, the Italian Ministry of Foreign Affairs and Leducq Foundation, represented a great incentive for nursing Ri.MED international cooperation strategies, reinforcing partnerships with USA and China.

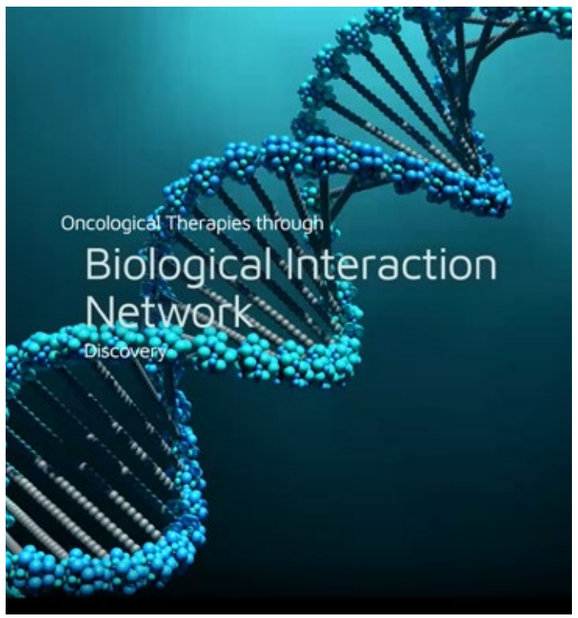


This year, researchers have also created strategic partnerships with SMEs and start-up, participating in the Ministry of Economics grant, for the development of new products. Not only collaborative grants, but also individual ones, as the applications submitted to the prestigious EU ERC Starting Grant, the Italian My First AIRC Grant and Cariplo-Telethon Alliance. Finally, two excellent researchers, from abroad, applied for the Marie Skłodowska-Curie Actions, selecting Ri.MED as host institution. In 2022, four new projects have been awarded to Ri.MED Foundation, three of them funded by the NRRP (managed by Ministry of Health and the Ministry of Research), and one funded by Ministry of Health.

Progetto

POTENZIAMENTO INFRASTRUTTURE DI RICERCA

GMP Facility, Laboratori di Ricerca e Servizi Diagnostici e Terapeutici dell'Istituto Mediterraneo per i Trapianti e le Terapie ad Alta Specializzazione (IRCCS-ISMETT)



UNIONE EUROPEA
Fondo europeo di sviluppo regionale

PO FESR SICILIA 2014-2020

Progetto SE.N.SO

Sensore strutturato per Stress Ossidativo

ASSE OT1 - AZIONE 1.1.5 "Sostegno all'avanzamento tecnologico delle imprese attraverso il finanziamento di linee pilota e azioni di validazione precoce dei prodotti e di dimostrazione su larga scala"

Beneficiari:
Impresa capofila: DIPIETRO GROUP srl
Partner industriali: CERTY CEQ srl; MICROSYSTEMS srl; RULETECH srl;
Organismi di Ricerca: Università degli Studi di Palermo; IRIB/CNR: Istituto di Biomedicina ed Immunologia Molecolare; Fondazione Ri.MED

Importo dell'intervento:	**3.467.224,38€**
Quota Fondazione Ri.MED	**390.550,00€**
Data di inizio intervento:	**12/01/2021**
Data prevista fine intervento:	**11/07/2023**

Se.N.So
Sensore Nanostrutturato per Stress Ossidativo

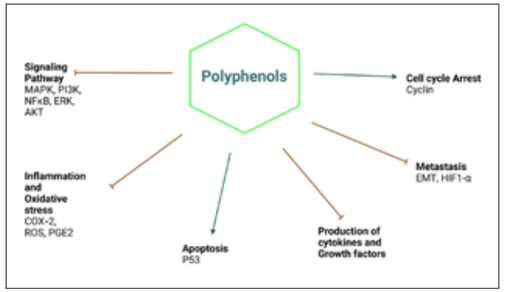
GRANTS

FOCUS ON 2022

Great scientific opportunities have been inaugurated in 2022 in Ri.MED Foundation, in terms of new strategic scientific collaborations and strengthening of relevant infrastructures for research activities. The focus is on: ISPEMI, the National Biodiversity Future Center and the National Center for Gene Therapy and Drugs based on RNA Technology.

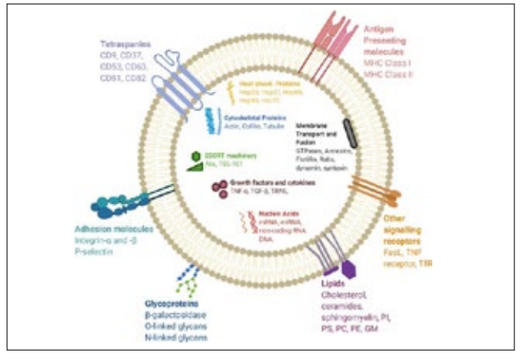
NATIONAL BIODIVERSITY FUTURE CENTER

Within the National Biodiversity Future Center, where Ri.MED Foundation is a full member of the HUB, strategic scientific collaborations are expected to be activated with the aim to integrate competences and skills in the natural products exploitation for therapeutic scopes. Inside this multidisciplinary context, Ri.MED researchers will contriubute with the creation of natural products libraries that will be computationally evaluated and then screened towards specific therapeutic targets (among them, the NLRP3 inflammasome and protein TDP-43). Natural products will be selected by virtual screening campaigns from Molecular Informatics and HTS groups, via retrosynthetic analysis. Moreover, the collaboration with the University of Verona, will lead to the creation of a public *in silico* platform for the molecular profiling. In this research line, the expected overall output will be new hit compounds, intended as starting point of medicinal chemistry programs towards the identification of novel potential modulators. In this setting, strategic inputs are also expected from Ri.MED Imaging and radiomics group that, in collaboration with IBFM-CNR, will work on the validation of (radio) biological cellular efficacy (*in-vitro* and *in-vivo*) and biodistribution (*in-vivo*), through preclinical testing, using **multimodal 3D imaging, artificial intelligence and radiomics techniques.**



GENE THERAPY NATIONAL CENTRE

New scientific results are expected inside the Gene Therapy National Centre too, where **Ri.MED Medicinal Chemistry scientific team**, will contribute to the set-up of the laboratory for oligonucleotides synthesis, with a special attention on ASO compounds (antisense oligonucleotides), with the final aim to identify those ASO most suitable to be used for treatment of aging-associated cardiac and vascular senescence and inflammation. To this scope, the **Advanced Data Analysis group** is developing computational tools to design the most suitable ASOs, whose efficacy will be experimentally validated in Ri.MED laboratories. Another clinical area on which Ri.MED will focus is non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH). **Ri.MED scientists at the Regenerative Medicine Unit** will share their expertise to produce extracellular vesicle (EV)-based therapeutics as treatment option for patients with an increased risk to develop liver fibrosis. The activities, in collaboration with the Lab of Biocompatible Polymers (Dept STEBICEF, University of Palermo), include design and synthesis of EV-laden biomaterials, such injectable hydrogels and polymer-coated extracellular vesicles. Furthermore, in collaboration with the Medical and Biotechnology Lab (Dept of Medical Sciences, University of Turin) a list of candidate anti-fibrotic microRNAs to be loaded into different cargos will be selected. Finally, a release of EVs according to Good Manufacturing Practice (GMP) compliant is planned in order to launch *in vivo* safety and efficacy preclinical studies.



Gurung et al.,Cell Commun Signal. 2021 Apr 23;19(1):47

INSTITUTE FOR PRECLINICAL STUDIES AND MOLECULAR IMAGING (ISPeMI)

The Institute for Preclinical Studies and Molecular Imaging (ISPeMI) was established in 2007, by a consortium of partners that include: the Ri.MED Foundation, the Experimental Zooprophylactic Institute of Sicily "A, Myrri" and Zootechnical Experimental Institute for Sicily, based on financial support provided by the European Commission, the Italian Ministry of Research, the Ministry of Economics (ONP Research and Competitiveness 2007-2013). The completion of the laboratories according to standards requested to obtain 6 a 7 Tesla magnetic resonance scanner.
The Institute inaugurates a new era for the Sicilian Life Science sector, reinforcing the scientific infrastructure necessary to ensure proper translation of biomedical innovation into patient care, making possible, within the same facility, the full process of preclinical testing throughout an integrated approach.

In 2022, ISPeMI was officially inaugurated with the concurrent joint signature among Ri.MED Foundation, IRCCS ISMETT, UPMC Italy and Zootechnical Experimental Institute for Sicily further strengthening the Consortium's attractiveness within the national and international framework for translation in Life Science. A further recognition has been received from the Sicilian Region (Dept. of Production Activities), with a new investment for the restructuring of the Ancient Stall, located within the same area, aimed at creating a prestigious high-education center, with conference halls, digitally equipped classrooms and world-class laboratories. These investments have the potential to make ISPeMI a major national asset for bio-medical high-education within the next few years. The advanced training program in pre-clinical studies and experimental surgery will also involve the active efforts of highly competitive researchers and doctors within the Ri.MED-ISMETT-UPMC cluster.

GRANTS

2022 ONGOING SCIENTIFIC PROJECTS

PROJECTS IN PARTNERSHIP WITH SCIENTIFIC AND/OR HEALTH CARE INSTITUTIONS AND SMEs/BIG ENTERPRISES

OBIND
Oncological therapies through Biological Interaction Network Discovery

Click here for more information

Funding Agency/Programme: Sicilian Region. OP ERDF 2014/2020. Thematic Objective 1: Research, Technological Development and Innovation.
Objective: developing a computerized system in the oncology field which allows for aggregation and analysis of different data sets, focused on studying the interaction between different biological molecules that are found to have changed in tumor diseases and in order to create a rational outline for planning new treatments

SENSO
Development of a miniaturized device for monitoring oxidative stress in cellular systems

Click here for more information

Funding Agency/Programme: Sicilian Region - OP ERDF 2014/2020 - Thematic Objective 1: Research, Technological Development and Innovation.
Objective: creating a nanosensor to detect hydrogen peroxide (H2O2) released *in vitro*/*ex vivo* cellular system culture. To this scope, the expected output is an innovative, robust, reliable, and small-sized lab tool to monitor the H2O2 release within the culture in real time, without affecting the cells' growth conditions.

4FRAILTY
Intelligent sensors, infrastructures and management models for the safety of fragile people

Click here for more information

Funding Agency/Programme: Ministry of Education, University and Research (Italy). NOP "Research and Innovation", 2014-2020. Directorial Decree n. 1735/Rec. (13.06.2017) "Notice for the presentation of Industrial Research and Experimental Development projects in the 12 specialization areas identified by the 2015 - 2020 RNP".
Objective: creating a computational tool able to simulate the sensory platform in its set of sensors and therefore including vital and environmental parameters that will be collected during the clinical work-up phases. The simplicity and versatility of the computational implementation will allow to quickly simulate different virtual scenarios of the possible alterations of the vital and environmental signals associated with a pathological condition.

PROGEMA
Green processes for the extraction of active ingredients and the purification of waste and non-waste matrices

Click here for more information

Funding Agency/Programme: Ministry of Education, University and Research - NOP Research and Innovation 2014-2020 and Cohesion Development Fund.
Objective: improving the treatment of vegetation waters of the Olive Oil Production Chain to extract and reallocate pharmaco- logically active organic compounds, reduce their polluting effect, and re-use treated waters in the production processes.

PROMETEO
Advanced Medicinal Products placenta-derived for liver and endometrial diseases

Click here for more information

Funding Agency/Programme: Sicilian Region - OP ERDF 2014/2020 - Thematic Objective 1: Research, Technological Development and Innovation.
Objective: developing cell-based products from stem cells isolated from the placenta for clinical applications. Through *in vitro* and *in vivo* studies, cellular therapies will be developed to treat acute and chronic liver diseases and reactivate the endometrium. The therapeutic effects of cells and products will be tested on *in vivo* models of acute and chronic liver injury and on *in vitro* models for endometrial reactivation. All tasks will be optimized according to the principles of Good Manufacturing Practices (GMP) to develop cellular therapies.

National Biodiversity Future Center

Click here for more information

Funding Agency/Programme: National Recovery and Resilience Plan (NRRP) - Mission 4 (Education and Research), Component 2 - Investment 1.4.
Objective: promoting the sustainable management of Italian biodiversity in order to improve the planet's health and return beneficial effects, essential for all people. Specific objective in the health sector is the exploitation of natural products for therapeutic scopes. Read the "Focus Section" to know more details about the expected impact and ongoing activities.

National Center for Gene Therapy and Drugs based on RNA Technology

Click here for more information

Funding Agency/Programme: National Recovery and Resilience Plan (NRRP) – Mission 4 (Education and Research), Component 2 - Investment 1.4.
Objective: increasing the technological know-how necessary to design and deliver RNA-based and gene therapy medicinal products and identifying promising candidate drugs/genes in five major areas of human diseases (genetic diseases, cancer, metabolic/cardiovascular diseases, neurodegenerative disorders and inflammatory/infectious diseases). Read the "Focus Section" to know more details about the expected impact and ongoing activities.

GRANTS

2022 ONGOING SCIENTIFIC PROJECTS

PROJECTS IN PARTNERSHIP WITH SCIENTIFIC AND/OR HEALTH CARE INSTITUTIONS

Exploiting the power of human induced pluripotent stem cell extracellular vesicles as a new anti- inflammatory drug for lung ischemia reperfusion injury

Click here for more information

Funding Agency/Programme: National Recovery and Resilience Plan (NRRP) – Mission 6 (Health), Component 2 – Investment 2.1.
Objective: assessing technical and commercial feasibility of an innovative cell-free nanomedicine strategy based on extracellular vesicles (EV), human induced pluripotent stem cells and a young cell source such as cord blood. The project is focused on a patent which highlights the strong anti-inflammatory role of EV isolated from cord blood mesenchymal stromal induced pluripotent stem cells.

Life Science TTO Network

Click here for more information

Funding Agency/Programme: Italian Ministry of Health. National Plan for complementary investments to the NRRP – "Innovative Health Ecosystem" Program – Investment E.3.
Objective: establishing and consolidating a nationwide open and sustainable Technology Transfer Office (TTO) Network, which will connect, coordinate and align Network Partners and stakeholders (Research and Care Institutes (IRCCS), Universities, National Health System, Investors, SMEs, Industry, National and International Consortia) as well offer world-class services in a synergistic valuable way at national and international level.

BIOMITRAL

Engineering the mitral valve: bioinspired control of structure and function for enhanced *in vivo* performance

Click here for more information

Funding Agency/Programme: European Commission HORIZON 2020 – ERC Consolidator Grant
Objective: developing an innovative prototype of a mitral valve by engineering the chordal apparatus and reconnecting the left ventricle with the valve leaflets.

iRhom2

A new therapeutic target in osteoarthritis

Click here for more information

Funding Agency/Programme: Fondazione con il Sud - Bando Capitale Umano ad Alta Qualificazione 2018.
Objective: validating iRhom2 as potential and innovative therapeutic target of osteoarthritis, using state-of the-art proteomics methods and *in vivo* models of the disease, including a study focused on iRhom2 inhibitors.

GMP FACILITY

Laboratori di Ricerca e Servizi Diagnostici e Terapeutici dell'Istituto Mediterraneo per i Trapianti e le Terapie ad Alta Specializzazione

Click here for more information

Funding Agency/Programme: Sicilian Region - OP ERDF 2014/2020 – Thematic Objective 1: Research, Technological Development and Innovation.
Objective: strengthening research, cell production, and diagnostic laboratories of Ri.MED - ISMETT cluster by the purchase of cutting-edge equipment and by infrastructural improvements.

PhD SCHOLARSHIPS CO-FUNDING

Funding Agency/Programme: National Recovery and Resilience Plan (NRRP) Mission 4 (Education and Research), Component 2, Investment 3.3

1. PHD FELLOWSHIP IN CHEMICAL, ENVIRONMENTAL, BIOMEDICAL, HYDRAULIC AND MATERIALS ENGINEERING.
A.A. 2022/2023/2024/2025 - Cycle XXXVIII°, coordinated by Prof. Giorgio Domenico Maria Micale. Innovative PhD fellowship managed in partnership with University of Palermo, and co-funded by Ri.MED Foundation.

2. PHD FELLOWSHIP IN MECHANICAL, MANUFACTURING, MANAGEMENT AND AEROSPACE INNOVATION.
A.A. 2022/2023/2024/2025 - Cycle XXXVIII°, coordinated by Prof. Giovanna Ivana Lo Nigro. Innovative PhD fellowship managed in partnership with University of Palermo, and co-funded by Ri.MED Foundation.

3. PHD FELLOWSHIP IN TRANSLATIONAL MOLECULAR MEDICINE AND SURGERY- CYCLE XXXVIII°
A.A. 2022/2023/2024/2025, coordinated by Prof Gaetano Caramori. Innovative PhD fellowship managed in partnership with University of Messina, and co-funded by Ri.MED Foundation.

INTELLECTUAL PROPERTY & TECHNOLOGY TRANSFER

PATENT PORTFOLIO

Research activity of Ri.MED is strongly patient oriented, but in order to ensure that scientific results reach clinical needs, it is necessary to correctly manage the intellectual property generated by our researchers as well as the process of technology transfer which derives from it.
From the laboratories, inventions are translated into patents and then into new solutions for the patients.

The protection of intellectual property is a fundamental value for Ri.MED to develop an innovative model of research sustainability. For this reason, Ri.MED has set up an Intellectual Property and Technology Transfer Office to support, promote and foster the progress of translational research through the enhancement of its application effects: patenting, patent license, industrial sponsorship and creation of technological spin-offs.

Furthermore, in 2021 Ri.MED and a multinational company operating in the cardiovascular area have finalized an important license and collaboration agreement having as object Ri.MED patent family "Triskele" and that is still in full force. This patent family consists of a heart valve prosthesis, a heart valve and their related delivery system and one of the inventors is Gaetano Burriesci, Ri.MED group leader in Bioengineering and Medical devices.

PATENT PORTFOLIO UP TO 31.12.2022

DRUG DISCOVERY

Nitro-oleic acid controlled release platform to induce regional angiogenesis in abdominal wall repair.
WO2019100021
Fondazione Ri.MED - University of Pittsburgh

Novel reversible nitroxide derivatives of nitroalkenes that mediate nitrosating and alkylating reactions
WO2018067709
University of Pittsburgh

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

NK-mediated immunotherapy and uses therefor
WO2018099988
Fondazione Ri.MED - IRCCS ISMETT

Mandrel-less electrospinning processing method and system, and uses therefor
WO2018175234
Fondazione Ri.MED - University of Pittsburgh

Extracts for the regeneration of ligaments
PCT/US2019/019119
Fondazione Ri.MED - University of Pittsburgh

TISSUE ENGINEERING AND BIOMEDICAL DEVICES

Method and system for the evaluation of the risk of aortic rupture or dissection in an ascending thoracic aortic aneurysm.
WO2018220573
Fondazione Ri.MED - IRCCS ISMETT

Transatrial access for intracardiac therapy
WO2017127682
University of Pittsburgh

Bi-layer extra cellular matrix scaffolds and uses thereof
WO2017044787
University of Pittsburgh

Hybrid Micro Molding-Fiber Deposition Substrate Processing for Cell Biology Manipulation and Local Anisotropy
US 63/091,462
Fondazione Ri.MED - University of Pittsburgh

Double components mandrel for electrospun stentless, multi-leaflet valves fabrication.
WO2016138416
University of Pittsburgh

Retrievable self-expanding non-thrombogenic lowprofile percutaneous atrioventricular valve prosthesis
WO2016138423
University of Pittsburgh

Multi-layered graft for tissue engineering applications
WO2019023447
Fondazione Ri.MED - University of Pittsburgh

Treating soft tissue via controlled drug release
WO2015134770
University of Pittsburgh

Microfluidic Tissue Development Systems
WO2017062629
University of Pittsburgh

A modular, microfluidic, mechanically active bioreactor for 3D, multi-tissue, tissue culture.
WO2015027186
University of Pittsburgh*

Recruitment of mesenchymal stem cells using controlled release systems
WO2014022685
University of Pittsburgh

Osteoarthritis treatment with chemokine-loaded alginate microparticles
U.S. Patent Appl. No. 16/241,112
Fondazione Ri.MED - University of Pittsburgh

Organ chip to model mammalian joint.
U.S. Patent Appl. No. 16/193,972)
University of Pittsburgh

Multi-well mechanical stimulation systems and incubators
WO2019079722
Fondazione Ri.MED - University of Pittsburgh

A stentless biopolymer heart valve replacement capable of living tissue regeneration.
WO2018156856
University of Pittsburgh

An expandable percutaneous cannula
PCT/US2018/017795
Fondazione Ri.MED - University of Pittsburgh

Biodegradable metallic - polymeric composite prosthesis for heart valve replacement
WO2019210059
Fondazione Ri.MED - University of Pittsburgh – UniversiTyOFCincinnati

Processing method and apparatus for micro-structured rope-like material
US provisional Patent Application 62/874,114
Fondazione Ri.MED - University of Pittsburgh

Semi-rigid annuloplasty ring and method of manufacturing
WO2019220365
Fondazione Ri.MED

Heart valve prosthesis
WO2010112844
Fondazione Ri.MED

Prosthesis delivery system
WO2012052718
Fondazione Ri.MED

Prosthetic heart valve
WO2016203241
Fondazione Ri.MED

Implant for heart valve repair
WO/2022/229667
Fondazione Ri.MED - Université de technologie de Compiègne - Centre National De La Recherche Scientifique (CNRS) - Assistance Publique/Hôpitaux De Paris

NOTE:
Also patents where University of Pittsburgh is the only applicant have been co-developed with Ri.MED.

BIOMEDICAL RESEARCH AND BOIOTECHNOLOGY CENTER

SOCIO-ECONOMIC IMPACT

The state-of-the-art center that the Foundation is building in Carini, a few miles from Palermo's international airport, will become a reference point for researchers from all over the world, playing a leading role in the scientific and medical industries.

Construction was started in 2020 and, despite unpredictable events, works are now in full swing on the 25,000-square meter area that will host the Biomedical Research and Biotechnology Center (BRBC). The COVID-19 pandemic had a strong impact on the project's timeline, and plans had to be modified along the way to meet restrictions, including a prolonged lockdown, new regulations, and frequent quarantines for entire teams of workers. The 2022 challenge was the ongoing Russian war on Ukraine, impacting Europe and causing a shortage of building materials. However, resilience combined with stubbornness and passion of all the people involved in the project, allowed us to soldier on. Today the layout and elevations are clearly visible in the campus. This project will improve the lives of patients and also open new job opportunities for hundreds of skilled resources, providing a strong economic impact and scientific connotation in the community.

The temporary association of enterprises (ATI) is led by Italiana Costruzioni, while the management of the construction is entrusted to a team led by Progetto CMR, part of the group team awarded the international design competition of the BRBC and led by HOK. The project is inspired by the "village street" model, and provides for an extremely flexible organization of space: 17,070 square meters of laboratories, common areas, meeting rooms, offices, an auditorium, and a guest house.

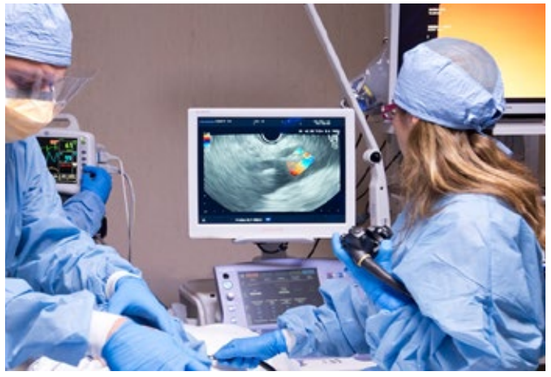
The BRBC's laboratories and technological platforms will be dedicated to the research and development of innovative vaccines and drugs, cell therapies, organ and tissue engineering for early diagnosis and targeted treatment of terminal organ failure, tumors, infectious diseases and pathologies related to aging, with particular attention to neurological diseases.
Once fully operational, in 2025, the BRBC will employ approximately 600 people creating resources to help innovators break the existing cycle and move their products further and faster through the tech transfer pipeline.

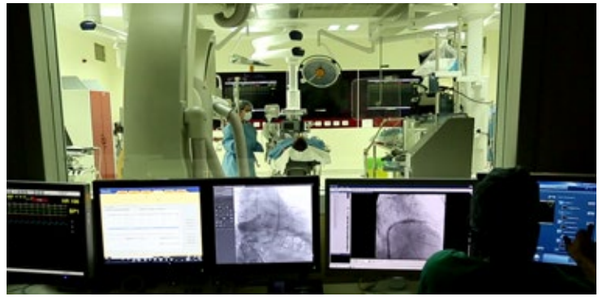
THE Ri.MED - ISMETT - UPMC CLUSTER

A STRATEGIC INTEGRATION

The challenge in Life Sciences is increasingly embodied in the ability to quickly translate results of scientific research into clinical applications. This involves becoming more and more focused on the complementary integration of different resources and skills: basic research, research and preclinical development of new therapies and medical devices, biomarkers, clinical trials. The project of an integrated center for translational research and highly specialized care confirms the strategic collaboration between UPMC, ISMETT, and Ri.MED.

Integrating complementary skills increases the potential of translational research and, in turn, the chances of success in competing for research line funding. For this reason, and given the increasing number of joint scientific projects, we have begun to identify "cluster" activities with shared objectives. In 2022, governance coordination by the respective leaders provided new management strategies and guidelines while on the administrative level our grants offices worked to improve access to financing. Scientific staff was constantly updated on the progress of joint research projects and new development opportunities. The communication departments of the three partner institutions collaborated to direct their respective efforts toward a shared image of the synergistic cluster.





THE Ri.MED - ISMETT - UPMC CLUSTER

2022 JOINT RESEARCH RETREAT

To maximize cluster synergies and identify complementary research topics, the 2022 edition of the annual Ri.MED Research Retreat was expanded to a "Joint Research Retreat" with ISMETT and UPMC.

Day one introduced the Ri.MED/ISMETT/UPMC **joint projects**:

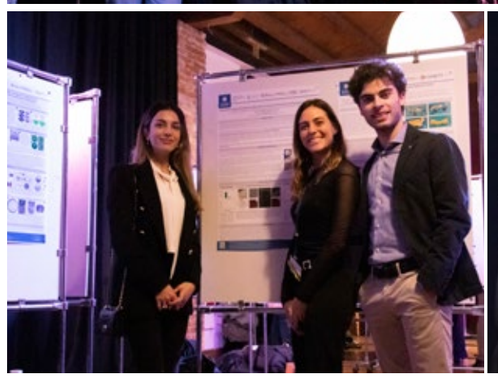
- Development and characterization of a bioengineered bile duct;
- Tissue engineering for treatment of benign esophageal diseases;
- A radiomic approach to predict end-stage liver failure and its implication on central nervous system.

The second part of the day was dedicated to the Grand **Poster Session**, an opportunity for all Ri.MED research groups to present scientific projects in progress, and for researchers of ISMETT to present ongoing joint projects with Ri.MED's Regenerative Medicine and GMP Cell Products teams.

Day two focused on **Ri.MED research opportunities** (presentation of the Ri.MED genomics-proteomics and bioengineering platforms and of funded projects for gene therapy and development of RNA technology drugs), and continued with presentations by ISMETT physicians of specific **clinical needs** which were then reviewed in depth during four **thematic roundtables:**

- Precision medicine for the management of neurodegenerative diseases in end-stage organ disease;
- The paradigm of patients on ECMO;
- The bidirectional relationship between diabetes and transplantation;
- Liver transplant oncology in primary and metastatic tumors.

The Joint Research Retreat aims to bring together physicians and researchers in a format that proved successful for maximizing relations and identifying joint research topics.



Ri.MED RESEARCH

Ri.MED's scientific projects are based on three main areas of interest: regenerative medicine and immunotherapy, aimed at developing advanced therapy medicinal products (ATMP); new drug discovery research; development and tissue engineering and biomedical bioengineering, focused on developing biomaterials, engineered tissues, and medical devices.
The activities range from identifying new biologically active molecules to developing cellular products for tissue repair and/or regeneration, and organotypic cultures for regenerative purposes and as models for pharmacology screening, and all the way to simulation of physiological systems and preclinical validation of new generation implantable organs and devices.

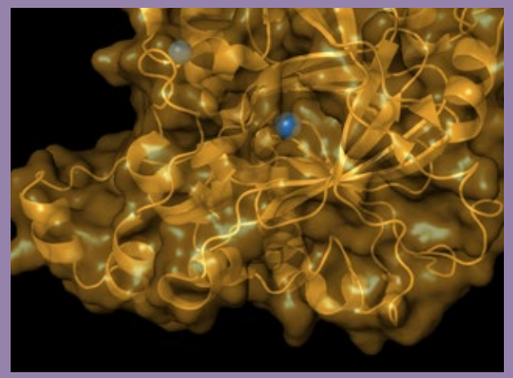
Ri.MED's translational research approach is based on therapeutic needs and developed on multiple levels, including the collaboration with our partners: from basic research and pre-clinical research and development, to clinical trials conducted with IRCCS ISMETT.
Ri.MED has a diversified and balanced project portfolio led by a multidisciplinary team with clear product development goals and a "bench-to-bedside" approach.
The translational research engine of Ri.MED Foundation envisages the development of skills and technology platforms supporting scientific projects.

DRUG DISCOVERY

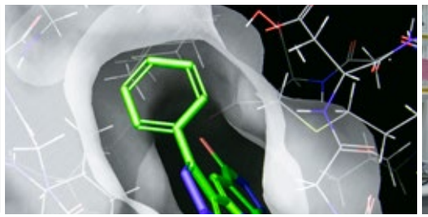
STRUCTURAL BIOLOGY AND BIOPHYSICS
MOLECULAR INFORMATICS
MEDICINAL CHEMISTRY
EXPERIMENTAL LUNG RESEARCH
PROTEOMICS
ADVANCED DATA ANALYSIS
IMAGING AND RADIOMICS

Ri.MED Foundation researchers are involved in drug discovery projects to identify new mechanisms underlying diseases of interest and to discover new biologically active molecules. Studying biomolecular pathways throughout classical methods integrated with multidisciplinary approaches including omics and in-silico tools, our researchers aim at discovering and validating new therapeutic targets in oncology and aging-associated diseases. The research groups operating in this unit, conduct translational research projects as well as early drug discovery projects. The expertise of the group covers all the phases of the early drug discovery process, starting with the study of target proteins through, biological assays, biophysical and structural biology approaches together with in-silico and medicinal chemistry contributions.

The unit has the expertise and ability to develop and perform biophysical, biochemical and cell-based screening assays as well as guide hit optimization and hit-to-lead processes. Radiobiology and Imaging complete the group expertise. During 2022, hundreds of molecules, selected using in-silico methods were tested on NLRP3 target. Preliminary actives are currently being validated through hit expansion and *in silico* binding mode rationalization. In the next phase, the most promising hit series will undergo SAR exploration and hit-to-lead optimization.
The medium-term goal is to define the *in vitro* profile of the promising hits (potency, efficacy, selectivity and toxicity) in order to identify a lead molecule. Researchers in this unit develop artificial intelligence algorithms for segmentation, extraction of radiomic features, and training predictive models to be applied to multimodal imaging. Significant results in preclinical molecular imaging were achieved in 2022, with a new preclinical decision support systems based on PET radiomics in studies on an innovative 64Cu-labelled chelator in mouse models for evaluation of non-invasive biodistribution.



3D structure of the C-terminal dimerization domain of SARS-CoV-2 Nucleocapside protein
Courtesy of the Structural Biology and Biophisics Group



PUBLICATIONS

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Anna Paola Carreca, Massimiliano Gaetani, Rosalia Busà, Maria Giovanna Francipane, Maria Rita Gulotta, Ugo Perricone, Gioacchin Iannolo, Giovanna Russelli, Claudia Carcione, Pier Giulio Conaldi, Ester Badami.
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Walter Arancio, Claudia Coronnello
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Pierpaolo Alongi , Riccardo Laudicella, Alessandro Stefano , Federico Caobelli , Albert Comelli , Antonio Vento , Davide Sardina , Gloria Ganduscio , Patrizia Toia, Francesco Ceci , Paola Mapelli , Maria Picchio , Massimo Midiri , Sergio Baldari , Roberto Lagalla , Giorgio Russo
Q J Nucl Med Mol Imaging 2022 Dec;66(4):352-360.
https://doi.org/10.23736/S1824-4785.20.03227-6

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Radiomics Analysis on Gadoxetate Disodium-Enhanced MRI Predicts Response to Transarterial Embolization in Patients with HCC
Roberto Cannella , Carla Cammà , Francesco Matteini , Ciro Celsa , Paolo Giuffrida , Marco Enea , Albert Comelli , Alessandro Stefano , Calogero Cammà , Massimo Midiri , Roberto Lagalla , Giuseppe Brancatelli , Federica Vernuccio
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"Deep Learning Network for Segmentation of the Prostate Gland With Median Lobe Enlargement in T2-weighted MR Images: Comparison With Manual Segmentation Method"
Giuseppe Salvaggio, Albert Comelli, Marzia Portoghese, Giuseppe Cutaia, Roberto Cannella, Federica Vernuccio, Alessandro Stefano, Nino Dispensa, Giuseppe La Tona, Leonardo Salvaggio, Mauro Calamia, Cesare Gagliardo, Roberto Lagalla, Massimo Midiri
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Pierpaolo Alongi , Riccardo Laudicella , Francesco Panasiti , Alessandro Stefano, Albert Comelli , Paolo Giaccone , Annachiara Arnone , Fabio Minutoli , Natale Quartuccio , Chiara Cupidi , Gaspare Arnone , Tommaso Piccoli , Luigi Maria Edoardo Grimaldi , Sergio Baldari Giorgio Russo
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Viviana Benfante , Alessandro Stefano, Albert Comelli , Paolo Giaccone , Francesco Paolo Cammarata , Selene Richiusa , Fabrizio Scopelliti, Marco Pometti, Milene Ficarra , Sebastiano Cosentino , Marcello Lunardon , Francesca Mastrotto , Alberto Andrighetto , Antonino Tuttolomondo , Rosalba Parenti , Massimo Ippolito, Giorgio Russo
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STRUCTURAL BIOLOGY AND BIOPHYSICS

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FOCUS

- Identification of new therapeutic targets
- Development of therapeutic proteins with particular emphasis on therapeutic antibodies
- Biophysical screening for the discovery of new therapeutic candidates for preclinical studies
- Structure determination of proteins and protein complexes
- Implementation of NMR-based diagnostic tools

AIMS

- Unravel molecular mechanisms behind human diseases
- Develop novel preclinical candidates for unmet medical needs
- Contribute to define next generation therapeutic treatments
- Facilitate the creation and consolidation of local biotech companies
- Contribute to consolidate Structural Biology in South Italy and Mediterranean Area

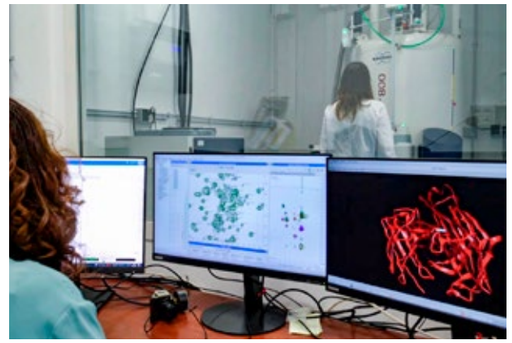
COLLABORATIONS

- National Research Council (CNR), Palermo, ITA
- University of Palermo, ITA
- University of Campania "Luigi Vanvitelli", Naples, ITA
- The Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT-IRCCS), Palermo, ITA
- European Brain Research Institute Rita Levi-Montalcini, Roma, ITA
- King's College London, Londra, UK

The Structural Biology and Biophysics group provides biophysical and structural information of biological phenomena guided by folding, aggregation, and interaction of proteins with the ultimate goal of understanding the molecular mechanisms underlying serious pathologies. The group is also engaged in small molecules-based drug discovery, and development of protein-based therapeutics with particular emphasis on therapeutic antibodies.

The main focus of the research activities of the group is on neurodegenerative diseases. Neurodegeneration is an increasing threat of our increasingly aging modern society. Current treatments are in the best-case palliative and non-specific, reflecting the fact that the detailed understanding of most of these diseases is still lacking. Our research aims at understanding the molecular mechanisms of protein misfolding and aggregation behind neurodegenerative diseases, and relies on the concept that knowledge of the normal function and of the interaction network of aggregating proteins is a key tool to design molecules which can specifically compete out pathological aggregation. Native protein-protein interactions could indeed provide important means of altering and controlling the function and assembly of proteins involved in neurodegenerative diseases, and could fulfil a protective role against aberrant aggregation.

In parallel, the group is engaged in the structural and biophysical characterization of Mussel Foot adhesive proteins, which, similarly to proteins involved in neurodegenerative diseases, undergo to phase transition and form stable protein aggregates. The final aim of this research project is the developing bioadhesives able to work in wet environment. In the last years, there is a growing interest focusing on the development of novel naturally-derived glues in several areas of clinical applications such as tissue engineering, implantation of medical devices and wound closure. The big challenge in developing new bio-adhesive molecules is to find molecules able to work in wet and hostile environment and capable of making tissues adhere together in an efficient way in those conditions. Proteins from sessile animals with adhesive properties in water, could overcome these difficulties.



MOLECULAR INFORMATICS

FOCUS

- **Creation of molecular libraries for biological screening**
- **Design of chronic inflammation disease modulators**
- **Design of anticancer agents**
- **Study of protein-protein interactions in biological processes**
- **Computational applications for biologics design**

AIMS

- **Understanding of molecular mechanisms behind different pathologies**
- **Design of chemical or biological therapeutic agents**
- **Creation of predictive *in silico* models to reduce from-bench-to-bedside process duration**

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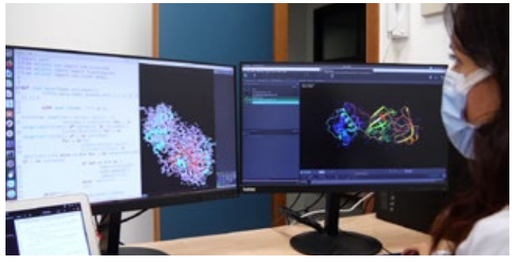
- University of Palermo, ITA
- University of Milan, ITA
- Université Paris Cité, FRA
- University of Vienna, AUT
- Institute of Translational Pharmacology (IFT) (CNR), ITA



The Molecular Informatics group at Ri.MED Foundation mainly deals with the creation and use of *in silico* tools for the design, identification and optimization of biologically active molecules.
The group aim is the creation of reliable *in silico* prediction models to be used for the understanding of molecular mechanisms behind different pathologies and the subsequent design of chemical or biological therapeutic agents. The approaches used rely on the classical molecular modeling tools for virtual screening as well as modern chemoinformatics tools based on artificial intelligence.
Over the years, the team has gained various experiences in the field of medicinal chemistry and computational chemistry.
The expertise acquired by the team members is synergistically exploited for the creation of molecular libraries and to create and validate reliable theoretical models to be used for subsequent virtual ligand screening (VLS).

The molecular informatics group is also involved in the exploration of the chemical space in addition to the enrichment processes of the available molecular libraries to create focused libraries to be used for biological screening campaigns.

The *in silico* outcomes are further validated experimentally through biological or biophysical assays. In recent years, the collaboration with academic groups from University of Palermo and Vienna has strengthen the development of approaches based on the use of deep learning for the activity prediction of small molecules. Furthermore, during the last year, the group has set a dedicated *in silico* platform which will be used for the design and optimization of biological drugs.

During 2022 the group has been involved in three main projects based on the design of chronic inflammation disease modulators, the development of a computational prioritization system to repurpose drugs on the human kinome and the study of protein-protein interactions applied to Host-guest recognition in infectious diseases. The group is also in charge to run the compound management platform with the aim of creating molecular libraries suitable for biological screening and draw up screening plate to be delivered at Ri.MED screening platform facility or to outside collaborators. The main outcomes for 2022 have been the Completion of the OBIND platform (Oncological therapies through Biological Interaction Network Discovery - https://www.obind.eu/, the publication of the recommendation system on protein kinases for the repositioning of anticancer drugs (KUALA: a machine learning-driven framework for kinase inhibitors repositioning) and a virtual screening system based on molecular fingerprints (EMBER-Embedding Multiple Molecular Fingerprints for Virtual Screening)

MEDICINAL CHEMISTRY

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FOCUS

- Design and synthesis of new chemotypes
- Identification of new hit compounds
- Family hit expansion and structure optimization
- Structure-activity relationship (SAR) investigation
- Hit-to-lead optimization
- Planning, set-up, optimization and scale-up of synthetic routes
- Retrosynthetic analysis
- Structure characterization of new chemotypes
- Design and synthesis of novel potential non-covalent inhibitors of NLRP3 inflammasome

AIMS

- Discovery of new potential targets modulators
- Early drug discovery toward disease therapeutic treatment

COLLABORATIONS
- University of Palermo, ITA
- University of Campania "Luigi Vanvitelli", Naples, ITA

Focus of Medicinal Chemistry group is the design and synthesis of novel small molecules, aiming at the discovery of new hits toward therapeutic targets of interest. The synthetic effort leads to the creation of compound libraries and building blocks collections. Main expertise include the design, synthesis, structural elucidation and analytical characterization of newly synthesized compounds.

To date, most efforts are dedicated to the identification of novel non-covalent and allosteric potential inhibitors of NLRP3 (nucleotide-binding domain leucine-rich-containing family, pyrin domain-containing 3) inflammasome, a validate target for chronic inflammation in age-related diseases, such as, neurodegeneration and autoimmune disorders. MCC950 is the best lead candidate (IC_{50} of 8 nM) but failed the clinical trials due to hepatic and renal toxicities.

Starting from MCC950 structure and from evidences in literature of the binding mode of a close analogue (pdbs: 7PZC and 7ALV), we designed new chemotypes trying to retain the electronic properties of this class and the key interactions within the NACHT domain, in a region proximal to Walker A, in order to identify novel potential inhibitors of the NLRP3 machinery.

In detail, during the year 2022, our research goal was the set-up and optimization of the synthetic route for the preparation of a small set of α,β-unsaturated sulfonamides. The desired compounds were obtained in high yield and high purity grade and were evaluated *in vitro* in a phenotypic assay by the HTS group of Ri.MED Foundation; however, none of these small molecules showed inhibition for the target and more research is ongoing. Indeed, we are exploring the synthetic feasibility of 3 more chemotypes and this will be the main task of next year research activities.

A second project of interest deals with the identification of novel inhibitors of Sirtuin-6 (Sirt6), a validated target for lymphoma treatment. In collaboration with University of Palermo and Molecular Informatics group of Ri.MED Foundation, docking studies and structural analysis combined to Molecular Dynamics simulations helped with the selection of Sirt6 crystal structure on which, a set of fragments were rationally designed as novel potential modulators of Sirt6. Planning and set-up of synthetic routes for the preparation of these fragments were the focus of the end of 2022, and are still ongoing.

The main goal is the validation of these new fragments in the biological phenotypic assay, to prove the anticancer activity in liquid lymphomas. During 2023, the synthetic route will be fully optimized and the newly obtained title compounds will be biologically evaluated.

Within the multidisciplinary Drug Discovery area, the Medicinal Chemistry group supports the screening campaigns, with hit structure confirmation, hit re-synthesis, hit series expansion and optimization, hit-to-lead phase. A screening campaign of 912 compounds selected by Virtual Screening (VS) on the NACHT and PYD domains of NLRP3 was completed in September 2022. The campaign led to the identification of 11 preliminary hits which were re-purchased and underwent quality control check held by the medicinal chemistry platform. The hits were confirmed and only one was selected for hit series expansion and structural optimization which will be one of the focus of next year research activities.

EXPERIMENTAL LUNG RESEARCH

FOCUS

- **Chronic Lung diseases**
- **Inflammation**
- **Innate immunity**
- **Advanced experimental models**

AIMS

- **Dissect novel mechanisms promoting chronic inflammatory lung diseases**
- **Discover new therapeutic targets**
- **Develop experimental models to study lung diseases**

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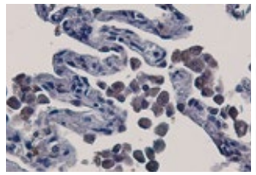
- University Medical Center Groningen, NL
- University of Palermo, ITA
- Institute of Translational Pharmacology (IFT) – National Research Council (CNR), ITA
- Institute for Biomedical Research and Innovation (IRIB) – National Research Council (CNR), ITA

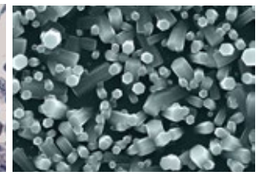
The Experimental Lung Research group studies the dysregulation of innate immune responses in chronic inflammatory airway diseases. A specific focus is on the role of macrophages and the mechanisms controlling their activation and death. In this respect, recent evidences suggest that dysregulation of cell death mechanisms impairs the resolution of inflammation and contributes to sustain chronic inflammatory reactions.

Several forms of regulated death mechanisms in addition to apoptosis have being reported, including pyroptosis, necroptosis and PANoptosis. Contrarily to the inflammatory silent apoptosis, other forms of lytic cell death fire up inflammatory reactions.

Therefore, fine tuning of cell death appears a novel strategy for the control and treatment of chronic inflammation. In addition, the cross-talk between macrophages and lung structural cells, including bronchial epithelial cells and fibroblasts, plays a key role in the progression of chronic lung diseases and represents a more recent research focus of our lab. Research conducted in the last years by our team has led to the discovery of novel mechanisms promoting dysregulated inflammatory response and cell death of human macrophages in disease-relevant models characterized by elevated oxidative stress. In particular, we have discovered that inflammasome-independent activation of caspases in response to cigarette smoke exposure promotes the cleavage of gasdermins (both D and E) and enhances pyroptosis upon exposure to pathogen-associated molecular patterns (PAMPs). More recently, the group has extended its area of interest by exploring the role of myeloid cell death in the context of viral infections.

We have consolidated expertise in the set-up of advanced experimental models using human primary cells isolated from peripheral blood or derived from lung resections in collaboration with ISMETT IRCCS. This has recently attracted the collaboration with the Engineering Department at the University of Palermo for the development of joint projects where our experimental models serve for the set-up and validation of nanostructured electrochemical sensors for monitoring oxidative stress and inflammation.





PROTEOMICS

FOCUS

- **Investigate ectodomain shedding in health and disease**
- **Dissect the role of ADAM17 and iRhoms in cancer and inflammatory diseases**
- **Investigate protein trafficking and secretion by using high-resolution mass spectrometry**

AIMS

- **Use unbiased proteomics to better understand pathophysiological processes**
- **Identify molecular targets and biomarkers to improve healthcare**
- **Develop new diagnostic and therapeutic approaches to better predict possible side effects of medication**
- **Support proteomics studies of any size on collaborative basis**

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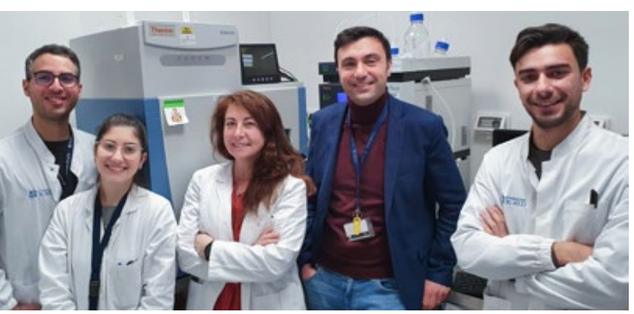
- St. George's, University of London UK
- University of Nottingham UK
- Università di Udine, ITA
- University of Padova, ITA
- University of Palermo, ITA
- IRIB-CNR, Palermo ITA

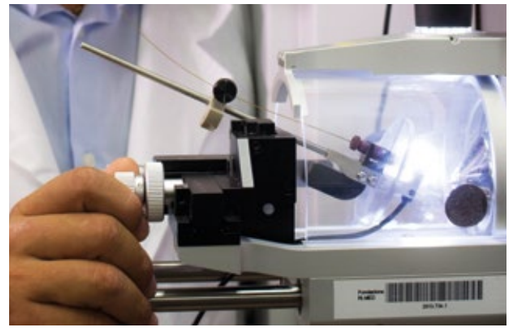


The Proteomics Lab of Fondazione Ri.MED has established a state-of-the-art proteomic platform, comprising a Vanquis Neo uHPLC System on-line coupled to an Exploris 480 mass spectrometer that allows top-level quantitative proteomic analysis. In details, this technology allows the chromatographic separation of different peptides derived from the proteolytic digestion of complex protein mixtures, electrospray ionization of such peptides and their fragmentation into a number of ions with a specific pattern of different mass/charge ratios, called mass spectra, that are a unique signature of each peptide. Mass spectra get computationally analyzed to infer each single protein contained in the starting mixture.

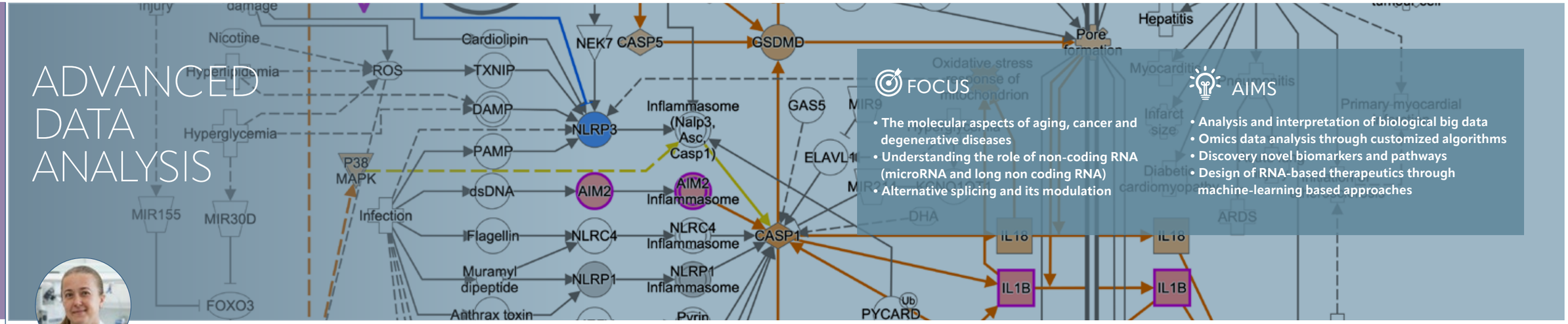
The Proteomics Lab uses high-resolution proteomics and cell biology-based approaches to investigate the proteolytic release of transmembrane proteins from the cell surface, the so-called ectodomain shedding, and elucidate the role of proteinases and their regulators in the development of diseases, such as arthritis.

Ectodomain shedding is a post-translational modification that plays a crucial role in cell-cell communication and other biological processes. ADAM17, a member of the "disintegrin and metalloproteinase" family that was first identified as the enzyme responsible for the proteolytic cleavage of TNFα mediates ectodomain shedding of over 80 proteins, spanning from signaling molecules, such as cytokines and growth factors, to cell receptors, adhesion molecules and endocytic proteins.

Functions of a protease strictly depend on its collections of substrates. We established an advanced workflow for secretome analysis to identify ADAM17 substrates in a systematic manner and, therefore, to explore novel functions of the proteinase. In addition, by using similar approaches in proteomics we investigate how the essential regulators of ADAM17, iRhom1 and iRhom2, can control its activity and drive the proteinase toward specific groups of substrates. In addition to internal projects, the lab supports the research within Ri.MED and actively collaborates with several national groups and overseas at projects spanning from the proteomic analysis of liquid biopsies of transplanted patients, in the pursue of biomarkers to predict graft rejection, to the proteomic characterization of the cancer-associated metalloproteinase ADAM15.



ADVANCED DATA ANALYSIS



FOCUS

- The molecular aspects of aging, cancer and degenerative diseases
- Understanding the role of non-coding RNA (microRNA and long non coding RNA)
- Alternative splicing and its modulation

AIMS

- Analysis and interpretation of biological big data
- Omics data analysis through customized algorithms
- Discovery novel biomarkers and pathways
- Design of RNA-based therapeutics through machine-learning based approaches

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Vincenzo Martorana
PhD Student in Economics and Statistics

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- National Research Council (CNR), ITA
- IRCSS ISMETT, Palermo, Italy
- University of Pittsburgh, PA, US

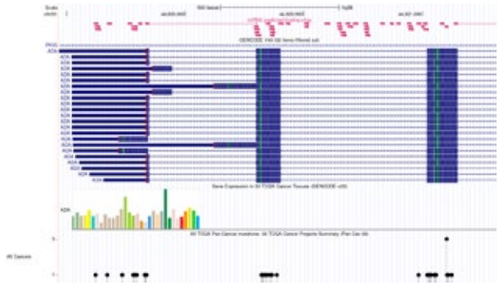
The Advanced Data Analysis group integrates different layers of expertise spanning from wet lab to big data analysis, with the goal of unraveling biological complex systems by developing customized experimental and computational pipelines. In fact, we constantly put at the service of other research groups our expertise on the analysis of biological big data, both leveraging established algorithms and developing new ones, also based on machine learning and deep learning approaches. We actively participate in the Drug Discovery area activities by supporting other groups in high throughput screening experimental design and their data analysis.

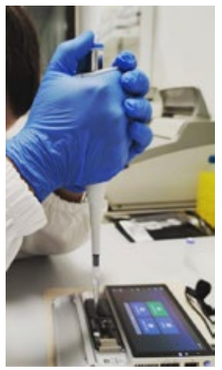
Our main interest is in biological regulatory networks. In particular, we study the regulatory role of non-coding RNA, e.g., microRNA and long non-coding RNA, in the context of aging, cancer and degenerative diseases. We seek the role of non-coding RNA in gene expression modulation and the occurrence of alternative splicing events, by integrating the analysis of multiple omics data platforms and network analysis methods.

Regulatory interactions that have been computationally predicted are validated in wet lab, e.g., with luciferase assays for microRNA-target interactions. For completeness, we are growing expertise with the OXFORD Nanopore technology, which provides a complete picture of the samples at transcriptomics level, useful for our investigations on gene expression and alternative splicing occurrences.

In 2022, we started to be involved in the newly born National Center for RNA-based Therapeutics, funded by PNRR, with a role in developing antisense oligonucleotides (ASO) to modulate the gene expression or the occurrence of alternative splicing of selected targets. In this scenario, we are developing machine learning based algorithms to design the optimal ASOs to be used on selected targets. Specifically, we are involved in two projects concerning aging-related diseases, SMART and SUNFOX, which aim to regulate the aberrant alternative splicing of LMNA and the expression of FOXO4, respectively.

During 2022, we joined the COST action "Mye-Infobank: Converting molecular profiles of myeloid cells into biomarkers for inflammation and cancer". Within this collaboration, we are growing our expertise in single cells transcriptomics and deconvolution from bulk RNAseq data.

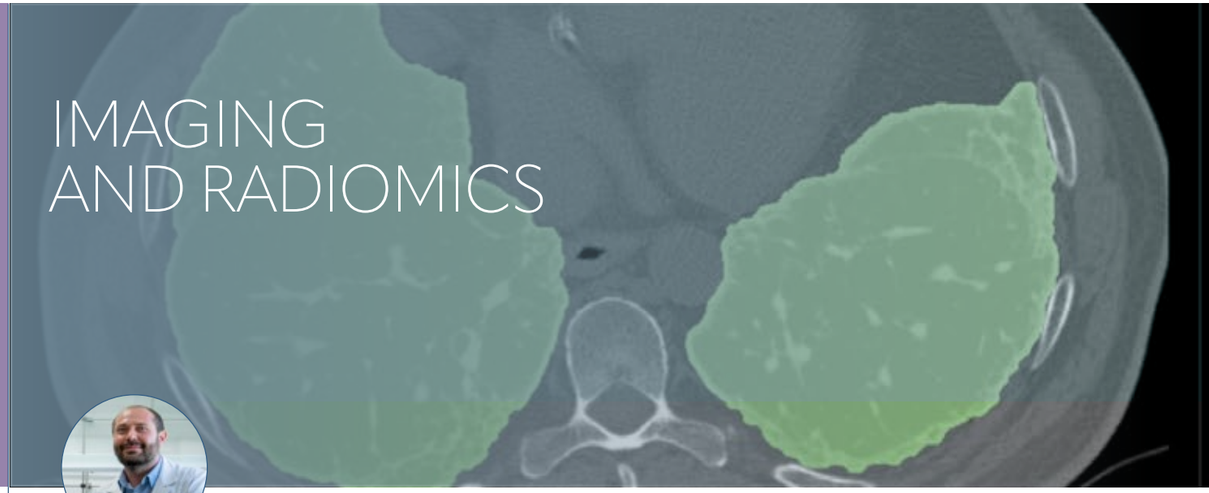




DRUG DISCOVERY

Ri.MED RESEARCH

IMAGING AND RADIOMICS



FOCUS

- **Artificial Intelligence in Precision Medicine**
- **Radiomics for the quantitative evaluation of the Efficacy of treatments**
- ***In Vitro* and *In Vivo* Theranostic studies: New Radiopharmaceuticals for Diagnosis and Therapy**
- **Radiobiology Studies for Dosimetry-Time Effectiveness of Radiopharmaceutical Therapy**

AIMS

- **To develop Artificial Intelligence systems to support Biomedical decision-making**
- **To develop tools for target Detection, Segmentation and Classification in Biomedical Imaging**
- **To develop Radiomics tools for Preclinical Biodistribution analysis of Radiopharmaceuticals**
- ***In Vitro* Quantification of Radioactive compounds**

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- University of Catania, Catania, ITA
- University of Messina, Messina, ITA
- Biomedical Campus University of Rome, Rome, ITA
- Cannizzaro Hospital, Catania, ITA
- University Hospital, Palermo, ITA
- Experimental Zooprophylactic Institutes of Sicily (IZS Sicily), Palermo ITA
- Georgia Institute of Technology, Atlanta, GA 30332, USA

The Imaging and Radiomics Group, a multidisciplinary group, focuses on a variety of research fields from biology and medicine to engineering and math until informatics. One part of the research team specializes in *in vitro* and *in vivo* radiobiology studies and biodistribution of new compounds with Artificial Intelligence (AI) tools; another group specializes in radiomics, segmentation, and classification using AI. All members of the team are united by a common interest in multimodal imaging.

During 2022, preliminary *in vitro* experiments using gamma counters to evaluate bound and internalized radiopharmaceuticals in cell line samples were performed. For the development of predictive systems for vascular classification, the Imaging and Radiomics group collaborated with Georgia Institute of Technology doing radiomic analysis on magnetic resonance angiography imaging of mice with special kind of anemia.

The group has conducted the following research lines in preclinical and clinical molecular imaging with positron emission tomography (PET):

- Analysis of [18F]FDG PET/CT images of metastatic colorectal cancer patients to establish radiomic models capable of predicting disease progression (PD) and survival outcomes;
- Radiomics predictive models of response analyzing [68Ga]DOTATOC PET/CT images for theragnostics in Gastroenteropancreatic (GEP) Neuroendocrine tumors (NETs), to support clinical decision;
- Semi-automatic strategies to identify several mouse organs, and to investigate, through radiomic analyses, possible changes over time in [64Cu] chelator biodistribution using micro-PET imaging;
- Radiomic analysis based on advanced imaging segmentation methods, complete with a Machine-Learning application to predict Alzheimer's Disease based on Amyloid-PET for final clinical diagnosis;
- Comparisons of algorithms using preclinical data from mice undergoing micro-PET/CT scans after chelator injection.

Clinical research activities in Magnetic Resonance Imaging included:

- Evaluations of the performance of MRI-based radiomic features for diagnosing and grading chronic hepatic encephalopathy in adult patients with cirrhosis;
- Radiomics analysis of MRI images was performed to distinguish cerebellopontine angle neurinomas from schwannomas that arise elsewhere in the neck;
- Radiomic workflow was developed for predicting hepatocellular carcinoma (HCC) response after transarterial embolization (TAE);
- Analysis of resting-state functional magnetic resonance (RS-fMRI) signals from WU-Minn HCP 1200 Subjects Data Release to investigate brain function at a high level.

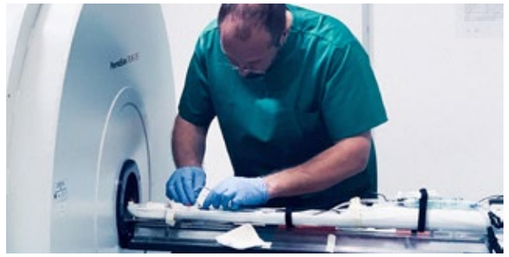
Additionally, the group was involved in the development of:

- Deep learning (DL) algorithms for accurate liver delineation in high-resolution computed tomography (CT) images of pre-transjugular intrahepatic portosystemic shunt (TIPS) cirrhotic patients;
- High-resolution semantic segmentations on Cardiac Tomography Angiography imaging, coupled with shape-prior-based segmentation that enforces anatomical correctness;
- Voxel-based Morphometries to quantify brain mass using both the K-means clustering algorithm and artificial neural network to quantify brain mass;
- A new free and user-friendly radiomics framework, namely matRadiomics.

Several research studies were conducted by the group in the field of:

- Fully automated deep learning networks, like Efficient Neural Network (ENet) for segmenting prostate glands with median lobe enlargement and ERFNet for retroperitoneal sarcoma (RPS) segmentations.

In 2022, simulations were conducted using a morphological and biophysically detailed model of a Cornu Ammonis 1 (CA1) pyramidal neuron.



REGENERATIVE MEDICINE AND IMMUNOTHERAPY

EXPERIMENTAL IMMUNOTHERAPY
HEPATOBILIARY REGENERATIVE MEDICINE
REGENERATIVE MEDICINE - CELLULAR THERAPIES
GMP CELL FACTORY
PRECLINICAL RESEARCH *IN VIVO*

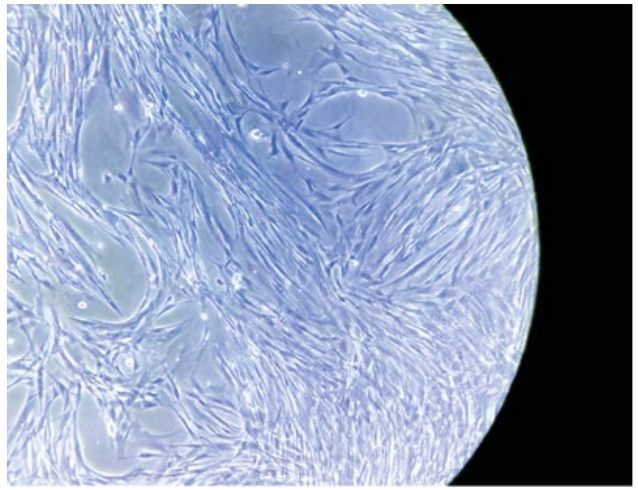
The focus of the Regenerative Medicine and Immunotherapy Area is the development of innovative cell-based therapies, biologicals and biomaterials to treat end-stage organ diseases and post-transplant complications.

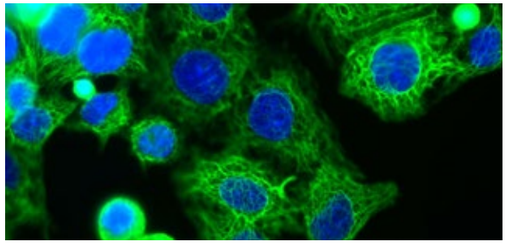
In the field of regenerative medicine, active projects aim to find innovative therapeutic solutions for (i) liver diseases, using products derived from cells (secretome and extracellular vesicles), modified to promote their release and their action in the liver; (ii) biliary diseases, through the development of engineered bile ducts. In the field of immunotherapy, we are developing (iii) naïve and engineered Natural Killer cells for the treatment of hepatocarcinoma recurrence, especially after transplantation, and (iv) tolerogenic dendritic cells derived from living donors to induce tolerance to transplantation in kidney or liver transplant recipients.
Another important research focus is the study and development of cell therapies for the treatment of post-transplant infections.

The research groups operating in this Area perform research projects including the final steps of product development, preclinical in vivo studies and advanced therapies production.
The expertise of the groups belonging this Area covers molecular and cellular biology, immunology, biomaterial science, quality assurance and quality control, as well as deep knowledge on animal care and small and big experimental animal models. This multidisciplinary and complementary knowledge has the potential to greatly contribute to a rapid translation of innovative therapies to the clinics.

Our activities mainly take place at Ri.MED laboratories in ISMETT IRCCS, with strong interaction with ISMETT researchers and clinicians, and in close collaboration with University of Pittsburgh and University of Pittsburgh Medical Center's teams, as well as with leading Italian/European research groups and cell factories

Three-dimensional biliary cell culture.
Courtesy of the Hepatobiliary Regenerative Medicine Group





PUBLICATIONS

Galectin-9 and Interferon-Gamma Are Released by Natural Killer Cells upon Activation with Interferon-Alpha and Orchestrate the Suppression of Hepatitis C Virus Infection

Anna Paola Carreca, Massimiliano Gaetani, Rosalia Busà **Maria Giovanna Francipane**, Maria Rita Gulotta , Ugo Perricone , Gioacchin Iannolo , Giovanna Russelli , **Claudia Carcione**, Pier Giulio Conaldi , **Ester Badami**
Viruses 2022 Jul 14;14(7):1538. https://doi.org/10.3390/v14071538

Hepatocellular carcinoma, hepatitis C virus infection and miRNA involvement: Perspectives for new therapeutic approaches

Ester Badami , Rosalia Busà , Bruno Douradinha , Giovanna Russelli, Vitale Miceli , Alessia Gallo , Giovanni Zito , Pier Giulio Conaldi , Gioacchin Iannolo
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HCV Interplay With Mir34a: Implications in Hepatocellular Carcinoma

Ester Badami , **Claudia Carcione** , **Cinzia Maria Chinnici** , Rosaria Tinnirello , Pier Giulio Conaldi , Gioacchin Iannolo
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Rosalia Busà , Matteo Bulati , **Ester Badami** , Giovanni Zito , Daniela Claudia Maresca , Pier Giulio Conaldi , Giuseppe Ercolano , Angela Ianaro
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Claudia Coronnello, **Maria Giovanna Francipane**
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Blood Vessel Detection Algorithm for Tissue Engineering and Quantitative Histology

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Composite Hydrogels of Alkyl Functionalized Gellan Gum Derivative and Hydroxyapatite/Tricalcium Phosphate Nanoparticles as Injectable Scaffolds for bone Regeneration

Giovanna Pitarresi, Fabio Palumbo, Calogero Fiorica, Flavia Bongiovì, Annalisa Martorana, Salvatore Federico, **Cinzia Maria Chinnici**, Gaetano Giammona.
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Human Amnion-Derived Mesenchymal Stromal Cells: A New Potential Treatment for Carbapenem-Resistant Enterobacterales in Decompensated Cirrhosis

Mariangela Pampalone, **Giampiero Vitale,** Salvatore Gruttadauria, **Giandomenico Amico**, Gioacchin Iannolo, Bruno Douradinha, Alessandra Mularoni, Pier Giulio Conaldi, Giada Pietrosi
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Salvatore Pasqua, Francesco Monaco, Francesca Cardinale, Simone Bonelli, Pier Giulio Conaldi, Danilo D'Apolito
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In Vitro Evaluation of Nanoerythrosome Cytotoxicity and Uptake in Pancreatic Endothelial Cells: Implications for β-Cell Imaging Applications

Vitale Miceli, Marco Fornasier, Matteo Bulati, **Giandomenico Amico**, Pier Giulio Conaldi, Anna Casu, Sergio Murgia 3
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Human Amnion-Derived Mesenchymal Stromal/Stem Cells Pre-Conditioning Inhibits Inflammation and Apoptosis of Immune and Parenchymal Cells in an *In Vitro* Model of Liver Ischemia/Reperfusion

Giovanni Zito, Vitale Miceli, **Claudia Carcione,** Rosalia Busà, Matteo Bulati, Alessia Gallo, Gioacchin Iannolo, Duilio Pagano, Pier Giulio Conaldi
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Specific Anti-SARS-CoV-2 Humoral and Cellular Immune Responses After Booster Dose of BNT162b2 Pfizer-BioNTech mRNA-Based Vaccine: Integrated Study of Adaptive Immune System Components

Rosalia Busà, Maria Concetta Sorrentino, Giovanna Russelli, **Giandomenico Amico**, Vitale Miceli, **Monica Miele**, **Mariangela Di Bella, Francesca Timoneri,** Alessia Gallo, Giovanni Zito, Daniele Di Carlo, Pier Giulio Conaldi, Matteo Bulati
Frontiers in Immunology, 2022 https://doi.org/10.3389/fimmu.2022.856657

Analysis of the Specific Immune Response after the Third Dose of mRNA COVID-19 Vaccines in Organ Transplant Recipients: Possible Spike-S1 Reactive IgA Signature in Protection from SARS-CoV-2 Infection

Mariangela Miele, Rosalia Busà, Giovanna Russelli, Maria Concetta Sorrentino, **Mariangela Di Bella**, **Francesca Timoneri**, **Giampiero Vitale,** Enrico Calzolari, Patrizio Vitulo, Alessandra Mularoni, Pier Giulio Conaldi. Matteo Bulati.
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Immune Response after the Fourth Dose of SARS-CoV-2 mRNA Vaccine Compared to Natural Infection in Three Doses' Vaccinated Solid Organ Transplant Recipients

Rosalia Busà, Giovanna Russelli, **Monica Miele**, Maria Concetta Sorrentino, **Mariangela Di Bella**, **Francesca Timoneri**, Giuseppina Di Mento, Alessandra Mularoni , Patrizio Vitulo , Pier Giulio Conaldi, Matteo Bulati
Viruses 2022, 14, 2299 https://doi.org/10.3390/v14102299

Long-Term Effectiveness of BNT162b2 Pfizer-BioNTech mRNA-Based Vaccine on B Cell Compartment: Efficient Recall of SARS-CoV-2-Specific Memory B Cells

Rosalia Busà, Monica Miele, Maria Concetta Sorrentino, Giandomenico Amico, Francesca Timoneri, Vitale Miceli, Mariangela Di Bella, Giovanna Russelli, Alessia Gallo, Giovanni Zito, Gioacchin Iannolo, Pier Giulio Conaldi, Matteo Bulati
International Journal of Molecular Sciences 2022-11-30
https://doi.org/10.3390/ijms232315046

Changes in the Transcriptome Profiles of Human Amnion-Derived Mesenchymal Stromal/Stem Cells Induced by Three-Dimensional Culture: A Potential Priming Strategy to Improve Their Properties

Alessia Gallo, Nicola Cuscino, Flavia Contino, Matteo Bulati, **Mariangela Pampalone**, **Giandomenico Amico**, Giovanni Zito, **Claudia Carcione,** Claudio Centi, Alessandro Bertani, Pier Giulio Conaldi, Vitale Miceli
Int J Mol Sci. 2022 Jan; 23(2): 863. https://doi.org/10.3390/ijms23020863

EXPERIMENTAL IMMUNOTHERAPY

FOCUS

- **Engineering of CAR-NK cells for the treatment of liver cancer**
- **Increasing the anti-tumoral fuction of NK cells by *ex vivo* activation with specific cytokines**
- **Understanding the role of dendritic cells in tolerance induction**
- **Development of GMP-compliant protocols for large scale cell production for clinical applications**
- **Production of Tolerogenic Dendritic Cells**
- **Preclinical studies and *in vivo* proof-of-concept of safety and efficacy of of cell-mediated therapies**

AIMS

- **Treatment of patients affected by liver cancer with innovative CAR-NK cells engineered to have enhanced specificity to hepatocellular carcinoma**
- **Early weaning off immunosuppressive therapy of Solid Organ Recipient using Tolerogenic Dendritic Cells**
- **Development of off-the-shelf cellular products**

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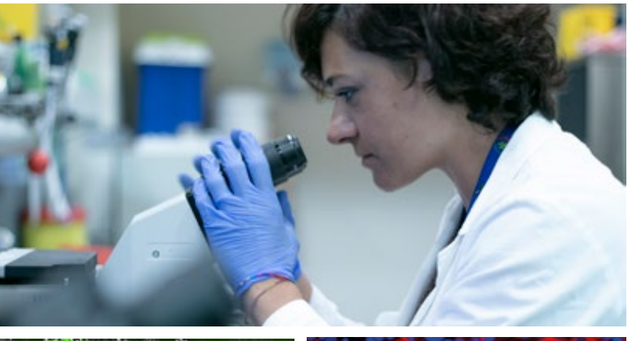
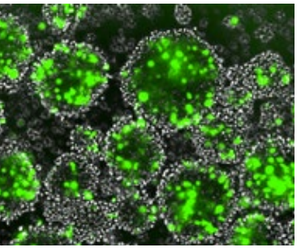
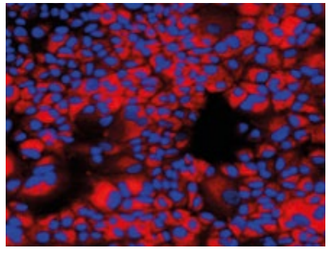
- Thomas E. Starzl Transplantation Institute, University of Pittsburgh School of Medicine, USA
- University Of Pittsburgh Medical Center (UPMC)
- The University of Pittsburgh, McGowan Institute of Regenerative Medicine (MIRM), USA
- Experimental Zooprophylactic Institutes of Sicily (IZS Sicily), IT
- National Research Council (CNR), ITA





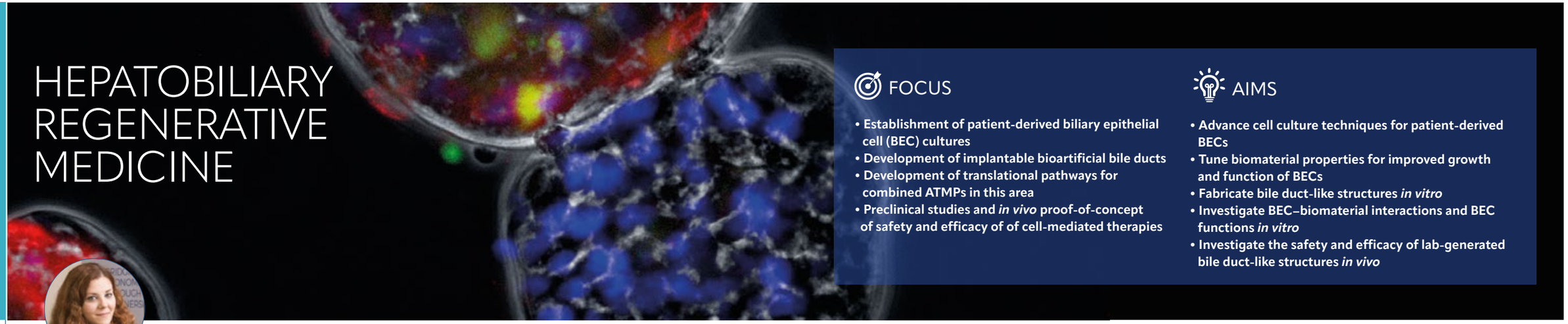


Our research is focused on the development of cell-mediated therapies aiming at increasing the life expectancy of patients in solid organ transplantation and cancer. During 2022 we focused our research on novel treatment to improve the prognosis of hepatocellular carcinoma (HCC), such cell-mediated immunotherapy. A second project developed in our lab investigates the role of tolerogenic Dendritic cells in the induction of operational immune tolerance in solid organ recipients.

HCC is a malignant epithelial tumor arising from hepatocytes. Treatments for HCC include hepatectomy, liver transplant or chemotherapy which are not always effective for advanced forms of HCC and the risk of recurrence is high. Therefore novel treatment strategies are urgently needed. Natural killer (NK) cells play an important role in the innate host immune response against viruses and tumors. The frequency and function of NK cells in the peripheral blood and liver are associated to the recurrence and survival rates of patients with resectable HCC. Thus, hepatic NK cells are thought to play an important role in mediating the immune function of the liver. We have patented a method to isolate high numbers of viable NK cells from an alternative source such as the liquid perfusate of the liver of deceased donors. We have also optimized a protocol to expand these cells obtaining clinically relevant cell concentrations. We have recently demonstrated that NK cell conditioning with cytokines such as interferon-alpha (IFN-α) significantly increase their anti-viral an anti-tumor response both *in vitro* and *in vivo*. Genetic modification techniques have been developed to improve the specificity and efficacy of NK cell cytotoxicity to tumor cells. For example, the approach using CAR for NK cells has enhanced the specificity and efficacy of NK cell therapy. In our laboratory we are investigating the use of a novel CAR construct to engineer NK cells with specificity for a tumor antigen described in HCC and known as Glypican-3 (GPC3). The 4th generation lentiviral construct is designed to also secrete soluble cytokines such as interleukin-15 (IL-15), known to sustain NK cells proliferation, function and survival, and IFN-α. The vector we designed also expresses the suicide gene Epidermal Growth Factor Receptor in a truncated form (EGFRt) as safety switch. Lately, we are exploring the role of the ADAM-17 in the NK-mediated innate response to cancer by knocking-out the expression of this metalloproteinase, which is involved in the stabilization of the surface protein CD16 that concurs to NK cell killing of target cells.

Our lab is also interested in developing a cell-based approach to induce operational tolerance in liver/kidney using tolerogenic Dendritic cells. One major caveat of organ transplantation is graft rejection. Accordingly, immunosuppressive therapy is provided for life to transplanted patients, though it is accompanied by severe side effects such as kidney failure. The use of cellular therapies such as the administration of donor derived tolerogenic Dendritic Cells for early weaning of liver transplanted patients is a novel strategy to prevent graft loss. Our strategy in the use of the liver perfusate from deceased donors as source of DCs precursors.

HEPATOBILIARY REGENERATIVE MEDICINE



FOCUS

- **Establishment of patient-derived biliary epithelial cell (BEC) cultures**
- **Development of implantable bioartificial bile ducts**
- **Development of translational pathways for combined ATMPs in this area**
- **Preclinical studies and *in vivo* proof-of-concept of safety and efficacy of of cell-mediated therapies**

AIMS

- **Advance cell culture techniques for patient-derived BECs**
- **Tune biomaterial properties for improved growth and function of BECs**
- **Fabricate bile duct-like structures *in vitro***
- **Investigate BEC–biomaterial interactions and BEC functions *in vitro***
- **Investigate the safety and efficacy of lab-generated bile duct-like structures *in vivo***

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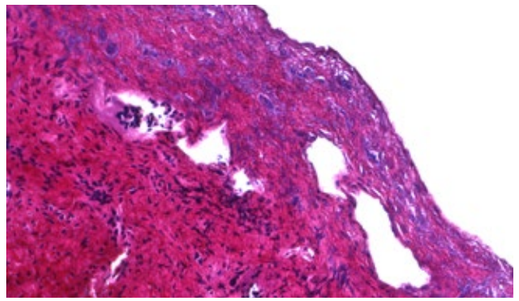

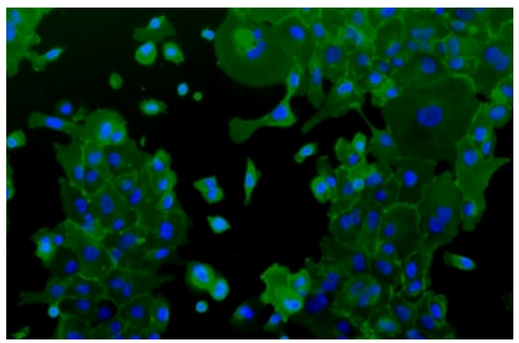

The engineering of extrahepatic bile ducts represents a crucial step towards new treatment options for congenital and iatrogenic structural abnormalities of the extrahepatic biliary tree, for which no definitive therapy exists. In recent years, considerable efforts have been devoted to this goal, and both cellular and acellular constructs have been described. However, only rarely have these constructs progressed beyond the *in vitro* stage, leaving unexplored their potential as therapies, and the *in vitro* characterization has often been incomplete.

While previous approaches were predominantly dominated by an engineering point-of-view, we applied **a rigorous approach to design, fabrication and characterization** that takes into consideration a set of biological, physiological, and biomechanical data of the native tissue, in order to build a construct featuring near-native tissue properties.

Specifically, we focused on regenerating a functional common bile duct (CBD), which is the most distal segment of the extrahepatic biliary tree connecting the biliary tree to the duodenum. The CBD-like construct that we are currently validating contains biliary cells, answering to a shared need to create biologically relevant devices, and it is also planned to incorporate, in the future, other cell types typically found in a native CBD, such as endothelial and smooth muscle cells. The biliary-like epithelium that we have developed is functional and it is surrounded by a biomaterial with mechanical properties. We have performed a deep and **multilevel characterization** of cell behavior in the construct: studies of cell metabolic activity and proliferation; enzymatic functional assays; studies of bile modification; proteomics. We have also performed several tests to confirm that the whole construct does not leak when it is passed by a fluid, and possesses adequate mechanical properties to ensure proper handling by the surgeon, implantability in the patient, as well as host integration and remodeling.

Noteworthy, our CBD-like construct is a monolithic structure with two (the biological and the mechanical) interpenetrating phases, and as such, it stands out from previously-described bilayer constructs, which have limited translational potential due to frequent interlayer delaminations and no-homogeneity.
While embracing a wide-ranging vision, we have also made important **considerations for the path to clinical translation**, including costs of the construct, ease of fabrication, and regulatory approval processes. An inexpensive and simple hydrogel casting method is exploited to generate the biological phase, while the mechanical phase is generated through electrospinning. We use biomaterials of which there are already investigational and FDA-approved precedents. Future preclinical studies will provide definitive information about the safety and biological efficacy of our construct.
Importantly, the systematic approach that we follow ensures that, even if the end-goal of engineering a clinically translatable surrogate CBD is not achieved, the knowledge and skills gained from our study will **impact the scientific field and push the state-of-the-art**, for example with respect to biliary cell biology and biofabrication technologies.

REGENERATIVE MEDICINE: CELLULAR THERAPIES

FOCUS

- **Identifying the best therapeutic agent by comparing secretome/EVs of different sources**
- **Creation of master cell banks according to GMP standards**
- **Manufacturing of secretome/EVs according to GMP standards**
- **Design and production of secretome/EV-laden biomaterials**
- ***In vivo* preclinical tests of efficacy and safety**

AIMS

- **Developing treatment options for chronic skin wounds**
- **Developing treatment options for NAFLD/NASH**
- **Improving the efficacy of secretome/EV therapeutics by providing scaffold biomaterials**

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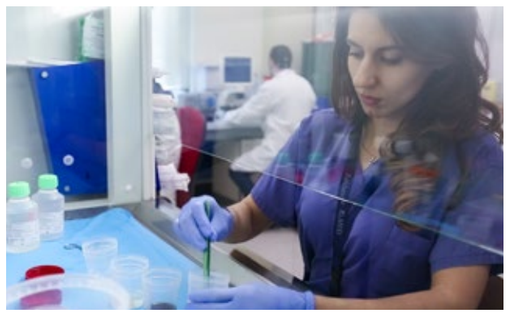
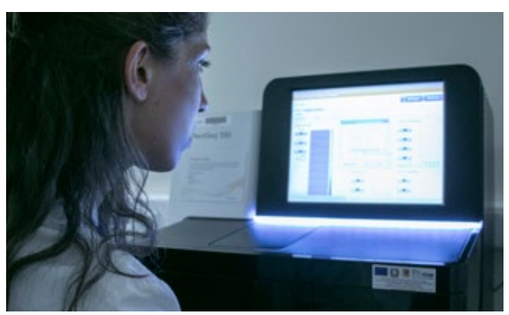
- National Center for Gene Therapy and Drugs based on RNA Technology, University of Padova, ITA

The Regenerative Medicine - Cell Therapy group is engaged in developing strategies focusing on the use of therapeutic cells, such as mesenchymal stromal cells (MSCs) and their secreted factors to restore/improve organ function after a damage. In this scenario, the role of secretome as a new booster for regenerative medicine is crucial. Bioactive molecules of secretome include a wide range of soluble proteins and nucleic acids (e.g., microRNAs) with anti-inflammatory, anti-oxidative and anti-fibrotic effects. Alternatively, bioactive molecules of secretome are also packed into extracellular vesicles, nanosized particles with the capacity to bring their cargos into target cells. MSC-derived secretome and extracellular vesicles recapitulate therapeutic features of parental cells with equal or even better efficacy, to the point that they are considered next generation therapeutics (cell-free therapy).

We seek to develop treatment options for chronic skin wounds, and for acute and chronic liver diseases. In particular, patients with cirrhosis complications or with an increased risk of developing liver fibrosis, such as those with obesity, type 2 diabetes and/or metabolic syndrome, could benefit from MSC-based therapeutics. Furthermore, improving the efficacy of cell-free therapy by polymer-based delivery systems is a central research topic. Delivery systems might preserve the stability of soluble agents by providing protection from clearance and from *in vivo* enzymatic degradation. Delivery systems will also sustain the release of soluble agents to target organ/cells. These systems can be fabricated either as sponges for topical application, or as injectable hydrogels and polymer-coated extracellular vesicles for systemic administration.
The main research activities of our group are listed below:

1. Selecting the appropriate quality control parameters for a GMP-compliant production of MSC-based therapeutics.
2. Releasing cells and secreted products according to the principles of manufacturing and quality control (QC) described in the guidelines for ATMP/biological therapeutics.
3. Design and synthesis of secretome/extracellular vesicle-laden biomaterials, such as injectable or 3D porous hydrogels, and polymer-coated extracellular vesicles.
4. Implementing *in vitro* approaches to evaluated the tuning of biomaterials with cell secreted factors.
5. Implementing in vitro approaches to evaluate the efficiency of extracellular vesicle delivery inside target cells.
6. Preclinical studies of efficacy and safety.





GMP CELL FACTORY

FOCUS

- **Cell based therapies for end stage organ failure (e.g. based on MSCs)**
- **Immunotherapies, e.g. Multivirus specific T lymphocytes**

AIMS

- **Contribute to translational medicine, providing new ATMPs to patients**
- **ATMPs release for Phase I studies and single patient use**
- **Optimization/automation of production processes and Quality Control (QC) tests**

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The Cell factory group is developing an advanced therapy medicinal product (ATMP) based on polyclonal multivirus specific T cells (MVT-cells) for autologous and allogeneic use. T lymphocytes are stimulated simultaneously by EBV, CMV, BK polyoma virus and Adenovirus peptides mix to generate virus-specific T cells to treat virus related complications after transplant.

The use of closed culture systems is an active challenge for our cell factory. We introduced G-Rex 10M devices to expand the T cells and obtained appropriate cell yield, expected immunophenotype and relevant specific activity in the final product. The production group is also involved in specific Anti-SARS-CoV-2 Immune Response studies. Understanding magnitude and quality of immune responses is important for the management and control of the pandemic, as well as for future vaccination strategies. We analyzed the cell-mediated response after BNT162b2 Pfizer-BioNTech mRNA-based vaccine or post-infection, both in solid organ transplant recipients and in immunocompetent donors.

In 2022, the Quality Control group investigated the protocols used in diagnostic laboratories to recover Aspergillus spp. from blood cultures, since the traditional method for the subculture of presumptively positive culture bottles did not result in an optimal recovery performance. To investigate how to overcome this issue, we compared three recovery approaches: (i) the routine method, using a sterile airway needle/subculture unit and collecting two drops onto SDA plates, (ii) the use of a sterile airway needle/subculture unit and collection of a larger aliquot (100 µL), following vigorous agitation of the vials, and (iii) decapsulating the bottle, withdrawing and centrifuging the sample, and collecting 100 µL of the pellet onto SDA plates. Our results showed that only the third procedure was able to recover all five Aspergillus spp. from all positive culture bottles, even with the lowest concentrations of CFU.

PRECLINICAL *IN VIVO* RESEARCH

FOCUS

- **Test surgical, pharmacological, metabolic, gene and immunological therapies on animal models that mimic terminal organ failure**
- ***In vivo* Proof-of-concept**
- **Training for doctors and veterinarians**

AIMS

- **Welfare of Laboratory animals**
- **Drafting of the protocol**
- **Standardization of animal model**
- **3R Application (Reduction, Replacement and Refinement)**
- **Training modules for researchers and personnel who perform procedures (function A), take care of animals (function C), euthanize animals (function D) and design procedures and projects (function B)**

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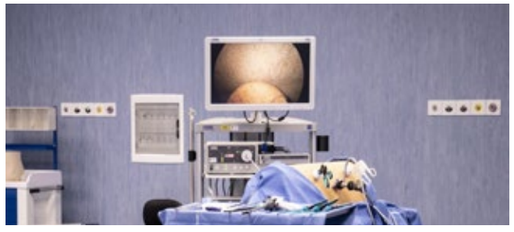
- Experimental Zootechnical Institute for Sicily (ISZS) Palermo, ITA



Our research is focused on: the development of animal pathological models, the improvement of housing conditions based on specie's needs and their microbiological condition and experimental refinement procedures, in order to improve animal wellbeing, and to standardize protocols to obtain robust data.

To date, as the Ri.MED Foundation does not have its own animal facility, the preclinical *in vivo* group uses animal facilities of our institutional partners (such as IZS Sicilia and ISZ) and acts as an interface between Ri.MED research groups and Competent Authorities to carrying out the *in vivo* projects and to obtain the mandatory authorization .

During 2022, we focused our research on transforming a conventional animal facility into a SPF-like (Specific Patogen Free) facility, in order to house a colony of immunosuppressed NOG mice for a study on human hepatocellular carcinoma (HCC).
Immunodeficient mice are often used in translational medicine because they allow the development of human tumors. They are used to test the safety of cell-based therapies (CTP) in order to avoid adverse phenomena due to the immune system and to ensure that the data can be translated to humans. Among the mouse lines, NOD/Shi-scid IL-2 Rynull (NOG) is the most used in this field, this because it carries mutations derived from three mouse strains, NOD/ShiJcl inbred, Prkdcscid (SCID) and IL-2Rynull.

Another characteristic of NOG mice is the deficiency of T, B and NK cells as well as dysfunction of dendritic cells and macrophages. This means that they can more easily develop infections leading to death or worsening animal health condition. With our study we demonstrated that it is possible to maintain the microbiological conditions suitable for this strain for at least 7 months in a non SPF facility.

Moreover in 2022, we started a project in which we studied in swine model how the preoperative "conditioning" could affect pig stress and postoperative outcome. We have, also, obtained the extension of the Ministerial authorization of the large animal facility IRCCS ISMETT to the ovine species, adapting the structure to the ethological needs of the sheep.
Furthermore, our group takes care of training external and internal personnel for functions a) b) c) d) of the directorial decree on training pursuant to art. 23 Legislative Decree 26/14.

BIOENGINEERING AND TISSUE ENGINEERING

BIOENGINEERING AND MEDICAL DEVICES
CARDIOVASCULAR TISSUE ENGINEERING
MUSCULOSKELETAL TISSUE ENGINEERING

The Bioengineering and Tissue Engineering macro-area is a multidisciplinary field that focuses on the development and translation of innovative therapeutic treatments based on the application of the most advanced engineering and regenerative approaches. This activity entails the modelling and analysis of physiological and pathological systems at multi-physics/multi-scale level; the development and characterisation of innovative biomaterials and scaffolds; the study and promotion of tissue regeneration; and the design, development, and preclinical validation of new generation implantable organs and devices. Researchers in this macro-area are engaged in the development new treatments for a wide range of diseases and conditions, including cardiovascular diseases and musculoskeletal disorders.

Scanning electron microscopy of human Saphenous Vein (SV) harvested by conventional technique. Colors highlight the layered structure of the SV. Blue: tunica adventitia; green: tunica media; orange: tunica intima.
Courtesy of the Cardiovascular tissue engineering Group

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BIOENGINEERING AND MEDICAL DEVICES

FOCUS

- **Development and translation of innovative therapeutic treatments**
- **Next generation medical devices**
- **Advanced diagnostic solutions**
- **Therapeutic planning**

AIMS

- **Assist local healthcare institutes**
- **Provide engineering support to clinical decisions**
- **Facilitate development of next generation therapies and diagnostics**
- **Medical devices development and assessment**

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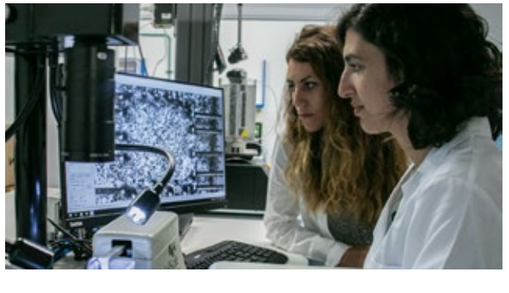
The Bioengineering group applies engineering principles and physical science to the analysis of biological systems and the design new generation biomedical technologies. The group operates mainly in the cardiovascular field, contributing to the conception and optimisation of advanced diagnostic solutions, support tools for therapeutic planning and new generation medical devices based on innovative approaches. The group is rapidly consolidating as a reference for regional healthcare, for the local academic institutions, and for the small and medium enterprises in the geographic area.

The group's researchers offer solid skills in numerical modelling, fluid-structural analysis, and medical device design and development. They are continuously expanding their portfolio of medical device technologies, which already includes a number of innovative technologies for surgical and minimally invasive heart valve repair and replacement. In collaboration with clinical and industrial partners, they are facilitating the introduction of these technologies into clinical practice to maximise their positive impact on patients' quality of life.

In 2022, the group made significant progress in fundamental and applied research. They implemented new numerical SPH-based approaches for modelling complex cardiovascular systems, including fluid-structure interaction problems and simulation of clot formation. They also contributed to enhance the understanding of thrombosis in patients with atrial fibrillation, potentially leading to a more accurate risk stratification of patients. They identified and implemented more accurate approaches for the characterisation of biomaterials and the improvement of clinical procedures.

The group expanded its patent portfolio in the area of medical devices and, in collaboration with academic and industrial partners, made major progress in the *in vitro* and *in vivo* preclinical evaluation of innovative cardiovascular solutions. They are actively participating in the translation of medical devices developed by their researchers into clinical practice.

The group's research has resulted in six scientific papers in leading peer-reviewed journals, eight presentations at major international conferences, and the filing of a new patent.



CARDIOVASCULAR TISSUE ENGINEERING

FOCUS

- **Tissue-engineered heart valves**
- **Tissue-engineered vascular grafts**
- **Tissue-engineered cardiac patches**
- **Bioreactors for enhanced extracellular matrix elaboration**
- **Native/engineered tissue image-based structural and histopathological analysis**
- **Native/engineered tissue numerical models for mechanics and tissue growth**

AIMS

- ***In silico*, *in vitro*, and *in vivo* models**
- **Quantitative histology and biomaterial micro-structure image-based analysis**
- **Structural modelling for cardiovascular tissue engineering**
- **Mechanical and topological conditioning to duplicate native tissue properties**
- **Endogenous tissue growth, vascular grafts, and engineered heart valves.**

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The cardiac tissue engineering group seeks to couple a mechanistic understanding of the relationship between scaffolds micro-structure, mechanics and endogenous tissue growth with the development of novel biomaterials for tissue engineering strategies. The focus of the D'Amore's lab is upon clinical applications where few effective solutions currently exist, with increasing emphasis upon unmet clinical needs in cardiovascular diseases.

The group can leverage on considerable experience (15+years) in structural characterization and bioprocessing, prototyping of medical devices, and pre-clinical development. The image analysis and numerical methods that we implemented have impacted the way several leading laboratories look at scaffold design and native tissue micro-structure characterization.

Leveraging this expertise further, we have developed experimental protocols and numerical models to correlate scaffold micro-structure with mechanical behavior across scales and relate this behavior with ECM elaboration. Our image analysis software and mechanical models have been utilized on a large spectrum of biomaterials including: ECM gels, polyurethane scaffolds, decellularized tissue, aneurism tissue, and human aorta. Motivated by the desire to fill the gap between material design, bioprocessing, and performance *in-vivo*, we sought the opportunity to lead several small and large animal studies.

The scaffolds we designed and fabricated have been tested in a variety of *in-vivo* scenarios (e.g., engineered pulmonary, tricuspid, mitral valve; cardiac patch; abdominal wall repair; engineered vascular graft) where in addition to the material bioprocessing we were responsible for the biomechanical, histological, immunohistochemical (IHC) assessment and project management. These efforts secured funding for >$11 M (Dr D'Amore role: PI or Co-I), and generated: 49 journal articles, 6 issued patents, 11 patent applications, 2 career awards and "Neoolife", a Pitt start-up (Neoolife) focusing on engineered heart valve technology.

The multidisciplinary research team we assembled has a successful history of collaboration in the fields of engineered cardiac tissue, biomechanics and biomaterials which is documented by multiple publications in top peer-reviewed journals (49+, average IF=7.8, h=28), competitive research awards (e.g. ERC consolidator award (PI), NIH R01s (co-I), STTR NIH) and shared intellectual properties (6 issued patents, 11 patent applications).
This includes two ongoing large animal studies focusing on pulmonary and mitral valve engineering and one rat model study which aims to assess host response to the implantation of an engineered vascular graft. The RiMED laboratory (24 scientists strong, 100 m2 of fully equipped independent lab space + 300m2 of office space and numerical lab) was established in 2020 and is located in buildings 16 and 18 at the University of Palermo.

The group's research has resulted in six scientific papers in leading peer-reviewed journals, eight presentations at major international conferences, and the filing of a new patent.

MUSCULOSKELETAL TISSUE ENGINEERING

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FOCUS

- **Cartilage and bone pro-regenerative technologies**
- **Bioactive materials stimuli-responsive**
- **Tendon/ligament-like scaffolds**
- ***Ex vivo* tissue culturing**
- **Reliable musculoskeletal diseases modeling**

AIMS

- **Biofabrication of active scaffolds for the cartilage focal lesions repair**
- **Production of engineered tendon-like constructs supporting the surgical tendon/ligament reconstruction**
- **Set-up of advanced *in vitro* models of musculoskeletal diseases**
- **Use of macrofluidic bioreactor for the *ex vivo* culturing of biphasic tissues**

COLLABORATIONS

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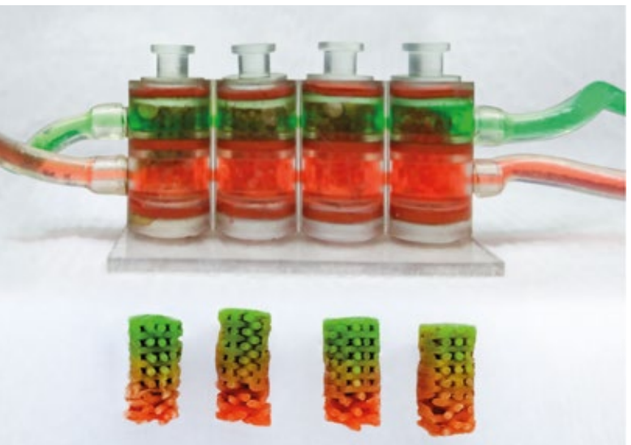
- Buccheri la Ferla clinic (ITA)
- University of Palermo (UNIPA) (ITA)
- The Children Hospital of Philadelphia (CHOP), USA

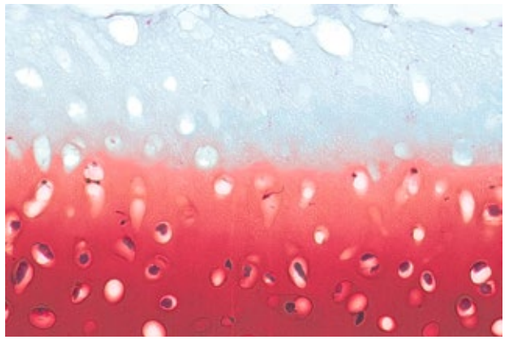
The MusculoSkeletal Tissue Engineering (MSTE) group is dedicated to pursuing as a main objective the advancement of groundbreaking strategies for the treatment of various pathologies that affect ligaments, tendons, cartilage, bone, and muscles.

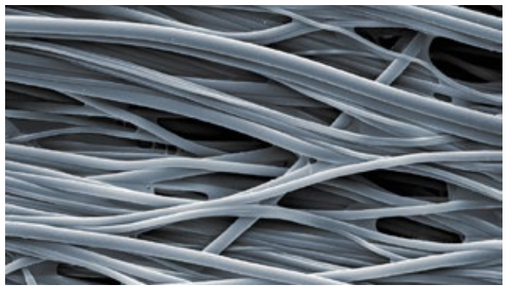
In order to achieve this goal, the MSTE group employs a wide range of innovative approaches relying on tissue engineering technologies. These approaches are based on the most advanced techniques of biofabrication, including but not limited to 3D printing, electric-field induced biofabrication, and bioprinting.

The primary focus of the MSTE group is to fabricate bioactive scaffolds able to provide pro-regenerative cues for cells that are either seeded onto or embedded within these kinds of 3D supports. Following this approach, we aim to support reconstructive surgery after tendons/ligaments injuries promoting the regeneration of functional tissue in the mid-term. Analogously, our activity aims to restore the functionality of joints affected by focal chondral defects developing functional scaffolds stimuli-responsive, with a vision toward a clinical translation in the near future. In addition, the MSTE group is leading several projects aimed to the development of highly realistic *ex vivo* models of pathologies affecting the musculoskeletal system. Such an activity is supported by the employment of a proprietary technology based on macro-fluidic bioreactors for the culturing of biphasic tissues in native-like conditions.

The MSTE group is actively involved in numerous collaborations with international partners, which allow the design and management of different interdisciplinary projects. These collaborations serve as a fertile ground for the exchange of knowledge, expertise, and resources, enabling the MSTE group to push forward the boundaries of innovation. In this direction, we are developing engineered models of muscle tissue with a specific focus on fill the gap in the knowledge of the intricate interplay between the pathologies affecting the nervous system and the musculoskeletal apparatus. This groundbreaking research provides a deeper insight into the mechanisms underlying various neuromuscular disorders and facilitates the exploration of innovative therapeutic approaches.







TECHNOLOGY PLATFORMS

With its strongly oriented translational approach, Ri.MED strategy provides for the development of skills and technological platforms, significantly enhanced in recent years, also thanks to the funding provided by the Sicilian Region and by the Dipartimento "Casa Italia" of Presidency of the Council of Ministers.

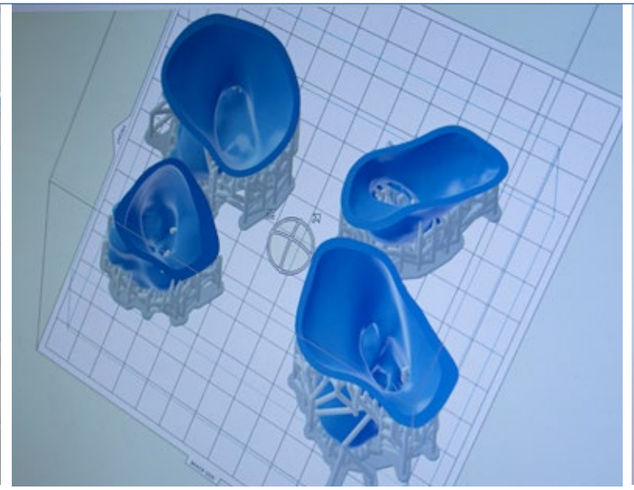
Some example are the automated system implemented for the storage and manipulation of molecule libraries for the **Screening laboratory**; the cardiac simulator and instrumentation supplied to the **Bioengineering Platform** for the characterization of biomaterials and medical devices; an 800 MHz magnetic resonance spectrometer at the **Biophysics and Structural Biology** platform, while the **Biomedical Imaging and Radiomics** platform uses 3T and 7T spectrometers and employs skills for the analysis of multimodal data and images, predictive diagnosis of pathologies and relapses.

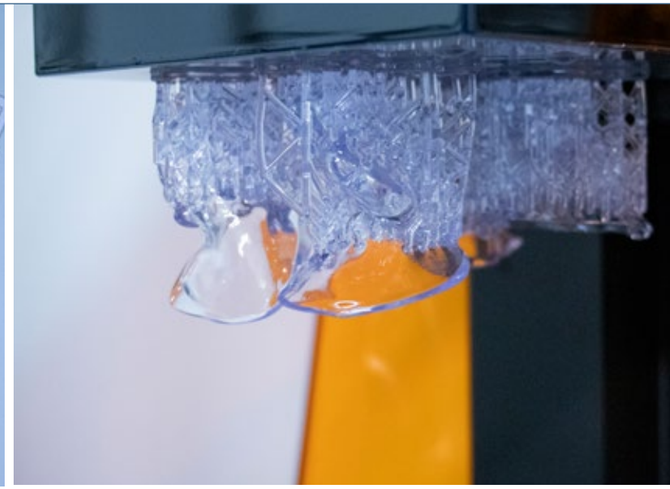
The **Tissue Engineering Platform** allows the mechanical and structural characterization of native and bioengineered tissues and the *in vitro* and *in vivo* study of de novo tissue development; it also has software for the elaboration of predictive numerical models for tissue growth and regeneration and instruments for the development of engineered heart valves through the use of a six-degrees-of-freedom robotic arm. The **Bioinformatics** and **Molecular Informatics** groups integrated hardware and software with a virtual screening speed of 5,000 molecules per minute, with proprietary algorithms to study molecular interactions at the cellular level, and with the infrastructure for analyzing chemical-physical properties.

The **Medicinal Chemistry** Platform allows for the structural validation of primary hits and expansion of the chemical family, as well as the structural optimization of biologically promising molecules, up to the identification of small molecules that will enter the preclinical development phase. At IRCCS ISMETT, the **Proteomics** group supports the identification of new pharmacological targets and biomarkers, as well as the study of potential side effects of particular therapeutic molecules, while the **Cell factory** allows for the production of ATMP.

- BIOENGINEERING
- BIOINFORMATICS
- CELL FACTORY
- IMAGING AND RADIOMICS
- MEDICINAL CHEMISTRY
- MOLECULAR INFORMATICS
- PROTEOMICS
- SCREENING
- STRUCTURAL BIOLOGY AND BIOPHYSICS
- TISSUE ENGINEERING

Bioengineering
PLATFORM





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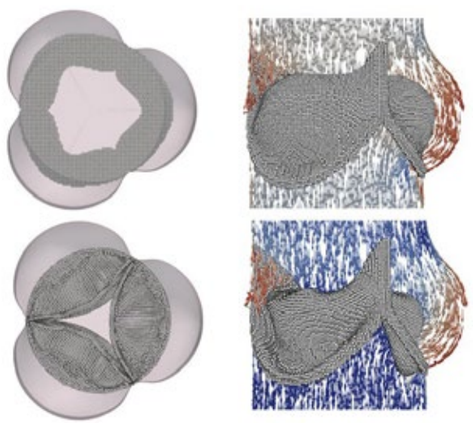
- Università degli Studi di Palermo, IT
- Institute Foundation G. Giglio, IT
- Università degli studi di Padova, IT
- Université de Technologie de Compiègne, FR
- Barts Heart Centre at St Bartholomew's Hospital, GB
- Great Ormond Street Hospital for Children, GB
- University College London, GB
- Queen Mary University of London, GB
- University of Bristol, GB
- University of Leeds, GB
- University of Pittsburgh, US
- University of Alabama at Birmingham, US
- MitrAssist, CN
- Adeka, JP

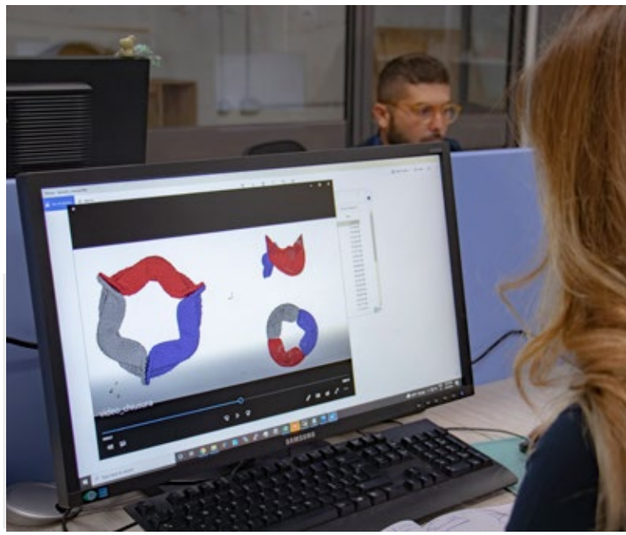
The Bioengineering Platform provides the treatment and characterisation of biomaterials, the numerical simulation of complex physiological systems, and the preclinical validation of medical devices of the different classes (from class I to class III).

Our research team offers solid expertise in numerical modelling, fluid-structure analysis, design optimisation of medical devices, and pre-clinical evaluations complying with regulatory requirements and good practice. The platform is consolidating as a reference for healthcare providers, academic groups and small and medium-sized enterprises in the region, contributing to stimulate the implementation of clinical innovations emerging from the local excellence and providing the necessary professional training to generate new technical and business competencies in the field.

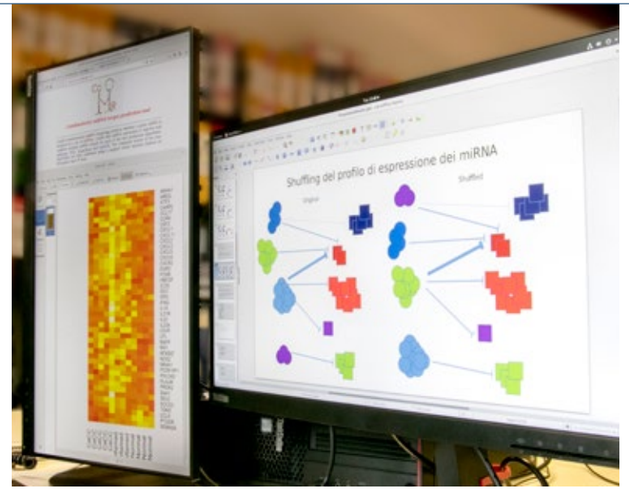


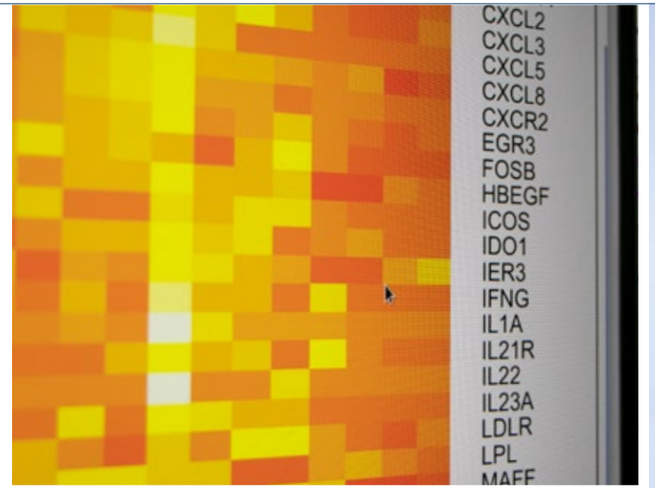
EXPERTISE
- Mechanical and thermo-mechanical and rheological characterisation of biomaterials and biofluids;
- Numerical simulation of physiological systems and their interaction with medical devices (by means of structural, fluid dynamic and fluid-structure interaction analyses);
- Development of numerical codes for the study of cardiovascular problems (e.g. simulation of thrombosis);
- Development of support tools for therapeutic planning;
- Development of advanced diagnostic solutions;
- Design of medical devices;
- Hydrodynamic and structural *in vitro* characterisation of physiological systems and cardiovascular implants.

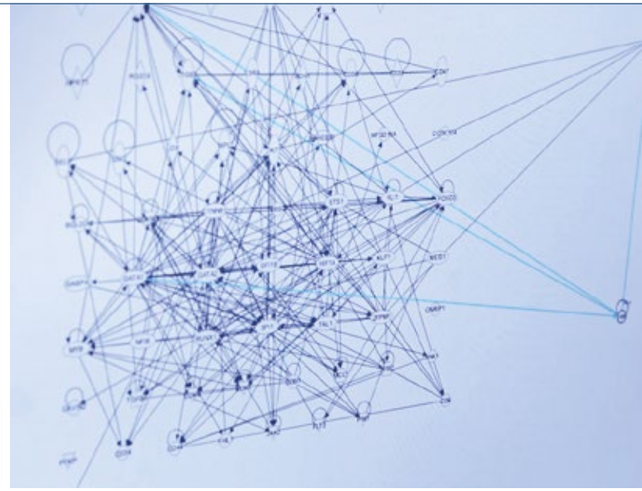
TECHNOLOGY PLATFORM
- Codes for the numerical simulation of complex physiological systems (developed in house and commercial);
- Equipment for the treatment and characterisation of biomaterials and biofluids;
- Tools for the basic manufacturing of components and prototypes;
- Instruments for the preclinical validation of cardiovascular medical devices.



Bioinformatics
PLATFORM







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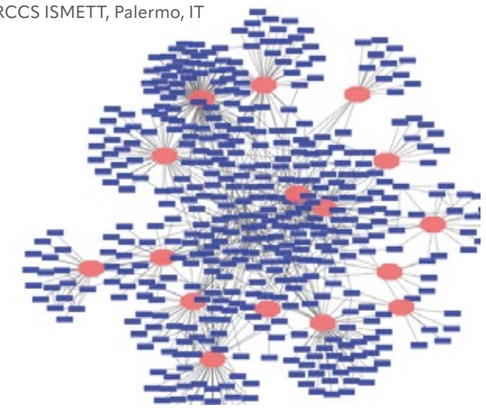
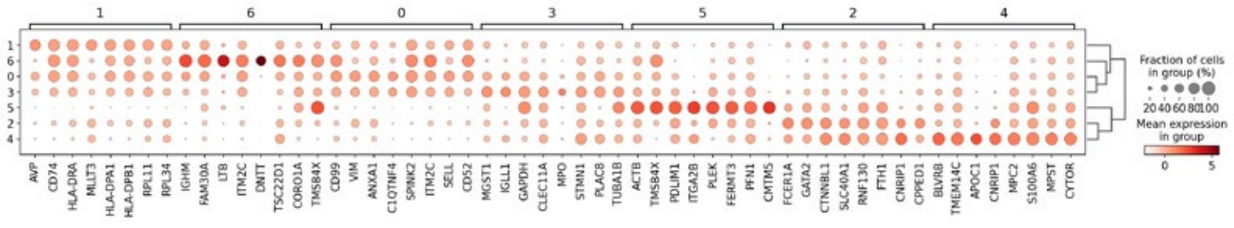
- Università degli Studi di Palermo, IT
- University of Pittsburgh, US
- IRCCS ISMETT, Palermo, IT



Bioinformatics platform is devoted to support Ri.MED researchers and collaborators to retrieve the most amount of information from their data, with a particular interest on Biological Big Data. For instance, it supports the Drug Discovery Unit in high throughput screening experimental design and data analysis. It performs standard high-throughput data analysis, applied on a wide range of data source technologies, e.g., microarray or next generation sequencing data, integrated with clinical data if available.

Very often, the biological questions of interest and the associated experimental designs cannot be analyzed by the commercial software available to the scientific community. In this case, the expertise on computer programming and big data management for analyzing high-throughput data in a customized way. The main scientific interest of the group is the study of biological interaction networks, analyzed by integrating many sources of data. For instance, the project is dedicated to describe the regulatory interaction network of the endogenous microRNA in a specific tissue of interest, by analyzing its microRNA and gene expression profiles.



EXPERTISE
- Molecular biology, Genetics, Systems biology
- Bulk and single cell RNAseq data analysis, Genomics, Epigenomics, Transcriptomics, Metagenomics
- Statistics, Bioinformatics, Machine Learning, Complex Systems, Network analysis
- Long-read Next Generation Sequencing (Oxford Nanopore Technologies)
- Programming with R, Python, Matlab, Galaxy

TECHNOLOGY PLATFORM

Software

Our scripts for data analysis are realized with open-source language, i.e., Python, R and Bioconductor libraries. Visualization of interaction network is performed with the software Pajek or Cytoscape. We use the software Knime to share user friendly pipelines for data analysis. In order to better satisfy the collaborators needs we are able to enrich our analysis by comparing them with the results obtained with the software Ingenuity Pathway Analysis.

Hardware
- 3 workstations
- Server - CPU: 2x Xeon Gold 6152 2.10 GHz 22 Cores RAM: 128GB
- Server - CPU: 2x AMD Epyc 7402 24 Cores 2.8GHz RAM: 256GB HDD: 3x 480GB SSD GPU: 2x Nvidia A100 40GB
- 1 MinION Mk1C, portable and real-time device for DNA and RNA sequencing, Oxford Nanopore Technologies.

ACTIVE RESEARCH PROJECTS

OBIND - Oncological therapies through Biological Interaction Network Discovery. This project is funded by Regione Sicilia within the program PO FESR – azione 1.1.5.
The aim of the project is the development of a technological platform useful for the analysis of biological interaction networks among proteins, messenger RNA, microRNA and small molecules. The focus is to find new therapeutic approaches to cancer treatment. Total funded Budget: 1.967.779,70 Euro; to Fondazione Ri.MED 540.000,00 Euro.

SUnFox - Senescence Undoing by FOXO4 knock down. This project is funded by National Center for Gene Therapy and Drugs based on RNA Technology" (CN3 – Spoke 4)
Abstract: This is a project that studies a novel senolytic approach for the treatment of aging-associated diseases and senescence-associated diseases. The aim is inducing the apoptotic death of the senescent cells by knocking down FOXO4 via systemic and local delivery of specific ASO/siRNA.

SMART: (Splicing Modulation by Advanced RNA Technologies)
Abstract: The project aims to study *in silico* and *in vitro* RNA-based approaches to manipulate pathological splicing events. Classical *in vitro* assays will be supported by imaging and omic studies. Splicing modulation can be obtained by means of i) canonical ASO (that inhibit selected splicing site by occupancy), ii) bifunctional ASO (targeted bifunctional oligonucleotide enhancer of splicing - TOES) and iii) engineered Cas13.

Cell Factory

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PARTNERSHIPS AND COLLABORATIONS

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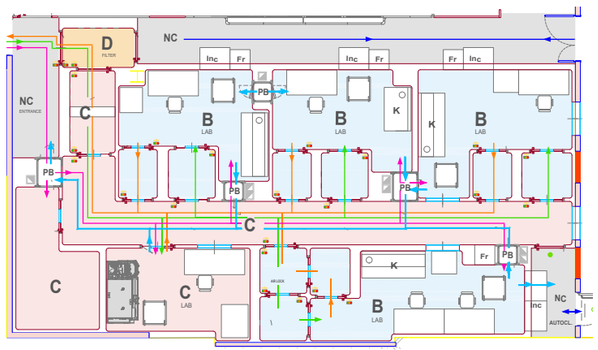
- ISMETT- IRCCS policlinico San Matteo, Pavia, IT
- ISMETT-IRCCS Ospedale Galeazzi- Sant'Ambrogio, IT
- University of Pittsburgh, US

The new cell factory guarantees flexibility in the type of production and functionality of the different areas. The design of production and quality control layouts for advanced therapies (gene therapy, cell therapy, tissue engineering and combined ATMPs) was approved by AIFA during a Scientific Advice meeting. There are 4 class B laboratories, one of which allows for a higher containment and has an autoclave for waste treatment.

The other 3 class B laboratories can be used in a totally independent way, for the simultaneous preparation of three different products. Alternatively, they can be connected two by two. In the last case, part of the operations can be performed in one lab and other manipulations can be performed in the second lab, passing the intermediate product through a pass box.



GMP Facility Layout, with personnel and material flows

An additional class C room is used for cell preparation in specific closed systems. Maintenance can be performed without access to the production rooms, as the engines of the equipment protrude into technical areas.

The Quality Control laboratories are equipped to conduct all the tests on raw materials, intermediates and final products required for product release. These labs can receive and adequately store reagents, materials and products according to GMP. Production areas and QC labs are equipped with a remote monitoring system.

EXPERTISE

- Set up of a GMP compliant Quality Assurance System
- Definition of GMP production protocols
- Development of Quality Control Methods
- Validation of environment, equipment, products
- GMP Training

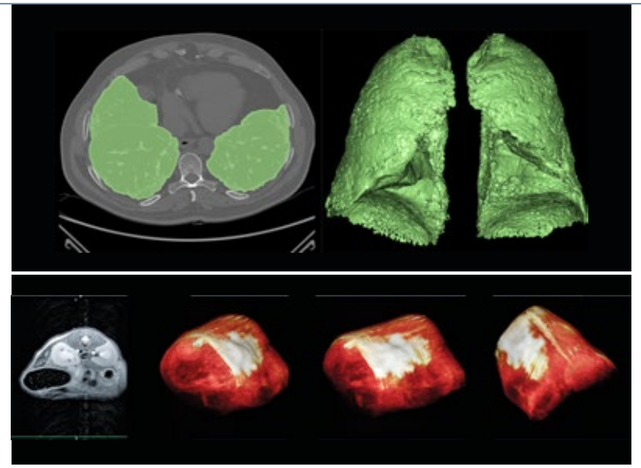
ACTIVITIES

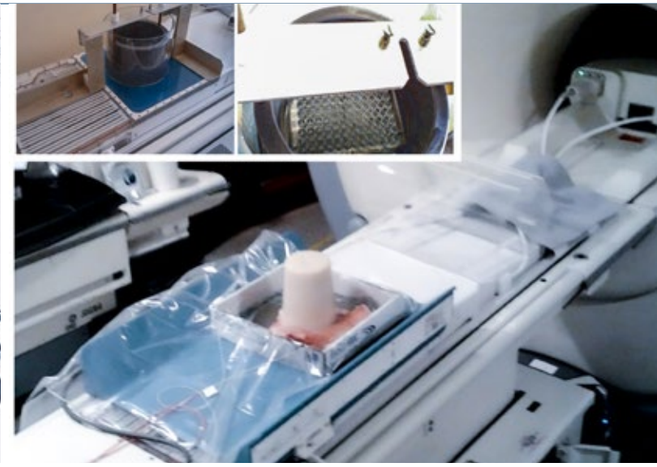
The facility is being qualified. The cell factory staff will carry out the validation of fundamental general processes (gowning validation, sanitization and clean hold time, passage of materials, etc.). Once the necessary development/ technology transfer data of the first advanced therapy products (adoptive immune therapies) are available, specific validation activities for the production process and related quality control methods will be carried out.

A complete dossier on the first advanced therapy product and its intended clinical use will be submitted as an integral part of the manufacturing authorization application of the new facility. Novel culture methods allowing minima operators' intervention and automatic QC tests are being tested.

Continuous activities include the maintenance of the GMP compliant Quality Assurance system and the periodic training of internal and external staff.

Imaging and Radiomics
PLATFORM





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PARTNERSHIPS AND COLLABORATIONS
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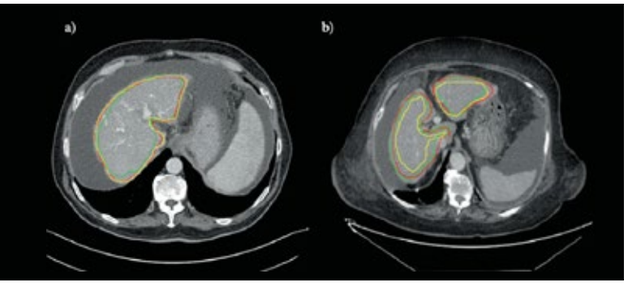
- Institute of Molecular Bioimaging and Physiology, (IBFM-CNR), Cefalù, IT
- Georgia Institute of Technology, (GIT), Atlanta, US
- Nuclear Medicine Unit, University of Messina, IT
- Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, (PROMISE), University of Palermo, IT
- Department of Biomedicine, Neuroscience and Advanced Diagnostics, (BIND) University of PalermoPalermo, IT
- Department of Engineering, University of Palermo, IT
- Department of Agricultural, Food and Forests Sciences University of Palermo, IT
- Medical Physics Unit, Cannizzaro Hospital, Catania, IT
- Nuclear Medicine Department, Cannizzaro Hospital, Catania, IT
- Istituto Zooprofilattico Sicilia (IZS), Palermo, IT



The Biomedical Imaging platform provides a crucial support to promote the translation of scientific results from *in vitro* test, through *ex vivo* and *in vivo* test to clinical applications, specifically for neuroscience and cancer research. The staff, currently increasing, is today composed of scientific figures who have Transversal skills suitable to support the entire translational workflow ranging from the processing *In-Vitro* tests (cell models) to *Ex-Vivo* tests (phantom, scaffold, tissues and organs) and In -Vivo (animal models), up to the Analysis through Artificial Intelligence, Machine Learning and Deep Learning algorithms applied to Multispectral Images in the Agri-food field and Biomedical Images (Cellular, Preclinical and Clinical). During 2022, the platform will be enriched with a WIZARD 2470 gamma counter with 10 PerkinElmer detectors and radiation protection devices (at ISMETT), a microCT Skyscan 1276 CMOS Bruker (at IZS) in order to offer more options for imaging and radiomics.

During 2022, the Biomedical Imaging platform made use of two magnetic resonances (3T and 7T), a Spectrum *In Vivo* Imaging System (Bioluminescence), a gamma counter with 10 detectors, a MicroCT and Positron Emission Tomography - Computed Tomography (PET/CT) imaging methods made available by participant institutions. Pre-processing, segmentation, radiomics and artificial intelligence (machine learning and deep learning) analysis tools from images per predictive diagnosis of pathologies, biodistribution analysis of radiopharmaceuticals and biomedical diagnosis support were developed in collaboration with GIT and IBFM-CNR.

EXPERTISE
- Image Processing Models (MR/PET/CT/IVIS, Microscopy and histological), 3D Segmentation, Deep Learning and Machine Learning to Extract, Classify and Delineate Tumor Volumes and Radiomics Features for Predictive Diagnosis of Pathologies (eg. Tumor, COVID19) and Relapses and Medical Decisions Support
- Magnetic Resonance Imaging (T1, T2, DP, DWI, ADC and DCE)
- Positron Emission Tomography/Computer Tomography (PET/CT)
- Spectroscopy on phantoms, *in-vivo* and *ex-vivo* samples
- *In Vitro* and *In Vivo* Radiobiology: Radiopharmaceuticals and Radio-labeled Chelators
- Biodistribution analysis of Radiopharmaceuticals: Preclinical Molecular Imaging
- PET/CT, MRI, HRCT, IVIS, Gamma Counter
- Python, Matlab, CUDA

TECHNOLOGY PLATFORM
At Istituto Zooprofilattico Sperimentale:
- Bruker Pharmascan 70/16 (7 Tesla). Coils available:
  - Mouse and rat brain 2x2 receive surface array coils
  - Mouse and rat transmit-receive volume coil (40 mm internal diameter and 75 mm external diameter)
  - Rat body 8x2 transmit volume array coil (72 mm internal diameter and 89 mm external diameter)
- IVIS Spectrum Advanced pre-clinical optical imaging
- microCT Skyscan 1276 CMOS Bruker
- Software: TopSpin, Paravision 6.1, Jmrui, Tarquin, Horos

At IRCSS ISMETT
- GE DISCOVERY MR 750 W 3 Tesla High-Field Magnetic Resonance 3.0 T (neuro, body, breast, angio, osteoarticular, cardio, etc.)
- WIZARD 2470 gamma counter with 10 PerkinElmer detectors and radiation protection devices

At Institute of Molecular Bioimaging and Physiology, National Research Council (IBFM-CNR):
- PET/CT Clinical and Preclinical
- Server - CPU: 2x Xeon Gold 6152 2.10 GHz 22 Cores RAM: 128GB
- Server - CPU: 2x AMD Epyc 7402 24 Cores 2.8GHz RAM: 256GB HDD: 3x 480GB SSD GPU: 2x Nvidia A100 40GB
- 1 MinION Mk1C, portable and real-time device for DNA and RNA sequencing, Oxford Nanopore Technologies.

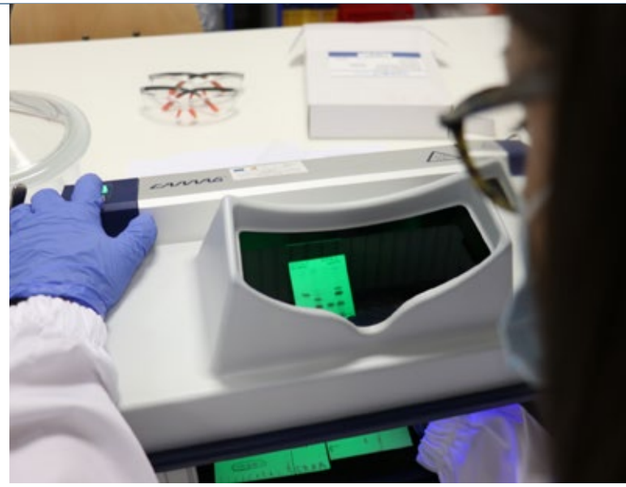
ACTIVE RESEARCH PROJECTS
In vivo small animals imaging supporting the Project Immuno- terapia NK-mediata per il trattamento e/o la prevenzione della recidiva HCC e/o HCV post-trapianto.
Supervised by Dr. Ester Badami.

In Vitro radiobiology studies on innovative radiopharmaceuticals through pharmacokinetic, trafficking and cell viability assays (ISOLPHARM project)
IBFM-CNR: Dr. Giorgio Russo e Dr. Alessandro Stefano.
Fondazione Ri.MED: Dr. Albert Comelli e Dr.ssa Viviana Benfante.

Diagnostic classification of the degree of portal hypertension in patients with cirrhosis using radiomics features and artificial intelligent algorithms on CT.
ISMETT: Dr. Roberto Miraglia and Dr. Giuseppe Mamone.
Fondazione Ri.MED: Dr. Albert Comelli and Dr.ssa Claudia Coronnello.

IN VIVO biodistribution studies for the evaluation of the efficacy of the treatment of site-directed radiopharmaceuticals on a preclinical mouse model in diagnosis and theranostics (ISOLPHARM project).
IBFM-CNR: Dr. Giorgio Russo , Dr. Francesco Cammarata e Dr. Alessandro Stefano. Fondazione Ri.MED: Dr. Albert Comelli e Dr.ssa Viviana Benfante

Medicinal Chemistry
PLATFORM



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PARTNERSHIPS AND COLLABORATIONS
- Università degli studi della Campania Luigi Vanvitelli, Naples, IT
- University of Palermo, Palermo, IT

The Medicinal Chemistry group is focused on the design and synthesis of novel small molecules with potential biological activity, together with the creation of compound libraries and building blocks collections; and aims at discovering new hits toward therapeutic targets of interest. Moreover, the platform supports the early drug discovery campaigns, with hit structure confirmation, hit re-synthesis, hit series expansion and optimization, hit-to-lead.
Design, organic synthesis, structural elucidation and analytical characterization of newly synthesized compounds are the main expertise. Furthermore, structure-activity-relationship (SAR) studies allow to explore the chemical space of hits to better define the *in vitro* biological profile.

The platform is fully equipped for reactions set-up; mixtures work-up and purification; isolation, structure characterization, and standard purity grade assessment.

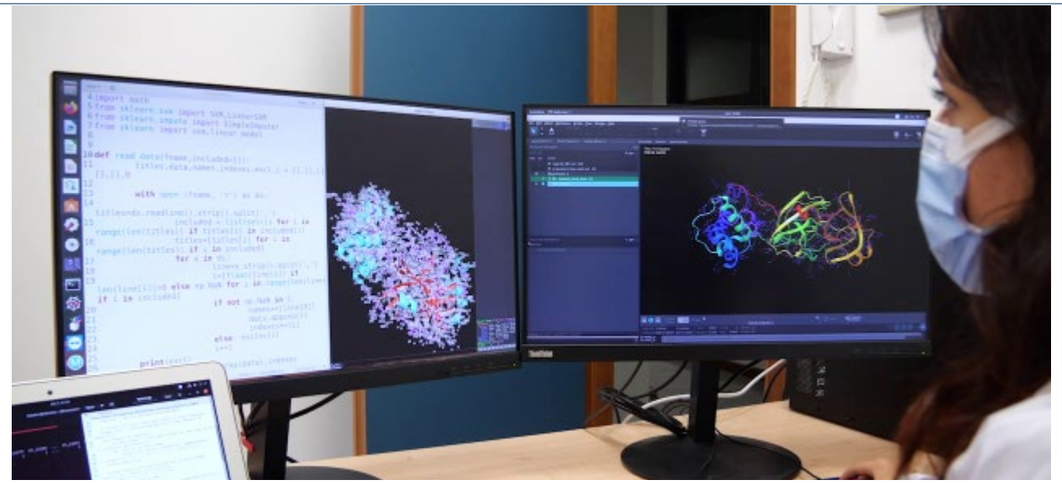
EXPERTISE
- Drug design
- Planning, development and optimization of synthetic routes
- (Microwave-assisted) organic chemistry
- Purification of complex mixtures (normal and reverse phase)
- Isolation of title compounds
- Structure elucidation and analytical characterization
- Purity grade assessment

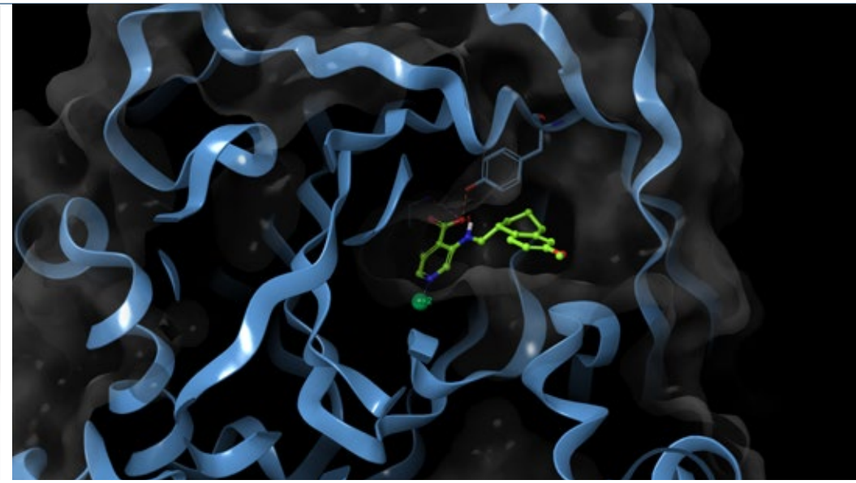
TECHNOLOGY PLATFORM
- Milli Q-3, Merck: Water purifier system for production of pure and ultra-pure water needed for analytical applications
- Isolera One, Biotage: Flash chromatography apparatus for isolation on normal and reverse phase of compounds of interest from complex reaction mixtures
- Nexera, Shimadzu: High-performance liquid chromatography (HPLC) platform for semi-preparative applications and purity grade assessment
- LC-MS 2020, Shimadzu: Liquid chromatography-mass spectrometry platform for reactions monitoring and analysis of organic compounds from complex samples
- Discover 2.0, CEM: Microwave reactor for homogenous and heterogenous catalytic transformations
- Genevac HT 4X, StepBio: Centrifugal vacuum evaporator for samples drying (from organic solvents) at low temperature, preserving stability and integrity
- Lyovapor L-200 Büchi: Lyophilizer for samples drying (from water) at low temperature, preserving the stability and integrity

Implementation:
H-Cube Mini-Plus, StepBio: Flow reactor for catalytic hydrogenations; able to generate high-pressure hydrogen with the electrolysis of water

ACTIVE RESEARCH PROJECTS
- Development of selective inhibitors of NOD-like receptor family, pyrin domain containing 3 (NLRP3)
- Design and synthesis of new Sirtuin-6 (Sir6) inhibitors, a validated target for lymphoma treatment.

Molecular Informatics
PLATFORM





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PARTNERSHIPS AND COLLABORATIONS
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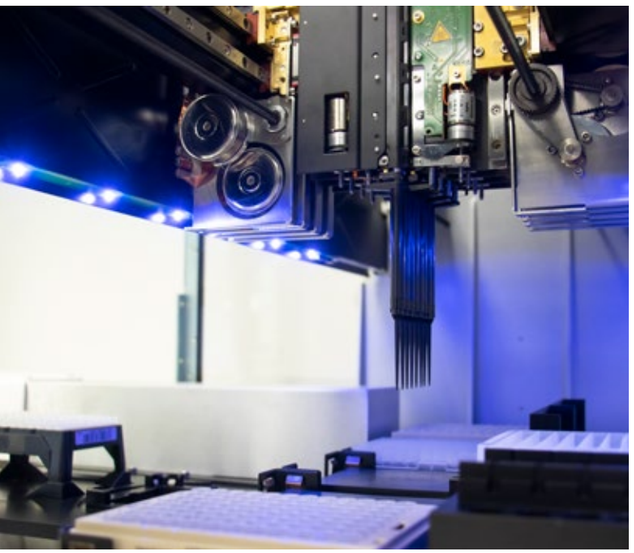
- University of Vienna (Pharmaceutical chemistry department)
- Italian National Council of Research (CNR), IT
- University of Verona, IT
- University of Paris citè, FR
- University of Palermo, IT



The Molecular Informatics group mainly deals with the identification and optimization of biologically active molecules through the use of *in silico* techniques.
The approaches used range over classical molecular modeling techniques for virtual screening to the combination of modern chemoinformatics in house developed tools. Over the years the team has developed various experiences in the field of medicinal chemistry and computational chemistry. The expertise acquired by team members is synergistically exploited for the creation of molecular libraries, creating and validating reliable theoretical models to be used for subsequent virtual screening of ligands (VLS).

The outcome of the models generated and optimised are further validated experimentally through biological or biophysical tests. The molecular informatics group is also involved in the exploration and enrichment of the chemical space to create the most suitable molecular libraries to be used for biological screening campaigns. In recent years, the collaboration with different academic groups from university of Palermo and Vienna allowed the development of approaches based on the use of deep learning for the activity prediction of small molecules. Furthermore, during the last year, the group has set the *in silico* Biologics platform that will be used for the design and optimisation of biological drugs.

EXPERTISE
- Structure based virtual screening (Docking and Pharmacophore)
- Ligand Based virtual screening (pharmacophore, molecular descriptors based models, QSAR and 3D QSAR)
- Molecular Dynamics
- Dynamic pharmacophore (hybrid technique based on the use of pharmacophores from the molecular dynamics trajectory)
- Chemical Database creation and management
- Chemical data mining
- Machine Intelligence in Drug Design
- Biologics design

TECHNOLOGY PLATFORM
Software
- Schrödinger suite for small molecule drug discovery
- Schrödinger suite for biologics drug discovery
- LigandScout expert suite
- Autodock and Autodock Vina
- AlvaDesc/AlvaModel
- DESMOND (OPLS2005 and OPLS3e, OPLS4)
- AMBER
- NAMD
- VMD
- GROMACS
- KNIME

Hardware
- 6 Workstations
- Server: 80 cores e 2 x NVIDIA Tesla K80
- Server: 96 cores e 2 x NVIDIA A100

Calculation capability:
- Library optimisation → ~ 6,000 molecules/min
- Virtual Screening HTVS → ~ 5,000 molecules/min
- Virtual Screening SP → ~ 1,500 molecules/min
- Molecular Dynamics → ~ 200 ns/day/Card (on 40,000 atoms system)

Integrated in Silico Platform
The group is actually working at the creation of an integrated platform for molecular network analysis in collaboration with the Bioinformatics group

ACTIVE RESEARCH PROJECTS
- Development of selective inhibitors of NOD-like receptor family, pyrin domain containing 3 (NLRP3)
- Machine learning-based drug repurposing of Kinases inhibitors.
- Modulation of protein-protein interaction modulators
- Design and development of Main protease (Mpro) SARS-CoV-2 inhibitors
- Structure- and ligand-based approaches for *in silico* profiling of small molecules

Proteomics
PLATFORM

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PARTNERSHIPS AND COLLABORATIONS
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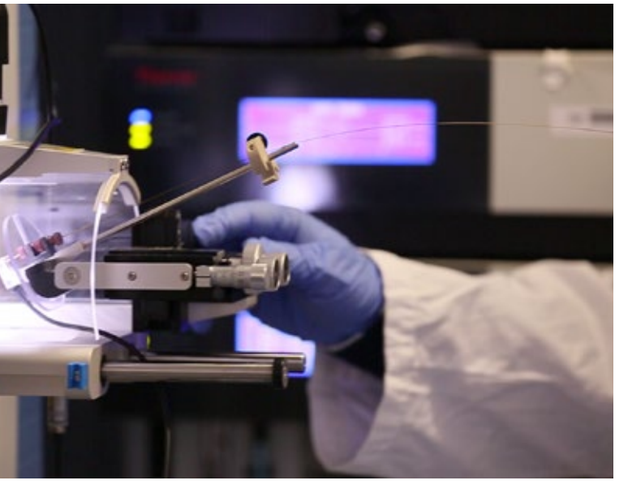
- University of East Anglia, Norwich UK
- University of Surrey, UK
- St. George's, University of London, UK
- University of Nottingham, UK
- Università di Udine, IT
- University of Padova, IT
- University of Palermo, IT
- IRIB-CNR, Palermo IT



The proteome is the entire set of proteins that is expressed by a cell, tissue or organism. The systematic high-throughput analysis of proteomes, known as proteomics, enables the identification of proteins and their relative content within biological samples. Furthermore, proteomics allows quantification of differentially regulated proteins across multiple conditions. Proteomics has broken through over the past decade with the evolution of several approaches, mainly mass spectrometry-based technologies for large-scale study of proteins. Proteomic applications in preclinical and clinical research are numerous. They span from identification of novel potential drug targets, to discovery of disease-associated biomarkers and prediction of drug-dependent side-effects.

Ri.MED has established a state-of-the-art proteomic platform, comprising a full-equipped laboratory for biochemistry and molecular biology, tissue culture facilities and a Vanquis Neo uHPLC system connected to an Orbitrap Exploris 480 mass-spectrometer, and an UltiMate 3000 RS LCnano System on-line coupled to a Q-exactive mass spectrometer, that allow top-level quantitative proteomic analysis. In details, this technology allows the chromatographic separation of different peptides derived from the proteolytic digestion of complex protein mixtures, electrospray ionization of such peptides and their fragmentation into a number of ions with a specific pattern of different mass/charge ratios, called mass spectra, that are a unique signature of each peptide. Mass spectra get computationally analyzed to infer each single protein contained in the starting mixture. Moreover, Ri MED instruments and the dedicated software allow quantitative proteomics, by which is not only possible to identify the unknown proteins of a biological samples, but also to quantify levels of the same protein in different biological samples.

In addition to support the forefront scientific research at Ri.MED, our proteomic platform aims to provide high-standard quantitative proteomic analysis for external research groups on collaborative basis, thus becoming a benchmark for the whole scientific research in the area.

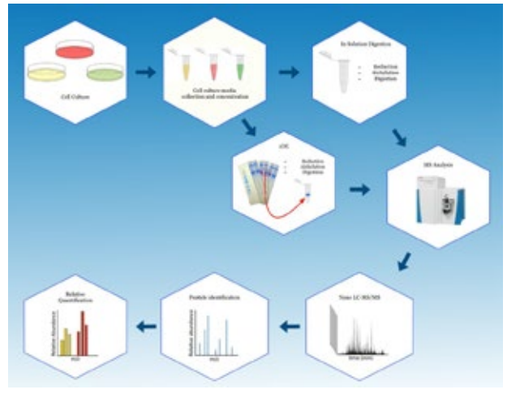
EXPERTISE
- Structure based virtual screening (Docking and Pharmacophore)
- Protein concentration from conditioned media
- Spectrophotometric Measurement (Bradford, BCA, micro BCA)
- Precipitaion and sample chemical processing
- In solution and in gel proteolysis
- Filter-aided sample preparation (FASP)
- STAGE (STop And Go Extraction) TIPS sample desalting
- Sample CleanUp
- pH fractionation
- Secretome protein enrichment with click sugars (SPECS)
- Label free quantitative proteomics
- Western Blot
- SDS-PAGE
- Quantitative and qualitative analysis of predicted and / or annotated proteins by liquid chromatography tandem mass spectrometry (LC-MS / MS) with Bottom Up and Shot-gun approaches.

Generalized mass spectrometry based proteomic workflow

TECHNOLOGY PLATFORM
The unit is equipped with:

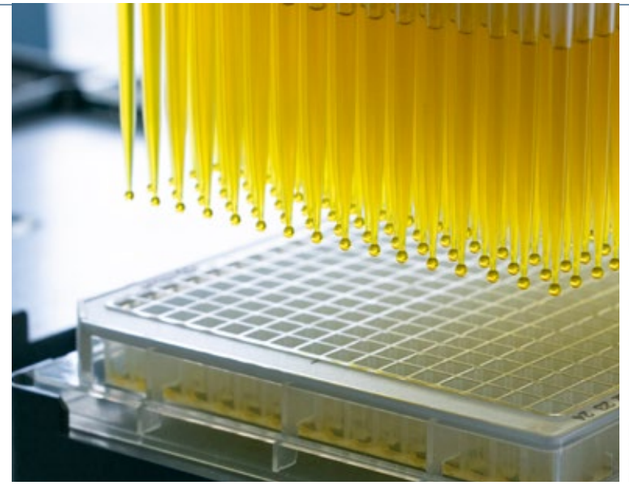
Hardware devices:
- Ultra-High Performance Liquid Cromatography: Vanquish Neo uHPLC, UltiMate 3000 UHPLC RSLC-nano System (Thermo Scientific).
- Mass Spectrometers: Exploris 480 and Q-Exactive (Thermo Scientific)

Software devices:
- Chromeleon
- Xcalibur
- Proteome Discoverer
- MAX QUANT
- DIANN
- Perseus for statistical analysis



Screening

PLATFORM



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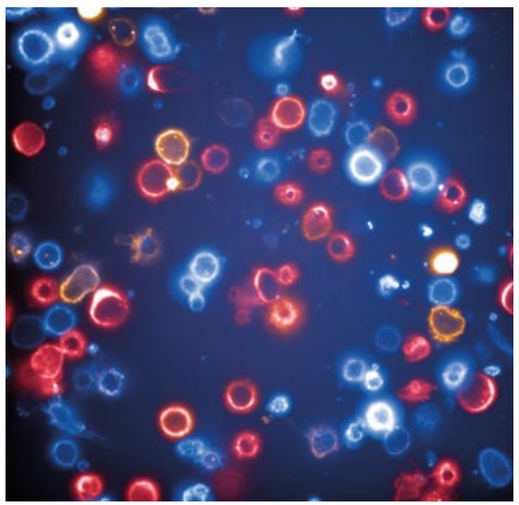
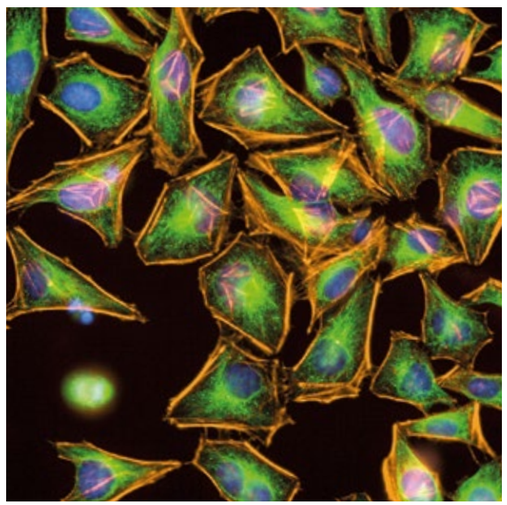
PARTNERSHIPS AND COLLABORATIONS

- Institut de la Vision, Parigi, FR
- Università degli Studi della Campania "Luigi Vanvitelli", Naples, IT

The high-throughput screening (HTS) platform provides labs and expertise for the development, miniaturization and validation of biochemical and cellular assays for the screening of libraries of compounds.

Our instrumentation allows the setup of flexible and partially automated protocols using a variety of readouts including absorbance, luminescence, fluorescence, TR-FRET, and imaging. Our lab is equipped with a high-content screening (HCS) system combined with software for image analysis and data evaluation. The platform supports Drug Discovery projects by performing both primary screening as well as dose-response curves, orthogonal and secondary assays.



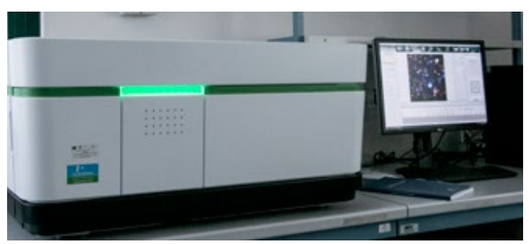


EXPERTISE

- Set-up and validation of primary assay (cell-free and cell-based);
- Different readouts possible including absorbance, luminescence and TR-FRET;
- Assay miniaturization (384-well plates);
- High-content imaging (HCI);
- Screening/high-content screening (HCS);
- Data analysis and primary active selection;
- Hit picking for primary hit validation through dose-response assays;
- Orthogonal and secondary assays;
- Toxicity tests.

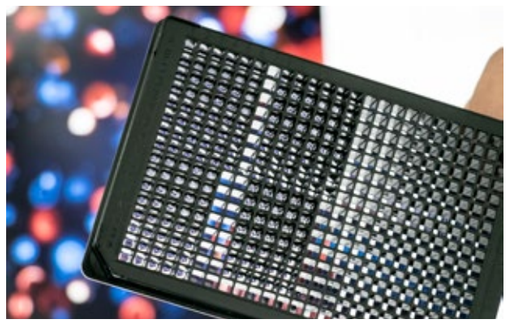
TECHNOLOGY PLATFORM

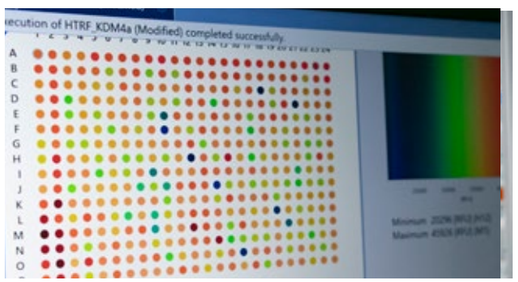
- Wet lab for cell and molecular biology;
- EL406 (Biotek) – automatic microplate washer/dispenser;
- Aquamax 4000 – automatic microplate washer for gentle cell washing;
- Operetta-CLS (Perkin Elmer) –high-content imaging (HCI) system;
- Spark (Tecan) - multimode microplate reader;
- In-Hood-Bravo (Agilent) - liquid handling system.



ACTIVE RESEARCH PROJECTS

Development of selective inhibitors of the intracellular NLRP3 receptor for the treatment of chronic inflammatory diseases associated with aging. The project aims at discovering new molecules able to selectively inhibit the activation of NLRP3. In 2022, our group successfully performed two screening campaigns (about 2000 compounds) that led to the discovery of some primary actives. Discovered compounds are currently being characterized for their mechanism of action and other key properties.





Structural Biology and Biophysics
PLATFORM

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PARTNERSHIPS AND COLLABORATIONS
- King's College London
- Scuola Normale Superiore of Pisa, IT
- European Brain Research Institute Rita Levi-Montalcini, Roma, IT
- Università degli studi della Campania "Luigi Vanvitelli", Naples, IT
- University of Palermo, IT
- Institute of Biophysics of the Italian National Research Council (IBF-CNR), Palermo, IT
- European Synchrotron Radiation Facility - Grenoble, FR
- International Covid-19 NMR Consortium

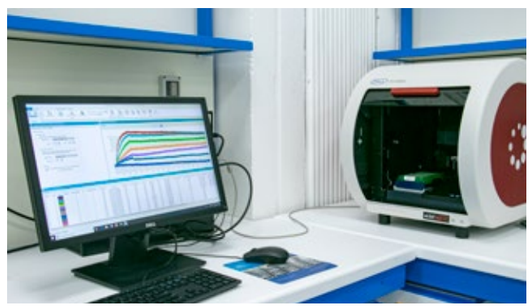
The Structural Biology and Biophysics Platform provides support for target identification, small molecules-based drug discovery, and development of protein-based therapeutics with particular focus on therapeutic antibodies. The platform is supplied with cutting-edge equipment that allow a multi-techniques approach, such as nuclear magnetic resonance, circular dichroism, calorimetry, and interferometry. The available technologies allow elucidating of the structure/function relationship of proteins, as well as structural, kinetic and thermodynamic studies of protein-protein and protein-ligand interactions.

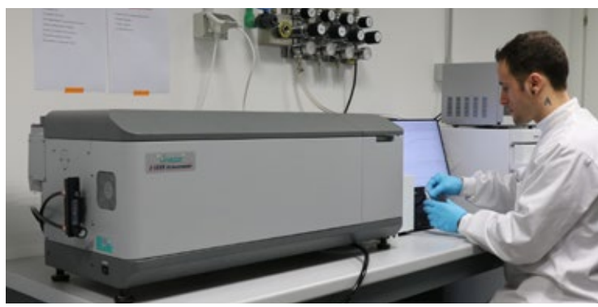
The Platform supports several research projects in several therapeutic area such as Neurodegenerative diseases, Cancer, and Infectious diseases. The diversity of all the active research projects well represents the potential of the Platform which can be used for basic research as well as translational science and can support transversally several research activities.

EXPERTISE
- Proteins Production: from cloning to purified and characterized proteins;
- Determination of size, structure and stability of macromolecules;
- Structural, kinetic and thermodynamic studies of protein-protein and protein-ligand interactions;
- BLI-based and NMR-based fragments screening;
- Development and application of customed analytical assays.

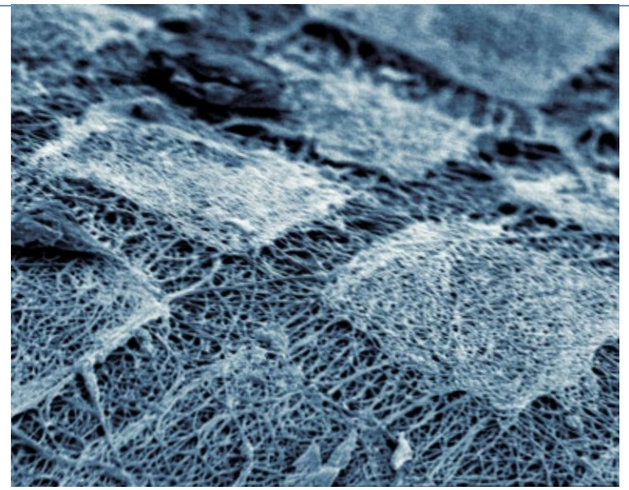
TECHNOLOGY PLATFORM
- Molecular biology laboratories for the production of recombinant proteins according to the most modern cloning, expression and purification techniques;
- AVANCE NEO 800 MHz NMR spectrometer equipped with cryo-probe – Bruker;
- MicroCal PEAQ_ITC – Malvern Panalytical;
- Bio-Layer Interferometer Octet Red96 – Sartorious;
- CD Spectropolarimeter J-1500 – JASCO;
- Multi-mode high-performance Microplate Reader CLARIOstar Plus – BMG Labtech

ACTIVE RESEARCH PROJECTS
- Development of selective inhibitors of NOD-like receptor family, pyrin domain containing 3 (NLRP3)
- BLI- and NMR-based fragment screening
- Development of neutralizing antibodies
- Identification and characterization of interactions among SARS CoV-2 RTC and LaRPs
- Molecular mechanisms of protein misfolding diseases
- Development of nontoxic bio-adhesives for wet environments
- Structure/function relationship studies of SARS-CoV-2 proteins





Tissue Engineering
PLATFORM



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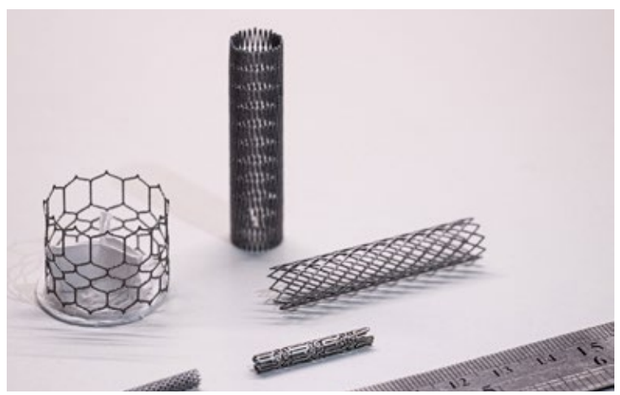
PARTNERSHIPS AND COLLABORATIONS
- McGowan Institute for Regenerative Medicine (MIRM), Pittsburgh, US
- University of Pittsburgh Medical Center (UPMC), Pittsburgh, US
- Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) IRCCS, Palermo, IT
- Universidad Abierta Interamericana (UAI), Buenos Aires, Argentina
- Advanced Technologies Network Center (ATeN Center), Palermo, IT
- Neoolife, Pittsburgh, USA
- TELEA BioTech, Sandrigo, Italy
- Columbia University Irving Medical Center (CUIMC), New York, US
- Technical University of Munich, (TUM), Munich, German
- University of California Irvine (UCI), Irvine, USA

The Tissue Engineering Group aims at establishing a world class and financially sustainable tissue engineering program at Ri.MED with a focus on clinical translation. The interest of this research group is upon clinical applications where few effective solutions exist, with an emphasis upon unmet clinical needs in cardiovascular diseases. The bioprocessing and tissue engineering core platform, in the process of being developed, offers disruptive tools for prototyping and assessing advanced scaffolds and biomaterials for tissue engineering applications.

The platform aims to address a broad spectrum of needs within the IRCCS-ISMETT, UPMC Italy and Ri.MED cluster as well as it aims to push forward collaborative efforts with investigators at the McGowan Institute, and Pitt departments of bioengineering and surgery.



EXPERTISE
- Advanced bio-fabrication;
- Polymer Synthesis;
- Decellularization of organs and tissues;
- Mechanical, physical, and chemical characterization of native and engineered tissues;
- Qualitative and quantitative histological evaluation of native and engineered tissues;
- Formulation and characterization of controlled drug-release medical devices;
- *In silico* and *in vitro* mechanobiology models;
- Structural deterministic models for tissue growth and scaffold degradation;
- Pre-clinical evaluation in small and large animal models';
- Numerical simulation of physiological systems and their integration with medical devices;
- FDA class II and III medical devices prototyping.

TECHNOLOGY PLATFORM
- Extraction of ExtraCellular matrix from organ and tissue to produce bio-hybrid medical devices such as cardiac patches and vascular grafts;
- Numerical codes for the development of simulation models of physiological and predictive systems;
- Numerical models to predict de novo tissue growth, native tissue remodeling, and polymeric degr adation;
- Software for quantitative histology analysis;
- Software for qualitative and quantitative analysis of microstructure from scanning electron microscopy and multi-photon images;
- Innovative methods of morphological analysis of micro and nanostructured materials;
- Bio-assembly Robot for the development and optimization of engineered heart valves;
- Electrodeposition equipment with and without mandrel;
- Design and development of bioreactor;
- Prototyping unit with Plastic and Metallic 3D Printers;
- Mechanical characterization of the tissue engineered prosthesis including cardiac patches, vascular grafts, and heart valves.

ACTIVE RESEARCH PROJECTS
- Development of a minimally invasive heart valve implantation system;
- Design and development of a minimally invasive cardiac patch implantation system;
- Design of a prototype esophageal graft;
- Development and evaluation of conductive cardiac patches;
- Quantitative analysis of
- Manipulation of the scaffold microarchitecture in collaboration with the company Telea Biotech
- Study of the tissue growth, native tissue remodelling and polymeric degradation;
- Study of mechanical, morphological properties and resistance to platelet deposition of a cardiac patch obtained from pericardium in association with the company ADEKA;
- Redesign electrospun scaffold microarchitecture;
- Development of a bioengineered chordal apparatus for heart valve repair;
- Development of a 3-layer vascular graft to limit hyperplasia of the intima and promote reendothelialization.

WORK IN PROGRESS






BRBC

Biomedical Research and Biotechnology Center

EDITORIAL AND GRAPHIC DESIGN PROJECT
Ufficio Comunicazione & Marketing Fondazione Ri.MED
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Printed by OFFSET STUDIO di Serraino Angelo & C.SNC. - Palermo
August 2023

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