Fondazione Ri.MED

SCIENTIFIC REPORT 2021

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SCIENTIFIC REPORT 2021

A MEDITERRANEAN **HUB** FOR TRANSLATIONAL RESEARCH

COVER PICTURE:
In vitro culture of human NK cells induced from liver perfusate of deceased donors. Image of cultured NK cells were taken on 21 under a microscope. NK cells like to grow as clusters.

FOUNDING PARTNERS

Presidenza del Consiglio dei Ministri

Regione Siciliana

Consiglio Nazionale delle Ricerche

University of Pittsburgh

UPMC
LIFE CHANGING MEDICINE

PARTNER

ISMETT Istituto di Ricovero e Cura a Carattere Scientifico

Alessandro Padova
DIRECTOR GENERAL

Fondazione Ri.MED consolidated its activities toward its statutory mission in 2021. BRBC construction work has continued at a steady pace, with all foundation work completed while, in parallel, the ISMETT 2 executive project is underway.

We have continued a transition toward a new organizational structure to embrace the challenges ahead, with the strategic vision of the UPMC-IRCCS ISMETT-Ri.MED Cluster. Administration and R&D organizational structures have been re-aligned, and in parallel a performance evaluation process was implemented for 2021. The effects of the pandemic on all fronts were minimized thanks to the effort, commitment, and passion of all Ri.MED employees.

Important results were achieved with respect to our institutional objectives in terms of translational research and training. Ri.MED research teams, supported by the administration offices, were highly productive in terms of R&D programs, receiving recognition for their excellence and attracting public and private funding. In 2021, Fondazione Ri.MED reached a milestone with a royalties-based License and Collaboration agreement for the Triskele cardiac implant.

As always, I would like to thank Ri.MED Scientific Director Dario Vignali for the fruitful collaboration and discussions, and the Scientific Committee members for their advice in developing a vision for Ri.MED translational research.

In 2021, following the vision of President Paolo Aquilanti and in close and productive collaboration with Dr. Angelo Luca, CEO of IRCCS ISMETT, recently appointed Vice President of Fondazione Ri.MED, a number of initiatives were launched to promote further integration of ISMETT-Ri.MED-UPMC in terms of R&D, project financing, human resources, and general administration.

Thanks must go out to all members of Ri.MED's Board of Directors, and in particular to the strategic guidance of President Paolo Aquilanti. A special recognition goes to Professor Bruno Gridelli, Vice President of Fondazione Ri.MED, who has recently retired. Professor Gridelli has had a unique role in shaping our past, present, and future. A final acknowledgement to our legal advisor Giuseppe Mazzarella and his office for constructive and daily discussions on all fronts, and in particular in the process towards the realization the BRBC.

Dario Vignali
SCIENTIFIC DIRECTOR

The mission of the Ri.MED Foundation is to translate biotechnology and biomedical research into improved therapies for patients, enhanced by our strategic partnerships with IRCCS ISMETT and our founding partners, such as UPMC. Ri.MED's mission is also to facilitate the recruitment, education, and training of the next generation of Italian biomedical scientists and clinical scientists. Current translational research projects aim to address therapeutic needs in four research areas: cancer (with an emphasis on immunotherapy), organ insufficiency (which includes organ transplantation and regenerative medicine), aging (with an emphasis on neurodegeneration), and infectious diseases (with an emphasis on diseases of relevance in the Mediterranean area).

We are grateful to the commitment and efforts of our Scientific Committee: Prof. Lucia Altucci, Prof. Ivet Bahar, Prof. Antonino Cattaneo, Prof. Francesco Dieli, and Prof. Fadi Lakkis. They work conscientiously and constructively to develop our strategies and to ensure our competitiveness at the national and international levels, to maximize the efficient translation of our research observations into clinical trials, and to deliver a meaningful socio-economic impact at the regional and national levels.

Despite the devastating COVID-19 pandemic throughout 2021, our Ri.MED scientists were eager to contribute to the ongoing global research effort. They contributed to scientific projects and collaborations focused on COVID-19 that were initiated in 2020, such as the structural characterization of SARS-COV-2 proteins, the selection of lead molecules targeted against SPIKE and Mpro, and the identification of microRNAs expressed in lung tissues. Ri.MED also organized its own virtual symposium focused on this theme: "COVID-19: Mechanistic Dissection, Drug Development and Clinical Evaluation."

Our commitment to excellence and the growth of our researchers, our international and multidisciplinary environment, and the rich network of collaborations are what make us proud to be part of Ri.MED and its mission.

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Ri.MED OVERVIEW UP TO 31.12.2021

Networking

4 Agreements for labs management

33 Active scientific collaborations and technology transfer agreements

7 Scientific collaborations and technology transfer agreements signed in 2020

Training & employment

61 Employees in 2021

62% 38%

41 Scholarships

18 University of Pittsburgh
Post- Doc Fellowships c/o University of Pittsburgh

36 Ph.D. Fellowships

40 Internships

Intellectual property

About **475** Scientific publications

28 Patent

Fundings for Research

19.190.031 €
Awarded through national and international GRANTS

8.000.000 €
Sicilian Region operational contributions for Ri.MED-ISMETT cluster

Scientific knowledge dissemination

39 Ri.MED scientific meetings

10 Ri.MED internal meetings

9 Ri.MED institutional events

35 Participations in scientific events or local development activities

Building the BRBC

17.070 mq of laboratories

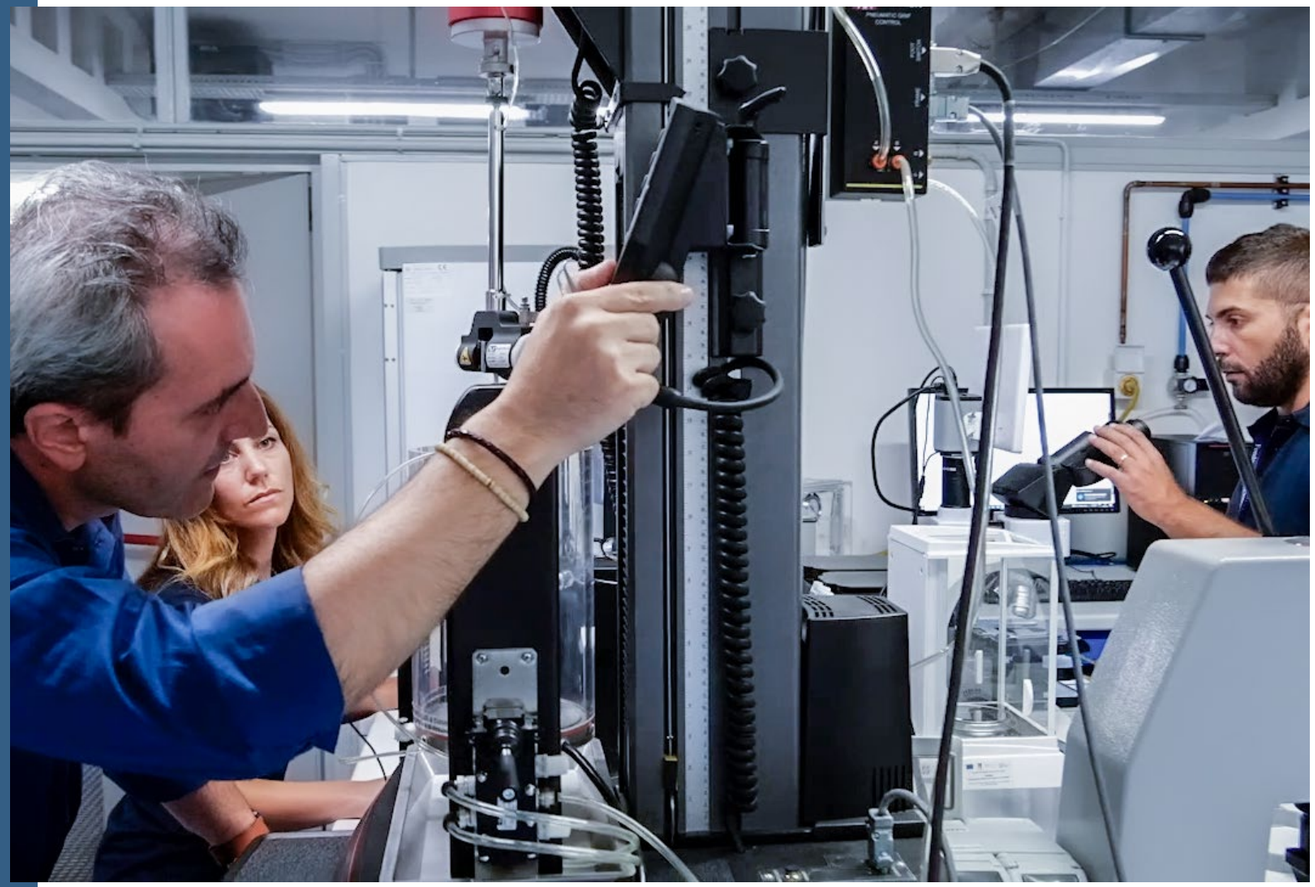
196.350.000 € Value of the investment

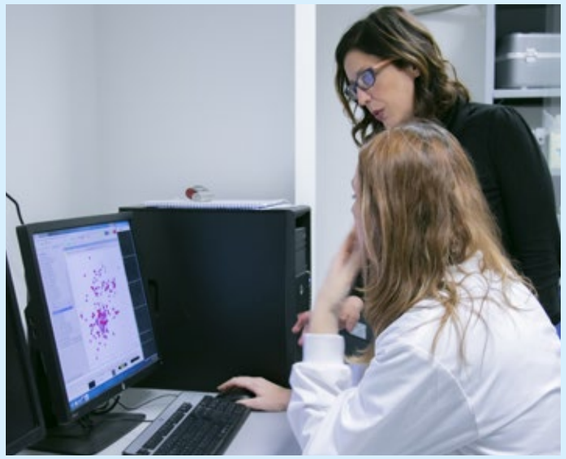
600 Planned occupancy opportunities

TRAINING
Nurturing new talents

Ri.MED puts great effort into training highly qualified staff, recognizing their decisive role in the successful outcomes of scientific challenges, and for the competitiveness and development of the entire territory.

Some of these programs were made possible thanks to the partnership with the University of Pittsburgh, which has already hosted 18 postdocs as part of the Ri.MED Fellowship program, and other training programs have been activated as part of the funding obtained through EU (for example Horizon 2020 "Oactive," and the ERC consolidator "Biomitral), ministerial ("Progema" and "4Frailty"), and regional calls ("Chemist", "Obind", "Senso", "Prometeo," and "iRhom2").



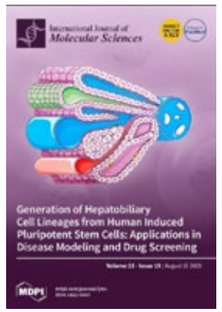


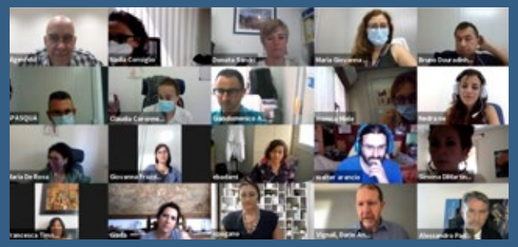
The year 2021 also saw a considerable increase in the number of selection procedures, double those of the previous year, highlighting the constant growth of investment in human resources made by the Foundation.

The data relating to training deserve to be highlighted, in particular: in 2021, 14 internships and 18 research doctorates were activated, meaning more than thirty young people who are developing skills that, in turn, will contribute to the growth and well-being of the territory.

DISSEMINATION OF SCIENTIFIC KNOWLEDGE

Activities related to scientific dissemination and sharing of research results are part of the Foundation's own mission. The first Ri.MED virtual symposium was held in June: "COVID-19: Mechanistic Dissection, Drug Development and Clinical Evaluation," which saw the participation of such important names as Rolf Hilgenfeld, Camillo Ricordi, Andrea Gambotto, under the scientific direction of Dario Vignali, Caterina Alfano, Giovanna Frazziano, and Monica Miele.





The Foundation participated in Innovabiomed, a network for biomedical innovation, which took place in July in Verona and saw the participation of over 50 companies and organizations, and hundreds of visitors. Ri.MED was present with a representation of researchers from the Bioengineering and Medical Devices group (the Group Leader Gaetano Burriesci, with Alessia Viola and Sofia Di Leonardo), the Tissue Engineering group (Arianna Adamo and Marzio Di Giuseppe), and the Immunotherapy group (Ester Badami), and was awarded as an "example of convergence between health, science and industry." Antonio D'Amore, Ri.MED group leader in Tissue Engineering, was also invited to present his innovative heart valve at a conference entitled "Il domani è già realtà: Dalla ricerca di base all'innovazione." (Tomorrow Is Already Here: From Basic Research to Innovation)."
D'Amore was also the lead figure of the "Cuore" episode of the Rai Cultura Information and Scientific Study program broadcast on Rai2, Rai3, and Rai Scuola.
This added to the typical seminar activity carried out by the researchers, who present their scientific activity, often as invited speakers, at the main scientific meetings of their specific area of interest. 2021 was an important year in terms of scientific publications, with over fifty articles in peer reviewed journals with relevant impact factors, a contribution to scientific dissemination in various sectors. The Foundation's press office also works to communicate the main research results to the wider public of non-experts, thus contributing to the dissemination of scientific knowledge.

PUBLIC ENGAGEMENT

Involving and inspiring a heterogeneous public is one of our priorities: we are working on a public engagement program to involve citizens of all ages, in collaboration with the principal figures in the territory. Our presence and interaction with the local community aims at developing activities that facilitate and promote knowledge, from science and health to investment and employment opportunities, legality, and meritocracy.

As with all event-related contact activities, 2021 was heavily penalized by the restrictions related to the COVID-19 pandemic; nevertheless, Ri.MED took part in the European Researchers' Night with the initiative "Ricerca, ingegno e tecnologia al servizio della salute" (Research, Ingenuity, and Technology at the Service of Health) of the Tissue Engineering group, which has been very popular with young and elders.
Collaborations with schools also continued: the "Research Friday" laboratories of applied sciences at the STEM high school of the CEI in Palermo and a collaboration with the Mursia Institute of Carini, and with the two-year course of the ITS Volta of Palermo, the first Industrial Technical Institute in Sicily specialized in "new technologies of life," aimed at training technicians specialized in the use of diagnostic and biomedical devices.

Ri.MED intends to gradually increase its presence, developing new educational programs with schools, and involving the local community.

NETWORKING

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

Creating networks that generate competitive research financing is crucial. For this reason, Ri.MED pays great attention to the continual development of its network of scientific collaborations and scientific agreements with bodies and institutions operating in its areas of interest: there are currently 31 agreements in place to develop technological innovation, promote research activities, and share laboratories and resources with European and U.S. institutions. Six agreements were signed in 2021.

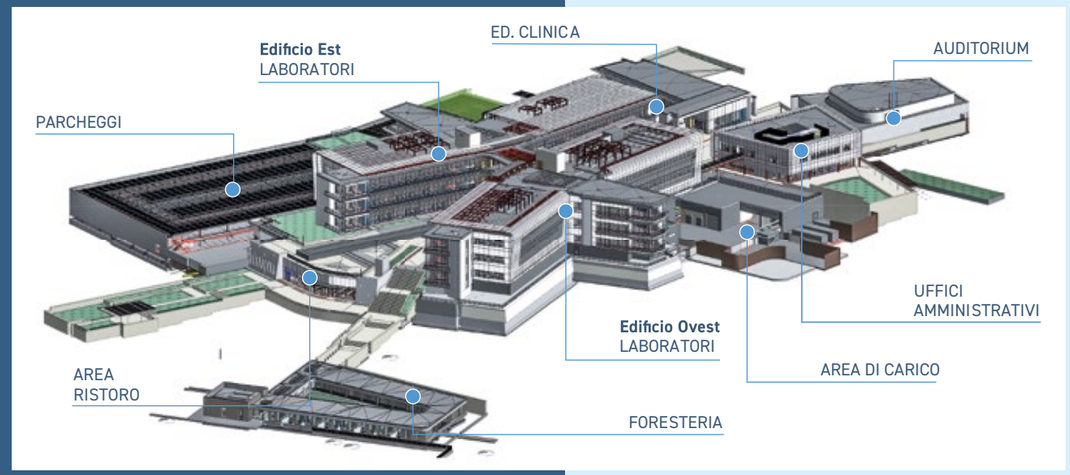
Ri.MED signed agreements for lab hosting with institutions with which it has signed collaboration agreements: the management of the Regenerative Medicine and Immunology laboratories at IRCCS ISMETT, of strategic importance for integrating basic and clinical research; the Structural Biology and Biophysics laboratories at ATeN Center; the Bioengineering and Medical Devices laboratory at the University of Palermo; and the High Throughput Screening laboratory at the CNR IRIB.

BRBC

Socio-economic impact on Sicily and Southern Italy

Ri.MED is building the Biomedical Research and Biotechnology Center (BRBC) in Carini, in the province of Palermo, a center capable of communicating with universities and research institutions, on the one hand, and with pharmaceutical and biotechnological companies on the other, developing strategic alliances and attracting research funding and investments.

BRBC's state-of-the-art laboratories and technological platforms will be dedicated to the research into and development of innovative vaccines and drugs, cell therapies, and the engineering of organs and tissues for the early diagnosis and targeted treatment of terminal organ failure, tumors, infectious diseases and pathologies related to aging, with particular attention to neurological diseases.



Construction at the site was started in 2020, and despite the difficulties related to the impact of the pandemic work is continuing steadily: the construction site in Carini now clearly shows the structure of the research campus.

The temporary association of enterprises (ATI) is led by Italiana Costruzioni, while management of the construction is entrusted to an RTI led by Progetto CMR, which belongs to the group winner of the international design competition of the center led by HOK.

During 2021, the foundations of all the buildings were completed, and the elevated structures are under construction (some of which are already completed), as well as some plant engineering works underway.

The project is inspired by the "village street" model, and provides for an extremely flexible organization of space: 17,070 square meters of laboratories, large common areas, meeting rooms, offices, an auditorium, a guesthouse, and direct connection with the new ISMETT hospital.

Once fully operational, the center will offer employment to approximately 600 people and will contribute to the generation of related activities linked to the technological transfer of research products.

AN INTEGRATED RESEARCH AND CARE CENTER

In 2021, the project for the construction of the new ISMETT hospital, designed by Renzo Piano, which will be built next to the BRBC on the same Carini site , was also presented. This will create an integrated center for highly specialized research and treatment in Italy, to the benefit of patients, of the economic development of Southern Italy, and of the entire country.

The discoveries made at BRBC will be used by the hospital partner IRCCS-ISMETT and other Italian hospitals, improving the availability of advanced diagnostic and therapeutic tools and protocols for patients.

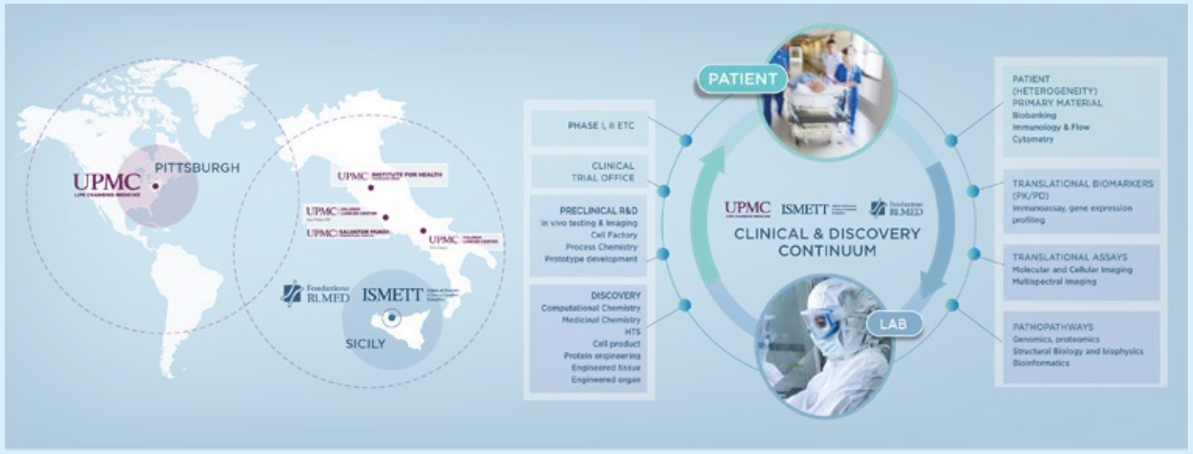
A policy of economic development based on biomedical sciences is being implemented, which in the coming years will lead Sicily to establish itself internationally as a pole of excellence in the Life Sciences, thanks also to the know-how provided by UPMC and the University of Pittsburgh.

The progress of the BRBC construction works, the project of the new ISMETT center, and the consequent strategic plan for the establishment of an integrated center for research and highly specialized care in Sicily were presented in October before the Italian Senate, in the presence of the President of the Republic Sergio Mattarella and of the President of the Senate Maria Elisabetta Alberti Casellati.

Strong involvement of the American partner UPMC, with the presence of Bruno Gridelli and Leslie Davis, as well as the Italian Ambassador to the United States Mariangela Zappia, and the transfer of know-how, public-private management models and international partnerships are the basis for the creation of an integrated center with complementary skills. Renzo Piano illustrated the project of the new hospital, and Sebastiano Musumeci, President of the Sicilian Region, shared the Region's commitment to the realization of structural interventions able to provide services of excellence, and to reduce the so-called passive mobility of patients towards other health services. The focus of Maria Chiara Carrozza, President of the National Research Council, was on networking among different entities and basic and applied research.

THE IMPORTANCE OF BEING A CLUSTER

The challenge in the Life Sciences is increasingly embodied in the ability to quickly translate the results of scientific research into clinical applications, and is therefore more and more focused on the complementary integration of resources and skills from different matrices, such as basic research, research and preclinical development of new therapies and medical devices, biomarkers, and clinical trials. The project to create an integrated center for translational research and highly specialized care in Carini will confirm the full completion of the strategic collaboration between UPMC, ISMETT, and Ri.MED, an international partnership that is becoming more concrete every year.



Integrating complementary skills increases the successful potential of translational research projects and, consequently, 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, characterized by the commonality of objectives.

During the year, periodic coordination meetings were held, and managed at different levels: governance coordination by the respective leaderships provided the strategies and guidelines for managerial activities; at the administrative level, by the grants offices to synergistically strengthen access to financing; in the scientific field, to update members on the progress of joint research projects and to identify new development opportunities.

In addition, the communication offices of the three entities have begun to collaborate to direct their respective efforts toward an image of the synergistic cluster.

Ri.MED RESEARCH RETREAT

After two long years of online meetings, on 17 December we finally managed to find ourselves attending a very special edition of the Ri.MED Research Retreat and the Annual Meeting, a completely new format, based on the idea of maximizing the relationship with ISMETT and identifying research topics useful to both.
The first part of the day was dedicated to the presentation by some ISMETT physicians of specific clinical needs, which were then examined in depth in five thematic roundtables:

"Mitigating the risk of infection in solid organ transplantation," "Healing the lung," "Addressing ischemic injury / reperfusion of solid organs via drugs or cells," "Current status and future role of liquid biopsy in cancer treatment," and "Coordinated projects in neuroscience."
The ISMETT / Ri.MED thematic roundtables brought together physicians and researchers, in a format that proved to be truly useful for maximizing the relationship between the two, and identifying joint research topics. Particularly significant is the fact that follow-ups by some groups are already in progress, and thanks to the Ri.MED Research Retreat they have had the opportunity to get to know each other and interact.
The event ended in the afternoon with a poster session, an opportunity for all the research groups of the Foundation to present the scientific projects in progress, and for the researchers of IRCCS ISMETT to present the joint projects already started with the Ri.MED groups of Regenerative Medicine and GMP Cell Products.

Ri.MED RESEARCH GROUP

The research activity of the Ri.MED Foundation is organized in research groups and platforms. At the moment, the groups are located at different sites, and are continually expanding, also in view of the forthcoming opening of the research center in Carini, where they will all converge. In addition to the structured staff, in 2021, 45 people were managed, including trainees, PhD students, fellows, and post-docs: training and enthusiasm are the basis of our growth.
More than 60% of the researchers are women.

ATMP - Advanced Therapy Medicinal Products

Regenerative Medicine and Immunotherapy

Ri.MED Lab c/o IRCCS ISMETT, Palermo

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Claudia Carcione
Laboratory Technician
Giampiero Vitale
Laboratory Technician
Salvatrice Lo Giudice
Laboratory Support

GMP CELL FACTORY & PRECLINICAL RESEARCH

GMP Cell Factory

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TISSUE ENGINEERING AND BIOENGINEERING

Tissue Engineering

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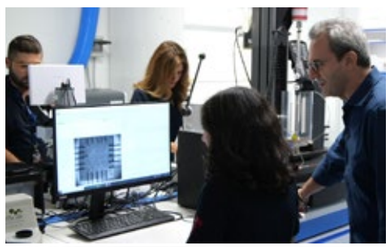

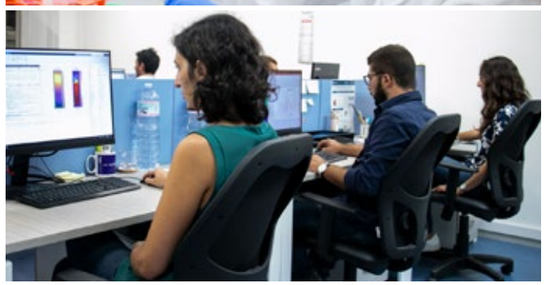

DRUG DISCOVERY

Molecular Informatics

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Salvatore Contino
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Camilla Pecoraro
PhD Student in Molecular and Biomolecular Sciences
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Viviana Benfante
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Simone Valenti
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Identification of Therapeutic Targets and Screening

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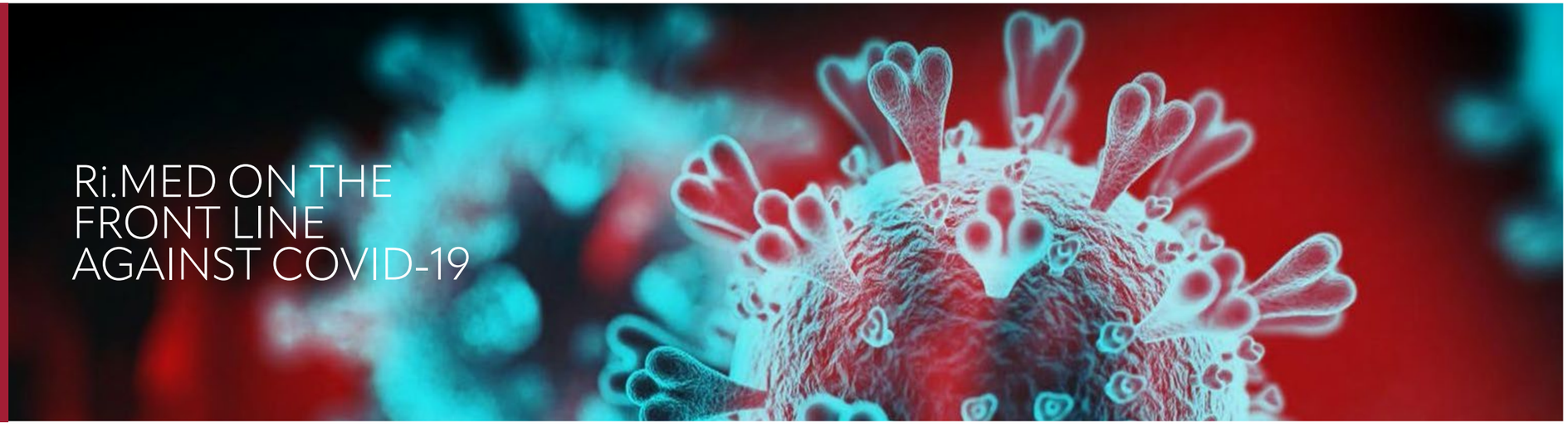


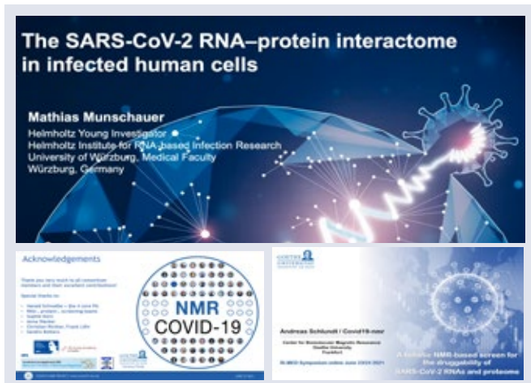
Ri.MED ON THE FRONT LINE AGAINST COVID-19

The state of emergency related to the COVID-19 pandemic lasted throughout 2021. In parallel with the Foundation's own research and development programs, our scientists wanted to make their contribution by undertaking scientific projects and collaborations focused on COVID -19 started last year: for example, the structural characterization of SARS-COV-2 proteins, identification of compounds as potential inhibitors of Mpro and Spike-ACE 2 interaction, and human micro RNAs against SAR-COV-2 in pulmonary tissues.

In June 2021, the Foundation organized its own virtual symposium focused on the topic: "COVID-19: Mechanistic Dissection, Drug Development and Clinical Evaluation." The meeting was attended by important names, such as Rolf Hilgenfeld, Camillo Ricordi and Andrea Gambotto, together with Ri.MED researchers Ugo Perricone, Caterina Alfano, Albert Comelli, and Monica Miele. Particularly relevant were the collaborations, primarily those arising within the Ri.MED - ISMETT - UPMC cluster between Italy and the U.S., but also those at the European level, in particular membership in the COVID-19 NMR International Consortium.

The Drug Discovery approach, presented on the first day, was aimed at the discovery of new and more effective therapies. Prof. Rolf Hilgenfeld, a structural biologist who has been studying coronavirus proteins since the early 2000s, presented his work in developing molecules capable of inhibiting the action of main protease, whose three-dimensional structure he was the first to provide. Caterina Alfano, Ri.MED Group leader in Structural Biology and Biophysics, has conducted several studies aimed at obtaining a complete picture of the mechanisms of SARS-CoV- 2, both to identify other SARS-CoV-2 protein targets different from the spike protein, and to understand which host cell proteins are essential for virus replication. In collaboration with the EBRI Rita Levi Montalcini Foundation, Dr. Alfano's group is developing neutralizing antibodies for the nsp-9 and N proteins of SARS-CoV-2, and is engaged in the study of the interactions between the viral RNA transcription complex and an important class of human proteins, the La-related-proteins (LaRPs).

The second day of work was focused instead on clinical research and experiences with hospitalized patients. Prof. Gambotto, from the University of Pittsburgh, presented the results of the clinical trials conducted in the U.S. on the vaccine he developed; Dr. Cillo's study on the predictive models generated by the transcriptional profiles recorded on the first day of hospitalization in the ICU was also from Pittsburgh; Prof. Ricordi, President of the Ri.MED Foundation for the previous five years, presented the data on the studies conducted in Miami in collaboration with Dr. Lanzoni on the administration of placental stem cells for the treatment of patients with acute respiratory distress. A fundamental contribution came from the Ri.MED Foundation's clinical partner: in fact, IRCCS ISMETT in Palermo activated the Level 3 Intensive Care Unit for the management of the pandemic. Dr. Panariello, head of the Intensive Care Unit, reported on the use of extracorporeal veno-venous membrane oxygenation (V-V ECMO) as a life-saving therapy in the treatment of hospitalized patients; Dr. Monica Miele, head of GMP production at the Ri.MED Foundation, also collaborated with Dr. Bulati in the efficacy study of the Pfizer vaccine on ISMETT transplanted and, in general, immunocompromised patients.



MOLECULAR INFORMATICS AND MEDICINAL CHEMISTRY

Mpro

Ri.MED Team: **Molecular informatics and Medicinal Chemistry**

ABSTRACT

The study concerns a later phase of the viral cycle, which involves the Main protease (Mpro), one of the enzymes responsible for the maturation of structural and functional viral proteins. Actually, Mpro promotes the cleavage of polyproteins into smaller mature proteins that assemble themselves, producing new infectious virions. Mpro therefore represents a potential target for a specific antiviral therapy for COVID-19 patients. The aim is to design new Mpro inhibitors in order to hinder and damage the viral replication machinery. The selection of potentially active compounds on MPro is nearing completion. In particular, the Molecular Informatics and Medicinal Chemistry groups have developed, in collaboration with the Advanced Data Analysis group, a classification model based on classical machine learning approaches with the aim of optimizing the computational prioritization of molecules predicted as active on target Mpro. In fact, this model is a useful support for the scoring functions to be used together with other classic virtual screening techniques.

RESULTS

Machine learning model release and 100 compounds selected to be tested on Mpro.

PUBLICATIONS

Nedra Mekni , **Claudia Coronnello,** Thierry Langer, **Maria De Rosa**, **Ugo Perricone**, Support Vector Machine as a Supervised Learning for the Prioritization of Novel Potential SARS-CoV-2 Main Protease Inhibitors, Int. J. Mol. Sci. 2021, 22(14), 7714

IMMUNOLOGY AND TRANSPLANTS

Specific Anti-SARS-CoV-2 Cellular Immune Responses study after BNT162b2 Pfizer-BioNTech mRNA-based vaccine in a solid organ transplant recipient cohort

Ri.MED Team: **GMP Cell Factory and Regenerative Medicine**
PARTNERSHIP: IRCCS ISMETT

ABSTRACT

SARS-CoV-2 vaccine is considered the primary health strategy able to end the COVID-19 pandemic. The viral infection has a worse impact on solid organ transplant recipients (SOTRs) than general population but the effect of vaccination in this subgroup of immunosuppressed patients is not known due to the exclusion of SOTRs from vaccination trials. To better characterize the effect of vaccination on the immune response in SOTRs, we assessed the B and T-cell responses in 80 SOTRs and in 42 immunocompetent subjects (ICs), after the second dose of BNT162b2 vaccine and after the booster dose. Blood samples of ICs and SOTRs were taken at defined time points in order to detect anti-Spike protein IgG using chemiluminescent assay, whereas *ex vivo* IFN-γ-ELISpot assay was used for evaluating the number of T cells specific to S protein among the peripheral blood mononuclear cells (PBMCs) of the vaccinated people.

RESULTS

Our results suggest that current vaccination Pfizer-BioNTech approach against SARS-CoV-2 might be less effective than expected in SOTRs. If confirmed in larger studies our results will support new approaches aimed to obtain a better stimulation of specific immunogenicity by the use of high-dose vaccines, repeated booster-dose or use of adjuvants. In this scenario, it is clear that clinical trials using specific clinical endpoints, to define the efficacy of the SARS-CoV-2 vaccine in SOTRs is an urgent need.

PUBLICATIONS

- **Monica Miele**, Rosalia Busà, Giovanna Russelli, Maria Concetta Sorrentino, **Mariangela Di Bella**, **Francesca Timoneri**, Alessandra Mularoni, Giovanna Panarello, Patrizio Vitulo, Pier Giulio Conaldi, Matteo Bulati. "Impaired anti-SARS-CoV-2 humoral and cellular immune response induced by Pfizer-BioNTech BNT162b2 mRNA vaccine in solid organ transplanted patients". Am J Transplant. 2021;21:2919–2921.

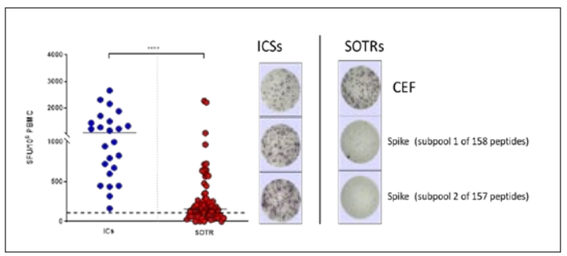


Fig 1. The dot plot describes the frequency of circulating IFN-γ-cells responding to overlapping peptides spanning of the SARS-CoV-2 Spike protein and CEF (as positive control peptide pool) after second dose of vaccination in SOTRs and ICs cohort.

STRUCTURAL BIOLOGY AND BIOPHYSICS

Structural characterization of SARS-CoV-2 proteins

Ri.MED Team: Structural Biology and Biophysics

PARTNERSHIP
- UK Dementia Research Institute (UK DRI) - King's College London, London, United Kingdom.
- European Brain Research Institute Rita Levi Montalcini (EBRI), Rome, Italy
- Scuola Normale Superiore di Pisa (SNS), Pisa, Italy
- Molecular Medicine Department – University of Pavia, Pavia, Italy
- European Synchrotron Radiation Facility (ESRF), Grenoble, France
- Covid19-NMR International Consortium

ABSTRACT
The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is already the third coronavirus infection that has occurred in the third millennium. The epidemic has afflicted the world population over all of the last two years and is still on-going. This situation underlines the importance of gaining a deep understanding of the rules that regulate viral internalization and reproduction and the necessity to translate this knowledge into treatments. As Structural Biology and Biophysics group, we focused on the non-structural protein 9 (nsp9), the Nucleocapside protein (N), and on the accessory protein 8 (orf8), all being of extreme biological and therapeutic relevance. Nsp9 is a dimeric ssRNA-binding protein highly conserved among Betacoronaviruses, and it is involved in the viral replication machinery. Deletion of nsp9 in the mouse models prevents the synthesis of RNA and productive infection, indicating that the mature form of nsp9 is fundamental for viral replication. Protein N, also dimeric, packages the positive strand of viral RNA and plays an important role in improving the transcription efficiency of subgenomic viral RNA. Orf8 is believed to be responsible for the evolution of Betacoronaviruses and their species jumps as well as to have a role in depressing the host response. In fact, orf8 reduces the transcription of the class I MHC complex, compromising the action of the host's T lymphocytes on infected cells. The validity of orf8 as a therapeutic target is further confirmed by the reduced aggressiveness of SARS-CoV-2 in patients infected with viruses with orf8 gene deletion.

RESULTS
We designed and produced the expressing gene constructs for recombinant nsp9, N full length, N-CTD, and orf8. We also developed successful purification protocols, performed the biophysical characterization of the protein by circular dichroism (CD) and nuclear magnetic resonance (NMR), and assigned the NMR backbone assignment of nsp9. The crystal structure of nsp9 from SARS-CoV-2 was recently published but the availability of the crystal structure does not, nevertheless, reduce the interest of studying the protein in solution as this is the prerequisite to fragment based drug screening and other experimentally-based drug design strategies. In collaboration with the EBRI Rita Levi Montalcini Foundation, we are also working on the identification and biophysical characterization of scFvs against SARS-CoV-2 proteins

PUBLICATIONS
- Altincekic N., Korn S.M., Qureshi N.S., Dujardin M., Ninot-Pedrosa, Abele R., Abi Saad M.J., **Alfano C.**,..,**Monaca E.**,..,**Sabbatella R.**,.., Schlundt A. (2021) Large-scale recombinant production of the SARS-CoV-2 proteome for high-throughput and structural biology applications. Frontiers in Molecular Biosciences, 8:89. doi: 10.3389/fmolb.2021.653148.
- Dudás E.F., Puglisi R., Korn S.M., **Alfano C.**, Bellone M.L., Dal Piaz F., Kelly G., **Monaca E.**, Schlundt A., Schwalbe H., Pastore A. (2021) Backbone chemical shift spectral assignments of SARS coronavirus-2 non-structural protein nsp9. Biomolecular NMR Assignments, 5(2):235-241. doi: 10.1007/s12104-021-10011-0.

Covid-19- NMR International Consortium

Ri.MED Team: Structural Biology and Biophysics

PARTNERSHIP
- Goethe University of Frankfurt
- Covid19-NMR International Consortium

ABSTRACT
The Covid19-NMR consortium was launched by Goethe University in Frankfurt in March 2020 and within a month it became an international consortium: scientists from all over the world, united by the ongoing pandemic emergency, are collaborating in an effort unique to study SARS-CoV-2 using NMR spectroscopy. The overall goal is to join forces to achieve scientific results as quickly as possible and make them immediately available online with weekly updates. The specific objective of the research is to determine the solution structures of SARS-CoV-2 RNAs and proteins and to carry out a fragment-based drug screening using NMR techniques. As part of the Covid19-NMR Consortium, the Structural Biology and Biophysics team of Ri.MED Foundation deals with the structural elucidation of the viral proteins orf8 and nsp9.

RESULTS
Within the Covid-19 NMR International Consortium, the Structural Biology and Biophysics team of Ri.MED Foundation contributed in compiling a compendium of more than 50 protocols for the production and purification of 23 of the 30 SARS-CoV-2 proteins or fragments thereof. The backbone assignment of the NMR resonances was also performed for the viral protein nsp9. On going studies aim at studying the oligomerization behavior of orf8.

PUBLICATIONS
- Altincekic N., Korn S.M., Qureshi N.S., Dujardin M., Ninot-Pedrosa, Abele R., Abi Saad M.J., **Alfano C.**,..,**Monaca E.**,..,**Sabbatella R.**,.., Schlundt A. (2021) Large-scale recombinant production of the SARS-CoV-2 proteome for high-throughput and structural biology applications. Frontiers in Molecular Biosciences, 8:89. doi: 10.3389/fmolb.2021.653148.
- Dudás E.F., Puglisi R., Korn S.M., **Alfano C.**, Bellone M.L., Dal Piaz F., Kelly G., Monaca E., Schlundt A., Schwalbe H., Pastore A. (2021) Backbone chemical shift spectral assignments of SARS coronavirus-2 non-structural protein nsp9. Biomolecular NMR Assignments, 5(2):235-241. doi: 10.1007/s12104-021-10011-0.

IMAGING AND RADIOMICS, ADVANCED DATA ANALYSIS AND IDENTIFICATION OF THERAPEUTIC TARGETS AND SCREENING

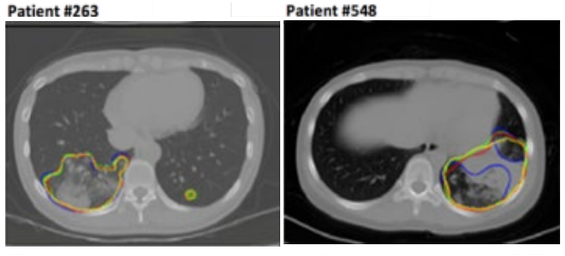
Customized Efficient Neural Network per l'identificazione della regione infetta da COVID-19 nelle immagini CT

Ri.MED Team: Imaging and Radiomics and Advanced Data Analysis

PARTNERSHIP
Institute of Bioimaging and Molecular Physiology (IBFM-CNR), Cefalù (PA), Italy.

ABSTRACT
In the field of biomedical imaging we present a customized deep learning approach aimed at addressing the real-time, and fully automated identification and segmentation of COVID-19 infected regions in computed tomography images. In a previous study, we adopted ENET, originally used for image segmentation tasks in self-driving cars, for whole parenchyma segmentation in patients with idiopathic pulmonary fibrosis which has several similarities to COVID-19 disease. To automatically identify and segment COVID-19 infected areas, a customized ENET, namely C-ENET, was implemented and its performance compared to the original ENET and some state-of-the-art deep learning architectures. The experimental results demonstrate the effectiveness of our approach. Considering the performance obtained in terms of similarity of the result of the segmentation to the gold standard (dice similarity coefficient ~75%), our proposed methodology can be used for the identification and delineation of COVID-19 infected areas without any supervision of a radiologist, in order to obtain a volume of interest independent from the user.

RESULTS
We demonstrated that the proposed customized deep learning model can be applied to rapidly identify, and segment COVID-19 infected regions to subsequently extract useful information for assessing disease severity through radiomics analyses.



CT images showing parenchyma with visible subregions (yellow for the gold standard, red for C-ENET, green for ENET, and blue for ERFNET) infected with COVID-19 disease.

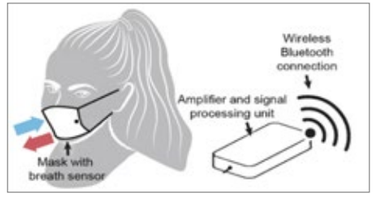
Mask-integrated sensor for oxidative stress (Sensomar)

Ri.MED Team: Experimental Lung Research group

PARTNERSHIP
- Università degli Studi di Palermo
- Istituto per la Ricerca e l'Innovazione Biomedica (IRIB-CNR)
- Istituto di Fisiologia Clinica (IFC-CNR)

FUNDS FISR 2020

ABSTRACT
The severe forms of Covid-19 present a pathophysiological picture similar to that found in other forms of respiratory distress characterized by an increase in oxidative stress (OS) markers. Clinical and experimental evidences recognize in the increase of OS a determining and early pathogenetic role of lung damage. Therefore, OS assessment in biological fluids may provide useful information to discriminate inflammatory parameters, predict exacerbations, evaluate the more severe pathological pictures, and monitor the epidemic phenomenon. The project aims at developing an an innovative, economic and miniaturized device for monitoring patients suffering from respiratory diseases, through the non-invasive detection of hydrogen peroxide (marker of OS) in respiratory exhalations. This device, integrated on a face mask, will be connected with a remote system and will provide information about the evolution of the disease to the subjects involved in the health supply chain (patient, doctor, institutions, etc.). As such, the device will have a direct impact for patients suffering from COVID-19, both in a home-monitoring scenario and in hospitalized patients in addition to traditional monitoring systems to identify rapidly evolving conditions and to monitor the response to treatments.



RESULTS
1. Realization of a mask-integrated nano-structured sensor for SO measurement and of a centralized monitoring device;
2. Development of a circular production system capable of producing the required volumes;
3. Development of a decision support system for diagnostic, therapeutic and epidemiological purposes.

DRUG DISCOVERY

Ri.MED Foundation researchers are involved in drug discovery projects to identify new biologically active molecules. Studying biomolecular pathways integrated with genomics, proteomics, metabolic and secretomics data, our researchers were able to arrive at the functional validation of new therapeutic targets for diseases in therapeutic areas of interest, such as oncology and aging-associated diseases.

Some of these projects are now in their screening phase for the discovery of new hit compounds. This process starts with the study of target proteins through biophysical and computational chemistry approaches, and developing biophysical, biochemical or cellular screening assays. Thanks to the integrated virtual screening platform, hundreds of molecules of synthetic and natural origin were selected using structure-based (docking) and ligand-based (pharmacophore) techniques. Last year saw the creation of a molecular database that today consists of around 2,000 molecules. Some of these have been biologically tested.

The active molecules, known as singletons, will be validated through QSAR (quantitative structure-activity relationship) studies. In the next phase, the most promising hit series in terms of druggability will be selected, and the hit-to-lead optimization phase will be entered. The medium-term goal is to select the lead molecule to be subjected to preclinical testing, then to evaluate the efficacy through in vivo studies integrated with molecular imaging, and characterize the pharmacokinetic and toxicological profile suitable for clinical experimentation on patients.

Development of neutralizing antibodies against SARS-CoV-2
Caterina Alfano, PhD

Identification and characterization of interactions among SARS CoV-2 RTC and LaRPs
Caterina Alfano, PhD

Molecular mechanisms of protein misfolding diseases
Caterina Alfano, PhD

Structural and biophysical studies probing the interaction of KDM4a with potential inhibitors
Caterina Alfano, PhD

The role of repetitive sequences in cellular senescence and aging
Walter Arancio, PhD

Role of inflammasomes in cigarette-smoke associated lung inflammation
Chiara Cipollina, PhD

Development of AI Algorithms for Extraction and Selection of Radiomics Features from Biomedical Imaging
Albert Comelli, PhD

Development of Automatic Fully 3D Algorithms for Biomedical Image Segmentation and Classification
Albert Comelli, PhD

Modeling microRNA-target interaction network
Claudia Coronnello, PhD

Design and synthesis of novel potential KDM4a inhibitors
Maria De Rosa, PhD

Design and synthesis of novel potential NLRP3 inflammasome inhibitors
Maria De Rosa, PhD

Design of non-covalent inhibitors of NLRP3 in the inflammation disease.
Ugo Perricone, PhD

Design of modulators of Histone lysine demethylase 4 (KDM4) as anticancer agents.
Ugo Perricone, PhD

iRhom2: a new therapeutic target in osteoarthritis
Simone Dario Scilabra, PhD

iRhom2 regulates ectodomain shedding of MHC class I molecules
Simone Dario Scilabra, PhD

Dissecting the role of iRhom2 in regulating levels of MHC-I molecules and responsiveness of PDAC to immunotherapy
Simone Dario Scilabra, PhD

OBIND – Oncological therapies through Biological Interaction Network Discovery
Claudia Coronnello, PhD Ugo Perricone, PhD

PRODUCTS: **Drugs - Biologics**

Development of neutralizing antibodies against SARS-CoV-2

Caterina Alfano, PhD
calfano@fondazionerimed.com

COLLABORATIONS

- European Brain Research Institute Rita Levi Montalcini (EBRI) - Rome, Italy
- Scuola Normale Superiore (SNS) - Pisa, Italy

THERAPEUTIC AREA

Infectious diseases

BRIEF DESCRIPTION

The COVID-19 pandemic that has spread over the last two years is still ongoing, despite the fact that various strategies have been developed to prevent infection or alleviate clinical manifestations. Most of the efforts have been invested in the development of vaccines, capable of inducing an immune response against the viral protein Spike, and neutralizing antibodies against Spike. However, considering the mutant nature of SARS-CoV-2 which mainly affects the Spike region, the effectiveness of the tools available to date is not demonstrated for current and future variants of the virus.

The goal of our research project is to find more durable and broad-spectrum treatments that are effective against the numerous variants of the virus and that can also counteract future coronavirus pandemics. In particular, we are involved in the development of antibodies, in the form of single chain fragment variable (scFv), against the Nucleocapsid protein (N) and the Nsp9 protein of SARS-CoV-2, rather than on the Spike protein whose rapid and high mutability results in a reduction in the effectiveness of the treatments available. Both chosen proteins are of extreme biological and therapeutic relevance. Nsp9 is a highly conserved ssRNA-binding dimeric protein among Betacoronaviruses and is involved in virus replication and transcription. Protein N, also dimeric, packages the positive strand of viral RNA and plays an important role in improving the transcription efficiency of subgenomic viral RNA.

IMPACT

Since the onset of the COVID-19 pandemic, most efforts have been invested in developing vaccines capable of inducing an immune response against the viral protein Spike, and neutralizing antibodies against Spike. However, one of the questions that researchers immediately tried to answer concerns the extreme variability of the SARS-CoV-2 virus, mainly due to the highly mutability nature of the Spike protein, which makes the therapeutic tools developed so far less and less effective. Therefore, it remains essential to investigate other stages of viral infection and replication. We aim to develop new molecules against proteins other than Spike, while implementing a platform and workflow for rapid and decisive intervention in the event of future coronavirus pandemics. The data produced so far on Nsp9 and the Nucleocapsid protein N provide the prerequisite for selecting and developing new neutralizing antibodies against these two proteins, thus preventing viral replication and/or attenuating the aggressiveness of the virus.

RESULTS ACHIEVED IN 2021

Cloning and setup of the expression and purification of the nucleocapsid protein N were carried out, both in the full-length form and in the only C-terminal domain (CTD) involved in the dimerization of the protein. The biophysical characterization through circular dichroism and nuclear magnetic resonance was performed on both constructs, which allowed to verify the correct folding of the produced proteins and evaluate their stability. The production and characterization of recombinant N protein was the prerequisite for the screening of biologically active fragments and in particular of antibodies in scFv format, selected by our collaborators from EBRI and SNS through IACT (Intracellular Antibody Capture Technology). Interaction studies were then carried out using Bio-Layer Interferometry (BLI) technology, between five selected antibodies and the N protein. Preliminary results show affinities in the order of nM for at least two scFvs, predicting a promising route in the development of antibodies against SARS-CoV-2.

GOALS FOR 2022

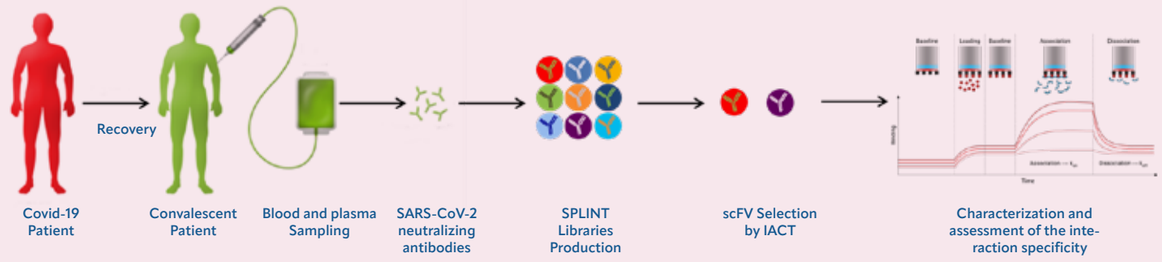
Quantities in the order of tens of mg of N protein will be produced, both unlabelled for crystallization tests, and $^{15}N/^{13}C$ doubly labelled for the determination by NMR Chemical Perturbation Analysis of the surfaces involved in the interaction with the scFv. The labelled samples will be used to acquire a large dataset of three-dimensional NMR experiments, using our Bruker NMR spectrometer operating at 800 MHz and equipped with a cryo-probe. Resonance assignment will be performed automatically, using dedicated software based on Artificial Intelligence, and manually validated. This will allow us to quickly reach our goals and will open the possibility to structural studies of N/scFv complexes. The selected scFvs will be produced by EBRI in quantities and purities such as to allow the necessary structural and interaction studies. At the same time, the selection of antibodies against the Nsp9 protein will be carried out, followed by the determination of the kinetic parameters of the interaction by BLI.

MEETINGS

- *Exploring the druggability of SARS-CoV-2-COVID-19 NMR Webinar*, June, 2021, online;
- Structural biology for medicine and biotechnology, December, 2021, online;
- *COVID-19: mechanistic dissection, drug development and clinical evaluation*. Ri.MED virtual symposium, June, 2021, online.

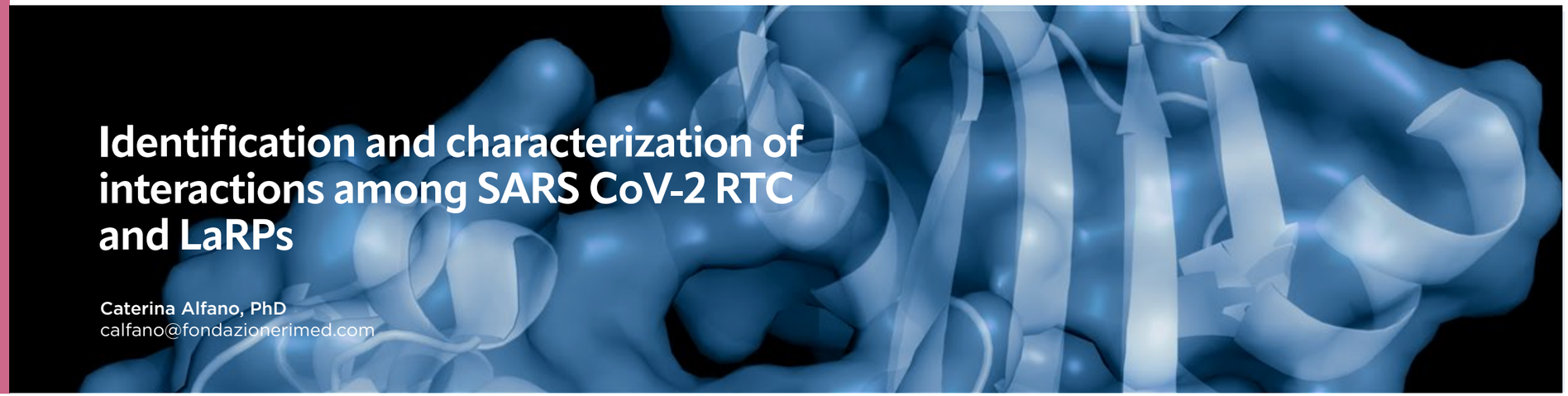
PUBLICATIONS

- Altincekic N., Korn S.M., Qureshi N.S., Dujardin M., Ninot-Pedrosa, Abele R., Abi Saad M.J., **Alfano C.,..,Monaca E.,..,Sabbatella R.,..**, Schlundt A. (2021) Large-scale recombinant production of the SARS-CoV-2 proteome for high-throughput and structural biology applications. Frontiers in Molecular Biosciences, 8:89. doi: 10.3389/fmolb.2021.653148.
- Dudás E.F., Puglisi R., Korn S.M., **Alfano C.**, Bellone M.L., Dal Piaz F., Kelly G., **Monaca E.**, Schlundt A., Schwalbe H., Pastore A. (2021) Backbone chemical shift spectral assignments of SARS coronavirus-2 non-structural protein nsp9. Biomolecular NMR Assignments, 5(2):235-241. doi: 10.1007/s12104-021-10011-0.



Schematic view of our workflow. From sera of convalescent patients to recombinant scFvs.
Adapted from Casadeval & Pirofski (2020) J. Clin. Invest., 130(4): 1545.

PRODUCTS: **Drugs - Biologics**



Identification and characterization of interactions among SARS CoV-2 RTC and LaRPs

Caterina Alfano, PhD
calfano@fondazionerimed.com

COLLABORATIONS

- Randall Division of Cell and Molecular Biophysics, King's College London (KCL), UK
- Institute of Biochemistry I, University of Regensburg, Germany

THERAPEUTIC AREA

Infectious diseases

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

SARS-CoV-2, analogously to the large part of viruses, requires an array of proteins from the host cells for its life cycle. The abduction and hijacking of eukaryotic proteins occur through RNA-protein or Protein-Protein Interactions (PPIs). The latter are, indeed, crucial for the assembling of the Replication-Transcription Complex (RTC) whom enzymatic core-unit is composed by three non-structural proteins (nsps): nsp12 (RNA-dependent-RNA-polymerase, RdRp) that mediates the nucleotides polymerization, and nsp7 and nsp8 as cofactor and primase, respectively. New findings based on proteomic and computational models, suggest that some La Related Proteins (LaRPs) play a crucial role in viral genome replication. The five members of the LaRPs superfamily (Genuine La, LaRP1, LaRP4A, LaRP4B and LaRP7), despite sharing the extremely conserved N-terminal motif (La Module, LaM), recognize different substrates and, consequently, are involved in different processes of regulation of the cellular RNA. Our goal is the identification and the structural characterizing of the interactions that involve the pivot triad nsp12-8-7 of the mini-RTC complex with one or more members of LaRPs family. Highlighting the key regions directly involved in the interaction will be useful for eventually designing PPIs inhibitors able to block virus replication.

IMPACT

Understanding the molecular mechanisms by which viruses sequester and exploit host systems to carry out their own infection cycle is of crucial importance for developing effective strategies aimed at countering the etiological agent. In this context, the in-depth knowledge of the role that LaRPs play in SARS-CoV-2 replication will contribute to the fight against the SARS-CoV-2 pandemic and any future coronavirus epidemics and/or pandemics. In particular, the biophysical and structural characterization of the interaction surfaces between a LaRP and the SARS-CoV-2 RTC will be useful for the development of inhibitors capable of reversing the sequestring of LaRPs by SARS-CoV-2 and inhibiting, consequently, viral replication.

RESULTS ACHIEVED IN 2021

In 2021 we have optimized the production protocols for recombinant LaRPs (both full length and LaM), and the members of the SARS-Cov-2 mini-RTC (nsp12-nsp8-nsp7). Some LaRPs and nsp12 have a considerable size for expression in bacterial systems, we so applied several molecular cloning strategies to obtain the maximum yield from E.coli cells. We also setup ad hoc purification procedures which allowed to get milligrams of high purity proteins suitable for biophysical studies. We then performed preliminary interactions experiments by BioLayer Interferometry technique (BLI). The binding analyses showed affinities of Larp7 for nsp8, in agreement with published proteomic data. Biophysical studies involving LaRP4 are now on going to evaluate if it also interacts with the RTC.

GOALS FOR 2022

For 2022, the goal will be to confirm the interactions observed in the preliminary studies and deepen the analysis for the other members of the LaRP family (LaRP4A, LaRP4B and Genuine La). Affinities towards SARS-CoV-2 mini-RTC will be tested by biophysical techniques such as BLI and Isothermal Titration Calorimetry (ITC). The interactions that will give positive results *in vitro* will then be studied in the cell using human cells with knock-out or knock-down for LaRPs proteins. The cells will be infected with SARS-Cov-2 virions at the group of Prof. Conte of the Randall Division for Cell & Molecular Biophysics of King's College of London (UK), and the viral replication capacity will be evaluated in the absence of LaRP.

MEETINGS

- AIC School 2021 - Fundamentals of Crystallography: the theory behind the crystal structure solution. August, 2021, online;
- Exploring the druggability of SARS-CoV-2-COVID-19 NMR Webinar, June, 2021, online;
- COVID-19: mechanistic dissection, drug development and clinical evaluation. Ri.MED virtual symposium, June, 2021, online.

PUBLICATIONS

- Altincekic N., Korn S.M., Qureshi N.S., Dujardin M., Ninot-Pedrosa, Abele R., Abi Saad M.J., **Alfano C.,...,Monaca E.,...,Sabbatella R.,...**, Schlundt A. (2021) Large-scale recombinant production of the SARS-CoV-2 proteome for high-throughput and structural biology applications. Frontiers in Molecular Biosciences, 8:89. doi: 10.3389/fmolb.2021.653148.
- Dudás E.F., Puglisi R., Korn S.M., **Alfano C.**, Bellone M.L., Dal Piaz F., Kelly G., **Monaca E.**, Schlundt A., Schwalbe H., Pastore A. (2021) Backbone chemical shift spectral assignments of SARS coronavirus-2 non-structural protein nsp9. Biomolecular NMR Assignments, 5(2):235-241. doi: 10.1007/s12104-021-10011-0.

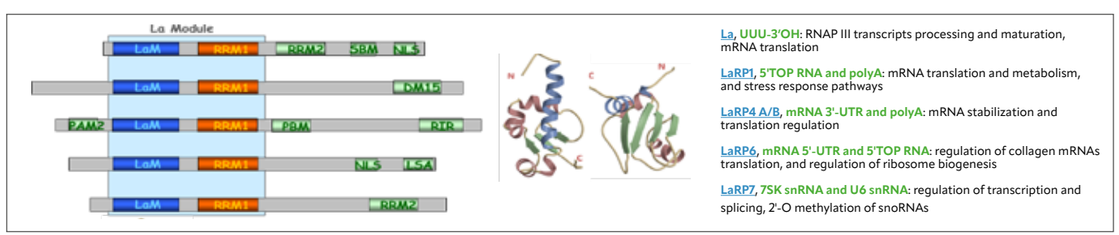
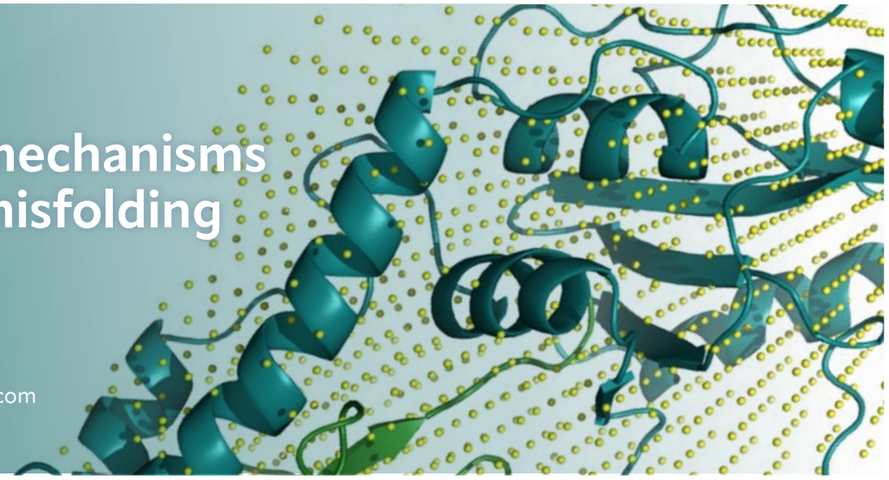


Fig.1: Domains organization of LaRPs proteins. (Left) Schematic representation of human LaRPs domains. (Middle) NMR structures of both La Motif and RRM1 domains of Human La protein (PDB IDs: 1S7A, 1S79). (Right) Description of the RNA molecule recognized by each LaRP (in green) and pathway in which the LaRP is involved (in gray).

PRODUCTS: **Drugs - Biologics**

Molecular mechanisms of protein misfolding diseases



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- Dipartimento di Fisica e Chimica (DiFC)- Università degli Studi di Palermo, Palermo, Italy
- European Synchrotron Radiation Facility (ESRF) - Grenoble, France
- Dipartimento di Matematica e Fisica E. De Giorgi - Università del Salento, Lecce, Italy
- Institute of Nanotechnology (CNR Nanotec) - CNR, Lecce, Italy
- Institute of Experimental Neurology (INSPE) - IRCCS San Raffaele, Milan, Italy

THERAPEUTIC AREA

Neuroscience

BRIEF DESCRIPTION

Neurodegenerative diseases, which prevalence is expected to increase rapidly in the coming decades, represent a major challenge for medicine and public health. Unfortunately, current treatments are palliative and poorly effective at best, reflecting a poor understanding of the molecular basis of most of these diseases.

Although each neurodegenerative disease is characterized by specific clinical symptoms and unique pathological characteristics, they all share a very similar molecular mechanism, characterized by the progressive formation of protein aggregates. Specifically, a mutation or a stochastic event is able to destabilize a specific protein, different for each neurodegenerative disease, which loses its folding, and self-organizes into insoluble amyloid structures, which progressively accumulate inside the cell forming neurotoxic inclusions. The study of protein aggregation mechanisms is, therefore, essential to design molecules able to compete specifically for aggregation.

In this context, our research is focused on the study of the molecular mechanisms leading to protein aggregation and on the characterization of specific protein-ligand interactions. In particular, our work is currently aimed at the patho-physiological study of Ataxin-3 and TDP-43 proteins, involved in Spino-cerebellar ataxia type 3 (SCA3) and in Amyotrophic Lateral Sclerosis (ALS), respectively.

IMPACT

The lack of a deep understanding of the molecular basis of neurodegenerative diseases leads to inefficient and poorly specific therapies. The present research project addresses crucial questions in both structural and cellular biology, that are essential to get new insights into the understanding of the pathological mechanisms of these diseases. The knowledge provided may eventually help to approach the design of specific therapies and can hold clues to the very fundamental phenomenon of protein folding and assembly. The biophysical approach used, which involves the use of spectroscopy, calorimetry and interferometry techniques, represents a powerful tool for the aims of the project. In fact, biophysics and structural characterization studies of highly aggregation-prone proteins are useful to better define the molecular basis of this kind of disorders under conditions that are easier to manage, by the means of *in vitro* assays using highly pure recombinant proteins. The information obtained can be used both for the design of more specific and more effective anti-aggregating compounds, able to interfere with the pathological behavior of these proteins, and to validate innovative *in vitro* models capable of mimicking the complexity of *in vivo* systems.

RESULTS ACHIEVED IN 2021

As part of the Ataxin-3 project, we have produced forms progressively more pathological of the protein. We then performed measurements of the aggregation kinetics, by means of fluorescence spectroscopy, of three forms of Ataxin-3 with increasing expansion of the polyQ tract at the C-terminal, corresponding to an increasing degree of toxicity. The results obtained show the same aggregation features for the three forms of Ataxin-3, confirming that, under the tested experimental conditions, the catalytic domain of Ataxin-3 (Josephin) acts as a nucleation center in the aggregation process of the whole protein. A similar result was obtained for the three forms of Ataxin-3 in which the catalytic site was inactivated by insertion of the C14A point mutation. This allowed us to validate the mutants as models for the study of Ataxin-3 aggregation, both *in vitro* and in cell, in the presence of poly-Ubiquitin chains, a natural ataxin-3 ligand that would undergo the proteolytic action in presence of wild-type forms.

As part of the TDP-43 project, the two RNA recognition motifs, RRM1 and RRM2, were successfully produced. These were then used in preliminary biophysical studies aimed at establishing whether there is a specific interaction between these isolated motifs and epigallocatechin gallate (EGCG), a natural polyphenol that has already demonstrated, in studies carried out on other proteins with a high aggregation tendency, to possess an anti-aggregant activity.

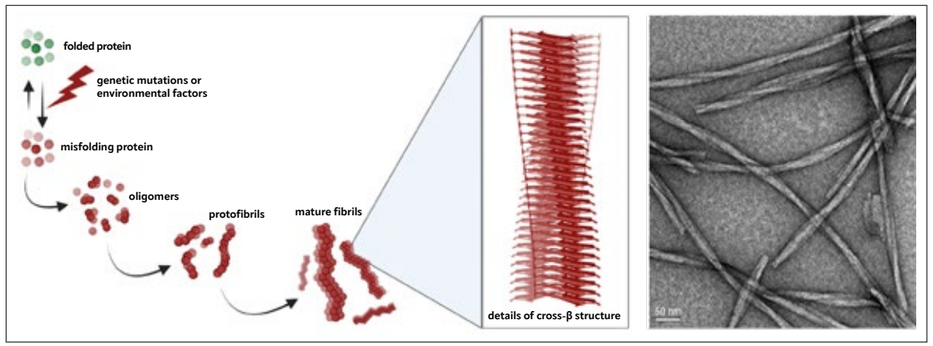
GOALS FOR 2022

Our goal is a better understanding of the role of protein-protein interactions in Ataxin-3 aggregation in order to use this information to design specific molecules with anti-aggregating properties. For this purpose, the kinetic and thermodynamic parameters of complexes Ataxin-3/poly-Ubiquitin chains will be determined, and it will also be evaluated the effect of the poly-Ubiquitin chains in Atx-3 aggregation. The results obtained from biophysical analysis, followed by in-cell studies, will constitute a fundamental step for the future design of specific peptide-mimetic compounds with anti-aggregating function.

As part of the TDP-43 project, *in vitro* studies will continue aimed at investigating the interaction between the different domains of TDP-43 and EGCG. Where the interaction is confirmed, they will be evaluated the anti-aggregating properties of the natural molecule on the individual TDP-43 domains, allowing to broaden our knowledge on the contribution of the individual domains in promoting or mitigating the aggregation phenomena of the whole protein. The data collected on the interaction and anti-aggregating effects of the natural molecule will guide a possible design of compounds deriving from EGCG capable of interfering more specifically with the aggregation process of TDP-43.

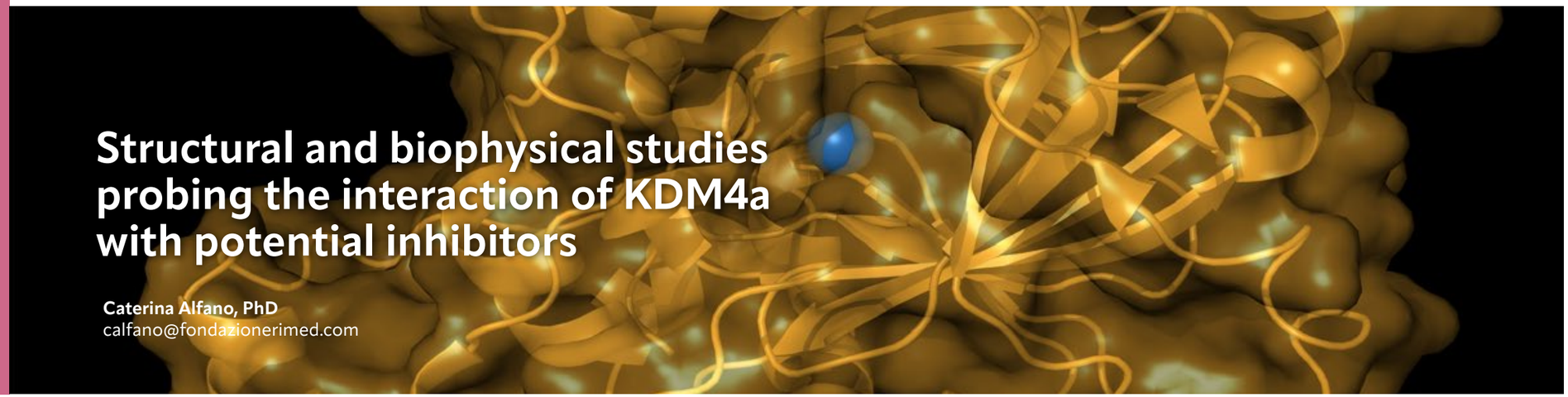
MEETINGS

- BraYn - 4th Research Assembly for Young Neuroscientists, October, 2021, Pisa (Italy)
- 5th e-Workshop CNR Nanotec, November, 2021, online



Representation of protein aggregation pathway. (Left) Neurodegenerative diseases-associated proteins loose their native structure and progressively aggregate, forming supramolecular insoluble assemblies [created by BioRender.com]. (Right) Image of amyloid-β mature fibrils captured by Transmission Electron Microscopy. *(Gras et al. 2011).*

PRODUCTS: **Drugs**



Structural and biophysical studies probing the interaction of KDM4a with potential inhibitors

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COLLABORATIONS

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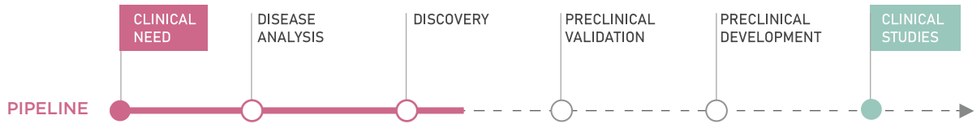
THERAPEUTIC AREA

Oncology

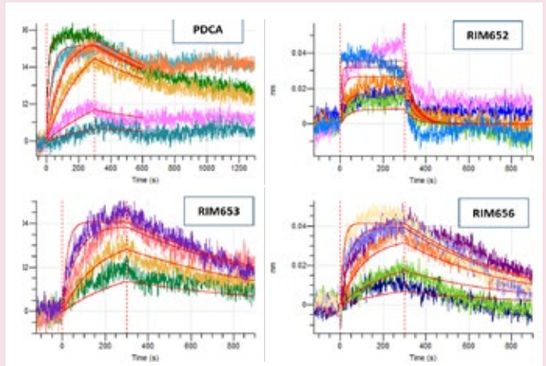


BRIEF DESCRIPTION

DNA and histones modifications are important components of epigenetic regulation and represent an essential process in controlling the structure and/or function of the chromatin, with different modifications yielding distinct functional consequences. DNA and histones post translational modifications include phosphorylation, acetylation, methylation, ubiquitination and O-GlcNAcylation. Among these, methylation is the most advanced epigenetic marker in the regulation of chromatin dynamics, and can affect both DNA and histones. Methylation of histones involves lysine and arginine residues, counting for three forms of methylation on lysine residues (monomethyl, dimethyl, and trimethyl), and three forms on arginine (monomethylated, symmetric and asymmetric demethylated). In the past, histones methylation modification was thought to be irreversible, but it is now well known that it can be reversed by histone lysine demethylase enzymes (KDMs), several of which are deregulated in cancer. Among KDMs, KDM4A is the only one able to demethylate trimethylated residues, and increasing evidence indicates that it plays a key role in tumor initiation, promotion, and progression. As part of the Drug Discovery Area of Ri.MED Foundation, we aim at identify lead compounds targeting KDM4a catalytic domain. In particular, we probed the interaction of KDM4A and several fragments and small molecules selected through virtual screening approach by the Ri.MED Molecular Informatics Group

IMPACT

Chromatin architecture is controlled by epigenetic mechanisms such as DNA and histones modifications. Aberrant alterations in the chromatin structure are common findings in tumors, and so several enzymes involved in DNA and histone modifications became important therapeutic targets in oncology. Among these targets, KDM4a has been reported to be deregulated in several cancer types, such as prostate, bladder, colorectal, squamous cell carcinoma, and lung and breast cancers. It is not surprising then there is a great interest in developing molecules able to modulate the demethylase activity of KDM4a. Several molecules targeting KDM4a have already been developed but most of them remain in the preclinical phase because they lack selectivity and specificity for KDM4a with respect other histone demethylases, so modulating other non-desirable targets.



Kinetic analysis by BLI of the interaction between KDM4a e molecules selected by virtual screening.

RESULTS ACHIEVED IN 2021

This project collects an interdisciplinary effort of several research groups in Ri.MED. As Structural Biology and Biophysics Group, we optimized the expression and purification of recombinant KDM4a catalytic domain, and performed stability studies by nuclear magnetic resonance and circular dichroism (Fig. 1). Bio-layer Interferometry technology was used to determine the equilibrium dissociation constant (KD) and the binding kinetic parameters K_{ass} and K_{diss} for fragments and small molecule inhibitors selected through virtual screening approach by the Ri.MED Molecular Informatics Group. Saturation-Transfer Difference (STD) NMR experiments have also been used as confirmation. Interestingly, few fragments showed both μM activity on KDM4 in the enzymatic assay, and μM value of the binding constant in the biophysical assay. The data were then validated by testing analogues compounds.

GOALS FOR 2022

Our goal is to complete a second screening campaign. If the latter will give molecules with potential inhibitory effect, our ambition is to give experimental structural information that can guide the optimization of drug design and synthesis so that to obtain a lead compound in a shorter time. Newly designed and synthesized molecules obtained by the Molecular Informatic and Medicinal Chemistry groups will be tested using biophysical techniques such as nuclear magnetic resonance and Bio-layer Interferometry. A deeper structural characterization will be performed for compounds that will show a nM affinity for KDM4.

MEETINGS

- CCPNMR Conference, August, 2021, online
- AIC School 2021 - Fundamentals of Crystallography: the theory behind the crystal structure solution. August, 2021, online;

PRODUCTS: **Biomarkers, Biologics**

The role of repetitive sequences in cellular senescence and aging

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THERAPEUTIC AREA

- **Aging diseases**
- **Neuroscience**

BRIEF DESCRIPTION

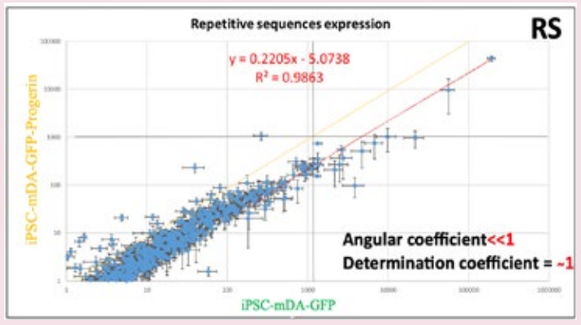
Repetitive DNA sequences (RS) represent about half of the human genome. Despite RS have been considered for long just "junk DNA", now it is clear that RS have a role in almost every aspect of human biology, especially in aging and aging related diseases, but also in cancer and development. The role of the RS has been often overlooked because RS are intrinsically difficult to study and dedicated experimental procedures and bioinformatics pipelines are needed; procedures and pipelines we are able to perform. Among many other roles in cell biology, RS seem to play a specific central role in triggering cellular senescence that in turn is one of the main factors driving the human aging process and progress. Cellular senescence is a state of permanent cell cycle arrest that induce a proinflammatory microenviroment in their surroundings, that is usually reset by the removal of senescent cells due to macrophage activity. During aging instead senescence cells accumulate, and in turn induce a chronic mild inflammatory state that is probably at the very root of many diseases of the elders, such as cardiovascular and degenerative diseases, but also tissue remodeling as fibrosis or even cancer. A better understanding of the crosstalk between RS and cellular senescence can shed light to novel biomarkers, therapeutic targets and clinical approaches for the treatment of aging and aging related diseases, and over.

IMPACT

A plethora of evidences are accumulating in suggesting that RS play a central role in aging and aging related diseases. In particular, RS at the telomeres are strictly interlaced with the replicative senescence and at centromeric level with chromosomal stability. It is known that RS are under a strict epigenetic control mechanism based on CpG DNA methylation. During aging, this epigenetic landscape is subverted, and the sequence homologies of RS became a hub for homology directed recombination thus causing in turn genomic instability, DNA damage, senescence and even cancer transformation. Moreover, RS usually carry regulatory sequences, thus their deregulation, and/or mobilization, can alter the transcriptional profile of the cell. RS activation has been suggested as a general feature of aging cells that acquire the senescent phenotype and recently it has been proved that RS repression in senescent cells triggers the inflammation associated with aging (inflammaging) via the cytosolic DNA sensing pathway (cGAS-STING), a component of the innate immune system against virus infection. By all these reasons, RS are relevant potential targets for the treatment of age-associated disorders.

RESULTS ACHIEVED IN 2021

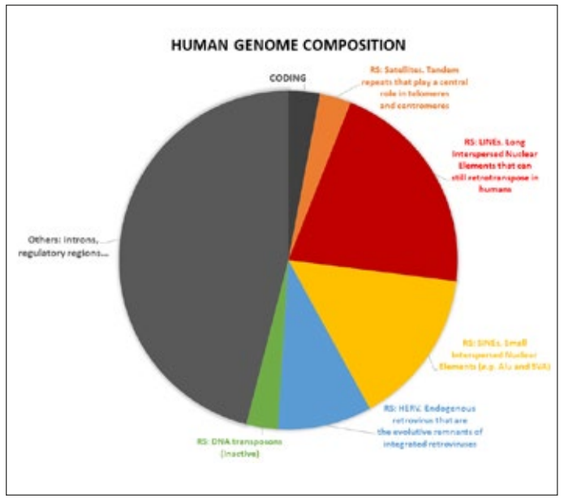
Alteration of the cellular homeostasis caused by RS can be mediated not only by their epigenetic state at chromatin level but also by their transcriptional activity. Even if RS were considered almost transcriptional silent, it is now evident that they are actively transcribed, and that their transcription is strictly regulated and have a large impact in the biology of the cell. The pipeline to analyze the transcriptional output of RS in Next Generation Sequencing transcriptome experiments has been refined and tested in different settings than the original one (iPSC-derived dopaminergic neurons). The studies on the Hutchinson-Gilford Progeria Syndrome have continued in parallel with studies on the molecular characterization of cellular senescence and possible therapeutic targets and novel pharmacological approaches. A grant application, namely 'Repetitive DNA sequences and the role of their transcripts in the Hutchinson-Gilford Progeria Syndrome' has been filed and submitted to Cariplo Telethon Alliance GJC2021 for its evaluation.



Progerin expression induces a significant downregulation of transcription from human repetitive sequences in iPSC-derived dopaminergic neurons.

GOALS FOR 2022

We aim to apply the pipeline we developed to analyze RS transcription in a panel of published Next Generation Sequencing transcriptomes. In details, in order to better understand the role of RS, we aim to analyze a fibroblast model of Hutchinson-Gilford Progeria Syndrome to evaluate the contribute of RS in an aging model different from a neuronal setting as we did before, but we want also to extend the analysis on specimens of non-aging related diseases such as breast cancer and autism. In the latter, our preliminary data are very promising. More and more evidences suggest that the role of RS is peculiar and differs from tissue to tissue, e.g., RS seem to have a physiological role in the development of brain while they possess a pathological effect when express in the endothelium and in many forms of cancer.



About half of the human genome is composed by Repetitive DNA Sequences (RS). RS have a central role in human biology, but are probably the part of the human genome that has been less studied and understood.

PRODUCTS: **Biomarkers**

Role of inflammasomes in cigarette-smoke associated lung inflammation

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COLLABORATIONS

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- Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IRCCS ISMETT), Palermo, Italy
- Institut de la Vision, Paris, France
- Ospedale Civico Di Cristina Benfratelli, Palermo, Italy
- Department of Engineering, University of Palermo, Palermo, Italy
- Department of Biomedicine, Neuroscience and Advanced Diagnostics, (BIND) University of Palermo, Palermo, Italy

THERAPEUTIC AREA

Aging diseases

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

Cigarette smoke (CS) is a major risk factor for chronic inflammatory airways diseases such as chronic obstructive pulmonary disease (COPD). Smoke exposure causes oxidative stress and tissue damage leading to lung epithelial barrier dysfunction, airways remodeling and impairment of innate immune responses thus increasing the risk of infection. Recent drug discovery efforts have focused on targeting inflammatory pathways. Recently, inflammasomes have emerged as immune regulators potentially involved in CS-associated lung diseases. Inflammasomes are multiprotein complexes composed by a receptor protein, an adaptor protein, known as ASC, and pro-caspase-1. Activation of the inflammasome complex promotes autocatalytic cleavage of caspase-1, which in turn cleaves pro-IL-1β, pro-IL-18 and gasdermin D (GSDMD).

Upon cleavage, GSDMD forms pores on cell membranes, allowing the release of mature cytokines and increasing cell permeability eventually leading to a lytic pro-inflammatory form of cell death known as pyroptosis.
We have recently reported that CS inhibits the NLRP3 inflammasome in human macrophages while promoting NLRP3-independent caspase-1 activation via the TLR4-TRIF-caspase-8 axis. In the presence of bacterial lipopolysaccharide (LPS), CS shifts the inflammatory response from MyD88-NF-κB axis (inhibited by CS) to TRIF-caspase-8 enhancing caspase-1 activation and leading to imbalanced immune responses. During 2021, we have evaluated the impact of CS on GSDMD and downstream events in human primary macrophages, lung macrophages and lung structural cells.

IMPACT

The project will contribute to the discovery and characterization of the role of inflammasomes, non-apoptotic caspases and gasdermin D in the pathogenesis of cigarette smoke-associated lung diseases. By expanding our investigation beyond immune cells, we will unveil whether inflammasome-related responses occur in lung structural cells, including bronchial epithelial cells and fibroblasts, and contribute to chronic inflammation, tissue damage and lung remodeling. All this work aims at identifying new potential therapeutic targets for the treatment of CS-associated lung diseases.
Of note, the impact of this work goes beyond the specific context of lung diseases. In fact, inflammasome activation contributes to the pathogenesis of several chronic diseases including atherosclerosis, type II diabetes, and neurodegenerative diseases. Therefore, findings derived from our current work will have a broader impact in different fields of research.

RESULTS ACHIEVED IN 2021

In 2021 we have investigated the impact of CS on GSDMD cleavage in human monocyte-derived macrophages (hMDMs) and in distal lung tissue sections from smokers and non-smoking controls. In hMDMs, we found that CSE±LPS increased cleaved GSDMD and cell permeability, in absence of cell death (Figure 1). Of note, GSDMD cleavage did not occur in response to LPS, while it was strongly enhanced upon stimulation with LPS+CSE, suggesting a synergistic effect between the two stimuli. We found that CSE promoted LPS internalization leading to caspase-4 activation, thus contributing to increased GSDMD cleavage. Caspase-dependent GSDMD cleavage was associated with mitochondrial damage. Increased cleaved GSDMD was found in lung macrophages of smokers compared to non-smoking controls (Figure 2). Our findings revealed that activation of caspase-1, -4, and -8 and GSDMD cleavage upon exposure to CS may promote lung macrophage dysfunction and contribute to the chronic and unresolved inflammation observed in the smokers' lung.

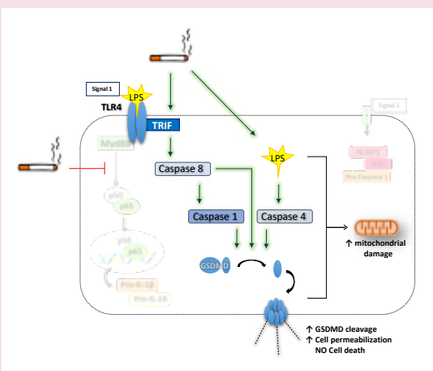

Figure 1. Proposed model. CS alters the inflammatory response to LPS: the TLR4-MyD88 pathway is inhibited and no NLRP3 inflammasome activation occurs. Activation of caspase-8, -1 and -4 occurs and leads to GSDMD cleavage, increasing cell permeability with no cell death. Activation of caspases and GSDMD leads to mitochondrial damage.

Furthermore, during 2021, we have successfully set-up several advanced experimental models to study lung diseases in collaboration with IRIB-CNR, University of Palermo and IRCCS-ISMETT. We have established protocols for isolation and culturing primary bronchial epithelial cells, primary fibroblasts and alveolar macrophages from fresh lung tissue samples. We are currently studying the role of inflammasomes in monolayer cultures and in 3D air-liquid interface (ALI) models exposed to CS.

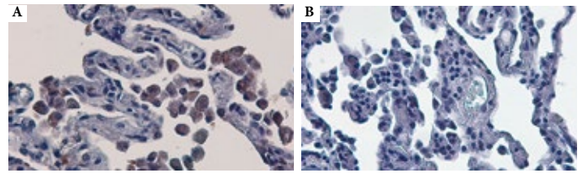

Figure 2. Cleaved GSDMD (immunohistochemistry staining) in distal lung tissue sections from Smokers (A) and Non-Smokers (B).

GOALS FOR 2022

During 2022, we will investigate the impact of long-term exposure to CS on cell death during bacterial infections. In particular, we will test the hypothesis that exposure to CS, in addition to dampen protective inflammatory responses, predisposes macrophages to pro-inflammatory cell death during bacterial infections. And this may sustain chronic inflammation and favors the increased risk of infection observed in smoking subjects.
Furthermore, we will investigate the impact of viral infection (using poly(I:C) as stimulation model) on inflammasome activation and gasdermin cleavage.
Finally, we will evaluate the impact of CS exposure on lung fibroblasts (using MRC5 cell line as well as primary fibroblasts) and primary bronchial epithelial cells grown in monolayer as well as in 3D ALI models with a focus on inflammasome-related responses.

MEETINGS

- *Cigarette smoke induces ASC-independent and caspase-dependent activation of Gasdermin D in human macrophages.* Cristaldi M, Buscetta M, Cimino M, Amato S, Aronica TS, Cipollina C. EMBO Workshop The inflammasomes: The next frontier 21 – 24 September 2021, Martinsried, Germany.
- *Development of a screening strategy for the identification of NLRP3 selective inhibitory compounds.* Dino P, Buscetta M, Zito G, Bucchieri F, Cipollina C. 93° Congresso Nazionale Società Italiana di Biologia Sperimentale (SIBS), 22-25 Aprile 2021 Palermo, Italy.

PUBLICATIONS

- Di Vincenzo S, Sangiorgi C, Ferraro M, **Buscetta M**, **Cipollina C**, Pace E (2021) Cigarette smoke extract reduces FOXO3a promoting tumor progression and cell migration in lung cancer. Toxicology, 454:152751. DOI: 10.1016/j.tox.2021.152751.
- Chiappara G, Di Vincenzo S, Sangiorgi C, Di Sano C, D'Anna C, Zito G, **Cipollina C**, Vitulo P, Bertani A, Pace E. (2022) Cigarette smoke upregulates Notch-1 signaling pathway and promotes lung adenocarcinoma progression. Toxicol Lett. 355:31-40. DOI: 10.1016/j.toxlet.2021.11.002.

PRODUCTS: **Biomarkers, Medical Devices & Tissue Engineering**

Development of AI Algorithms for Extraction and Selection of Radiomics Features from Biomedical Imaging

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THERAPEUTIC AREA

Oncology

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

Radiomics is a new frontier of medicine based on the extraction of quantitative data from radiological images which can not be seen by radiologist's naked eye and on the use of these data for the creation of clinical decision support systems. The long-term goal of radiomics is to improve the non-invasive diagnosis of focal and diffuse diseases of different organs by understanding links between extracted quantitative imaging data and the underlying molecular and pathological characteristics of lesions. In the last decade, several studies have highlighted the enormous potential of radiomics in both tumoral and non-tumoral diseases of many organs and systems including brain, lung, breast, gastrointestinal and genitourinary tracts. The enormous potential of radiomics needs to be pursued with the methodological rigor of scientific research and by integrating radiological data with other

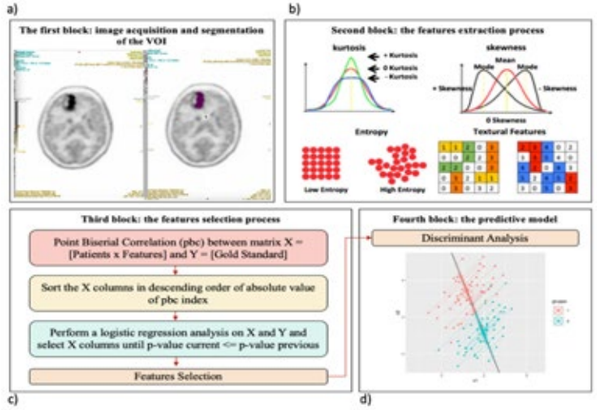
Figure 1. The radiomics workflow of the study. (a) PET image with lesion segmentation based on the thresholding method (see Section 2.1). (b) Some examples of the 44 features extracted using LifeX software, such as kurtosis, skewness, entropy and two textural features in the form of matrix (see Section 2.2), (c) Feature selection using a mixed descriptive-inferential sequential approach (see Section 2.2.3), (d) Representative figure of biplanar feature classification (low and high tumour grade) were used for training purpose. Successively, the classifier was used to predict the label in the test set, where instances were without corresponding labels.

IMPACT

Radiomics is utilized to improve the prediction of patient overall survival and/or outcome, identifying personalized predictive and/or prognostic models to support the medical decision process. Target segmentation, feature extraction, feature selection, and classification model are the fundamental blocks of a radiomics workflow. The good diagnostic performance of the combination of multiple radiomic features for the diagnosis of cancer may help predicting lesions where aggressive management may be warranted. The project proposes a novel radiomics workflow to identify a relevant prognostic model concerning a real clinical problem. In the specific, we propose an operator-independent segmentation system with the consequent automatic extraction of radiomics features, and a novel feature selection approach to create a relevant predictive model in patients underwent different imaging methods like magnetic resonance, computer tomography and positron emission tomography.

RESULTS ACHIEVED IN 2021

The project contributed to:
-Develop a novel method for feature selection in radiomics based on a correlation matrix and point-biserial-correlation coefficient for features reduction and selection, and Discriminant analysis (DA) use for features classification in a whole sample and sub-groups for primary tumor or local relapse (T), nodal disease (N), and metastatic disease (M);
-Investigate the potential application of texture analysis of Cho-PET/CT images in prostate cancer and to propose a system incorporating a new machine-learning radiomics model to select PET imaging features able to predict disease progression in prostate cancer (PCa) in patients with same class of risk at re-staging;
-Observe the variability of positron emission tomography (PET) radiomics features under the impact of co-registration with magnetic resonance imaging (MRI) using the difference percentage coefficient, and the Spearman's correlation coefficient for three groups of images: (i) original PET, (ii) PET after co-registration with T1-weighted MRI and (iii) PET after co-registration with FLAIR MRI;
-Determine the diagnostic performance of texture analysis of prostate MRI for the diagnosis of prostate cancer among Prostate Imaging Reporting and Data System (PI-RADS) 3 lesions.



GOALS FOR 2022

During the 2022, we aim to develop a new 3D radiomics matrix to discover new prognostic indices to perform an early assessment of treatment response to therapy using imaging methods. this new 3D radiomic matrix will have to characterize tumor lesions from the healthy tissue. we will rely on the experience we have gained in the segmentation of lesions and on the extraction, selection and reduction of radiomic descriptors for tissue classification and diagnosis support. This matrix will be completely 3D and will take on a spherical shape to make it totally unchanged in spatial positioning. we will use our previous works to compare and test the new matrix on datasets already used with state-of-the-art radiomic matrices. Our goal will be to generate a new radiomics matrix that takes the advantages of 3D for the extraction and combination of features capable of supporting precision medical diagnosis.

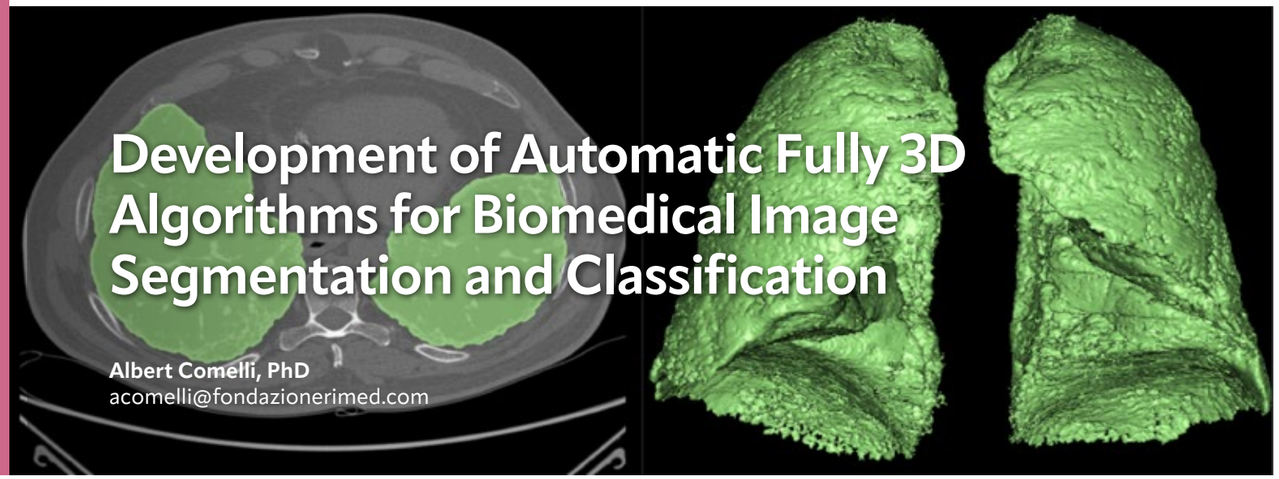
MEETINGS

24rd Conference on Medical Image Understanding and Analysis, July, 2020, Oxford (UK).

PUBLICATIONS

- G. Russo, A. Stefano, P. Alongi, **A. Comelli,** B. Catalfamo, C. Mantarro, C. Longo, R. Altieri, F. Certo, S. Cosentino, MG. Sabini, S. Richiusa, GMV. Barbagallo, and M.Ippolito. Feasibility on the Use of Radiomics Features of 11[C]-MET PET/CT in Central Nervous System Tumours: Preliminary Results on Potential Grading Discrimination Using a Machine Learning Model. Current Oncology. 2021; 28(6):5318-5331. DOI 10.3390/curroncol28060444.
- F. Vernuccio, F. Arnone, R. Cannella, B. Verro, **A. Comelli**, F. Agnello, A. Stefano, R. Gargano, V. Rodolico, G. Salvaggio, R. Lagalla, M. Midiri and A. Lo Casto. Diagnostic performance of qualitative and radiomics approach to parotid gland tumors: which is the added benefit of texture analysis?. The British Journal of Radiology, 94, 20210340. DOI 10.1259/bjr.20210340.
- S. Barone, R. Cannella, **A. Comelli,** A. Pellegrino, G. Salvaggio, A. Stefano and F. Vernuccio. Hybrid descriptive-inferential method for key feature selection in prostate cancer radiomics. Applied Stochastic Models in Business and Industry, 37(5), 961-972. DOI 10.1002/asmb.2642.
- G. Cutaia, G. La Tona, **A. Comelli**, F. Vernuccio, F. Agnello, C. Gagliardo, L. Salvaggio, N. Quartuccio, L. Sturiale, A. Stefano, M. Calamia, G. Arnone, M. Midiri, G. Salvaggio. Radiomics and Prostate MRI: Current Role and Future Applications. Journal of Imaging. 2021; 7(2):34. DOI 10.3390/jimaging7020034.
- A. Stefano, A. Leal, S. Richiusa, P.T rang, **A. Comelli, V. Benfante**, S. Cosentino, MG. Sabini, A .Tuttolomondo, R.Altieri, F.Certo, GMV.Barbagallo, M.Ippolito, G.Russo. Robustness of PET Radiomics Features: Impact of Co-Registration with MRI. Applied Sciences. 2021; 11(21):10170. DOI 10.3390/app112110170.
- P. Alongi, A. Stefano, **A. Comelli**, **R. Laudicella**, S. Scalisi, G. Arnone, S. Barone, M.Spada, P. Purpura, T. V. Bartolotta, M. Midiri, R. Lagalla and G. Russo. Radiomics analysis of 18F-Choline PET/CT in the prediction of disease outcome in high-risk prostate cancer: an explorative study on machine learning feature classification in 94 patients. Eur Radiol (2021). DOI 10.1007/s00330-020-07617-8.

PRODUCTS: **Biomarkers, Medical Devices & Tissue Engineering**



Development of Automatic Fully 3D Algorithms for Biomedical Image Segmentation and Classification

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COLLABORATIONS

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- Georgia Institute of Technology, (GIT), Atlanta, USA
- Nuclear Medicine Unit, University of Messina, Messina, Italy
- Unit of Nuclear Medicine Fondazione Istituto G.Giglio, Cefalù, Italy
- Department of Engineering, University of Palermo, Palermo, Italy
- Medical Physics Unit, Cannizzaro Hospital, Catania, Italy
- Nuclear Medicine Department, Cannizzaro Hospital, Catania, Italy
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THERAPEUTIC AREA

Oncology

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

In the field of biomedical imaging, target segmentation is routinely used as the first step in any automatized disease diagnosis system (i.e. radiotherapy system) and, in the last few years, in radiomics studies to obtain great volumes of quantitative data from medical images. These data are then used as imaging biomarkers to identify any possible associations with patient outcome. The first step of a radiomics workflow is the target (e.g. tumor or organ) delineation in such a way as to avoid distortions in parameter extraction. Although manual delineation seems like the most intuitive and easily implemented way of obtaining target volume, it is a time consuming process and it is subject to the greatest inter- and intra-observer variability. This variability causes irreproducible results in the radiomics signature that is highly influenced by the region of interest drawn to identify the tumour. For this reason, an automatic and operator-independent target delineation method is mandatory.

Figure 1. Examples of correct segmentation for the deep learning networks for the whole gland (left column), transition (middle column)
and peripheral zone (right column). The manual segmentation (yellow), ENet (red), ERFNet (blue), and U-Net (green) are superimposed.

Figure 2. CT images showing parenchyma with visible subregions (yellow for the gold **standard, red for C-ENET, green for ENET, and blue for ERFNET,) infected with COVID-19 disease.**

IMPACT

The segmentation process remains a popular and challenging area of research. Nowadays clinical activity places a high level of demand on segmentation algorithms, which are required to produce repeatable results, to be independent by the choices performed by the user and capable of processing in real-time. The project proposes a segmentation system specifically engineered to reach the maximum level of automation and capable of obtaining an operator independent segmentation. In the specific, we propose two methodology:
- A fully 3D active surface (AS) driven by a 3D machine learning component (i.e., 3D tissue classification) for segmentation of the tumours in brain districts in PET;
- A deep learning framework for segmentation of the whole-gland and zonal prostate, of the aneurysmal aorta and its valve but also parenchyma with idiopathic pulmonary fibrosis that provide accurate and fast segmentation results after being trained with a small image dataset of High-Resolution Computerized Tomography.

RESULTS ACHIEVED IN 2021

The project contributed to:
-Develop a customized deep learning approach aimed at addressing the real-time, and fully automated identification and segmentation of COVID-19 infected regions in computed tomography images;
-Design a deep-learning approaches whose aim is to tackle the fully-automated, real-time, and 3D delineation process of the prostate whole-gland, central zone + anterior stroma + transition zone (TZ), and peripheral zone (PZ) segmentation on T2-weighted MRI;
-Demonstrate that deep learning models can rapidly segment and quantify the 3D geometry of ascending thoracic aortic aneurysm (ATAAs) with high accuracy, thereby facilitating the expansion into clinical workflow of personalized approach to the management of patients with ATAAs;
- Design efficient and operator-independent segmentation algorithms capable of reconstructing the brain lesion three-dimensional (3D) shape for accurate diagnosis and radiotherapy treatment planning.

Figura 1

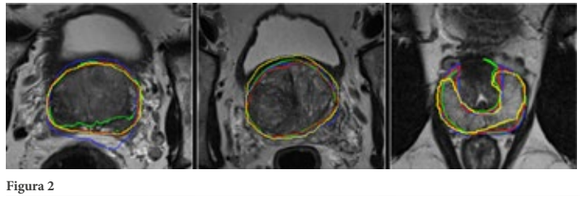
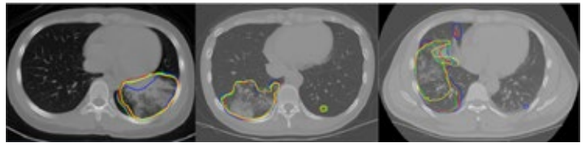


Figura 2



GOALS FOR 2022

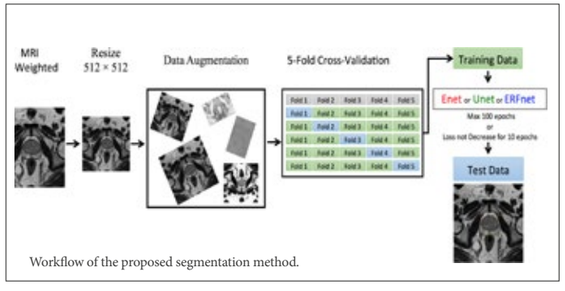
In 2022 the main goal will be to develop efficient and operator-independent segmentation methods capable of reconstructing the 3D lesion shape. These new methods will be inspired by local active contour segmentation algorithms guided by artificial intelligence algorithms such as convolution neaural networks. A deep learning model, trained by examples provided by expert human operators, is capable of labeling the tissues into two or more categories like: lesion/organ and background. This method is consistent and fast to reproduce the same result every time and incorporating some of the trainer's wisdom in the process of driving the local active contour segmentation process. Our aim will be to hybridize the best of the two methods to obtain an automatic segmentation algorithm, operator independent and acquisition method independent.

MEETINGS

25rd International Conference on Pattern Recognition, January, 2021, Milano (IT)

PUBLICATIONS

- **A. Comelli,** N. Dahiya, A. Stefano, F. Vernuccio, M. Portoghese, G. Cutaia, A. Bruno, G. Salvaggio, A. Yezzi. Deep Learning-Based Methods for Prostate Segmentation in Magnetic Resonance Imaging. Applied Sciences. 2021; 11(2):782. DOI 10.3390/app11020782.
- **R. Laudicella, A. Comelli,** A. Stefano, MSzostek, L. Crocè, A. Vento, A. Spataro, A. Comis, F. La Torre, M. Gaeta, S. Baldari and P. Alongi. Artificial neural networks in cardiovascular diseases and its potential for clinical application in molecular imaging. Current Radiopharmaceuticals, 14(3), 209-219. DOI 10.2174/1874471013666200621191259.
- A. Stefano, **A. Comelli.** Customized Efficient Neural Network for COVID-19 Infected Region Identification in CT Images. Journal of Imaging. 2021; 7(8):131. DOI 10.3390/jimaging7080131.
- R. Cuocolo, **A. Comelli,** A.Stefano, **V. Benfante,** N. Dahiya, A. Stanzione, A. Castaldo, D.R.De Lucia, A. Yezzi and M. Imbriaco. Deep learning whole-gland and zonal prostate segmentation on a public MRI dataset. Journal of Magnetic Resonance Imaging. 2021. DOI 10.1002/jmri.27585.
- **A. Comelli** and A. Stefano. Active Surface for Fully 3D Automatic Segmentation. In: Del Bimbo A. et al. (eds) Pattern Recognition. ICPR International Workshops and Challenges. ICPR 2021. Lecture Notes in Computer Science, vol 12661. Springer, Cham. DOI: 10.1007/978-3-030-68763-2_27
- **R. Laudicella,** A. Iagaru, **A. Comelli,** S. Baldari, I.A. Burger. Artificial Intelligence Clinical Application in Prostate Cancer Molecular Imaging. Hematology, 1(7), 28-35. DOI 10.36000/hbT.OH.2021.07.028.



Workflow of the proposed segmentation method.

PRODUCTS: **Biomarkers, Biologics**

Modeling microRNA-target interaction network

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COLLABORATIONS

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- Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) – University of Palermo, Palermo, Italy
- Dipartimento di Scienze Economiche, Aziendali e Statistiche (SEAS) – University of Palermo, Palermo, Italy
- Department of Computational and Systems Biology (CSB) – University of Pittsburgh, Pittsburgh (PA), US

THERAPEUTIC AREA

- Oncology
- Infectious diseases

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

MicroRNA are short RNA molecules with an important role in post-transcriptional regulation of the gene expression. By now, approximately 2.000 microRNA have been identified, and each of them can regulate the expression of thousands of mRNA targets. Since the human genomes counts for approximately 20.000 mRNAs, we have to unravel a tight and complex biological interaction network, called mirnome. In addition, the scenario is complicated by the fact that each cellular tissue is characterized by a specific gene expression profile. As a consequence, the actual interaction network is tissue specific. In this project, we aim to model any tissue specific interaction network, focusing our studies on cancer tissues, in order to detect the anomalies in the interaction network with respect to the normal tissues' behavior.
MicroRNA and mRNA expression profiles necessary to model the tissue specific interaction network can be obtained by high throughput data analysis techniques, based on microarray or NGS technologies.
These technologies provide quantitative information about all microRNAs and mRNAs endogenously expressed in the analyzed tissue. It is our aim to develop algorithms to model and compare the microRNA-target interaction network of tissues in different conditions.

IMPACT

Biological Big Data repositories are rapidly growing, partly due to the fact that in order to publish results in the most important journals, it is mandatory to make available to the public the original data used to obtain the results described on the paper. When data archives for gene expression profiles are needed, researchers use data repositories as Gene Expression Omnibus or ArrayExpress. As a consequence, if a researcher is interested in specific cellular tissues, it is highly probable that such data repositories contain a huge collection of suitable set of gene expression profiles. This kind of data contains the information of the expression of the entire genome in the tissues of interest and it is generally useful to perform the initial screening to decide on which features focus the research. In the face of a huge amount of available data, what is missing is data analysis algorithms useful to integrate many sources of biological big data. While it is common practice to detect differentially expressed microRNAs or mRNAs among two different tissue conditions in order to detect anomalies in the expression profiles, it doesn't still exist an established method to detect which of these anomalies affect the interactions among microRNAs and mRNAs. We aim to develop such methods, in order to bring new instruments useful to understand cancer causes, moving from asking "which genes are involved" to the more functional question "which interaction are affected".

RESULTS ACHIEVED IN 2021

In 2021 we focused our studies on two proteins involved in the RISC complex, i.e. AGO2 and GW182. In collaboration with IRIB-CNR and STEBICEF-UNIPA, we collected a dedicated gene expression profiles database. Specifically, we collected data by analyzing two cell lines, i.e., MCF7 and MDA. In both cases, we performed the following experimental procedures in triplicates: 1) AGO2 protein immunoprecipitation, 2) AGO2 protein silencing, followed by GW182 protein immunoprecipitation, 3) GW182 protein immunoprecipitation and 4) GW182 protein silencing, followed by AGO2 protein immunoprecipitation. All the samples have been analyzed with whole genome microarray chip (Agilent technology) in order to identify and quantify the precipitated RNA specimens. We are currently analyzing the data collected so far. On the other end, in collaboration with IRCSS ISMETT, we are optimizing experimental protocols devoted to *in vitro* validation of microRNA-mRNA target interactions, through luciferase-based assays.

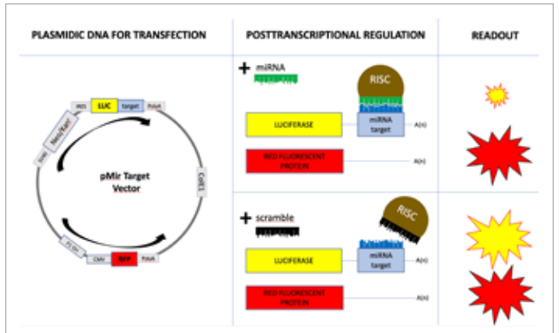

Fig. 1: The transfection of an appropriate plasmid in cultured human cells allows to detect the posttrascitional regulation carried out by the RISC complex by evaluating the downregulation of the activity of the luciferase whose mRNA carries the 3 UTR of the gene of interest during cotransfection experiments with suitable miRNAs previously selected in silico. Normalization is carried out via a reporter gene encoded by the same plasmid but insensitive to the same post-transcriptional regulation.

GOALS FOR 2022

In 2022 we aim to develop an algorithm useful to construct the interaction network of microRNA and mRNA. Each microRNA-mRNA pair will be associated with a p-value based on the correlation between their expression profiles. The main difficulty is due to the fact that currently it is impossible to simultaneously detect and validate all the interactions between microRNAs and mRNAs occurring in a specific tissue. As a consequence, the validation of a predicted network is not straightforward. In order to continue with the algorithm validation, we will include the interaction network obtained with our algorithm as new input to ComiR, a microRNA target prediction tool we aim to upgrade. Up to now, ComiR uses as input the microRNA expression profile and predicts their targets. The new version of ComiR will use the messenger RNA expression profile too, by computing the microRNA – target interaction network with our algorithm. We expect to improve the target prediction of the original version of ComiR, first because we will focus on the genes actually expressed in the examined tissue. Secondly, also the microRNA - mRNA interactions will be limited to the ones predicted as functional by our algorithm, and we aim to validate its efficiency by proving an additional increase of the performance in detecting microRNA targets.

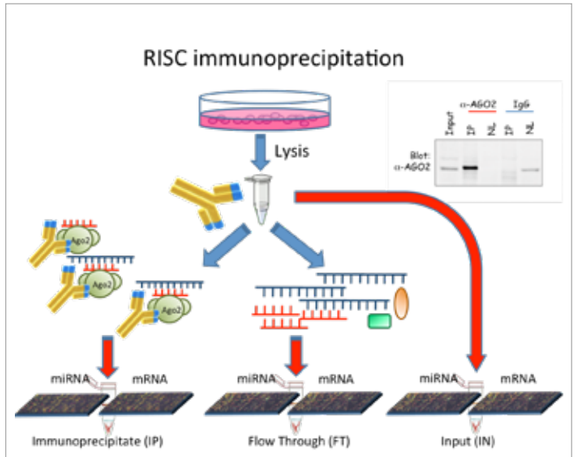

Fig. 2: : RIP-Chip Experimental design. The cell lysate is divided in two samples through the immunoprecipitation of a RISC protein. The RNA of three samples is extracted and analyzed with microarray technology. The three samples are the total cell lysate (IN), the immunoprecipitated sample (IP) and the flow through (FT).

PRODUCTS: **Drugs**

Design and synthesis of novel potential KDM4a inhibitors

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COLLABORATIONS

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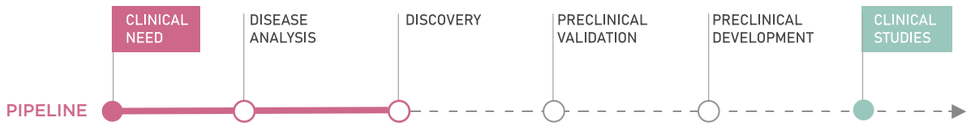
THERAPEUTIC AREA

Oncology



BRIEF DESCRIPTION

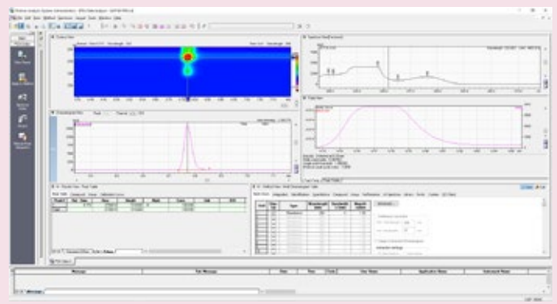
The discovery of new, more effective and selective drugs is a challenging, time and money consuming process. The early drug discovery phase needs the interplay of several expertise and joint skills. In Ri.MED, we implemented an inter-disciplinary Drug Discovery area aimed at identifying new potential active ingredients. Recently, a first screening campaign was completed for the target KDM4a. This enzyme belongs to the 2-oxoglutarate-dependent JmjC subfamily (KDM2-7) and catalyzes the removal of di- and trimethyl marks from H3K9 and H3K36 histones. DNA and histones modifications are important components of the epigenetic regulation representing essential events in controlling the chromatin structure and function; methylation is indeed the most advanced epigenetic marker. Thus, KDM4a is an appealing target and has been found frequently over-expressed in several human cancers, such as, ovarian and colon carcinoma. The virtual fragment screening campaign led to the identification of a preliminary active (IC50 of 7.1 μM) targeting the KDM4a catalytic domain. This primary hit was used as the starting point for the design and synthesis of novel chemotypes. In detail, the carboxypyridine core was included in bicyclic scaffolds using a bioisosteric approach and aiming at identifying innovative and more potent hit compounds.

IMPACT

Aberrant alterations in the chromatin structure and its disfunction are common causes in tumors, and several enzymes involved in DNA and histone modifications, such as, acetylation, methylation, demethylation, and ubiquitination have been validated as therapeutic targets in oncology. Of note, drugs targeting epigenetic enzymes can act preventing the cancer progenitor cells formation, as well as killing the cancer cells usually resistant to other classical therapeutic treatments. KDM4a deregulation has been reported for several cancer types, such as prostate, bladder, colorectal, squamous cell carcinoma, lung and breast cancers. There is indeed a great interest for the scientific community in developing small molecule drugs able to inhibit the demethylase activity of KDM4a. Several small molecules targeting KDM4a have already been developed but none of them managed to reach the market as they failed the clinical trials due to lack of selectivity and specificity. To date, there is no drug available in the market targeting KDM4a.

RESULTS ACHIEVED IN 2021

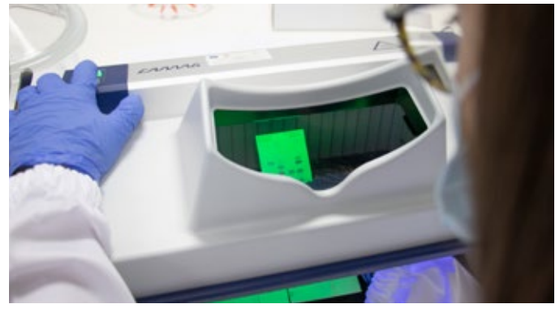
During the year 2021, we mainly focussed on the rational design of innovative chemotypes starting from the carboxypyridine structure of the fragment that emerged as a preliminary hit compound from the screening campaign. In detail, three novel scaffolds were designed, namely, i) the tetrahydrocarbolines, ii) the naphthyridinones and iii) the pyrazole-pyridazines. For each class, a list of hundred close analogues was provided, and a common short and versatile synthetic route was planned. A selection of analogues for each scaffold was studied *in silico* in collaboration with the Molecular Informatics group. The idea was to explore whether the new structures could reproduce the binding mode of the lead compound QC6352; or if they somehow could occupy a hydrophobic pocket identified within the catalytic active site, in order to obtain allosteric inhibitors which are different from the classical competitive inhibitors oxochetoglutarate analogues. For this purpose, five pdbs of the KDM4a protein in complex with known inhibitors were used for the docking studies. The synthesis activities were organized on the basis of the compound prioritization given by the computational study. In particular, we focused on the study and optimization of the synthetic route of tatrahydro-carbolines.



UHPLC outcome of the analytical run of a reaction mixture sample for monitoring and verification of the expected product molecular ion.

GOALS FOR 2022

During the next year, we aim at completing the optimization of the synthetic routes for the three chemotypes. The synthesis activities foresee, for each step, an evaluation of the reactivity of the reaction intermediates and of the best reaction conditions, in order to obtain the expected products with the highest yields and using as much green conditions as possible. For each newly synthesized intermediate, a complete analytical characterization will be performed through NMR spectroscopy techniques (1H and 13C) and mass spectrometry. The title compounds that will be biologically evaluated, will be purified in high resolution liquid chromatography, in order to obtain them with a purity greater than 96%. The goal is to complete the synthesis of a mini series of compounds representative of each chemotype, and then teste them *in vitro*, in the primary enzymatic assay, by the HTS group. The biological screening outcomes will be essential for identifying potential hits. The structural optimization of the most promising molecules will follow and through an in-depth study of structure-activity relationships (SAR) we will explore the chemical space of biologically interesting compounds.



PUBLICATIONS

- **Mekni, N.; Coronnello, C.**; Langer, T.; **De Rosa, M.; Perricone, U.** (2021). Support Vector Machine as a Supervised Learning for the Prioritization of Novel Potential SARS-CoV-2 Main Protease Inhibitors. Int. J. Mol. Sci., 22, 7714 (1-20). doi.org/10.3390
- Sharma, A.; **De Rosa, M.**; Singla, N.; Singh, G.; Barnwal, R. P.; Pandey, A. (2021). Tuberculosis: An Overview of the Immunogenic Response, Disease Progression, and Medicinal Chemistry Efforts in the Last Decade toward the Development of Potential Drugs for Extensively Drug-Resistant Tuberculosis Strains. J. Med. Chem. 64(8), 4359–4395. doi.org/10.1021/acs.jmedchem.0c01833

PRODUCTS: **Drugs**

Design and synthesis of novel potential NLRP3 inflammasome inhibitors

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COLLABORATIONS

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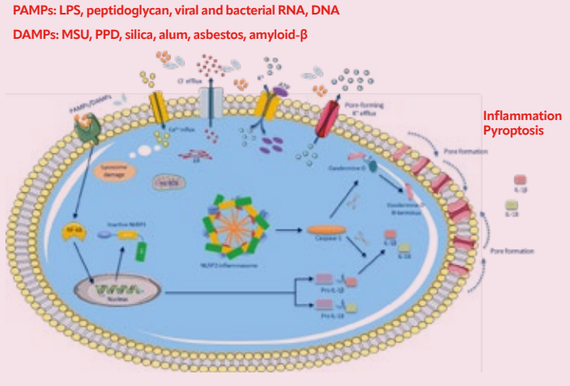
THERAPEUTIC AREA

Aging diseases

BRIEF DESCRIPTION

The NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome is a cytosolic multiprotein complex of cells of the innate immune system and mediates the inflammatory response versus a plethora of several stimuli, deriving from pathogens or molecular damages. An overactivation of NLRP3 has been associated with a state of chronic inflammation and has been found in a number of age-related pathological conditions, such as neurodegenerative, cardiovascular, autoimmune and metabolic diseases. Thus, NLRP3 is a validated target and is appealing for the discovery of new anti-inflammatory drugs. To date, MCC950 is the best lead candidate (IC50 of 8 nM) but failed the clinical trials due to its hepatic and renal toxicity. This inhibitor seems to bind the target in a non-covalent and non-competitive manner at a region proximal to the Walker B, within the NACHT domain. In light of these evidences (pdb 7ALV), we focussed on the design and synthesis of new chemotypes, maintaining the electronic properties of the lead compound, in order to identify novel potential inhibitors of the NLRP3 machinery. In detail, five chemotypes were designed, and for each class of structures a feasible, cheap and no-time consuming synthetic route was developed.



Right:
Possible mechanisms of activation of the NLRP3 Inflammasome.

IMPACT

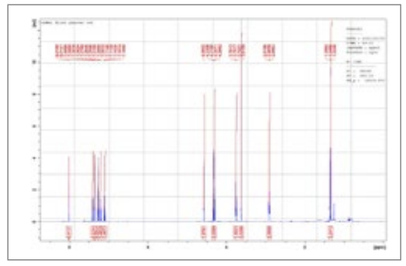
In a highly competitive context, this project aims at discovering new drugs capable of treating chronic inflammation associated with age-related diseases. Currently, no drugs of small molecule nature targeting NLRP3 are available on the market, and only monoclonal antibodies (Anakinra, Canakinumab, Rilonacept) are used for the therapeutic treatment. However, several drawbacks, such as high costs, poor patient compliance, very short plasma half-life (a daily injection is required), the increased risk of contracting infections due to immune system suppression, and poor blood brain penetration are associated to these biologic drugs. The discovery of small molecule drugs selectively targeting NLRP3 is the goal of several pharmaceutical companies, and undoubtedly represents a breakthrough in overcoming the aforementioned limitations, and in improving the quality of life of patients suffering from chronic inflammation. A strength of the project is the presence within the Ri.MED Foundation of a Drug Discovery area that brings together very different expertise and joint skills necessary to face the early discovery phase, helping in the identification of new hits, with the structural optimization of the most promising ones, up to the identification of a lead to be taken further onto the preclinical studies.

RESULTS ACHIEVED IN 2021

During the year 2021, starting from mid-April, when officially the Medicinal Chemistry laboratory activities started, we focussed on the study and optimization of the synthetic route of four chemotypes: i) the tatrahydro-carbolines; ii) 3-sulfonylindoles, iii) α,β-unsaturated sulfonamides, iv) thiazinoazepine. The investigation of the synthetic pathways involved, for each step, an evaluation of the reactivity of the synthesis intermediates and of the best reaction conditions, in order to obtain the title compounds with the highest yields while using green conditions. A scale-up feasibility study was performed for each step. Complete analytical characterization was performed for each reaction intermediate using NMR spectroscopy techniques (1H and 13C) and mass spectrometry. The final compounds that will be biologically screened in the *in vitro* primary assay were purified in high resolution liquid chromatography, and obtained with a purity greater than 96%. To date, for the (i) tetrahydrocarbolines, the whole synthetic route was optimized. As soon as we have a representative number of samples per chemotype, we will send them to the HTS group of Ri.MED for their biological evaluation.

GOALS FOR 2022

During the next year, we aim at completing the optimization of the synthetic routes of the other chemotypes. The first goal is to finalize the synthesis of a mini series of representative compounds of each chemotype, which will be tested *in vitro*, in the primary phenotypic assay, by the HTS group. The results of biological screening will be essential for identifying potential hits. The rational design of analogues will allow the series expansion and the structural optimization of the promising molecules. Through an in-depth study of structure-activity relationships (SAR), we will explore the chemical space of biologically interesting compounds. In collaboration with the Molecular Informatics group, we will learn about the binding mode of the new chemotypes within the NACHT site of the NLRP3 target. The rational design will be accompanied hand in hand by the synthesis of molecules and by their biological evaluation in the primary assay, which will give indications on the success or failure of the chemical manipulations. The ultimate goal is to identify compounds with a defined pharmacokinetic profile in terms of potency and efficacy. Secondary and orthogonal biological assays developed by the HTS group, will help us to address non-specificity and selectivity issues of the newly synthesized compounds and to implement the information deriving from the use of an indirect primary cell type assay.



Mono-dimensional proton spectrum (^{1}H NMR) of a title compound

MEETINGS

Gli Inflammasomi: La frontiera futura, Settembre, 2021, Martinsried (Germania) – (online Embo workshop)

PUBLICATIONS

- **Mekni, N.; Coronnello, C.**; Langer, T.; **De Rosa, M.; Perricone, U.** (2021). Support Vector Machine as a Supervised Learning for the Prioritization of Novel Potential SARS-CoV-2 Main Protease Inhibitors. Int. J. Mol. Sci., 22, 7714 (1-20). doi.org/10.3390
- Sharma, A.; **De Rosa, M.**; Singla, N.; Singh, G.; Barnwal, R. P.; Pandey, A. (2021). Tuberculosis: An Overview of the Immunogenic Response, Disease Progression, and Medicinal Chemistry Efforts in the Last Decade toward the Development of Potential Drugs for Extensively Drug-Resistant Tuberculosis Strains. J. Med. Chem. 64(8), 4359–4395. doi.org/10.1021/acs.jmedchem.0c01833

PRODUCTS: **Drugs**

Design of non-covalent inhibitors of NLRP3 in the inflammation disease

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COLLABORATIONS

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THERAPEUTIC AREA

- Aging diseases
- Neuroscience

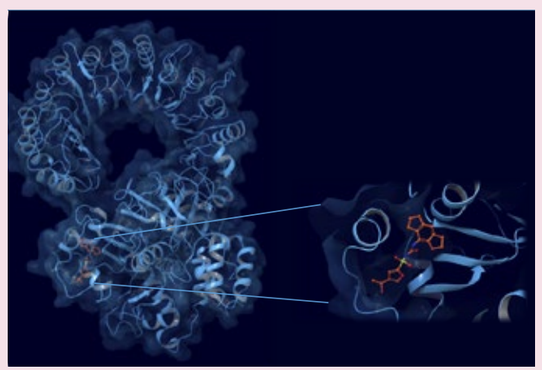
PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

The NLRP3 (NOD-like receptor family, pyrin domain-containing protein 3) inflammasome is a cytosolic complex that coordinates innate immune responses by detecting a wide range of molecules associated with damage and pathogens (DAMP and PAMP). Following activation, the NLRP3 protein assembles with the ASC adapter and pro-caspase-1. This promotes the caspase-1 dependent cleavage of pro-IL-1, pro-IL-18 and gasdermin D leading to release of cytokines, pore formation and finally piroptosis. Greater activation of NLRP3 has been linked to several chronic conditions, including neurodegenerative diseases, atherosclerosis, type II diabetes, fibrosis and rheumatoid arthritis. Preclinical evidence supports the fact that the inhibition of NLRP3 can restore the physiological condition in various pathological diseases, with a reduced impairment of the host's immune defences. Therefore, NLRP3 represents an attractive drug target. Recently, many research groups have focused on the development of selective modulators of the NLRP3 inflammasome, that is, molecules that do not interfere with the protective activity of other types of inflammasome. However, the lack of structural information on the protein and an unclear molecular mechanism of the few known inhibitors hinders the task of designing new selective modulators.

IMPACT

The chronic inflammation-related diseases have been rising in the western world in the last decade and to date not all the molecular mechanisms causing these diseases have been clarified. The currently used anti-inflammatory drugs are used to dampen the immune response involved in the onset of chronic inflammation, suppressing the symptoms of inflammation, but with a rare complete remission of the disease. The pharmacology of chronic inflammation focuses mainly on four groups of anti-inflammatory drugs: prostaglandin inhibitors (NSAIDs), glucocorticoids (GC), disease-modifying drugs (e.g. Methotrexate and Sulfasalazine) and inflammatory cytokine blocking agents. Most of the current therapies act on the immune system in an attempt to inhibit the production of pro-inflammatory chemical mediators, without however resolving the causes of the pathology. This project has the objective of elucidating the molecular mechanisms behind the activation of the NLRP3 complex and the design of non-covalent and non-ATP-competitors' selective inhibitors so as to act on the process causing the inflammatory pathology mediated by NLRP3.



A focus insight on the NLRP3 protein putative binding site.

RESULTS ACHIEVED IN 2021

In 2021, the Molecular Informatics group integrated several computational structural biology approaches in order to create a full 3D model of the NLRP3 protein that can be used for virtual screening campaigns and for the rational design of modulators in collaboration with the medicinal chemistry group. In particular, starting from the cryoEM model of the protein as a template, an ab initio approach was employed to insert the missing parts of the NACHT domain through threading and multiple alignment to insert the portion of the PYD domain available as an X-ray resolved structure. This completed model was subjected to various energy minimization protocols and in-depth studies of molecular docking with known inhibitors. This process made it possible to create a computational model that can be used for virtual screening and rational design campaigns. The rationalization of the binding mode of active compounds was in fact the first step to ensure that the model was able to reproduce the experimental poses of the known inhibitors. During 2021, the analogues of two hit compounds were also tested which had been found from the previous biological screening campaign, but which, despite having shown biological activity, gave rise to qualitative problems. These molecules come from the design of protein-protein inhibitors that would act on the PYD domain of NLRP3.

GOALS FOR 2022

In 2022, the goal will be to carry out biological assays on 1000 molecules acquired in 2021 from virtual screening campaigns and to find two hit families with patentable chemotypes. For this reason, the molecular informatics group will work alongside the medicinal chemistry group for the rational design of new synthetic molecules and for the rationalization of the binding mode of the molecules proposed by synthetic chemistry. The previous work done on the computational model of protein will allow to rationalize the different chemotypes that will potentially bind the NACHT domain of the protein. At the same time, the group will continue the study of protein-protein inhibitors (PYD-PYD) by expanding the series of analogues of the two preliminary actives found in previous screenings.

PRODUCTS: **Drugs**

Design of modulators of Histone lysine demethylase 4 (KDM4) as anticancer agents

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COLLABORATIONS

Department of Precision Medicine - Luigi Vanvitelli University of Campania, Naples , Italy

THERAPEUTIC AREA

Oncology

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

Epigenetic processes are essential mechanisms in the development and physiological functioning of cellular gene expression patterns. Global changes in the epigenetic scenario are distinctive signs of cancer initiation and progression. N-Methylation of lysine and arginine residues is one of the most frequent mechanisms of transcriptional epigenetic regulation in eukaryotes. In humans there are two families of enzymes that catalyse the demethylation of lysine residues (KDMs). The KDM2-7 family is the largest class of demethylases, consisting of 20 enzymes. In particular, KDM4A is frequently amplified and over-expressed in various types of human cancers, for example in ovarian cancer, colon or squamous cell carcinoma. The main objective of the research project is the rational design and the synthesis of small molecules able to modulate the epigenetic mechanisms regulated by Histone lysine demethylase 4 (KDM4) at the base of tumour pathologies. The rational design of the molecules provides for different approaches including the creation of *in silico* models created on the target proteins, object of our study, and their validation in a retrospective way. These models are used for virtual screening and molecular modeling in order to identify potential Hit compounds, through computational techniques and to guide chemical synthesis towards compounds that go from a hit compound profile to a lead compound profile

IMPACT

Recent advances in the field of cancer epigenetics have highlighted the importance of epigenetic mechanisms in the development of tumour pathology. Particular importance has been given to DNA methylation, histone modifications, and microRNA expression modifications. The reversible nature of epigenetic aberrations in tumour cells has, since the beginning of the related discoveries, underlined the promising aspect of epigenetic therapy as a valid therapeutic strategy in the field of oncology. In this context, drugs with epigenetic targets act in two ways, preventing the formation of cancer progenitor cells, and killing, at the same time, the cancer cells usually resistant to other therapeutic agents. Although in recent years several epigenetic drugs have been approved by the public institutions responsible for regulatory activity, many clinical trials are currently underway, and therefore there are numerous possibilities for developing new drugs that act at the level of epigenetic mechanisms.

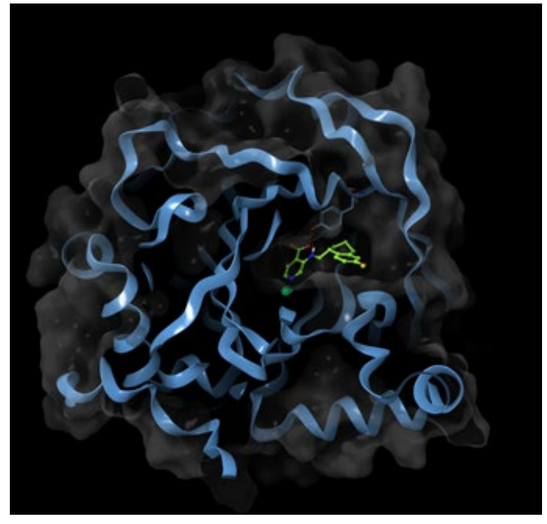
RESULTS ACHIEVED IN 2021

During 2021, hybrid computational models (structure- and ligand-based) were created. These models were capable to perform massive virtual screening on commercial libraries, prioritizing chemotypes with patentability space. In particular, we focused on the use of molecular docking algorithms, structure- and ligand-based pharmacophores and chemoinformatic approaches based on the use of molecular fingerprints. The activity data of previous biological screenings were also used to set structural constraints to the models used for virtual screening. The approaches used were combined in a consensus-type scoring function that allowed to optimize the selection of molecules recognized as active potentials with complementary approaches.

From this virtual screening campaign, 350 molecules have been selected that will be tested during 2022 with a biochemical assay on the KDM4a protein. Computational models were also used for the rational design of in-house designed molecules. Together with the medicinal chemistry group, the binding modes of the series designed by the medicinal chemistry group were theorized

GOALS FOR 2022

For 2022 the main objective is the biological evaluation of the molecules selected by virtual screening in order to find hit compounds to be used as a starting point for the design and synthesis of analogue molecules of the preliminary hits in order to improve their potency and the toxicity profile. Computational models will also be used to rationalize the results found for the newly synthesized molecules. The Machine learning approaches recently developed at the molecular informatics group, will be used to evaluate the selectivity towards other demethylases of the designed compounds. This approach will be useful in the SAR evaluation process and in guiding the structural optimization of molecules in the hit-to-lead phase.



Inside the KDM4 binding site, an active inhibitor in action.

PRODUCTS: **Drugs and Biologics**

iRhom2: a new therapeutic target in osteoarthritis

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COLLABORATIONS

- Institute of Aging and Chronic Diseases, University of Liverpool, UK
- Pharmacy Department, Università di Pisa, Pisa, Italy
- German Center for Neurodegenerative Diseases (DZNE), Munich, Germany

THERAPEUTIC AREA

Aging diseases

BRIEF DESCRIPTION

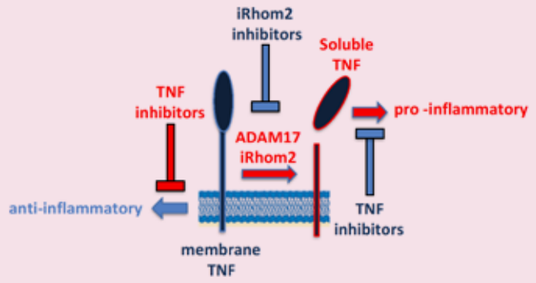
Osteoarthritis (OA) is a debilitating disease causing pain and stiffness. OA is characterized by breakdown of articular joint, due to the aberrant activity of MMPs and ADAMTSs. The endocytic receptor LRP1 controls turnover of these proteinases, thus its inactivation by ectodomain shedding contributes to development of the disease. In addition, the proinflammatory cytokine TNF plays a role in its progression by enhancing the expression of metalloproteinases. Similar to LRP-1, TNF is proteolytically released by ADAM17, and this cleavage elicits its pro-inflammatory potential. It is clear how inhibition of ADAM17 may lead to beneficial effects in OA progression by preventing LRP-1 and TNF shedding, thus enhancing metalloproteinase turnover and diminishing their expression, respectively. Nevertheless, ADAM17 cleaves more than 80 different proteins, and, as a consequence, its complete inhibition leads to their dysregulation with detrimental side-effects. iRhom1 and iRhom2 are essential regulators of ADAM17, in that they guide the enzyme maturation through the secretory pathway and direct its proteolytic activity towards specific substrates. By using unbiased secretome analysis, we found that ADAM17-mediated shedding of TNF and LRP-1 is specifically mediated by iRhom2, with iRhom1 that is not able to compensate. Thus, pharmacological inhibition of iRhom2 can be protective in OA, with lower risk of side effects.

IMPACT

The proposed project will investigate the role of iRhom2 in the context of OA. iRhom2 is an ER trafficking protein that guides the maturation of ADAM17, a protease with a crucial role in development and inflammation. Although based on solid proteomic data, the proposed project is highly innovative. The major expected result will be the amelioration of OA progression in the absence of iRhom2. In addition, this study plans to generate a molecule that is able to block function of iRhom2, and therefore TNF release. It is expected, that upon a positive outcome of the primary objectives, multiple applications will arise. Indeed, implication of TNF and iRhom2 on the pathogenesis of inflammatory and neurodegenerative diseases, such as rheumatoid arthritis and Alzheimer's, is already proven, and our inhibitory molecule can find an application in the therapy of these diseases. This will significantly promote multidisciplinarity among different medical specialties and research topics. It has recently emerged that iRhom2 and its homologue iRhom1 can direct ADAM17 activity towards specific substrates, but this area of investigation is still on its infancy. Our study will lead to a comprehensive analysis of those proteins that are processed by ADAM17 in an iRhom1 or iRhom2-dependent manner. Thus, it will provide further insight into the iRhom biology, revealing new functional and structural properties of these proteins and the mechanism by which they regulate ADAM17 substrate-selectivity.

RESULTS ACHIEVED IN 2021

After finalizing an MTA with the University Health Network (UHN, Toronto, Canada) for the use of the transgenic iRhom2 knockout mouse, we began the study *in vivo* on these mice, in collaboration with George Bou-Gharios at the University of Liverpool. Preliminary results showed evident differences in cartilage and bone development in iRhom2 KO mice. These mice will be subjected to the surgical induced model of OA in 2022. In addition to *in vivo* experiments, our PhD student Simone Bonelli, is using CRISPR-Cas9 to ablate the expression of iRhom2 in a chondrocyte-like cell line to characterize the turnover of specific proteases involved in the degradation of cartilage and the molecular mechanisms regulated by iRhom2 in cartilage.



Genetic ablation of iRhom2 leads to inactivation of ADAM17 in immune cells. As a consequence, the membrane-tethered TNF, which has anti-inflammatory properties, cannot be converted in soluble TNF, which is a pro-inflammatory cytokine. This suggests that iRhom2 inhibitors may be more efficient than anti-TNF inhibitors, which block both membrane-tethered and soluble TNF, in the therapy of inflammatory diseases.

GOALS FOR 2022

2022 will be crucial for the accomplishments of two milestones of the project. Firstly, we will complete the *in vivo* study and understand the role of iRhom2 in the development of osteoarthritis. We expect that iRhom2 deletion will improve disease progression by reducing LRP-1 shutdown and extracellular levels of MMP-13, ADAMTS-4 and ADAMTS-5. Furthermore, we will use proteomics to analyze the cartilage of OA mice. This study will provide interesting information on alterations in the balance between catabolic/anabolic factors of the extracellular matrix, and therefore on the potential molecular pathways regulated by iRhom2 that induce OA. Potentially, this study could identify new mechanisms, in addition to the turnover of metalloproteinases, which may be involved in the pathogenesis of OA and therefore new pharmacological targets.

PUBLICATIONS

- Spanò D. P. and **Scilabra S. D.**; Tissue inhibitor of metalloproteases 3 (TIMP-3): in vivo analysis underpins its role as a master regulator of ectodomain shedding, Membranes, *in press.*
- **Calligaris M**, Cuffaro D, **Bonelli S**, Spanò DP, Rossello A, Nuti E, **Scilabra SD**. Strategies to Target ADAM17 in Disease: From its Discovery to the iRhom Revolution. Molecules. 2021 Feb 10;26(4):944. doi: 10.3390/molecules26040944. PMID: 33579029; PMCID: PMC7916773.
- Yamamoto K, de Groot R, **Scilabra SD**, Kwok HF, Santamaria S. Editorial: ADAM, ADAMTS and Astacin Proteases: Challenges and Breakthroughs in the -Omics Era. Front Mol Biosci. 2021 Oct 12;8:780242. doi: 10.3389/fmolb.2021.780242. PMID: 34712699; PMCID: PMC8546316.
- **Carreca AP**, Pravatà VM, **D'Apolito D, Bonelli S, Calligaris M, Monaca** E, Müller SA, Lichtenthaler SF, Scilabra SD. Quantitative Proteomics Reveals Changes Induced by TIMP-3 on Cell Membrane Composition and Novel Metalloprotease Substrates. Int J Mol Sci. 2021 Feb 27;22(5):2392. doi: 10.3390/ijms22052392. PMID: 33673623; PMCID: PMC7957584.

iRhom2 regulates ectodomain shedding of MHC class I molecules

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COLLABORATIONS

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THERAPEUTIC AREA

- Oncology
- Infectious diseases

PIPELINE

CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

The proteolytic release of transmembrane proteins, also known as 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. We established an advanced workflow for secretome analysis to identify ADAM17 substrates in a systematic manner. This approach allowed the identification of 24 potentially novel substrates of ADAM17, including the major histocompatibility complex (MHC) class I molecules. MHC-I molecules play a crucial role in presenting oncogene- and virus-derived antigens to cytotoxic limphocytes (CTLs) and natural killer cells (NKs). In addition, we found that iRhom2, which is an essential regulator of ADAM17, also regulates shedding of MHC class I molecules (H2-D1 in mouse) and their surface levels. Understanding the functional consequences in immunity of the regulation of MHC class I molecules by iRhom2 is the central aim of the project.

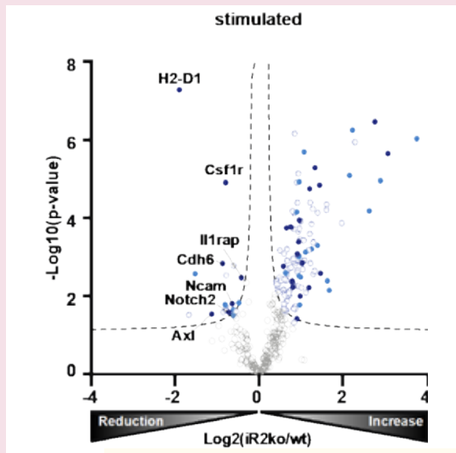


Figura a destra: Volcano plot showing the −log10 of p-values versus the log2 of protein ratio between iRhom2 KO and wild-type bone marrow derived macrophages of 512 proteins (n=6). Proteins above the two dashed hyperbolas are considered significantly regulated (blue dots for transmembrane proteins, light blue dots for secretedproteins). H2-D1, one of the murine MHC-I aplotypes, is the most reduced protein in the secretome of iRhom2 KO macrophages.

IMPACT

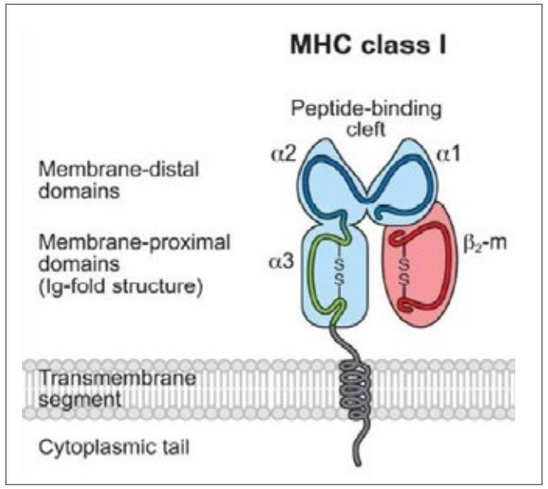
iRhom2 was first described as essential regulator of ADAM17, a protease with a key role in immunity for its ability to release TNF. Our results show an additional major role of iRhom2 in immunity, which is regulating the ectodomain shedding of MHC class I molecules, which are crucial proteins in immune responses in that they present peptide antigens to immune cells. It is clear that this research may contribute to enlarge our knowledge about iRhoms and their functions in immunity. In addition, the proteomic method that we have developed can be applied to investigate proteins that get sequestered by the extracellular matrix, including growth factors and chemokines, which are difficult to investigate by standard methods for secretome analysis.

RESULTS ACHIEVED IN 2021

In 2021 we have characterized the mechanism leading to the iRhom2-dependent shedding of MHC class I molecules and these results will soon be published in peer-review journals and presented at international conferences. In detail, by using high-resolution proteomics we found that iRhom2 KO macrophages failed to release MHC-I molecules. We developed a method, called "secretome analysis of heparin-treated cells (hep-sec)" which allowed the identification of a higher number of ADAM17 substrates than standard secretome analysis, including MHC-I molecules.

GOALS FOR 2022

The major goal for 2022 is understanding whether the iRhom2-dependent regulation of MHC class I shedding has functional consequences in immunity. Thus, we aim to ablate the expression of iRhom2 in selected cancer and immune cells by using CRISPR-Cas technology, and analyze CTL and NK activation when primed with these iRom2 KO cells. CTLs or NKs will be co-cultured with cancer and immune cells where iRhom2 is ablated, and their activation evaluated by a proteomic procedure that enables secretome and cell surface proteome analysis of cells when in a co-culture with a different cell line. This proteomic approach, based on a previously published procedure called "secretome protein enrichment with click sugars" (Khun et al., EMBO J, 2012), consists of metabolically labeling CTL or NK cells with azido-sugars that are incorporated into glycoproteins, before co-colturing them with target cells. This strategy enables pulling down only proteins that have incorporated azido-sugars, therefore coming from CTLs or NKs, thus separating them from proteins released by target cells which have not been previously labeled.



Schematic representation of a MHC class I molecule

PUBLICATIONS

Calligaris M, Cuffaro D, **Bonelli S,** Spanò DP, Rossello A, Nuti E, **Scilabra SD.** Strategies to Target ADAM17 in Disease: From its Discovery to the iRhom Revolution. Molecules. 2021 Feb 10;26(4):944

PRODUCTS: **Biomarkers - Drugs and Biologics**

Dissecting the role of iRhom2 in regulating levels of MHC-I molecules and responsiveness of PDAC to immunotherapy

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COLLABORATIONS

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THERAPEUTIC AREA

Oncology

BRIEF DESCRIPTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers and generally resistant to immunotherapy. A major cause of PDAC limited sensitivity to immunotherapy is loss of MHC-I cell surface expression. Therapies to augment the sensitivity of PDAC to immunotherapy are urgently needed. iRhom2 is a ER-associated protein strongly linked with TNFα and IFN-I signalling pathways for its ability to mediate trafficking and activation of the TNFα-convertase and the stimulator of interferon gene (STING), respectively. We hypothesize that iRhom2 can increase MHC-I cell surface levels in PDAC cells, thereby playing a beneficial role in enhancing PDAC sensitivity to immunotherapy. The major aim of this proposal is to dissect the role of iRhom2 in the regulation of MHC-I in the context of PDAC.

IMPACT

This project proposes to study the role of iRhom2 in PDAC and its potential involvement in regulating MHC-I levels in this tumor. Accomplishment of its objectives by using innovative techniques in proteomics, such as hiSPECS and suSPECS, and established collaborations between Fondazione Ri.MED, University of Palermo and Technical University of Munich, may have a terrific impact on the scientific field and society.Indeed, this multidisciplinary project may be translated into clinical benefits for PDAC patients, whose prognosis is dismal at the moment. Furthermore, upon positive outcome of the proposed project, similar proteomic approaches could be used to identify biomarkers that can be useful for the treatment of other types of tumors, including colon and lung cancer.

RESULTS ACHIEVED IN 2021

The project has recently begun. This will be carried out by Donatella Pia Spanò, who applied to and has been awarded a scholarship to start a PhD. In 2021 we set up all the necessary collaborations to accomplish the objectives of the project, including the University of Palermo.

GOALS FOR 2022

We expect to analyse the expression of iRhom2 in different PDAC cell lines and correlate its expression with levels of MHC-class I. Then, iRhom2 will be ablated by CRISPR-Cas9 or overexpressed by retroviral vectors in these cell lines, and STING activation and MHC I levels evaluated by an array of biochemical and proteomic methods.

MEETINGS

Spanò D. P. and Scilabra S. D.; Tissue inhibitor of metalloproteases 3 (TIMP-3): in vivo analysis underpins its role as a master regulator of ectodomain shedding, Membranes, in press.

PUBLICATIONS

Calligaris M, Cuffaro D, **Bonelli S,** Spanò DP, Rossello A, Nuti E, **Scilabra SD**. Strategies to Target ADAM17 in Disease: From its Discovery to the iRhom Revolution. Molecules. 2021 Feb 10;26(4):944

PRODUCTS: **Digital platform for data set aggregation and analysis**

OBIND - Oncological therapies through Biological Interaction Network Discovery

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COLLABORATIONS

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  - Dipartimento di Scienze Economiche, Aziendali e Statistiche (SEAS)
  - Dipartimento di Innovazione Indistriale e Digitale (DIID)
  - Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF)
- **Consiglio Nazionale delle Ricerche, Istituto per la Ricerca e l'Innovazione Biomedica (IRIB), Palermo, Italy**
- **SecurProject SRL, Palermo, Italy**
- **2038 Innovation Company, Palermo, Italy**
- **Os2 SRL, Palermo, Italy**

THERAPEUTIC AREA

Oncology

Oncological Therapies through Biological Interaction Network Discovery

Il Progetto n. 086202000366 – "OBIND", CUP G29J18000700007 è stato cofinanziato dalla Regione Siciliana, Assessorato delle Attività Produttive nell'ambito dell'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" del PO FESR Sicilia 2014/2020

BRIEF DESCRIPTION

The study of complex pathologies, such as tumors, requires an integrated data analysis approach. The development of tumor pathology, passes through the regulation of pathways involving a large number of biological molecules. To date, more than 500 genes have been identified as being strongly involved in the process of transforming normal cells into cancer cells. The OBIND project was born with the aim of creating an information platform in the oncology field that allows a rapid and effective aggregation and subsequent analysis of heterogeneous data sets. The aggregated information is exploited as a starting point for the study of the interactions between different biological molecules, which are altered in tumor pathologies in order to create a rational design in the design of new therapies. To date, there is no IT tool that integrates the information available relating to these disciplines, although the topics are strictly interlaced. The collection and analysis of the data described in a single environment would facilitate the design of highly specialized and personalized therapies. The main objective of the project is therefore the creation of an expert system for the enhancement and integration of data already in the possession of the partners, of data from public domain databases, and of data that will be produced during the project phases through proprietary prediction algorithms.

IMPACT

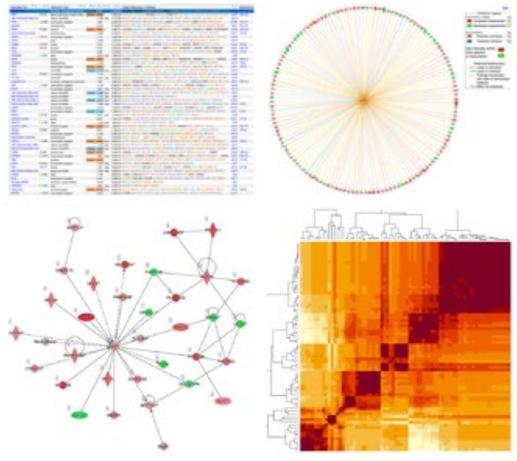
The OBIND platform will represent a hub to which researchers from the medical-scientific community, companies, sector contributors and suppliers of data useful for the results and indications achieved will be able to access. High value is given by the possibility for the medical-scientific community to obtain a holistic view of the oncogenic process. Indeed, revealing interactions between biological molecules that can open the doors to new applications in the therapeutic field. The system will be centered on the analysis of the biological interactions involved in the onset of a tumor. The main focus will be on the interactions between proteins, RNA and small molecules, enhancing the presence on the web of numerous data sets that can be used for such analyzes. One of the strengths of the platform is represented by the validation of biological data with *in vitro* methodologies that will help to further improve the predictive capacity of the algorithms used within it. Through personalized access to the platform it will be possible to consult the repositories of interest (data, results obtained by integrating and processing the information collected, suggestions for further data collections aimed at optimizing the solutions already identified or identifying new solutions), from one point any of the Internet through modern webGUI, through the apps for smartphones and tablets prepared as part of the project, through open interfaces that open access to other systems.

RESULTS ACHIEVED IN 2021

In 2021, the back-end infrastructure of the platform was completed, creating the data repository and the connections between the various DBs that power the platform. Furthermore, from the chemoinformatics side, both the study systems of protein-protein interactions and the creation of models based on machine intelligence for the prediction of affinity between small molecules and therapeutic targets in the oncological field have been optimized, e.g., Kinase family.
As regards the data collection to support the bioinformatics component of the platform, the panel of experiments aimed at characterizing the gene expression profiles of two breast cancer cell lines (MCF7 and MDA) was completed. In particular, two proteins of the RISC complex (AGO2 and GW172) were studied, analyzing the gene expression profiles obtained following their inhibition and / or immunoprecipitation. These data will be used for the realization of new algorithms for the prediction of microRNA targets

GOALS FOR 2022

2022 provides for the closure of the regional loan (PO FESR, Azione 1.1.5) obtained for the OBIND project. The online platform will be released by the end of 2022. In particular, we will proceed with a test phase of a beta version that can be consulted internally by the project partners to validate all the components in order to then make the platform safely usable externally. In the test phase, both the part of the integrated public databases consultation and the part of integrated predictive algorithms will be evaluated. The goal for 2022 is also to promote the platform through social media and participation to national and international conferences. The promotion of the project has already begun through the website available online at
https://obind.os2service.com/il-progetto/.



PUBLICATIONS

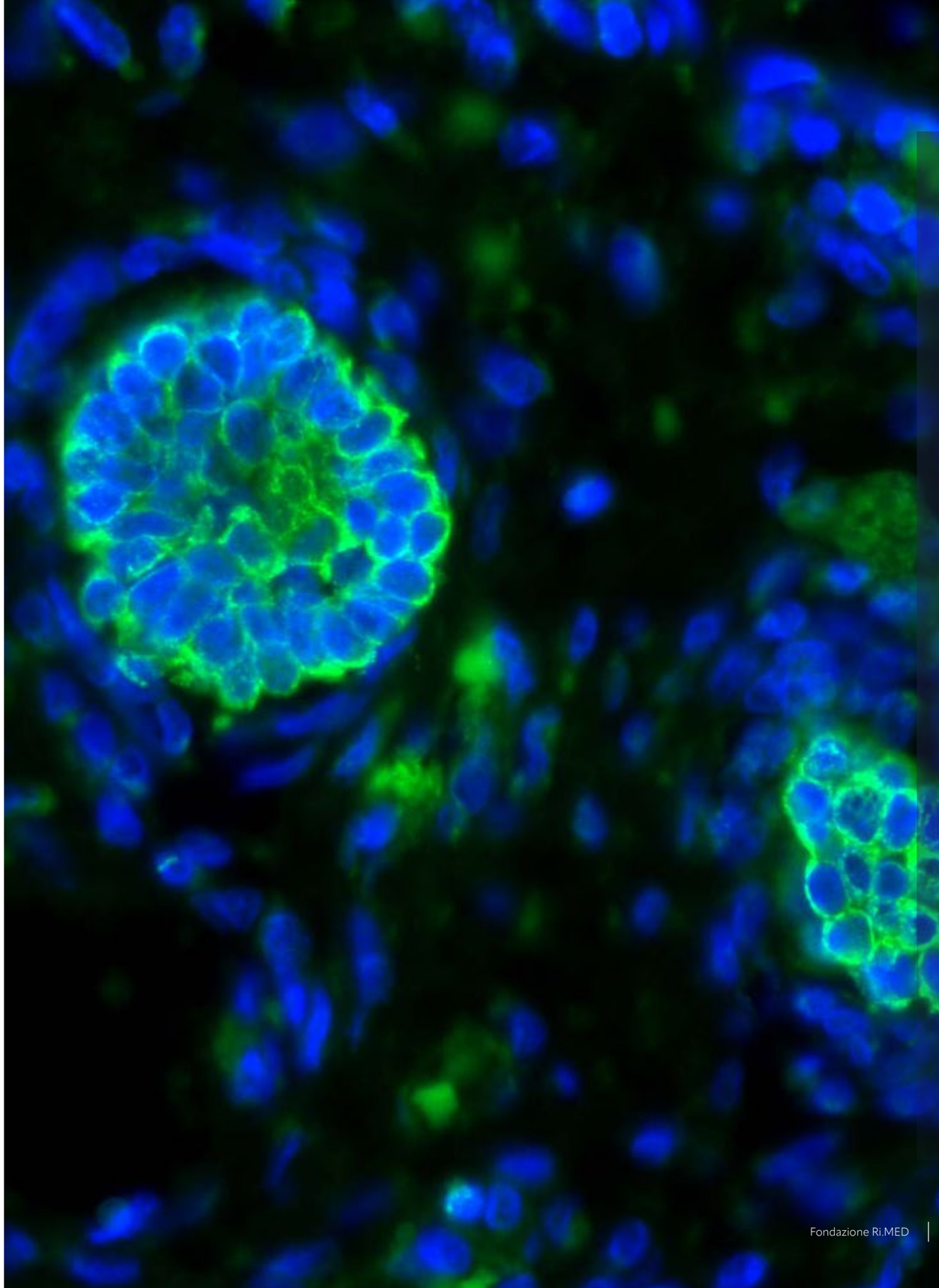
- **Gulotta M.R., De Simone G.**, John J., **Perricone U.**, Brancale A., (2021) A Computer-Based Methodology to Design Non-Standard Peptides Potentially Able to Prevent HOX-PBX1-Associated Cancer Diseases, Int. J. Mol. Sci., 22(11), 5670 doi: 10.3390/ijms22115670
- **Gulotta M.R.**, Vittorio S., Gitto R., **Perricone U.**, De Luca L., (2021), Exploring molecular contacts of MUC1 at CIN85 binding interface to address future drug design efforts, Int. J. Mol. Sci., 22(4), 2208, doi: 10.3390/ijms22042208

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

The Regenerative Medicine and Immunotherapy laboratories are at IRCSS ISMETT, and are focused on developing new cellular therapies for end-stage organ diseases and post-transplant complications. The projects undergoing preclinical phase aim at developing cellular products for tissue repair and/or regeneration, and developing organotypic cultures to be used for regenerative purposes and as models for pharmacological screening.

The team was trained to operate according to Good Manufacturing Practice (GMP) and Good Clinical Practice (GCP) for designing and performing preclinical/clinical trials and producing advanced therapies.
Another important research focus is the study and development of cellular therapies for the prevention of disease recurrence and the treatment of post-transplant infections. Some projects are developed in close collaboration with the UP and UPMC teams in Pittsburgh. This allows researchers to accelerate the preclinical development process towards the patient, thanks to the transfer of protocols and know-how.

The new generation of vaccines, composed of recombined proteins, aims at treating hospital-acquired infections of different etiology. Thanks to the "Research Infrastructure (IR)" project, co-funded by the Sicilian Region through the 2014-2020 PO FESR Sicilia, during 2021 the research, cell production and diagnostic laboratories of the Ri.MED-ISMETT cluster were upgraded through modernization works and acquisition of state-of-the-art equipment and instruments.



CAR-NK cells engineering for hepatocellular carcinoma cell therapy
Ester Badami, PhD

Tolerogenic Dendritic Cells therapy for early weaning of Liver Transplanted patients
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Biomaterial-based delivery solutions for secretome of human mesenchymal stromal cells (MSCs) for the treatment of chronic skin wound
Cinzia Chinnici, PhD

Creation of a bank of patient-specific induced pluripotent stem (iPSCs) from children with monogenic liver disease: a platform for gene editing and drug discovery
Cinzia Chinnici, PhD

Optimization of an anti-fibrotic therapy for liver: delivery solutions for secretome, extracellular vesicles and miRNAs based on biomaterials
Cinzia Chinnici, PhD

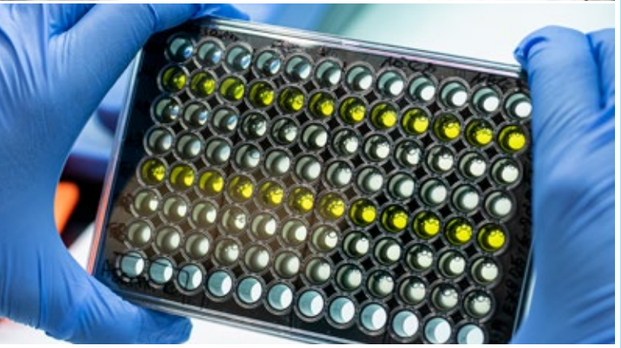
The human umbilical cord. From discarded tissue to a possible source of care (PROMETEO)
Simona Corrao, PhD

Development and characterization of a bioengineered bile duct
Maria Giovanna Francipane, PhD Mattia Pasqua, PhD

Development of a reliable *ex vivo* osteoarthritis model responsive to mTORC1-inhibitors via oxidative stress reduction and autophagy promotion
Riccardo Gottardi, PhD Roberto Di Gesù, PhD

Multi virus-specific T cells to treat post-transplant viral infections
Monica Miele, PhD

Mesenchymal Stem Cell for Liver Disease: A Possible Therapy for Preventing Spontaneous Bacterial Peritonitis
Mariangela Pampalone



The Ri.MED **Regenerative Medicine and Immunotherapy** laboratories with **IRCCS ISMETT**

Since its inception, the Ri.MED Foundation has collaborated with IRCCS ISMETT for the use and management of the Regenerative Medicine laboratories.
In 2017 Ri.MED joined the corporate structure of IRCCS ISMETT, thus laying the foundations for the creation of an integrated research and highly-specialized care center for the benefit of patients, and for the economic development of Southern Italy and the whole country.

Preclinical research, translational research, and cell therapy production programs (GMP Facility) are conducted in the IRCCS ISMETT Regenerative Medicine laboratories. Alongside projects strongly oriented towards clinical application, we proceed with the translation of innovative therapies based on cellular products, such as pancreatic islet transplantation and adoptive immunotherapy.

By creating the coexistence of laboratories and research staff within the highly specialized institute, we intend to facilitate the exchange of knowledge and ideas between physicians and researchers.
The Ri.MED/ISMETT cluster will culminate in the project for the construction of a new 250-bed hospital integrated with the BRBC of the Ri.MED Foundation in Carini (Palermo): a center where research results will be quickly translated, with a full interaction between doctors and researchers.

PRODUCTS: **ATMP** (Advanced Therapy Medicinal Products)

CAR-NK cells engineering for hepatocellular carcinoma cell therapy

Ester Badami, PhD
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COLLABORATIONS

- Istituto Zooprofilattico Sicilia IZS, Palermo, Italy
- Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) IRCCS, Palermo, Italy

THERAPEUTIC AREA

Oncology

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

HCC is a malignant epithelial tumor arising from hepatocytes and is often associated with chronic hepatitis and cirrhosis caused by hepatitis B or hepatitis C virus infections. Treatments for HCC include hepatectomy, liver transplant or chemotherapy are not effective for advanced forms of HCC and the risk of recurrence is high. Therefore novel treatment strategies are needed to improve the prognosis of HCC.

Immunotherapy is one such therapy that functions differently from conventional treatments. 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 with 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 and immunological defense mechanisms against HCC.

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 improved the specificity and efficacy of NK cell therapy. In this study, we propose the use of a novel CAR construct that combines the induced tumor antigen specificity of a Tumor Associated Antigen (TAA) described in HCC into NK cell.
In addition, NK cells will be engineered to produce IFN that our

IMPACT

CAR-engineering has found more ground in T cell-mediated therapies. However, CAR-NK cells have several advantages over CAR-T cells. CAR-NK cells reportedly reduce the risks of autoimmune response and neoplastic transformation because they have a shorter lifetime than CAR-T cells. In addition, cytokines released from NK cells, such as IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF), are considered safer than the cytokine storm that results from CAR-T cell therapy.
Unlike CAR-T cells, CAR-NK cells retain an intrinsic capacity to recognize and target tumour cells through their native receptors, making the escaping of tumour cells through downregulation of the CAR target antigen less likely. Lastly, NK cells do not require strict HLA matching and lack the potential to cause graft-versus-host disease, an important risk imposed by CAR-T cell immunotherapy, which make it possible for CAR-NK cells to be an "off-the-shelf" allogeneic therapeutic option. In our study, we will genetically engineer human primary NK cells with a CAR protein specific for a Liver Tumor Associated Antigen. Our goal is to obtain CAR-NK cells using a GMP-compliant scalable virus-free method of gene editing.

RESULTS ACHIEVED IN 2021

In 2021, we found that the protocol of generation of CAR-NK using lentiviral supernatant was the most efficient. Using this protocol, we obtained above 25% of primary Nk cells expressing the CAR. In order to further augment the efficiency of transduction, vectofusing was added to the viral supernatant. In order to increase NK cells viability, retronecting was used to coat culture plates, obtaining up to 80% of viable cells **(Fig. 1)**. Last, we observed that the protocol of nucleofection, a virus-free process of gene editing, did not confer a satisfactory level of CAR NK cell transfection and transfected cells were mostly dead, likely due to the fact that the protocol of nucleofection remains too stressful for primary NK cells.
To address cell cytotoxicity and specificity against the specific tumor associated antigen, NK cell expression then CAR molecule were first isolated by using a late technology instrument which allowed achievement of CAR+ NK cells with a purity above 95%
(Fig. 2). These high pure NK cells also retained great vitality and ewer used for following functional assays. This recently acquired instrument was the MACSTyto (Miltenyi Biotec). CAR+ NK cells also expressed the suicide gene tEGFR **(Fig. 2)**. Classic chromium release assay was used to test CAR+ Nk cells cytotoxicity and antigen specificity against two different tumor target cells: K562, which expressed the GPC3 tumor antigen at low levels; Huh7.5 which by contrast were characterized by high level of the cell surface of the target antigen **(Fig. 3)**.
As shown in **Figure 4**, CAR-NK cells acquire more specific cytotoxicity against GPC3high Huh7.5 target cells compared to GPC3dim K562. Our preliminary data show that CAR+ NK cells are more potent effector cells than CAR- counterpart against hepatocellular carcinoma cells positive for the GPC3 tumor antigen.

preliminary data have proven to significantly enhance NK cells response to tumor and infection. The vector we designed is a fourth generation construct harboring the signaling domain specific for TAA antigen followed by a transmembrane signaling domain, CD28 and 4-1BB co-stimulatory intracytoplasmic domains and the suicide gene Epidermal Growth Factor Receptor in a truncated form (EGFRt).
The truncated form is inert as it has lost its function and does not respond to the growth factor, thus avoiding unwanted and uncontrolled responses. Furthermore, the receptor specifically binds to the antibody Cetuximab and *in vivo* binding to EGFRt leads to death of target cells by complement fixation.

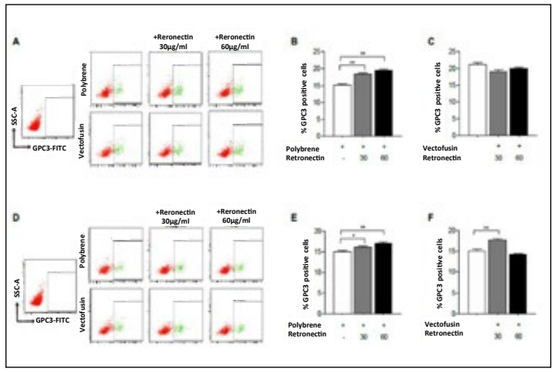


Fig.1 Comparative lentiviral transduction of NK cells using Polybrene, Vectofusin and Retronectin. (A) Gating strategy to estimate the transduction efficacy of NK cells transduced with CAR-IL15 pseudotyped lentiviral particles. (B) NK cells from one donor were transduced with CAR-IL15 pseudotyped lentiviral particles using polybrene alone or with retronectin 30-60µg/ml and (C) with vectofusin alone or with two concentrations of retronectin. (D) Gating strategy to estimate the transduction efficacy of NK cells transduced with CAR-IFNα-IL15 pseudotyped lentiviral particles. (E) NK cells from one donor were transduced with CAR-IFNα-IL15 pseudotyped lentiviral particles using polybrene alone or with retronectin 30-60µg/ml and (F) with vectofusin alone or with two concentrations of retronectin. We achieved about 20-25% of CAR expression using lentiviral transfection on primary NK cells, with 80% of viability.

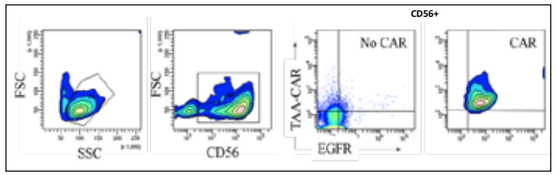


Fig.2 GPC3-CAR effector cell sorting and phenotype. Transfected cells were stained with anti-TAA mAb FITC conjugated, CD45-PerCP and CD56-APC and sorted with MACSTyto sorter. Sorted cells were >95% pure and expressed the EGFR suicide gene. Non-transfected cells were negative for GPC3 and EGFR.

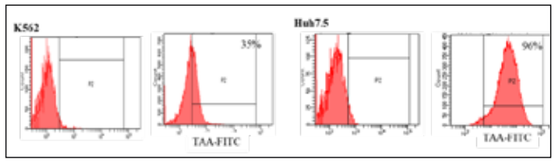


Fig.3 Flow cytometry analysis of GPC3 expression by two cell lines K562, low expressors, and Huh7.5 that were GPC3^{bright}

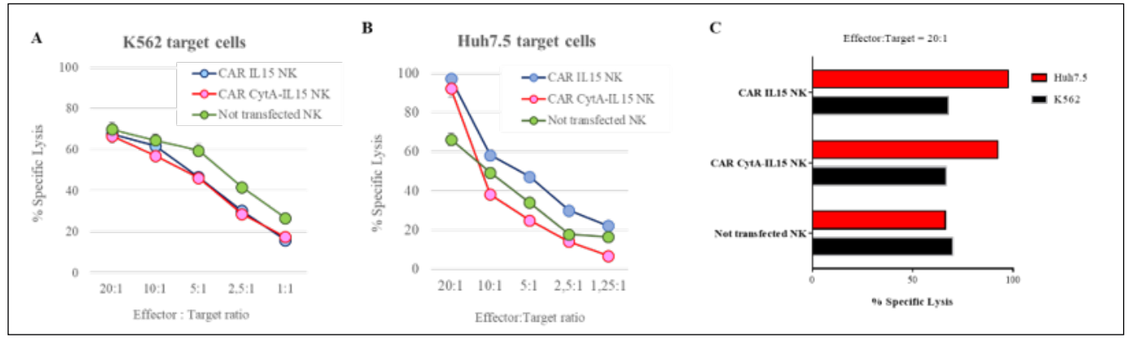


Fig.4 . CAR-NK cells have enhanced cytotoxicity against GPC3+ target cells. CAR IL15-NK and CAR-IFNα-IL15-NK were challenged in a Chromium release assay against K562 GPC3^{dim} (A, C) or Huh7.5 GPC3^{bright} target cells (B, C). Non-transfected NK cells were used as control. CAR+ effector showed increased cytotoxicity against TAA expressing cells. No significant difference was observed regarding the expression of IFNα by NK cells.

GOALS FOR 2022

Test the efficiency of IFNα-activated NK cells *in vivo*
We will use a model of immune-compromised NGS mice ortothopically xeno-grafted with human hepatocarcinoma HepG2-Red-FLuc, a cell line that stably expresses the reporter gene Luciferase. Tumor growth will be traced by Bioluminescence Imaging (BLI) using the newly acquired IVIS Spectrum Instrument (*In Vivo* Imaging System) a high-sensitivity, low noise, *in vivo* imaging technology platform that enables non-invasive visualization and tracking of cellular and genetic activity within a living organism in real time (Fig 3). This protocol has been accepted by the Italian Ministry of Health and the aim is to study the anti-tumor efficacy of NK cells activated with IL2/IL15 or IFNα derived from 2 patients on a total of 100 mice. Being immuno-suppressed, mice need clean conditions for housing. NSG mice will be housed in SPF areas/cages at Istituto Zooprofilattico Sicilia (IZS).

To develop and validate direct *in vivo* delivery of mRNA encoding for the GPC3-CAR by inoculation of lipid nanoparticles dressed with CD56 receptors.
Alternatively to *ex vivo* transfection of NK cells followe dby infusiopn in the patient, a novel gene therapy it has been recently proposed with direct delivery of the mRNA encoding for the CAR sequence by mean of lipid nanoparticles (LNP) dressed with receptor specific for target cells. In our study, we will use LNP expressing the CD56, the archetypal marker specific for human NK cells, containing the mRNA for GPC3-CA.

MEETINGS

Annual Meeting of the International Society of Cell&Gene Therapy - ISCT 2021, VIRTUAL

PUBLICATIONS

- *Additive Effects of Type I Interferons and Galectin-9 on the Suppression of Hepatitis C Virus Infection by Interferon-alpha—activated Natural Killer Cells.* **Anna Paola Carreca,** Massimiliano Gaetani, Rosalia Busà, **Maria Giovanna Francipane**, Giovanna Russelli, **Claudia Carcione,** Pier Giulio Conaldi, **Ester Badami**
Under review - Cellular and Molecular Immunology (Nature press).
- HC*V Interplay with Mir34a: Implications in Hepatocellular Carcinoma* **Ester Badami, Claudia Carcione, Cinzia Maria Chinnici,** Rosaria Tinnirello, Pier Giulio Conaldi and Gioacchin Iannolo
Front. Oncol. - Gastrointestinal Cancers: Hepato Pancreatic Biliary Cancers. In Press.
- *A Radioactive-Free Method for the Thorough Analysis of the Kinetics of Cell Cytotoxicity.* **Coronnello C**, Busà R, Cicero L, **Comelli A, Badami E.** J Imaging. 2021 Oct 23;7(11):222.
- *Donor Preconditioning with Inhaled Sevoflurane Mitigates the Effects of Ischemia-Reperfusion Injury in a Swine Model of Lung Transplantation*
- Alessandro Bertani, Vitale Miceli, Lavinia De Monte, Giovanna Occhipinti, **Valeria Pagano**, Rosa Liotta, **Ester Badami**, Fabio Tuzzolino, Antonio Arcadipane - Biomed Res Int. 2021 Jan 8; 2021

INTELLECTUAL PROPERTY

Patent n. 16/464,823
Patent extension: Europa; USA
Fondazione Ri.MED, IRCCS ISMETT
Title: NK-mediated immunotherapy and uses thereof
Inventor: Ester Badami

PRODUCTS: **ATMP** (Advanced Therapy Medicinal Products)

Tolerogenic Dendritic Cells therapy for early weaning of Liver Transplanted patients

Ester Badami, PhD
ebadami@fondazionerimed.com

COLLABORATIONS

Thomas E. Starzl Transplantation Institute, University of Pittsburgh, USA

THERAPEUTIC AREA

Organ insufficiences

CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

PIPELINE

BRIEF DESCRIPTION

One major caveat of organ transplantation is the occurrence of an unwanted immune response to the graft. Accordingly, immunosuppressive therapy is provided for life to transplanted patients, though it is accompanied by severe side effects such as kidney failure and others. It is therefore important to explore alternative curative methods based, for instance, on the use of cellular therapies based on the administration of donor derived tolerogenic Dendritic Cells for early weaning of liver transplanted patients. The aim of this study is the optimization of a cell mediated therapy to promote operational immune tolerance in liver recipients using tolerogenic Dendritic Cells (DCreg) obtained from the liver perfusate of deceased-donors. During liver procurement from brain-dead-heart-beating donors, the aorta is clamped and liver perfused though the hepatic vein. The liver perfusate thus obtained is routinely discarded. However, liver perfusate contains an incredible amount of blood borne cells circulating to/from tissues, among which DCs precursors. These CD14+ monocytes are cultured for 7 days with IL4/GM-CSF/IL-10 and Vitamin-D3 and examined *in vitro* for their immunosuppressive potential against alloreactive donor-derived T cells.

Figure 1

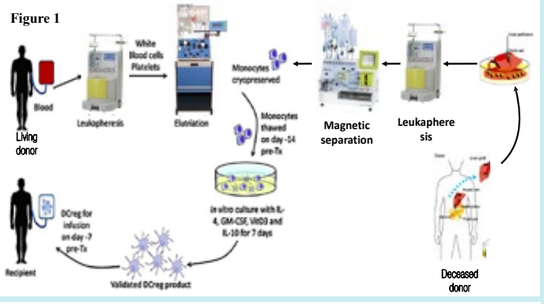
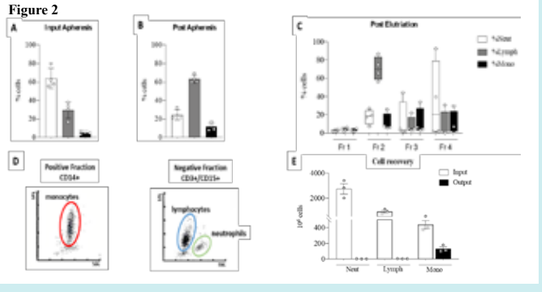


Figure 1.Cartoon depicting the workflow of generation of Tol-DC from elutriated blood monocytes of living donor of the prospective liver allograft donor in GM-CSF, VitD3, and IL-10, and infusion of the validated cell product into the graft recipient 7 days before transplant (1). Alternatively, monocytes are derived from the liver perfusate of deceased liver donors by apheresis and separation with antibodies conjugated to magnetic beads

Figure 2



IMPACT

Patients who undergo solid organ transplant require lifelong immunosuppression to prevent organ rejection. Immunosuppressive therapy are associated with life-threatening side effects such as infection, malignancy, diabetes, cardiovascular disease and renal failure. In organ transplantation, the ideal form of immunosuppression is to induce donor specific tolerance without impairing the host defenses or increasing the susceptibility to infection from all types of organisms. Dendritic Cells, if opportunely redirected, can serve to induce long term tolerance to donor alloantigen by inducing donor-specific T cell hypo-responsiveness and memory to donor alloantigen. DCreg functionally prevent organ rejection and early weaning from immunosuppressive therapy in transplanted patients. The Phase I/II protocol optimized by our collaborators in Pittsburgh (Prof AW Thomson) consists in the use of tolerogenic Dendritic cells obtained from the peripheral blood of liver living donors.

The frequency of living donors transplants is drastically lower than deceased donors. The possibility to use DCreg for the early weaning of immunosuppressive therapy in the cohort of patients that receive livers from decease donors would increase the number of treatable individuals using infusion of DCreg. The choice of liver transplantation is promising as the liver is an immune-tolerant organ per se. to date, no alternative therapies for the induction of operational immune tolerance have been proposed. The therapy proposed in this study could increase the number of retained transplants and could be applied also to other solid organs different than liver such as kidneys.

RESULTS ACHIEVED IN 2021

Optimize the protocol of isolation of CD14+ precursors by elutriation.

The product of liver perfusion contains Neutrophils (45-90%), Lymphocytes (20-45%) and Monocytes (5-10%). According to Thomson's protocol, Tol-DC are produced starting from monocytes isolated from the apheresis product of living donors by elutriation. Processing the perfusate from deceased donors as close as possible to the time of procurement greatly enhances cell viability and performance. All samples were importantly contaminated by neutrophils/granulocytes. We found that this subpopulation could not be separated from the PBMC fraction even by density gradient centrifugation, which suggested that contaminating neutrophils shared the same morphological and density features as lympho-monocytes. This led us to the conclusion that the contaminating residue was composed by a population of low density granulocytes (LDGs) that had degranulated thus losing original density. This population has been recently described being highly represented in patients with ongoing statuses of chronic inflammation, such as SLE, asthma, tuberculosis, psoriasis and last but not least severely symptomatic COVID-19 patients. LDGs have been implicated in the pathogenesis of SLE by producing type I interferons and undergoing spontaneous NETosis (Neutrophils Extracellular Traps) involved in the release of granulocyte intracellular content among which DNA that induces clumping of cells in the surrounding micro-environment. We did experience, indeed, important clump formation and cell loss in our cellular product. So at first, we tried to reduce activation/degranulation of granulocytes during perfusate procurement in the attempt to reduce the population of contaminating LDGs, by adding to the liquid of collection the anticoagulant EDTA (50mM). We have obtained a conspicuous reduction of LDGs which however was not sufficient to be processed by elutriation. To further reduce degranulation by neutrophils, we have also added DNaseI to all buffers, obtaining only marginal improvement (**Fig 2A**). The product of apheresis still contained >15% of neutrophils (**Fig 2B**). Although we succeded in reducing the contamination of this population, as aforementioned the percentage of neutrophils still remains too high to use elutriation to obtain monocyte pure population (**Fig 2C**). In the presence of >3% of granulocytes, the process of elutriation failed and we could not separate monocytes in any fraction (**Fig 2C**).

We therefore tried to directly purify monocytes using CD14-monoclonal antibodies conjugated to magnetic beads. Cells were isolated using the large scale research-grade instrument AutoMACS (Miltenyi Biotec) obtaining a >90% pure population of monocytes (**Fig 2D**). As expected, the number of purified monocytes that we obtained (50-200x106) was below the estimated number (about 400x106) **(Fig 2E).** However, the number of pure monocytes thus obtained is still a remarkable quantity for clinical downstream applications.

Figure. 2. Cellular composition of liver perfusate (n=4) before (A) and after apheresis, using Com. TEC (B); elutriation, using ELUTRA (C); cellular isolation through magnetic beads separation, using AutoMacs. SFL/SSC plot of CD14+ fraction (left panel) and CD3+CD15+ fraction (right panel)(D). Subpopulations of Neutrophils, lymphocytes and monocytes after beads-conjugated depletion (E). Means ± SEM.

GOALS FOR 2022

Characterization of tolerogenic signature of liver perfusate derived Tol-DCs. DCreg and DC will be tested for secretion of IL10, IL12, IL-6 and TNFα. To determine the cytokine signature and citotoxicity induced by DCreg on effector cells, cytokines IFNγ, IL-17A, IL-4, Perforin, Granzyme-B, IL-10, IL-6, IL-12p70, IL-1β, IL-6 and TNFα secreted in the supernatant of Mixed Lymphocytes Reaction (MLR) co-cultures of DC/DCreg and alloactivated CD4+ and CD8+ T cells will be quantified Expansion of Treg population will be assessed in DCreg/MLR by flow cytometry with CD4+CD25+CD127-Foxp3high antibodies.

Large-scale/clinical-grade isolation of monocytes from the product of perfusion of deceased liver donors.

In 2021, we demonstrated that it is possible to obtain on a small scale Tol-DC starting from the monocytes isolated from liver perfusate of deceased donors. The aim of this proposal is to scale up the protocol of generation of Tol-DC on a large scale. We will optimize the apheresis procedure trying to reduce as much as possible the proportion of granulocytes contaminating the final product. Next, CD14+ monocytes will be separated using MACSProdigy cell separator (Miltenyi Biotec), available in our laboratories **(Fig 2) (4).**

For large-scale clinical-grade Tol-DC generation, we propose to learn the know-how in the laboratories of Prof Angus Thomson.

Optimize the protocol of isolation of CD14+ precursors by magnetic beads.

We have improved and partially optimized the protocol of monocyte isolation form the liver perfusate. One constraint is the fact that the starting cellular product originates from a deceased donor. Consequently, the quality of cell is variable and is closely related to the amount of contaminating granulocytes. During the course of 2022, we will attempt a different approach, trying to first deplete granulocytes and low density granulocyte by using two different markers such as CD15 and CD66b antibodies conjugated to magnetic beads. This because CD15 is specifically expressed by neutrophils that have not degranulated and CD66b is upregulated after degranulation. This should increase the possibilities to obtain a more efficient depletion of contaminating cell fraction.

PRODUCTS: **Biologics**

Biomaterial-based delivery solutions for secretome of human mesenchymal stromal cells (MSCs) for the treatment of chronic skin wound

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COLLABORATIONS

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- Lab of Biocompatible Polymers, STEBICEF, Università di Palermo, Italy
- Fondazione IRET, Ozzano dell'Emilia, Bologna, Italy

THERAPEUTIC AREA

Organ insufficiences

BRIEF DESCRIPTION

The therapeutic efficacy of MSCs is actually attributed to their secretome, a mixture of bioactive and immunomodulatory products (i.e., growth factors, cytokines, chemokines, enzymes, genetic material, and extracellular vesicles) stimulating the endogenous repair processes. Nevertheless, the *in vivo* administration of secretome needs a delivery device sustaining its release over time. This last point is crucial, since the way by which soluble products are "presented" to the target tissue might influence their therapeutic efficacy. The project is organized in three phases:

PHASE 1. *In vitro* collection and characterization of cell soluble factors. The aim of phase 1 is to identify the "best secretome" for specific applications of regenerative medicine, such as the treatment of chronic skin wounds. The selected product will undergo to phase 2.

PHASE 2: development of biomaterial-based delivery solutions of MSC secretome. The starting biomaterial is a HA derivative (HA-EDA), formulated as a 3D hydrogel with a sponge-like structure. The hydrogel is integrated with secretome of MSCs from two different sources: fetal dermis and umbilical cord (UC). The aim of phase 2 is to obtain a ready-to-use HA formulation with defined composition and mechanism of action, stabilizing the secretome itself, and sustaining its delivery with the expected doses. The biofunctional product should be also suitable for fabrication scalability and clinical translation. Products having the most appropriate biological properties to treat chronic skin wounds (topical application) will pass to phase 3.

PHASE 3. *In vivo* preclinical proof of concept (efficacy test of the product) in a validated model of diabetic foot ulcer. The aim is to test the efficacy of two different formulations (hydrogel integrated with secretome of fetal dermal cells and hydrogel integrated with secretome of UC-MSCs) in dbdb diabetic mice with a pressure ulcer. The selected therapeutic product will undergo to preclinical validation. Moreover, a GMP release of the product is planned in order to launch future *in vivo* preclinical safety tests.

IMPACT

As a cell-free product, secretome-based therapy has the advantage to limit potential risks related to conventional cell transplantation (tumorigenicity, transmission of infections, immunoreactions). In addition, secretome collected as cell culture conditioned medium (CM) is easier to handle than cells as a ready-to-go biological product, and can be more easily produced as a drug for clinical applications. It was estimated that approximately 50% of chronic wounds fails to heal. The HA hydrogel sponge integrated with secretome might represent a valid therapeutic option for the treatment of diabetic skin ulcers. The approach is low-cost and easy to handle.

RESULTS ACHIEVED IN 2021

We conducted the first efficacy study (preclinical proof of concept) of the product "HA hydrogel integrated with secretome" of two cell types in a murine model of diabetic foot ulcer. The results are promising since suggest that adding MSC secretome to HA might improve its therapeutic efficacy. Moreover, they provide implications for a future preclinic validation of the product. In particular, only the topical application of hydrogel + secretome of fetal dermal cells induced a complete wound closure (re-epitelialization), while the other two treatments (hydrogel + secretome of UC-MSCs and hydrogel alone) determined a partial wound closure, even though significant. Interestingly, the residual de-epithelialized area was significantly lower compared to vehicle in ulcers treated with hydrogel + secretome of fetal dermal cells, but not in ulcers with the other two treatments. In addition, the epidermal layer was significantly thicker compared to vehicle only in ulcers treated with hydrogel + both secretomes, and not in ulcers treated with hydrogel alone. Neo-vascularization, re-innervation and molecular analysis are ongoing.

GOALS FOR 2022

The most promising product, identify during the preclinical proof of concept (HA hydrogel + fetal dermal cell secretome), will be subjected to preclinical validation to test its efficacy in a different model of skin wound (surgical wound) to better reproduce ISMETT's patients with a non-healing surgical site. During the preclinical validation, additional parameters, such as the dose-dependent effect and the quality of the healed skin, will be evaluated in larger groups of animals. In addition, a GMP release of materials is also planned before to launch the *in vivo* preclinical safety tests.

PUBLICATIONS

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- **Chinnici CM**, Russelli G, Bulati M, Miceli V, Gallo A, Busà R, Tinnirello R, Conaldi PG, Iannolo G. Mesenchymal stromal cell secretome in liver failure: Perspectives on COVID-19 infection treatment. World J Gastroenterol. 2021 May 7;27(17):1905-1919. doi: 10.3748/wjg.v27.i17.1905.
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- Miceli V, Bertani A, **Chinnici CM,** Bulati M, **Pampalone M, Amico G, Carcione C**, Schmelzer E, Gerlach JC, Conaldi PG. Conditioned Medium from Human Amnion-Derived Mesenchymal Stromal/Stem Cells Attenuating the Effects of Cold Ischemia-Reperfusion Injury in an In Vitro Model Using Human Alveolar Epithelial Cells. Int J Mol Sci. 2021 Jan 6;22(2):510. doi: 10.3390/ijms22020510

Fig. 2. Hyaluronic acid (HA) hydrogel sponge formulation for topical application.

PRODUCTS: **ATMP** (Advanced Therapy Medicinal Products)

Creation of a bank of patient-specific induced pluripotent stem (iPSCs) from children with monogenic liver disease: a platform for gene editing and drug discovery

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COLLABORATIONS

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THERAPEUTIC AREA

Organ insufficiences

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

Human iPSC-based technology holds great promise for many disease modeling. By reprogramming patient somatic cells, we can generate a potentially unlimited source of patient-specific iPSCs carrying the disease that offers the chance of investigating the disease without the need of animal models. Platforms based on patient-specific iPSCs can be established for approaches of gene editing, drug discovery or to study the pathogenic mechanism underlying the disease. The goal of the project is a creation of banks of iPSCs derived from ISMETT's pediatric patients with metabolic liver diseases caused by a point mutation in a single gene. ISMETT's pediatricians will help to select those diseases where the defect is solely expressed within the liver without extrahepatic manifestations (e.g., tyrosinemia type I, alpha-1-anti-tryspin deficiency, phenylketonuria, Wilson's disease). In fact, this will allow to use the base editing approach, that directly convert one base or base pair into another, enabling the efficient correction of point mutations in non-dividing cells such as hepatocytes, and potentially reverting the diseased phenotype. Banked patient-specific iPSCs will represent a cell source available to establish platforms for gene editing and for drug discovery.

IMPACT

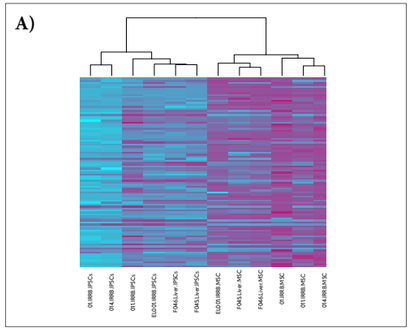
The liver transplant program at IRRCS-ISMETT includes pediatric patients with metabolic liver diseases. This is based on the fact that the majority of enzymes involved in various metabolic pathways are present within the liver and the transplant can cure or at least control the disease manifestation. If genome editing will become reality to treat these patients, this will dramatically improve their quality of life. Moreover, an in-house bank of patient-specific iPSCs offers a great opportunity to start new studies or establishing new collaborations, in fields such as gene therapy field or drug discovery, o to study pathogenic mechanisms.

RESULTS ACHIEVED IN 2021

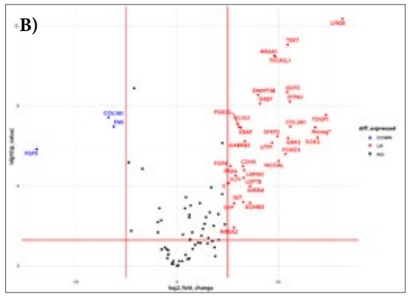
During this year, we've acquired an expertise in generation, expansion and banking of human iPSCs. Cell lines were generated by reprogramming fibroblasts of fetal dermis (4 samples) and MSCs of fetal liver (2 samples) with the non-integrating Sendai virus method. We obtained good quality iPSC colonies, stable during cultivation since the first passages (no manual cleaning required). Interesting, cell lines from the first reprogramming experiments (2-year ago) showed a prompt recovery after thawing (approx. 90% viability) and were highly proliferating, indicating their potential for banking. The induced pluripotency in reprogrammed cells was evaluated by gene expression analysis in comparison with the somatic cells of origin (TaqMan pluripotency card) and by immunofluorescence analysis of pluripotency markers (e.g., SOX2, TRA-1-60 and SSEA4). In addition, the miRNA profile of extracellular vesicle (EV- iPSCs was distinct from that of the somatic cells of origin and similar to that of embryonic stem cells, thus confirming the induced pluripotency. The *In vitro* differentiation toward endodermal lineages (definitive endoderm) was conducted in all samples and revealed 70-85% of differentiated cells.

GOALS FOR 2022

Obtaining skin biopsies from pediatric patients with metabolic liver disease to generate and bank patient-specific iPSCs (protocol submission to ISMETT's Internal Institutional Review Board for donation). A high-throughput sequencing of genomic DNA in each cell line will perforemd to confirm the genetic disease (point mutation in a single gege). Moreover, secretome and EVs will be collected from cultured iPSC colonies, and stored for future studies (e.g., disease biomarkers).

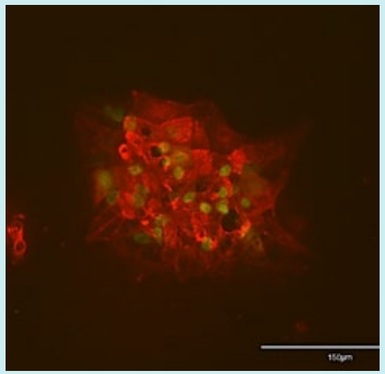


A) Hierarchial clustering of iPSC samples vs. the somatic cells of origin (skin fibroblasts).

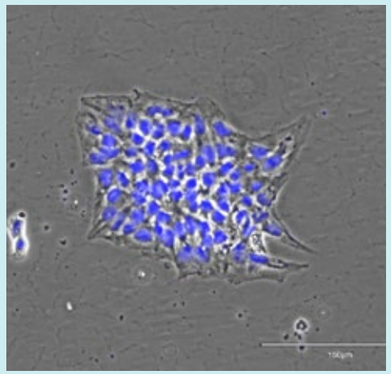


B) Volcano plots showing pluripotency genes expressed in iPSCs vs fibroblasts (MSCs)

A) Cultured iPSC colonies at passage 20.



B) Double immunofluorescence analysis with anti-SSEA4 (red) and anti-TRA-1-60 (green) antibodies, showing a positive iPSC colony.



C) DAPI for nuclear conterstain of the same colony

PRODUCTS: **Biologics**

Optimization of an anti-fibrotic therapy for liver: delivery solutions for secretome, extracellular vesicles and miRNAs based on biomaterials

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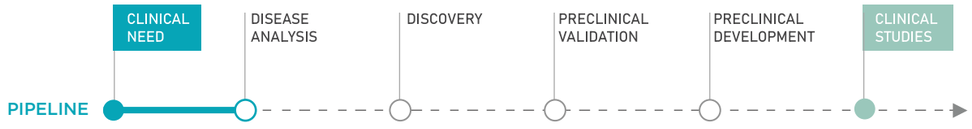
- **Lab of Biocompatible Polymers, STEBICEF, Università di Palermo, Italy**
- **Fondazione IRET, Ozzano dell'Emilia, Bologna, Italy**

THERAPEUTIC AREA

Organ insufficiences



BRIEF DESCRIPTION

Hepatic fibrosis is a chronic condition with the potential to be reverted before its progression to end-stage liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC). The current treatments do not fully reverse hepatic fibrosis, though they significantly reduce it. In addition, there is still a lack of FDA-approved methods to prevent or revert hepatic fibrosis. Due to the strong clinical needs, anti-fibrotic drugs are on phase II and III clinical trials, and lots of efforts have be made to optimize the clinical outcomes of the treatments. One reason of the low effects could be the low numbers of hepatic stellate cells (HSCs) in the liver, which only account for 5-8% of total liver cells, so that the therapeutic agents cannot easily reach them. Since activated HSCs are considered the main therapeutic target of the several anti-fibrotic treatments, a targeted delivery of therapeutic agents to activated HSCs is considered critical to enhance the effectiveness of the anti-fibrotic treatments. For example, the hyaluronic acid (HA)-based targeted delivery to activated HSCs has been tested for drug and miRNA delivery. HA is the natural ligand for CD44 receptor, which is overexpressed by activated HSCs. The approach is safe and has not been used yet to deliver secretome, extracellular vesicles (EVs) or other biological agents. Secretome/EV-based anti-fibrotic therapy for liver has been tested in several animal models (20-30% reduction). Except for one study, the administration was done without delivery devices. Therefore, the aim of the project is optimizing the efficacy of these treatments by providing HA-based delivery solutions. HA will be formulated for a targeted delivery (nanocarriers) or for a sustained release (hydrogel depot). The second formulation may work in case the first approach will result in a poor effect. Our proposed treatments include: 1) secretome; 2) EVs; 3) cocktail of specific miRNAs (e.g., anti-fibrotic and anti-inflammatory,). The project is organized in three phases:

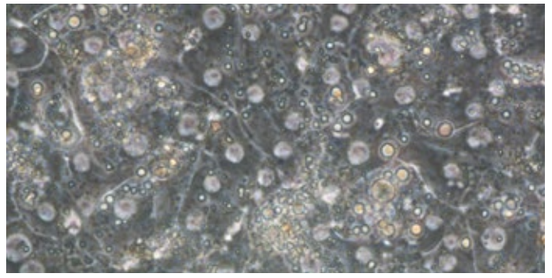
PHASE 1. Production and characterization of secretome and EVs of MSCs and iPSCs; comparative analysis of EV-miRNAs of MSCs vs. EV-miRNAs of iPSCs; selection of specific miRNAs for cocktail; validation of the efficacy in cellular models of hepatic fibrosis; computational analysis (ligand-receptor interaction) to select the HA derivative properly interacting with CD44 receptor.

PHASE 2. Production of HA-based biomaterials; integration of biomaterials with soluble products (secretome, EVs and miRNAs); *in vitro* release kinetics.

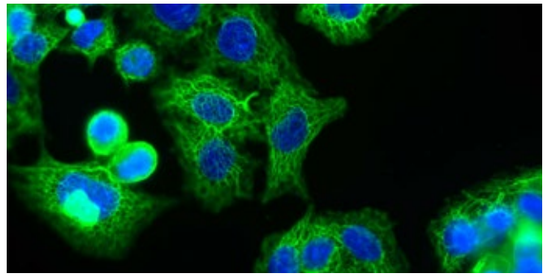
PHASE 3. Efficacy study (preclinical proof of concept) in a mouse model of hepatic fibrosis.

IMPACT

Due to the drawbacks associated with liver transplant, including organ shortage and a life-time immunosuppression, there is an urge for anti-fibrotic treatments capable to revert the hepatic fibrosis.



Human adult hepatocytes in culture



Immunofluorescence analysis of cultured human adult hepatocytes stained with anti-CK18 antibody (green) and DAPI for nuclear counterstain (blue).

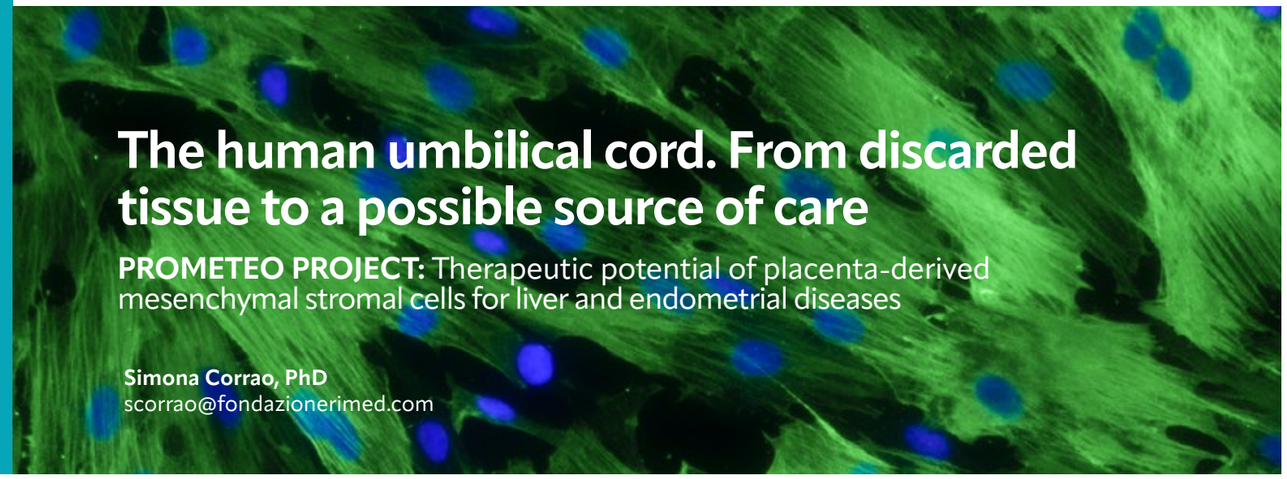
RESULTS ACHIEVED IN 2021

Comparative analysis between EV-MSCs and the EVs from the correspondent induced pluripotent stem cells (iPSCs) generated by reprogramming, revealed an enrichment in miRNAs considered anti-fibrotic and anti-inflammatory according to literature, in both EV types. Nevertheless, the higher RNA contents of EV-iPSCs versus EV-MSCs, together with the higher number of EV-iPSCs (10-fold higher) compared to EV-MSCs, obtained by processing equal volumes of secretome, suggests higher scalability for a larger production of EV-iPSCs vs EV-MSCs.

GOALS FOR 2022

In vitro efficacy tests of secretome, EVs and miRNA cocktail (dose-dependent effect) in cellular models of hepatic fibrosis; designing HA-based delivery systems (injectable nanocarriers and hydrogel depot) for a targeted delivery to HSCs and a sustained release of the several treatments; selection of the "best" HA derivative following computational analysis (ligand-receptor interaction: HA-CD44); fabrication of specific nanocarriers and hydrogel depots; integration of biomaterials with cellular products and *in vitro* release kinetics studies.

PRODUCTS: **ATMP** (Advanced Therapy Medicinal Products)



The human umbilical cord. From discarded tissue to a possible source of care

PROMETEO PROJECT: Therapeutic potential of placenta-derived mesenchymal stromal cells for liver and endometrial diseases

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COLLABORATIONS

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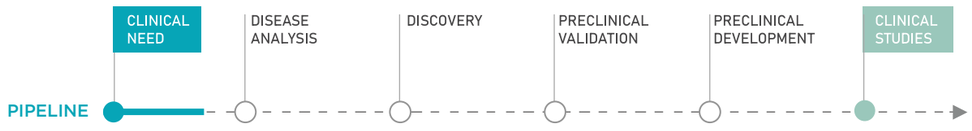
THERAPEUTIC AREA

Organ insufficiences



BRIEF DESCRIPTION

The PROMETEO Project aims to evaluate the use of perinatal tissue-derived cells for the treatment of chronic inflammatory pathologies causing organ failure. Among these, in addition to liver disease (a major causes of public health problems and death worldwide), there are also dysfunctions of the uterine wall that negatively affect the success of a pregnancy due to a thinning of the endometrial component, poorly receptive.
Umbilical cord mesenchymal stromal cells (hUC-MSCs) are extensively used in recent studies on the treatment of inflammatory states (for example, the consequences of Covid-19). The high number of stromal cells in a tissue considered as a waste, the positive impact related to ethical problems, and their immunoregulatory, anti-inflammatory and, presumably, stimulating cell regeneration, even by releasing of extracellular vesicles (EVs), make the cord a promising source of cellular products for advanced therapies.
The analyses on EVs and microRNAs (miRNAs) contained therein, in addition to the soluble factors released by hUC-MSCs in the extracellular environment, and the development of experimental models that reflect the clinical pathological aspect of the endometrium, will allow establishing a possible reparative phenomena and functional recovery of the damaged tissue.

IMPACT

If the experiments on *in vitro* models show that hUC-MSCs and their derivatives (EVs and/or soluble factors) are capable of promoting tissue regeneration and modulating inflammatory processes affecting the endometrial tissue, hUC-MSCs could be used in clinical settings for curing patients otherwise subjected to treatments that have not yet given full efficacy (endometrial scratch, immunoglobulins, glucocorticoids, anticoagulants). The high number of MSCs obtainable from each cord, the ability to activate the reparative, anti-fibrotic, anti-bacterial, and pro-angiogenic mechanisms generated by the cells or, possibly, by their secretion products (immunosuppressive molecules and miRNAs contained in EVs), could be at the basis of a high-number cell production for repairing therapy programs and restoration of tissue function. Developing cellular products, according to GMP standards, would lead to the generation of a medicinal product alternative to the usual pharmacological therapies that, in the end, can still induce serious cell damage. This would therefore favor not only a clear improvement in the quality of patients' life but also a reduction of therapeutic failures, the number of embryonic implants, and health and hospital management costs.

RESULTS ACHIEVED IN 2021

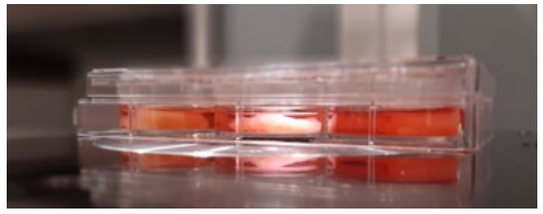
Umbilical cords, provided by the Casa di Cura Candela, were used to isolate, characterize, expand, and create a bank of hUC-MSCs for research purposes (Fig.1A). The cells obtained at p0 were by both immunofluorescence (Fig.1B) and flow cytometry analysis, using the following panel of markers:
Negative markers: HLA-DR, CD34, CD31, CD45, CD14, CD324;
Positive markers: CD105, CD90, CD73, CD166, CD146, CD29, CD13, CD44.

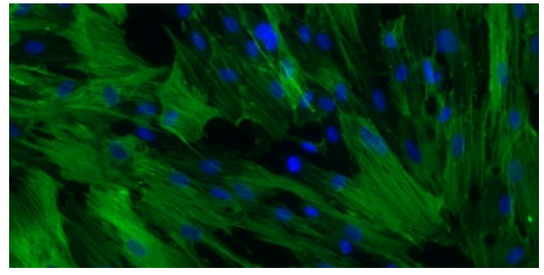
The subcultures at p1 and p2 have given information for the 'scale up' in the case of production under cGMP standards.
The EVs released from n=3 batches of hUC-MSCs at p3 were evaluated by NanoSight. The content of miRNA has been evaluated by RT-PCR in order to determine those common to all the three batches (donor-independent) and, mainly, the differential miRNAs (donor-dependent), which could lead to the creation of a donor-specific 'fingerprint'.
In collaboration with Dr. Giovanni Zito (IRCCS-ISMETT), an *in vitro* model of pathological endometrium has been devised considering the co-culture of hormone-responsive stromal and epithelial cells on which testing the repairing properties of perinatal MSCs.

Isolamento di MSCs da cordone ombelicale umano mediante tecnica non-enzimatica e follow-up della coltura al microscopio ottico

GOALS FOR 2022

The hUC-MSCs will be subjected to a highly inflammatory environment (cytokines typical of many pathologies that lead to organ dysfunction) for the subsequent analysis of release factors and miRNAs that may show a repairing, anti-inflammatory, immunomodulatory properties. Since the response given by cell-cell interaction could be significantly different, compared to the molecules in the conditioned medium alone, the hUC-MSCs should be placed in direct co-culture with appropriate cell types (endometrial, but not only). This information can open new scenarios on their use for the treatment of endometrial dysfunctions, but also of other organs extremely compromised by inflammatory events (liver, lung, cardiovascular system, nervous system). These investigations will be conducted in collaboration with IRCCS-ISMETT, and it will be of fundamental importance in the understanding of the possible tissue repair mechanisms induced by hUC-MSCs.
The creation of model with hormone-responsive endometrial cells will occupy an important part of the project for the year 2022.
Since the PROMETEO project also involves MSCs from other sources, the investigations on hUC-MSCs will be carried out in parallel for a faster achievement of the final aims of the project in its entirety and complexity. This will take into consideration the activity of a team of researchers who work in both Ri.MED and IRCCS-ISMETT.





Immunofluorescenza per l'actina del muscolo liscio (α-SMA), tipica di un sottotipo di cellule fibrogeniche attivate (miofibroblasti), espressa dalle hUC-MSCs.

PUBLICATIONS

- **Pampalone M, Corrao S, Amico G, Vitale G**, Alduino R, Conaldi PG, Pietrosi G. Human Amnion-Derived Mesenchymal Stromal Cells in Cirrhotic Patients with Refractory Ascites: A Possible Anti-Inflammatory Therapy for Preventing Spontaneous Bacterial Peritonitis. Stem Cell Rev Rep. 2021 Jun;17(3):981-998. doi: 10.1007/s12015-020-10104-8. Epub 2021 Jan 3. PMID: 33389680; PMCID: PMC8166706.

PRODUCTS: **ATMP** (Advanced Therapy Medicinal Products) - **Medical Devices & Tissue Engineering**

Development and characterization of a bioengineered bile duct

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COLLABORATIONS

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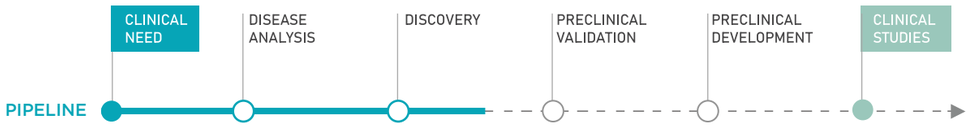
THERAPEUTIC AREA

Organ insufficiences



BRIEF DESCRIPTION

This research program focuses on the reconstruction of portions of the biliary tree using tissue engineering approaches, in which cells and biomaterials are combined to generate an implantable medical device. Our goal is to reconstruct the choledocus, which is the distal region of the extrahepatic biliary tree connecting the liver to the duodenum. The clinical need is related to a set of cholangiopathies that include biliary atresia, distal cholangiocarcinoma, choledochal cysts, and injuries resulting from liver transplantation or other procedures performed on the biliary tract or other organs of the epigastrium. These conditions mainly cause strictures that progressively lead to an inflammatory responseand possibly liver failure. Therapeutic options are limited and often require liver transplantation. However, due to the shortage of organ donors and given substantial mortality, morbidity, and economic costs associated to these diseases, new therapeutic alternatives are urgently needed. Efforts have been directed towards the bioengineering of bile ducts, however, the results of this research have been often inconclusive and poorly translatable. Our research group is currently working on this direction, optimizing both the cellular source and the biomaterials, with the prospect of creating an implantable bioartificial duct.

IMPACT

The generation of a bioartificial choledocus would represent a significant breakthrough in the field of advanced therapies for the treatment of cholangiopathies requiring the use of choledocus substitutes. Despite the need for alternatives to standard therapies, to date there is no bioengineered bile duct in the clinic for several reasons. Most of the challenges concern cells, in terms of type and source, and biomaterials, which must demonstrate unique properties in terms of biocompatibility and biodegradability. The scientific approach that we pursue aims to generate a device that can be implanted in patients. Therefore, the choice of tools to be used for its development, especially cells and biomaterials, are designed in perspective of a clinical application. Many of the previous approaches relied primarily on acellular constructs, but while some showed encouraging results, they were often inconclusive with insufficient preclinical characterization. As also highlighted by other authors, for a successful therapeutic outcome, the bioengineered bile duct must couple the biomaterial(s) with a cellular source. Unlike previous studies, our goal is to perform a deep preclinical analysis over the long term, which will pave the way for a future clinical trial.

RESULTS ACHIEVED IN 2021

During 2021, we selected the most suitable biomaterials for our device and optimized their concentration based on mechanical properties and cellular response in terms of colonization, viability and functionality. Cell density was also optimized by seeding different concentrations of cells. For preliminary characterization, we used a cholangiocarcinoma cell line, but future experiments will be performed with primary cells that we will isolate from clinical samples, thanks to the approval of the ethical protocol that we have submitted early this year.
To make a bioartificial choledochus, we have generated a poly-L-lactic acid mold by 3D printing. The mold consists of two outer walls and a central stick, and it has the same size of a human choledochus. We used two techniques to fabricate the bioartificial duct. We mixed cholangiocytes with photocollagen, and poured this mixture into the mold. After incubation at 37 °C for 30 min, we removed the outer walls of the mold while we left the stick. Following photopolymerization of the photocollagen by UV exposure, we soaked the tube in another polymer with mechanical properties. We then removed the stick, thus obtaining a tube coated with agarose only externally. The biological and mechanical properties of this construct are under investigation.

GOALS FOR 2022

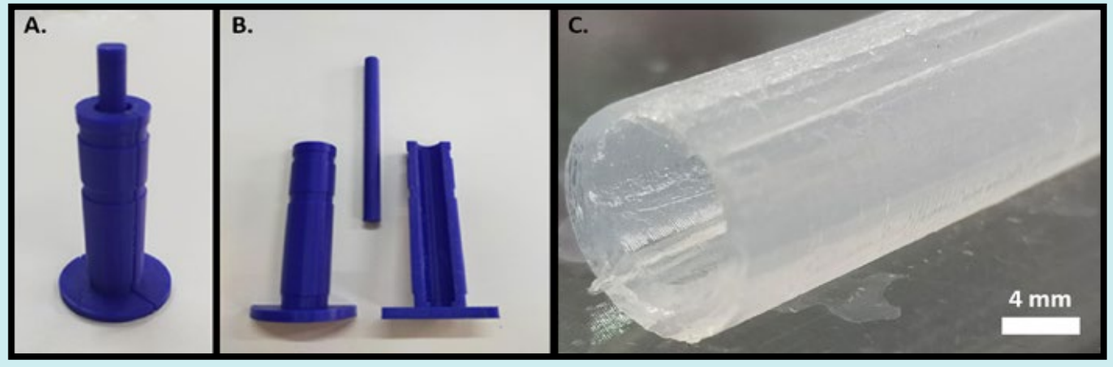
Once cell culture parameters are optimized using cholangiocarcinoma cells, our goal is to isolate primary human cells from clinical specimens, and to study cell behavior when seeded onto the biomaterials optimized during previous experiments.
The biomedical device will be further characterized from a biological and mechanical perspective. In addition, in anticipation of testing the biocompatibility, immune response, safety and efficacy of our medical device in animal models, we will write a protocol to be submitted to the O.P.B.A.

MEETINGS

Ri.MED Research Retreat, Dicembre, 2021, Palermo. Pasqua, M; Di Gesù, R; Francipane, M. G. Development and Characterization of a Bioengineered Bile Duct.

PUBLICATIONS

- **Pasqua, M., Di Gesù, R., Chinnici, C.M.,** Conaldi P.G., **Francipane, M.G.** (2021). Generation of Hepatobiliary Cell Lineages from Human Induced Pluripotent Stem Cells: Applications in Disease Modeling and Drug Screening. Int J Mol Sci. 22(15):8227. DOI: 10.3390/ijms22158227



Pictures of mold (A and B) and bioartificial choledocus (C).

PRODUCTS: **Medical Devices & Tissue Engineering**

Development of a reliable *ex vivo* osteoarthritis model responsive to mTORC1-inhibitors via oxidative stress reduction and autophagy promotion

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COLLABORATIONS

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THERAPEUTIC AREA

Organ insufficiences

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

Osteoarthritis (OA) is a chronic joint disease, which causes pain, swelling, deformation, and in the most severe cases, hypomobility of the joint. Intuitively, these pathologic conditions seriously get in the way for daily movements, and cause invalidating conditions due to the need for supporting assistance1. Hyaline cartilage is the more heavily involved joint area. However, the underlying mechanisms responsible of osteoarthritis are complex and interconnected, and interest all joint structures such as subchondral bone, synovium, muscles, and ligaments2,3. Recent epidemiologic analyses4 pointed out the age-related nature of osteoarthritis, and highlighted that the OA incidence in 75 years old population is 4 times higher than in 40 years old people for hip osteoarthritis, and 14 times higher for knee osteoarthritis. Moreover, age-related comorbidities with an incidence between 14% and 20% has been reported5. The point of contact was found in the intestinal disorder known as leaky gut (LG), which is one of the most frequent OA comorbidities age-related6. The etiology of leaky gut is reportedly connected with a dysregulation of the homeostasis in the intestinal epithelium, which is mainly composed of enterocytes covered by a layer of protective mucus. Here, cells are interconnected each others through tight junctions, a cell-cell connection which reduce to zero intercellular gaps blocking paracellular translocation of substances (Figure 1). LG is caused by the partial or total laxity of tight junctions, which promote the diffusion in the bloodstream of LPS produced by resident microbiota triggering systemic inflammatory responses (SIR) that damage joint cartilages

IMPACT

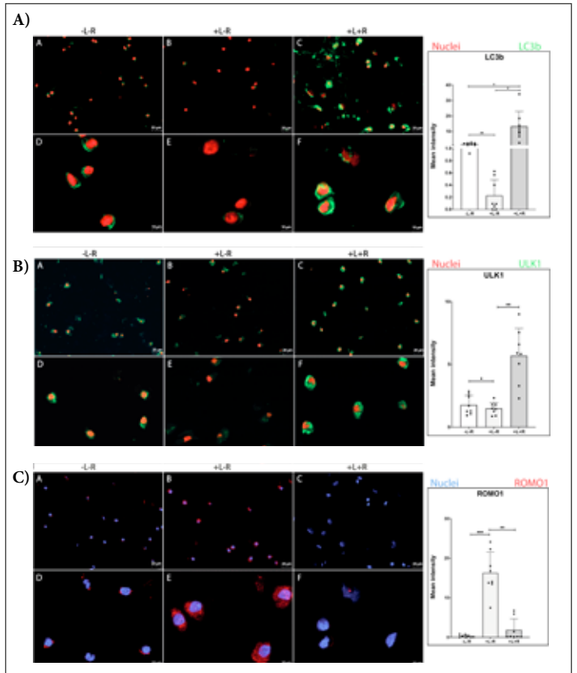
In the last decade, interdisciplinary studies involving regenerative medicine, molecular biology, and pharmaceutical chemistry, are directed to discover new pathways involved in OA to be used as target for innovative pharmacological approaches. However, the challenge is particularly hard due to heterogenous OA-related molecular mechanisms involving a wide plethora of biochemical factors. Very recently, the pathways activated by the interaction of lipopolysaccharide (LPS) with its extracellular receptor (toll like receptor 4, TLR4) in chondrocytes has been strictly correlated with the onset of osteoarthritis. Our work is included in this complex scenario, and aims to create a stimuli responsive 3D *ex vivo* model mimicking the leaky gut-related OA. Our encouraging results suggest that our model will have a strong impact in the development of new pharmacological therapy targeting osteoarthritis secondary to TLR-4 receptor activation. The model will be used as a reliable and realistic bench test for new small molecules as well as to test innovative drug delivery systems (DDS).

RESULTS ACHIEVED IN 2021

We successfully induced OA-like condition after induction a pro-inflammatory stress mimicking systemic inflammatory responses (SIR) on viable porcine osteochondral tissues. The model exhibited macroscopic modifications compatible with osteoarthritis secondary to leaky-gut condition *in vivo*. We thoroughly analyzed the TLR-4 related molecular mechanisms after the pro-inflammatory stimulation through immunofluorescence. Interestingly, we found that pathways involving autophagy-promoting factors as LC3b and ULK1 were deactivated after pro-inflammatory stimulation. Conversely, the pathway involving oxidative stress mediated by ROMO1 enzymatic complex was induced after the same stimulation. Remarkably, all modifications were reversible after treatment with an mTORC-1 inhibitor, which restored the pathways autophagy-related and inhibited the pathways responsible of the oxidative stress.

GOALS FOR 2022

The goal for 2022 is to move forward through the development of small molecules able to interact with the pathways modulated by the activation of the TLR-4. New molecules will be tested using our model as pre-clinical screening. Moreover, the model will be further optimized using an updated version of our biphasic bioreactor based on a new set-up involving the collecting systems of the apparatus.



Induction of reversible pathways associated with Osteoarthritis in vivo. Pathways involving autophagy-promoting factors as LC3b (A-green) and ULK1 (B-green) were activated after Rapamycin treatment. Conversely, the pathway involving oxidative stress mediated by ROMO1 enzymatic complex (C-red) was inhibited after Rapamycin administration

MEETINGS

29th Annual Meeting EUROPEAN ORTHOPAEDIC RESEARCH SOCIETY (EORS), September 15th-21th, 2021, Rome (Italy). Development of a realistic stimuli-responsive ex vivo OA model to study the gut-joint axis.

PRODUCTS: **ATMP** (Advanced Therapy Medicinal Products)

Multi virus-specific T cells to treat post-transplant viral infections

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COLLABORATIONS

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THERAPEUTIC AREA

Organ insufficiences

BRIEF DESCRIPTION

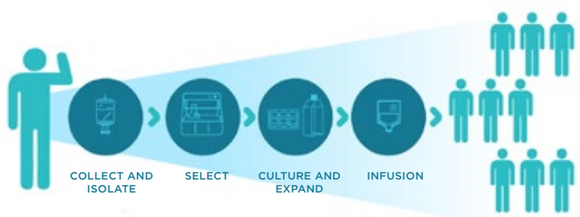
Infusion of virus-specific T lymphocytes represents a valid alternative therapeutic strategy to conventional anti-viral drugs for the treatment of virus-related complications in organ transplanted patients. In order to increase the clinical potential of this cell-based immunotherapy, we are developing in our research laboratories innovative approaches to generate and select specific multi-virus T clones.

The T lymphocyte clones, generated from healthy donors' blood, are activated *in vitro* against Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Adenovirus (ADV), BK Poliomavirus (BKV) and Herpesvirus-8 (HHV-8) using a mixture of immunodominant and interleukin viral peptides.

The creation of a multi-virus specific heterologous T lymphocytes bank, which is our final objective, would guarantee the availability of a "ready-to-use" product: multi virus specific T cells, derived from a donor who is compatible in terms of major HLA histocompatibility, to be infused into the patient when a post-transplant virus-related complication is diagnosed.

IMPACT

Infectious disease, particularly those caused by viral agents, are the main cause of post-transplant morbidity and mortality. Up to 75% of transplanted patients develop infections during the first year after transplantation. The primary cause of this phenomenon is the inhibition of the cell-mediated virus-specific immune response induced by the immunosuppressant drugs used to prevent rejection. Since T cells play a key role in the control and clearance of viral infections, the state of immunodepression promotes primary infection, reinfection or reactivation of viral agents with high prevalence, such as herpes viruses (eg EBV, CMV and HHV-8), with possible development of systemic or organ diseases. The treatment of these infections is a significant challenge because of the scarcity of antiviral drugs and their associated toxicity. An alternative treatment, now clinically validated, is the infusion of virus-specific T lymphocytes, an advanced Therapy Medicinal Product (ATMP) that enables the patient to develop *in vivo* a cytotoxic response against infected cells: an effective therapy both as a prophylaxis and as cure of virus-induced pathological manifestations which could be lethal for the patient.



RESULTS ACHIEVED IN 2021

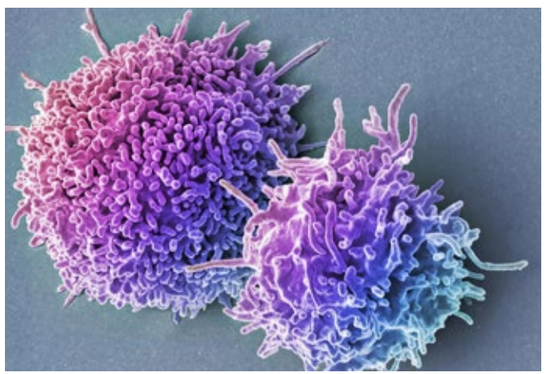
During 2021 we optimized the *ex vivo* expansion of 6 batches of multivirus-specific T cells through the use of two innovative systems for cell culture (G-Rex®10 and G-Rex®10M Open System) that allow to obtain a higher yield of the final product with minimal handling and a reduction in the consumption of reagents and growth factors. Some biological parameters, such as the concentration of glucose and lactate dehydrogenase in the culture medium, were analyzed at regular time intervals, and were used as growth indicators in alternative to the classical cell count. The quality control, specificity and potency assays (Elispot test, immunophenotype and cytotoxicity test) were also performed, which allowed us to define the best culture system for optimal growth among those tested..

GOALS FOR 2022

After the qualification of the equipment, our team will participate to the completion of basic process validation (gowning, material passage etc) and will begin the validation activity required for multivirus-specific T lymphocyte production.

PUBLICATIONS

- Complete intra-laboratory validation of a LAL assay for bacterial endotoxin determination in EBV-specific cytotoxic T lymphocyte **Salvatore Pasqua**, Maria Concetta Niotta, Giuseppina Di Martino, Davide Sottile, Bruno Douradinha, **Monica Miele**, **Francesca Timoneri**, **Mariangela Di Bella**, Nicola Cuscino, **Chiara Di Bartolo**, Pier Giulio Conaldi, and **Danilo D'Apolito** Mol Ther Methods Clin Dev. 2021 Sep 10; 22: 320–329. PMID: 34514024
- Successful Use of Heterologous CMV-Reactive T Lymphocyte to Treat Severe Refractory Cytomegalovirus (CMV) Infection in a Liver Transplanted Patient: Correlation of the Host Antiviral Immune Reconstitution with CMV Viral Load and CMV miRNome. **Miele M**, Gallo A, **Di Bella M**, **Timoneri F**, Barbera F, Sciveres M, Riva S, Grossi P, Conaldi PG. Microorganisms. 2021 Mar 26;9(4):684. doi:10.3390/microorganisms9040684. PMID: 33810329



PRODUCTS: **ATMP** (Advanced Therapy Medicinal Products) - **Biologics**

Mesenchymal Stem Cell for Liver Disease: A Possible Therapy for Preventing Spontaneous Bacterial Peritonitis

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COLLABORATIONS

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THERAPEUTIC AREA

Organ insufficiences

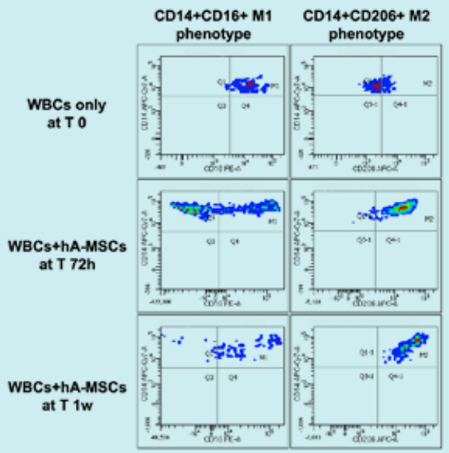
PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

Patients with liver cirrhosis have an increased risk of developing multiple organ failure due to infections caused by bacterial translocation caused by the passage of bacteria and their products, such as endotoxins, from the lumen to the intestinal wall and from the mesenteric lymph nodes to the bloodstream. Bacteria and their products are able to activate the immune system with increased release of mediators capable of inducing the systemic inflammatory response syndrome (SIRS) whose progression culminates in multiple organ failure (MOF). The functional alterations of the bacterial defenses of nonspecific and cell-mediated humoral immunity facilitate the engraftment of infections in the various sites, including ascitic fluid with the risk of developing PBS (Spontaneous Bacterial Peritonitis). In this study, was evaluated the effect of human amniotic mesenchymal cells, hA-MSCs, on the ascitic fluid of patients with liver cirrhosis in the presence of infection.

IMPACT

In patients with advanced cirrhosis, DNA-bacterial translocation induces the activation of the complement system in both plasma and ascitic fluid and activates the cell-mediated immune response and the overproduction of nitric oxide by peritoneal macrophages with a higher production of pro-inflammatory cytokines (IL-6 and TNF-α). Macrophages, innate immunity cells, represent the first line of defense against microbes and could be used as targets for the treatment of ascites in basal conditions or in the presence of over infection.



A representative dot plot showing CD14+CD16+ M1- and M2-like macrophages from total WBCs in post-paracentesis (T 0) AF, in which M1-like cells are prevalent compared with M2-like cells after 72 h of co-culture with hA-MSCs, when the high increase of M2 macrophages was observed, and after 1 week of co-culture with hA-MSCs, in which M2-like cells increased more than M1, even though the latter were still present

RESULTS ACHIEVED IN 2021

Studies were conducted to evaluate the effect of amniotic mesenchymal cells (hAMSCs) on ascitic fluid from cirrhotic patients (Child-Turcotte-Pugh B and C) undergoing paracentesis and infected with carbapenem-resistant Enterobacterales. Previous results have shown that cells in contact with ascitic fluid do not show morphological variations, proliferation inhibitions, phenotypic variations or significant necrosis /apoptosis values and a decrease in the production of pro-inflammatory cytokines associated with a greater anti-inflammatory polarization of the component. M2-like macrophage in the presence of hA-MSCs. However, the data did not show a significant decrease in the M1-like macrophage component which could determine a continuous phagocytic activity for the restoration of physiological conditions. Further studies were conducted in order to evaluate both the final bacterial load of the ascitic fluid following treatment with hA-MSCs, and tsecretome concentration of some complement proteins (MBL, Ficoline, C3a) responsible for the immune paralysis. The obtained results could be the basis for further investigating the therapeutic role of hA-MSCs in bacterial clearance and macrophage phagocytosis in PBS.

GOALS FOR 2022

Proteomic and molecular investigation of cellular components (hA-MSCs and WBCs) following *in vitro* co-culture treatment.

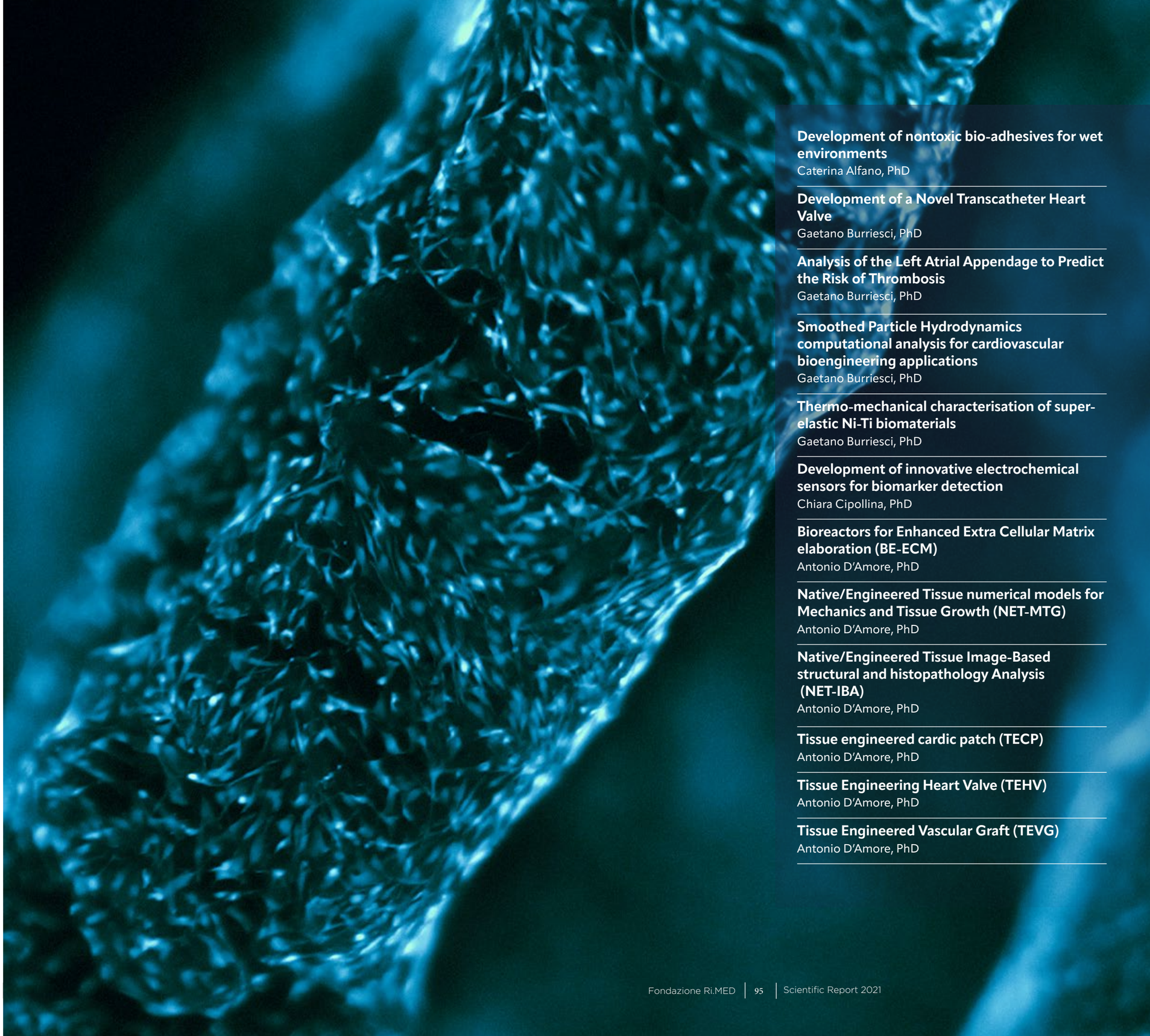
PUBLICATIONS

- **Pampalone, M., Corrao, S., Amico, G., Vitale, G.**, Alduino, R., Conaldi, P. G., & Pietrosi, G. (2021). Human Amnion-Derived Mesenchymal Stromal Cells in Cirrhotic Patients with Refractory Ascites: A Possible Anti-Inflammatory Therapy for Preventing Spontaneous Bacterial Peritonitis. Stem cell reviews and reports, 17(3), 981–998. https://doi.org/10.1007/s12015-020-10104-8
- Miceli, V., Bertani, A., **Chinnici, C.** M., Bulati, M., **Pampalone, M., Amico, G., Carcione, C.**, Schmelzer, E., Gerlach, J. C., & Conaldi, P. G. (2021). Conditioned Medium from Human Amnion-Derived Mesenchymal Stromal/Stem Cells Attenuating the Effects of Cold Ischemia-Reperfusion Injury in an In Vitro Model Using Human Alveolar Epithelial Cells. International journal of molecular sciences, 22(2), 510. https://doi.org/10.3390/ijms22020510
- Lo Nigro, A., Gallo, A., Bulati, M., **Vitale, G.**, Paini, D. S., **Pampalone, M., Galvagno, D.,** Conaldi, P. G., & Miceli, V. (2021). Amnion-Derived Mesenchymal Stromal/Stem Cell Paracrine Signals Potentiate Human Liver Organoid Differentiation: Translational Implications for Liver Regeneration. Frontiers in medicine, 8, 746298. https://doi.org/10.3389/fmed.2021.746298

BIOENGINEERING AND TISSUE ENGINEERING

The third macro-area of interest, Bioengineering and Tissue Engineering is focused on the simulation of physiological systems, the study of biomaterials and engineered tissues to improve the understanding of pathological mechanisms and tissue regeneration, and for the development of new therapeutic solutions. Researchers in this macro-area can design, develop, and arrive at preclinical validation of next-generation implantable organs and devices, particularly in the cardiovascular field. Significant results were achieved in 2021, both in terms of basic research and in terms of direct applications for patients. In particular, new approaches to characterize biological tissues and biomaterials and scaffold fabrication were investigated.

A greater understanding of the biological and physiological mechanisms responsible for adhesion in wet environments, cell proliferation, and the onset of thromboembolic events in pathological and post-procedural situations has been gained. In addition, important developments have been found in the preclinical, in vitro and in vivo evaluation of innovative cardiovascular solutions, including engineered cardiac patches and novel heart valves made using biostable polymers, engineered tissues or genetic engineering applications.



Development of nontoxic bio-adhesives for wet environments
Caterina Alfano, PhD

Development of a Novel Transcatheter Heart Valve
Gaetano Burriesci, PhD

Analysis of the Left Atrial Appendage to Predict the Risk of Thrombosis
Gaetano Burriesci, PhD

Smoothed Particle Hydrodynamics computational analysis for cardiovascular bioengineering applications
Gaetano Burriesci, PhD

Thermo-mechanical characterisation of super-elastic Ni-Ti biomaterials
Gaetano Burriesci, PhD

Development of innovative electrochemical sensors for biomarker detection
Chiara Cipollina, PhD

Bioreactors for Enhanced Extra Cellular Matrix elaboration (BE-ECM)
Antonio D'Amore, PhD

Native/Engineered Tissue numerical models for Mechanics and Tissue Growth (NET-MTG)
Antonio D'Amore, PhD

Native/Engineered Tissue Image-Based structural and histopathology Analysis (NET-IBA)
Antonio D'Amore, PhD

Tissue engineered cardic patch (TECP)
Antonio D'Amore, PhD

Tissue Engineering Heart Valve (TEHV)
Antonio D'Amore, PhD

Tissue Engineered Vascular Graft (TEVG)
Antonio D'Amore, PhD

PRODUCTS: **Medical Devices & Tissue Engineering**

Development of nontoxic bio-adhesives for wet environments

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COLLABORATIONS

- UK Dementia Research Institute (UK DRI) - King's College London, Londra, Regno Unito
- Istituto di Biofisica (IBF) – Consiglio Nazionale delle Ricerche (CNR), Palermo, Italy
- European Synchrotron Radiation Facility (ESRF), Grenoble, France

THERAPEUTIC AREA

Organ insufficiences

BRIEF DESCRIPTION

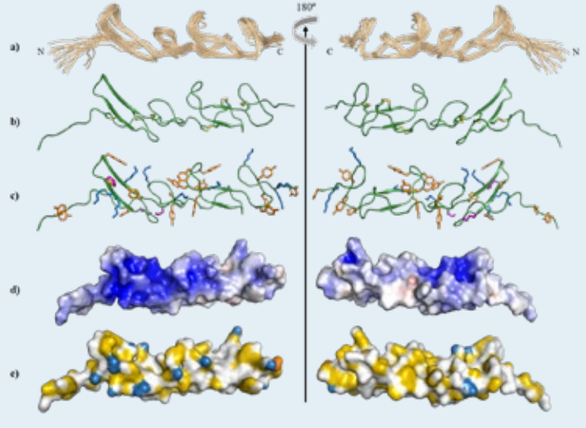
Mussels have developed a strategy for strong underwater adhesion through the secretion of a protein-based stringy appendage called byssus, formed by bundles of fibers inter-twinned together. Each filament ends with a protein-rich plaque, containing the mussel foot proteins (mfps) characterized by adhesive features, that acts as a water-resistant glue and allows mussels to firmly anchor to different substrates. A peculiarity of mfps is that they contain catecholic amino acid 3,4-dihydroxy-l-phenylalanine (DOPA), a derivative of tyrosine obtained by post-translational modification which is able to bind to a wide variety of substrates. However, it is becoming increasingly clear that the presence of DOPA is not necessary to produce strong wet-resistant adhesion, and DOPAminated proteins do not have higher adhesive properties than the corresponding non-DOPAminated versions. We have previously characterized Pvfp-5β, one of the mfp proteins from the Asian green mussel Perna viridis. Pvfp-5β is first to be secreted and to establish interaction with the substrate making it a system of particular interest. We demonstrated that it is possible to produce recombinant Pvfp-5β in bacteria and that it has low toxicity and intrinsic adhesive properties also in the absence of DOPA. We then made another important step forwards the possibility of understanding the structural determinants of the adhesive properties of Pvfp-5β by solving its 3D structure in solution and characterizing the dynamics of the protein, in view of using it for biological and biotechnological applications.

IMPACT

The development of novel naturally-derived glues has a great impact in areas such as tissue engineering, implantation of medical devices, regenerative medicine and surgery. Indeed, there are situations where more traditional techniques such as suturing are impracticable and the use of tissue adhesives becomes particularly crucial. In the last 30 years, bio-adhesives have been changing the surgical process with increasing importance and rapid development. Indeed, bio-adhesives are less invasive, have less possibility to damage the tissues, better immobilization and the ability to promote wound healing through different mechanisms, thanks to their antibacterial, anti-inflammatory, and antioxidant properties. 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. In nature several marine organisms, such as mussels, manage to adhere so firmly to wet surfaces to resist the strength of tides and stormy waves. Bio-inspired proteins from sessile animals with adhesive properties in water are then very attractive, also because their properties of being biodegradable, usually nontoxic to the human body and do not easily elicit strong immune response. The full understanding of the adhesive mechanisms involving mfps is thus paramount for developing biomaterials with properties that could be exploited not only in regenerative medicine, tissue engineering but also material science.

RESULTS ACHIEVED IN 2021

We solved the NMR solution structure of Pvfp-5β, one of the mpfs secreted by the Asian green mussel Perna viridis. Among the proteins produced by this organism, which include also Pvfp-3α and Pvfp-6, Pvfp-5β is first to be secreted and to establish interaction with the substrate making it a system of particular interest. Pvfp-5β structure, highly rigid except for a few residues affected by slow local motions in the µs-ms time scale, comprises of an elongated β-rich fold formed by two tandem EGF-like motifs. The overall rigidity, favored by the presence of disulfide bridges, could be a main structural feature to perform its function, favoring the persistent exposure of tyrosine and lysine residues to the solvent, maximizing the interaction with surfaces and/or other proteins. We also generated docking models of all the three possible protein-protein interactions: Pvfp-5β/Pvfp-5β, Pvfp-5β/Pvfp-3α e Pvfp-5β/Pvfp-6. These preliminary studies, allowed to identify the surfaces and type of interactions (cation−π, π−π, hydrogen bonds) involving the three proteins during the coacervation process occurring in mussel adhesion. Overall, our work constitutes a direct structural attempt to understand the molecular recognition of mussel proteins at the molecular level.



Struttura NMR in soluzione di Pvfp-5β:
a) Sovrapposizione delle 20 strutture a più bassa energia. **b)** Rappresentazione cartoon della struttura a più bassa energia con ponti disolfuro in giallo. **c)** Rappresentazione cartoon della struttura a più bassa energia con tirosine, lisine e arginine evidenziate rispettivamente in arancione, blu e magenta. **d)** Potenziale elettrostatico superficiale con residui acidi in rosso e basici in blu. **e)** Superficie idrofobica.

GOALS FOR 2022

Several questions remain open on the coacervation process in mussel adhesion: we do not know, for instance, the complex stoichiometry or the relative contribution of the different components, nor the precise kinetics of events that may take place. We also do not know precisely how the presence of DOPA could influence the binding mode. More work is then necessary to address these important open questions. Our next step will consist in studying the self-assembly process of Pvfp-5β and, in parallel, in expressing and purifying Pvfp-3α to determine the 3D structure in solution and to obtain experimental evidences of the interaction mode with Pvfp-5β. To this aim, we will use several biophysical techniques such as Circular Dichroism, Mass Spectrometry, Nuclear Magnetic Resonance, and Isothermal Titration Calorimetry.

MEETINGS

- CCPNMR Conference, August, 2021, *online*
- Workshop on Integrative modelling of biomolecular complexes with HADDOCK, 2021, *online*

PUBLICATIONS

- **Morando M.A., Venturella F., Monaca E., Sabbatella R.**, Rosa Passantino, Pastore A., **Alfano C.** (2021) The solution structure of recombinant Pvfp-5β reveals new insights into mussel adhesion. Comm Biol. Submitted

PRODUCTS: **Medical Devices & Tissue Engineering**

Development of a Novel Transcatheter Heart Valve

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COLLABORATIONS

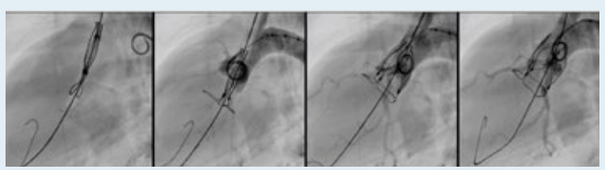
- University College London (UCL), London, UK
- Barts Heart Centre, London, UK

THERAPEUTIC AREA

- Organ insufficiences
- Aging diseases

BRIEF DESCRIPTION

Though standard open heart surgical aortic valve replacement has represented an effective treatment in the past, it is not ideal for the new patients' population. In fact, degenerative aortic stenosis due to senile valve calcification has now become the most common valvular disease, affecting more than 10% of adults older than 75 years. Due to the patients' age, this condition is often associated with relevant comorbidities and previous surgery, that increase dramatically the risks of mortality from surgery. As a result, about one third of elderly patients with symptomatic aortic stenosis are currently declined for surgery; and this number is rapidly rising due to the increasing longevity of the population. Transcatheter aortic valve implantation (TAVI) represents an ideal response to the needs of this rapidly expanding patients' population, as it allows delivering a valve substitute into the anatomical site through the vascular system, avoiding the need of open-heart surgery and its associated risks. Clinical experience with this novel approach has clearly indicated that it is effective, though it still requires substantial design improvements to enhance the safety and effectiveness of the treatment. This project involves the development of a novel prosthetic aortic valve suitable for TAVI implantation, which would overcome the main limitations experienced with currently available solutions.



Sequence of implantation of the device

IMPACT

The work performed as part of this project demonstrated the feasibility of a new transcatheter heart valve concept, the TRISKELE system, characterised by a self-expanding nitinol wireframe, polymeric leaflets and a sealing cuff. This device offers significant improvements compared to current products used in TAVI practice, by providing a simpler and more reliable solution at a significantly lower cost. Moreover, the anchoring of the device, which the animal models have demonstrated to be achievable without presence of calcification calcification, reveals the potential for this system to expand the therapeutic advantages of transcatheter valve implantation to the class of patients suffering from aortic insufficiency, for which first generation TAVI is unsuitable.

Picture of the TRISKELE transcatheter heart valve system

RESULTS ACHIEVED IN 2021

During 2021, an important collaboration agreement with cardiovascular industries and venture capital firms was defined, resulting in the licensing of the intellectual properties related to the device and its delivery system owned by the Foundation. This marks the passage of the project to the technology transfer phase, which will see the Foundation work in direct cooperation with the industrial partners to facilitate the finalisation of the development of the device and its introduction in the market. The optimisation and industrialisation phase and the setting of the chronic and acute preclinical *in vivo* tests necessary for the subsequent clinical phase have already been started.

GOALS FOR 2022

It is expected that the optimisation and industrialization of the device will be completed in 2021. The delivery system will be redesigned to adapt it to industrial production and the *in vivo* evaluation phase will be undertaken. The collaboration with the industrial partners will also expedite the analysis of related non-clinical aspects (shelf life, packaging, accessories etc.), the final design dossiers compilation, and the organisation of the clinical investigation to successfully complete the translation of the device to the clinic.
In addition, the bioengineering group will collaborate on extending the operating principles of the TRISKELE to other valve districts, defining with the partners a new device suitable for transcatheter replacement of the mitral valve.

INTELLECTUAL PROPERTY

- Seifalian, A. M., Zervides, C., **Burriesci, G**. (2019) Heart Valve Prosthesis. Patent US10357358 B2
- **Burriesci, G.**, Bozkurt, S., Rahmani, B., Mullen, M.J. (2018) Prosthesis heart valve. Patent US2018161155 A1
- **Burriesci, G.**, Bozkurt, S., Rahmani, B., Mullen, M.J. (2018) Prosthesis heart valve. Patent EP3310301 A1
- **Burriesci, G.**, Tzamtzis, S., Seifalian, A. M. (2017) Prosthesis delivery system. Patent US9597211 B2
- **Burriesci, G.**, Bozkurt, S., Rahmani, B., Mullen, M.J. (2018) Prosthesis heart valve. Patent US2018161155 A1
- **Burriesci, G.**, Bozkurt, S., Rahmani, B., Mullen, M.J. (2018) Prosthesis heart valve. Patent EP3310301 A1
- **Burriesci, G.**, Tzamtzis, S., Seifalian, A. M. (2017) Prosthesis delivery system. Patent EP2629700 B1
- **Burriesci, G.**, Tzamtzis, S., Mullen, M. J., Seifalian, A., Yap, J. (2017) Vascular implant. Patent EP3043746 B1
- **Burriesci, G.**, Seifalian, A. M., Zervides, C. (2017) Heart Valve Prosthesis. Patent EP2413842 B1
- **Burriesci, G.**, Bozkurt, S., Rahmani, B., Mullen, M.J. (2016) Prosthetic heart valve. Application Number: WO2016203241 A1
- **Burriesci, G.**, Tzamtzis, S., Seifalian, A. M. (2012) Prosthesis delivery system. Patent WO2012052718
- **Burriesci, G.**, Seifalian, A. M., Zervides, C. (2010) Heart valve prosthesis. Patent WO2010112844 A1

PRODUCTS: **Biomarkers - Medical Devices & Tissue Engineering**

Analysis of the Left Atrial Appendage to Predict the Risk of Thrombosis

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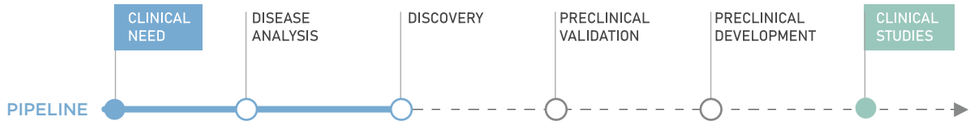
COLLABORATIONS

- University College London (UCL), London, UK
- Great Ormond Street Hospital (GOSH), London, UK - Università degli Studi di Palermo, Palermo, Italy

THERAPEUTIC AREA

- Organ insufficiences
- Aging diseases



BRIEF DESCRIPTION

Atrial fibrillation (AF) is a pathological condition characterised by an irregular heart contraction. AF can lead to serious complications such as stroke, ischemic attacks and dementia, due to related thromboembolic events, 90% of which originate in the left atrial appendage (LAA). This is a sac of muscle tissue protruding from the left atrium (LA). A number of studies were recently conducted by few research groups, focusing on LA patient specific morphologies. However, the causes and mechanisms responsible for thromboembolism still remain unclear.

In this project, patient specific models of the LAA integrating the wall motions typical of the cardiac process (neglected in previous CFD studies) were created. These allowed a more comprehensive analysis of the hemodynamic phenomena that occur in normal conditions and after the alterations produced by AF.

This project involves the participation of Dr **Alessandra Monteleone** and **Danila Vella** (Ri.MED) and of **Mr Giulio Musotto** (Ri.MED and University of Palermo).

IMPACT

Currently available literature mostly focuses on the analysis of the role of the appendage morphology (this is highly variable from patient to patient) on thrombus formation, relying on rigid-wall models which neglect the changes in wall contractility produced by fibrillation. In this study, instead, computational fluid dynamics approaches are applied to model the contribution of the wall motions of the appendage in both healthy and pathological conditions. The study clearly indicates the contractions of the left atrial appendage as an essential functional factor to maintain healthy fluid dynamic features, identifying its impairment as the primary factor enforcing flow conditions typically associated with clot formation.

Regarding the LAA inversion, this study represents the first systematic analysis of the phenomenon. Its results are providing important indications to identify its causes and provide clinicians with useful information both to prevent it and to intervene promptly to restore the physiological conditions of the heart.

RESULTS ACHIEVED IN 2021

Four patient specific models of the left atrium were generated to describe healthy and AF altered conditions, modelling active contractility through the setting of a virtual contraction calibrated on clinical evaluations of filling and emptying the chamber. These were synchronised with boundary conditions imposed on the fluid to replicate the physiological atrial pressure typical of healthy and AF conditions. The study has indicated that the hemodynamics in the LAA is strictly influenced by the active contraction phase, which appeared to be substantially more critical than the effect of the LAA shape.

The inversion of the four appendages was also simulated, applying a variable suction inside the chambers. The results indicate that, in this case, LAA morphology has a dominant role, and suggest new percutaneous maneuvers able to restore physiological conditions.

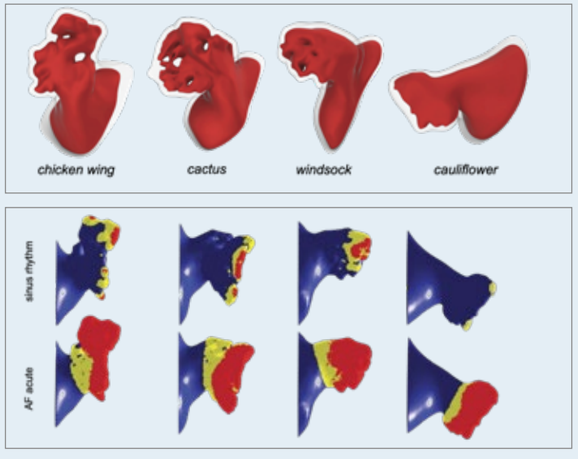
GOALS FOR 2022

The next step will require refining the models further and verifying the effectiveness of the current classification, based the global gross shape of the LAA rather than on the local topological features that our analysis indicates as more relevant. Then, more relevant descriptors will be identified, able to support a more effective stratification of the thromboembolic risks in AF patients.

Regarding the LAA inversion, the study will be extended to other patient specific models, to clarify the morphological factors that promote the phenomenon, with particular attention to the role of trabeculations, whose distribution and size have resulted very variable from patient to patient.

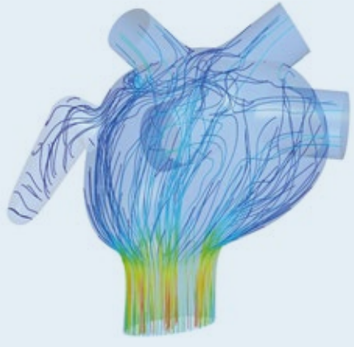
PUBLICATIONS

Vella, D., Monteleone, A., Musotto, G., Bosi, G.M., **Burriesci, G.** (2021) Effect of the Alterations in Contractility and Morphology Produced by Atrial Fibrillation on the Thrombosis Potential of the Left Atrial Appendage. Front. Bioeng. Biotechnol. 9: 586041. doi: 10.3389/fbioe.2021.586041



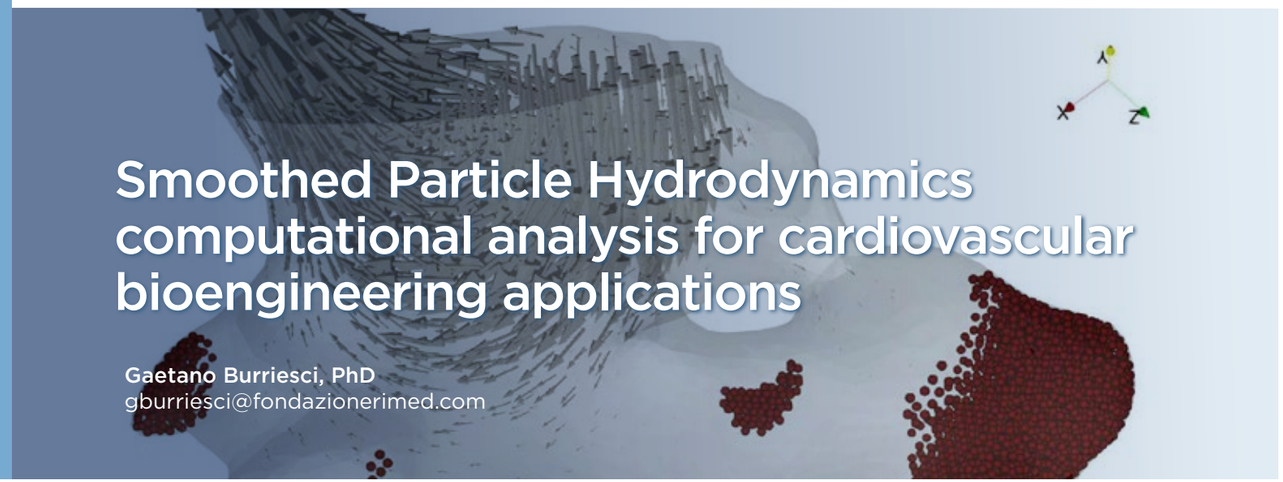
Patient specific models of the LAA implemented and analised in the described study.

Regions of the LAA where our study indicates higher risk thrombosis for the different patients, in heathy (top) and AF (bottom) conditions.



Streamlines predicted numerically in the aortic chamber for healthy operating conditions.

PRODUCTS: **Biomarkers - Medical Devices & Tissue Engineering**



Smoothed Particle Hydrodynamics computational analysis for cardiovascular bioengineering applications

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COLLABORATIONS

- Università degli Studi di Palermo, Palermo, Italy
- University College London (UCL), Londra, Regno Unito

THERAPEUTIC AREA

- Organ insufficiences
- Aging diseases

BRIEF DESCRIPTION

Thrombosis is a pathology leading to clot formation, that can result into arterial obstructions and, eventually, migrate through the cardiocirculatory system causing heart attack, stroke or pulmonary embolism. It is a complex process whose mechanisms are still unclear, due to the contribution of various factors including platelet activation and aggregation, chemical interaction of the involved reactants and hemodynamics. Since the available analytical solutions are often inadequate and too complex to find practical application, research is increasingly evolving towards the use of computational methods, stimulated by the recent advances in computational processing.
This study aims at analysing the formation, growth and evolution of thrombus by means of Smoothed Particle Hydrodynamics (SPH) numerical method coupled with a fluid structure interaction (FSI) model. Contrary to traditional and widely adopted Eulerian methods, SPH is a meshless Lagrangian approach. This makes it particularly suitable to realistically capture the multi-physical interaction between blood flow and thrombi. Moreover, the FSI model allows to study the thrombus formation by means of forces between particles which are linked through spring law. The concentration of the biochemical species involved in the coagulation cascade can be modelled by means of convection-diffusion equations.

This project involves the participation of Dr Alessandra Monteleone (Ri.MED) and Dr Alessia Viola (Ri.MED and University of Palermo).

IMPACT

Thrombosis is a relevant problem in the design and implementation of vascular protheses and artificial organs such as artificial heart valves. The main challenge for prevention and treatment of this pathology is represented by the poor knowledge of the mechanisms involved. The study of the hemodynamics can provide an effective support to identify and prevent the risk of thrombosis.
This research project aims to implement a numerical platform to support the diagnosis and treatment of cardiovascular diseases by simulating hemodynamics and thrombus formation. In future, employing patient-specific models, this diagnostic tool could support the development of new devices by allowing the evaluation of their performance, safety and potential improvements, prior to prototyping. Furthermore, the accurate modelling of several pathologies (such as atrial fibrillations) would provide further clarifications and indications for their mitigation and treatment.

RESULTS ACHIEVED IN 2021

In 2021, several new modules were implemented and integrated into the open-source code Panormus (developed at the University of Palermo) which contains a SPH solver. In particular, a module enabling computational structural mechanics was introduced, which allows the representation of solid structures and their response. This structural model was then coupled with the fluid dynamic model described with the SPH method by implementing a FSI partitioned approach. Moreover, a monolithic FSI approach has been implemented, able to describe formation, growth and lysis of thrombi and the interaction with blood flow. The thrombus formation is modelled through the main enzymatic reactions of the coagulation cascade. In particular, the model considers two biochemical species, thrombin and fibrin, and two states for the platelets, activated and bound aggregated. When specific conditions are satisfied, fluid particles can be turned into a solid phase by means of the introduction of internal spring connections.

The new developed modules have been validated against suitable reference cases available in the literature.
The complexity of the thrombosis phenomena is amplified in regions where the fluid is affected by the large deformation of functional immersed structures interacting with a pulsatile blood flow. In order to analyse the whole process, the monolithic FSI approach was extended to describe the aortic valve behaviour to evaluate the effect of the leaflets dynamics on the haemodynamics.

GOALS FOR 2022

Next steps of the research project will involve the enrichment and improvement of the modules recently implemented in the SPH code for bioengineering applications. To this end, the aspects concerning the fluid-structure interaction and the modelling of thrombus formation, growth and dissolution will be deepened and enriched. As regard the structural model, complex non-linear constitutive behaviour characterising soft tissues will be introduced as well as the anisotropic behavior of such materials. With regard to the thrombus model, it is planned to introduce the modelling and analysis of two different types of clot: the first resulting from platelet adhesion and the second induced by fibrins. Furthermore, it is planned to introduce the description of anticoagulant and antiplatelet agents and their effect on the different type of clot. Finally, the model will be applied to describe complex real conditions such as heart valve disfunctions, arterial stenosis, cerebral aneurysm, aortic abdominal or for the evaluation of the thromboembolic risk in the left atrial appendage when atrial fibrillation condition occur.

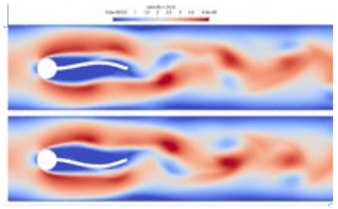

Figure: Benchmark test case for the validation of the partitioned FSI approach: interaction between an elastic beam and laminar incompressible fluid flow

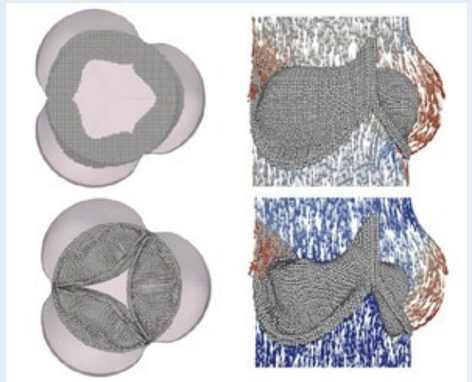

Figure: Application of the monolithic FSI approach to describe the aortic valve dynamics (leaflets represented with grey points)

Figure: Formation and evolution over time of a blood clot (brown region) immersed in the blood stream (red region).

PRODUCTS: **Medical Devices & Tissue Engineering**

Thermo-mechanical characterisation of super-elastic Ni-Ti biomaterials

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COLLABORATIONS

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- University College London (UCL), London, UK

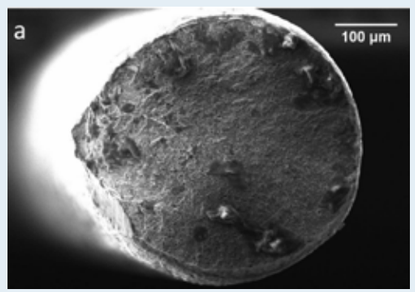
THERAPEUTIC AREA

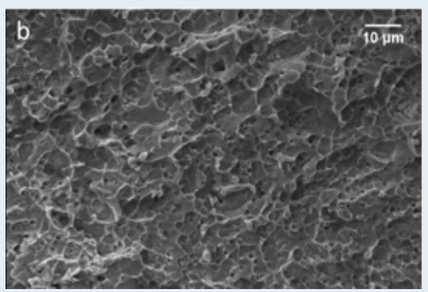
- Organ insufficiences
- Aging diseases

BRIEF DESCRIPTION

Nitinol is a biocompatible alloy commonly used in a number of medical implants, such as angioplasty stents, transcatheter heart valves and dental implants, due to its unique super-elastic behaviour. However, the mechanisms responsible for its uncommon mechanical response are still unclear and, therefore, not fully exploited. In particular, the super-elastic behavior is due to a reversible stress-induced transformation from an austenitic to a martensitic crystal configuration, associated with a release/absorption of heat. In this study we exploit these thermal effects to gain a better understanding of the phenomenon and a more accurate characterisation of nitinol.

This project involves the participation of Ms **Sofia Di Leonardo** (Ri.MED and University of Palermo). and Ms **Valentina Pinto** (Ri.MED and University of Palermo).





SEM image of the fracture surface of a nitinol wire, in proximity of a micro laserwelded region. The analysis of the micromorphology indicates a ductile behaviour, confirming that the adopted welding methodology preserves the mechanical characteristics of the super-elastic alloy.

IMPACT

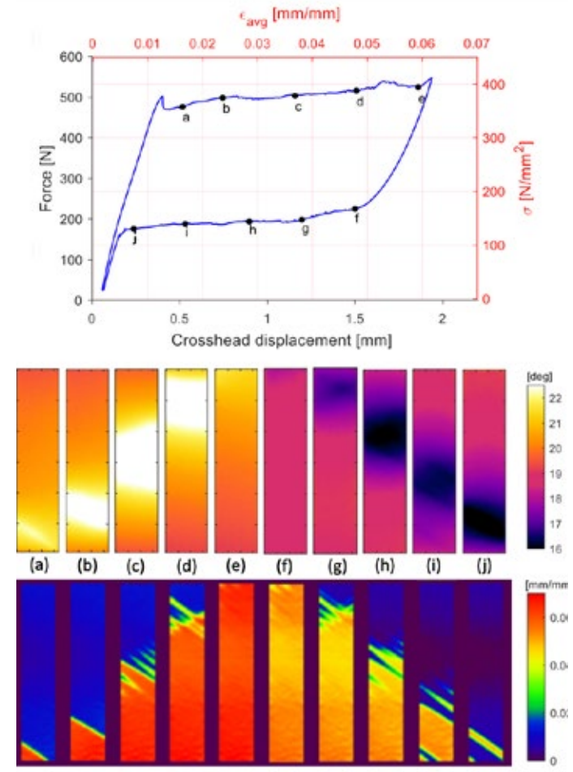
A more accurate understanding and characterisation of nitinol's behavior would contribute to increase the safety of medical devices based on this material. The combined implementation of Digital Image Correlation and Infrared Thermography optical techniques, adopted in this study, can support the evaluation of the thermo-mechanical behaviour of this complex material in critical biomedical applications, extending their potential durability.

RESULTS ACHIEVED IN 2021

The complex thermomechanical nitinol behaviour was investigated through the combined implementation of full field techniques, in order to identify the local mechanical characteristics of the material. In particular, the Digital Image Correlation technique (DIC), an innovative optical technique that allows the measurement of the strain state, was integrated with the Thermoelastic Stress Analyses (TSA). The latter allows to detect the temperature variations of components under cyclic loading, through infrared camera acquisition. This will facilitate the identification critical surface stress states through the analysis of the thermal signal in the frequency domain. The combined application of the DIC and TSA techniques has shown the ability to analyse complex local responses of the material, highlighting spurious bending moments related to the specimen inhomogeneity under load and the inadequacy of common thermoelastic formulations.

GOALS FOR 2022

Future studies will focus on the thermoelastic effect interpretation in the frequency domain. In particular, the study will aim to identify the most suitable formulations for the description of Nitinol's thermomechanical behaviour, obtaining full-field stress maps from temperature and deformation maps. This will allow the study of the regions subjected to high mechanical stress, providing a much-needed tool for the experimental analysis of medical implants and for the implementation of new, more appropriate numerical models of the material response in computational analyses.

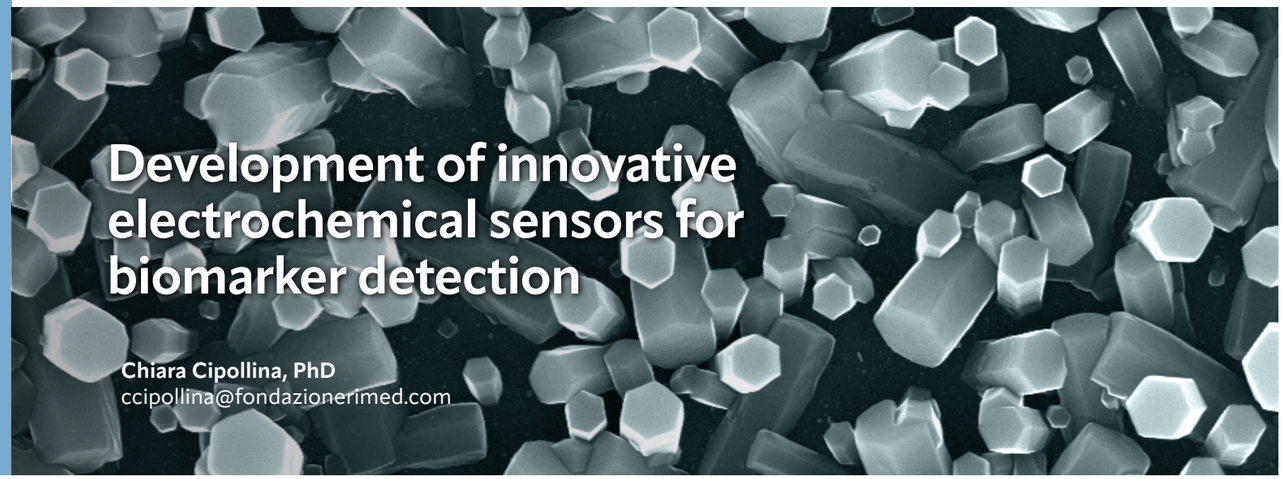


Load-displacement / stress-strain curve of a quasi-static test (top row) and thermal (middle row) and deformation (bottom row) maps related to selected points along the upper (a-e) and lower (g-j) plateau of phase transformation.

PUBLICATIONS

S. Di Leonardo, R. Cappello, **G. Burriesci,** G. Pitarresi, Investigation of the Thermomechanical Response of Cyclically Loaded NiTi Alloys by Means of Temperature Frequency Domain Analyses, Materials (Basel). 14 (2021) 7866. https://doi.org/10.3390/ma14247866.

PRODUCTS: **Medical Devices & Tissue Engineering**



Development of innovative electrochemical sensors for biomarker detection

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COLLABORATIONS

- Istituto per la Ricerca e l'Innovazione Biomedica (IRIB) - CNR, Palermo, Italia
- Istituto di Farmacologia Traslazionale (IFT) - CNR, Palermo, Italia
- Dipartimento di Ingegneria - Università degli Studi di Palermo, Palermo, Italia

THERAPEUTIC AREA

Aging diseases

Sensore Nanostrutturato per Stress Ossidativo

Il Progetto n. 082651290364 – "Se.N.S.O.", CUP G78I18000930007 è stato cofinanziato dalla Regione Siciliana, Assessorato delle Attività Produttive nell'ambito dell'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" del PO FESR Sicilia 2014/2020."

BRIEF DESCRIPTION

Nanostructured electrochemical sensors for quantitative detection of biomarkers in biological fluids are attracting growing attention. In an electrochemical sensor, the signal is generated from the redox reactions that takes place on the electrode surface. Electrochemical techniques possess several advantages compared to current approaches such as low cost, ease of use, even for unskilled personnel, high sensitivity and fast response times. Recently, wearable devices, implemented with wireless technology for signal transduction, have been also proposed for the direct and non-invasive detection of biomarkers in biological fluids (e.g., sweat, saliva and exhaled breath condensate). This may represent a valuable approach in telemedicine to easily monitor disease progression and response to therapy.
In collaboration with the Engineering Department (University of Palermo) and IRIB-CNR Institute, we are currently working to develop and validate in real operating conditions a nanostructured electrochemical sensor for the quantification of the hydrogen peroxide released by living cells. This activity has been funded under the call P.O. FESR Sicilia 2014/2020 – project Se.N.S.O. We are also working on the development of an innovative biosensor for the detection of asthma biomarkers in biological fluids (Industrial PhD project funded by PON R&I, 2014-2020).

IMPACT

The trend towards personalized medicine requires the development of innovative approaches to finely define patient characteristics and monitor the response to therapy. Under this scenario, the development of innovative biosensors able to quantify biomarkers in biological fluids, will provide new tools that will positively impact the progresses of personalized medicine and telemedicine.
Of note, the impact of our current work goes beyond the field of chronic airways diseases. In fact, oxidative stress is a condition that characterizes most inflammatory and age-related diseases. Also, the technology that we will develop for the detection of asthma biomarkers, can be easily extended to any other biomarker of interest.

RESULTS ACHIEVED IN 2021

During 2021, we have completed the set-up of all the biochemical assays and experimental models required for the validation of the electrochemical sensor for hydrogen peroxide developed by the colleagues at the Engineering Department within the project Se.N.S.O. Preliminary validation assays in real operating conditions have confirmed that the newly developed sensor is able to quantify hydrogen peroxide release from living human macrophages and bronchial epithelial cells (both primary cells and cell lines).
Furthermore, we have set-up the experimental platform required for the development and validation of electrochemical immunosensors for detection of asthma biomarkers.
In 2021, we have been awarded a research grant for a project entitled "Sensomar – sensor integrated in a mask" under the call FISR 2020 (56K EUR).

GOALS FOR 2022

During 2022, following the timeline of the project Se.N.S.O. we will continue with the validation of the sensor for hydrogen peroxide detection by assessing its performance under different experimental conditions and using different models. We will correlate hydrogen peroxide release with parameters of inflammation and cell damage. These activities will lead to the development and validation of a first portable device that will be fabricated in collaboration with our industrial partners.
During 2022, we aim at completing the proof-of-concept production of a nanostructured immunosensor for the quantification of a selected asthma biomarker in biological fluids. Finally, we will conduct the activities planned within the project Sensomar that will result in the creation of a proof-of-concept sensor for hydrogen peroxide integrated in a mask.

MEETINGS

- *Electrochemical sensor for evaluating oxidative stress in airway epithelial cells*, Di Vincenzo S, Patella B, Ferraro, Bollaci L, Buscetta M, Cipollina C, Aiello G, Inguanta R, Pace E. European Respiratory Society (ERS) Congress 2021, 5-8 September 2021, virtual. Published on European Respiratory Journal 58 (suppl 65) PA3704; DOI: 10.1183/13993003.congress-2021.PA3704
- *Electrochemical quantification of oxidative stress in airway epithelial cells*, Patella B, Di Vincenzo S, Ferraro M, Buscetta M, Pace E, Cipollina C, Aiello G, Bollaci L, Inguanta R. 72nd Annual Meeting of the International Society of Electrochemistry, 29 August - 03 September 2021, Jeju Island, Korea

PUBLICATIONS

Patella B, **Buscetta M**, Di Vincenzo S, Ferraro M, Aiello G, Sunseri C, Pace E, Inguanta R, **Cipollina C** (2021) Electrochemical sensor based on rGO/Au nanoparticles for monitoring H2O2 released by human macrophages. Sensors and Actuators B: Chemical, 327:128901. https://doi.org/10.1016/j.snb.2020.128901.

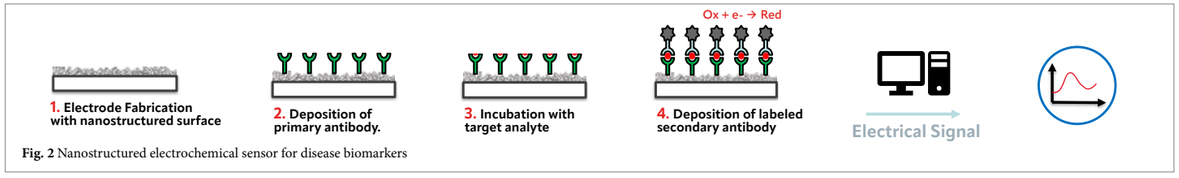

Fig. 2 Nanostructured electrochemical sensor for disease biomarkers

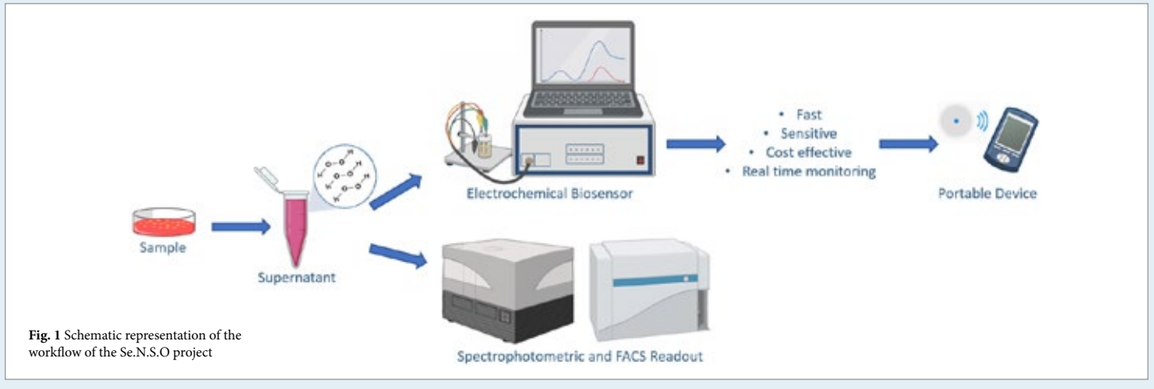

Fig. 1 Schematic representation of the workflow of the Se.N.S.O project

PRODUCTS: **ATMP - Medical Devices & Tissue Engineering**

Bioreactors for Enhanced Extra Cellular Matrix elaboration (BE-ECM)

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COLLABORATIONS

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- University of Pittsburgh, Pittsburgh, USA
- Telea Biotech – Tissue Engineering Biomedical Technologies, Italy

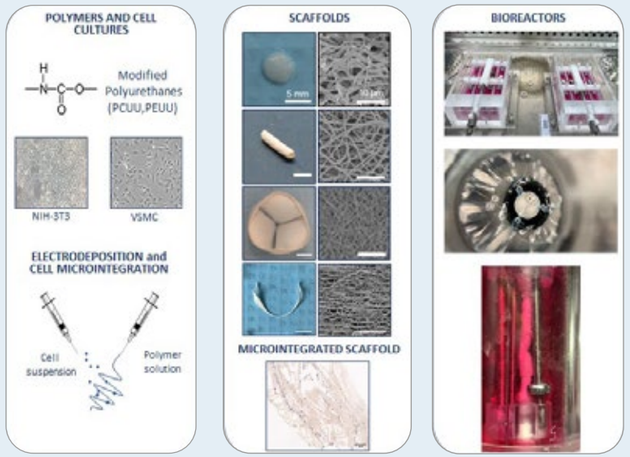
THERAPEUTIC AREA

- Organ insufficiences
- Aging diseases
- Oncology

BRIEF DESCRIPTION

Numerous studies have stressed that external mechanical forces can regulate tissue remodeling and development. Forces are ubiquitous *in vivo* and regulate critical biological functions, including cell migration, phenotypic profile, adhesion to extracellular matrix (ECM), and ECM production. Although we have some clues to how forces evocate different cellular responses in a natural environment, clarifying these mechanisms' complexity from a bioengineered point of view remains poorly investigated. BE-ECM research line, integrated by NET-IBA and NET-MTG, tries to address these critical topics by introducing and perfecting models able to study tissue growth, cell behavior, and biomaterials degradation *in vitro.*

This project involves the participation of Dr. Arianna Adamo, Expert scientist in mechanobiology.



BE-ECM: Integrated empirical and numerical approach to study extracellular matrix synthesis and elaboration in soft tissue. Electrospun polymeric scaffolds microintegrated with cells are generally accepted as in vitro model to elucidate the complex mechanism of extracellular matrix (ECM) synthesis in vivo. Examples of cardiac tissue surrogates based on biocompatible fibrous scaffolds include cardiac patches, vascular grafts, heart valves and engineered chordae tendineae processed by electrospinning and micro-integrated by electrospray. Custom made bioreactors are used to investigate the influence of mechanical load on ECM elaboration. Both mechanical and topological cues are widely recognized as a decisive factors in ECM formation and elaboration. Previous results have shown that de novo collagen production is sensitive to the applied strain level, and it is also a function of the mesoscopic niche created by the scaffold micro-architecture. ECM formation and elaboration is evaluated with a multi-scale empirical and numerical approach that includes in-plane mechanical response of the material, micro-architecture characterization via electron microscopy and digital image analysis, histological evaluation and nuclear aspect ratio estimate.

IMPACT

The potential impact of this research might improve the capacity to:
- simulate endogenous tissue growth on engineered scaffolds under mechanical load and deformation;
- simulate *in vivo* degradation of engineered scaffolds;
- investigate the impact of material topological and mechanical cues on ECM elaboration.
- investigate how cell signaling and biochemistry interact to guide cell behavior in biomaterials

The *in vitro* modeling ability will expand the understanding of biomaterials mechanobiology and allow to assess, using simplified tissue surrogates, the efficacy of novel tissue engineering strategies. These strategies include mechanisms to accelerate tissue growth, modulating material degradation characteristics, topological cues to dictate cell differentiation and lineage.

RESULTS ACHIEVED IN 2021

The BE-ECM research line was started in 2009 by the PI and his collaborators. BE-ECM is based on the use of bioreactors systems to mimic the native environment and understand cellular processes. Elastomeric, fibrous biodegradable scaffolds micro integrated with cells are produced in a variety of forms to suit a range of specific applications. In particular, BE-ECM research direction was designed to assist the development of tissue engineered heart valve (TEHV), engineered vascular graft (TEVG), cardiac patch (TECP), and bioengineered chordae tendineae (BECT). The aim for the year 2021 was to address fundamental questions regarding mechano-transduction mechanisms by utilizing simplified systems that stimulate ECM synthesis. The implementation of this concept allowed:
- to identify the unreported mechanism for enhancing ECM formation given a specific macroscopic load, the notion applies to TECP, TEVG, BECT design;
- to validate a novel apparatus for BECT mechanical conditioning;
- to implement a novel apparatus to induce accelerated degradation conditions on polymeric heart valves
- to study conditioning regimen for BECT able to duplicate mass and mechanical properties of native chordae tendineae.

GOALS FOR 2022

To perfect and promote the BE-ECM experimental platform, broaden the knowledge of biological complexity and use biophysical models to explore the cellular environment, in particular:
- to study cellular growth and tissue formation in conditioned BECT;
- to assess degradation curves of engineered atrioventricular valves developed in research line TEHV;
- to evaluate the effects of topology of engineered tunica intima (TEHV research line) on endothelial cell proliferation and stability.
- to evaluate the protein expression, gene expression and mecha-no-signaling of cells seeded inside the scaffold.
- to assess a hybrid micro-molding electrodeposition platform for cell manipulation.

MEETINGS

- *Mandrel-less fabrication of biomimetic microfiber wires for soft tissue engineering applications* - **Arianna Adamo,** Joseph G. Bartolacci, Marco G. Traina, Seng-guil Kim, Simon C. Watkins, William R. Wagner , Stephen F. Badylak, **Antonio D'Amore.** Congresso Nazionale Biomateriali 2021, Luglio, 2021, Lecce (Italy)
- *A continuous microfiber wire mandrel-less biofabrication for soft tissue engineering applications* - **Arianna Adamo**, Joseph B. Bartolacci, Marco Traina, William R. Wagner, **Antonio D'Amore**. 6th World Congress 2021 Tissue Engineering and Regenerative Medicine International Society (TERMIS), Novembre, 2021, Maastricht (Olanda)
- *Biomimetic engineered chordae tendineae generated with mandrel-less fabrication* - **Arianna Adamo**, Giovanni Spiaggia, William R. Wagner, **Antonio D'Amore.** 6th World Congress 2021 Tissue Engineering and Regenerative Medicine International Society (TERMIS), Novembre, 2021, Maastricht (Olanda)

Lezioni su invito:
- *Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation*, invited seminar for the Department of Physics and Chemistry Emilio Segrè, University of Palermo, Italy, April 19th 2021.

PUBLICATIONS

Arianna Adamo, Joseph G. Bartolacci, Marco G. Traina, Seungil Kim, Antonio Pantano, Giulio Ghersi, Simon C. Watkins, William R. Wagner , Stephen F. Badylak, and **Antonio D'Amore** "Continuous microfiber wire mandrel-less biofabrication for soft tissue engineering applications." Submitted to Advanced Healthcare Materials (IF 9.933)

INTELLECTUAL PROPERTY

US patent application PCT/US21/054767 filed on 10/2021, topic: topic: bioprocessing methods, title: "Hybrid micro molding-fiber deposition substrate processing for cell biology manipulation and local anisotropy". **Arianna Adamo**, **Pietro Terranova**, William R. Wagner, **Antonio D'Amore.**

PRODUCTS: **ATMP - Medical Devices & Tissue Engineering**

Native/Engineered Tissue numerical models for Mechanics and Tissue Growth (NET-MTG)

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COLLABORATIONS

- University of Pittsburgh, Pittsburgh, USA;
- University of Pittsburgh Medical Center, Pittsburgh, USA;
- Politecnico di Milano, Milan, Italy.

THERAPEUTIC AREA

- Organ insufficiences
- Oncology
- Aging diseases

PIPELINE: CLINICAL NEED | DISEASE ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

BRIEF DESCRIPTION

Main topic: NET-MTG, development of structural deterministic numerical models to predict mechanics, endogenous tissue formation and degradation of engineered and native tissue. Three macro-areas, which are widely recognized as relevant for the tissue engineering approach, still need more effective numerical models: I) mechanical models able to correlate the macro, meso and micro scales, II) tissue growth models with the ability to correlate mechanics and tissue elaboration, III) scaffold degradation model able to correlate mass loss with mechanical loads. This research line tries to address these three critical topics by introducing and by perfecting structural deterministic models for engineered and native tissues.

IMPACT

This research line has potential implications on a number of topics in computational biomechanics and scaffold design, more specifically:

- development of tools to assist engineered tissue and biomaterials design;
- development of tools to elucidate the interrelation between multiscale mechanics, de-novo ECM elaboration and scaffold degradation;
- development of tools and methods to study the relationship between macro-meso and - micro scale in engineered and native tissue. Targeted applications: TEHV, TEVG, TECP;
- development of numerical tools to elucidate mechanobiology of ECM aging;
- development of numerical tools to elucidate the mechanisms of pathological remodeling and fibrotic tissue formation.

RESULTS ACHIEVED IN 2021

- 4FRAILTY project was founded by Italian Minister of University and Research. The numerical part of 4FRAILTY project aims to create a numerical model of the sensory platform developed in the project, involving environmental and clinical data.
- Experimentally-derived computational simulations based on previously developed numerical models, have been used to analyze differences of fiber network mechanical behavior between young and aged muscles. Results will be published soon.

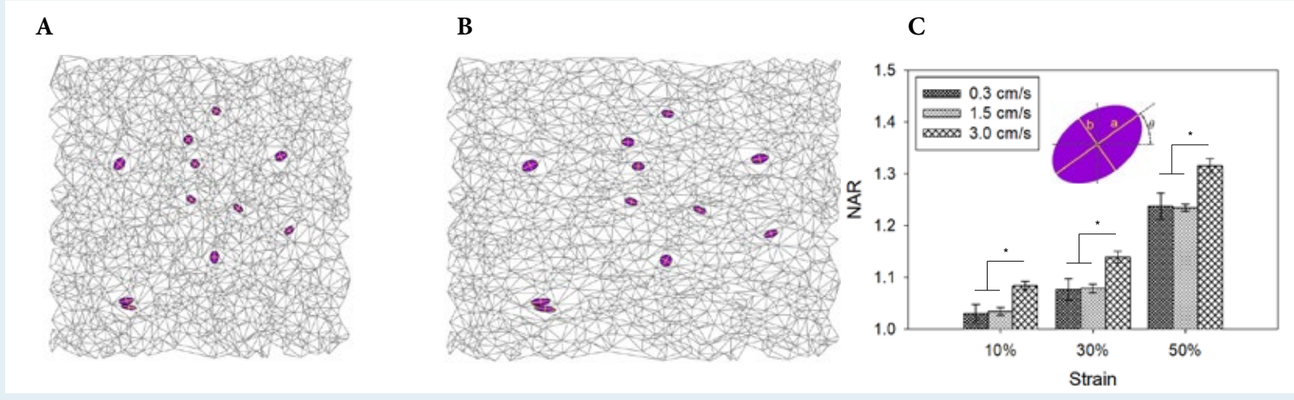
GOALS FOR 2022

Goals set for the 2022 reflect the ancillary nature of this research line within the more broad scheme the PI envision for the cardiac tissue engineering program at RiMED and the collaborations with our clinical partners, more specifically:

- to assist scaffold design utilized in TEHV, TECP and TEVG;
- to support *in vitro* modeling planned in BE-ECM;
- development of tissue growth predictive models based on experimental data provided in:
  - D'Amore, T. Yoshizumi., S. K. Luketich, M. T. Wolf, X. Gu, M. Cammarata, R. Hoff, S.F. Badylak, and W. R. Wagner. Bi-layred polyurethane-extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model. Biomaterials 2016 (107), 1–14, 5Y-IF 8.97;
  - D'Amore, M. Fazzari, H. Jiang, S. K. Luketich, M. E. Luketich, R. F. Hoff, D. L. Jacobs, X. Gu, S. F. Badylak, B. A. Freeman, W.R. Wagner. Nitro-oleic acid (NO2-OA) release enhances regional angiogenesis in a rat abdominal wall defect model. Tissue Engineering Part A 2018, IF 3.58;
- sviluppo modelli di simulazione degradazione di scaffold *in vivo*;
- sviluppo modelli di simulazione crescita cellulare.

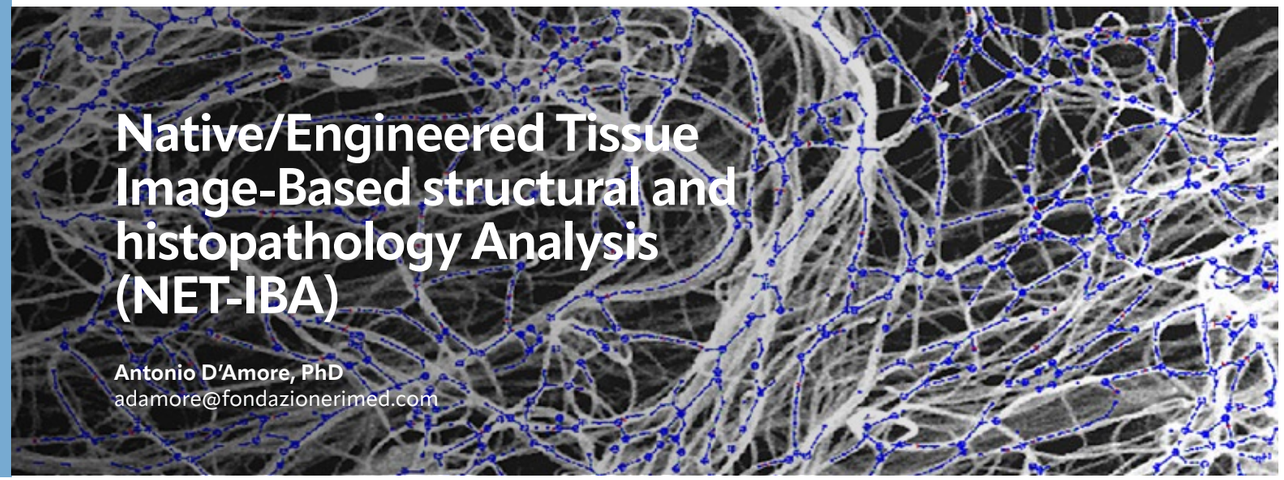
PUBLICATIONS

Y. Kawakami, K. Nonaka, N. Fukase, **A. D'Amore**, Y. Murata, P. Quinn, S. Luketich, K. Takayama, T. Matsumoto, J. H. Cummins, M. Kurosaka, R. Kuroda, W. R. Wagner, F. H. Fu, J. Huard. (2021). A Cell-free Biodegradable Synthetic Artificial Ligament for the Reconstruction of Anterior Cruciate Ligament (ACL) in a Rat Model. Acta Biomaterialia, 121, 275-287, 5Y-IF 7.16.



NET-MTG: Connecting scaffold large scale and cell meso scale deformations. Fiber network model of polyurethane scaffold seeded with vascular smooth muscle cells showing both un-deformed **(A)** and strip-biaxial deformation at 30 % strain **(B)**. Cell nuclei are shown in purple. Quantification of Nuclear Aspect Ration (NAR) for three different scaffold types (0.3, 1.5, 3.cm/s) fabricated via electrospinning at three different rastering speeds **(C)**. Scaffolds differed only in terms of fiber intersection with the 0.3 cm/s being the most dense material in terms of fiber intersection density. This structural feature while not affecting the macro-scale mechanics affected the cellular deformations inducing a significantly higher deformation (NAR, defined as the ratio between the major and minor axis of the nucleus) for the least dense material.

PRODUCTS: **ATMP - Biomarkers - Medical Devices & Tissue Engineering**



Native/Engineered Tissue Image-Based structural and histopathology Analysis (NET-IBA)

Antonio D'Amore, PhD
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COLLABORATIONS

- University of Pittsburgh
- Università degli Studi di Palermo
- University of Nagoya, Japan

THERAPEUTIC AREA

- Organ insufficiences
- Oncology
- Aging diseases

IMPACT

The software tools we developed and that we are advancing have the potential to impact two main categories of problems:

- **Innovative methods for quantitative histology**, potential applications include: biomaterial-host interactions, evaluation of drugs effects on tissue, inflammatory response evaluation, oncology, tissue elaboration *in vitro* and *in vivo*, big data;
- **Innovative methods for morphological analysis of micro and nano-structured materials**, potential applications within the context of chemical, process engineering or material science. These applications include process control, process characterization, structure-function characterization.

RESULTS ACHIEVED IN 2021

- Validation of techniques to angiogenesis. This tool can quantify the number, area and spatial distribution of blood vessels in the analyzed sections. The overall method consists of eight steps: averaging spatial filtering and thresholding on one of the color channels; extraction of connected components and removal of small connected components; morphological operations and vessel tunica check; circle detection and vessel lumen area check; detection of holes, connected components and vessel tunica; morphological operations; connected components labeling; blood vessel centroid detection. This tool has been set up as an open-source software tool working on different operating systems supported by a user-friendly graphical interface and facilitates large-scale analysis;
- Activity conducted to support funded NIH-R01 projects in collaboration with University of Pittsburgh;
- Utilized algorithm to support the research lines: BE-ECM, TECP e TEHV.

GOALS FOR 2022

The goals will be to realize a 3D upgrade of 2D analysis methods developed for micro and nanomaterials. For the realization of this software, we will use state-of-the-art microscopic techniques. Multiphoton microscopy represents an advancement in the field of 3D fluorescence imaging. This technique enables non-invasive three-dimensional analysis and quantitative analysis of fundamental structural constituents for engineered heart valves, engineered vessels and engineered myocardial walls. Based on this technique, a series of tests at multiphoton for engineered or native tissues will be performed. The software will develop using Matlab language-based code.

MEETINGS

Ri.MED Research Retreat, Dicembre, 2021, Palermo. Poster Session: "Characterization of the complete fiber network topology of planar fibrous tissues and scaffolds".

Lezioni su invito

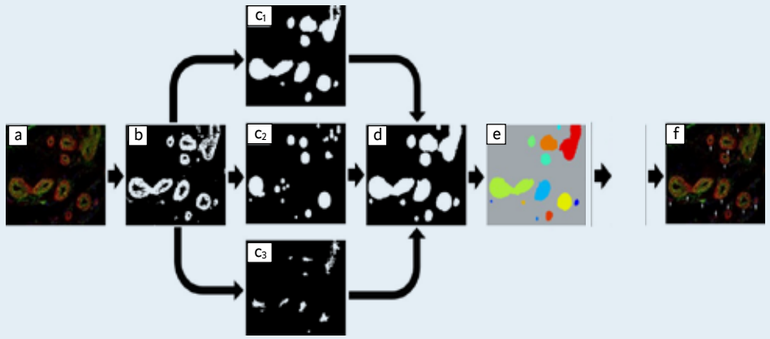
(1/2021) "Advancing Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", school of engineering Polytechnic University of Turin, Italy, January 7th 2020.

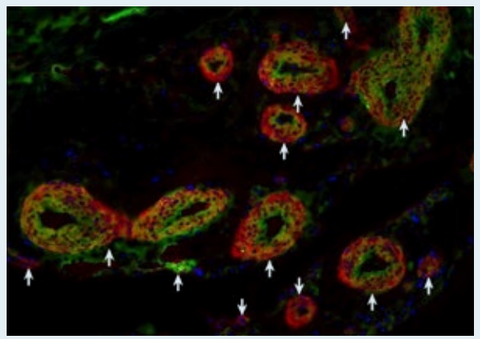
PUBLICATIONS

S. K. Luketich, **F. Cosentino, M. Di Giuseppe,** G. Menallo, G. Nasello, M. Maneschi, F. Gulizzi, P. Livreri, W. R. Wagner, and **A D'Amore**. Controlling in-plane mechanics of electrospun polyurethane scaffolds for cardiac tissue engineering applications. To be submitted to Journal of Mechanical Behavior of Biomedical Materials, IF: 3.23.

BRIEF DESCRIPTION

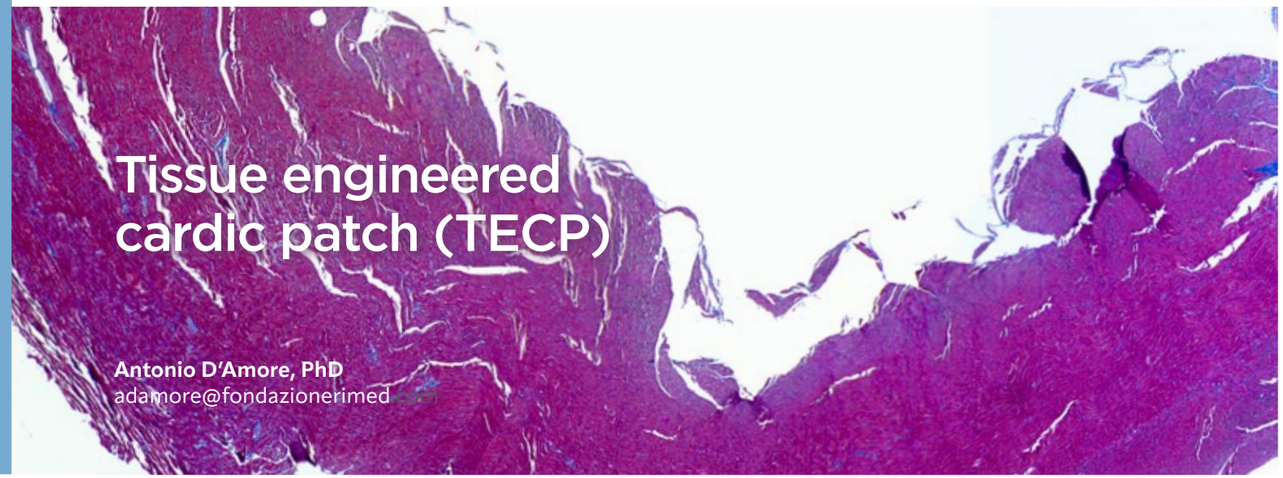
Main topic: Development of algorithms and automatic methods for structural and morphological analysis of native and engineered tissues. The core research activity is to use image-based structural and histopathological analyzes to obtain full control over the structure and function of biomaterials. This aim is achieved through tools capable of quantifying the structure of engineered and native tissues at different observation scales. Histopathology does not currently benefit from the advantages provided by image-based quantitative structural analysis. Most of the histological evaluations are still conducted with qualitative or semi-qualitative assessments. Similarly, digital image analysis tools for material science applications or process engineering are not designed with a potential clinical focus. This research line acts as the interface between these two disciplines and fills this knowledge gap. More specifically, our group aims to define novel software analysis tools and methods that can solve common problems currently faced in both clinical practice and material science.





NET-IBA:Algorithm for quantitative angiogenesis evaluation. Accurate identification and quantification of blood vessels can be labour intensive, time consuming and heavly dependet on the operator experience. An automated, objective method has been developed and validated, the block diagram illustrates the structure of the algorithm. (from left to right): **a)** input image, **b)** filtering and thresholding on red or green color channels, **c1)** detection of connected components , **c2)** morphological segmentation based on size and shape, **c3)** additional detection of connected components, **d)** segmentation criteria in **c1,c2,c3** are combined together using morphological operators, **e)** labeling of connected components , **f)** algorithm' result including vessel area quantification and spatial distribution (right).

PRODUCTS: **Medical Devices & Tissue Engineering**



Tissue engineered cardic patch (TECP)

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COLLABORATIONS

- University of Pittsburgh, Pittsburgh, USA
- UPMC, Pittsburgh, USA
- IRCCS ISMETT, Palermo, Italy
- UAI, Universidad Abierta Interamericana, Buenos Aires, Argentina
- JOMDD, Japanese Organization for Medical Device Development, Tokyo, Japan
- ATeN Center, University of Palermo, Palermo, Italy

THERAPEUTIC AREA

Organ insufficiences

BRIEF DESCRIPTION

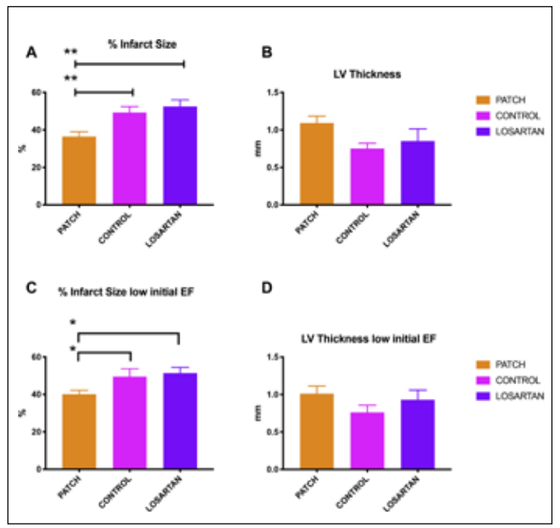
TECP, development of devices to support cardiac function of patients affected by myocardium infarction (MI). The cardiac restrain devices potentially offer an alternative therapy to the pharmacological and surgical treatments or to the adoption of ventricular aided devices (VAD). The general notion of cardiac patching is to provide mechanical support to the ventricle by surgically implanting TECP on the infarcted epicardium (local approach) or around the entire ventricle (global). The patch can be made of degradable or non degradable material. The scaffold utilized in this research line is designed to promote endogenous tissue growth and ideally induce regeneration or protection of healthy tissue in proximity of the infarcted region. As such, our approach involves two main strategies: designing polymeric patch able to reproduce the native ventricle mechanics, utilizing a multi-layer composite scaffold where the layer facing the epicardium is composed of bioactive extracellular matrix. The project presents a translation outlook and seek to develop and produce a patentable new device for patch catheter-based implantation for a minimally-invasive approach.

IMPACT

The main objective of this research line is the introduction of innovative strategies to mitigate the pathological remodeling induced by myocardium infarction. The World Health Organisation (WHO) estimated that ischemic heart disease (IHD) or ischemic cardiomyopathy (ICM) constitute 13.3% of global mortality. MI triggers a cascade of events that impacts both cells viability and functions, and this effect continues causing a non-ischemic expansion of the infarct. The propagation of the series of events following the cell death cycle stimulates a remodelling mechanism that results in scar formation, thus myocardium thickness reduction and dysfunction. The biodegradable cardiac restrain devices potentially offer a viable bridge therapy for patients waiting for full heart transplant. A secondary potential application is the ventricle patching to mitigate effects of pulmonary hypertension. Further steps should be conducted for the highest translational potential despite the advanced results achieved by the bio-fabrication of the biohybrids patch. So far, patch implantation requires to be applied by a sternotomy which is an open heart highly invasive technique and cardiac surgery urges minimally invasive procedures such as catheter-based implantation.

RESULTS ACHIEVED IN 2021

Bio-fabrication of a bilayered poly(ester carbonate urethane) urea (PECUU)–extracellular matrix (ECM) biohybrid patch which improved echocardiographic cardiac function and angiogenesis in rats when implanted post-MI. Studies were conducted in-vivo on a rat model and the results showed the patch altered the progression of maladaptive remodelling by decreasing left ventricular global mechanical compliance, inhibiting echocardiographically-measured functional deterioration, mitigating scar formation and left ventricular wall thinning, and promoting angiogenesis. Moreover, it was observed that the patch increased wall thickness and presented better morphological and functional echocardiographic endpoints after 8 weeks of implant.



TECP: effect of cardiac patch on left ventricle thickness and infarct size. (A) Percent infarct size related to healthy tissue was lower in patch-treated animals than in control and losartan animals. (B) LV thickness was not significantly different among groups. (C, D) In animals with initial very low EF (<35%), percent infarct size was lower in patch-treated animals than in both nonpatched animals (C), whereas LV thickness did not show differences (D).

Awards achieved

[G. 2] 4FRAILTY National Operative Program (PON) from Italian Ministry for Education and Research, "Sensoristica intelligente, infrastrutture e modelli gestionali per la sicurezza di soggetti fragili", €250k for 3 years for 2021-2023. Principal-Investigator: A. D'Amore, Fondazione RiMED, Italy;

[G.1] Industrial collaboration with JOMDD Medical Innovation and Adeka Corporation, "Characterization and comparative analysis of Adeka's pericardium patch" $424k for 9 months (2021). Principal-Investigator: A. D'Amore (20% efforts), Dr Wagner Co-I. University of Pittsburgh.

GOALS FOR 2022

The long-term goal of this research is the translation of the technology which is classified as a class III FDA medical device. Goals set for the year 2022 include:

- Assessment of cardiac patch scaffold on large animal model, primary goals: (I) sustain ventricular function; (II) induce endogenous tissue growth\ reduce fibrotic tissue; (III) mitigate wall thinning;
- Italian Ministry of health research grant application, project in collaboration with ISMETT.
- Preclinical study on chronic myocardial infarct swine model
- Development of a minimally invasive deployment, project in collaboration with Drs Pilato, e Raffa (ISMETT);
- Development of a robotic platform for advanced processing, protected by IP, in collaboration with Advanced Solution;
- Assess the effects, methods and therapeutical potential of right ventricle patching, project in collaboration with Drs Coyan, Silveira-Filho and Sciortino (UPMC).

PUBLICATIONS

[J. A. 1] Lindemberg M. Silveira-Filho, Garrett N. Coyan, **Arianna Adamo**, Samuel K. Luketich, Giorgio Menallo, **Antonio D'Amore** and William R. Wagner. "Can a biohybrid patch salvage ventricular function at late time point in post-infarction remodeling process?" In press on Journal of American College of Cardiology, Basic to Translational Research. IF 2.43

INTELLECTUAL PROPERTY

US provisional patent application. Pitt invention disclosure ID#05360 filed on 05/2020, topic: biomedical device/controlled release system, title: "Shape memory, polymeric, degradable drug eluting platform for nitro-fatty acid release". Lead innovator/developer: **A D'Amore.**

PRODUCTS: **Medical Devices & Tissue Engineering**

Tissue Engineering Heart Valve (TEHV)

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COLLABORATIONS

- University of Pittsburgh, Pittsburgh,USA
- UPMC, Pittsburgh, USA
- University of Cincinnati, Cincinnati, USA
- IRCCS ISMETT, Palermo, Italy
- West Virginia University, Morgantown, USA
- Harvard Medical School, Boston, USA
- Universidade Estadual de Campinas, Campinas, Brazil
- University of Texas at Austin, Austin, USA

THERAPEUTIC AREA

Organ insufficiences

BRIEF DESCRIPTION

Main topic: TEHV, to develop engineered tissue and valve prostheses for heart valve repair and replacement. Specific objectives:

- To characterize and duplicate human heart valve structure and mechanics;
- To design, prototype, and validate innovative valve prostheses with the ability to:
  - *Induce endogenous tissue growth;*
  - *Increase resistance to calcification;*
  - *Reduce thrombogenicity;*
- To develop technologies and strategies for a minimally invasive trans-catheter delivery approach.

The method utilized is based on a novel polymer processing technique developed by Dr. D'Amore's group, which is named double component deposition (DCD). DCD allows for the fabrication of fibrous valve prostheses to induce in-situ tissue growth. The fabrication method can also control the engineered construct's micro/macro structure and mechanical properties.

TEHV: Figure 1. Engineering the mitral valve. A) biaxial response of porcine aortic, pulmonary, tricuspid, mitral valve leaflets (AV, PV, TV, MV), circumferential (CD) and radial directions (RD). B) Collagen fiber diameter of porcine leaflets. Based on this characterization structure and function of the native valve and chordal apparatus can be duplicated with two novel bioprocessing techniques: Double Component Deposition (DCD) and Mandrel-Less Deposition (MLD) C) DCD controls valve mechanics: anisotropy ratio vs. mandrel tangential velocity and range of interest for the MV, ranges of interest to duplicate MV anisotropy are shown in red. D) DCD controls valve micro-structure: fiber bundle diameter vs. fabrication configurations. Native vs. engineered MV, ranges of interest to duplicate native MV bundle diameter are shown in red. Structural biomimicry for MV E) and chordae tendineae F).G) Complete engineered mitral valve with chordal apparatus. Data is presented as mean ± standard error, $p<0.05$.

IMPACT

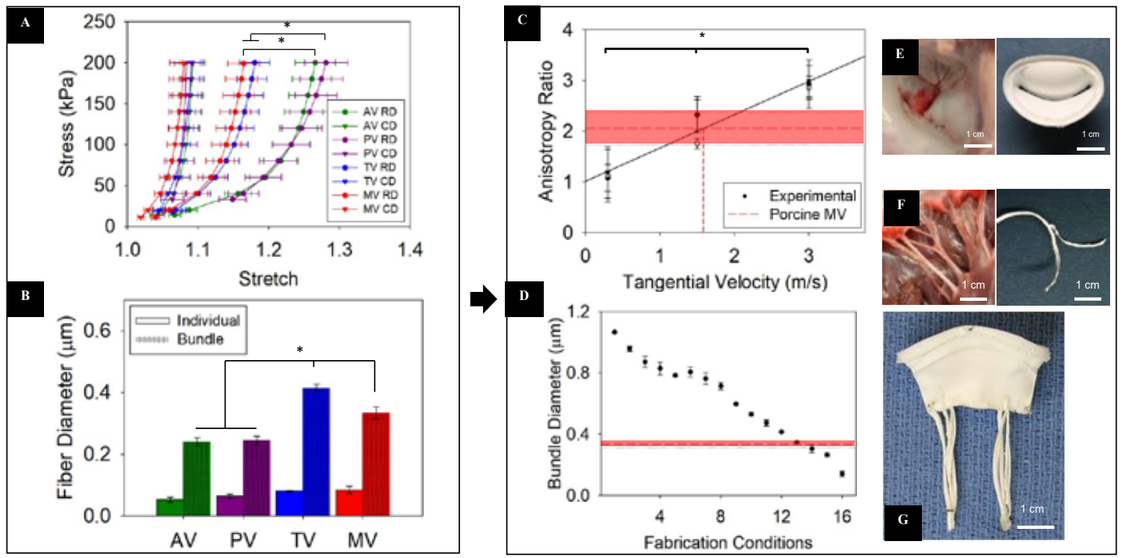
Nearly 80000 patients/year require a life-saving valve replacement in the US only. Current clinical practice for valve replacement involves two different classes of devices: mechanical valve prostheses and bio-prostheses. The mechanical valve has good longevity but requires chronic anticoagulation therapy, which is associated with a number of risk factors and affects the patients' quality of life. The second category does not require chronic anticoagulation therapy and yet suffers a number of failure mechanisms, with calcific degeneration being one of the most frequent. Technologies developed by Dr. D'Amore's s team aim to overcome the limitations of these two classes of medical devices by introducing engineered heart valves that can re-adjust to somatic growth, resist calcification, and do not require anticoagulants. This research line is functional to develop advanced polymer processing techniques, which can be utilized for different applications. Last, these research efforts also focus on prototyping novel hybrid medical devices based on combined biodegradable metallic and polymeric components.

RESULTS ACHIEVED IN 2021

The start-up Neoolife is further consolidated. The evaluation of the mitral valve prototype with chordal apparatus included continued. *In vivo* tests continued on the Large Animal Model to evaluate progress on the mitral valve and the tricuspid valve with and without a stent. Published a study of the mechano-biology of the tendon cords. Continued study on the transfer of a micro-pattern on the surface of an electrospun fabric. Consolidated and extended IP for the fabrication of scaffolds with integrated micro-topology capable of influencing cellular activity. Purchased a new biofabrication platform (BioAssemblyBot®) in collaboration with the company Advanced Solutions Life Sciences (ASLS).
Collaborations with clinicians and researchers on the national territory for publications and potential joint IPs have been started. Consolidated a research unit, with 13 researchers, in Tissue Engineering RiMED in Palermo (ITA) in collaboration with ATeN Center and Uni-PA. Consolidated international collaborations Italy / USA.

Funded grant proposals

- [G.4] (2020-2025) European Research Council Consolidator Award # 101002561, Engineering the mitral valve: bioinspired control of structure and function for enhanced *in vivo* performance, (BIOMITRAL) €1,993,786 for 5 years. Sole Principal-Investigator: A. D'Amore, Host institution: Fondazione RiMED, Italy, partners: University of Pittsburgh and Istituto Mediterraneo per i Trapianti e le Terapie ad Alta Specializzazione (ISMETT-IRCCS).
- [G.3] (2021-2023) National Operative Program (PON) from Italian Ministry for Education and Research, Sensoristica intelligente, infrastrutture e modelli gestionali per la sicurezza di soggetti fragili, (4FRAILTY) €250,000 for 3 years. Principal-Investigator: A. D'Amore, Fondazione RiMED, Italy;
- [G.2] (2020-2023) Start-up package as Head of the Cardiac Tissue Engineering Program, total of €3,187,434 in direct cost: €1,985,634 for 2021, €596,400 for 2022, €596,400 for 2023, Sole Principal-Investigator: A. D'Amore, Fondazione RiMED, Italy;
- [G.1] (2019-2021) Pitt Innovation Challenge (PInCh) program. OneValve: The Self Generating Heart Valve, the team (Drs Coyan, D'Amore, Wagner) was ranked #1st and was awarded for $ 100,000. PI: A. D'Amore, Univ. of Pittsburgh.



GOALS FOR 2022

The long-term goal is the translation of technology (class III FDA). Specific objectives for 2021 are defined as follows:
- Introduction of new rapid prototyping systems for the in-house creation of customized tools;
- Launch of the BIOMITRAL project funded by the ERC Consolidator Grant;
- Consolidation of a pipeline for polymer synthesis, ECM extraction, scaffold processing, histology, immunofluorescence, biomechanics, and advanced microscopy;
- Project proposal formulation to finance a chronic study on mitral prosthesis DCD: NIH R01;
- Review publication on bio-processing models for TEHV;
- Development of a new platform for electrodeposition on complex surfaces,
- Completion of FEM modeling of the chordal apparatus;
- Evaluation of new selective fiber deposition strategies for DCD;
- Start acute study for mitral valve with tendon cords,
- IP protection and consolidation;
- Further development of Neoolife and completion of the projects financed on the TEHV line;
- Personal training: four master's degree students, five doctoral students;
- Launch of McGowan Institute-UNIPA joint projects.
- Avvio di progetti congiunti McGowan Institute-UNIPA.

MEETINGS

- [C. P. 3] D. Pedersen, F. Madonia, A. D'Amore, W. R. Wagner. Tissue to Organ Level Evaluation of Heart Valve Scaffold Performance under Dynamic Loading Conditions. Tissue Engineering and Regenerative Medicine International Society (TERMIS) 2021 World Congress. November 15-19, Maastricht, Nederland.
- [C. P. 2] A. Arianna, J. B. Bartolacci, M. Traina, W. R. Wagner, A. D'Amore. A continuous microfiber wire mandrel-less biofabrication for soft tissue engineering applications. Tissue Engineering and Regenerative Medicine International Society (TERMIS) 2021 World Congress. November 15-19, Maastricht, Nederland.
- [C.P. 1] A. Adamo, J. Bartolacci, M. Traina, W. Wagner, A. D'Amore. Mandrel-less fabrication of biomimetic microfiber wires for soft tissue engineering applications Italian chapter of the European Society for Biomaterials annual meeting, SIB 2021, Lecce 11th – 14th July, Italy.

Invited speech

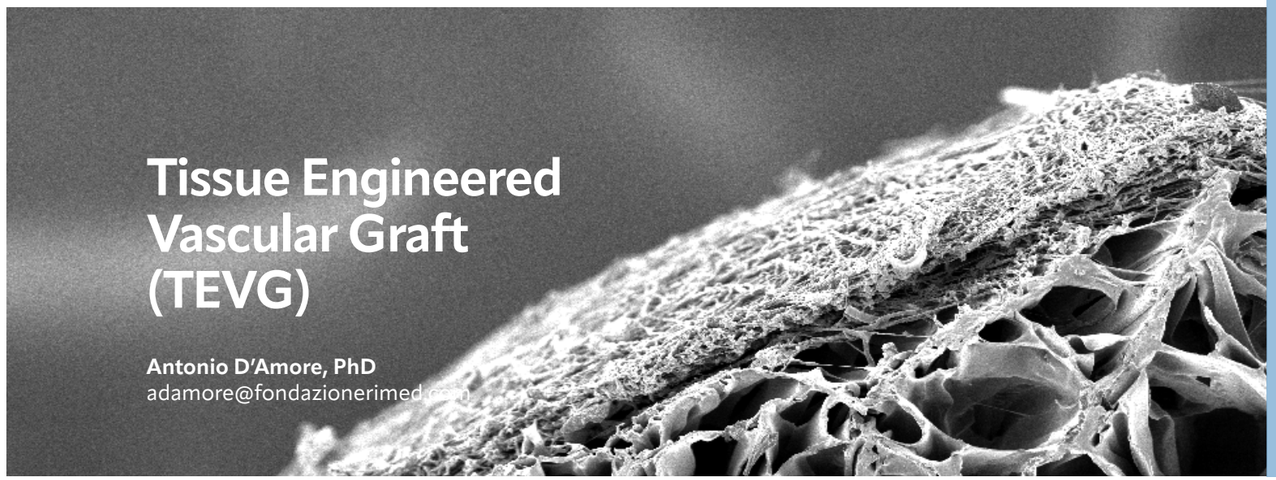
- [I. S. 6] (6/2021) "Engineering the mitral valve: bioinspired design to better address functional regurgitation", invited workshop, Innovabiomed "Il network place per l'innovazione biomedica". Verona, Italy July 3rd 2021;
- [I. S. 5] (4/2021) " Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation ", invited seminar for the Department of Physics and Chemistry Emilio Segrè, Univ. of Palermo, Italy, April 19th, 2021;
- [I. S. 7] (9/2021) "RiMED Cardiac Tissue Engineering Program: Overview On Technology Transfer And Translation" invited talk at ISMETT/UPMC Italy advanced therapies hospital facility located in Palermo Italy;
- [I. S. 4] (3/2021) "An overview on RiMED Tissue Engineering program", invited seminar for the Adolphe Merkle Institute, University of Fribourg, Switzerland, March 29th, 2021;
- [I. S. 3] (3/2021) "An overview on RiMED Tissue Engineering program", seminar series for the International PhD program on Biomedicine and Neuroscience, University of Palermo, Palermo, Italy March 27th, 2021

PUBLICATIONS

- [J. A. 4] Y. Matsumura, L. M. Silveira-Filho, G. Coyan, **A. D'Amore**, W. R. Wagner. Elastomeric tissue engineered template based tricuspid valve. To be submitted to J. of Thoracic and Cardiovascular Surgery, IF 4.88.
- [J. A.3] Z. Machaidze*, A. S. Bayoumi*, **A. D'Amore**, K. Feaver, W. Zang, B. Rego, D. Cooper, S. Shimada, K. Rich, J. Wen, D.W. Brown, R. Padera, F. J. Schoen, E. Aikawa, W. R. Wagner, M. S. Sacks and J. E. Mayer. Tissue formation and remodeling of acellular elastomeric scaffold in ovine single pulmonary leaflet replacement model. Submitted to Tissue Enginnering, IF 3.5. *equal contribution.
- [J. A.2] S. K. Luketich, G. Menallo, G. Nasello, M. Maneschi, F. Gulizzi, P. Livreri, W. R. Wagner, and **A. D'Amore**. Controlling in-plane mechanics of electrospun polyurethane scaffolds for cardiac tissue engineering applications. To be submitted to Journal of Mechanical Behavior of Biomedical Materials, IF: 3.23.
- [J. A. 1] **A. Adamo**, J.Bartolacci, M. Traina, A. Pantano, G. Ghersi, W. Wagner, S.F. Badylak, **A. D'Amore**. Bioprocessing, structure, mechanics and evaluation of micro-fiber based biodegradable suture material. Submitted to Biomaterials, 5Y-IF 8.97.

INTELLECTUAL PROPERTY

- [I. D. 3] US patent application PCT/US21/054767 filed on 10/2021, topic: topic: bioprocessing methods, title: "Hybrid micro molding-fiber deposition substrate processing for cell biology manipulation and local anisotropy". Lead innovator/developer: **A D'Amore**.
- [I. D. 2] US patent application PCT/US20/42115, filed on 07/2020, topic: biomedical device, title: "Processing method and apparatus for micro-structured rope-like material". Lead innovator/developer: **A D'Amore**.



Tissue Engineered Vascular Graft (TEVG)

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COLLABORATIONS

- University of Pittsburgh - Pittsburgh, USA
- University of Medical Center Pittsburgh (UPMC) - Pittsburgh, USA
- Advanced Technologies Network Center - ATeN Center, Palermo, Italy
- LivaNova – Mirandola, Italy

THERAPEUTIC AREA

Organ insufficiences

BRIEF DESCRIPTION

Main topic: TEVG, development of engineered vascular graft for coronary bypass.
Solutions clinically available to replace or treat a stenotic blood vessel include the auto-transplant (for example utilizing a section of the saphenous vein) or the adoption of synthetic materials such as Dacron or Teflon. The first class of intervention is limited by the availability of sufficient viable autologous tissue. The second category utilized synthetic materials that induce re-stenosis of the vessel up to the 50% of the treated cases. These issues could be potentially addressed by the tissue engineering approach.

The tissue engineering paradigm proposes the use of biodegradable scaffolds able to induce in-situ regeneration and lead to the formation of autologous, functional, non-thrombogenic tissue.
In this research line our group has identified two main targets:

1) to design grafts able to reproduce structure and mechanics of the native tissue;
2) to reduce the tunica intima hyperplasia by the adoption of ad-hoc scaffold surface morphology and structure.

IMPACT

The main target of this research line is to introduce innovative strategies and technologies for coronary bypass and for the treatment of critical limb ischemia. Given the limitations of current artificial vascular grafts and surgical procedures, the introduction and validation of a technology based on biodegradable graft capable to promote in-situ tissue growth has a profound innovative value as well as a potential commercial value. Applications involved with the development of this technology extend far beyond the coronary bypass. Other examples include engineered urethra or the endothelialization of cannula utilized in FDA class II and III medical devices.

RESULTS ACHIEVED IN 2021

Completed rat study assessing bilayer vascular graft and same day scaffold seeding. Perfected fabrication technique and initiated large animal study. Prototyped engineered vascular graft with three layers recapitulating the structure of tunica intima, media and adventitia (IP "Multi-Layered Graft for Tissue Engineering Applications", 2019). Developed different graft's layer configuration to demonstrate the hypothesized mechanism to mitigate tunica intima hyperplasia. Introduced novel technique for polymer surface modification at the micro and meso scale. Design and development of patterns to manipulate cells attached/seeded into the scaffold (IP "Hybrid micro molding-fiber deposition substrate processing for cell biology manipulation and local anisotropy disclosure in progress", 2021). Preliminary *in vitro* study, thanks to a custom-made bioreactor, to evaluate cellular infiltration into three layers graft.

GOALS FOR 2022

The final goal of this research line is the translation of the technology (FDA class III).
Targets set for the year 2022 include:

- Development of innovative engineered vascular grafts with the following specific aims:
  - *to recapitulate physiological mechanics of arteries and veins;*
  - *to achieve endogenous tissue growth/vessel patency/low thrombogenicity;*
  - *to reduce intimal hyperplasia;*
- Biomechanical characterization of human coronary arteries, in collaboration with the Core Foundation;
- Pre-clinical studies continue on rat chronic model in collaboration with the University of Pittsburgh;
- To mimic the native basal membrane architecture and structure to guarantee a suitable endothelialization of the inner part limiting the coagulation cascade and thrombus formation (that are huge limitations in the clinical application of TEVG);
- To investigate the degradation profile of the TEVG mimicking physiological conditions;
- To optimize the design of the pattern and to investigate cell adhesion and proliferation induced by superficial pattern;
- To corroborate the industrial partnership with LivaNova to support the hypothesis to enhance the endothelium formation to mitigate intimal hyperplasia;
- To assess *in vivo* the potential of the developed IP ("Multi-Layered Graft for Tissue Engineering Applications") and its capacity to reduce tunica intima hyperplasia.

Research funds

[G.1] Start-up package as Head of the Cardiac Tissue Engineering Program, $1.75 million for 3 years. Sole Principal-Investigator: A. D'Amore, Fondazione RiMED, Italy (2020-2023).

MEETINGS

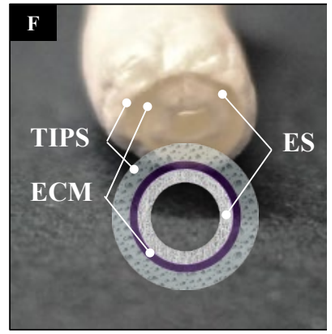
- [C.1.] "RiMED Cardiac Tissue Engineering Program: Overview On Technology Transfer And Translation" invited talk at ISMETT/UPMC Italy advanced therapies hospital facility located in Palermo Italy. September, 2021. Speaker: **A. D'Amore**.
- [C.2] "Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation ", invited seminar for the Department of Physics and Chemistry Emilio Segrè. April, 2021, University of Palermo, Palermo, Italy. Speaker: **A. D'Amore**.
- [C.1] "An overview on RiMED Tissue Engineering program", invited seminar for the Adolphe Merkle Institute. March, 2021. University of Fribourg, Switzerland. Speaker: **A. D'Amore**.

Invited speech

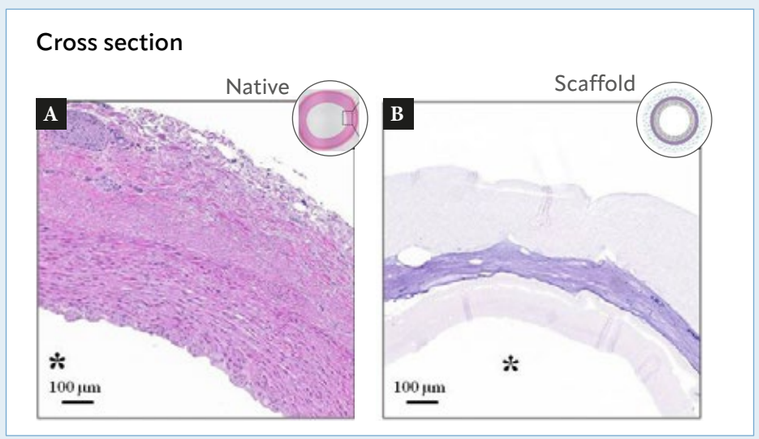
- [I. S. 6] (6/2021) "Engineering the mitral valve: bioinspired design to better address functional regurgitation", invited workshop, Innovabiomed "Il network place per l'innovazione biomedica". Verona, Italy July 3rd 2021;
- [I. S. 5] (4/2021) " Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation ", invited seminar for the Department of Physics and Chemistry Emilio Segrè, Univ. of Palermo, Italy, April 19th, 2021;
- [I. S. 7] (9/2021) "RiMED Cardiac Tissue Engineering Program: Overview On Technology Transfer And Translation" invited talk at ISMETT/UPMC Italy advanced therapies hospital facility located in Palermo Italy;
- [I. S. 4] (3/2021) "An overview on RiMED Tissue Engineering program", invited seminar for the Adolphe Merkle Institute, University of Fribourg, Switzerland, March 29th, 2021;

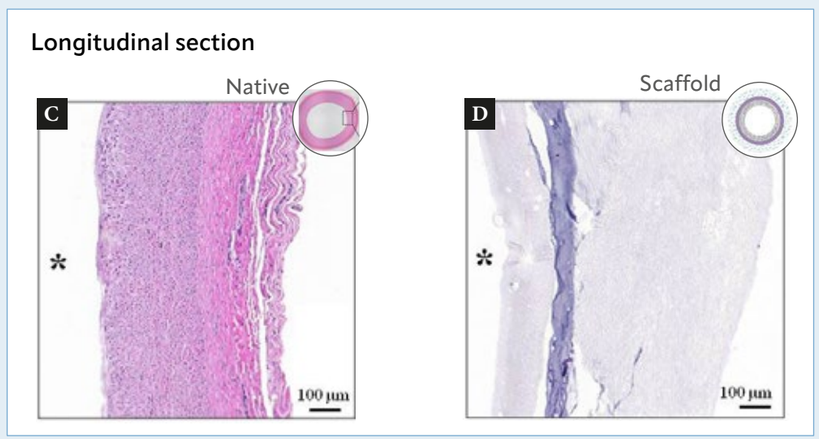
INTELLECTUAL PROPERTY

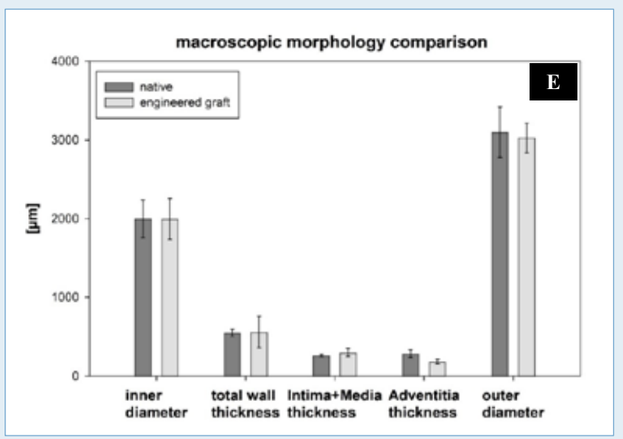
- [I.D.2] US patent application PCT/US21/054767 filed on 10/2021, topic: topic: bioprocessing methods, title: "Hybrid micro molding-fiber deposition substrate processing for cell biology manipulation and local anisotropy". Lead innovator/developer: **A. D'Amore.**
- [I.D.1] US patent PCT/US2018/043889, published date: 03/2021, topic: biomedical device, title: "Multi-layered graft for tissue engineering applications". Lead innovator/developer: **A. D'Amore.**



TEVG: Three-layer vascular graft mimicking coronary arteries's structure and mechanics. (A-D) Native and engineered vessel cross-section and longitudinal section comparison showing a three-layer arrangement mimicking the tunica intima, media, and adventitia. Asterisk indicates the vessel's lumen. (E) Quantitative comparison between the artificial and native vessel tunica thickness, quantification based on H&E staining in A-D. (F) Macro scale view of the scaffold cross-section showing the engineered tunica intima processed by electrospinning (ES), the engineered tunica media made of cardiac derived extracellular matrix (ECM) gel, and the engineered tunica adventitia processed by thermally induced phase separation (TIPS). Related publications: Krawiec, H. Liao, A. Josowitz, J. Weinbaum, A. D'Amore, P. Rubin, W. R. Wagner, D. Vorp. In Vivo Functional Evaluation of Tissue Engineered Vascular Grafts Fabricated Using Human Adipose-Derived Stem Cells from High-Cardiovascular Risk Populations. Tissue Engineering Part A, 2016 22 (9-10), 765-775, IF 3.89. Related intellectual property: PCT/US2018/043889, "Multi-Layered Graft for Tissue Engineering Applications".







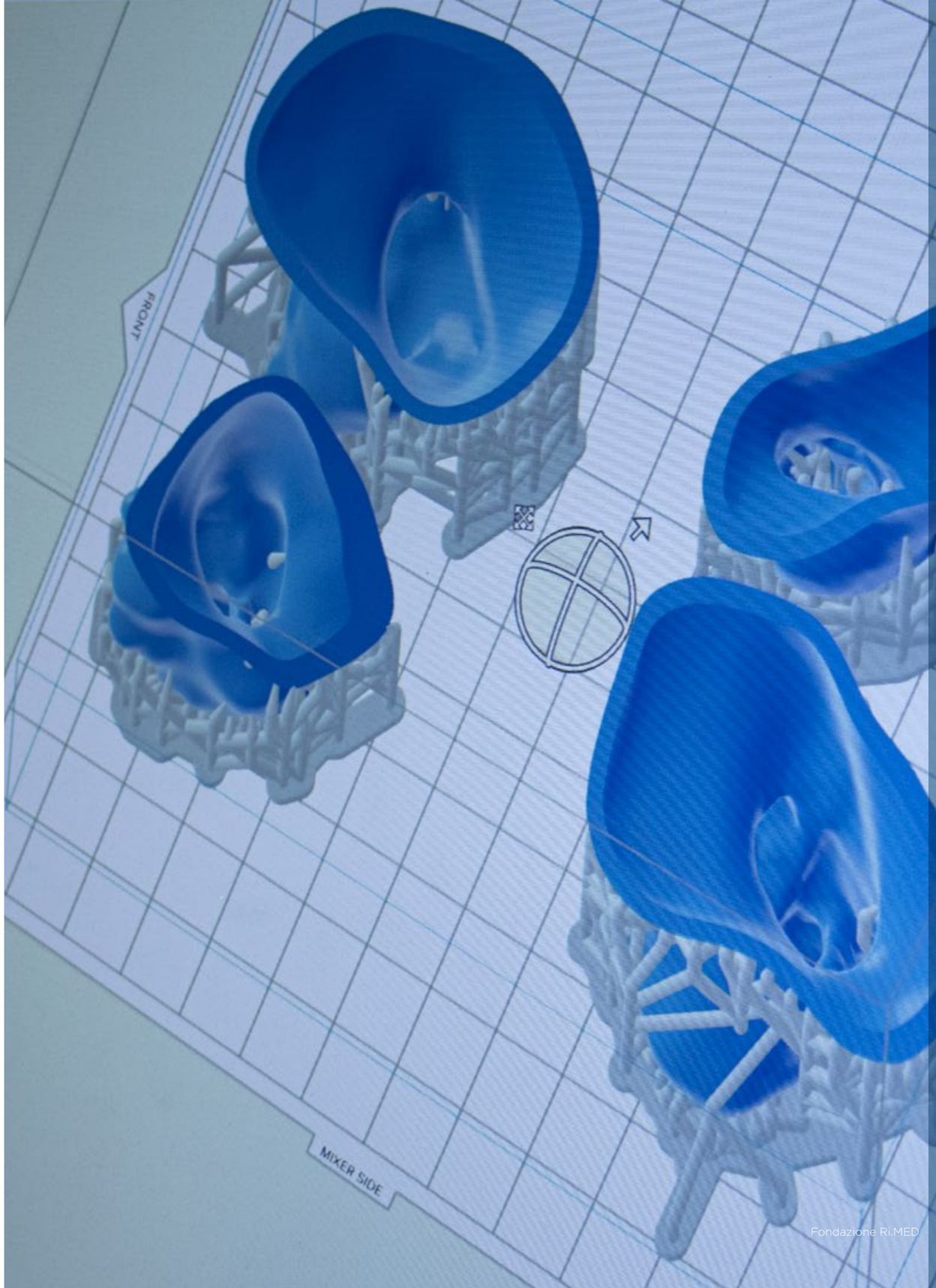
TECHNOLOGY PLATFORMS

In recent years, thanks to the funding provided by the Sicilian Region and by the Dipartimento Casa Italia of Presidency of the Council of Ministers, the technological equipment of the Ri.MED platforms has been significantly enhanced:

- 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;
- an automated system was also implemented for the storage and manipulation of molecule libraries for the **High Throughput Screening** laboratory;
- a cardiac simulator and instrumentation was acquired for the characterization of biomaterials and medical devices, which the **Bioengineering** group uses for the development of new solutions for patients;
- the **Biophysics and Structural Biology** platform, dedicated to the production and purification and three-dimensional study of proteins of therapeutic interest, can boast an 800 MHz magnetic resonance spectrometer, 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;
- 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. In 2021, the **Cell factory** for the production of ATMP was completely renovated.

The important news of 2021 was the setting up of the **Tissue Engineering** Platform, made possible also thanks to the funding obtained with the ERC awarded to Antonio D'Amore. This 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.

Finally in 2021, the **Medicinal Chemistry** Platform was completed, allowing 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.



- Bioengineering
- Bioinformatics
- Biomedical Imaging and Radiomics
- Cell Factory
- High-throughput Screening (HTS)
- Medicinal Chemistry
- Molecular Informatics
- Proteomics
- Structural Biology and Biophysics
- Tissue Engineering

Bioengineering

Platform

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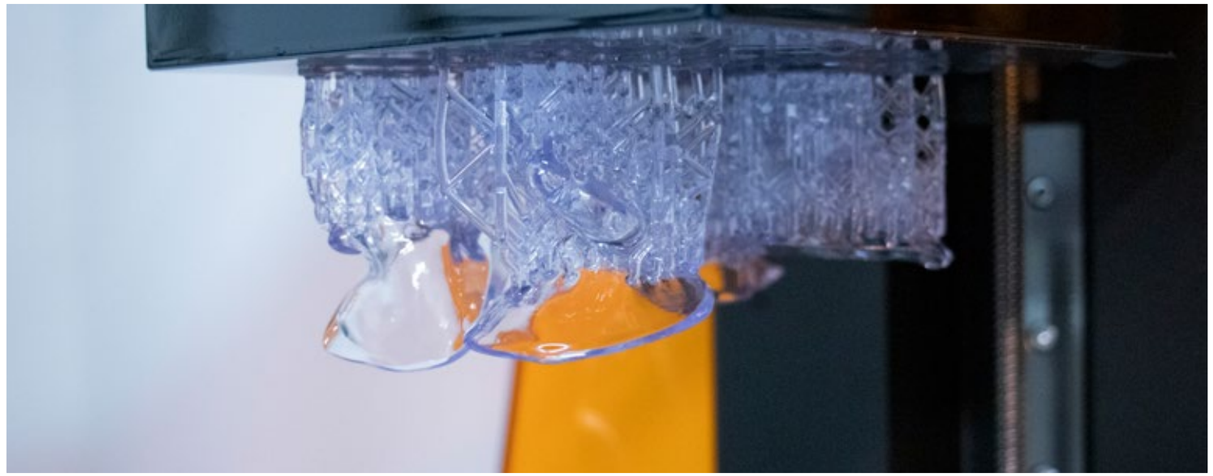
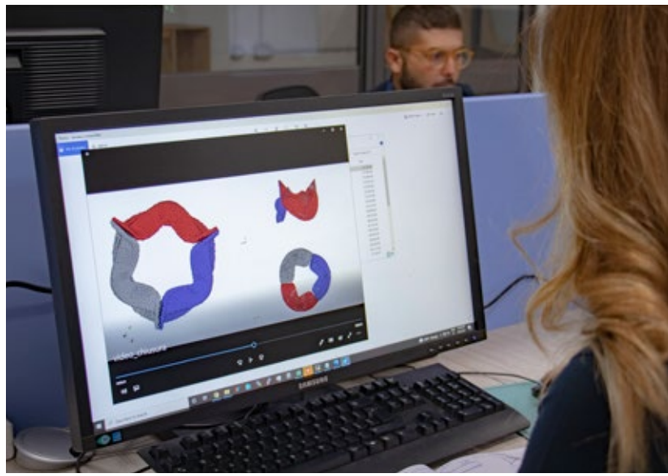
The Bioengineering Platform provides the treatment and characterisation of biomaterials, the numerical simulation of complex physiological systems; and the preclinical validation of medical devices of the different classes (from class I to class III). Our research team offers solid expertise in numerical modelling, fluid-structure analysis, design optimisation of medical devices, and pre-clinical evaluations complying with regulatory requirements and good practice. In the medium term, the division aims to establish 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.

PARTNERSHIPS & COLLABORATIONS

- ISMETT- IRCCS, Palermo, Italy
- Policlinico Giaccone, Palermo, Italy
- Università degli Studi di Palermo, Italy
- Institute Foundation G. Giglio, Italy
- Università degli studi di Padova, Italy
- Université de Technologie de Compiègne, France
- Barts Heart Centre at St Bartholomew's Hospital, UK
- Great Ormond Street Hospital for Children, UK
- University College London, UK
- Queen Mary University of London, UK
- University of Bristol, UK
- University of Leeds, UK
- University of Pittsburgh, USA
- University of Alabama at Birmingham, USA
- Adeka, JP





Expertise

- Development of cardiovascular medical implants;
- Mechanical and thermo-mechanical characterisation of biomaterials;
- Numerical simulation of physiological systems and their interaction with medical devices (by means of structural, fluid dynamic and fluid-structure interaction analyses);
- Development of patient-specific holistic decision-making processes;
- Determination of non-invasive prognostic markers for the monitoring and diagnosis of cardiovascular diseases;
- Hydrodynamic *in vitro* characterisation of physiological systems and cardiovascular implants;
- Functional life prediction for cardiovascular medical implants.

Technology platform

- codes for the numerical simulation of complex physiological systems;
- 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.

PUBLICATIONS

Di Leonardo, S., Cappello, R., **Burriesci, G.**, Pitarresi, G. (2021) Investigation of the thermomechanical response of cyclically loaded NiTi alloys by means of temperature frequency domain analyses. Materials, 14: 7866. https://doi.org/10.3390/ma14247866.

McGregor, C., Salmonsmith, J., **Burriesci, G.**, Byrne, G.W. (2021) Biological Equivalence of GGTA-1 Glycosyltransferase Knockout and Standard Porcine Pericardial Tissue using 90-day Mitral Valve Implantation in Adolescent Sheep. Cardiovascular Engineering and Technology. https://doi.org/10.1007/s13239-021-00585-0.

McGregor, C., Salmonsmith, J., **Burriesci, G.**, Byrne, G.W. (2021) Biological equivalence of GGTA-1 glycosyltransferase knockout and standard porcine pericardial tissue using 90-day mitral valve implantation in adolescent sheep. Xenotransplantation 28(5): Abstract 310.4

Annio, G., Torii, R., Ducci, A., Muthurangu, V., Tsang, V., **Burriesci, G**. (2021) Experimental validation of Enhanced Magnetic Resonance Imaging (EMRI) using Particle Image Velocimetry (PIV). Annals of Biomedical Engineering 49: 3481–3493. https://doi.org/10.1007/s10439-021-02811-1

Tango, A.M., Ducci, A., **Burriesci, G.** (2021) *In silico* study of the ageing effect upon aortic valves. Journal of Fluids and Structures, 103:103258. https://doi.org/10.1016/j.jfluidstructs.2021.103258.

Vella, D., **Monteleone, A.**, **Musotto, G.**, Bosi, G.M., **Burriesci, G.** (2021) Effect of the Alterations in Contractility and Morphology Produced by Atrial Fibrillation on the Thrombosis Potential of the Left Atrial Appendage. Front. Bioeng. Biotechnol. 9: 586041. doi: 10.3389/fbioe.2021.586041.

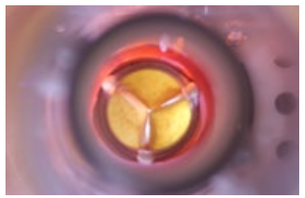
Burriesci, G., Rahmani, B., Byrne, G., McGregor, C. (2021) Bioprosthetic heart valve. Patent US11109962 B2 (granted)

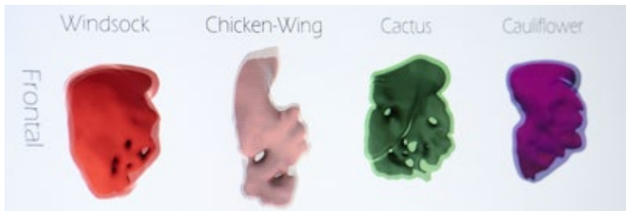
Burriesci, G. (2021) 半刚性瓣环成形环及其制造方法. Patent Application CN112437650

Burriesci, G. (2021) Semi-rigid annuloplasty ring and method of manufacturing. Patent Application EP3793479 A1.

ACTIVE RESEARCH PROJECTS

Analisi dell'appendice atriale sinistra per la predizione del rischio di trombosi.
- Simulazioni numeriche Smoothed Particle Hydrodynamics (SPH) per applicazioni di bioingegneria cardiovascolare.
- Caratterizzazione termomeccanica di biomateriali superelastici Ni-Ti.





Bioinformatic

Platform

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Bioinformatics and Data Analysis platform is devoted to help 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.

PARTNERSHIPS & COLLABORATIONS

- IRIB-CNR, Palermo, Italy
- Università degli Studi di Palermo, Italy
- University of Pittsburgh, USA
- IRCCS ISMETT, Palermo, Italy

4264	2615	15618	13233	17192	16093	16208	22978	9559	16621	16629	4543	11884	10779	4545	11939	13998	2444	12364	14436	14814	27676
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6741	2735	10245	9503	16398	37993	18784	14577	12919	15471	3054	3744	10837	3529	12978	11201	12231	12059	14499	13980	12608	13575
8687	2727	19429	15864	14548	16823	15891	5625	19131	14667	16213	13482	11276	12455	14174	15970	13134	13684	13112	13138	12919	12716
19412	2826	16243	17558	9518	16357	22227	12605	14097	15924	18246	17745	12608	27950	14372	13600	16445	12974	13717	15052	14988	5999
10380	2622	15333	17777	6138	16000	15517	17038	15956	7366	15277	17363	7438	14546	13936	51778	11726	13548	14067	30200	13207	14674
11711	2621	15744	14009	16134	13331	15269	19779	15458	14712	17402	14202	10954	13015	13524	13719	13539	10909	14066	15395	15913	13567

Expertise

- Descriptive statistics and inferential statistics.
- High throughput data analysis, i.e. Next Generation Sequencing or microarray based technologies.
- Machine Learning based predictive algorithms.
- Big Data management and analysis
- Network analysis

Technology platform

Software

Our scripts for data analysis are realized with open-source language, i.e. R and Bioconductor libraries. Visualization of interaction network is performed with the software Pajek or Cytoscape. We use the software Knine 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.

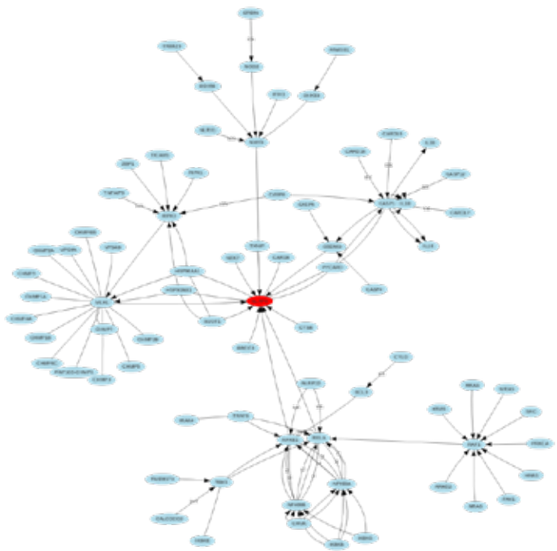
Hardware

- 3 workstation
- 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

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.



Example of network of biological interactions

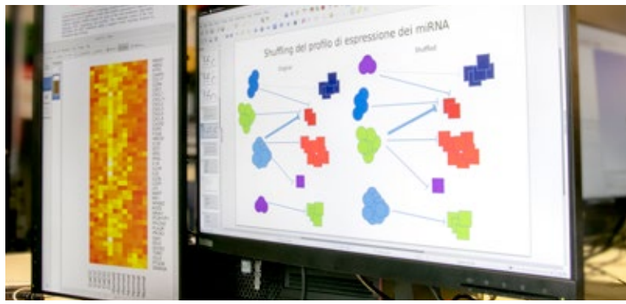
PUBLICATIONS

Coronnello, C, **Francipane MG** (2021) Moving Towards Induced Pluripotent Stem Cell-based Therapies with Artificial Intelligence and Machine Learning, Stem cell reviews and reports 1-11. doi.org/10.1007/s12015-021-10302-y

Coronnello C, Busà R, Cicero L, **Comelli A, Badami E** (2021) A Radioactive-Free Method for the Thorough Analysis of the Kinetics of Cell Cytotoxicity, Journal of Imaging 7 (11), 222. doi.org/10.3390/jimaging7110222

Cilluffo D, Chiavetta RF, Bivona S, Contino F, **Coronnello C**, Feo S, Di Leonardo A, Barra V
(2021) Transcriptomic Changes Following Partial Depletion of CENP-E in Normal Human Fibroblasts. Genes 12 (9), 1322. doi.org/10.3390/genes12091322

Iannolo G, Sciuto MR, Cuscino N, **Carcione C**, **Coronnello C**, **Chinnici CM,** Raffa GM, Pilato M, Conaldi PG,
miRNA expression analysis in the human heart: Undifferentiated progenitors vs. bioptic tissues—Implications for proliferation and ageing, Journal of Cellular and Molecular Medicine. 25 (18), 8687-8700, doi.org/10.1111/jcmm.16824



Biomedical Imaging and Radiomics

Platform

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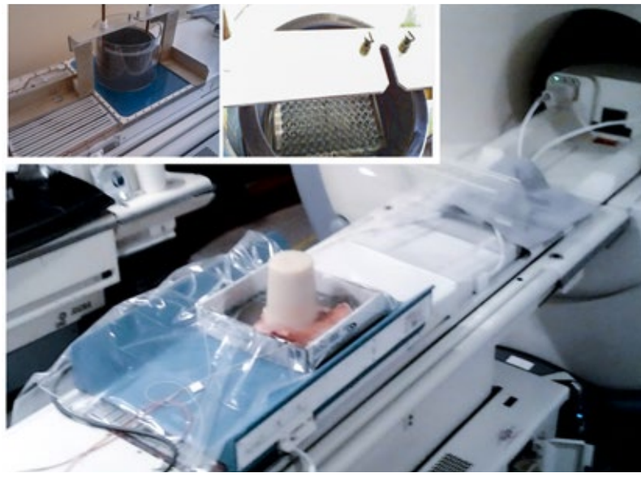
acomelli@fondazionerimed.com

During 2021, the Biomedical Imaging platform made use of two magnetic resonances (3T and 7T), a Spectrum *In Vivo* Imaging System (Bioluminescence) 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 and medical diagnosis support were developed in collaboration with GIT and IBFM-CNR.

The Biomedical Imaging platform provides a crucial support to promote the translation of scientific results in clinical applications, specifically for neuroscience and cancer research. The staff, currently increasing, is today composed by a computer scientist expert in biomedical image processing and analysis, and in acquisition of clinical and preclinical magnetic resonance, a physics, a veterinary and a doctoral student in nuclear medicine. During 2021, the platform was enriched with a WIZARD 2470 gamma counter with 10 PerkinElmer detectors and radiation protection devices (at ISMETT), a microCT Skyscan 1276 CMOS Bruker and a Magnetic Resonance guided Focused UltraSound Surgery (MRgFUS) - ExAblate 2100 - InSightec (at IZS) in order to offer more options for imaging and radiomics.

PARTNERSHIPS & COLLABORATIONS

- IRCCS ISMETT, Palermo, Italy
- Istituto di Bioimmagini e Fisiologia Molecolare, (IBFM-CNR), Cefalù, Italy
- Georgia Institute of Technology, (GIT), Atlanta, USA
- Unità di Medicina Nucleare, Università di Messina, Italy
- Dipartimento di Promozione della Salute, Assistenza Madre e Infanzia, Medicina Interna e Specialità Mediche, (PROMISE), Università di Palermo, Italy
- Dipartimento di Biomedicina, Neuroscienze e Diagnostica avanzata (BIND), University of Palermo, Palermo, Italy
- Dipartimento di Ingegneria, Università di Palermo, Italy
- Dipartimento di Scienze Agrarie, Alimentari e Forestali
- Università degli Studi di Palermo Unità di Fisica Medica, Ospedale Cannizzaro, Catania, Italy
- Dipartimento di Medicina Nucleare, Ospedale Cannizzaro, Catania, Italy
- Istituto Zooprofilattico Sicilia (IZS), Palermo, Italy



Expertise

- Image Processing Models (MR/PET/CT/IVIS 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 studies on innovative radiopharmaceuticals
- *In vitro* and *in vivo* Magnetic Resonance Guided Focused Ultrasound Surgery (MRgFUS)

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
- 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.)

At Institute of Molecular Bioimaging and Physiology, National Research Council (IBFM-CNR):

- PET/CT Clinicale Preclinical

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, Dr.ssa Claudia Coronello, Dr.ssa Monica Miele, Dr. Walter Arancio 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 Coronello.

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.

In vitro and in vivo functional imaging studies using Magnetic Resonance Guided Focused Ultrasound Surgery (MRgFUS) on tumor cells and mouse preclinical model for personalized medicine treatments with and without scaffolds.
IBFM-CNR: Dr. Giorgio Russo e Dr.Alessandro Stefano.
Fondazione Ri.MED: Dr. Albert Comelli, Dr.ssa Claudia Coronello, Dr. Walter Arancio, Dr. Simone Valenti e Dr.ssa Viviana Benfante.

PUBLICATIONS

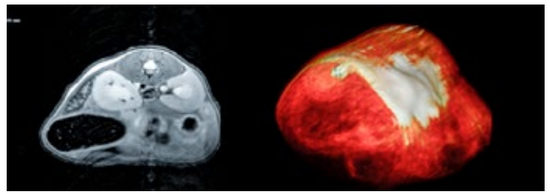
C. Coronnello, R. Busà, L.Cicero, **A. Comelli, E. Badami.** A Radioactive-Free Method for the Thorough Analysis of the Kinetics of Cell Cytotoxicity. Journal of Imaging. 2021; 7(11):222. DOI 10.3390/jimaging7110222.

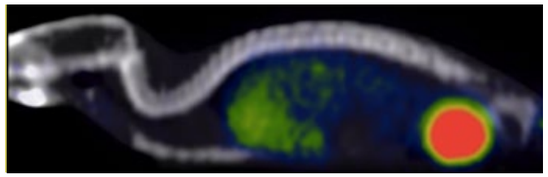
A. Stefano, P. Pisciotta, M. Pometti, **A. Comelli**, S. Cosentino, F. Marletta, S.Cicero, MG. Sabini, M. Ippolito, G. Russo. Early Monitoring Response to Therapy in Patients with Brain Lesions Using the Cumulative SUV Histogram. Applied Sciences. 2021; 11(7):2999. DOI 10.3390/app11072999.

D. Giambelluca, R. Cannella, F. Vernuccio, **A. Comelli**, A. Pavone, L. Salvaggio, M. Galia, M. Midiri, R. Lagalla and G. Salvaggio. PI-RADS 3 Lesions: Role of Prostate MRI Texture Analysis in the Identification of Prostate Cancer. Current Problems in Diagnostic Radiology. DOI: 10.1067/j.cpradiol.2019.10.009

G. Salvaggio, **A. Comelli**, M. Portoghese, G. Cutaia, R. Cannella, F. Vernuccio, A. Stefano, N. Dispensa, G. La Tona, L. Salvaggio, M. Calamia, C. Gagliardo, R. Lagalla and M. Midiri. Deep learning network for segmentation of the prostate gland with median lobe enlargement in T2-weighted MR images: comparison with manual segmentation method. Current Problems in Diagnostic Radiology. DOI 10.1067/j.cpradiol.2021.06.006.

A. Comelli, N. Dahiya,A. Stefano, **V. Benfante**, G. Gentile, V. Agnese, G.M. Raffa, M. Pilato, A. Yezzi, G. Petrucci and S. Pasta. Deep learning approach for the segmentation of aneurysmal ascending aorta. Biomed. Eng. Lett. (2021). DOI 110.1007/s13534-020-00179-0.

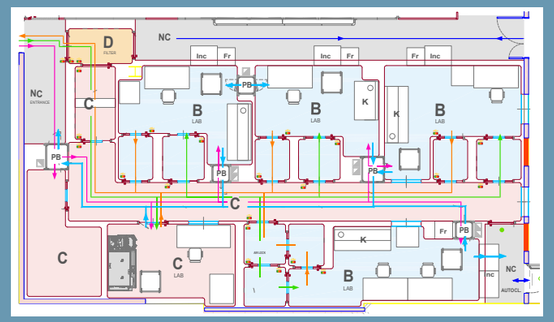




Cell Factory

CONTACTS:
Chiara Di Bartolo, MSC
cdibartolo@fondazionerimed.com

The Cell Factory Group includes Production, Quality Assurance and Quality Control staff (Ri.MED Foundation), as well as a Qualified Person (ISMETT) for the release of Advanced Therapy Medical Products for clinical use. The group can provide support for the definition of Good Manufacturing Practice (GMP) compliant production processes and quality control tests.
The new Cell Factory at IRCCS ISMETT hospital will allow to produce advanced therapies developed by Ri.MED Foundation and ISMETT researchers, and to use these therapies in clinical trials or for the specific treatment of single patients.
Moreover, thanks to Technology Transfer agreements with the University of Pittsburgh and with other Cell Factories/ companies in Italy and Europe, it will be possible to produce and test, at our facility, externally developed products to be used for ISMETT patients or to be sent to other hospitals.



GMP Facility Layout, with personnel and material flows

PARTNERSHIPS & COLLABORATIONS

- IRCCS ISMETT, Palermo, Italy
- University of Pittsburgh, Pittsburgh, USA
- UPMC, Pittsburgh, USA

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

FACILITY DESCRIPTION

The new cell factory, completed in 2021, 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, or 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, after passing the intermediate product through a pass box. An additional class C room is used for cell preparation through 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 the QC labs are equipped with a remote monitoring system.

ACTIVITIES

The renovation works of the new cell factory and the Quality Control laboratories were completed in December 2021. Most of the equipment is installed and the furnishings are being finalized. In 2022, the qualification of the equipment will be completed.
The cell factory staff will carry out in 2022 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.
Continuous activities include the maintenance of the GMP compliant Quality Assurance system and the periodic training of internal and external staff.

High-throughput Screening

Platform

CONTACTS:
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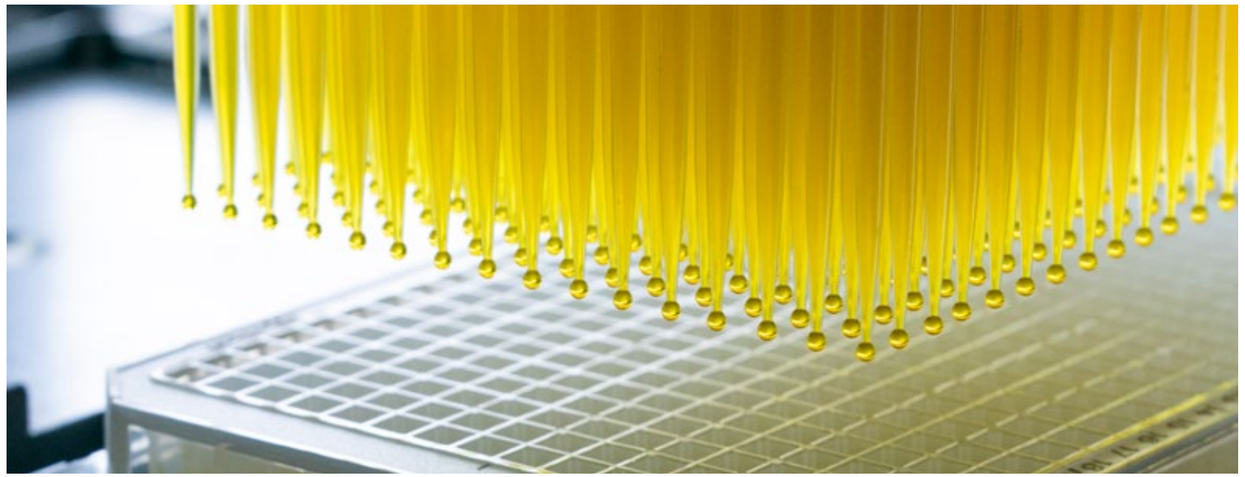
The high-throughput screening (HTS) platform provides labs and expertise for the development, miniaturization and validation of biochemical and cellular assays for the screening of libraries of compounds. Our instrumentation allows the setup of flexible and partially automated protocols using a variety 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.

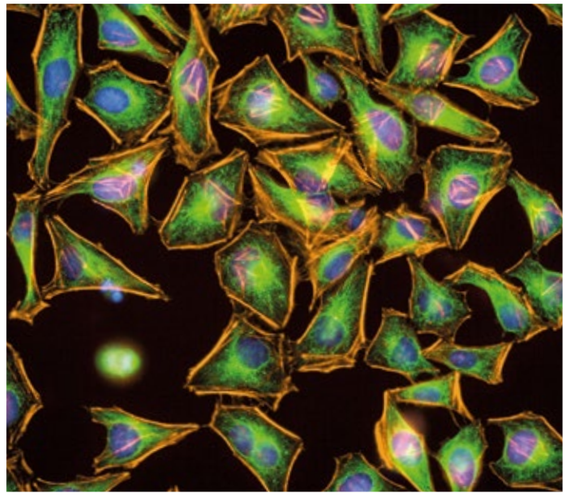


Expertise

- 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

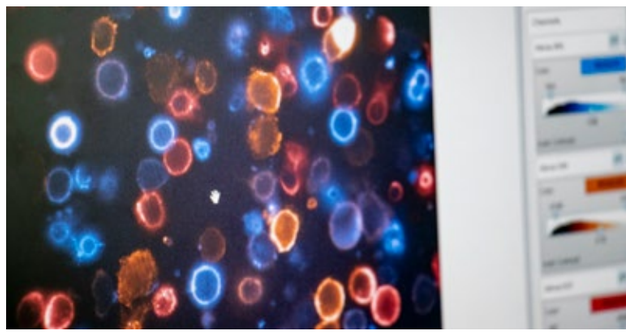
- Wet lab for cell and molecular biology;
- EL406 (Biotek) – automatic microplate washer/dispenser;
- Aquamax 4000 – automatic microplate washer for gentle cell washing;
- Operetta-CLS (Perkin Elmer) –high-content imaging (HCI) system;
- Spark (Tecan) - multimode microplate reader;
- In-Hood-Bravo (Agilent)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



ACTIVE RESEARCH PROJECTS

Development of inhibitors of the Lysine-specific demethylase 4A (Kdm4A) for new anticancer therapies. The project aims at discovering new molecules able to effectively and selectively inhibit the Kdm4A enzyme. To this purpose, a primary enzymatic assay has been developed for the screening of selected libraries of compounds. The first screening campaign was completed in January 2021 and led to the identification of a validated hit currently under study;

Development of selective inhibitors of the intracellular NLRP3 receptor for the treatment of chronic diseases associated with aging. The project aims at discovering new molecules able to selectively inhibit the activation of NLRP3. In 2021, our group completed the validation of the primary assay, a phenotypic assay in which the release of the cytokine IL-1β from human macrophages is measured following selective activation of the NLRP3 inflammasome. The first screening campaign was completed, and results are currently being validated.



PARTNERSHIPS & COLLABORATIONS

- Istituto per la Ricerca e l'Innovazione Biomedica (IRIB) - CNR, Palermo, Italy
- Istituto di Farmacologia Traslazionale (IFT) - CNR, Palermo Italy
- Institut de la Vision, Parigi, France
- Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy
- Università degli Studi di Palermo, Palermo, Italy

Medicinal Chemistry
Platform

CONTACTS:
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The Medicinal Chemistry group is focused on the design and synthesis of novel small molecules, together with the creation of compound libraries and building blocks collections and aims at discovering new active ingredients towards the theraputic target of interest. The Medicinal Chemistry platform supports the early phases of drug discovery programs, with hit structure confirmation, hit re-synthesis, hit series expansion; and in the later stages of hit optimization, and hit-to-lead. The expertise include the design, the organic synthesis, the structural elucidation, and analytical characterization of newly synthesized compounds. Moreover, the structure-activity-relationship (SAR) studies allow to explore the chemical space of compounds of interest and to better define their biological profile in terms of potency and efficacy. The platform is fully equipped and can handle each step of the work-flow, such as, i) reactions set-up; ii) reaction mixtures work-up and purification; iii) isolation of compounds of interest; iv) structure elucidation and characterization; v) standard purity grade assessment for testing compounds.

PARTNERSHIPS & COLLABORATIONS

- Università degli studi della Campania "Luigi Vanvitelli", Naples, Italy
- Istituto per la Ricerca e l'Innovazione Biomedica (IRIB) - CNR, Palermo, Italy

Expertise

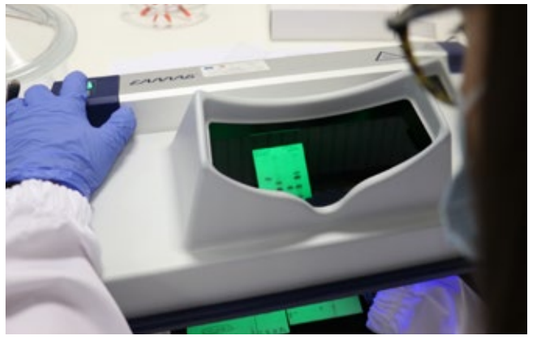
- Drug design
- Organic synthesis
- Planning, development and optimization of synthetic routes
- Microwave-assisted chemistry
- Purification of complex reaction mixtures in normal and reverse phase
- Structure elucidation
- Analytical characterization
- Purity grade assessment

Technology platform

- Water purifier system: Production of pure and ultra-pure water for analytical applications
- Flash chromatography apparatus: Isolation on normal and reverse phase of compounds of interest from complex reaction mixtures
- High-performance liquid chromatography (HPLC): Semi-preparative applications and standard purity grade
- Liquid chromatography-mass spectrometry (LC-MS): Reaction monitoring and analysis of organic compounds commonly found in complex samples

- Microwave reactor: Homeogenous and heterogenous catalytic transformations
- Centrifugal vacuum evaporator suitable for freeze-drying

Implementation:
- Solvents purification system: Safe and rapid production of anhydrous solvents, required in air-free reactions
- Peptide synthesizer for parallel synthesis



ACTIVE RESEARCH PROJECTS

- Design and synthesis of new allosteric inhibitors of NLRP3 inflammasome, validated target for chronic inflammation in age-related diseases
- Design and synthesis of new KDM4a inhibitors, as potential anti-cancer drugs

PUBLICATIONS

- **Mekni, N.; Coronnello, C.**; Langer, T.; **De Rosa, M.; Perricone, U**. (2021). Support Vector Machine as a Supervised Learning for the Prioritization of Novel Potential SARS-CoV-2 Main Protease Inhibitors. Int. J. Mol. Sci., 22, 7714 (1-20). doi.org/10.3390

- Sharma, A.; **De Rosa, M.**; Singla, N.; Singh, G.; Barnwal, R. P.; Pandey, A. (2021). Tuberculosis: An Overview of the Immunogenic Response, Disease Progression, and Medicinal Chemistry Efforts in the Last Decade toward the Development of Potential Drugs for Extensively Drug-Resistant Tuberculosis Strains. J. Med. Chem. 64(8), 4359–4395. doi.org/10.1021/acs.jmedchem.0c01833

Molecular Informatics

Platform

CONTACTS:
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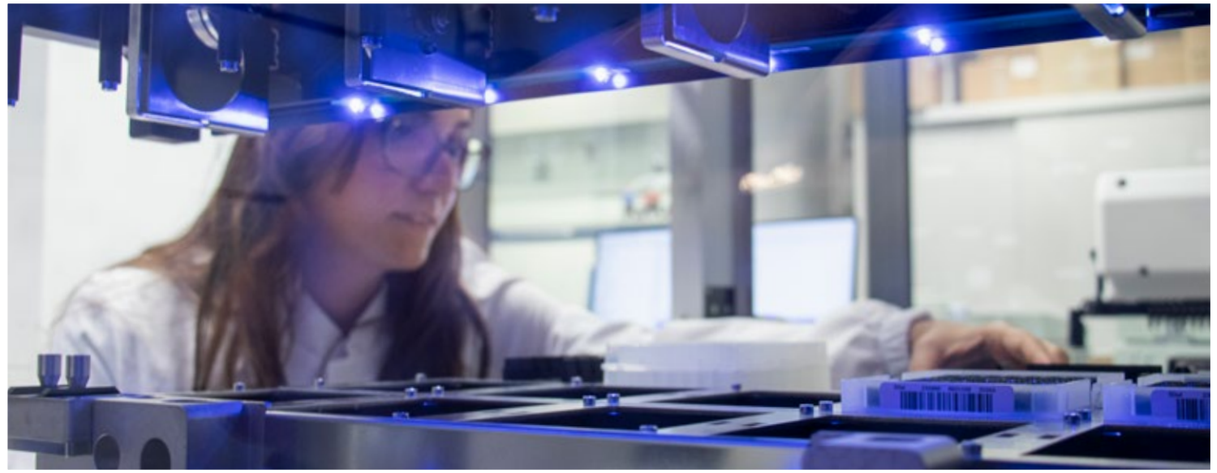
uperricone@fondazionerimed.com

The Molecular Informatics group of the Fondazione Ri.MED mainly deals with the identification and optimization of biologically active molecules through the use of *in silico* techniques usually used for virtual screening or for different chemoinformatic approaches. 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 results obtained through the created models are further validated experimentally through biological or biophysical tests. The group of computational chemistry is also involved in the exploration of the chemical space and in the optimization of the enrichment processes of the virtual molecular libraries available to be used for screening campaigns in High-Throughput (HTS) mode. In recent years, collaboration with the computer engineering group of the University of Palermo has allowed the development of approaches based on the use of artificial intelligence for the prediction of activity and toxicity of biologically active small molecules. Furthermore, during the last year, the group was able to launch the *in silico* Biologics platform that will be used for the design and planning of biological drugs.

PARTNERSHIPS & COLLABORATIONS

- Institut de La Vision, Paris, France
- Università di Vienna (Dip. di Chimica Farmaceutica), Austria
- University of Pittsburgh, USA
- Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy
- Consiglio Nazionale delle Ricerche IRIB-CNR, Palermo, Italy
- Università di Palermo, Italy



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
- QSARINS
- 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 molecole/min
- Virtual Screening HTVS → ~ 5,000 molecole /min
- Virtual Screening SP → ~ 1,500 molecole /min
- Molecular Dynamics → ~ 200 ns/giorno/scheda (su un sistema medio di 40,000 atomi)

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

- Design of inhibitors of **NLRP3** as targets of inflammatory pathology
- Research of protein modulators involved in the epigenetic regulation of tumor pathology (**KDM4**)
- Design and development of **CDK1** inhibitors involved in tumor diseases.
- Modulation of protein-protein interaction modulators with particular reference to **MUC1-CIN85** complexe
- Design and development of Main protease (Mpro) **SARS-CoV-2** inhibitors
- Deep learning and machine learning approaches for *in silico* profiling development

PUBLICATIONS

- **Maria Rita Gulotta** , Riccardo Brambilla, **Ugo Perricone**, Andrea Brancale, A Rational Design of α-Helix-Shaped Peptides Employing the Hydrogen-Bond Surrogate Approach: A Modulation Strategy for Ras-RasGRF1 Interaction in Neuropsychiatric Disorders, Pharmaceuticals 2021, doi: 10.3390/ph14111099
- Ornella Randazzo, Stella M. Cascioferro, Camilla Pecoraro, Widad Ait Iddouch , Amir Avan, Barbara Parrino , Daniela Carbone , **Ugo Perricone**, Godefridus J. Peters, Patrizia Diana, Elisa Giovannetti, SF3B1 modulators affect key genes in metastasis and drug influx: a new approach to fight pancreatic cancer chemoresistance, Cancer Drug Resist 2021, doi: 10.20517/cdr.2021.61
- **Nedra Mekni** , **Claudia Coronnello**, Thierry Langer, **Maria De Rosa**, **Ugo Perricone**, Support Vector Machine as a Supervised Learning for the Prioritization of Novel Potential SARS-CoV-2 Main Protease Inhibitors, Int. J. Mol. Sci. 2021, doi:10.3390/ijms22147714
- **Maria Rita Gulotta** , **Giada De Simone**, Justin John, **Ugo Perricone,** Andrea Brancale, A Computer-Based Methodology to Design Non-Standard Peptides Potentially Able to Prevent HOX-PBX1-Associated Cancer Diseases, Int. J. Mol. Sci. 2021, doi: 10.3390/ijms22115670
- **Maria Rita Gulotta** , Serena Vittorio, Rosaria Gitto, **Ugo Perricone**, Laura De Luca, Exploring molecular contacts of MUC1 at CIN85 binding interface to address future drug design efforts, Int. J. Mol. Sci. 2021, doi: 10.3390/ijms22042208

Proteomics
Platform

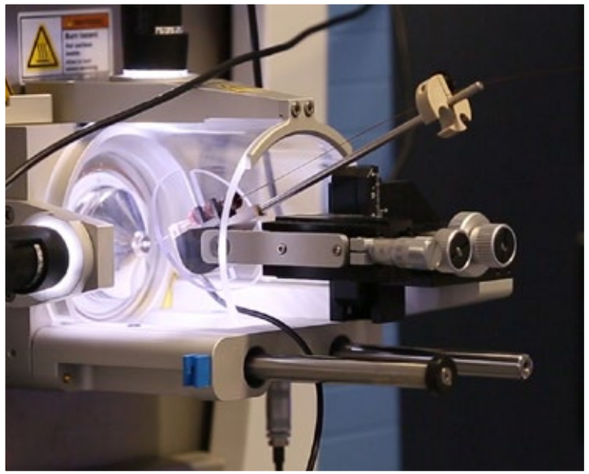
CONTACTS:
Simone Dario Scilabra, PhD
sdscilabra@fondazionerimed.com

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 an UltiMate 3000 RS LCnano System on-line coupled to a Q-exactive 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. 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.

PARTNERSHIPS & COLLABORATIONS

- Institute for Aging and Chronic diseases, University of Liverpool, UK
- German Center for Neurodegenerative Diseases (DZNE), Munich, Germany
- Queen Mary University of London, UK
- Dipartimento di Scienze Farmacologiche, Università di Pisa, Italy
- STEBICEF, Università di Palermo, Italy



Expertise

- 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.

Technology platform

Hardware devices:
- Ultra-High Performance Liquid Cromatography, UHPLC UltiMate 3000 UHPLC RSLCnano System (Thermo Scientific).
- Mass Spectrometer Q-Exactive (Thermo Scientific)

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

ACTIVE RESEARCH PROJECTS

iRhom2: a new therapeutic target for osteoarthritis

- iRhom2: a new therapeutic target for osteoarthritis

Osteoarthritis (OA) is a debilitating disease causing pain and stiffness. At molecular level, osteoarthritis in characterized by breakdown of articular joint, due to the aberrant activity of matrix metalloproteinases (MMPs) and their related disintegrin metalloproteinases with thrombospondin domains (ADAMTSs). The low-density lipoprotein receptor-related protein 1 (LRP1) controls turnover of these proteinases, thus its inactivation by ectodomain shedding contributes to development of the disease. Although the etiology of OA has been traditionally classified as non-inflammatory, the proinflammatory cytokine TNF plays a role in its progression by enhancing the expression of metalloproteinases. Similarly to LRP-1, TNF is proteolytically released by ADAM17, and this cleavage elicits its pro-inflammatory potential. It is clear how inhibition of ADAM17 may lead to beneficial effects in OA progression by preventing LRP-1 and TNF shedding, thus enhancing metalloproteinase turnover and diminishing their expression, respectively. Nevertheless, ADAM17 cleaves more than 80 different proteins, and, as a consequence, its complete inhibition leads to their dysregulation with detrimental side-effects.

Two inactive cognates of rhomboid proteinases, known as iRhom1 and iRhom2, are essential regulators of ADAM17, in that they guide the enzyme maturation through the secretory pathway and direct its proteolytic activity towards specific substrates. By using unbiased secretome analysis, we found that ADAM17-mediated shedding of the large majority of its substrates is supported by either iRhom1 or iRhom2. Interestingly, shedding of TNF and LRP-1 is specifically mediated by iRhom2, with iRhom1 that is not able to compensate. Thus, pharmacological inhibition of iRhom2 can be protective in OA, with lower risk of side effects. Investigating this hypothesis is the central aim of this project.

iRhom2 regulates ADAM17

dependent shedding of surface levels of MHC class I molecules

Major histocompatibility complex (MHC) class I molecules, which are found on the cell surface of all nucleated cells, play a pivotal role in the adaptive immune system by presenting peptide antigens to immune cells.

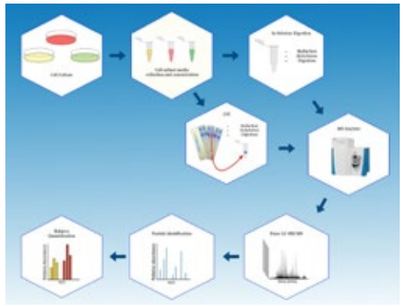
By using unbiased protemics we found that surface levels of MHC class I molecules are regulated by iRhom2-dependent ectodomain shedding. Investigating the functional consequences *in vivo* of this regulatory pathway is the major aim of the project.

Dissecting the role of iRhom2 in the regulation of MHC-I molecules and responsiveness of PDAC to immunotherapy

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers and generally resistant to immunotherapy. A major cause of PDAC limited sensitivity to immunotherapy is loss of MHC-I cell surface expression, mainly as a consequence of MHC-I degradation through autophagy. Therapies to augment the sensitivity of PDAC to immunotherapy are urgently needed. iRhom2 is a ER-associated protein strongly linked with TNFα and IFN-I signalling pathways for its ability to mediate trafficking and activation of the TNFα-convertase and the stimulator of interferon gene (STING), respectively. We hypothesize that iRhom2 can increase MHC-I levels in PDAC cells by activation of STING. Thus, iRhom2 may play a beneficial role in enhancing PDAC sensitivity to immunotherapy. The major aim of this proposal is to dissect the role of iRhom2 in the regulation of MHC-I in the context of PDAC. This study will dissect iRhom2 function, a master regulator of immune responses, in the immunology of PDAC. MHC-I loss in PDAC is the main cause of failure of immunotherapies, thus, this research will provide new insights into this process and lead to new potential targets to augment PDAC sensitivity to immunotherapy.

PUBLICATIONS

- Spanò D. P. and **Scilabra S. D.**; Tissue inhibitor of metalloproteases 3 (TIMP-3): *in vivo* analysis underpins its role as a master regulator of ectodomain shedding, Membranes, in press.
- **Calligaris M**, Cuffaro D, **Bonelli S**, Spanò DP, Rossello A, Nuti E, **Scilabra SD**. Strategies to Target ADAM17 in Disease: From its Discovery to the iRhom Revolution. Molecules. 2021 Feb 10;26(4):944. doi: 10.3390/molecules26040944. PMID: 33579029.
- Yamamoto K, de Groot R, **Scilabra SD**, Kwok HF, Santamaria S. Editorial: ADAM, ADAMTS and Astacin Proteases: Challenges and Breakthroughs in the -Omics Era. Front Mol Biosci. 2021 Oct 12;8:780242. doi: 10.3389/fmolb.2021.780242. PMID: 34712699; PMCID: PMC8546316.
- Carreca AP, Pravatà VM, **D'Apolito D**, **Bonelli S**, **Calligaris M**, **Monaca E**, Müller SA, Lichtenthaler SF, **Scilabra SD**. Quantitative Proteomics Reveals Changes Induced by TIMP-3 on Cell Membrane Composition and Novel Metalloprotease Substrates. Int J Mol Sci. 2021 Feb 27;22(5):2392. doi: 10.3390/ijms22052392. PMID: 33673623; PMCID: PMC7957584.



Generalized mass spectrometry based proteomic workflow

Structural Biology and Biophysics

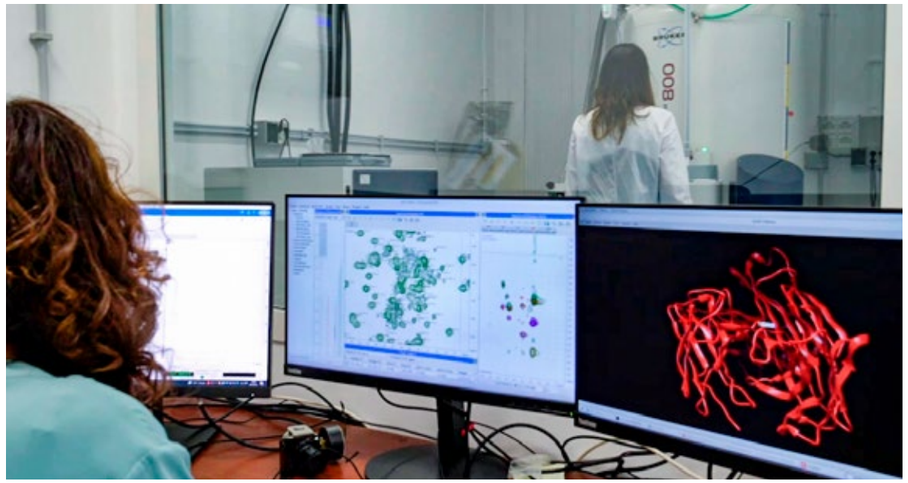
Platform

CONTACTS:
Caterina Alfano, PhD
calfano@fondazionerimed.com

The Structural Biology and Biophysics Platform provides crucial support to small molecules-based drug discovery processes, development of therapeutic antibodies, and development of recombinant protein vaccines. It is also paramount in studying molecular mechanisms at atomic level by elucidating the structure/function relationships of proteins involved in pathological pathways. To gain these purposes, the platform is supplied with cutting-edge equipment that allow a multi-techniques approach such as nuclear magnetic resonance, circular dichroism, calorimetry, and interferometry. In 2021 the Platform will be further implemented for proteins crystallization, and for protein oligomerization and aggregation studies.
The Platform supports several research projects in several therapeutic area such as Aging diseases, Cancer, Infectious diseases and Biomedical application. The diversity of all the active research projects well represents the potential of Structural Biology which can be applied to basic research as well as translational science and can support transversally several research activities.

PARTNERSHIPS & COLLABORATIONS

- King's College London, London, UK
- Scuola Normale Superiore of Pisa, Italy
- Fondazione EBRI Rita Levi Montalcini
- Università degli Studi di Perugia, Italy
- Università degli studi della Campania "Luigi Vanvitelli", Naples, Italy
- Università degli Studi di Palermo, Italy
- Istituto di Biofisica (IBF-CNR), Palermo, Italy
- European Synchrotron Radiation Facility (ESRF)
- International Covid-19 NMR Consortium



Expertise

- Proteins Production: from cloning to purified and characterized proteins;
- Determination of size, structure and stability of macromolecules;
- Structure, kinetic and thermodynamic studies of protein-protein and protein-ligand interactions;
- BLI-based and NMR-based fragment screening;
- Analytical assays, application and development.

Technology platform

- Wet lab for cloning, expression and purification of recombinant proteins;
- 800 MHz triple-resonance NMR spectrometer with cryogenically-cooled probe;
- Isothermal Calorimeter;
- Bio-Layer Interferometer;
- CD Spectropolarimeter;

ACTIVE RESEARCH PROJECTS

- Development of neutralizing antibodies against SARS-CoV-2
- Identification and characterization of interactions among SARS CoV-2 RTC and LaRPs.
- Molecular mechanisms of protein misfolding diseases.
- Structural and biophysical studies probing the interaction of KDM4a with potential inhibitors.
- Development of nontoxic bio-adhesives for wet environments.
- Role of the interaction alfa-synuclein/membranes in Parkinson disease.
- BLI- and NMR-based fragment screening.
- Structure/function relationship studies of SARS-CoV-2 proteins.

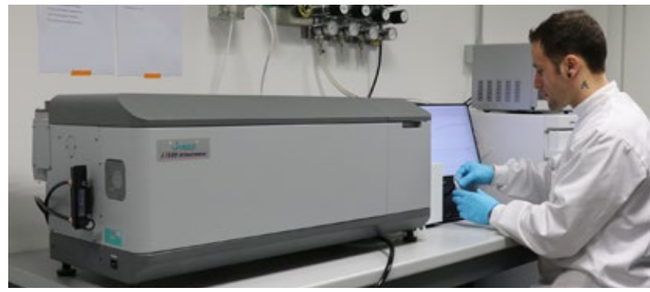
PUBLICATIONS

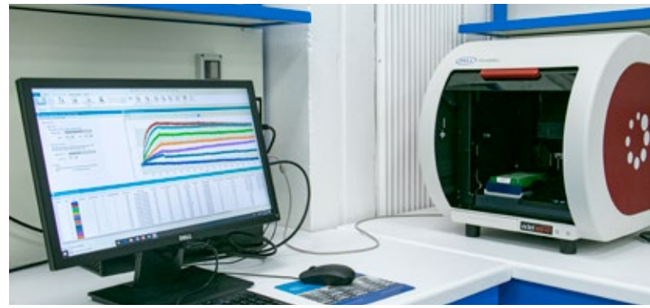
Altincekic N., Korn S.M., Qureshi N.S., Dujardin M., Ninot-Pedrosa, Abele R., Abi Saad M.J., **Alfano C.**,...,**Monaca E.**,...,**Sabbatella R.**,..., Schlundt A. (2021) Large-scale recombinant production of the SARS-CoV-2 proteome for high-throughput and structural biology applications. Frontiers in Molecular Biosciences, 8:89. doi: 10.3389/fmolb.2021.653148.

Dudás E.F., Puglisi R., Korn S.M., **Alfano C.**, Bellone M.L., Dal Piaz F., Kelly G., **Monaca E.**, Schlundt A., Schwalbe H., Pastore A. (2021) Backbone chemical shift spectral assignments of SARS coronavirus-2 non-structural protein nsp9. Biomolecular NMR Assignments, 5(2):235-241. doi: 10.1007/s12104-021-10011-0.

Morando M.A., **Venturella F.**, **Monaca E.**, **Sabbatella R.**, Rosa Passantino, Pastore A., **Alfano C.** (2021) The solution structure of recombinant Pvfp-5β reveals new insights into mussel adhesion. Comm Biol. Submitted.

Passantino R., Mangione M.R., Ortore M.G., Costa M.A., Provenzano A., Amenitsch H., **Alfano C.**, Martorana V., Vilasi S. (2021) Investigation on a MMACHC mutant from cblC disease. BBA - Proteins and Proteomics - Submitted.





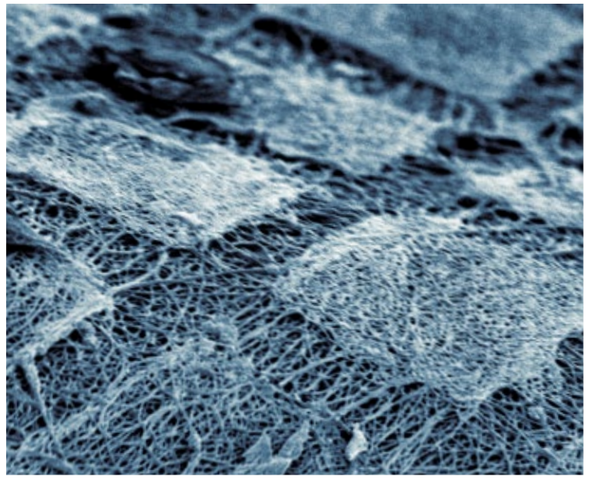
Tissue Engineering
Platform

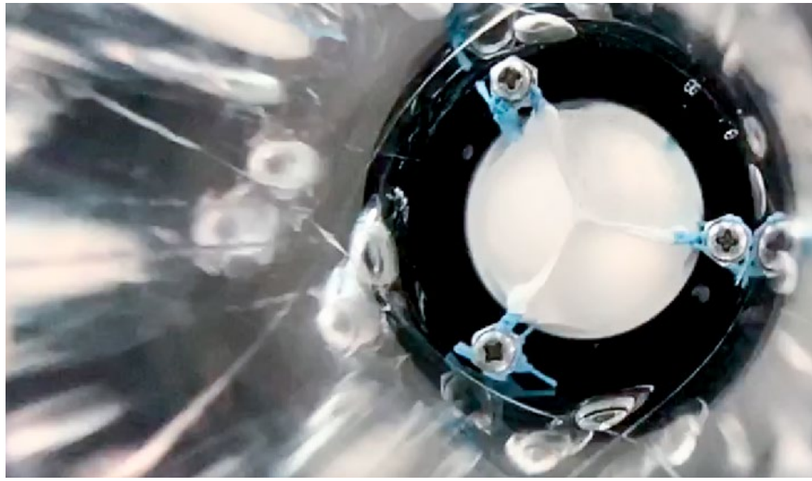
CONTACTS:
Antonio D'Amore, PhD
adamore@fondazionerimed.com

The tissue engineering platform is focused on two main aspects: basic science and translational medicine, applied, in particular, to the cardiovascular research area. This platform enables the mechanical and structural characterization of native and bioengineered tissues and provides a wide range of *in vitro* and *in vivo* assays to study the de novo tissue development. Moreover, offers deterministic structural models for tissue growth and regeneration, customized software for tissues' structural characterization and quantitative histology. From a translational point of view, the tissue engineering platform focuses on the medical device development and the study of minimally invasive mechanisms of deployment especially. In particular, the platform focuses on developing cardiac patches, engineered heart valves, vascular grafts, and bioengineered chordae tendineae. The new tissue engineering laboratories are equipped with the latest generation systems and consist of two macro areas: the biofabrication unit, whose instrumentation includes a bioprinting platform based on a robotic arm with six degrees of freedom, tools for electrospinning and electrodeposition, and the division of materials characterization, that includes machinery for mechanical tests, histological characterization and characterization of the micro and macro structure.

PARTNERSHIPS & COLLABORATIONS

- University of Pittsburgh (PITT), Pittsburgh, USA
- University of Pittsburgh Medical Center (UPMC), Pittsburgh (PA), USA
- Politecnico di Milano, Milano, Italy
- Advances Solutions Life Sciences (ASLS) Inc. - Louisville (KY), USA
- IRCCS ISMETT, Palermo, Italy
- UAI, Argentina
- Aten, University of Palermo
- LivaNova – Mirandola, Italy
- University of Cincinnati, Cincinnati, USA
- West Virginia University, Morgantown, USA
- Harvard Medical School, Boston, USA
- University of Campinas, Campinas Brazil
- Univ. of Texas at Austin, Austin USA
- Telea Biotech – Tissue Engineering Biomedical Technologies, Italy
- University of Nagoya, Japan
- JOMDD, Tokyo, Japan





Expertise

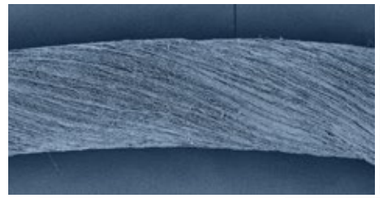
- Development of cardiovascular devices such as cardiac patch, vascular graft and heart valve;
- Synthesis of polymers with a large degree of degradation for the development of medical devices;
- Decellularization of healthy organs;
- Histological characterization of native and engineered tissues;
- Structural characterization of native and engineered tissues;
- Simulation models of physiological systems and their integration with medical devices;
- Development and prototyping of systems for positioning and implantation in a minimally invasive way of medical devices;
- Deterministic structural models for tissue growth and regeneration.

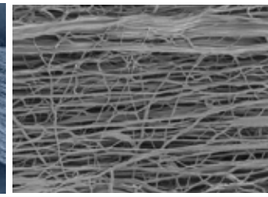
Technology platform

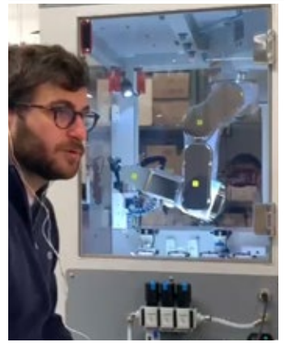
- Numerical codes for the development of simulation models of physiological and predictive systems;
- Software for quantitative histology analysis;
- Software for generating stochastically generated fibrous nets;
- Innovative methods of morphological analysis of micro and nanostructured materials;
- Bio-assembly Robot for the development and optimization of engineered heart valves;
- Electrodeposition equipment with and without mandrel;
- Instrumentation for the mechanical characterization of the devices engineered within the TECP, TEHV and TEVG projects.

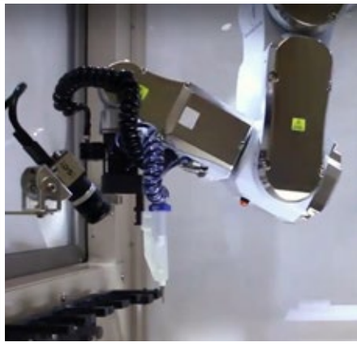
ACTIVE RESEARCH PROJECTS

- Development of a minimally invasive heart valve implantation system;
- Development of a minimally invasive cardiac patch implantation system;
- Study of mechanical, morphological properties and resistance to platelet deposition of a cardiac patch obtained from pericardium in association with the company ADEKA;
- Development of a 3-layer vascular graft to limit hyperplasia of the intima and promote endothelialization.









PUBLICATIONS

Published

[J. A. 2] L. M. Silveira-Filho, G. Coyan, **A. Adamo**, S. K. Luketich, G. Menallo, A. **D'Amore**, W. R. Wagner. 2021 Can a biohybrid acellular patch salvage ventricular function at late time point post-infarction remodeling process? Journal of American College of Cardiology Basic Trans. Science, 6 (5), 447-463 IF 3.7.

[J. A. 1] Y. Kawakami, K. Nonaka, N. Fukase, **A. D'Amore**, Y. Murata, P. Quinn, S. Luketich, K. Takayama, T. Matsumoto, J. H. Cummins, M. Kurosaka, R. Kuroda, W. R. Wagner, F. H. Fu, J. Huard. 2021 A Cell-free Biodegradable Synthetic Artificial Ligament for the Reconstruction of Anterior Cruciate Ligament (ACL) in a Rat Model. Acta Biomaterialia, 121, 275-287, 5Y-IF 716.

Submitted

[J.A.4] Y. Matsumura, L. M. Silveira-Filho, G. Coyan, **A. D'Amore**, W. R. Wagner. Elastomeric tissue engineered template based tricuspid valve. To be submitted to J. of Thoracic and Cardiovascular Surgery, IF 4.88.

[J. A.3] Z. Machaidze*, A. S. Bayoumi*, **A. D'Amore**, K. Feaver, W. Zang, B. Rego, D. Cooper, S. Shimada, K. Rich, J. Wen, D.W. Brown, R. Padera, F. J. Schoen, E. Aikawa, W. R. Wagner, M. S. Sacks and J. E. Mayer. Tissue formation and remodeling of acellular elastomeric scaffold in ovine single pulmonary leaflet replacement model. Submitted to Tissue Enginnering, IF 3.5. *equal contribution.

[J. A.2] S. K. Luketich, G. Menallo, G. Nasello, M. Maneschi, F. Gulizzi, P. Livreri, W. R. Wagner, and **A D'Amor**e. Controlling in-plane mechanics of electrospun polyurethane scaffolds for cardiac tissue engineering applications. To be submitted to Journal of Mechanical Behavior of Biomedical Materials, IF: 3.23.

[J.A.1] **A. Adamo**, J.Bartolacci, M. Traina, A. Pantano, G. Ghersi, W. Wagner, S.F. Badylak, A. D'Amore. Bioprocessing, structure, mechanics and evaluation of micro-fiber based biodegradable suture material. Submitted to Biomaterials, 5Y-IF 8.97.

GRANTS

Ri.MED Foundation supports its scientific activity through funding opportunities offered by public and private entities, regional, national, and international institutions.

Accessing research funds is a strategic activity for the Foundation. For this reason, a Grant Area was established to select financial programs supporting biomedical research, specialized training, and the dissemination of activities, submitting proposals - also in collaboration with other entities - and managing funding programs, as well as coordinating and supervising the approved projects.

In 2021, activities continued on the 8 multi-year projects funded in 2019 and 2020 and currently in progress. In addition, two new projects received funding under national funding (FISR-MUR COVID 19 and MISE) program.

It should be noted that 2021 marked the transition to a new programming cycle that will cover the next seven-year period 2021-2027 and, following the pandemic period, the launch of a transition program covering the three-year period 2021-2023, through the National Recovery and Resilience Plan (NRP). The Grants Area has focused its activities on deepening the requirements for participation in the offer of funding opportunities, dictated by the new European, national, and regional Program Plans.

Among the 2021 activities aimed at obtaining research funds, it is worth mentioning the submission of one project for the HORIZON 2020 EU Calls, 3 projects of Research and Development for national funding (Ministry of Health and MUR), and 2 projects with grant foundations.
In addition, 13 proposals for Innovative and Green Doctorates were submitted for PON 2014-2020 - REACT-EU funds, in partnership with the University of Palermo.

Ongoing scientific project in 2021

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"

Funded by: Assessorato alle Attività Produttive della Regione Siciliana

The goal of the project is to strengthen research, cell production, and diagnostic laboratories of the Ri.MED - ISMETT cluster by the purchase of cutting-edge equipment and by infrastructural improvements

PROGEMA

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

Funded by: Ministero dell'istruzione, dell'Università e della Ri- cerca PON Ricerca e Innovazione 2014-2020

The goal is to improve the treatment of vegetation waters of the oil production chain to extract and reallocate pharmaco- logically active organic compounds, reduce their polluting ef- fect, and re-use treated waters in the production processes.

OBIND

Oncological therapies through Biological Interaction Network Discovery

Funded by: Regione Siciliana nell'ambito del bando PO FERS Azione 1.1.5

The project focuses on the study of biological interactions influencing tumor diseases using new statistical and computational data processing methods, and multiple source integrated data analysis model application.

BIOMITRAL

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

Funded by: European Commission HORIZON 2020 – ERC Con- solidator Grant

The goal of the project is to develop an innovative prototype of a mitral valve by engineering the chordal apparatus and reconnecting the left ventricle with the valve leaflets.

SENSO

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

Funded by: Regione Siciliana nell'ambito del bando PO FERS Azione 1.1.5

The project aims at creating a nanosensor to detect hydrogen peroxide (H2O2) released in vitro/ex vivo cellular system culture. The project aims at making available 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.

PROMETEO

Advanced Medicinal Products placenta-derived for liver and endometrial diseases

Funded by: Regione Siciliana nell'ambito del bando PO FERS Azione 1.1.5

The main goal of the project is to develop 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 task will be optimized according to the principles of Good Manufacturing Practices (GMP) to develop cellular therapies.

iRhom2- a new therapeutic target in osteoarthritis

Funded by: Fondazione con il Sud – Bando Capitale Umano ad Alta qualificazione 2018

The goal of the project is to validate iRhom2 as potential and innovative therapeutic target of osteoarthritis, using state-of the-art proteomics methods and in vivo models of the disease. The project also aims at studying iRhom2 inhibitors.

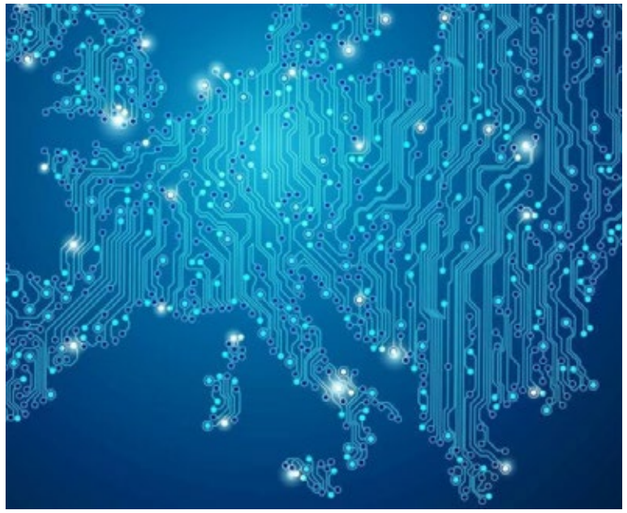
4FRAILTY

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

Finanziato da: Ministero dell'istruzione, dell'Università e della Ricerca PON Ricerca e Innovazione 2014-2020

The goal is to create a computational tool to simulate the sensory platform, including all sensors and vital and environmental signs collected during the clinical work-up. The simplicity and versatility of the computational implementation will allow to quickly simulate different virtual scenarios of any alteration of vital and environmental signs associated with a disease.

Projects eligible for funding in 2021



NABUCCO

New Drugs and Biomarkes of pharmacological response and resistance in colorectal cancer

Call: Accordo di Innovazione - Ministero dello Sviluppo Economico (MISE)

The goal is to create a diagnostic, prognostic and therapeutic network from the collaboration between big pharma (Merck Serono), SMEs (BIOVIIIX), IRCCS and academic world (Universita "Vanvitelli", Ri.MED, IEO) for deconvolution of pathogenic mechanisms underlying the causes of colorectal carcinoma.

The project was approved in 2020 but due to technical problems it will start project in 2022

SENSOMAR

Sensore nano-strutturato per stress ossidativo integrato in mascherina per valutazione in remoto

Call: FISR/MUR 2020 Coordinator IRIB-CNR e IFC-CNR, partners: Uniiversità di Palermo; the RiMED Foundation participates as a support subject to the CNR

The proposal aims to develop an original methodology and an innovative, cost-effective and miniaturised device for monitoring patients suffering from respiratory diseases through the non-invasive detection of hydrogen peroxide in the respiratory exhale. The device, integrated on a mask, will interface with a remote system and will provide information on the evolution of the disease to subjects involved in the health supply chain.

EIDH

European Digital Innovation Hub.

Adhesion of the Foundation to the regional call for interest, (coordinator the Technological Park of Sicily), for the network of Poles for European Digital Transformation (Digital Europe Program)

The network takes the name of European Digital Innovation Hubs (Edih) and aims to ensure the digital transition of the PA and industry through the adoption of advanced technologies (Cyber security, Artificial Intelligence, High Performance Computing).

The candidacy has been approved by the MISE, the opening of negotiations with the EU is awaited

Scientific projects submitted in 2021

Regional Project: HUB BIO-INNAVATHE, BIG & OPEN DATA FOR INNOVATIVE THERAPIES

Call: Bando Nazionale Piano Operativo Sanitario - POS 2014-2020 -Traiettoria 4 "Biotecnologia, bioinformatica e sviluppo farmaceutico"

The project aims to establish the first interregional Hub for Life Sciences. The Hub will allow to develop new innovative technologies and to bring new products to the market, as well as to launch an ambitious Higher Education program.
Coordinator: IRCCS-ISMETT, several national partners of the BIO-INNOVATHE HUB. Ri.MED Foundation in involved in the project through scientific collaboration with subjects of the network

Regional Project: Genesis-Ati

Study of GENEtic events at the carcInogenesi baSe in areas with a high pollution rate by type of production

Call: Bando Nazionale Piano Operativo Sanitario - POS -2014-2020 – Traiettoria 3 "Medicina rigenerativa, predittiva e personalizzata"
Coordinator: ARPA Sicilia, partners UNIPA, INFN, USL Ragusa. Ri.MED Foundation in involved in the project through scientific collaboration with INFN.

Dissecting the role of iRhom2 in the autophagy of MHC-I molecules to enhance responsiveness of PDAC to immunotherapy.

Call: Bando Fondazione AIRC. Fellowships call for applications 2021.
Principal Investigator: Simone Scilabra

Introduction of application of advanced 3D printing tools in the field of cardiovascular therapies.

Call: Bando della Banca d'Italia
Purchase of a 3D printer to be used in collaborative activities between the Research Group in Bioengineering of the Ri.MED Foundation and clinical partners in local hospitals. The availability of equipment it would contribute decisively to raising the standard of care and assistance of cardiovascular diseases, defining more effective therapeutic prototypes and offering the patient a more human and less traumatic experience.
Principal Investigator: Gaetano Burriesci.

PRIME

HORIZON-HLTH-2021-DISEASE-04-02: Building a European innovation platform for the repurposing of medicinal products.

The project aims to create an innovative drug repositioning platform that allows in silico screening and prioritization of candidate drugs for the treatment of paediatric diseases. The platform will provide an efficient R&D environment where users will have immediate access to a system of integrated databases and services as well as tools for evaluating the legislation for the reuse and repositioning of drugs in the pediatric field.

The project was promoted by a European partnership with the consortium for Biological and Pharmacological Evaluations (IT). The RiMED Foundation participates in the project as a "third party" of the CNR.
Principal Investigator: Chiara Cipollina.

REACT-EU. Innovative and Green Doctorates

Call: PON 2014-2020

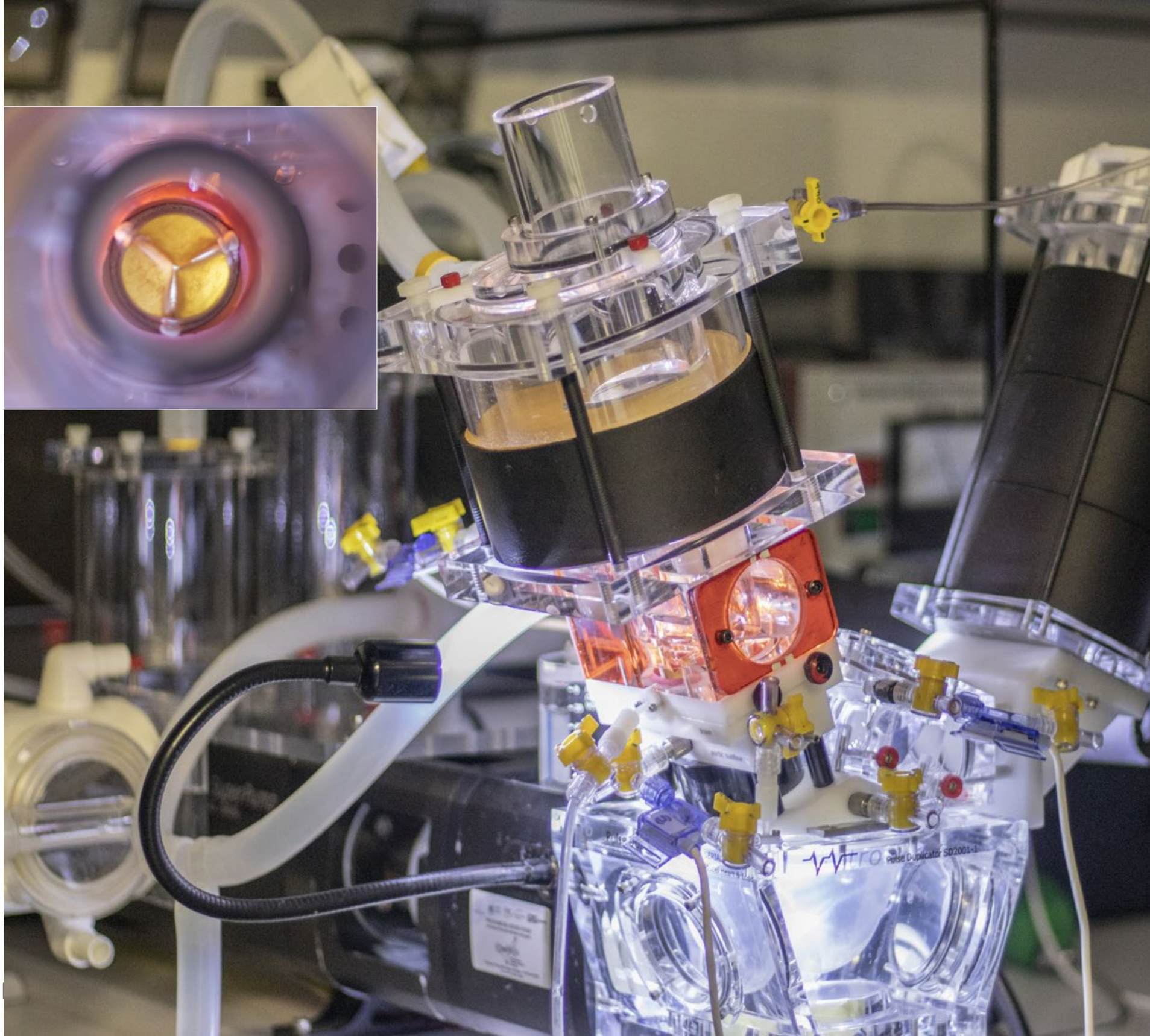
The project aims at the creation of a diagnostic, prognostic and therapeutic network, obtained from the collaboration between big pharma (Merck Se- rono SpA), SME (BIOVIIIX SRL), IRCCS and the academic world (University "Vanvitelli", Ri.MED, IEO), for the deconvolution of the pathogenetic mechanisms underlying the etiogenesis of colorectal cancer. UNIPA in partnership with the Foundation has submitted applications for 13 projects of Innovative Doctorates.

INTELLECTUAL PROPERTY AND TECHNOLOGY TRANSFER

The research activity of Ri.MED is strongly patient oriented, but in order to ensure that scientific results meet 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 IP&TT (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 licensing, industrial sponsorship, and creation of technological spin-offs.

Furthermore, in 2021 Ri.MED and a multinational company operating in the cardiovascular area finalized an important license and collaboration agreement having as its object the Ri.MED patent for the Triskele cardiac implant. This family of patent consists of a heart valve prosthesis, a heart valve, and a related delivery system. One of the inventors is Gaetano Burriesci, Bioengineering group leader of the Foundation.



Patent portfolio up to 31.12.2021

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
Fondazione Ri.MED - University of Pittsburgh

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

NK-mediated immunotherapy and uses thereof
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

MEDICAL DEVICES & TISSUE ENGINEERING

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
Fondazione Ri.MED - University of Pittsburgh

Bi-layer extra cellular matrix scaffolds and uses thereof
WO2017044787
Fondazione Ri.MED - 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
Fondazione Ri.MED - University of Pittsburgh

Retrievable self-expanding non-thrombogenic lowprofile percutaneous atrioventricular valve prosthesis
WO2016138423
Fondazione Ri.MED - 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
Fondazione Ri.MED - University of Pittsburgh

Microfluidic Tissue Development Systems
WO2017062629
Fondazione Ri.MED - University of Pittsburgh

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

Recruitment of mesencymal stem cells using controlled release systems
WO2014022685
Fondazione Ri.MED - 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)
Fondazione Ri.MED - 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
Fondazione Ri.MED - 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 – University of Cincinnati

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

Valved stent for the treatment of tricuspid regurgitation
US provisional Patent Application 62/868,275
Fondazione Ri.MED - University of Pittsburgh

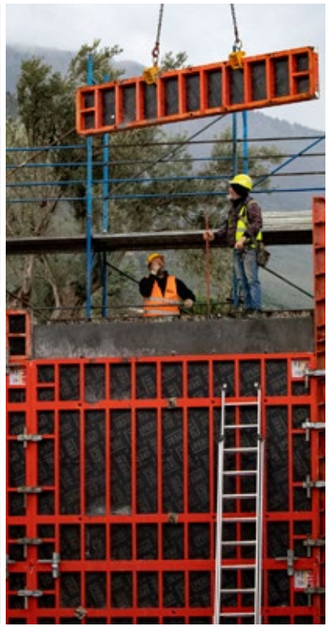
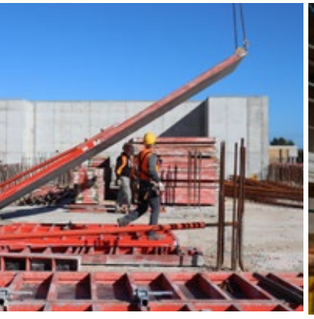
Semi-rigid annuloplasty ring and method of manufacturing
WO2019220365
Fondazione Ri.MED

Heart valve prosthesis
WO2010112844
Fondazione Ri.MED

Prosthesis delivery system
WO2012052718
Fondazione Ri.MED

Prosthetic heart valve
WO2016203241
Fondazione Ri.MED

WORK IN PROGRESS

BRBC

Biomedical Research and Biotechnology Center

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