Fondazione Ri.MED

SCIENTIFIC REPORT 2020

Fondazione Ri.MED

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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

COVER PICTURE:
Human mesenchymal stem cells bone-marrow derived (hBMSCs) adherent onto poly-(D)-lactic acid hyperelastic fibers (FITC-phalloidin staining).

SCIENTIFIC REPORT 2020

A MEDITERRANEAN HUB FOR TRANSLATIONAL RESEARCH

Alessandro Padova

DIRECTOR GENERAL

Fondazione Ri.MED opens successfully the year 2020 with the **ground-breaking milestone for the BRBC construction**. A success for all the hard work over the years of everyone involved with the continuous support of all the Italian founders, the Presidency of the Council of Ministers, the Region of Sicily and the Italian National Research Center (CNR) and of the US partners, the University of Pittsburgh and University of Pittsburgh Medical Center (UPMC). An important recognition must be acknowledged also to ANAC (Italian Anti-Corruption Agency) and Palermo Prefecture for the **legality and anticorruption protocol agreements**.

In 2020, we have started concomitantly **a transition towards a new organization structure** to embrace the challenges ahead with the strategic vision of the **UPMC-IRCCS ISMETT-Ri.MED Cluster.** Administration and R&D organization structures have been consolidated and the process will continue during 2021. It is important to underline **the great effort, commitment and passion of all Ri.MED people** during this challenging year.

Important results have been achieved towards our institutional objectives in terms of translational research and training: Ri.MED research teams, supported by the administration offices, have been productive in terms of R&D programs, receiving recognition for their excellence and attracting public and private funding; A start-up stemmed during 2020 based on IP jointly developed with University of Pittsburgh.

I am grateful to Ri.MED Scientific Director Dario Vignali for the fruitful collaboration and continuous leadership, the Scientific Committee Members for their advice in developing a vision for Ri.MED translational research and Dr Angelo Luca, CEO of IRCCS ISMETT for a very productive year of partnership. Thanks to all members of Ri.MED Board of Directors, and in particular to the **strategic guidance of our President Paolo Aquilanti and Vice President Bruno Gridelli**. A final acknowledgement to our legal advisor Giuseppe Mazzarella and his team for constructive and daily discussions always aimed at Ri.MED vision and ensure all necessary actions with founders, relevant institutions and other stakeholders for a sustainable future.

Dario Vignali

SCIENTIFIC DIRECTOR

Welcome to the 2020 edition of our annual Scientific Report, which offers an overview of our investigators and their research, activities and available technologies, key elements of the Ri.MED mission: **translating biomedical and biotechnological research into innovative therapies for patients**, and **facilitating the recruitment, education and training of the next generation of italian biomedical scientists and physician-scientists**.

Ri.MED concentrates on five research areas: **cancer**, with an emphasis on immunotherapy, **organ insufficiency,** which includes organ transplantation and regenerative medicine, **diseases of aging**, with an emphasis on **neurodegeneration**, and **infectious diseases**, with an emphasis on diseases of relevance to the Mediterranean area.

In 2020, we continue to be grateful to the commitment and effort of our Scientific Committee - Prof. Lucia Altucci, Prof. Ivet Bahar, Prof. Antonino Cattaneo, Prof. Francesco Dieli, Prof. George Fadi Lakkis. The COVID19 pandemic presented enormous challenges for everyone, including Ri.MED. Although we were unable to hold our annual Ri.MED Scientific Symposium and Ri.MED Research Retreat, we continued our research efforts including **new projects on COVID19**.

Communication between the **Scientific Committee** and Ri.MED investigators was enhanced and expanded by establishment of the **Ri.MED R&D Committee** (Dr. Caterina Alfano, Group Leader in Structural Biology and Biophysics; Prof. Gaetano Burriesci Group Leader in Bio Engineering; Prof. Antonio D'Amore Group Leader in Tissue Engineering; Dr. Giovanna Frazziano, Group Coordinator for Regenerative Medicine; Dr. Ugo Perricone Group Leader), with members attending all Scientific Committee meetings. One particularly significant event in 2020 was breaking ground on the Ri.MED Biomedical Research and Biotechnology Center in Sicily.

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

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Ri.MED OVERVIEW UP TO 31.12.2020

Networking

3 Agreements for labs management

31 Active scientific collaborations and technology transfer agreements

12 Scientific collaborations and technology transfer agreements signed in 2020

Training & employment

61 Employees in 2020

59%

41%

30 Scholarships

18 University of Pittsburgh

Post- Doc Fellowships c/o University of Pittsburgh

24 Ph.D. Fellowships

27 Internships

Intellectual property

About **300** Scientific publications

27 Patent

Fundings for Research

19.174.193€

Awarded through national and international GRANTS

8.000.000€

Sicilian Region operational contributions for Ri.MED-ISMETT cluster

Scientific knowledge dissemination

9 Ri.MED internal meetings

38 Ri.MED scientific meetings

9 Ri.MED institutional events

33 Participations in scientific events or local development activities

Building the BRBC

28.000 sq m of laboratories

196.350.000 € Value of the investment

600 Planned occupancy opportunities

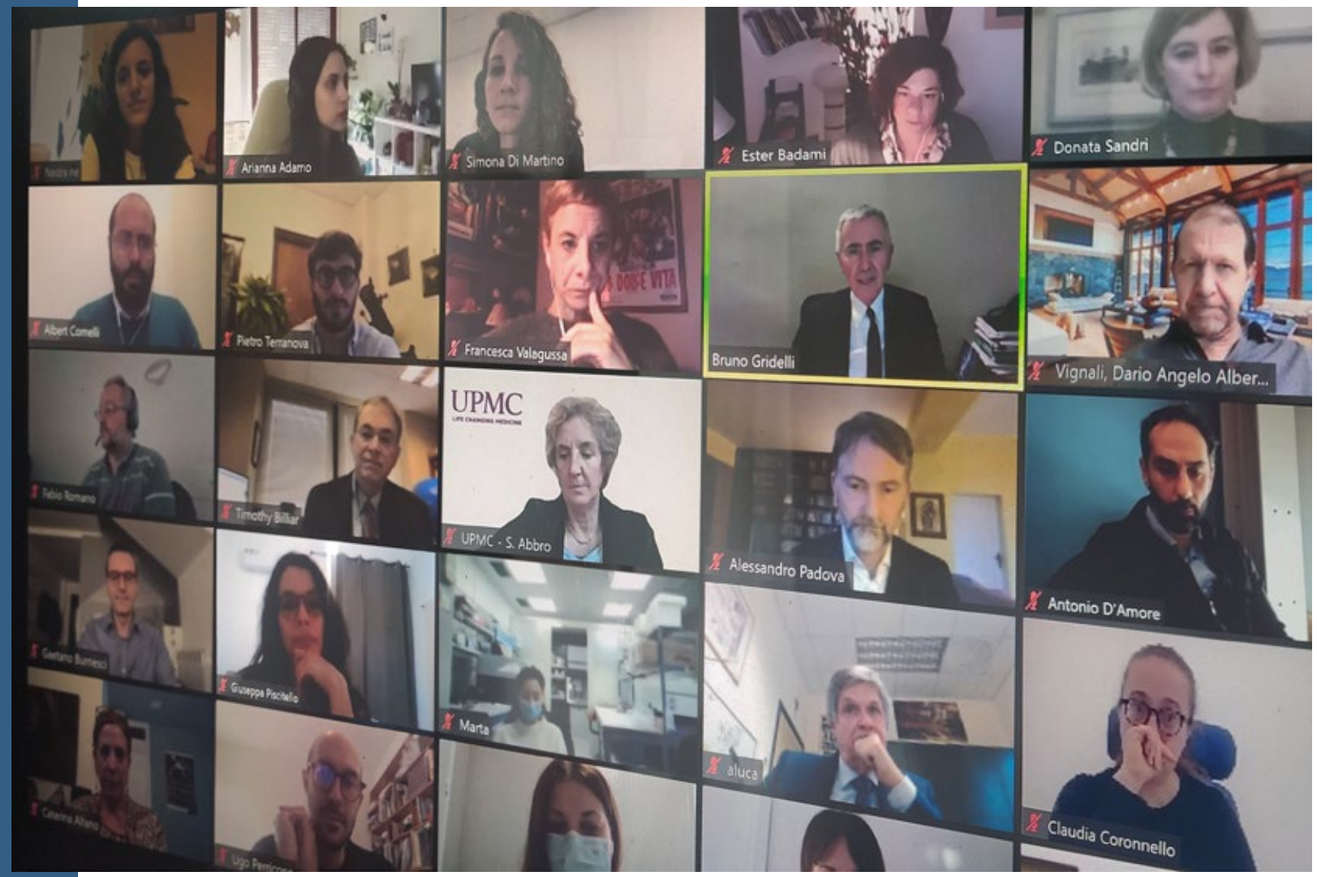
TRAINING
Nurturing new talents

Ri.MED has always placed great effort in training highly-qualified staff, recognizing their key role for successfully accomplishing the scientific challenges, and the competitiveness and development of the whole territory. To date, Ri.MED has activated 27 traineeships, 30 scholarships, 24 PhD and 18 Post-Doc fellowships: a trend destined to increase in view of the creation of the Biomedical Research and Biotechnology Center (BRBC).

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, and other training programs have been activated with the funding obtained through European, national, and regional calls.
Ri.MED obtained approval from FonARCom for the "Innovation and Development" training program for its staff. During 2020, 5 courses were held alternating the in-class presence - where possible - with remote mode: Leadership and Effective HR Coordination; Team Working; Video Editing; and Statistics applied to Biomedicine , for a total of 42 participants and 98 hours of training.

DISSEMINATION OF SCIENTIFIC KNOWLEDGE

Activities linked to scientific dissemination and sharing research results are part of the Foundation's own missions.
The 2020 edition of the annual Ri.MED Scientific Symposium was canceled due to COVID-19 restrictions that unfortunately affected all activities this year and heavily influenced the organization of all our planned scientific events..
The Ri.MED Foundation participated with two events at the European Week of Regions and Cities, which took place remotely in October:



the talk "Science: driving force for the Mediterranean", led by Ri.MED, detailed the role of science to offer centrality and growth opportunities to geographically isolated territories, such as the islands of Cyprus and Sicily. The “Communicating Science #EngageAudience” workshop also saw the participation of Ri.MED on the topic of disseminating scientific knowledge in the territory.

2020 was a profitable year in terms of scientific publications: over thirty articles on peer review journals with relevant impact factors, a contribution to scientific popularization in the sector. Also, the press office worked to convey the main research results to a wider audience of non-experts, thus contributing to the dissemination of scientific knowledge.

NETWORKING

The aim of the collaborations was to integrate complementary competences with joint translational research projects, increasing their critical mass and potential for success. Also, creating networks that generate competitive research financing is crucial.

Ri.MED focuses on the continuous development of its network of scientific collaborations and scientific agreements with bodies and institutions in its areas of interest. There are currently 31 ongoing agreements to develop technological innovation, promote research activities and share laboratory spaces and resources with European and U.S. institutions. Twelve agreements were signed in 2020.

Ri.MED signed agreements for lab hosting and has being managing four laboratories for several years: the Regenerative Medicine and Immunology Laboratory at IRCCS ISMETT, of strategic importance for integrating basic and clinical research; the Structural Biology and Biophysics Laboratory 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

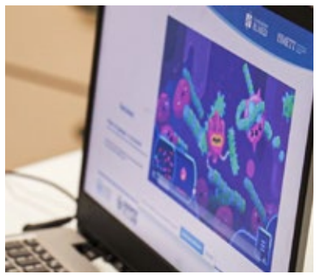
Social and economic impact on Sicily and southern Italy

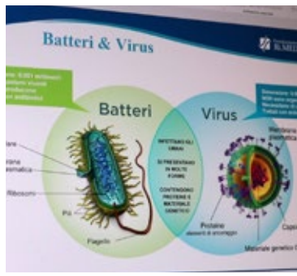
Ri.MED is engaged in the creation of the Biomedical Research and Biotechnology Center (BRBC) in Carini, near Palermo. The BRBC, which will also host a business incubator, is a management model of public-private partnership with universities and research centers, and pharmaceutical and biotechnological companies, developing strategic alliances and attracting funds and investments for research, with a positive impact on the economy of southern Italy.

During 2020, construction works started: on February 14 the land was officially delivered and - despite the interruption due to the COVID-19 restrictions - the construction site finally started.
First of all, the secular olive trees present on the land were explanted and re-planted, then the first structural interventions were carried out: the excavations and casting of the foundation slabs, the assembly of the perimeter formwork of the offices, auditorium, clinics and parking lots, assembly of all six cranes, etc. The temporary association of enterprises (ATI) led by Italiana Costruzioni continues under the direction of the group headed by HOK, winner of the international design competition of the center.
The BRBC will attract members of the international scientific community in Palermo, retaining the best Italian doctors and scientists in our country, also thanks to the collaboration with UPMC and the University of Pittsburgh (UP).

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 main players 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.





Ri.MED intends to gradually increase its presence developing new educational programs with schools, involving the local community.
As with all event-related contact activities, 2020 was heavily penalized by the COVID-19 restrictions. However, Ri.MED attended the European Researchers' Night with the in-remote initiative "La cura è dentro di te", organized the "Research Friday" laboratories of applied sciences at the STEM High School of CEI in Palermo, and started a new project with the Industrial Technical Institute “Volta” of Palermo to train technicians specialized in using diagnostic and biomedical devices.

ATMP - Advanced Therapy Medicinal Products

Regenerative Medicine and Immunotherapy

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GMP Cell Factory

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DRUG DISCOVERY

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Identification of Therapeutic Targets and Screening

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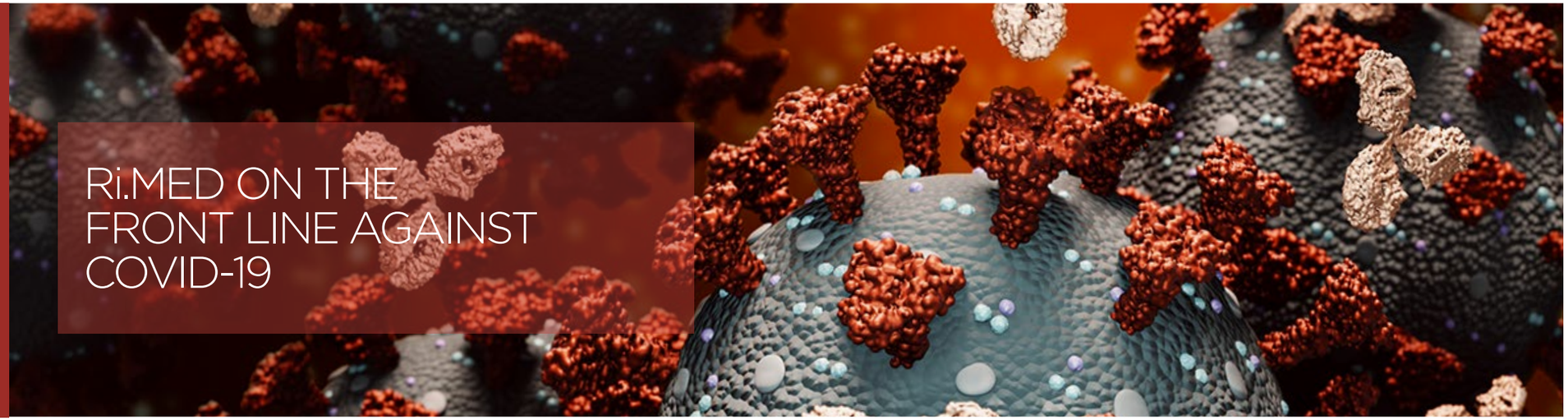


Ri.MED ON THE FRONT LINE AGAINST COVID-19

The year 2020 was marked worldwide by the terrible COVID-19 pandemic, caused by the SARS-CoV-2 virus, which had serious social and economic consequences. In parallel with the Foundation's own research and development programs, our scientists wanted to make their contribution, undertaking new scientific projects and collaborations focused on COVID-19.

Ri.MED FOCUS
COVID-19

MOLECULAR INFORMATICS AND MEDICINAL CHEMISTRY

SPIKE

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

ABSTRACT

The study is focused on the initial phase of the viral cycle, i.e. the entry of the virus into the host cell. Actually, different computational approaches have been adopted to study the mechanism by which the virus binds the host cell through the protein-protein interaction between the viral glycoprotein Spike and the human protein ACE2 (Angiotensin-converting enzyme 2). The project goal was to identify key residues and launch a drugdesign campaign of new chemotypes on this therapeutic target. The in-silico techniques adopted on Spike, including alanine scanning and molecular dynamics, allowed to identify hot spots of the Spike protein and evaluate, from a qualitative-quantitative point of view, its involvement in the stabilization of the host-guest complex. A virtual screening campaign of 2 million molecules was carried out on the target region identified. The screening permitted the identification of a series of molecules capable of potentially inhibiting the interaction of the Spike protein with the human ACE 2 receptor.

RESULTS

Identification of 1000 compounds as putative inhibitors of Spike-ACE 2 interaction

PUBLICATIONS

Gulotta et al., ChemMedChem 2020, 15, 1921–1931

Mpro

Ri.MED Team: Molecular informatics group , Medicinal Chemistry group and Advanced Data Analysis group

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 are collaborating with the Advanced Data Analysis group for the adoption of quantitative approaches based on machine learning techniques that, used in combination with classic ligand-based virtual screening techniques, can optimize the computational model reliability.

RESULTS

Identification of 1000 compounds as putative inhibitors of Mpro.

JEDI Grandchallenge Billion molecules against Covid-19

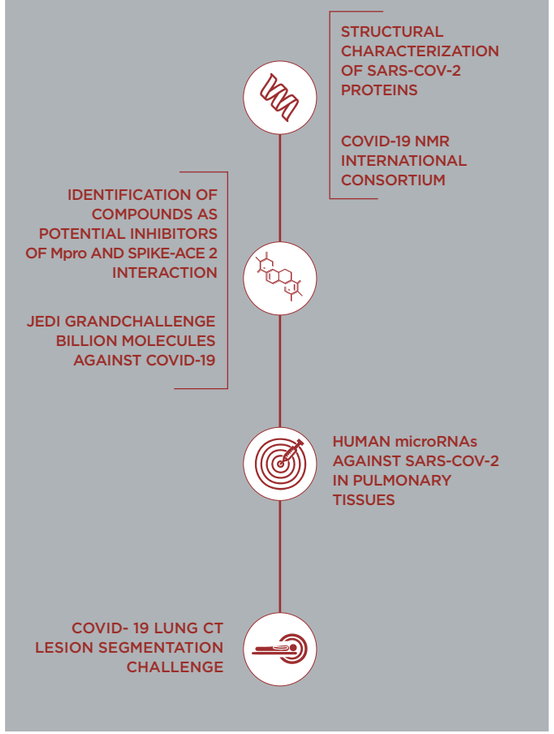
Ri.MED TEAM: Molecular informatics group and Medicinal Chemistry group

ABSTRACT

The Ri.MED group of Molecular informatics, led by Ugo Perricone, with the collaboration of Maria De Rosa, Principal Investigator in Medicinal Chemistry, participated in the challenge JEDI Grandchallenge Billion molecules against Covid-19, sending the compounds identified as the most promising series. The JEDI Commission collected and compared the results obtained from research teams from all over Europe: the common molecules selected by the various groups were then evaluated in a first step, on the basis of the computational methods used and grouped in similarity clusters to eliminate redundancy of chemotypes. The second evaluation step was the synthetic feasibility study of the compounds, that is to say whether they can actually be made and - if so - that they are not toxic. The strategy was to create a computational workflow based on the consensus of several algorithms that created filters capable of minimizing the false positives found by virtual screening. This approach allows to have very stringent molecule selection criteria. The real strength of the approaches used then lies in the continuous comparison with the Medicinal Chemistry group, in order to assess that the results obtained with in silico methodologies are aligned with Medicinal Chemistry.

RESULTS

From one billion starting molecules, 1000 potentially useful ones have been identified, coming from all over Europe. Of these, 65 belong to the Ri.MED team. This is a truly gratifying result, demonstrating the excellent teamwork carried out by the Computational and Medicinal Chemistry group.



STRUCTURAL BIOLOGY AND BIOPHYSICS

Structural characterization of SARS-CoV-2 proteins

Ri.MED TEAM: Structural Biology and Biophysics group

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 of Pisa (SNS), Pisa, Italy
- Molecular Medicine Department – University of Pavia, Pavia, Italy
- 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 2020 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), and on the accessory protein 8 (orf8), both 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. 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 both recombinant nsp9 and orf8. We also developed a successful purification protocol for nsp9, performed the biophysical characterization of the protein by circular dichroism (CD) and nuclear magnetic resonance (NMR), and assigned the NMR backbone assignment. 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.

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, Accepted.
- 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, Accepted.

Covid-19 NMR International Consortium

Ri.MED TEAM: Structural Biology and Biophysics group

PARTNERSHIP

- Goethe University of Frankfurt
- UK Dementia Research Institute (UK DRI) - King's College London, UK
- Covid19-NMR International Consortium

ABSTRACT

The Covid19-NMR consortium was launched by Goethe University in Frankfurt last March 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.

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, Accepted.

ADVANCED DATA ANALYSIS AND IDENTIFICATION OF THERAPEUTIC TARGETS

Covid-19 Lung CT Lesion Segmentation Challenge

RI.MED TEAM: Advanced Data Analysis group

PARTNERSHIP

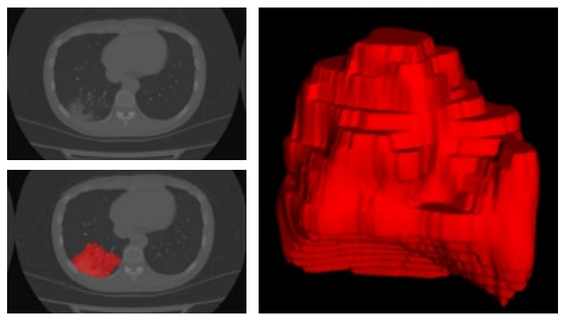
- Institute of molecular bioimaging and physiology (IBFM-CNR), Cefalù (PA), Italy.
- Georgia Institute of Technology, Atlanta, USA.

ABSTRACT

The Advanced Data Analysis Team participated in the COVID-19 LUNG CT LESION SEGMENTATION CHALLENGE (COVID-19-20), organized by the Medical Image Computing and Computer Assisted Intervention Society. It was an international challenge for the development of artificial intelligence (AI) algorithms for the segmentation and quantification of lung lesions caused by SARS-CoV-2 infection from multicenter, multinational, and patients of different age, gender, and disease severity. Participants - 221, including our Scientist in Nuclear Magnetic Resonance, Dr. Albert Comelli - were provided with the same non-contrast CT data-set of the chest of 199 and 50 patients, respectively, for training and validation task, with positive RT-PCR for SARS-CoV-2 and annotations of COVID-19 lung lesions provided by the NIH Multinational Consortium for CT AI in COVID-19. Thirty days to train AI algorithms and test them on a data-set of 46 additional patients with SARS-CoV-2 positive RT-PCR and COVID-19 lesion annotations not visible to participants. The challenge committee assessed the performance of the algorithms by comparing their results with actual diagnoses.

RESULTS

The AI algorithm trained by the Advanced Data Analysis Team of the Ri.MED Foundation is ranked first in Italy.



CT study of the lung, detection of the COVID-19 lesion, and 3D reconstruction of the lesion COVID-19

Human microRNAs against SARS-CoV-2

RI.MED TEAM: Advanced Data Analisys group and Therapeutic Target Identification group

PARTNERSHIP

- Dipartimento di Scienze Economiche, Aziendali e Statistiche, UNIPA;
- Department of Computational and Systems Biology, University of Pittsburgh

ABSTRACT

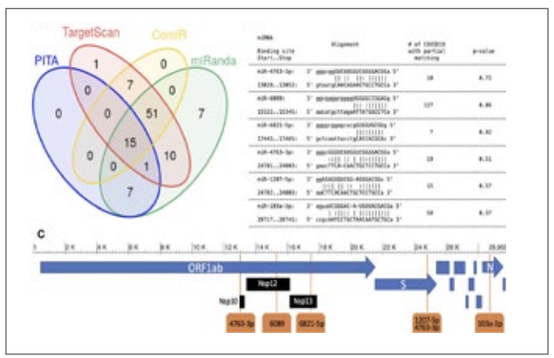
The aim of the project is to discover the interactions between the endogenous microRNA in lung tissues and the SARS-CoV-2 virus. The virus, by infecting the cells, could dysregulate the complex network of biological interactions between microRNA and messenger RNA, acting as a "sponge". Specifically, we predicted a strong affinity between the hsa-miR-1271, highly expressed in the bronchial ephithelium, and a region of the SPIKE RNA sequence. This interaction, by lowering the hsa-miR-1271 availability, could cause the rising of the expression of the Colony Stimulating Factor 1 (CSF1) protein, and, consequently, the enhancing of the high inflammatory response revealed in the most severe cases of COVID-19.

RESULTS

The prediction of 5 microRNAs, expressed in pulmonary tissues, able to regulate viral proteins and to putatively suppress the virus' reproduction.

PUBLICATIONS

Bertolazzi Giorgio, Cipollina Chiara, Benos Panayiotis V., Tumminello Michele, Coronnello Claudia, miR-1207-5p Can Contribute to Dysregulation of Inflammatory Response in COVID-19 via Targeting SARS-CoV-2 RNA, Frontiers in Cellular and Infection Microbiology 10, 663, (2020); https://www.frontiersin.org/article/10.3389/fcimb.2020.586592



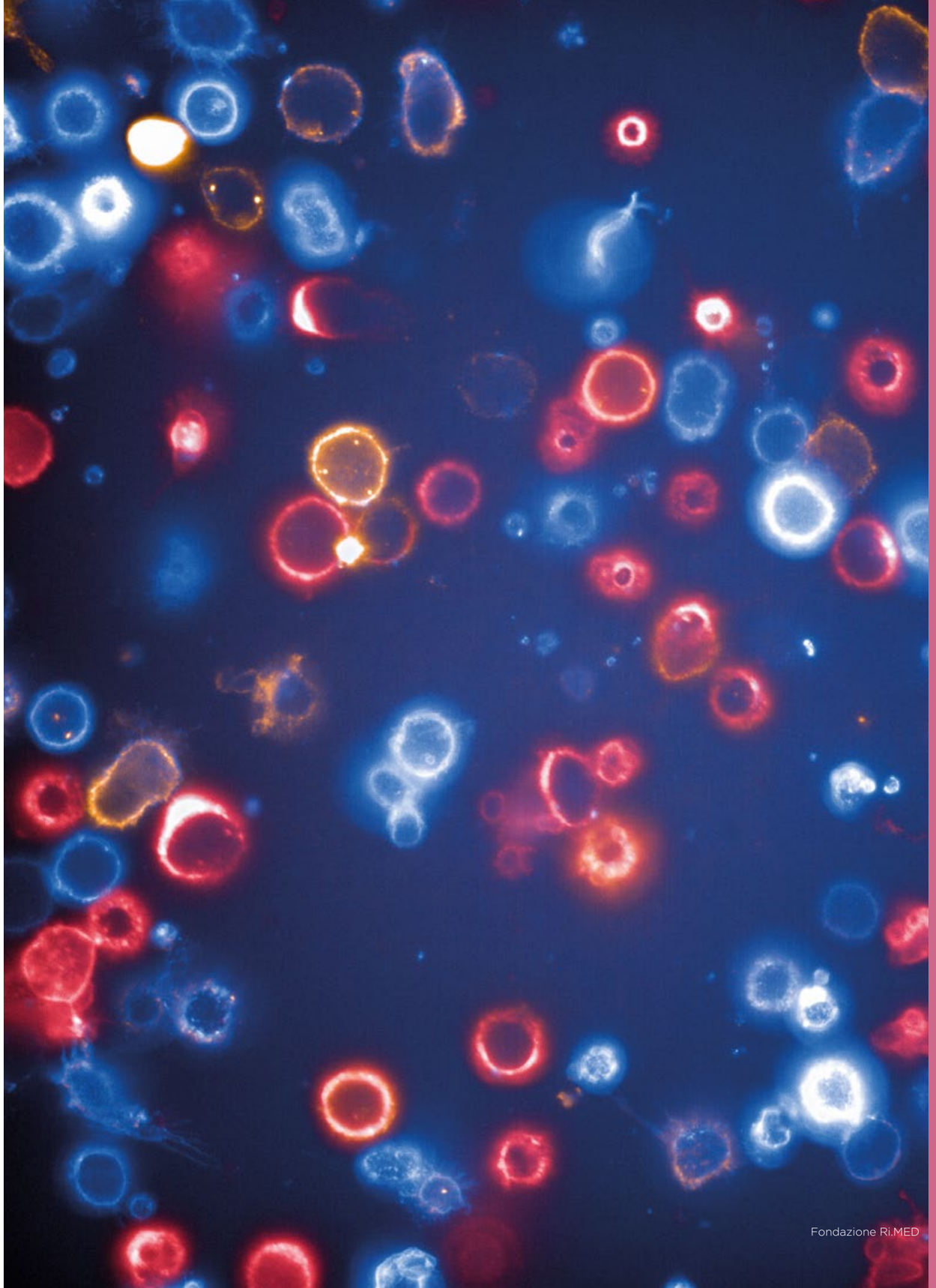
DRUG DISCOVERY

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 accomplish 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.



Structural characterization of SARS-CoV-2 proteins
Caterina Alfano, PhD

Molecular mechanisms of protein misfolding diseases
Caterina Alfano, PhD

Structural studies to elucidate the oligomerization mechanism of nucleophosmin protein NPM1
Caterina Alfano, PhD

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

Role of the NLRP3 inflammasome and immuno-metabolic alterations in chronic obstructive pulmonary disease (COPD)
Chiara Cipollina, PhD

Development of AI algorithms for extraction and selection of radiomics features from biomedical imaging
Albert Comelli, PhD

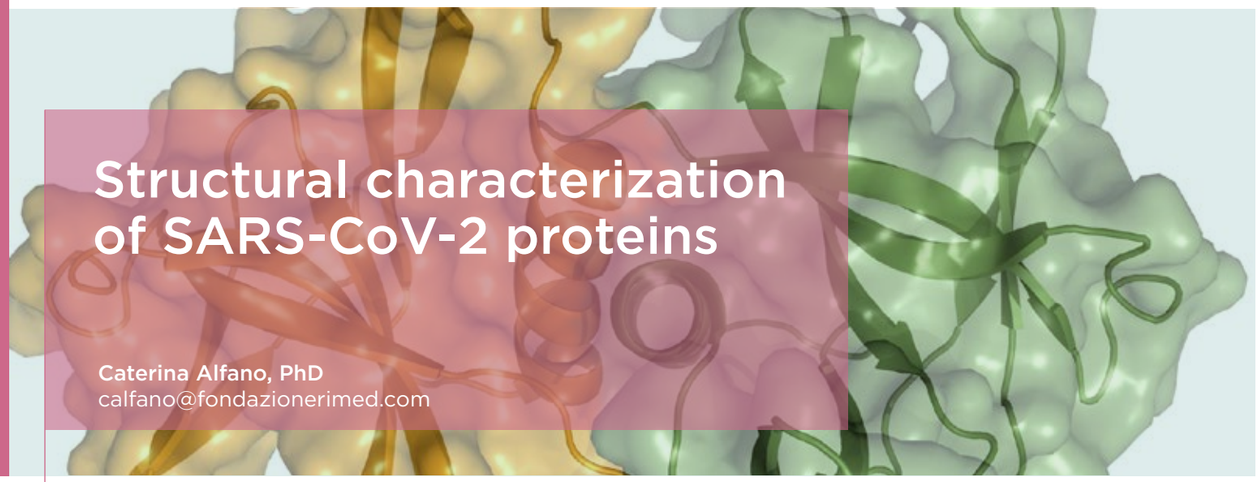
Development of automatic 3D algorithms for biomedical image segmentation and classification
Albert Comelli, PhD

Modeling microRNA-target interaction network
Claudia Coronnello, 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

PRODUCTS: **DRUGS - BIOLOGICS**



Structural characterization of SARS-CoV-2 proteins

Caterina Alfano, PhD
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COLLABORATIONS

- 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
- Covid19-NMR International Consortium

THERAPEUTIC AREA

Infectious diseases

PIPELINE

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

BRIEF DESCRIPTION

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 2020 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 part of an International consortium (Covid19-NMR) aiming at the characterization by NMR the SARS-CoV-2 proteins, we focused on the non-structural protein 9 (nsp9), and on the accessory protein 8 (orf8), both 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. 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.

IMPACT

Since the outbreak of the pandemic, most of the efforts have been invested in the development of vaccines against the viral protein Spike, but they are usable on healthy individuals and their long-term coverage is still unknown. In parallel, numerous neutralizing antibodies have been isolated, all against Spike. However, considering the mutant nature of SARS-CoV-2 which mainly affects the Spike region, the activity of these antibodies is not demonstrated for virus variants. Therefore, it remains essential to investigate other stages of infection and viral replication. We aim at developing new molecules against proteins other than Spike, implementing at the same time a platform and a work-flow for a rapid and decisive intervention in the event of future coronavirus pandemics. The data we produced so far on nsp9 and orf8 provide the prerequisite for further studies by NMR aimed at identifying fragments able to interfere with nsp9 and orf8 function, thus preventing viral replication and/or attenuating virus aggressiveness. Our data are also the prerequisite to select and develop new neutralizing antibodies against these two proteins.

RESULTS ACHIEVED IN 2020

We performed the backbone NMR resonance assignment of nsp9 protein (Fig. 1). 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. Moreover, as a part of an International consortium aimed at the study of all SARS-CoV-2 proteins by NMR, we 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. This resource has been generated in a coordinated and concerted effort between more than 30 labs worldwide, with the aim of providing pure mg amounts of SARS-CoV-2 proteins to further investigate their structure/function relationship and to investigate the drugability of those structures by small molecules. The resulting data are made publicly available throughout the project prior to publication to ensure a unique worldwide effort in fighting the actual pandemic.

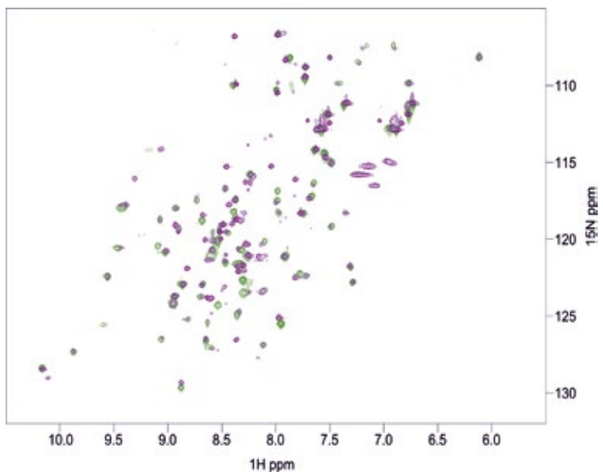


Fig. 1. Comparison of 2D ^{15}N HSQC NMR spectra of nsp9 protein recorded for a 100 µM sample at two pH conditions using a 800 MHz Bruker NMR Spectrometer. Spectrum in green is related to sample at pH 7.0, while spectrum in purple is related to sample at pH 5.0.

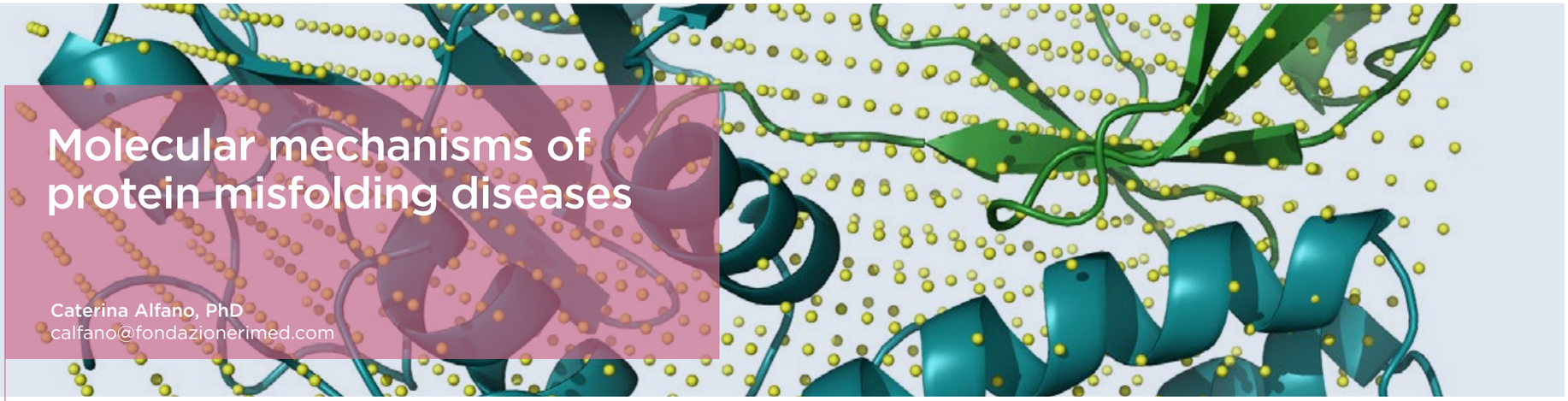
GOALS FOR 2021

We will produce recombinant nsp9 and orf8 to verify the specificity of single-chain variable fragment antibodies types (scFv) selected by our collaborators EBRI and SNS. Moreover, we will carry out structural and thermodynamic studies of orf8 and nsp9. We have already produced the cDNAs coding for orf8 and nsp9 and implemented the protocols to obtain pure mg amounts of samples. We will produce the unlabelled proteins, for stability studies and for initial crystallization tests, and doubly 15N/13C labelled samples for the structure determination by three-dimensional NMR. The labelled samples will be used to acquire an extensive dataset of three-dimensional NMR experiments, using our 800 MHz Bruker NMR spectrometer equipped with cryo-probe. Resonance assignment and structural calculation will be performed automatically, using dedicated software, and validated manually. This will allow to reach our goals quickly and will open the possibility for studies on the interactions between the two viral proteins and the selected scFv. The latter will be produced by EBRI in quantities and purities that will allow us to carry out structural and interaction studies of antigen/antibody complexes.

PUBLICATIONS

- Astoricchio E., **Alfano C.**, Rajendran L., Temussi P., Pastore A. (2020) The Wide World of Coacervates: From the Sea to Neurodegeneration. Trends Biochem Sci, 45(8):706-717.
- Puglisi R., Brylski O., **Alfano C.**, Martin S.R., Pastore A., Temussi P. (2020) Quantifying the thermodynamics of protein unfolding using 2D NMR spectroscopy. Comm Chemistry, doi: 10.1038/s42004-020-00358-1.
- 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, Accepted.
- 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, Accepted.

PRODUCTS: **DRUGS - BIOLOGICS**



Molecular mechanisms of protein misfolding diseases

Caterina Alfano, PhD
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COLLABORATIONS

- UK Dementia Research Institute (UK DRI) - King's College London, London, United Kingdom
- Department of Physics and Chemistry (DiFC) - University of Palermo, Palermo, Italy

THERAPEUTIC AREA

Aging diseases

PIPELINE

BRIEF DESCRIPTION

Neurodegeneration is an increasing threat of our increasingly aging modern society. Current treatments are in the best-case palliative and non-specific, reflecting the fact that the detailed understanding of the molecular basis of most of neurodegenerative diseases is still lacking. Our research aims at understanding the molecular mechanisms of protein misfolding diseases and rely on the concept that knowledge of the normal function and of the interaction network of aggregogenic proteins is a key tool to design molecules which can specifically compete out aggregation. Native protein-protein interactions could indeed provide important means of altering and controlling the function and assembly of those proteins involved in neurodegenerative diseases and they could fulfil a protective role against aberrant aggregation. We selected ataxin-3 (atx-3), the protein responsible for the inherited Machado-Joseph disease (MJD), also known as Spinocerebellar Ataxia type 3, as a model system in this project.

IMPACT

The research project addresses key unanswered questions in structural and cell biology that are essential to get new insights into the understanding of neurodegenerative disease. 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 cellular importance of the protein chosen as model system, ataxin-3, is testified by its involvement in a disease which is part of the steadily increasing family of incurable diseases caused by protein misfolding and aggregation, including Alzheimer and Parkinson's diseases. If successful, the proof of concept gained in the project will be highly beneficial more in general to understand the events that lead to pathology of misfolding diseases and provide new tools to prevent them.

RESULTS ACHIEVED IN 2020

In the latest studies we have focused on the effect of polyubiquitin chains in the growth of fibrils of the N-terminal catalytic domain of ataxin-3, called Josephin. The latter, although it does not contain the polyglutamine trait and is spatially distant from it, is involved in misfolding and is the element that determines the properties and morphology of ataxin-3 aggregates. Our results confirmed our hypothesis that the formation of thioflavin-positive aggregates is strongly inhibited by the presence of polyubiquitin chains, natural partners of ataxin-3 (Fig. 1). This is in agreement with the known aggregogenic role of Josephin and with the hypothesis that when ataxin-3 is in the free state it exposes surfaces located on the Josephin domain responsible for binding to ubiquitin. The hydrophobic nature of these surfaces triggers aggregation and explains Josephin's role as a nucleation center in the aggregation process of even whole ataxin-3. Our results with polyubiquitin chains demonstrate that obscuring the hydrophobic patches on Josephin by interaction with its natural binder prevents from aberrant aggregation.

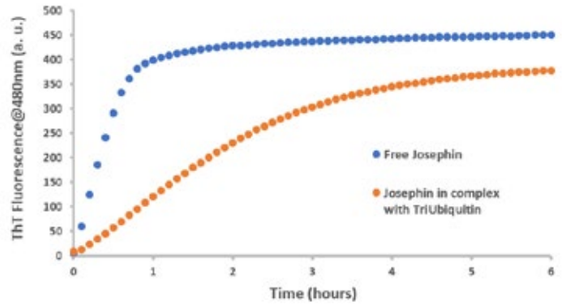


Fig. 1. Time evolution of ThT fluorescence emission @480nm of both free Josephin and Josephin in complex with tri-Ubiquitin chain.

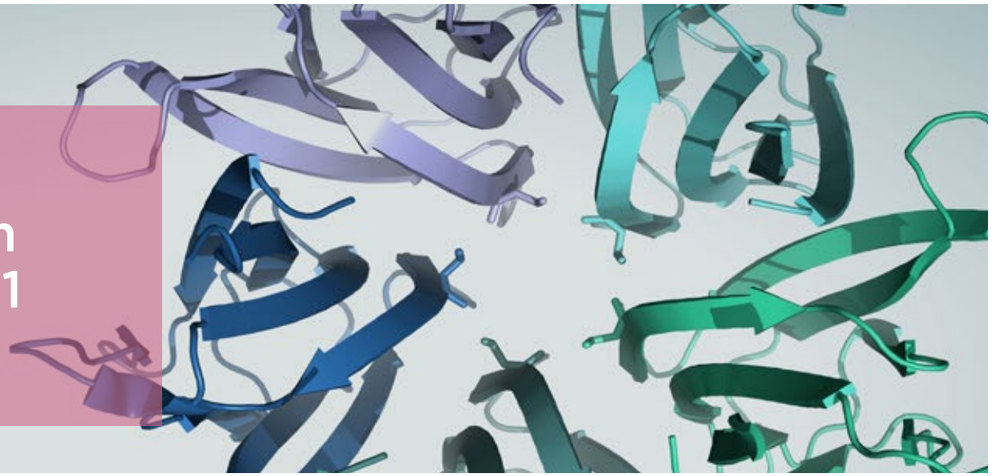
GOALS FOR 2021

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 anti-aggregation drugs. For this purpose, the kinetic and thermodynamic parameters of the ataxin-3/poly-ubiquitin chain complexes will be determined and the effect of the poly-ubiquitin chains in the aggregation of ataxin-3 will be evaluated.

PUBLICATIONS

- Astoricchio E., **Alfano C.**, Rajendran L., Temussi P., Pastore A. (2020) The Wide World of Coacervates: From the Sea to Neurodegeneration. Trends Biochem Sci, 45(8):706-717.
- Puglisi R., Brylski O., **Alfano C.**, Martin S.R., Pastore A., Temussi P. (2020) Quantifying the thermodynamics of protein unfolding using 2D NMR spectroscopy. Comm Chemistry, doi: 10.1038/s42004-020-00358-1.
- Fricano A., Vetri V., Vannocci T., Pastore A., **Alfano C**. Role of autocleavage in ataxin-3 aggregation process. Under preparation.

PRODUCTS: **DRUGS - BIOLOGICS**



Structural studies to elucidate the oligomerization mechanism of nucleophosmin protein NPM1

Caterina Alfano, PhD
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COLLABORATIONS

- **TES Pharma, Perugia, Italy**
- **Department of Medicine, Section of Haematology and Clinical Immunology – University of Perugia, Perugia, Italy.**

THERAPEUTIC AREA

Oncology

PIPELINE

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

BRIEF DESCRIPTION

About one-third of patients affected by Acute myeloid leukemia (AML), an aggressive cancer of the myeloid cells, has an aberrant cytoplasmic expression of nucleophosmin protein (NPM1) due to heterozygous mutations at the C-terminus domain of the protein. Several functions have been attributed to this highly abundant nucleolar phosphoprotein, such as ribosome biogenesis, maintenance of genome stability, nucleolar stress response, and regulation of apoptosis, and they all rely of the capability of NPM1 to translocate among the nucleolus, the nucleus and the cytoplasm. In AML blasts, translocation is very limited but it is still functioning due the heterozygous nature of the mutations and this allows the survival of malignant cells. Abolishing the NMP1 translocation could therefore interfere with the survival of AML cells. In the last decades, several approaches have been explored to impair the translocation in AML cells by targeting the N-terminal domain of NPM1 responsible of the oligomerization process which leads to translocation. Despite these efforts an effective therapy is not yet available, probably due to the lack of a deeper understanding to the molecular mechanisms behind the oligomerization process of NPM1. Our goal is to elucidate those mechanisms so that to identify molecules able to impair NPM1 translocation in AML blasts and interfere with the life path of the only malignant cells.

IMPACT

Acute myeloid leukemia is the most common acute leukemia in adults. Patients aged 18-60 years affected by AML can be cured using conventional chemotherapy only in 50% of the cases, while older patients (>60 years) can be curable only in a small percentage (5-10%). The therapy of this disease remains therefore an urgent medical need in particular considering our increasingly aging modern society. It is expected that the research project would play a key role in developing new therapeutic strategies targeting NPM1-mutated AML cells resulting in a strong impact on health care, both because AML is the most common acute leukaemia in adults and because in about one-third of cases the disease is associated to NPM1 mutations.

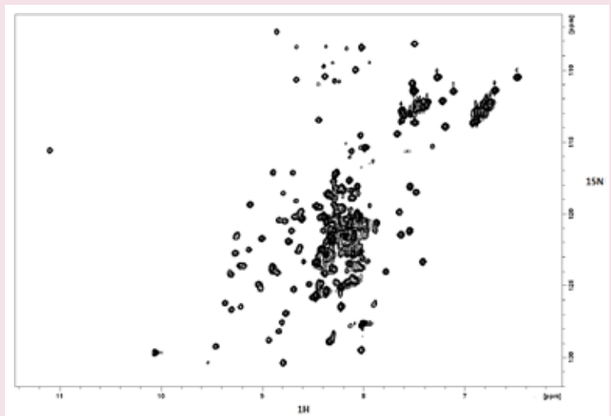


Fig. 1. 2D 15N HSQC NMR spectrum of NPM1 L18E S88E recorded at 25°C with a 800MHz Bruker NMR Spectrometer.

RESULTS ACHIEVED IN 2020

To elucidate the molecular mechanisms of NPM1 oligomerization, we performed Nuclear Magnetic resonance (NMR), Circular Dichroism (CD), and Size Exclusion Chromatography combined with multi-angle light scattering (SEC-MALS) on four different constructs of the N-terminal domain of NPM1: 1-130, 1-130 S88E, 1-130 L18A-S88E and L18E-S88E. Currently, all our results consistently highlight the presence of several oligomeric forms for 1-130, 1-130 S88E, 1-130 L18A-S88E constructs, confirming our first hypothesis that a small molecules drug discovery workflow has high probability of failure if the constructs above are used in the experimental screening steps. In contrast, On the contrary, NMR and SEC-MALS data performed on L18E-S88E mutant reveal that this folds as an isolated monomer (Fig. 1) and could therefore be suitable for a biophysical screening campaign of fragments/small molecules designed to inhibit NPM1 oligomerization. A deep understanding of the molecular bases that drive NPM1 oligomerization process remains far, but our results allow for an opening towards a new Drug Discovery Program within Ri.MED.

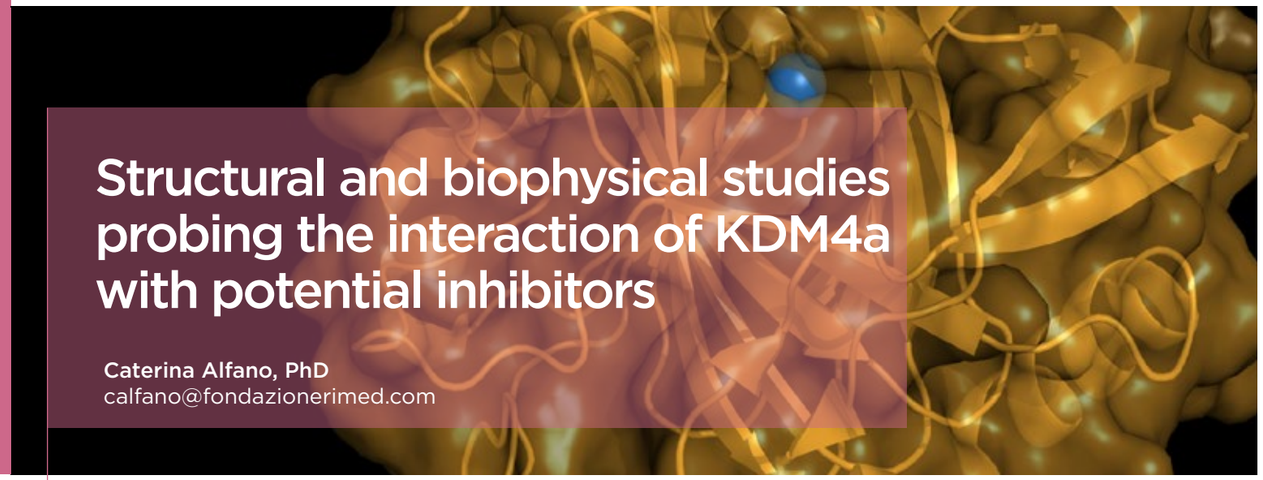
GOALS FOR 2021

We aim at determining the three-dimensional structure in solution of NPM1 L18E-S88E by nuclear magnetic resonance. This will be then compared with the structure of the monomeric subunit of wild type pentameric NPM1. Indeed, the confirmation of a high structure similarity among the two species is crucial to assess if the Ri.MED Drug Discovery workflow can be applied on NPM1 target.

PUBLICATIONS

- Astoricchio E., **Alfano C.**, Rajendran L., Temussi P., Pastore A. (2020) The Wide World of Coacervates: From the Sea to Neurodegeneration. Trends Biochem Sci, 45(8):706-717.
- Puglisi R., Brylski O., **Alfano C.**, Martin S.R., Pastore A., Temussi P. (2020) Quantifying the thermodynamics of protein unfolding using 2D NMR spectroscopy. Comm Chemistry, doi: 10.1038/s42004-020-00358-1.
- Morando M.A., Monaca E., Sabbatella R., Giacchè N., Passeri D., **Alfano C**. Molecular insights of the oligomerization process of NPM1 N-terminal domain. Under preparation.

PRODUCTS: **DRUGS**



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

Caterina Alfano, PhD
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COLLABORATIONS

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

THERAPEUTIC AREA

Oncology

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

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.

RESULTS ACHIEVED IN 2020

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. We are now in process of testing analogues compounds so that to confirm the hits.

GOALS FOR 2021

Our goal is to confirm the primary hits found so far by deep analysis of their binding mode with KDM4a. Few analogues of the primary hit compounds are already under test and if successful we aim at determining the three-dimensional structure of KDM4a/hit complexes by X-ray crystallography. Our ambition is to give experimental structural information that can guide a better 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.

PUBLICATIONS

- Puglisi R., Brylski O., **Alfano C.**, Martin S.R., Pastore A., Temussi P. (2020) Quantifying the thermodynamics of protein unfolding using 2D NMR spectroscopy. Comm Chemistry, doi: 10.1038/s42004-020-00358-1.

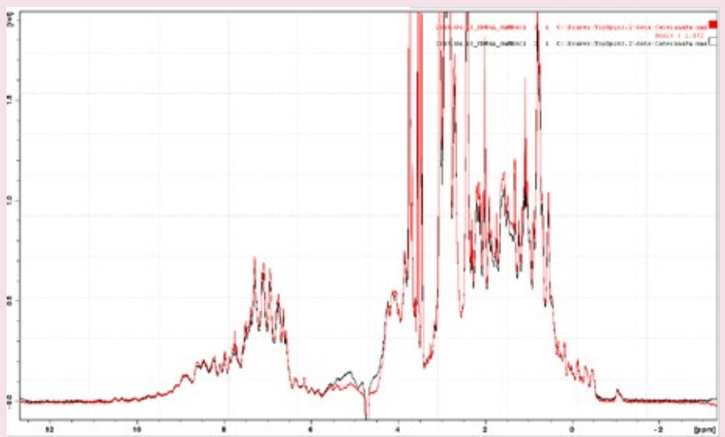
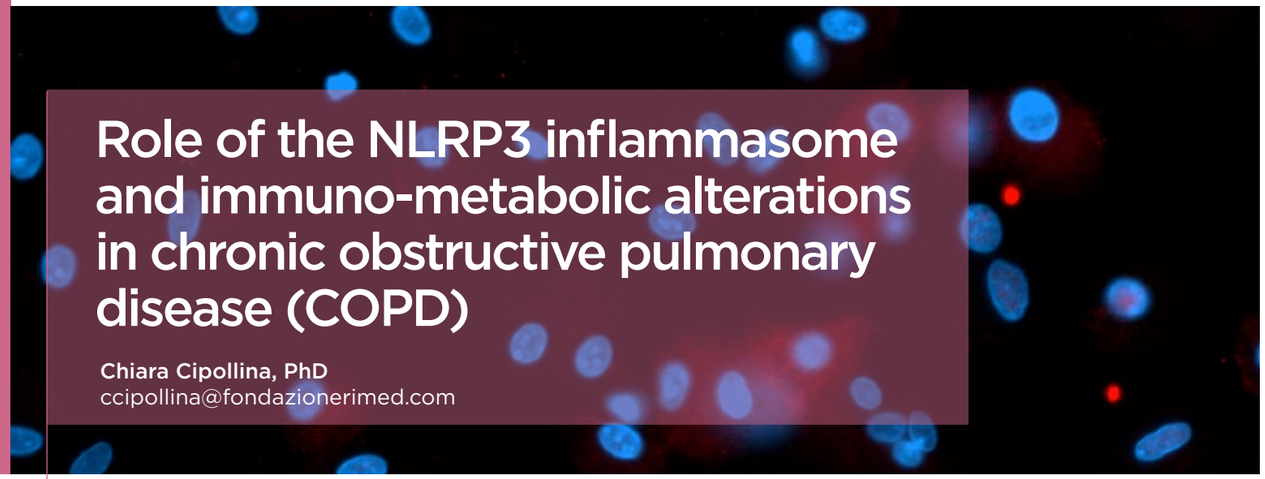


Fig. 1:: ^{1}H monodimensional NMR spectra of KDM4a in different buffer conditions.

PRODUCTS: **DRUGS - BIOMARKERS**



Role of the NLRP3 inflammasome and immuno-metabolic alterations in chronic obstructive pulmonary disease (COPD)

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COLLABORATIONS

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- Ospedale Civico Di Cristina Benfratelli, Palermo, Italy
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THERAPEUTIC AREA

Aging diseases

PIPELINE

CLINICAL NEED → DISEASES ANALYSIS → DISCOVERY → PRECLINICAL VALIDATION → PRECLINICAL DEVELOPMENT → CLINICAL STUDIES

BRIEF DESCRIPTION

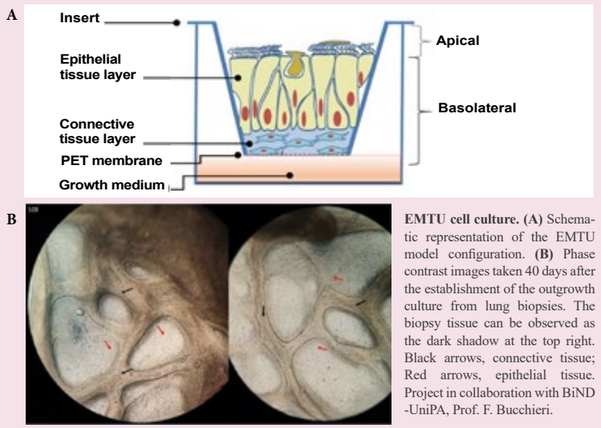
Chronic obstructive pulmonary disease (COPD) is a leading cause of death in the world. It is a disease associated with aging and exposure to cigarette smoke and pollutants. COPD is characterized by a progressive and irreversible reduction of the airflow.
To date there is no therapy able to block disease progression, and therefore new drugs are urgently required.
Airway remodeling, cellular senescence, activation of the bronchial epithelium and immune dysfunction together with chronic inflammation contribute to the pathogenesis of COPD. These events involve both structural cells (such as epithelial cells and fibroblasts) and immune cells (such as macrophages).
In recent years, we have studied the mechanisms of immune dysfunction in experimental models of COPD where cigarette smoke is used as a stimulus to simulate what happens in the COPD lung. Our goal is to test the hypothesis that the NLRP3 inflammasome and its related pathways play a role in the pathogenesis of COPD. Starting from the NLRP3 inflammasome, our studies further extend to other inflammasomes and to the non-apoptotic role of caspases as well as to the pathway of interferon β and Gasdermin D (GSDMD).
In parallel, since NLRP3 is a validated target for several chronic inflammatory diseases, in collaboration with the Drug Discovery Unit at Fondazione Ri.MED, we are working to the development of new selective inhibitors of NLRP3.

IMPACT

The project will contribute to the discovery and characterization of the role of the NLRP3 inflammasome, the non-apoptotic caspases and gasdermin D in the pathogenesis of COPD. This, in turn, will allow the identification of new potential therapeutic targets.
Moreover, the work done in collaboration with the Drug Discovery Unit will contribute to the development of selective NLRP3 inhibitors to be used for the development of new drugs. The impact of this work goes beyond the specific context of COPD. In fact, activation of NLRP3 contributes to the pathogenesis of several chronic diseases including atherosclerosis, type II diabetes, and neurodegenerative diseases.

RESULTS ACHIEVED IN 2020

The use of experimental models of inflammation associated with cigarette smoke is able to mimic the inflammatory context typical of COPD airways. In 2020, our study on the effects of exposure of primary human monocyte-derived macrophages (hMDMs) to cigarette smoke has revealed the presence of a strong imbalance of innate immune responses.
We discovered that smoking profoundly alters macrophage response to bacterial infection by inhibiting the TLR4-MyD88 axis (leading to a reduced release of inflammatory cytokine) and at the same time leaving unaltered the TLR4-TRIF axis, which leads to the activation of caspase-8/-1 and to interferon β release. This causes an imbalance of the immune response which could explain why exposure to cigarette smoke increases the risk of infection. With regards to the development of innovative experimental models for the study of lung diseases, one of the objectives achieved in 2020 has been the start of the collaboration with the University of Palermo - Department of Biomedicine, Neuroscience and Advanced Diagnostics (BiND), Prof. Fabio Bucchieri, who has developed a novel 3D epithelial-mesenchymal trophic unit (EMTU) model of the bronchial mucosa (Figure 1). Finally, another important goal achieved in 2020 has been the approval by the ISMETT IRCCS Ethics Committee of the monocentric prospective observational study entitled "Investigating the role of inflammasomes in chronic obstructive pulmonary disease (COPD)" (PI: Dr. Alessandro Bertani).



EMTU cell culture. (A) Schematic representation of the EMTU model configuration. **(B)** Phase contrast images taken 40 days after the establishment of the outgrowth culture from lung biopsies. The biopsy tissue can be observed as the dark shadow at the top right. Black arrows, connective tissue; Red arrows, epithelial tissue. Project in collaboration with BiND -UniPA, Prof. F. Bucchieri.

GOALS FOR 2021

One of the main objectives for 2021 will be the validation and the expansion of current data using alveolar macrophages and lung tissue samples isolated from COPD patients (smokers and former smokers) and in control groups (smokers and non-smokers) recruited from ISMETT IRCCS. We will also evaluate the activation of the pathways associated with the NLRP3 inflammasome in lung structural cells (epithelial and fibroblasts) using the lung EMTU 3D model, air-liquid interface cultures and primary cultures. The achievement of this goal will be possible thanks to the collaboration with IRIB-CNR, ISMETT IRCCS and with the University of Palermo - Department of Biomedicine, Neuroscience and Advanced Diagnostics. These experimental models will also be used for the set-up and validation of nanostructured sensors for oxidative stress detection in collaboration with the Engineering Department of the University of Palermo, in order to pursue the objectives of the project Se.N.S.O. (funded under the PO FESR program 2014/2020 - Action 1.1.5). With regard to the discovery of novel selective NLRP3 inhibitors, the main objective will be the validation of the primary assay for the screening campaign, which will be followed by screening leading to the identification of primary hits and their validation through orthogonal and secondary assays.

MEETINGS

- Cigarette Smoke upregulates Notch1 signaling pathway in lung adenocarcinoma". Chiappara G, Sangiorgi C, Di Sano C, D'Anna C, Di Vincenzo S, Zito G, **Cipollina C**, Bertani A, Vitulo P, Pace E. European Respiratory Journal 2020 56: 1974; DOI: 10.1183/13993003.congress-2020.1974.
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PUBLICATIONS

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- Cigarette smoke inhibits the NLRP3 inflammasome and leads to caspase-1 activation via the TLR4-TRIF-caspase-8 axis in human macrophages". Buscetta M, Di Vincenzo S, Miele M, Badami E, Pace E, **Cipollina C**. FASEB J. 2020, 34(1):1819-1832. doi: 10.1096/fj.201901239R. PMID: 31914643.

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 — DISEASES 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 medical disciplines, in order to improve personalized patient management.

IMPACT

Radiomics is used 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 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 2020

The project contributed to:

- 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;
- Obtain biological tumor volume (BTVs) from cerebral metastases in patients who underwent L-[11C]methionine (11C-MET) PET, using a fully automatic procedure and to use these BTVs to extract radiomics features to stratify between patients who respond to treatment or not;
- Improve the prediction of patient overall survival and/or outcome in 46 patients with prostate lesion underwent magnetic resonance imaging.

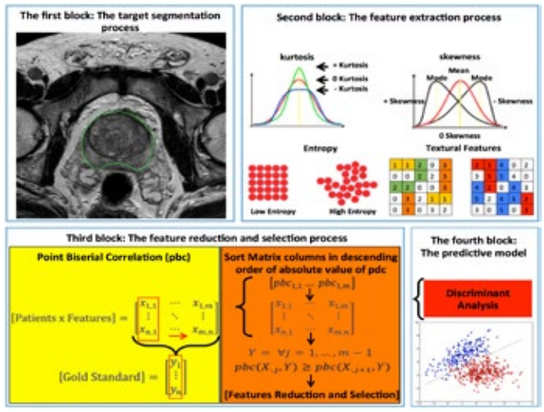

Figura 1. The proposed radiomics system. I) The segmentation algorithm based on the enhanced active contour; II) The MaZda texture analysis software to extracted radiomics features for each patient study; III) A novel mixed descriptive-inferential sequential approach to identify the most discriminative features reducing redundancy; IV) The prediction model based on the discriminant analysis.

GOALS FOR 2021

During the 2020, we aim to develop a new 3D radiomics matrix. 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

- **Comelli A**, Stefano A, Coronnello C, Russo G, Vernuccio F, Cannella R, Salvaggio G, Lagalla R, Barone S (2020). Radiomics: A New Biomedical Workflow to Create a Predictive Model. In: Papież B., Namburete A., Yaqub M., Noble J., Medical Image Understanding and Analysis. COMMUNICATIONS IN COMPUTER AND INFORMATION SCIENCE, vol. 1248, p. 280-293, ISBN: 978-3-030-52790-7, ISSN: 1865-0929, doi: 10.1007/978-3-030-52791-4_22
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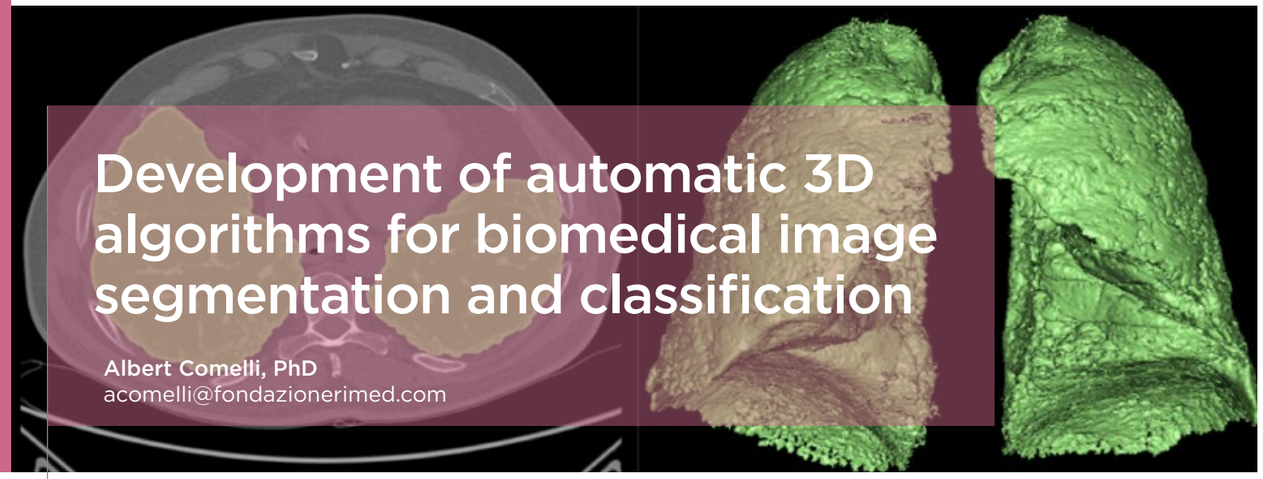
- Stefano A, Gioe M, Russo G, Palmucci S, Torrisi S.E, Bignardi S, Basile A, **Comelli A,** Benfante V, Sambataro G, Falsaperla D, Torcitto A.G, Attanasio M, Yezzi A, Vancheri C. (2020). Performance of radiomics features in the quantification of idiopathic pulmonary fibrosis from HRCT. DIAGNOSTICS, vol. 10, diagnostics10050306, ISSN: 2075-4418, doi: 10.3390/diagnostics10050306

PRODUCTS: **BIOMARKERS - MEDICAL DEVICES & TISSUE ENGINEERING**



Development of automatic 3D algorithms for biomedical image segmentation and classification

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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
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THERAPEUTIC AREA

Oncology

PIPELINE

CLINICAL NEED → DISEASES 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.

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 lung, head and neck, and brain districts in PET.
- A deep learning framework for segmentation 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 2020

The project contributed to:

- Identify an automatic, accurate, and fast deep learning segmentation approach, applied to the parenchyma, using a very small dataset of high-resolution computed tomography images of patients with idiopathic pulmonary fibrosis;
- Design efficient and operator-independent segmentation algorithms capable of reconstructing the tumour three-dimensional (3D) shape for accurate diagnosis and radiotherapy treatment planning;
- 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.

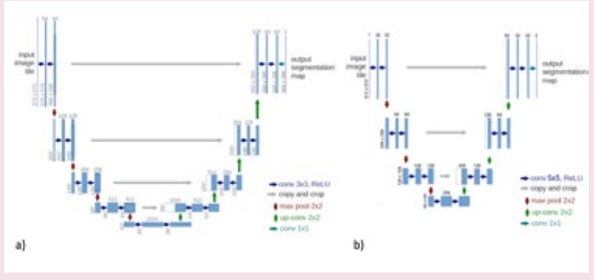
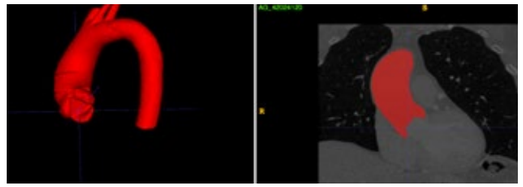


Figura 1. Comparison between the original UNet architecture (a) and our UNET implementation (b). Each blue box is a multi-channel feature map with the number of channels denoted at the top of the box. The x-y size is denoted at the bottom lower left edge of the box. White boxes represent copied low-resolution features.

GOALS FOR 2021

In 2020 the main goal will be to develop new hybridized 3D segmentation methods. 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..



3D segmentation of the aneurysmal ascending aorta realized through Deep learning models.

MEETINGS

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

PUBLICATIONS

- **Comelli A** (2020). Fully 3D Active Surface with Machine Learning for PET Image Segmentation. JOURNAL OF IMAGING, vol. 6, p. 113-125, ISSN: 2313-433X, doi: 10.3390/jimaging6110113
- **Comelli A**, Coronnello C, Dahiya N, Benfante V, Palmucci S, Basile A, Vancheri C, Russo G, Yezzi A and Stefano A. Lung Segmentation on High-Resolution Computerized Tomography Images Using Deep Learning: A Preliminary Step for Radiomics Studies. J. Imaging 2020, 6, 125.
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PRODUCTS: **BIOMARKERS - BIOLOGICS**

Modeling microRNA-target interaction network

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THERAPEUTIC AREA

- Oncology
- Infectious diseases

PIPELINE

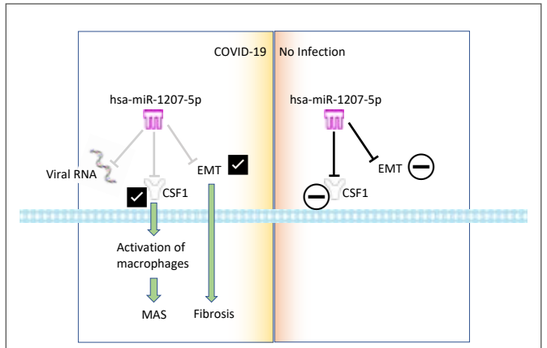
CLINICAL NEED | DISEASES ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

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 detected, 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 with 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 useful to obtain the results described on the paper. When data accounts for gene expression profiles, researchers use data repositories as Gene Expression Omnibus or ArrayExpress. As a consequence, if a researcher is interested in a 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, doesn't exists 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".



Caption: Proposed effects of hsa- miR-1207-5p interaction with SARS-CoV-19. With no infection, the microRNA regulates the expression of CSF1 - a cytokine that controls the production, differentiation and function of macrophages – and inhibits the epithelial mesenchymal transition (EMT) pathway. With COVID-19 infection, the viral RNA sequesters hsa-miR-1207-5p and, as a consequence, CSF1 and EMT genes are over-expressed. This over-expression might contribute to accelerate the fibrosis and macrophage activation syndrome (MAS), both associated with severe COVID-19 outcome.

RESULTS ACHIEVED IN 2020

In 2020 we focused on the update of the web-tool ComiR, a tool usefull to predict the targets of a set of microRNAs, given their expression profile. Currently, the ComiR algorithm includes the microRNA-mRNA binding sites computationally predicted in the 3'UTR of the mRNA. We discovered that, by including also the binding sites predicted in the coding region of the mRNA, the ComiR predictions are more performant and coherent with the experimentally detected targets.
The new algorithm has been validated by using a dataset of validated microRNA targets in D. melanogaster, and we aim to prepare a similar dataset on human cell line, in order to validate the results also in human applications. In addition, the algorithm has been applied to predict the interactions among the human microRNAs and the SARS-CoV-2 viral sequence. We identified a binding site of hsa-miR-1207 into the SPIKE RNA sequence. This interaction is probably involved in inflammation processes and we aim to further investigate on it.

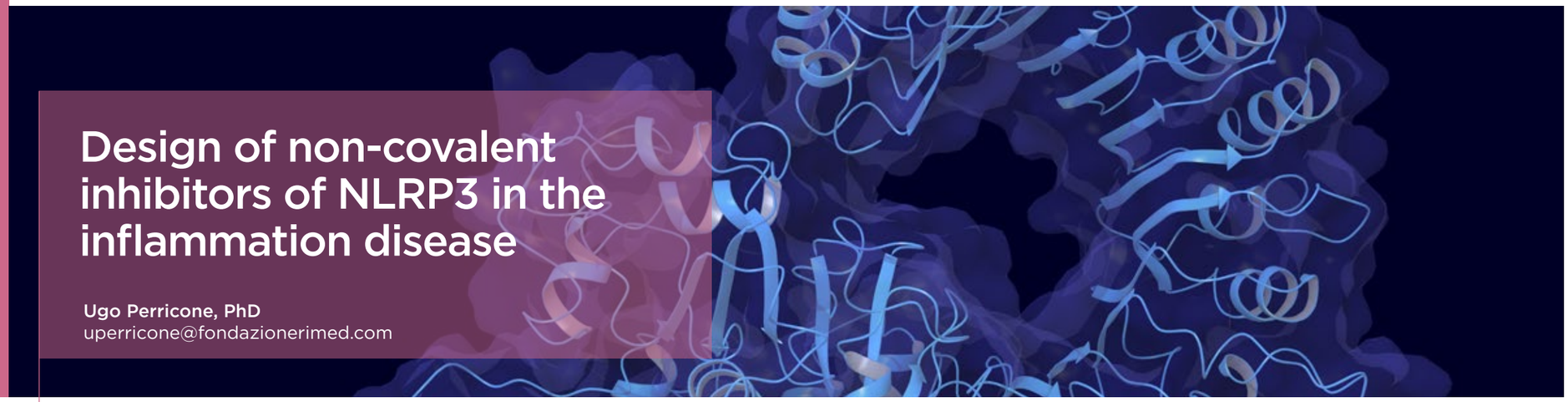
GOALS FOR 2021

In 2020 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.

PUBLICATIONS

- Bertolazzi, G, Cipollina, C, Benos, PV, Tumminello, M, **Coronnello, C.** (2020). miR-1207-5p Can Contribute to Dysregulation of Inflammatory Response in COVID-19 via Targeting SARS-CoV-2 RNA. Frontiers in cellular and infection microbiology, 10. 673-681. doi.org/10.3389/fcimb.2020.586592
- Bertolazzi, G, Benos, PV, Tumminello M, **Coronnello C** (2020) An improvement of ComiR algorithm for microRNA target prediction by exploiting coding region sequences of mRNAs, BMC Bioinformatics, 21 (8), 1-10. doi.org/10.1186/s12859-020-3519-5

PRODUCTS: **DRUGS**



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

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THERAPEUTIC AREA

Aging diseases

PIPELINE

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.

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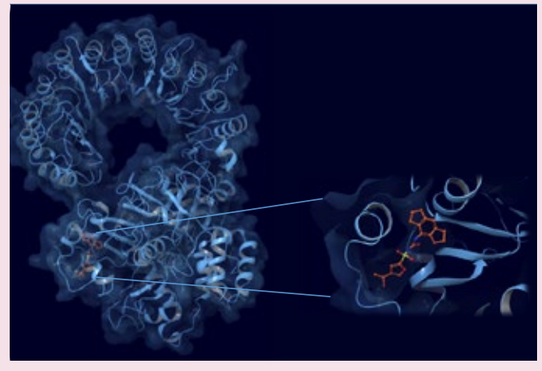
IMPACT

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 2020

In 2020, the Molecular Informatics group developed two computational approaches aimed at the rational design of NLRP3 inflammasome modulators. In particular, the first approach concerns the inhibition of the NACHT domain of the NLRP3 protein through a mechanism that is not competitive with ATP and with non-covalent binding. The second approach, on the other hand, concerns the design of protein-protein inhibitors (PPIs) that act at the level of the NLRP3 PYD domain, preventing its binding with the protein partner ASC which would lead to the recruitment of procaspase 1 and the consequent activation of the complex responsible for the cascade inflammasome. The main difficulty related to the design of active molecules on this protein is related to the absence or limited availability of structural data, which is why it was necessary to create a homology model of the protein and optimize the only available cryo-EM resolved structure. The use of advanced homology modeling and hybrid threading techniques have now made it possible to obtain a reliable model on which to base virtual screening campaigns for the selection of compounds to be tested on a primary and orthogonal functional assay. The virtual screening campaigns allowed the acquisition of a library of 500 molecules to be tested on NLRP3.

GOALS FOR 2021

In 2021 the goal will be to carry out biological assays on the 500 molecules acquired in 2020 and through joint design approaches with the medicinal chemistry group, to find at least two hit families (one for each approach considered) that have biological activity below 10μM confirmed in primary and orthogonal assays. In order to obtain families of hit compounds, we will start from the singletons found in the screening and which present the appropriate chemical characteristics to expand and confirm the activities found. The expansion of a series will also allow a first study of the SAR with the consequent launch of synthetic strategies aimed at maximizing the power and optimizing the ADMET profile of the designed molecules. In addition to the virtual screening of small molecules and peptidomimetics (in the case of inhibitors of the PYD domain), we will also proceed with the analysis of libraries of fragments in order to better explore the hot spots present on the surface of the protein.

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

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

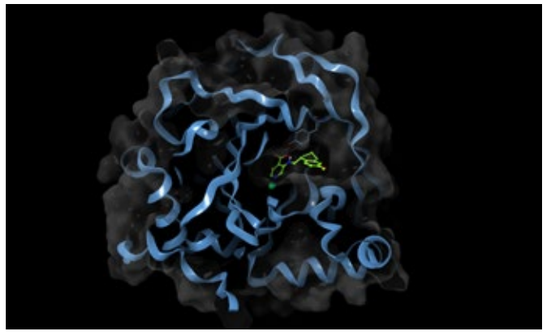
Recent advances in the field of cancer epigenetics have highlighted the importance of epigenetic mechanisms in the development of tumour pathology. Particular importance has been given to DNA methylation, histone modifications, and microRNA expression modifications. The reversible nature of epigenetic 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 2020

During 2020 the computational models that could be used for the rational design of molecules with inhibitory activity on the target were optimized. 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. Retrospective validation of computational models and their application in virtual screening campaigns on commercial fragment libraries allowed the identification of hits that showed uM activity on KDM4 on preliminary biochemical assays. The same fragments have been tested with biophysical techniques (BLI) and have shown Kd data in the uM range. Based on the results obtained from the screening, we moved on to the confirmation of the hit series through the selection of analogues and the design of new structures, also based on isosteric substitutions with the aim of improving the activity profile of the molecules found in the screening phase.

GOALS FOR 2021

For 2021, the main goal is the design and synthesis of molecules similar to the preliminary hits found in 2020 in order to improve their potency and toxicity profile. In addition, new virtual screening and joint design campaigns will be conducted with the medicinal chemistry team, with the aim of finding new molecules with newly designed scaffolds, which show appreciable activity. In particular, at least two hit families will be developed and in order to be able to carry out a SAR analysis aimed at further improvement processes of the chemical structure. The next step, currently scheduled for the end of 2021, is the design of a lead compound. The activity of the molecules will be evaluated both from the biological point of view with enzymatic and cellular assays and biophysical techniques (BLI) that will allow to evaluate the Kd of the compounds in an experimental way and to elucidate the portions most involved in the host-guest. This will also allow the further elucidation of the binding mode and the consequent prospective validation of the *In Silico* models used for the prediction of the binding mode of the molecules.



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

PUBLICATIONS

- Jose A Souto, Federica Sarno, Angela Nebbioso, Chiara Papulino, Rosana Álvarez, Lucia Altucci, Jessica Lombino, **Ugo Perricone**, Alessandro Padova, Ángel R. De Lera, A new family of JmjC-containing domain KDM inhibitors inspired on natural product purpurogallin, 2020, Front Chem May 25;8:312. doi: 10.3389/fchem.2020.00312

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

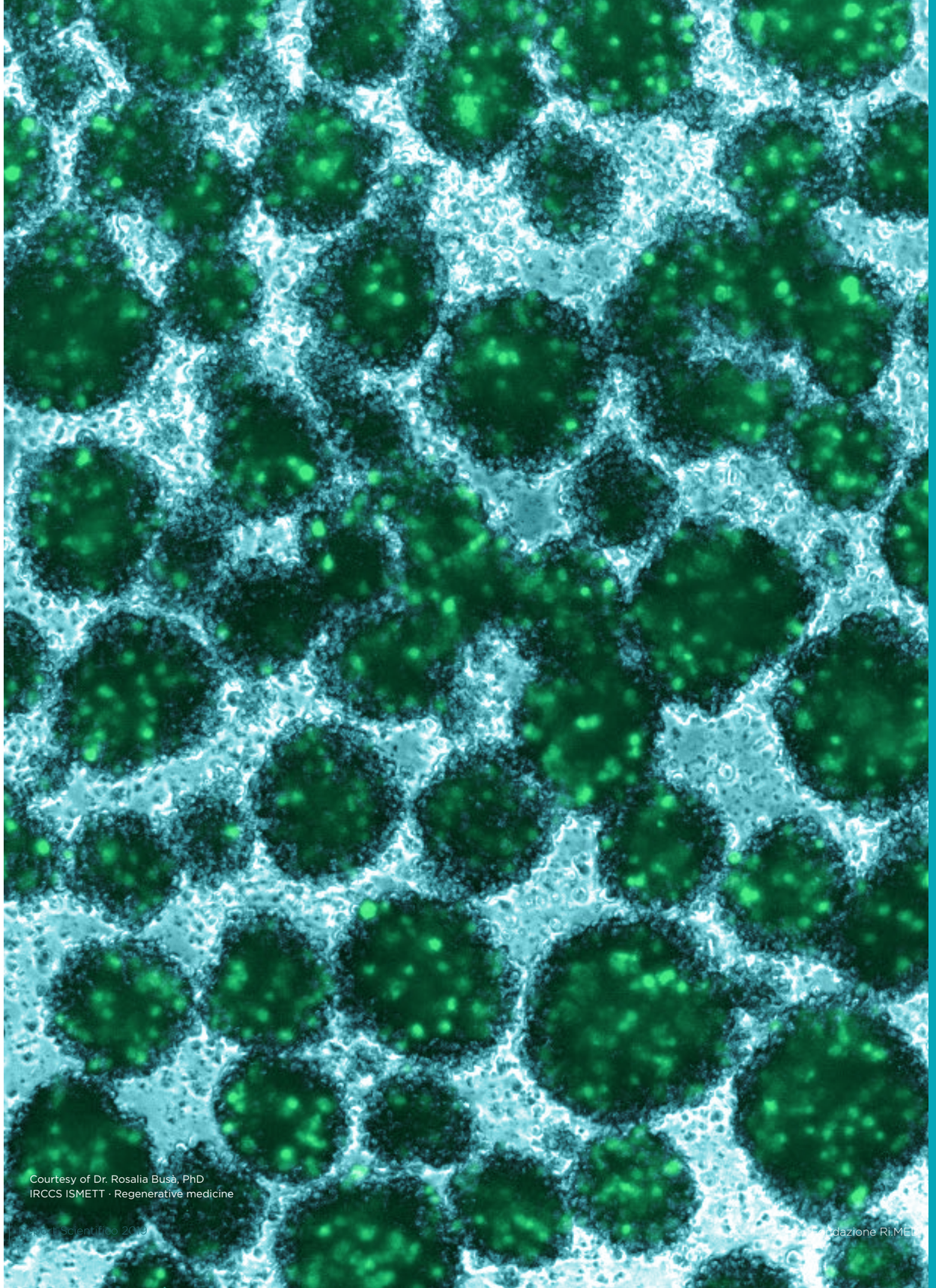
The regenerative medicine and immunotherapy laboratories are focused on developing new cellular therapies for end-stage organ diseases and post-transplant complications, and validating new vaccination strategies for infectious diseases.

The activities are shared with the IRCCS ISMETT team. The team includes researchers and technical staff specialized in research and development (*in vitro*, *in vivo* and first-in-man studies) and manipulation of biological samples of human origin. 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.

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.

Another important research focus is the study and development of cellular therapies for the prevention of disease recurrences and the treatment of post-transplant infections. Some projects are developed in close collaboration with the UP and UPMC teams in Pittsburgh.

This allows to accelerate preclinical development process towards the patient, thanks to the transfer of protocols and know-how. The new generation of vaccines, made up of re-combined proteins, aims at treating hospital-acquired infections of different etiology.



Courtesy of Dr. Rosalia Busà, PhD
IRCCS ISMETT · Regenerative medicine

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

Tolerogenic Dendritic Cells therapy for early weaning of Liver Transplanted patients
Ester Badami, PhD

Optimization of cell-based approaches for wound repair in diabetic foot: focus on biomaterial-based delivery solutions
Cinzia Chinnici, PhD

Generation of human iPSCs for disease modeling
Cinzia Chinnici, PhD

Development of a vaccine against *Klebsiella pneumoniae*
Bruno Douradinha, PhD

Mechanisms of immunoevasion of *Klebsiella pneumoniae*
Bruno Douradinha, PhD

Surveillance and characterization of multidrug resistant bacterial strains of clinical relevance
Bruno Douradinha, PhD

Fat-associated lymphoid clusters as expandable niches for ectopic liver development
Maria Giovanna Francipane, PhD

OActive - Advanced computational model, personalized, and multiscale to prevent OsteoArthritis
Riccardo Gottardi, PhD

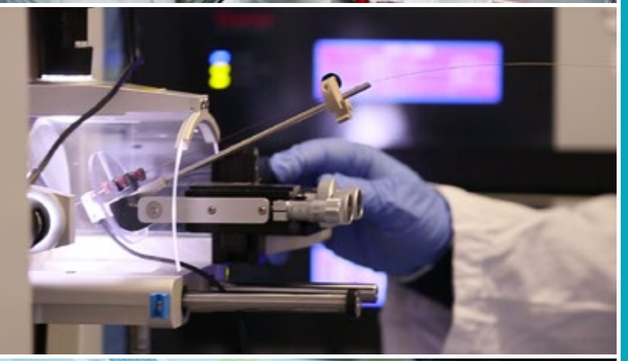
Development of an *ex vivo* stimuli-responsive osteoarthritis model for drug testing
Riccardo Gottardi, PhD

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

Study of mesenchymal stromal cells from human placenta for applications in regenerative medicine and possible hepatic therapies
Mariangela Pampalone

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

iRhom2 regulates surface levels of MHC class I molecules and immune responses
Simone Dario Scilabra, PhD



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

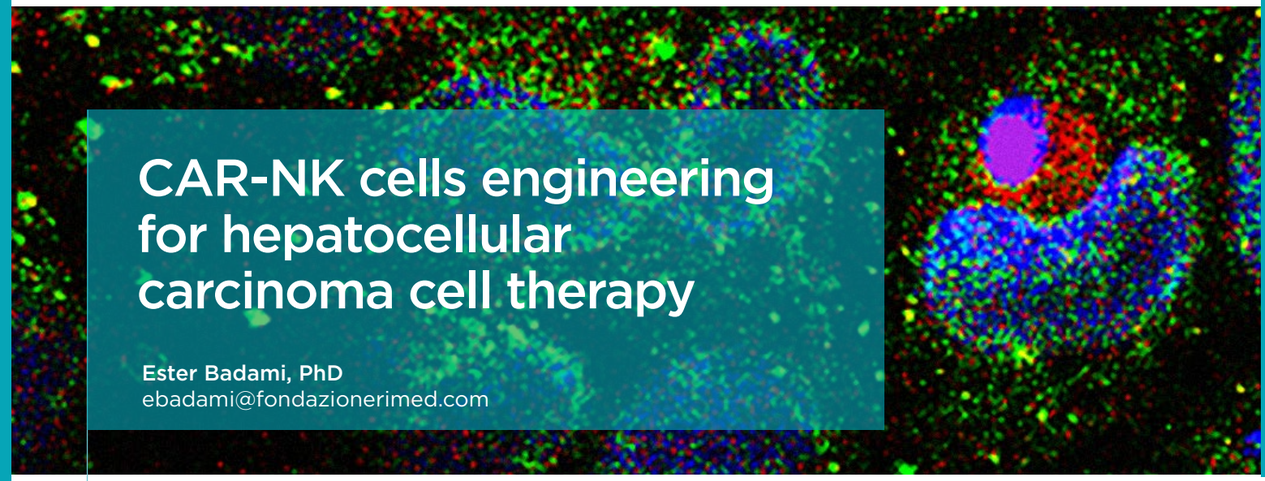
Since its inception, the Ri.MED Foundation has collaborated with IRCCS ISMETT for the use and management of the Regenerative Medicine laboratories.

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, 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 hospital, we intend to facilitate the exchange of knowledge and ideas between doctors 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.



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 (IRCCS ISMETT), Palermo, Italy

THERAPEUTIC AREA

Oncology

PIPELINE

CLINICAL NEED — DISEASES 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 preliminary data have proven to significantly enhance NK cells response to tumor and infection. The vector we

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 2020

In 2020, we compared different methods of gene transfer: lentiviral, retroviral transduction and nucleofection in different culture conditions.
Lentivirus and retrovirus were generated by transfection of 80% confluent (HEK) 293T cells and by packaging cell line Phoenix respectively together with the packaging constructs (LentiArtTM Virus Packaging Kit) and Lipofectamine3000 transfection reagent. To improve the transduction efficiency of virally-resistant NK cells were used: Polybrene, Retronectin, Vectofusin and BX795.
Lentiviral transduction. NK cells isolated from liver perfusate were transduced with our two pseudotyped lentiviral TAA-IL15-CAR and TAA-IFNα-CAR vectors comparing Polybrene alone and with Retronectin and Vectofusin alone or with the use of Retronectin. One week later the day of the transduction we performed flow cytometry analysis both TAA-specific CAR was successfully introduced on the surface of NK primary cells with a transduction yield between 12 to 22% (Fig 1).

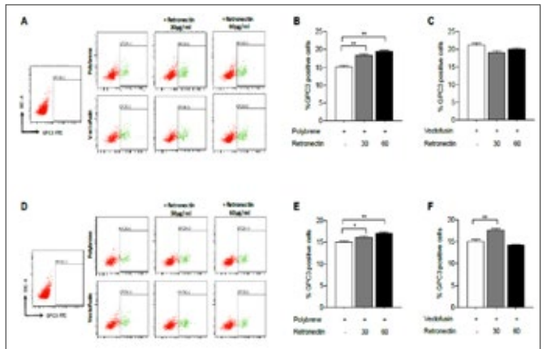

Figure 1. Comparative lentiviral transduction of NK cells using Polybrene, Vectofusin and Retronectin. (A) Gating strategy to estimate the transduction efficacy of NK cells transduced with TAA-IL15-CAR pseudotyped lentiviral particles. (B) NK cells from one donor were transduced with TAA-IL15-CAR pseudotyped 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 TAA-IFNα-CAR pseudotyped lentiviral particles. (E) NK cells from one donor were transduced with TAA-IFNα-CAR pseudotyped lentiviral particles using Polybrene alone or with Retronectin 30-60μg/ml and (F) with Vectofusin alone or with two concentrations of Retronectin.

Nucleofection. NK were transfected with P3 Primary cell 4D-Nucleofector X Kit L using 4D Nucleofector core X Unit by Lonza and plasimd control pmaxGFP and testing the following preset programs: EH100, EL100, FA100, EK100, DK100, DK125, CZ125 and CM137. Cell viability was between 8% to 36%, while the percentage of GFP+ cells ranked between 1% to 38%. We can conclude that DK100 has shown higher transfection efficiency and/or viability compared to the other programs (Fig. 2).

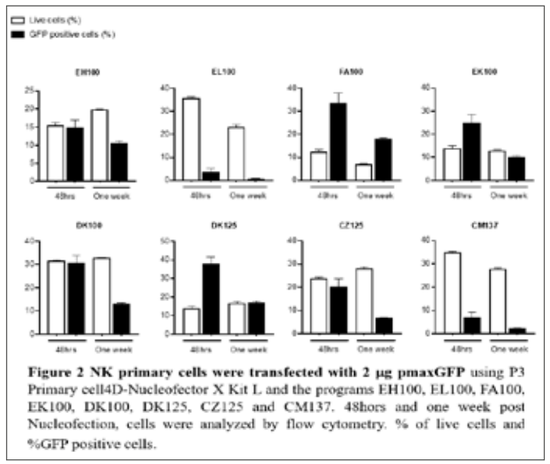

Figure 2 NK primary cells were transfected with 2 μg pmaxGFP using P3 Primary cell4D-Nucleofector X Kit L and the programs EH100, EL100, FA100, EK100, DK100, DK125, CZ125 and CM137. 48hors and one week post Nucleofection, cells were analyzed by flow cytometry. % of live cells and %GFP positive cells.

designed is a fourth generation construct harboring the signaling domain specific for TAA antigen followed by a transmembrane signaling domain, CD28 and 4-1BB costimulatory 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.

GOALS FOR 2021

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).

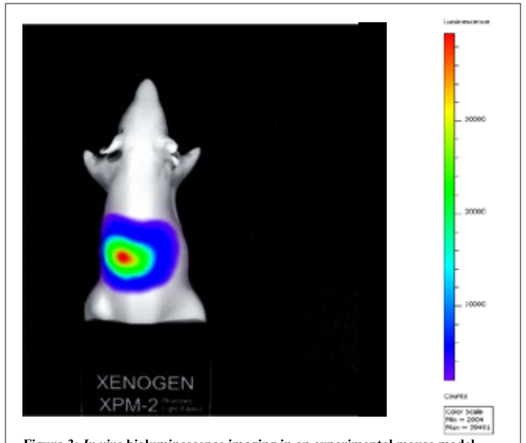

Figura 3: *In vivo* bioluminescence imaging in an experimental mouse model

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).
Test transfection efficiency on Cord-blood derived NK cells as alternative source of cells
CB-NK cell are progenitors of adult peripheral blood NK cells and therefore less differentiated. CB-NK cells proliferate at higher rates than PBMC NK cells and are described in the literature to be more permissive to genetic engineering. NK cell progenitors will be thus isolated from cord blood samples by magnetic separation and CD34+ beads and expanded with growth factors. We will test protocol to induce CAR expression with lentivirus, retrovirus and nucleofection.

MEETINGS

Virtual ISCT2020 Meeting

PUBLICATIONS

- Human NK cells activated with IFNα fully eradicate HCV infection by releasing Galectin-9 and IFNγ, **Ester Badami,** Anna Paola Carreca, Massimiliano Gaetani, Rosalia Busà, Giovanna Russelli, Diego Paini, Claudia Carcione, Pier Giulio Conaldi - *Submitted*
- 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 EPO n. 178039749
Ri.MED, ISMETT
Title: NK-mediated immunotherapy and uses thereof

PRODUCTS: **ATMP** (ADVANCED THERAPY MEDICINAL PRODUCTS)

Tolerogenic Dendritic Cells therapy for early weaning of Liver Transplanted patients

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COLLABORATIONS

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

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

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.

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 2020

In 2020, n=37 samples have been processed of which n=7 healthy donors PBMC and n=30 deceased donors liver perfusates.

On a small scale, we demonstrated that DCreg are obtained starting from CD14+ monocytes isolated from deceased donors liver perfusates with comparable tolerogenic phenotype and function than DCreg produced from apheresis products. By Flow cytometry we addressed the expression of markers of maturation such as CD40, CD1c, CD80, HLA-DR, CD14, DC-SIGN, CD86 and PD-L1.

As shown in Fig. 1A, markers likes CD40, CD1c, CD80 and HLA-DR were not differentially expressed between DC or DCreg. By contrast, CD14 and DC-SIGN were downregulated by mDC but not by DCreg, as expected as CD14 is downregulated by monocytes derived DCs. One of the release criteria for clinical use of Tol-DCs is the level of expression of co-inhibitory ligand PD-L1 compared to CD86 with a cutoff ratio of 2 or above (Fig 1B). Functionally, we showed by CFSE proliferation assay that DCreg were weak stimulators of allogeneic CD4+ and CD8+ T cell proliferation (Figure 2A-B). Further, DCreg were resistant to maturation induced by a potent immunostimulant of bacteria origin MPLA. To conclude, we demonstrated than CD14+ monocytes obtained from the liver perfusate of deceased donors can be used as a starting cellular input for the generation of Tol-DCs with phenotypic and functional characteristics compliant to the DCregs described by Thomson's et al.

GOALS FOR 2021

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-Foxp3^{high} antibodies.

Large-scale/clinical-grade isolation of monocytes from the product of perfusion of deceased liver donors. In 2021 we will scale up the protocol of generation of DCreg GMP-compliant. We will optimize the apheresis procedure trying to reduce as much as possible the proportion of granulocytes contaminating the final product.

Optimize the protocol of isolation of CD14+ precursors by elutriation. The apheresis product of liver perfusate of deceased concentrate will be processed by elutriation for the isolation of a pure population of monocytes. ISMETT and Ri.MED personnel will be trained by TERUMO specialists. The elutriated fraction of monocytes will be cultured with IL4/GM-CSF/Vit-D3/IL10 to generate DCreg. These cells will be tested phenotypically and functionally to induce tolerance into allogeneic T lymphocytes.

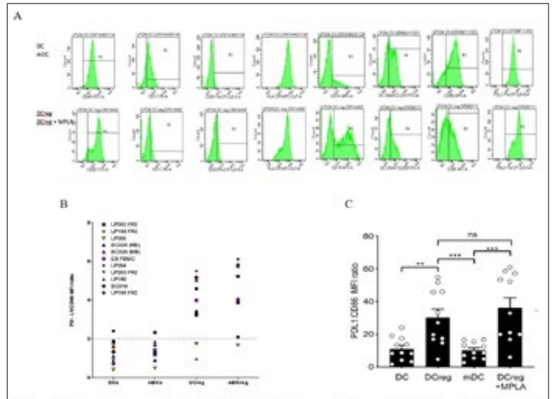

Figure 1. Flow cytometry analysis of DC/mDC (pooled on top panels) versus DCreg/DCreg+MPLA (pooled on the bottom panels) **(A)**. PDL-1:CD86 MFI ratio of DC cultured from bead-isolated monocytes from PMBC (n=4) and liver perfusate (n=7). Tol-DC preparations under the cutoff ratio of 2 are not compliant to release criteria **(B)**. PDL-1:CD86 MFI ratio of DC cultured from bead-isolated monocytes from liver perfusate (n=11). Significances of differences were determined using Wilcoxon t-test. ***p<0.05. Data are means ±SEM. mDC, LPS-stimulated DC; DCreg+LPS, DCreg stimulated with LPS **(C)**.

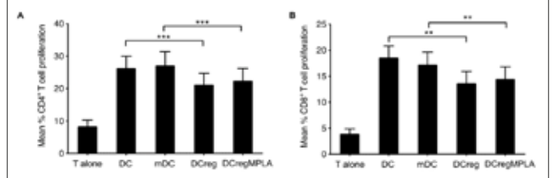

Figure 2. Proliferation of allogeneic T cells stimulated with DCreg. DC were generated from bead-isolated monocytes (*n*=22). CFSE-labeled allogeneic T cells were co-cultured for 5 days with various populations of DC (1DC:10T cells). DC or DCreg were stimulated for 20 hr with MPLA to generate mDC and DCregMPLA, respectively. T cell proliferation was measured by CFSE dilution. Data are means ±SEM. Significances of differences were determined using Wilcoxon t-test. *** p<0,05.

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

Optimization of cell-based approaches for wound repair in diabetic foot: focus on biomaterial-based delivery solutions

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COLLABORATIONS

- Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IRCCS ISMETT), Palermo, Italy
- Laboratory of Biocompatible Polymers, STEBICEF – University of Palermo, Palermo, Italy
- IRET Foundation - University of Bologna, Bologna, Italy

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

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

BRIEF DESCRIPTION

The therapeutic efficacy of MSCs is actually attributed to cell secretome, a mixture of bioactive and immunomodulatory products (growth factors, cytokines, chemokines, enzymes, and genetic material) capable to stimulate the endogenous repair processes. Nevertheless, the delivery of secretome needs to be coupled with biomaterials to achieve a controlled release over time. This last point is of crucial importance, since the way by cells and soluble factors are "presented" to the tissue influences therapeutic efficacy. The project is organized in 3 phase as follows:

PHASE 1: characterization of cell source and cell-derived product. The aim of phase 1 is to establish the "best secretome" for specific applications of regenerative medicine, such as the treatment of chronic ulcers in diabetic subjects. Products from the "best source" pass to phase 2.
PHASE 2: development of biomaterials as delivery solutions of MSC secretome. The aim of phase 2 is to obtain ready-to-use biofunctional formulations with defined composition and expected mechanism of action, able to stabilize the properties of the biological product itself, and to deliver the expected doses. Formulations should be also suitable for fabrication scalability and clinical translation. Products having the most appropriate biological properties for skin wound healing will pass to phase 3. Biomaterials are based on a hyaluronic acid derivative (HA-EDA) hydrogels functionalized or not with heparin (HP).
PHASE 3: *in vivo* preclinical testing in animal models of wounds. The aim of phase 3 is to test and validate efficacy of the proposed therapeutic solution in well established animal models of diseases, such as pressure ulcers in dbdb diabetic mice.

HA-EDA hydrogel sponge

IMPACT

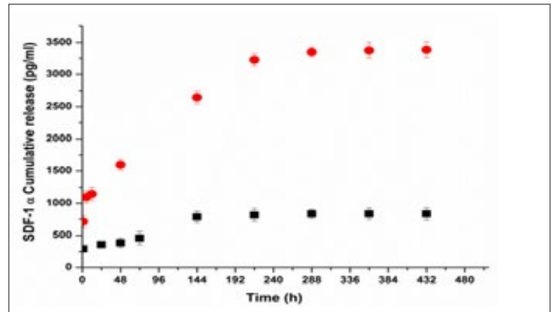
As a cell-free product, secretome-based therapy has the advantage to limit potential risks related to conventional cell transplantation (tumorigenicity, transmission of infections, immunoreactions). In addition, secretome collected as cell culture conditioned medium (CM) is easier to handle than cells as a ready-to-go biological product, and can be more easily produced as a drug for clinical applications.

RESULTS ACHIEVED IN 2020

In collaboration with the lab of Biocompatible Polymers at STEBICEF, we have produced a brand new biomaterial formulation based on hyaluronic acid hydrogel. The goal is to use a patent-free biomaterial in future preclinical validation studies. This new formulation will be validated *in vitro* by studying the release kinetics of MSC secretome. Thanks to the collaboration with the Department of Farmacia e Biotecnologie at the University of Bologna, we will soon test the new hydrogel formulation in a murine model of diabetic ulcer (preclinical proof of concept).
The manuscript "Small extracellular vesicles from human fetal dermal cells and their microRNA cargo: KEGG signaling pathways associated with angiogenesis and wound healing" (Chinnici CM et al.) has been published on Stem Cells Int (doi: 10.1155/2020/8889379).
A second manuscript has been recently submitted to Int J Mol Med and is currently under revision ("Extracellular vesicle-derived microRNAs of human Wharton's jelly mesenchymal stromal cells may activate endogenous VEGF-A to promote angiogenesis", Chinnici CM et al.).
A third manuscript (Chinnici CM et al., "Hyaluronic acid/heparin-based hydrogels as delivery solutions for mesenchymal stromal cells and their secretome") is still holding because is secondary to the conduction of the preclinical *in vivo* study.

GOALS FOR 2021

Preclinical proof of concept in collaboration with IRCCS-ISMETT, STEBICEF and University of Bologna. Testing HA-EDA hydrogels integrated with MSC secretome in a murine model of diabetic wound (the actual title is to be defined).

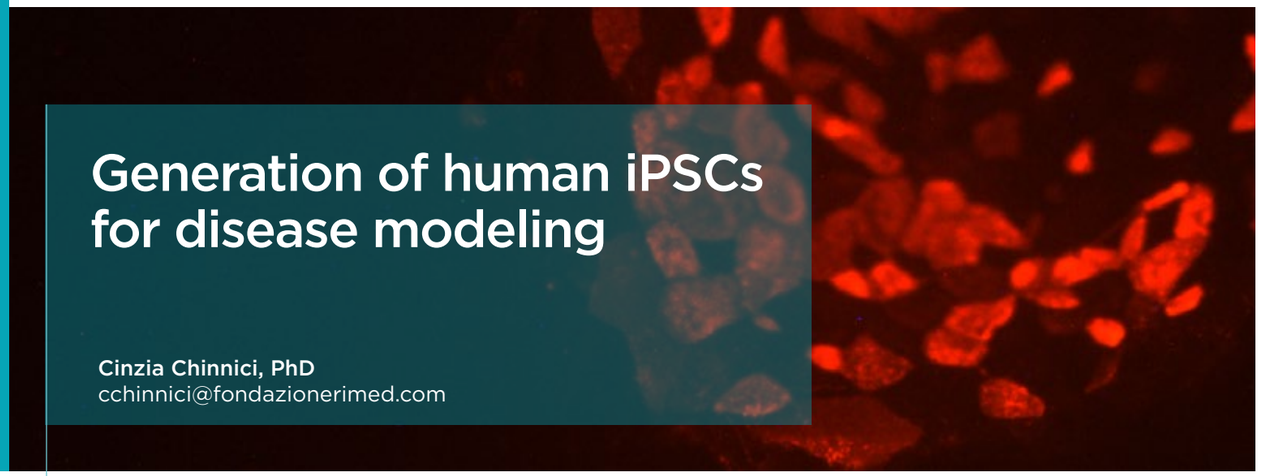


Sustained release of SDF1 - alpha from HA-EDA hydrogel

PUBLICATIONS

- Miceli V, Bertani A, **Chinnici CM**. et al. 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. Accepted: Int. J. Mol. Med.
- **Chinnici CM**, Amico G, Gallo A et al. Small Extracellular Vesicles from Human Fetal Dermal Cells and Their MicroRNA Cargo: KEGG Signaling Pathways Associated with Angiogenesis and Wound Healing. Stem Cells Int. 2020 Aug 13;2020:8889379. doi: 10.1155/2020/8889379.
- Schmelzer E, Miceli V, **Chinnici CM** et al. Effects of Mesenchymal Stem Cell Coculture on Human Lung Small Airway Epithelial Cells. Biomed Res Int. 2020 Mar 27;2020:9847579. doi: 10.1155/2020/9847579.

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



Generation of human iPSCs for disease modeling

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COLLABORATIONS

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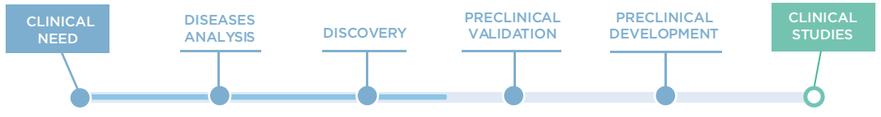
THERAPEUTIC AREA

Organ insufficiences

PIPELINE



BRIEF DESCRIPTION

Human induced pluripotent stem cells (iPSCs) hold the promise in many research areas, especially in disease modeling. By reprogramming somatic cells exhibiting disease morphology, a potentially unlimited source of iPSCs offers the ability to expand, differentiate, and study affected human cells without another animal model.

Physiologically relevant cellular models can accelerate the discovery of disease mechanisms, to screen drugs or to perform genome editing. Genome editing in iPSCs, in particularly, has been demonstrated to be highly effective for generating disease models for monogenic liver disorders.

The goal of the project is the establishment of patient-specific iPSC cell lines carrying the gene defecting as an *in vitro* model of metabolic liver disease. We aim to test *in vitro*, on iPSC-derived hepatocytes, the efficiency of gene editors in correcting a defective gene/enzyme essential for hepatocyte metabolism.

IMPACT

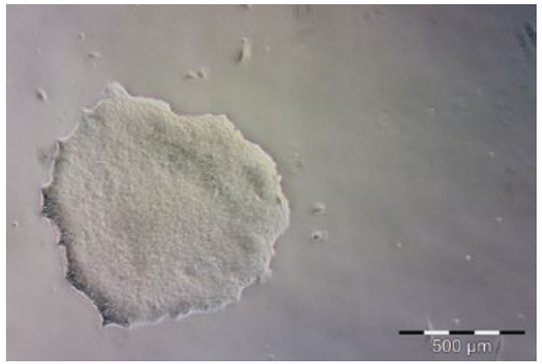
The liver transplant program at IRRCS-ISMETT includes pediatric patients affected with metabolic liver diseases. If we could use genome base editing approaches to treat c patients with metabolic liver diseases, and treat them with a single, systemic injection of vector/base editors, this will dramatically improve patient quality of life.

RESULTS ACHIEVED IN 2020

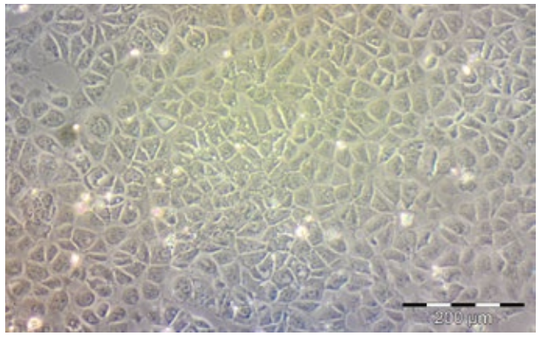
With the goal to acquire an expertise in iPSC technology. We have obtained by reprogramming MSCs of fetal dermis and umbilical cord using the non-integrating, Sendai virus method. The obtained iPSCs have been expanded and cryopreserved. The first attempt of differentiation toward hepatocyte lineage has been made (e.g., from iPSCs to definitive endoderm to hepatoblasts).

GOALS FOR 2021

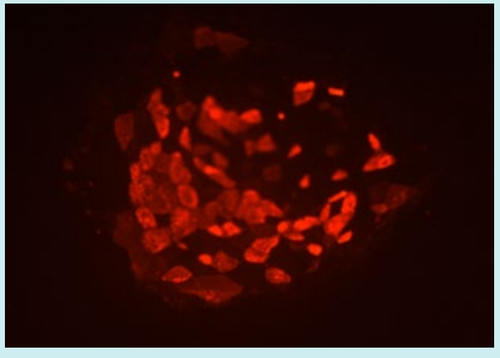
Optimize the protocol for generation, expansion and banking of human iPSCs. *In vitro* differentiation of iPSCs toward hepatic lineages (e.g., hepatocytes or cholangiocytes, this latter in collaboration with Maria Giovanna Francipane). Establishment of functional assays on mature cells obtained from iPSCs. Characterization of iPSCs with different approaches (molecular biology and biochemistry).



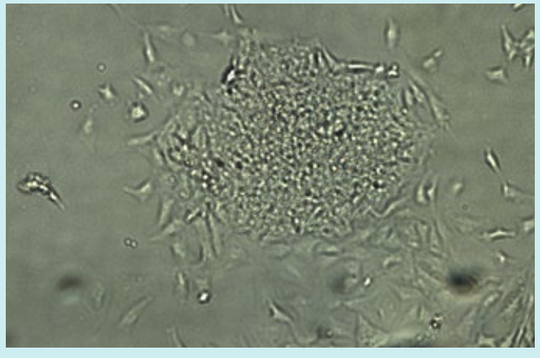
iPSC colony at passage 3



Definitive endoderm obtained from differentiated iPSCs



A - Live stain with anti-TRA-1-60 antibody (red) specific for iPSCs



B - Bright field image of an iPSC colony

Development of a vaccine against *Klebsiella pneumoniae*

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COLLABORATIONS

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- Careggi University Hospital, Florence, Italy
- University of Messina, Messina, Italy
- GSK Vaccines, Siena, Italy

THERAPEUTIC AREA

Infectious diseases

PIPELINE

BRIEF DESCRIPTION

In this project, we propose the development of a vaccine against *K. pneumoniae* using a reverse vaccinology approach. Through genome mining, we will identify conserved antigens in the genomes of reference strains available online and confirm their presence in the sequenced genomes of clinical *K. pneumoniae* isolates.
Computer analysis will allow us to identify surface proteins present in the majority of clinical isolates of *K. pneumoniae*, which will be components of a potential vaccine. Thus, this vaccine will also work for multidrug-resistant strains of *K. pneumoniae*, since the mechanisms responsible for the resistance do not impair a direct immune response against these bacteria.
We are convinced that this new approach will be effective against *K. pneumoniae* and will help fight this multidrug-resistant bacterium.

IMPACT

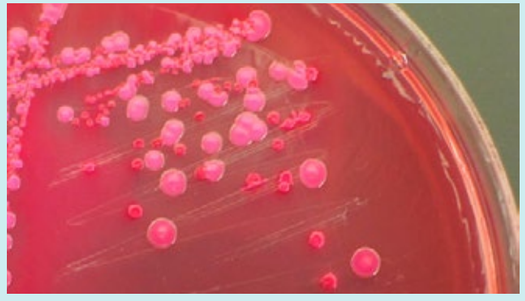
K. pneumoniae is a Gram-negative bacterium of clinical importance, which readily colonizes mucosal surfaces and, from there, has access to other tissues and establishes serious infections. Resistance to several antibiotics has been reported, reducing the number of effective treatments.
K. pneumoniae is increasingly becoming a public health problem. Indeed, the World Health Organization has published a list of antibiotic-resistant bacteria in which is stated the need of new interventions against *K. pneumoniae* is considered crucial.
To date, no vaccine is available against *K. pneumoniae*. Our approach to develop a vaccine against this multidrug-resistant pathogen could be an effective immunotherapy against *K. pneumoniae*, since it would also work for the multidrug-resistant strains responsible for nosocomial infections.

RESULTS ACHIEVED IN 2020

During this year, in collaboration with IRCCS-ISMETT, we identified sera from patients with at least one *K. pneumoniae* infection and/or colonization case. We selected about 100 samples for each of the conditions: infected (blood, internal fluids, urinary infections with high bacterial load), colonization (urinary infections with low bacterial load, rectal swab) and negative (patients without no cases of *K. pneumoniae*, as patent in the hospital management software of IRCCS-ISMETT). An ELISA immunoassay using protein extracts of *K. pneumoniae* allowed us to identify hyperimmune sera against *K. pneumoniae*, i.e, with a high titer of antibodies against this pathogen and negative sera, i.e, which had no response against *K. pneumoniae*. With these sera, and using 3 of the potential antigens as coating, we verified by ELISA that antibodies against those antigens were developed during infection or colonization with *K. pneumoniae*. In particular, one of the antigens induces a strong response of CD8 + T cells producing interferon-gamma *in vitro* by previous activation of dendritic cells, as demonstrated by ELISpot.

GOALS FOR 2021

Our goals for 2021 are to purify the remaining potential antigens identified using reverse vaccinology. Once the recombinant antigens have been obtained, we will perform ELISA assays using sera from patients mentioned above and we will verify the presence of antibodies developed against the antigens studied. This will tell us if the chosen antigens are immunogenic per se, that is, if during the natural course of an infection with *K. pneumoniae* if natural antibodies against these antigens are developed. We have submitted the authorization procedure for carrying out animal studies. This will allow us to obtain mouse serum containing antibodies against the chosen antigens and to study the immunogenicity of these antigens, for example, their potential to induce a humoral immune response. The obtained antibodies will also be used in other assays, for example, in a passive immunization approach and opsonophagocytosis.



Clinical isolate of *K. pneumoniae*

PRODUCTS: **BIOLOGICS**

Mechanisms of immunoevasion of *Klebsiella pneumoniae*

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COLLABORATIONS

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- Careggi University Hospital, Florence, Italy
- Vaxxilon gmbH, Berlin, Germany

THERAPEUTIC AREA

Infectious diseases

PIPELINE

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

BRIEF DESCRIPTION

Currently, several pathogenic microorganisms possess resistance to multiple antibiotics leading to a huge increase in nosocomial infections, some of them fatal. Among these, *Klebsiella pneumoniae* infections are widespread, mainly due to the plastic ability of this bacterium to acquire resistance genes, including to carbapenems. To establish efficient infections, *K. pneumoniae* must overcome several host immune defenses. Lipopolysaccharides (LPS) play an ambiguous role, as they both activate immune responses but can also play a role in immunoevasion. *K. pneumoniae* LPS can be classified into different serotypes by their O antigen. Interestingly, O2a and O2afg serotypes are prevalent in most multidrug-resistant *K. pneumoniae* strains. In this project, we aim to unravel if LPS O2a and O2afg serotypes could be part of an immuneevasion mechanism that allows multidrug-resistant strains of *K. pneumoniae* to avoid cells from the immune system and spread throughout the body.

IMPACT

As previously mentioned, *K. pneumoniae* is a pathogen of clinical importance due to its ability to develop resistance to all classes of antibiotics. Furthermore, *K. pneumoniae* has been shown to be able to evade immune responses via some surface proteins, such as fimbriae, and their LPS. However, these mechanisms are not yet fully understood. Once we understand how this bacterium manages to evade the immune system, new immunotherapies could be developed that would prevent the spread of *K. pneumoniae* in the body, thus preventing new infections. These therapies will be very suitable for patients in hospitals such as IRCCS-ISMETT, who have to undergo immunosuppressive regimens, thus becoming more susceptible to bacterial infections.

RESULTS ACHIEVED IN 2020

Genetic analysis showed that most of the carbapenem-resistant clinical isolates of *K. pneumoniae* from our Institute come from the O2afg serotype. As previously demonstrated, O2a and O2afg are less immunogenic, as confirmed by ELISA using *K. pneumoniae* infection sera and inpatient colonization. We observed that, when human monocytes are incubated with LPS extracted from O1, O2a or O2afg strains, both O2a and O2afg, but not O1, they failed to induce inflammatory cytokine production, suggesting a role in immunoevasion. However, the phagocytosis levels of *K. pneumoniae* strains showing these three O antigens by human monocytes and granulocytes are similar.
Our results indicate that the LPS O2a and O2afg serotypes allow the multidrug-resistant clinical isolates of *K. pneumoniae* to avoid an initial inflammatory immune response and, consequently, promote their systemic spread, leading to the various pathologies associated with this bacterium.

GOALS FOR 2021

Our goals for 2021 are to understand the role of the NF-κB molecule in the LPS-induced inflammation process studied. This molecule has been known to translocate to the monocytes' nucleus, thus inducing the inflammatory process. We want to check that monocytes, if incubated with LPS O2a and O2afg, do not induce an inflammatory response and that NF-κB remains in the cytoplasm of the cells. We will also want to study phagocytosis by monocytes and expression of bacterial proteins that allow to resist phagocytosis by RT-PCR. We will also study the expression of small non-coding RNAs in reference strains for LPS serotypes O1, O2a and O2afg, to understand if there are any differences in transcription.

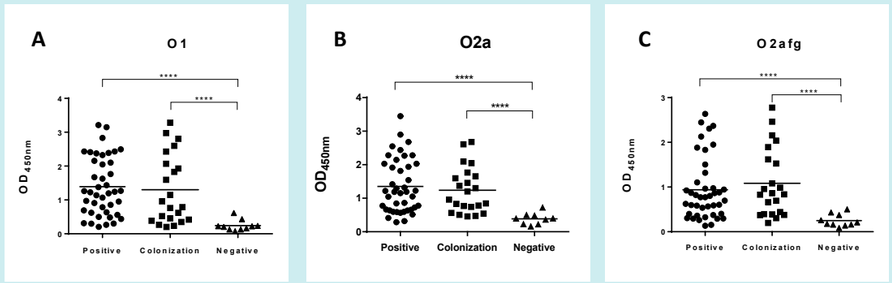

Fig. 1. Following *K. pneumoniae* infection or colonization, antibodies against LPS O1 (A), O2a (B) and O2afg (C) are present in human patients' sera. Sera samples were diluted 1:1000 (O1) or 1:100 (O2a and O2afg). Statistical analysis was done using the Mann–Whitney U test, **** $p < 0{,}0001$.

PRODUCTS: **BIOLOGICS**

Surveillance and characterization of multidrug resistant bacterial strains of clinical relevance

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COLLABORATIONS

- **Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IRCCS ISMETT), Palermo, Italy**
- **University of Siena, Siena, Italy**
- **Careggi University Hospital, Florence, Italy**

THERAPEUTIC AREA

Infectious diseases

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

BRIEF DESCRIPTION

Within this project, we intend to characterize particular and/or multidrug resistant bacterial strains that arise from continuous microbiological surveillance at our clinical partner, IRCSS-ISMETT. Their pathogenic potential will be assessed *in vitro*, to understand if these bacterial isolates can become strains of clinical relevance.
Bacterial clinical isolates which display a particular phenotype, e.g., resistance to a particular drug or set of drugs, will have their genomic material sequenced and matched against the information currently available in public databases. Once the drug resistance patterns are identified, these clinical isolates will be classified accordingly and, if a particular sequence type (ST) or novel species is found, it will be further characterized by *in vitro* assays, e.g., abiotic and cellular biofilm formation ability and human serum resistance.

IMPACT

By assessing both their clinical relevance and predominance, we can understand if novel interventions are required against these specific strains, such as a vaccine or an immuno-therapy. The predominance of such bacterial clinical isolates will be compared to what is currently observed in both the national and international sceneries, to understand if the Sicilian reality reflects what is presently observed at epidemiological level worldwide or if novel ST are arising in our clinical partner IRCCS-ISMETT. This is highly important since it will allow the definition of correct and/or novel prophylaxis and treatment regimens for patients who must suffer a transplantation and thus, have their immune system suppressed to avoid organ rejection.

RESULTS ACHIEVED IN 2020

We are currently analyzing all the data from the 208 genomes of *K. pneumoniae* strains that produce the KPC enzyme that is capable of cleaving carbapenems. These strains come from the IRCCS-ISMETT bacterial stock and were collected from 2008 to 2017, from various episodes of infection or colonization of patients who were admitted to the ISMETT for various types of treatment. We confirmed that, in the early years, the sequence types (STs) of these clinical isolates were, in the vast majority ST258 or ST512, the most predominant STs in Italy. In the following years, we found an enormous variation in STs, including STs that have not been observed before in Italy, and also new clinical isolates, that is, without an ST yet attributed. We are collecting data from those patients from whom these clinical isolates have been isolated, with respect to gender, type of treatment they have had, whether they have undergone a transplant surgery and other information that may be relevant.

GOALS FOR 2021

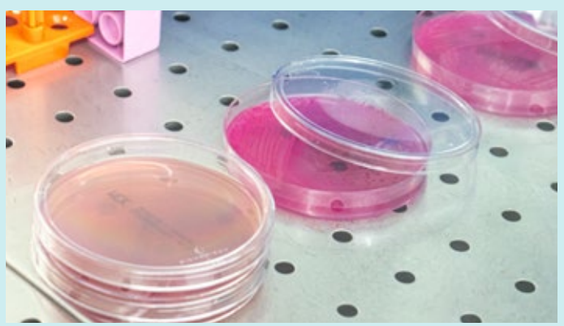
Our goals for 2021 are to finish and publish the data described above. We will make a heat map graph to understand the evolution of resistance genes, virulence genes and other factors that we will find relevant. The work will be very interesting because it will include the epidemiological evolution of the different *K. pneumoniae* strains during the last few years at the IRCCS-ISMETT and will be very useful for researchers working on the epidemiology of antibiotic-resistant bacteria.
Our data may indicate potential new STs that may cause future outbreaks in hospitals. We have already found and published in the past an ST that has the potential to become a pathogen of nosocomial importance, ST392, which has already spread to Sicily and Campania. Thus, our results may help predict how some *K. pneumoniae* ST are spreading and how to combat them.

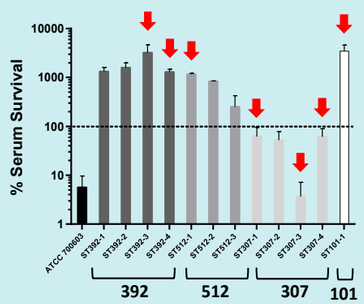
MEETINGS

- 14th Vaccine Elsevier Congress, September 28-29° 2020 (online)
- New advances in basic and applied virology, September 21st 2020 (online)
- Flanders Vaccine COVID-19 webinars, June 18th and 25th 2020 (online)
- eSymposia Keystone Vaccinology in the Age of Pandemics: Strategies Against COVID-19 & Other Global Threats, June 15-16th 2020 (online)

PUBLICATIONS

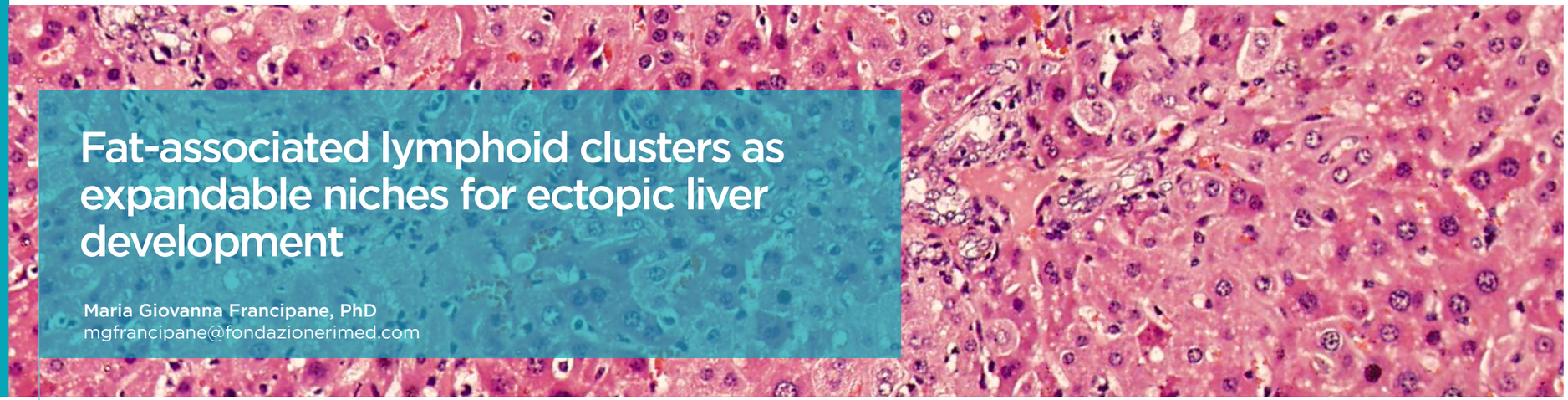
- D'Apolito D, Arena F, Conte V, De Angelis LH, Di Mento G, Carreca AP, Cuscino N, Russelli G, Iannolo G, Barbera F, Pasqua S, Monaco F, Cardinale F, Rossolini GM, Conaldi PG, **Douradinha B**. Phenotypical and molecular assessment of the virulence potential of KPC-3-producing *Klebsiella pneumoniae* ST392 clinical isolates. Microbiol Res. 2020 Nov;240:126551. doi: 10.1016/j.micres.2020.126551. Epub 2020 Jul 6.
- D'Apolito D, D'Aiello L, Pasqua S, Pecoraro L, Barbera F, **Douradinha B**, Di Martino G, Di Bartolo C, Conaldi PG. Strategy and validation of a consistent and reproducible nucleic acid technique for mycoplasma detection in advanced therapy medicinal products. Biologicals. 2020 Mar;64:49-57. doi: 10.1016/j.biologicals.2020.01.001. Epub 2020 Jan 22.





K. Pneumoniae ST392 KPC-3 producing clinical isolates are resistant to human serum

PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**



Fat-associated lymphoid clusters as expandable niches for ectopic liver development

Maria Giovanna Francipane, PhD
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COLLABORATIONS

- Lagasse Lab, McGowan Institute for Regenerative Medicine, Pittsburgh, USA
- Saint Barnabas Medical Center, Livingston, New Jersey, USA
- Mayo Clinic, Rochester, Minnesota, USA

THERAPEUTIC AREA

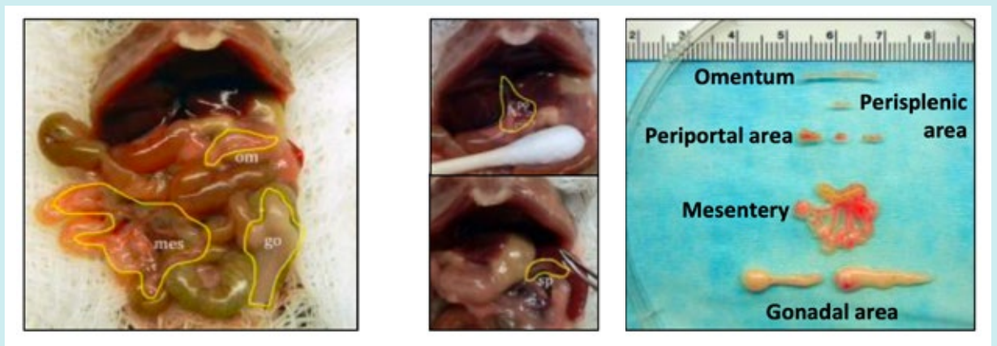
Organ insufficiences

PIPELINE

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

BRIEF DESCRIPTION

Hepatocyte transplantation holds great promise as an alternative approach to whole organ transplantation. Intraportal and intrasplenic cell infusions are primary hepatocyte transplantation delivery routes for this procedure. However, patients with severe liver disease often have disrupted liver and spleen architecture, which introduce risks in the cell engraftment process. Our group has previously demonstrated that lymph nodes are excellent sites for the development of ectopic tissues. Direct injection of hepatocytes into lymph nodes resulted in the formation of a functional ectopic liver both in small and large animal models. More recently, we tested whether other niches in the abdominal cavity could support the survival and proliferation of transplanted hepatocytes. We found that hepatocytes transplanted by intraperitoneal injection engraft and generate ectopic liver tissues in fat-associated lymphoid clusters (FALCs), which are adipose tissue-embedded, tertiary lymphoid structures located throughout the peritoneal cavity.



Left and middle panels: Gross pictures showing 5 major areas that contain FALCs in mouse abdominal cavity (om: omentum; mes: mesentery; go: gonadal area; po: periportal area; sp: perisplenic area). Right panel: Dissected omentum, perisplenic area, periportal area, mesentery, and gonadal area. Ruler indicates the size of the tissues (centimeters).

IMPACT

Liver transplantation is currently the only effective treatment for patients with severe hepatic failure. Unfortunately, there is an exacerbating liver transplant shortage due to many factors, ranging from the limited number of liver donors to the increasing number of patients awaiting a transplant. Therefore, the development of new therapies is a critical and urgent need. Our previously reported study identified the lymph nodes as a favorable site for cell engraftment in the mouse. More recently, we provided preclinical proof of concept for hepatocyte transplantation into lymph nodes as a cure for liver failure in a large-animal model with hereditary tyrosinemia type 1 (HT1), a metabolic liver disease caused by deficiency of fumarylacetoacetate hydrolase (FAH) enzyme. Our additional finding that FALCs in the abdominal cavity can also support the survival and proliferation of transplanted hepatocytes provides a further approach for regenerating functional ectopic livers.

RESULTS ACHIEVED IN 2020

We found that FALCs are receptive loci for hepatocytes transplanted intraperitoneally. Under selective pressure in the FAH-/- tyrosinemic mouse, FALC-engrafted wild-type hepatocytes expanded, showed similar transcriptome profiles as normal liver, and rescued the mice that would have otherwise died of liver failure. In FRGN mice which lack FALCs, intraperitoneal transplantation of hepatocytes failed to rescue the mice from liver failure, but FALCs could be restored following bone marrow cell transplantation from wild-type mice. Finally, inducing peritoneal inflammation via zymosan increased FALCs numbers, thereby improving hepatocyte engraftment and accelerating the recovery of tyrosinemic mice from liver failure. In conclusion, abdominal FALCs are essential extra-hepatic sites for hepatocytes engraftment after intraperitoneal transplantation, and as such, represent an easy-to-access and expandable niche for ectopic liver regeneration when adequate growth stimulus is present. To our knowledge, this is the first evidence regarding the definite fate of hepatocytes following injection into the peritoneal cavity.

GOALS FOR 2021

Future studies will focus on methods to safely and efficiently induce more abdominal FALCs, as well as the cellular and molecular mechanisms supporting the engraftment and proliferation of transplanted hepatocytes in abdominal FALCs.

PUBLICATIONS

- Nicolas CT, Kaiser RA, Hickey RD, Allen KL, Du Z, VanLith CJ, Guthman RM, Amiot B, Suksanpaisan L, Han B, **Francipane MG,** Cheikhi A, Jiang H, Bansal A, Pandey MK, Garg I, Lowe V, Bhagwate A, O'Brien D, Kocher JA, DeGrado TR, Nyberg SL, Lagasse E, Lillegard JB. (2020). Ex Vivo Cell Therapy by Ectopic Hepatocyte Transplantation Treats the Porcine Tyrosinemia Model of Acute Liver Failure. Mol Ther Methods Clin Dev., 18:738-750. doi: 10.1016/j.omtm.2020.07.009.-

PRODUCTS: **BIOMARKERS - MEDICAL DEVICES & TISSUE ENGINEERING**

OActive - Advanced computational model, personalized, and multiscale to prevent OsteoArthritis

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COLLABORATIONS

- Partner of the European project OActive - H2020: Lead partner: University of Nicosia; other partners on https://www.oactive.eu/partners/
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- Dept. of Pediatrics, Perelman School of Medicine, University of Pennsylvania (UPenn), Philadelphia, USA
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THERAPEUTIC AREA

Aging diseases

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

BRIEF DESCRIPTION

Osteoarthritis (OA) is an inflammatory frequent joint disease, mainly affecting middle-aged and elderly population. A systematic epidemiologic study, estimate that about 27% of people ≥ 65 years old suffer a symptomatic osteoarthritis worldwide, and it has been predicted that up to 25 million of people will be affected by osteoarthritis within the 2020. Recent findings point out as osteoarthritis involves the osteochondral complex, which includes the articular cartilage, and the subchondral trabecular bone. The focus of this work is to clarify the role of subchondral bone in the development of osteoarthritis, and to study the underneath biochemical mechanism. The strong point of our research is the innovative approach based on the *ex vivo* culture of human tissues, which permits to study cartilage and bone simultaneously. Tissues were obtained from surgical waste of total knee replacement surgery, and were cultured within our advanced bioreactors under dynamic flow conditions.

IMPACT

Our research aims to study the Osteoarthritis using a realistic *ex vivo* model based on human tissues. The employ of tissue is fundamental to analyze cartilage-bone crosstalk phenomena, and to establish their role in the development of osteoarthritis. Remarkably, human tissues are cultured within our advanced *in vitro* systems composed of biphasic bioreactors, which ensures tissues viability up to several week after harvesting and allows to investigate the osteoarthritis mechanism directly onto viable tissues. Our system represents an evolution of *in vitro* cell cultures, and may limits the employ of animal model to the preclinical trials. Interestingly, our system can also be adopted as drug screening platform, and open new perspective on the study of drugs pharmacokinetic.

RESULTS ACHIEVED IN 2020

We were able to determine and quantify proinflammatory mediators involved in the development of osteoarthritis, both in cartilage and subchondral bone. In particular, we found high levels of cytokines as IL-1β and IL-6 in subchondral bone, which suggest an active role of this tissue in osteoarthritis development. Moreover, we also highlighted the role of PIK3-AKT in maintaining the homeostasis in OA cartilage and bone.
This evidence, revert the conventional hypothesis attributing a passive role to the bone in the development of osteoarthritis. Moreover, the use of whole osteochondral plugs allows to investigate on the role of cartilage-bone crosstalk in the osteoarthritis progress. To perform our studies, we obtained osteoarthritic human tissues from surgical waste of total knee arthroplasty.
Therefore, we developed an experimental protocol to extract viable osteochondral units (plugs), and to maintain plugs under dynamic culture *ex vivo* using our biphasic bioreactors.

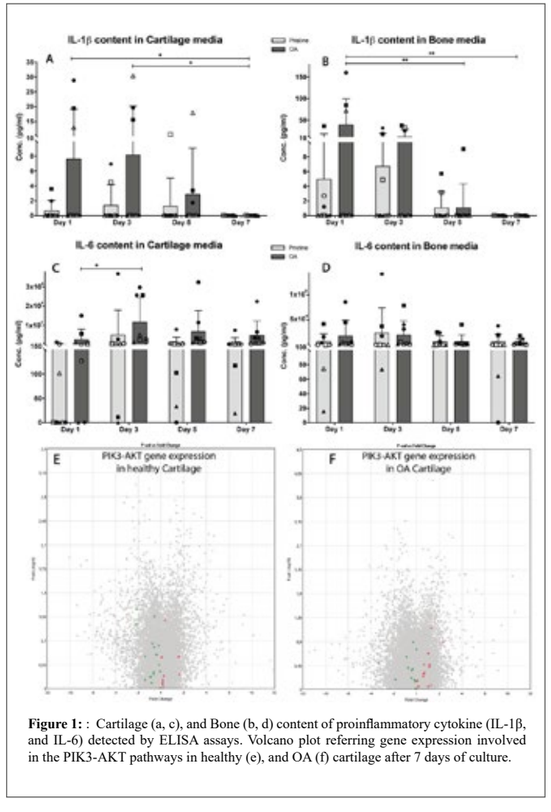

Figure 1: : Cartilage (a, c), and Bone (b, d) content of proinflammatory cytokine (IL-1β, and IL-6) detected by ELISA assays. Volcano plot referring gene expression involved in the PIK3-AKT pathways in healthy (e), and OA (f) cartilage after 7 days of culture.

GOALS FOR 2021

Our results confirmed the dynamic nature of osteoarthritic pathology, emphasizing the importance of crosstalk phenomena between cartilage and bone tissue. In the next steps we will realize a more realistic *ex vivo* model, which will include synovial tissue in addition to the osteochondral plugs.
The final goal is to determine the role of the synovial membrane in the production of proinflammatory cytokines, and their interaction with cartilage and subchondral bone. In the meantime, we are identifying potential biologically active molecules that could be tested within our *in vitro* system, evaluating possible interactions between tissues. This will offer further validation of the system to support its commercialization.

MEETINGS

- I. Chiesa, C. De Maria, A. Lapomarda, G.M. Fortunato, R. Di Gesù, F. Montemurro, G. Vozzi, **R. Gottardi**. Endothelial cells promote osteogenesis in an in vitro vascularized bone model developed by 3D bioprinting. Summer Biomechanics, Bioengineering, and Biotransport Conference - SB3C, virtual conference, June 2020
- I. Chiesa, C. De Maria, A. Lapomarda, G.M. Fortunato, R. Di Gesù, S. Aliakbarighavimi, F. Montemurro, R.S. Tuan, G. Vozzi, **R. Gottardi**. Endothelial cells support osteogenesis in a vascularized 3D bioprinted in vitro bone model. Orthopaedic Research Society Annual Meeting, Huston, TX, February, 2020

PUBLICATIONS

- I. Chiesa, C. De Maria, A. Lapomarda, G.M. Fortunato, F. Montemurro, R. Di Gesù, R.S. Tuan, G. Vozzi, **R. Gottardi**. Endothelial cells support osteogenesis in an in vitro vascularized bone model developed by 3D bioprinting. Biofabrication. 2020, 12(2):025013. DOI: 10.1088/1758-5090/ab6a1d.

PRODUCTS: **DRUGS** - **BIOMARKERS** - **MEDICAL DEVICES & TISSUE ENGINEERING**

Development of an *ex vivo* stimuli-responsive osteoarthritis model for drug testing

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COLLABORATIONS

- Partner of the European project OActive - H2020: Lead partner: University of Nicosia; other partners on https://www.oactive.eu/partners/
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THERAPEUTIC AREA

Aging diseases

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

BRIEF DESCRIPTION

Osteoarthritis (OA) is a multifactorial disease involving all joint tissues causing degeneration of articular cartilage, subchondral bone sclerosis and synovial inflammation.
The tissues damage seriously compromise the overall functionality of the articulation, culminating in joint pain and disability. Osteoarthritis is characterized by a complex etiology which both genetic and acquired factors seem be involved in. In particular, it is well note as the OA onset is connected to pro-inflammatory stimuli that drive the modifications that lead to cartilage and bone damage.
Recent findings highlighted the relevant influence of gut-resident microbiome in the regulation of inflammatory process in the body. In the particular case of the OA, the scientific community has given importance to the direct axis connecting gut and joint. Several studies found a correlation between pro-inflammatory microbiome-derived metabolites and osteoarthritis in humans. Interestingly, lipopolysaccharide (LPS) is among the most active bacterial metabolite in evoke an immune-mediated inflammatory response in joints.
In this context, our work is intended to deeply explore the LPS induced damage at tissue and cellular level and its role in osteoarthritis.

IMPACT

Several *in vitro* studies have been conducted on chondrocytes cells culture to clarify the correlation between LPS and osteoarthritis. However, this approaches lack in relevant information regarding the effect of LPS at macroscopic scale.
With this work we aim to develop an OA model inducing inflammation on healthy native osteochondral explants to observe the effect of a pro-inflammatory stimuli at cellular and tissue level. Remarkably, our model realistically reproduces at the micro and macro scale the physiological joint environment, configuring as an advanced drug screening platform. Such technology opens the way to improve the drug screening process, promoting the discovery of new potential pharmacological therapies toward OA.

RESULTS ACHIEVED IN 2020

We successfully developed a responsive *ex vivo* model of osteoarthritis based on healthy porcine osteochondral (OC) plugs harvested from non-loading area of the femoral trochlea. As our aim was to study the influence of gut microbioma on the onset of osteoarthritis, we used LPS to induce an OA-like inflammatory response in osteochondral plugs.
After 3 days of LPS exposure, the cartilage of OC plugs showed characteristic osteoarthritis modifications that are commonly noticed in native OA tissues.
We found a loss in proteoglycans content and a massive cartilage fibrillation among the most evident modifications. Notably, we obtained a fully reversion of the LPS-induced modifications after supplementing an anti-inflammatory drug to the damaged tissues.
These results suggest that our *ex vivo* OA model closely mimics the dynamics occurring in joints during an inflammation triggered by LPS. Moreover, used a drug screening platform our model may contribute to the development of an innovative pharmacological treatment toward OA.

GOALS FOR 2021

Our responsive *ex vivo* OA model is a promising system that can give a relevant contribution in the development of a therapy towards the treatment of OA. In our future studies, we plan to develop an even more realistic model using human tissues to screen new anti-OA drugs. Moreover, we aim to use our model as a pre-clinical platform to test an advanced cell-free approach exploiting the immunomodulatory properties of stem cells.

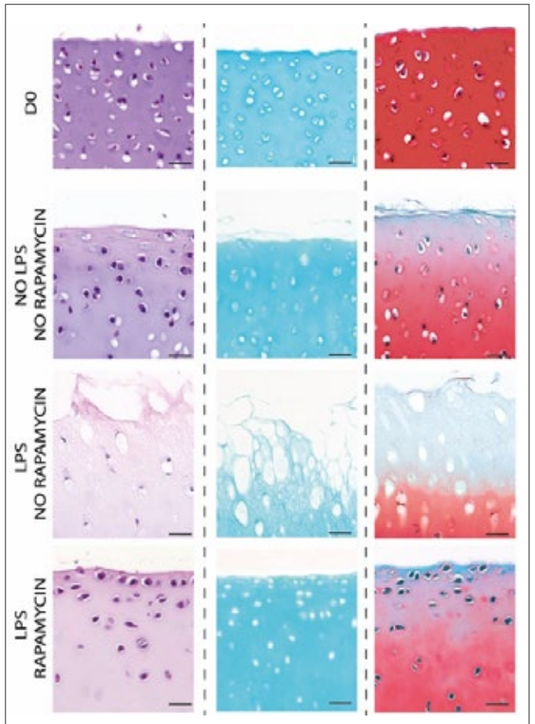

Figure 1: Histological sections of cartilage treated using our *ex vivo* OA model. Tissues subjected to an LPS-induced damage show a loss in proteoglycans and a surface fibrillation (LPS/NO RAPAMYCIN). The treatment with an anti-inflammatory drug repaired the tissue damage and reestablished a native-like morphology (LPS/RAPAMYCIN). Not-treated tissue (NO LPS/NO RAPAMYCIN) were not affected from our culturing platform and were similar to native tissues (D0).

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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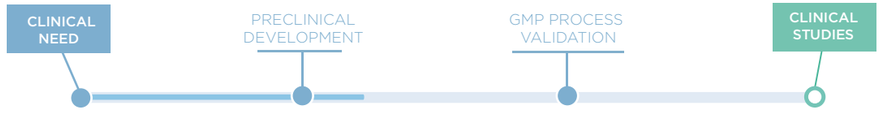
THERAPEUTIC AREA

Organ insufficiences

PIPELINE

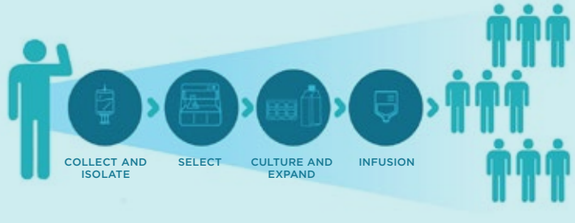


BRIEF DESCRIPTION

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 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 , which can be used both as a prophylaxis and as cure of virus-induced pathological manifestations which could be lethal for the patient.

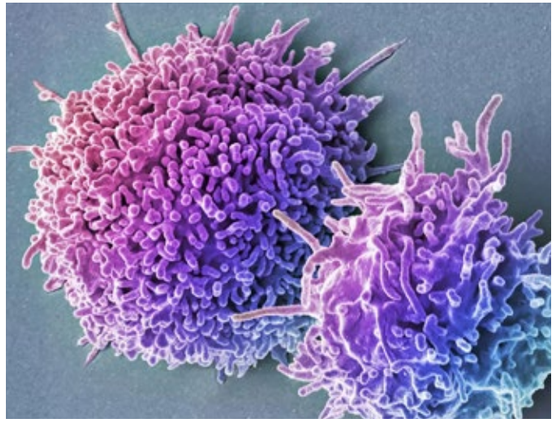


RESULTS ACHIEVED IN 2020

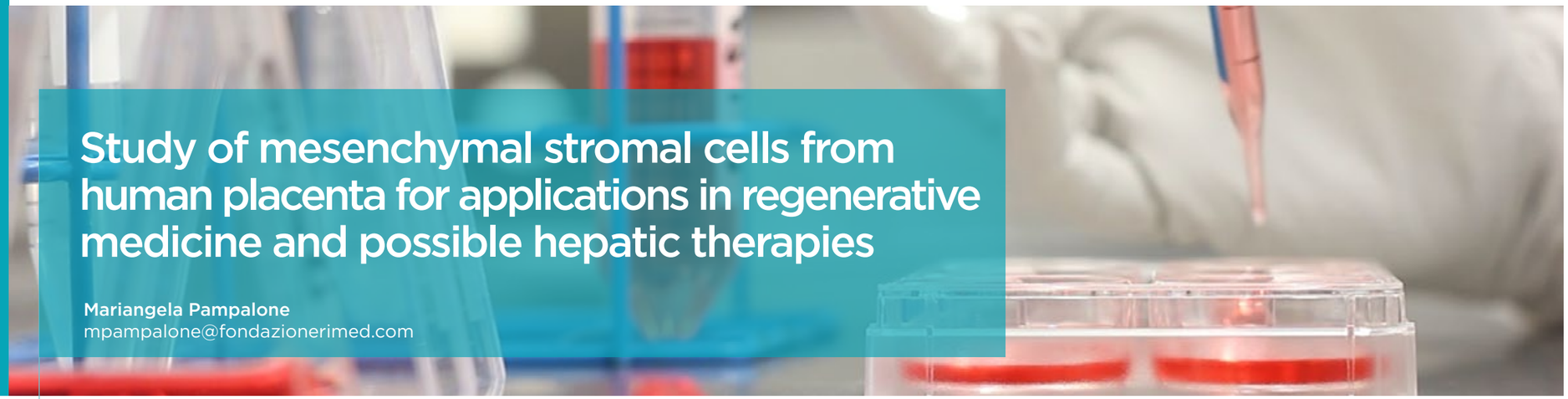
After optimizing the virus specific T cells production protocol (2019), during 2020 we focused on the use of new large-scale clinical grade culture devices, necessary to obtain a sufficient number of T lymphocytes to treat patients. This technology may allow to achieve a higher yield of the final cell product with few passages. In particular, we analyzed and compared cells grown in traditional multiwell plates versus cells grown into innovative systems such as G-Rex®6M Well Plate and G-Rex®10. Biological parameters, such as glucose and lactate dehydrogenase concentrations in the culture medium (used as cell proliferation indicators) were also evaluated at different time points as an alternative to the classic cell count.
This approach would allow the optimization of the timing of culture medium replacement and integration of growth factors / cytokines. The values of these parameters were correlated with cell growth in each tested culture system. As part of HHV-8 specific T lymphocytes project, we analyzed at different time points T cell response of patients with viremia/clinical manifestations such as KICS (KSHV inflammatory cytokine syndrome) following HHV-8 infections, after stimulation with mixed viral peptide pools.

GOALS FOR 2021

Once the new manufacturing labs are ready and after completing facility validations, the primary focus for 2021 will be to begin the required validations related to of multivirus-specific T lymphocyte production in order to obtain the authorization for clinical use



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



Study of mesenchymal stromal cells from human placenta for applications in regenerative medicine and possible hepatic therapies

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COLLABORATIONS

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THERAPEUTIC AREA

Organ insufficiences

PIPELINE

CLINICAL NEED | DISEASES 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 non-specific 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).

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. The obtained results could be the basis to further investigate the therapeutic role of hA-MSCs in bacterial clearance and macrophage phagocytosis as well as their cross talk with immune cells in SBP.

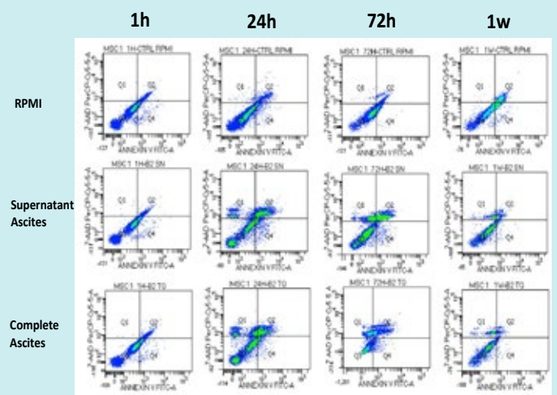

Fig.1: Representative panel of FC analyses showing how hA-MSCs were able to respond to AF exposure reducing apoptosis and necrosis at 1w suggesting a resistance to the stress (revealed at 24 and 72h), also compared with cells grown in RPMI

RESULTS ACHIEVED IN 2020

- Study to evaluate the effect of ascitic fluid from cirrhotic patients (Child-Turcotte-Pugh B) undergoing paracentesis on 3 different lots of amniotic mesenchymal cells (hA-MSCs) at different times of *in vitro* culture. The results obtained showed that the cells in contact with the ascitic fluid do not show morphological variations, inhibitions to proliferation, phenotypic variations or significant necrosis / apoptosis values.
- Study of conditioned media for the evaluation of pro and anti-inflammatory cytokines released and subsequent study of the macrophage component isolated from the ascitic fluid in order to evaluate the variation of the M1 or M2-like state following co-culture with hA-MSCs.

We found that hA-MSCs viability is not affected by ascitic fluid and, interestingly, hA-MSCs diminished the pro-inflammatory cytokine production, and promoted anti-inflammatory M2 macrophage polarization. Moreover, we found that there was no simultaneous significant decrease in the M1-like component, allowing a continual phagocytosis activity of macrophages and NK cells to restore a physiological condition. These data highlight the plasticity of hA-MSCs' immunomodulatory capacity, and pave the way to further understanding their role in conditions such as spontaneous bacterial peritonitis.

GOALS FOR 2021

Evaluation of the M1 / M2-like macrophage component and of the bacterial load of ascitic fluid with different degrees of bacterial infection following co-culture with placental mesenchymal stromal cells.

PUBLICATIONS

- *Human Amnion-derived Mesenchymal Stromal Cells in Cirrhotic Patients with Refractory Ascites: A Possible Anti-Inflammatory Therapy for Preventing Spontaneous Bacterial Peritonitis*
Mariangela Pampalone, Simona Corrao, Giandomenico Amico, Giampiero Vitale, Rossella Alduino, Pier Giulio Conaldi, Giada Pietrosi. Stem Cell Rev and Rep. https://doi.org/10.1007/s12015-020-10104-8
- *Human amniotic stem cells improve hepatic microvascular dysfunction and portal hypertension in cirrhotic rats*
Giada Pietrosi, Anabel Fernández-Iglesias, **Mariangela Pampalone**, Martí Ortega-Ribera Juan J. Lozano Héctor García-Calderó Laia Abad-ordà Pier G. Conaldi Ornella Parolini Giovanni Vizzini Angelo Luca Jaime Bosch Jordi Gracia-Sancho. Liver International https://doi.org/10.1111/liv.14610
- *The Immunomodulatory Properties of the Human Amnion-Derived Mesenchymal Stromal/Stem Cells Are Induced by INF--Produced by Activated Lymphomonocytes and Are Mediated by Cell-To-Cell Contact and Soluble Factors*
Matteo Bulati, Vitale Miceli, Alessia Gallo, Giandomenico Amico, Claudia Carcione, **Mariangela Pampalone** and Pier Giulio Conaldi. Front Immunol. 11: 54 doi: 10.3389/fimmu.2020.00054

PRODUCTS: **DRUGS - BIOLOGICS**

iRhom2: a new therapeutic target in osteoarthritis

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- Pharmacy Department, University of Pisa, Pisa, Italy
- German Center for Neurodegenerative Diseases (DZNE), Munich, Germany.

THERAPEUTIC AREA

Aging diseases

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

BRIEF DESCRIPTION

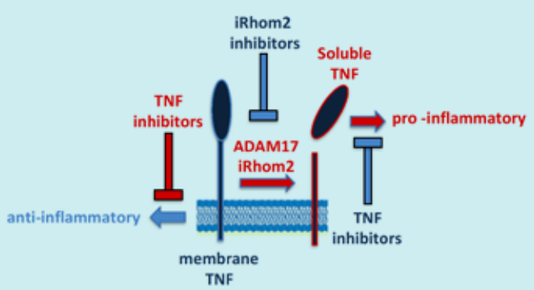
Osteoarthritis (OA) is a debilitating disease causing pain and stiffness. OA is characterized by breakdown of articular joint, due to the aberrant activity of MMPs and ADAMTSs. The endocytic receptor LRP1 controls turnover of these proteinases, thus its inactivation by ectodomain shedding contributes to development of the disease. In addition, the proinflammatory cytokine TNF plays a role in its progression by enhancing the expression of metalloproteinases. 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. 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 2020

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. The final results will be available before summer 2021. In addition to *in vivo* experiments, the team of Proteomics performed a number of experiments to characterize the turnover of specific proteases involved in the degradation of cartilage. These results have been published in renown international journals.



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 2021

2021 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.

MEETINGS

- Brainstorming in Metalloproteinases, Online Meeting, 21.10.2020
- British Society for Matrix Biology (BSMB) Autumn Meeting, University of East Anglia, Norwich, UK.
- Gordon Research Conference (GRC) in metalloproteinases , Il Ciocco Resort, Lucca,Italy (Poster).

PUBLICATIONS

- Carreca AP, Pravatà VM, Markham M, Bonelli S, Murphy G, Nagase H, Troeberg L, **Scilabra S.D**. TIMP-3 facilitates binding of target metalloproteinases to the endocytic receptor LRP-1 and promotes scavenging of MMP-1. Sci Rep. 2020 Jul 21;10(1):12067. doi: 10.1038/s41598-020-69008-9. PMID: 32694578; PMCID: PMC7374751.
- Yang, C.Y., L. Troeberg, and **S.D. Scilabra**, Quantitative Mass Spectrometry-Based Secretome Analysis as a Tool to Investigate Metalloprotease and TIMP Activity. Methods Mol Biol, 2020. 2043: p. 265-273.
- Interleukin 13 (IL-13)-regulated expression of the chondroprotective metalloproteinase ADAM15 is reduced in aging cartilage. C. Y. Yang, A. Chanalaris, S. Bonelli, O. McClurg, G. L. Hiles, A. L. Cates, J. Miotla Zarebska, T L Vincent, M L Day, S A Müller, S F Lichtenthaler, H Nagase, **S.D. Scilabra**, L Troeberg. Osteoarthr Cartil Open 2020 Vol. 2 Issue 4 Pages 100128
- Calligaris M, Cuffaro D, Bonelli S, Spanò DP, Rossello A, Nuti E, **Scilabra S.D**. Strategies to Target ADAM17 in Disease: From its Discovery to the iRhom Revolution. Molecules. 2021 Feb 10;26(4):944.

PRODUCTS: **DRUGS - BIOLOGICS**

iRhom2 regulates surface levels of MHC class I molecules and immune responses

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COLLABORATIONS

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THERAPEUTIC AREA

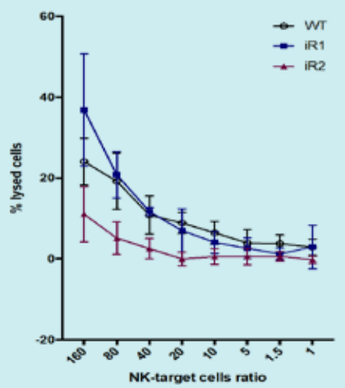
Oncology

PIPELINE

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

BRIEF DESCRIPTION

Immunotherapy has emerged as a promising treatment for cancer and a number of different approaches to boost the immune system to fight cancer cells is currently under development. Major histocompatibility complex (MHC) class I molecules are crucial in preventing tumor growth by presenting oncogene-derived antigens to cytotoxic limphocytes (CTLs) and natural killer cells (NKs). However, cancer cells have developed mechanisms to lower levels of MHC class I molecules in order to escape immunosurveillance.
iRhom1 and 2 essential regulators of the TNF alpha converting enzyme (ADAM17). We found that iRhom2 has an additional function to supporting ADAM17 maturation, which is regulating surface levels of MHC class I molecules (H2-D1 in mouse). Our preliminary results clearly display that ablation of iRhom2 in mouse embryo fibroblasts (MEFs) leads to decreased levels of H2D1 on the cell surface. Furthermore, iRhom2 KO cells were almost insensitive to NKs, indicating a clear functional consequence of iRhom ablation on NK activation. Interestingly, levels of H2D1 transcripts are similar in iRhom1 KO, iRhom2 KO and WT MEFs, suggesting that iRhoms regulate surface levels of H2D1 in a post-transcriptional manner. Understanding the mechanisms underlying the regulation of MHC class I molecules by iRhoms, and their potential roles in cancer progression is the central aim of the project.



Genetic deletion of iRhom2 in target cells decreases their natural killers-mediated lysis.
WT, iRhom1 KO or iRhom2 KO mouse embryo fibroblasts were radiolabeled with 51Chromium and incubated with serial dilutions of natural killers (NKs, 160-1:1). NKs-mediated cell lysis was measured by 51Chromium release.

IMPACT

iRhoms were first described as essential regulators of ADAM17, a protease with a key role in immunity and other biological processes. Interestingly, a number of ADAM17-independent activities of iRhoms are emerging, indicating that our understanding of these proteins is still at its infancy and that their biology is far more complex than what it was previously believed.
Our results show a strong link between iRhoms and MHC class I molecules, which are crucial proteins in immune responses in that they present peptide antigens to immune cells. Regulation of MHC class I molecules by iRhoms is ADAM17-independent. We hypothesize that iRhoms can control surface levels of MHC I molecules in a similar manner as they control ADAM17 maturation. It is clear that this research may contribute to enlarge our knowledge about iRhoms and their functions in immunity. In addition, this research may have a great translational potential, leading to novel immunotherapeutic approaches for those cancers that have not seen clear clinical benefits and treatments for rejection in organ transplantation.

RESULTS ACHIEVED IN 2020

Although this project has just started, several key accomplishments have been already obtained. In addition to preliminary results that are described in the previous paragraph, we set up a number of collaborations and measure for successfully carrying out the project. First, we completed the MTA agreement with the University Health Network (UHN, Toronto, Canada) for the use of the iRhom2 knockout transgenic mouse which will allow us to investigate the role of iRhom *in vivo* models of xenograft transplantation. This murine model will allow us to isolate iRhom2 knockout immune cells that are necessary to carry out the study and accomplish all goals of this project. In addition, we set up collaborations with Carl Blobel at the Weill Cornell University of New York and Stefan Lichtenthaler at the DZNE Munich, who provided materials and reagents that are necessary for the project, including iRhom knockout cells, constructs and antibodies targeting iRhoms for immunoblotting analysis.

GOALS FOR 2021

The major goal for 2020 is understanding how mechanistically iRhoms regulate surface levels of MHC class I molecules. To do so we aim to ablate the expression of iRhom1 and 2 in selected cancer cells by using CRISPR-Cas technology and analyze trafficking of H2D1 in these cancer cells. Then, we aim to investigate functional consequences of iRhom1 or 2 ablation on CTL and NK activation. CTLs or NKs will be co-cultured with cancer cells where iRhom1 or 2 are ablated, and their activation evaluated by using standard assays (including "induction of ovoalbumin-specific CTLs" and "51Cr release assay for NK citoxicity"). In order to investigate at an omic level effects of iRhom1 or 2 KO cancer cells on CTLs and NKs, we plan to establish a proteomic procedure that enables secretome and cell surface proteome analysis of CTLs or NKs, 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.

PUBLICATIONS

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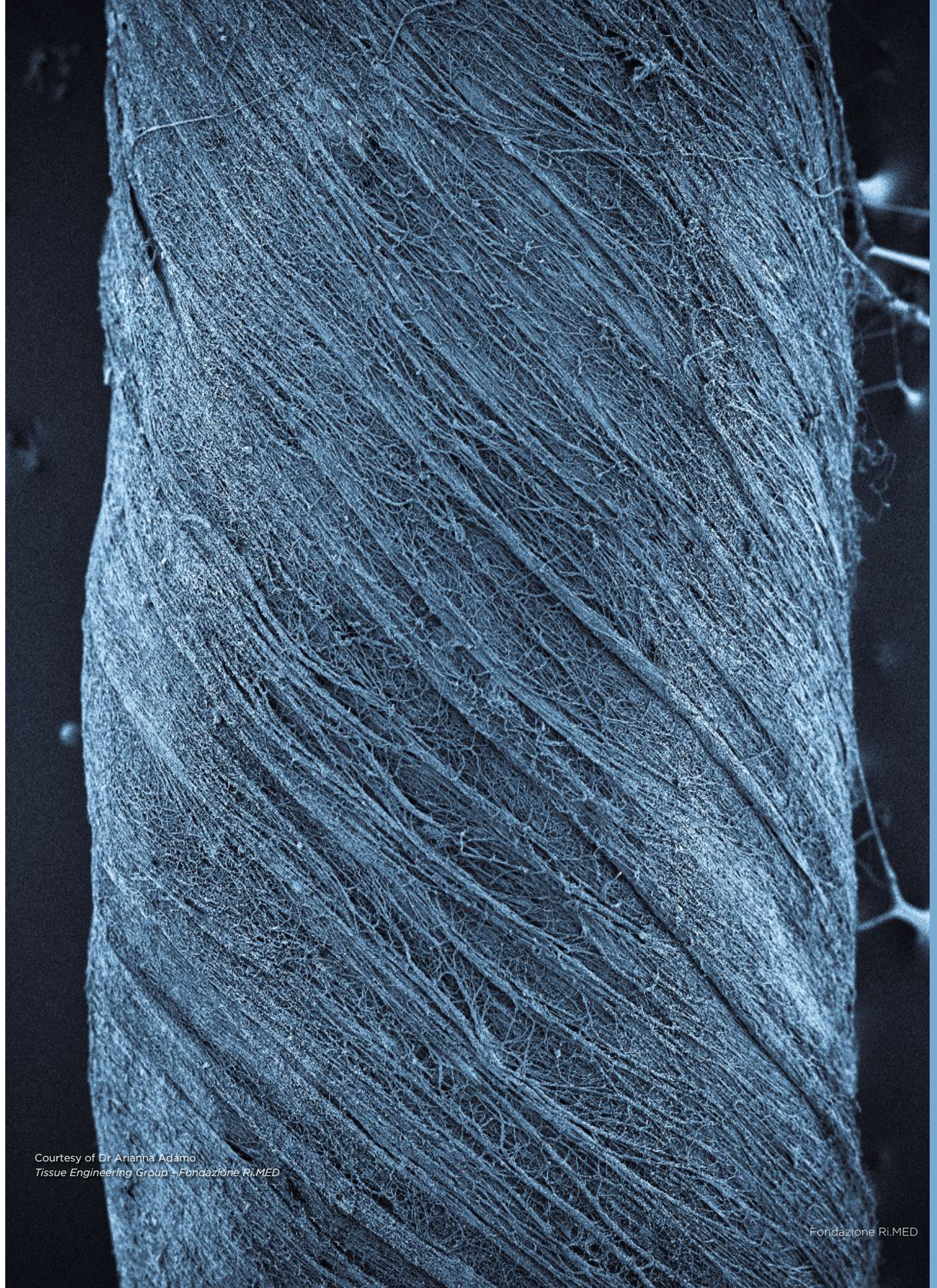
MEDICAL DEVICES & TISSUE ENGINEERING

The researchers of the Ri.MED Foundation, in close collaboration with clinical partners, are engaged in the simulation of physiological systems, studying biomaterials and engineered tissues, and developing new therapeutic solutions. Our Bioengineering and Tissue Engineering platforms are equipped with facilities enabling the synthesis and characterization of biomaterials and scaffolds for tissue engineering applications, and the design, development and preclinical validation of novel medical devices.

In 2020, the "BIOMITRAL" project by Antonio D'Amore, group leader in Tissue Engineering at Ri.MED Foundation, was awarded the ERC Consolidator Grant of the European Commission , and the Foundation was elected as host institution for the development of project that will be launched in 2021. New approaches are being studied for the characterization of biological tissues and biomaterials, and scaffold fabrication.

Important developments were accomplished in preclinical evaluation, in vitro and in vivo, of innovative cardiovascular solutions including engineered heart patches and new heart valves made using biostable polymers, engineered tissues and genetic engineering applications.
The possibility of in-house development and validation of clinical solutions, and the collaborations with the clinical centers, will facilitate the introduction of these new treatments in clinic.



Courtesy of Dr Arianna Adamo
Tissue Engineering Group - Fondazione Ri.MED

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

Development of a novel transcatheter heart valve
Gaetano Burriesci, PhD

Development of a novel Alfa-Gal Free Xenograft heart valve
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Analysis of the left atrial appendage to predict the risk of thrombosis
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Smoothed Particle Hydrodynamics computational analysis for cardiovascular bioengineering applications
Gaetano Burriesci, PhD

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

R-CaRe - Regenerative cartilage reheabilitation
Riccardo Gottardi, PhD

Development of an engineered hyperelastic scaffold for tendon enthesis regeneration
Riccardo Gottardi, PhD

PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**

Development of nontoxic bio-adhesives for wet environments

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COLLABORATIONS

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- Institute of Biophysics (IBF) – Consiglio Nazionale delle Ricerche (CNR), Palermo, Italy

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

BRIEF DESCRIPTION

Marine sessile animals have developed adaptive strategies to overcome obstacles that inhibit their adhesion to marine surfaces in water (pH, hydration layers and dielectric properties). This made these animals great potential sources of bio-compatible adhesives applicable in surgery, regenerative medicine, and tissue engineering. Among all sea organisms able to secrete adhesive products, mussels have received significant attention especially because of their ability to adhere tightly to their substrates also in turbulent tidal conditions. Mussel adhesion is possible through the secretion of a protein-based holdfast (byssus), chemically composed of proteins which mature in the mussel foot (mussel foot proteins, Mfps). Many efforts have been done to create bio-adhesive polymers that could mimic Mpfs, but with no success so far. More studies are needed to fulfil the lack of a complete understanding of the mechanism that presides over the adhesion in Mfps. In particular, there are not structural information on any Mfp and thus a complete understanding of the structure/function relationship for these proteins is missing. We aim at characterizing the three-dimensional structure of Mfps thus to identify key residues involved in the adhesion process and use this information to derive bio-compatible engineered proteins to be used as bioadhesives in surgery, regenerative medicine and tissue engineering 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. The big challenge in developing new bio-adhesive molecules is to find molecules able to work in wet and hostile environment and capable of making tissues adhere together in an efficient way in those conditions. Proteins bio-inspired from sessile animals with adhesive properties in water, could overcome these difficulties. They also have the attractive properties of being biodegradable, usually nontoxic to the human body and do not easily elicit strong immune response.

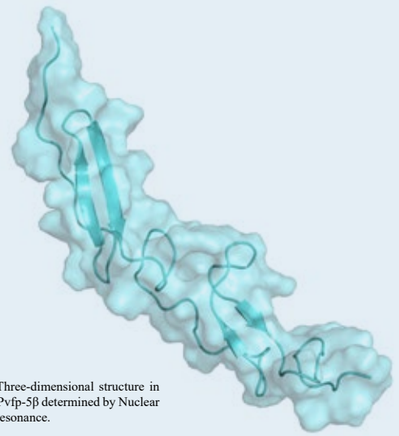

Figure 1: Three-dimensional structure in solution of Pvfp-5β determined by Nuclear Magnetic Resonance.

RESULTS ACHIEVED IN 2020

Recent studies showed that, among the three mfps recently identified in the Asian green mussel Perna viridis foot, Perna viridis foot protein type 5β (Pvfp-5β) is secreted first and it was then hypothesized that this protein is the first protein to initiate adhesion with the marine substrate. In the recent years we performed the first implementation of the successful production of recombinant Pvfp-5β and this allowed us to finalize this year the first structure of a Mfp and investigate its dynamic proprieties in solution by nuclear magnetic resonance (Fig. 1). Pvfp5-β structure determined by NMR, is consistent with our previous mass spectrometry data and also confirms the high homology with two tandem EGF motifs. The structure shows that the protein is an elongated monomer mainly dominated of random coil, and includes two coupled antiparallel β-sheets held together by the presence of five disulfide bridges. Despite the abundance of randomly coiled segments, the data suggest that PVFP-5β is well ordered and rigid as confirmed by relaxation NMR data (T1, T2, and HetNOE) that show fluctuations in the order of pico-nano- seconds. Key amino acid residues such as Tyr, Lys, and Arg, result well exposed at the protein surface so ready for contact with other surfaces. This represents the first structural insight to rationalize Mfps adhesive proprieties.

GOALS FOR 2021

Future studies want to address a detailed comparison of the properties of recombinant Pvfp-5β and those of the native and post-translationally DOPA modified protein. We then aim at implementing the enzymatic reaction to produce Dopa-modified Pvfp-5β and sequentially at determining its three-dimensional structure by nuclear magnetic resonance. Biodegradability and mechanical properties of the two Pvfp-5β forms will be also tested.

PUBLICATIONS

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- Puglisi R., Brylski O., **Alfano C.**, Martin S.R., Pastore A., Temussi P. (2020) Quantifying the thermodynamics of protein unfolding using 2D NMR spectroscopy. Comm Chemistry, doi: 10.1038/s42004-020-00358-1.
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PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**

Development of a novel transcatheter heart valve

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COLLABORATIONS

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THERAPEUTIC AREA

- Organ insufficiences
- Aging diseases

PIPELINE

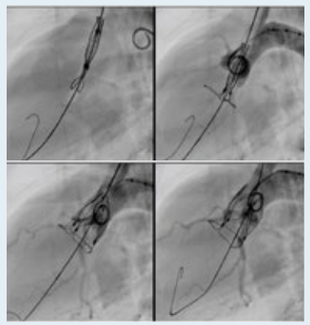
CLINICAL NEED → DISEASES ANALYSIS → DISCOVERY → PRECLINICAL VALIDATION → PRECLINICAL DEVELOPMENT → CLINICAL STUDIES

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 and pre-clinical assessment of a novel prosthetic aortic valve suitable for TAVI implantation, which would overcome the main limitations experienced with currently available solutions.

Picture of the TRISKELE transcatheter heart valve system and of its surgical version.



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, 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.

RESULTS ACHIEVED IN 2020

A new surgical version of the TRISKELE was designed, manufactured and successfully implanted in two sheep, reaching the target 90 days survival with both animals. Transvalvular pressure gradients, blood velocity and effective orifice areas remained good and stable for both study animals, after implantation until sacrifice. For the whole in-life period of both animals, there was no sign of turbulent flow, no alteration of the leaflet motion and very limited or undetectable prosthetic regurgitation. The local tolerance of the valve material was excellent as no inflammatory cells were observed on the leaflets.

There were no mineral deposits in any of the examined sections stained with HE&S and Alizarin Red, and no bacteria in any of the examined sections. The plasma free haemoglobin values kept within the reference range at each time point of the measurement. Hence, the valve has confirmed its potential to provide a new tool allowing improved safety, procedural simplification and reduced costs.

In 2020, Ri.MED Foundation was reassigned the intellectual property for the delivery system of the device (whose patent is granted both in Europe and USA) and its mitral version, gaining full freedom to operate for future developments.

GOALS FOR 2021

The next stage of this project will involve establishing partnership with cardiovascular companies and/or ventures capital firms, complete the development of the device and facilitate the route to market. In particular, the synthesis of the leaflets material will be scaled up and industrialised, requiring the testing and verification of its properties. In parallel, implementation of a valve manufacturing facility will be initiated.

The delivery system will need to be redesigned to enable industrial manufacturing, and the in-animal evaluation will be planned. Partnering with cardiovascular companies will also help addressing non-clinical aspects (shelf life, packaging, accessories etc.), compile the final design dossiers, and organising the clinical investigation to complete successfully the translation of the device to the clinic.

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PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**

Development of a novel Alfa-Gal Free Xenograft heart valve

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COLLABORATIONS

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- University of Alabama, Birmingham, USA

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

BRIEF DESCRIPTION

Bioprosthetic heart valves fail because they build up calcium deposits which weaken the valve, leading to tears, or obstruct blood flow because they impair the opening of the valve. Scientists and commercial valve companies have long sought to produce bioprosthetic heart valves which do not calcify, because these could be used in younger patients without the need for blood thinners. However, the calcification blocking treatments which have been developed so far have not been successful in younger adults. Our partners at UCL and UAB have identified an immune driven inflammation which accelerates calcification of biological heart valve materials. This inflammation is unique to humans because a portion of our immune system reacts with a substance called Gal, not made by humans but common in animals (including pigs and cows) and present on the bioprosthetic tissue.
To block this immune inflammation, our partners have genetically altered pigs, so they no longer make Gal.
Now, we are using the pericardial tissue from this new class of animals to develop a bioprosthetic heart valve which resists calcification, broadening the patient population suitable for bioprosthetic valves and improving the quality of life of recipients who receive this improved therapy.

IMPACT

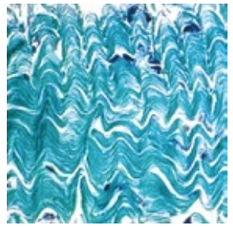
Approximately 300,000 valve replacements are performed annually worldwide. Two types of replacement valves are available, mechanical heart valves which require lifetime anticoagulation and bioprosthetic heart valves (BHVs) made from biological tissues, typically human or porcine heart valve leaflets, or animal pericardium. BHVs are preferred in older patients (> 65 years), where they are more durable. Younger patients generally receive MHVs due to rapid age-dependent BHV degeneration. In patents under 35 years of age, up to 100% structural valve deterioration occurs within 5 years. More durable BHVs would advance the standard of care by eliminating the need for anticoagulation in younger recipients and extending access to this therapy to more patients.

RESULTS ACHIEVED IN 2020

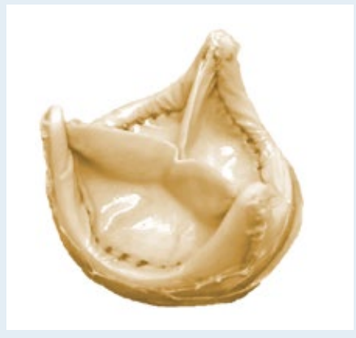
Five Gal-knockout (GalKO) and five standard bioprostheses made from porcine pericardium were implanted in juvenile sheep for 90 days. Echocardiography at 30-day intervals established valvular haemodynamic performance. After explantation, valves were examined macroscopically and microscopically for pannus, vegetation, inflammation, thrombus formation, and calcification. Calcification was further analysed by x-ray and quantitative atomic spectroscopy. There was no difference between study groups for echocardiographic data taken, with all animals exhibiting excellent haemodynamic performance and no adverse valve-related events. Examination of explanted prostheses identified mild pannus integration, minimal thrombus, and minimal leaflet deposits, with no difference between the groups. Neither group showed evidence of infection, vegetation, tissue inflammation, or structural valve degeneration. This implies that the mutation in GalKO pigs has no intrinsic detrimental biological impact, indicating GalKO pericardium would be suitable for use in clinical bioprosthetic heart valves.

GOALS FOR 2021

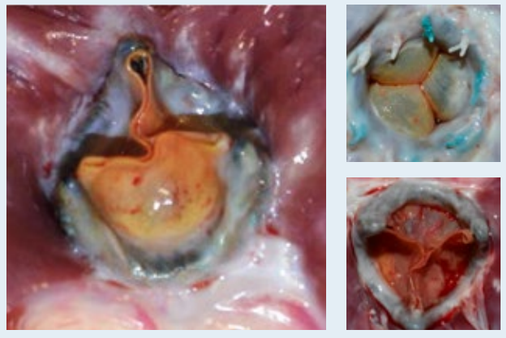
We will examine the possibility of using tissues extracted from other genetically modified animal species, such as bovine pericardium, that offers a longer and more established clinical experience compared to porcine pericardium. Also, we will verify the possibility of moving forward to preclinical stage with the currently developed solution.



Microstructure of the α1,3-galactosyltransferase gene-knockout porcine pericardium.

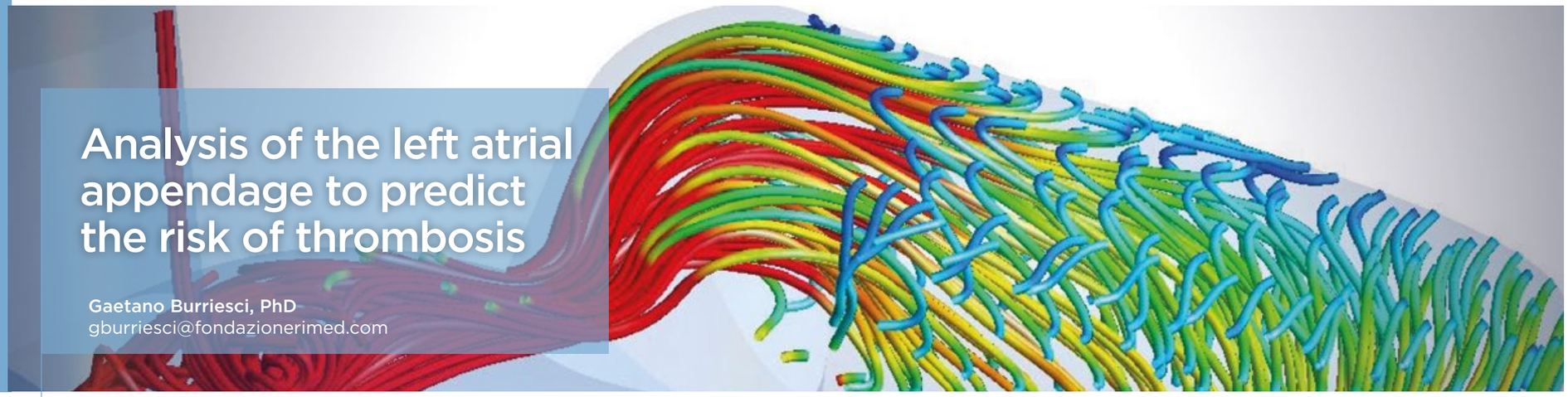


Prototype of the valve made from transgenic porcine pericardium.



Ovino Valve prototype after 90 days of implant in the mitral position of an ovine model.

PRODUCTS: **BIOMARKERS - MEDICAL DEVICES & TISSUE ENGINEERING**



Analysis of the left atrial appendage to predict the risk of thrombosis

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COLLABORATIONS

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- University of Palermo, Palermo, Italy

THERAPEUTIC AREA

- Organ insufficiences
- Aging diseases

PIPELINE

CLINICAL NEED → DISEASES ANALYSIS → DISCOVERY → PRECLINICAL VALIDATION → PRECLINICAL DEVELOPMENT → CLINICAL STUDIES

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, models of LA and 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

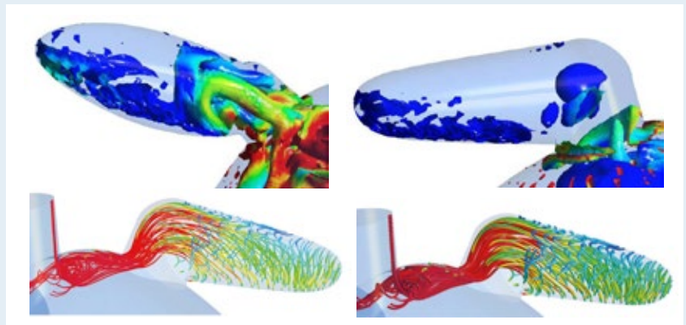
The currently available literature mostly focuses on the analysis of the role of the appendage morphology (this is highly variable from patient to patient) on thrombus formation, relying on rigid-wall models which neglect the changes in wall contractility produced by fibrillation. In this study, instead, 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.

RESULTS ACHIEVED IN 2020

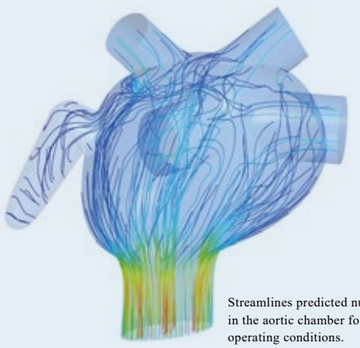
Two idealised models of the left atrium with a common LAA morphology were generated to describe healthy and AF altered shapes. Motions of the atrial and LAA walls were applied to simulate sinus and fibrillation contractility. The different scenarios were analysed and compared in terms of shear strain rate and vorticity produced in the different regions of the LAA.
The study clearly indicates that the contractile alterations associated with AF pathologies play a primary role in establishing haemodynamic conditions which promote higher incidence of ischemic events, consistently with the clinical evidence.
This factor, commonly neglected in the numerical studies of the LAA presented in the literature, needs to be simulated to model realistic flow. The enlargement of the cardiac chambers that progressively occurs in AF patients, can also have some worsening effect, confirming that the large morphological variations characterising the LAA in different patients may play a role.

GOALS FOR 2021

The described model is based on a number of assumptions, such as the laminar Newtonian description of blood and the use of a simplified anatomy. The contribution of patient specific anatomies will be investigated, enforcing a more physiological approach to simulate the wall dynamics, based on muscle contraction rather than on enforced displacements. For this purpose, the geometry of the model will be modified by integrating more complex patient specific morphological parameters and contractile activation of the appendage wall.

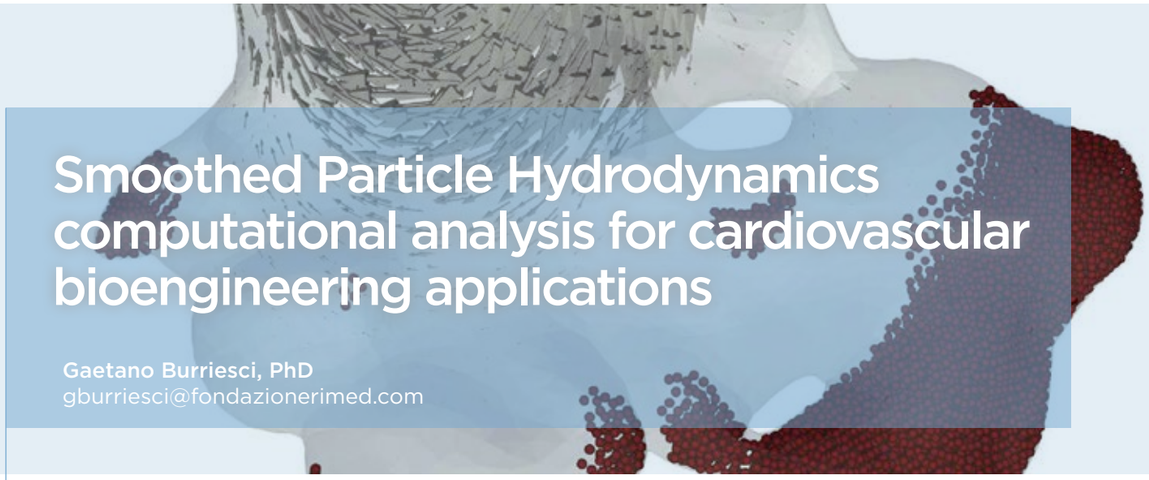


Streamlines predicted numerically in the atrial appendage for healthy operating conditions.



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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THERAPEUTIC AREA

- Organ insufficiences
- Aging diseases

PIPELINE

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

BRIEF DESCRIPTION

Thrombosis is a pathology leading to thrombus 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 mechanism is 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 far 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 a Smoothed Particle Hydrodynamics (SPH) numerical method coupled with a fluid structure interaction (FSI) model. Contrary to standard 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, FSI coupling allows to study platelets aggregation by means of internal particle attractive forces. Concentration of the biochemical species involved in the process can be modelled by advection-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 2020

In 2020, several new modules were implemented and integrated into the existing open-source SPH code Panormus (developed at the University of Palermo). 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 an FSI approach.

A rheological non-Newtonian model for blood, based on Casson's description, was introduced, thus initiating thrombus formation modelling. In particular, the increase in viscosity is taken as a key factor responsible for the hemodynamic changes leading to clot formation. .

GOALS FOR 2021

Next steps of the research project will involve the adjustment of the modules recently implemented in the SPH code for the modelling of the blood coagulation process. The FSI approach, already tested and validated, will be integrated to simulate particles adhesion. Moreover, the ability to model the biochemical reactions for the different species involved in the process will be introduced, by incorporating appropriate diffusion-advection equations. Subsequently, the effect of anticoagulant agents will be included in the code. These reactions will then be linked to the evolution of the attraction forces acting on the solid particles, so as to evaluate their transformation over time. Finally, the model will be validated against reference cases available in the literature and applied to describe complex real conditions such as heart valve disfunctions or arterial stenoses.

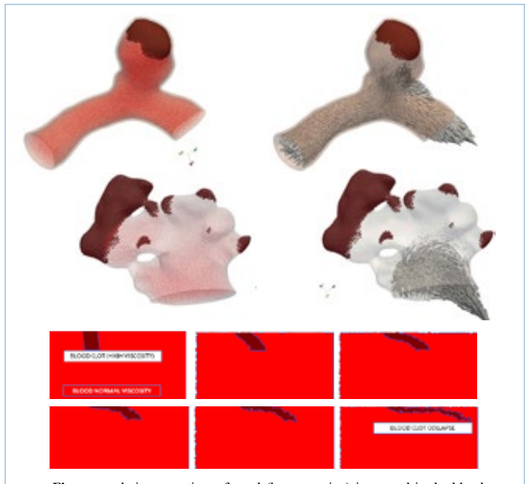
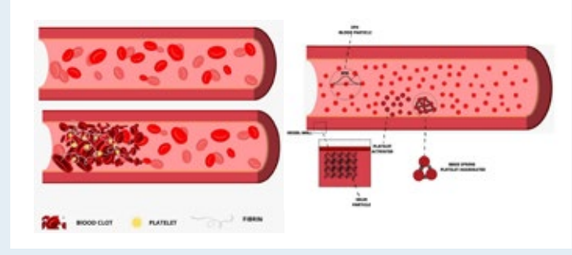


Figure: evolution over time of a gel (brown region) immersed in the blood stream (red region). The gel, which represents the first stage of thrombus formation, was modelled as a fluid with highly increased viscosity..



PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**

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

Gaetano Burriesci, PhD
gburriesci@fondazionerimed.com

COLLABORATIONS

- University of Palermo, Palermo, Italy
- University College London (UCL), London, United Kingdom

THERAPEUTIC AREA

- Organ insufficiences
- Aging diseases

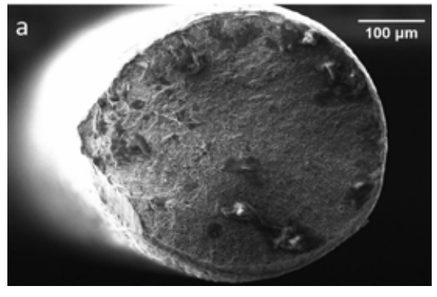
PIPELINE

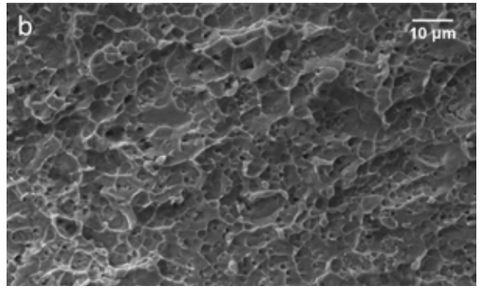
CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

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 project we exploit these 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).





SEM image of the fracture surface of a nitinol wire, in proximity of a micro laser-welded 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

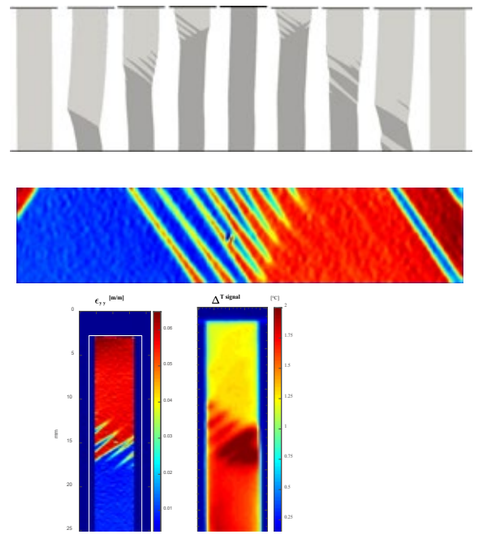
A more accurate understanding and characterisation of nitinol's behavior would contribute to increase the safety of medical devices based on this material. The combined implementation of Digital Image Correlation and Infrared Thermography optical techniques, adopted in this project, can support the evaluation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

RESULTS ACHIEVED IN 2020

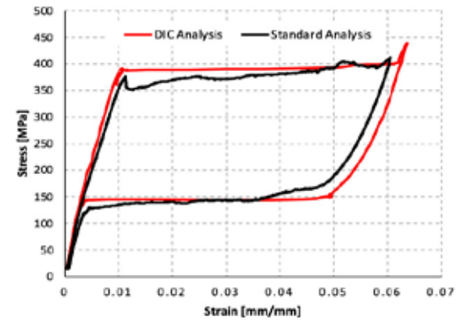
The main sources of error affecting the reliability of standard uniaxial tension tests for the mechanical characterisation of nitinol material were identified and analysed. In particular, the stress induced transformation that provides the super-elastic behaviour also causes the presence of regional inhomogeneities in the test specimen, associated with the formation of Lüders bands, lateral displacements and spurious bending moments superposed to the tensile load. These phenomena limit the reliability of standard characterisation methods to provide parameters sufficiently accurate to achieve an adequate description of the material and exploit its features optimally. DIC analysis, by allowing a full field detection of the strains, can help to overcome these limitations.
The implemented approach provides a substantially improved characterisation of the material, much needed to support the selection of specific alloys and thermomechanical treatments, and to achieve optimised levels of safety and efficacy in the most critical applications where nitinol is employed.

GOALS FOR 2021

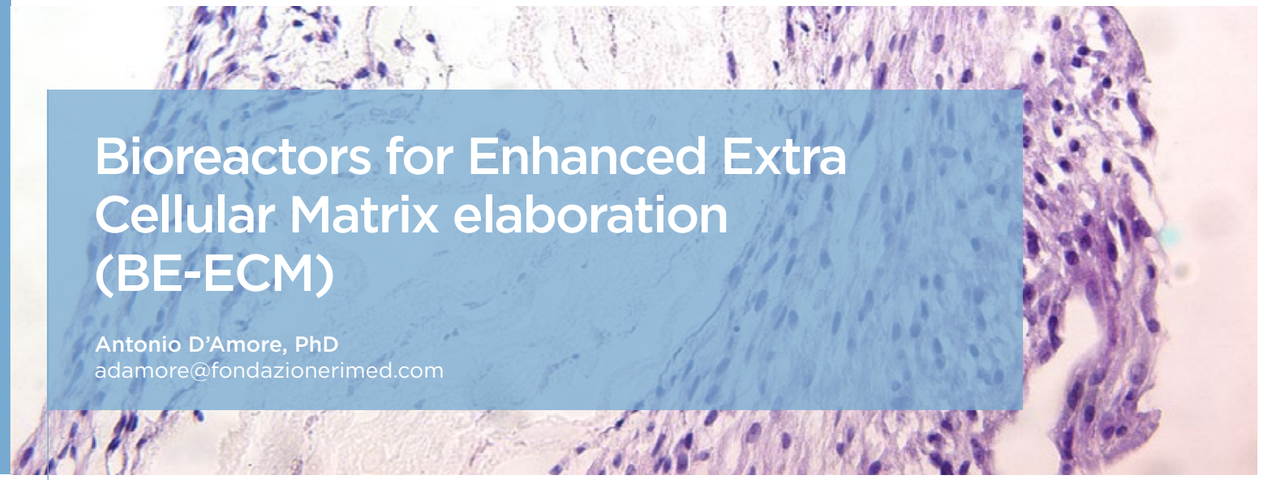
In order to enhance the developed methodology, thermoelastic Stress Analysis (TSA), commonly used to correlate the temperature change to the stress change in convention material, will be integrated in the analysis, examining the phase signal of the thermo-elastic effect. Then, the implemented approach will be used to perform the thermo-mechanical characterisation of complex medical devices, obtaining the full-field stress map from temperature and deformation maps. This will allow the identification of the regions subjected to higher mechanical stress, providing a much-needed tool for the experimental analysis of implantable device prototypes and their optimisation.



Deformation (left) and reversible temperature variation (right) maps acquired from a nitinol specimen subjected to cyclic tensile load.



PRODUCTS: **ATMP - MEDICAL DEVICES & TISSUE ENGINEERING**



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

Antonio D'Amore, PhD
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COLLABORATIONS

- **Politecnico di Milano, Milan, Italy**
- **University of Pittsburgh, Pittsburgh, USA**
- **Universidad de Zaragoza, Zaragoza, Spain**

THERAPEUTIC AREA

- **Organ insufficiences**
- **Aging diseases**
- **Oncology**

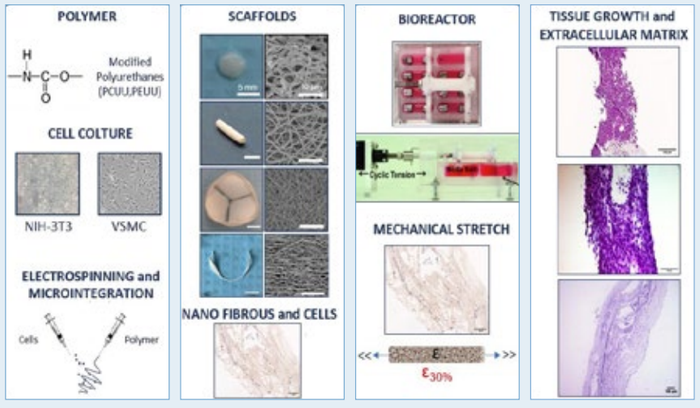
PIPELINE

CLINICAL NEED → DISEASES ANALYSIS → DISCOVERY → PRECLINICAL VALIDATION → PRECLINICAL DEVELOPMENT → CLINICAL STUDIES

BRIEF DESCRIPTION

Main topic: in vitro elastomeric models to investigate soft tissue mechanobiology.

Three macro-areas are recognized as crucial to understand the factors that drive tissue repair and de novo tissue formation: 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 models able to correlate mass loss with mechanical loads and deformations. The BE-ECM research line, integrated by NET-IBA and NET-MTG, tries to address these three critical topics by introducing and perfecting physical, *in-vitro* models able to study tissue growth, cell behavior and biomaterials degradation.



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 microintegrated 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

Potential impact of this research might involve improved 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

This *in vitro* modeling ability might allow to expand the understanding of biomaterials mechanobiology and might allow to assess, using simplified tissue surrogates, the efficacy of novel tissue engineering strategies. Examples of these modeling efforts include: mechanisms to accelerate tissue growth, solutions to modulate material degradation characteristics, topological cues to dictate cell differentiation and lineage.

RESULTS ACHIEVED IN 2020

The in-silico platform developed by the PI and his collaborators starting in 2009 is based on elastomeric, fibrous polyurethane scaffolds combined with cells. 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 platform utilizes stretch bioreactors and biodegradable polyurethane (e.g. PEUU, PCUU, PECUU) micro-integrated with cells via electro-spray. The aim for the year 2020 was to address fundamental questions regarding mechano-transduction mechanism *in vivo* by utilizing simplified systems *in silico* able to stimulate ECM synthesis. The implementation of this concept allowed:

- to identify unreported mechanism for enhancing ECM formation given a specific macroscopic load, the notion is applicable 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.

Teaching activity

- Mentor for the Institute for Clinical Research Education (ICRE). Career development program training the next generation of clinical and translational scientists, Univ. of Pittsburgh. Trainee: Casey, Tompkins-Rhoades, 2020;
- Guest lecturer for the biomedical engineering master of science and PhD program, BIOENG 2810 – Biomaterials & biocompatibility. Department of bioengineering, University of Pittsburgh. Title: "A brief overview on polymers processing methods for soft tissue engineering";
- Guest lecturer for the biomedical engineering master of science and PhD program, MSCMP 3735 – Extracellular matrix in tissue biology and bioengineering. Department of bioengineering, University of Pittsburgh. Title: "Cardiac ECM: structure - function, damage mechanism, and tissue engineering approaches to facilitate constructive remodeling".

Mentoring activity

- A. Adamo, PhD candidate, University of Palermo Italy, engineering chordae tendineae, mechanical conditioning and mechano-biology.

Invited speech

- "Ri.MED cardiac tissue engineering program, an overview to foster joint proposal applications", Monzino Cardiology Center, Milan, December 1st 2020;
- "Advancing Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", school of engineering Polytechnic University of Turin, Turin, December 17th 2020.

GOALS FOR 2021

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, submit manuscripts as senior author, based on A. Adamo's PhD project;
- 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 mechano-signaling of cells seeded inside the scaffold;
- to assess a hybrid micro-molding electrodeposition platform for cell manipulation;
- ERC and NSF proposals submissions;
- R01 and R21 proposals submissions.

MEETINGS

- Y. Kawakami, K. Nonaka, N. Fukase, **A. D'Amore**, J. Cummins, R. Kuroda, W. Wagner, J. Huard. A New Cell-free Biodegradable Synthetic Artificial Ligament for The Reconstruction Of Anterior Cruciate Ligament (ACL) In A Rat Model. Orthopedic Research Society (ORC) 2020 Annual Meeting. February 8-11, 2020 Phoenix, Arizona.

PUBLICATIONS

- N. Kashiyama, R. Kormos, Y. Matsumura, **A. D'Amore**, S. Miyagawa, Y. Sawa, W. R. Wagner. An adipose derived stem cells sheet combined with an elastic synthetic patch incorporating cardiac ECM enhanced cell survival and preserved cardiac function in rats following subacute myocardial infarction. In press on J. of Thoracic and Cardiovascular Surgery, IF 4.46.
- Controlling in-plane mechanics of electrospun polyurethane scaffolds for cardiac tissue engineering applications. S. K. Luketich, G. Menallo, G. Nasello, M. Maneschi, F. Gulizzi, P. Livreri, W. R. Wagner, and **A D'Amore**. To be submitted to Journal of Mechanical Behavior of Biomedical Materials, IF: 3.23
- A. Adamo, J.Bartolacci, M. Traina, W. Wagner, S.F. Badylak, **A. D'Amore**. Bioprocessing, structure, mechanics and hevaluation of micro-fiber based biodegradable suture material. To be submitted to Biomaterials, 5Y-IF 8.97.
- A Cell-free Biodegradable Synthetic Artificial Ligament for the Reconstruction of Anterior Cruciate Ligament (ACL) in a Rat Model. 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. on Acta Biomaterialia, 5Y-IF 7.16.

PRODUCTS: **ATMP - MEDICAL DEVICES & TISSUE ENGINEERING**

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

Antonio D'Amore, PhD
adamore@fondazionerimed.com

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 | DISEASES 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 2020

- BioMitral project was founded by ERC Consolidator Grant. The numerical part of BioMitral project aims 1) to evaluate via finite element method (FEM) *in silico* mechanics and organ level function of stentless, engineered mitral valves with chordae tendineae and 2) to identify the most effective number and locations of chordae – valve junction points.
- 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.

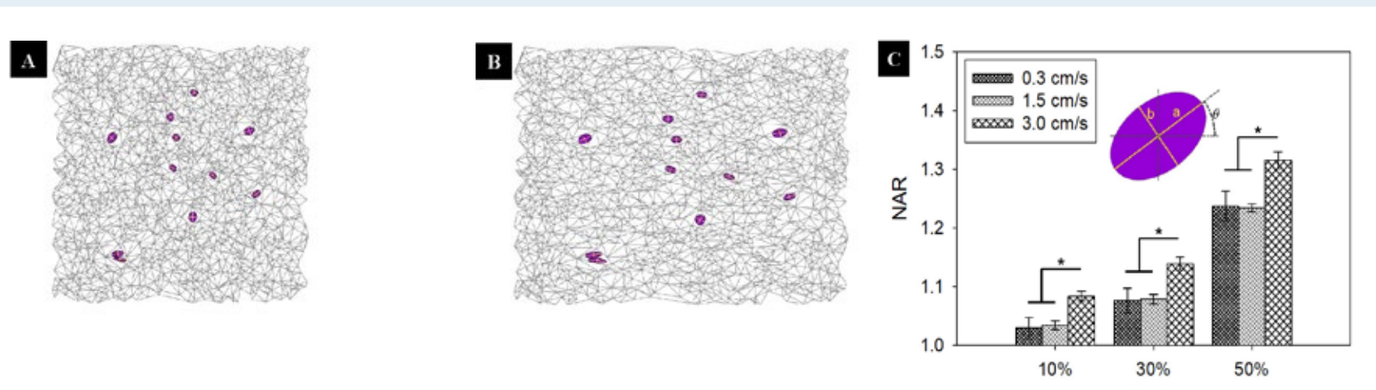
GOALS FOR 2021

Goals set for the 2021 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;
- development of numerical models to simulate *in vivo* scaffold degradation.
- development of numerical models to simulate influence of biomaterials topology on cellular migration.

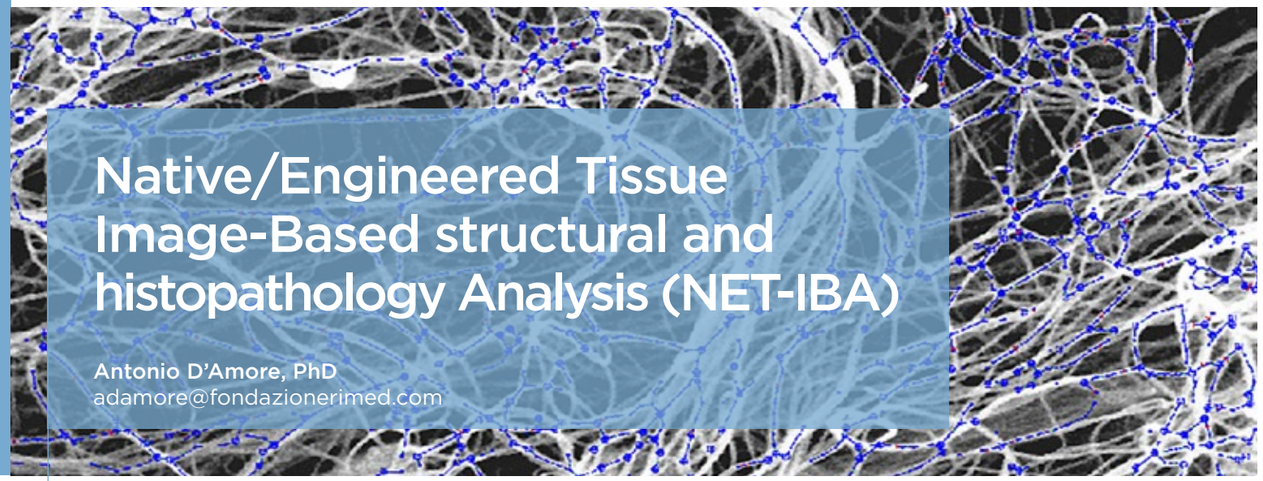
PUBLICATIONS

-G. Coyan, L. Silveira Filho, Y. Matsumura, S. Luketich, W. Katz, V. Badhwar, W. Wagner, **A. D'Amore**. Acute in vivo functional assessment of a stentless elastomeric biodegradable tricuspid valve. Journal of Cardiovascular Translational Research, DOI: 10.1007/s12265-020-09960-z, IF 2.75.



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** - **MEDICAL DEVICES & TISSUE ENGINEERING**



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

Antonio D'Amore, PhD
adamore@fondazionerimed.com

COLLABORATIONS

- University of Pittsburgh, Pittsburgh, USA
- University of Palermo, Palermo, Italy

THERAPEUTIC AREA

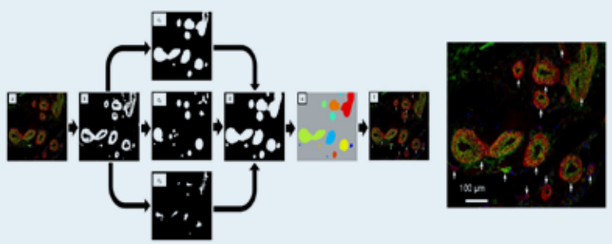
- Organ insufficiences
- Aging diseases
- Oncology

PIPELINE

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

BRIEF DESCRIPTION

Main topic: NET-IBA, development of an algorithms and automatic methods for structural and morphological analysis of native tissue and scaffolds. 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 assessment. Similarly, digital image analysis tools developed for material science applications or process engineering provide incomplete description of material morphology at micro and meso scales. This research line acts at the interface between these two disciplines. More specifically, our group aims to define novel image based software analysis tools and methods which can be utilized to address common problems currently faced in both clinical practice and material science. Examples include topological mapping of host cell recruitment in proximity to an implant, quantitative analysis of de-novo vessel formation. extracellular matrix morphological evolution due to a disease or to a treatment.



NET-IBA: Blood vessel detection algorithm on immunohistochemical staining. 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)

IMPACT

The software tools we developed and that we are tryaing to advance have the potential to impact on two main categories of problems:
- 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;
- morphological analysis of micro and nano-structured materials, potential applications within the context of chemical, process engineering or material science, include: process control, process characterization, structure-function characterization.

RESULTS ACHIEVED IN 2020

- Assisted McGowan institute affiliated faculty with methods for quantitative histology, this includes 2 funded NIH R01s in collaboration with Dr Ambrosio;
- Completed the implementation of an automatic code for the morphological segmentation of the identified blood vessels, classified by categories as demonstrated experimentally in: "A. D'Amore, M. Fazzari, H. Jiang, SK Luketich, ME Luketich, RF Hoff , DL Jacobs, X. Gu, SF Badylak, BA Freeman, WR Wagner. Nitro-oleic acid (NO2-OA) release enhances regional angiogenesis in a rat abdominal wall defect model. Published on Tissue Engineering Part A. ".
- Completed the validation of the software developed in collaboration with Dr Bruno. The algorithm is capable of processing data from fluorescence microscopy and segment/identify neo-vascularization;

Research grants obtained and/or managed
- European Research Council (ERC) "Consolidator Award 2020": "BIOMITRAL", $ 2 milion for 5 years. Sole Principal-Invistigator: A.D'Amore, Fondazione Ri.MED, Italy, (2020-2025).
- Fraction of start-up package as Head of the Cardiac Tissue Engineering Program, $ 1.75 million for 3 years. Sole Principal-Investigator: A. D'Amore, Fondazione Ri.MED, Italy (2020-2023).

Teaching activity
- Guest lecturer for the biomedical engineering master of science and PhD program, BIOENG 2810 – Biomaterials & biocompatibility. Department of bioengineering, University of Pittsburgh. Title: "A brief overview on polymers processing methods for soft tissue engineering", 2016-2020;
- Guest lecturer for the biomedical engineering master of science and PhD program, MSCMP 3735 – Extracellular matrix in tissue biology and bioengineering. Department of bioengineering, University of Pittsburgh. Title: "Cardiac ECM: structure - function, damage mechanism, and tissue engineering approaches to facilitate constructive remodeling", 2015-2020.

Mentoring activity
- Dr A. Adamo, Ri.MED Foundation - Expert Scientist in mechanobiology
- Dr F. Cosentino 2020, Ri.MED Foundation, Expert Scientist in numerical modelling

Invited speech
- "Advancing Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", school of engineering Polytechnic University of Turin, Turin, December 17th 2020;
- "Advancing electrodeposition technologies to enhance control of polymeric heart valve structure and function", Pathway to Market for TEMP Companies Workshop - a BioFab and Mayo Clinic Collaboration, Cleveland, December 7th 2020;
- "Ri.MED cardiac tissue engineering program, an overview to foster joint proposal applications", Monzino Cardiology Center, Milan, December 1st 2020;
- "Does bioinspired control of structure – function matter for tissue engineering heart valves? ", Division of pediatric cardiothoracic surgery, Children Hospital, University of Pittsburgh, Pittsburgh, March 6-2020.

GOALS FOR 2021

- 3D upgrade of 2D analysis methods developed for micro and nano-materials. The current version of the software developed "Gordium" relies on scanning electron microscopy 2D data, the research will include the upgrade of this methodology to 3D confocal microscopy data;
- Development software for failure models;
- Explore opportunities for spin-off company formation line NET-IBA.

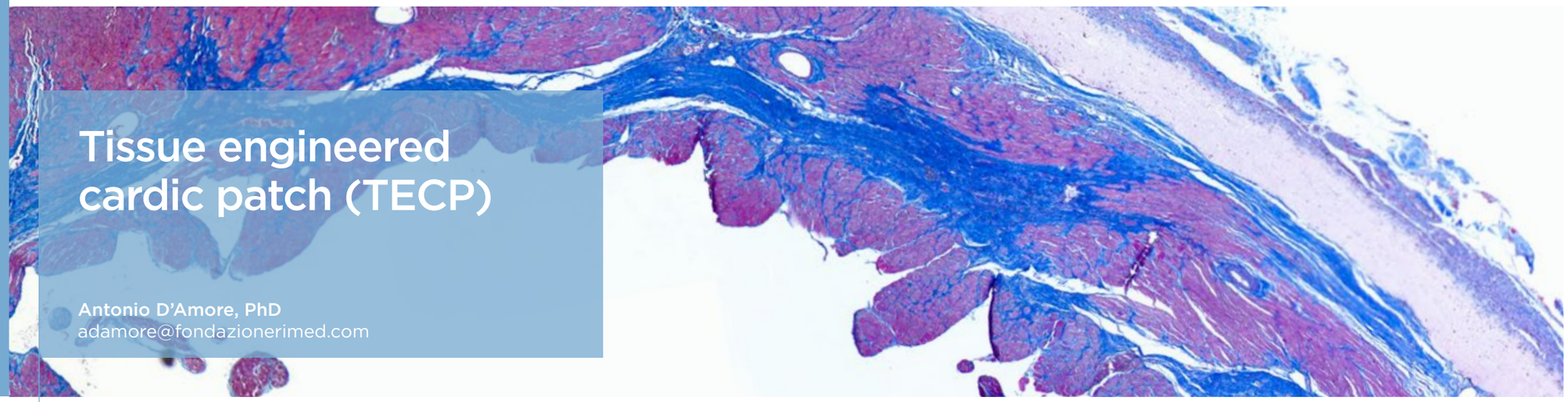
MEETINGS

- Kawakami, K. Nonaka, N. Fukase, **A. D'Amore**, J. Cummins, R. Kuroda, W. Wagner, J. Huard. A New Cell-free Biodegradable Synthetic Artificial Ligament For The Reconstruction Of Anterior Cruciate Ligament (ACL) In A Rat Model. Orthopedic Research Society (ORC) 2020 Annual Meeting. February 8-11, 2020 Phoenix, Arizona.

PUBLICATIONS

- A. Adamo, J. Bartolacci, M. Traina, W. Wagner, S.F. Badylak, **A. D'Amore**. Bioprocessing, structure, mechanics and hevaluation of micro-fiber based biodegradable suture material. To be submitted to Biomaterials, 5Y-IF 8.97.
- A. Adamo, A. Bruno, G. Menallo, M.G. Francipane, M. Fazzari, E. Ardizzone, W. Wagner, **A. D'Amore**. An automatic blood vessel detection algorithm for tissue engineering applications and quantitative histology. To be submitted to Annals of Biomedical Engineering, IF.3.47.

PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**



Tissue engineered cardic patch (TECP)

Antonio D'Amore, PhD
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COLLABORATIONS

- University of Pittsburgh, Pittsburgh, USA
- University of Pittsburgh Medical Center, Pittsburgh, USA
- University of Cincinnati, Cincinnati, USA
- Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IRCCS ISMETT), Palermo, Italy
- Mario Negri Institute of Pharmacological Research IRCCS
- Universidade Estadual de Campinas, Campinas, Brazil
- University of Texas, Austin, USA
- Virginia Commonwealth University, Richmond, USA
- ATeN Center, University of Palermo, Italy

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

CLINICAL NEED → DISEASES ANALYSIS → DISCOVERY → PRECLINICAL VALIDATION → PRECLINICAL DEVELOPMENT → CLINICAL STUDIES

IMPACT

The main objective of this research line is the introduction of innovative strategies to mitigate the pathological remodeling induced by myocardium infarction. In spite of the advancement made by pharmacological therapies, surgical treatment or VADs, congestive heart failure (CHF) remains a major cardiovascular disease in terms of epidemiology (2.1% of the US population) and mortality rate.
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.

RESULTS ACHIEVED IN 2020

Evaluated the effects of the intervention timing for bi-layered cardiac patch. Main results of cardiac patching with biohybrid approach: mitigates wall thinning, facilitates angiogenesis, reduces fibrotic tissue, sustains ventricle function up to 10 weeks from the infarction, decrease inflammation and improve M2 macrophage phenotype switch.

Research grant obtained and/or managed

- MIUR DOT1720429, "Dottorati di ricerca innovativi a caratterizzazione industriale", PhD student salary support of ~ €21k/year for 01/2018 - 12/2020. Co-PI: A. D'Amore, University of Pittsburgh, Co-PI: G. Ghersi, Universita' di Palermo.

Teaching activity

- Guest lecturer for the biomedical engineering master of science and PhD program, BIOENG 2810 – Biomaterials & biocompatibility. Department of bioengineering, University of Pittsburgh. Title: " A brief overview on polymers processing methods for soft tissue engineering";
- Guest lecturer for the biomedical engineering master of science and PhD program, MSCMP 3735 – Extracellular matrix in tissue biology and bioengineering. Department of bioengineering, University of Pittsburgh. Title: "Cardiac ECM: structure - function, damage mechanism, and tissue engineering approaches to facilitate constructive remodelling";

Mentoring activity

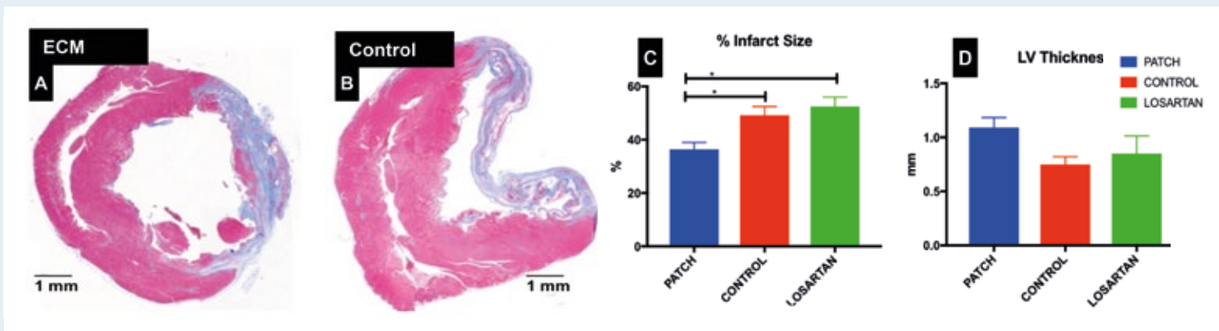
- A. Adamo, PhD candidate, University of Palermo Italy, cardiac patch development and biochemiocal characterization.

Invited speech

- "Ri.MED cardiac tissue engineering program, an overview to foster joint proposal applications", Monzino Cardiology Center, Milan, December 1st 2020.
- "Advancing Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", school of engineering Polytechnic University of Turin, Turin, December 17th 2020.

BRIEF DESCRIPTION

Main topic: TECP, development of restrain devices to support cardiac function of patients affected by myocardium infarction. The cardiac restrain devices potentially offer an alternative therapy to the pharmacological and surgical approach 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 engineered patch 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.



TECP: Long term effects of extracellular matrix-polymeric patch on infarct size and left ventricle thickness. **(A-B)** Typical MT stained whole heart sections and infarct/patch regions at the 16 wk time point. The ECM scaffold explants showed higher host cell infiltration and reduced fibrosis. **(C-D)** Quantitative comparison of infarct size and ventricle thickness in histological sections between patch treated, infarction control and Losartan treated groups. n=6, mean ± sem, *p<0.05.

GOALS FOR 2021

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 2021 include:

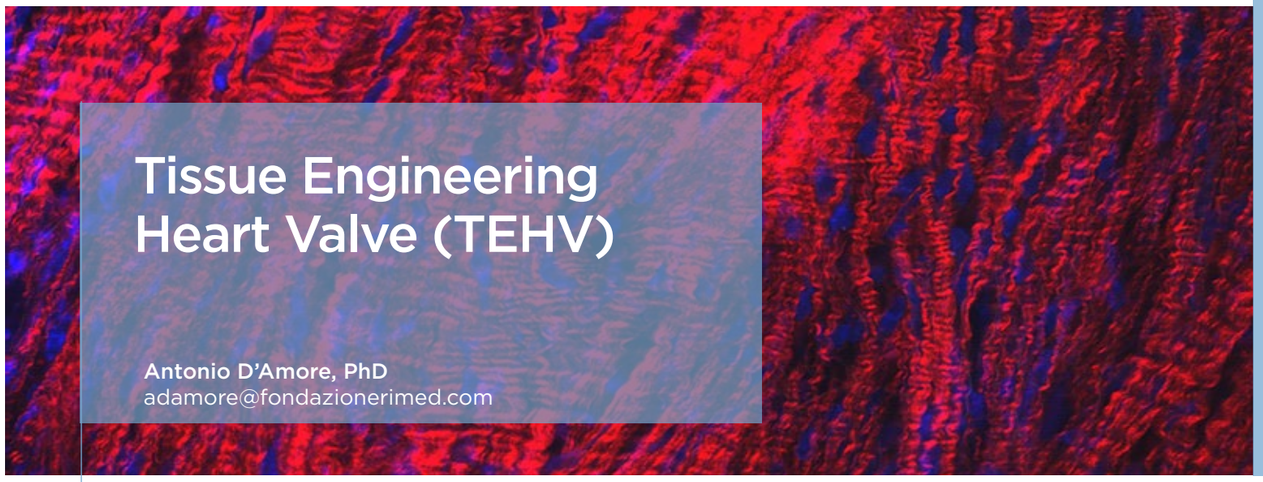
- Assessment of cardiac patch scaffold on large animal model, primary goals: (I) sustain ventricular function; (II) induce endogenous tissue growth\ reduce fibrotic tissue; (III) mitigate wall thinning;
- Italian Ministry of health research grant application, project in collaboration with Centro Cardiologico Monzino and ISMETT.
- R01 and R21 submissions
- Preclinical study on chronic myocardial infarct ovine model
- Development of a minimally invasive deployment, project in collaboration with Drs Pilato, Morsolini, Raffa (ISMETT) and Drs Coyan, Silveira Filho (UPMC and Univ. of Campinas Brazil);
- Development of a robotic platform for advanced processing, protected by IP, in collaboration with Advanced Solution
- Assess the therapeutical potential of right ventricle patching, project in collaboration with Drs Coyan, Silveira-Filho and Sciortino (UPMC).

PUBLICATIONS

- N. Kashiyama, R. Kormos, Y. Matsumura, **A. D'Amore**, S. Miyagawa, Y. Sawa, W. R. Wagner. An adipose derived stem cells sheet combined with an elastic synthetic patch incorporating cardiac ECM enhanced cell survival and preserved cardiac function in rats following subacute myocardial infarction. J. of Thoracic and Cardiovascular Surgery, IF 4.46.
- 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 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 Engineering Heart Valve (TEHV)

Antonio D'Amore, PhD
adamore@fondazionerimed.com

COLLABORATIONS

- **University of Pittsburgh, Pittsburgh, USA**
- **University of Pittsburgh Medical Center, Pittsburgh, USA**
- **University of Cincinnati, Cincinnati, USA**
- **Mediterranean Institute for Transplantation and Advanced Specialized Therapies (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

PIPELINE

BRIEF DESCRIPTION

Main topic: TEHV, development of engineered tissue and valve prostheses for the 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 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 able to induce in-situ tissue growth. The fabrication method has also the ability to control micro/macro structure and mechanical properties of the engineered construct.

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 have good longevity but require chronic anticoagulation therapy, which is in turn associated to 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 team aim to overcome the limitations of these two classes of medical devices by introducing engineered heart valves able to re-adjust to somatic growth, resist to 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 are also focusing the prototyping of novel hybrid medical devices based on combined biodegradable metallic and polymeric components.

RESULTS ACHIEVED IN 2020

Neoolife start-up formation (the first Pitt-Ri.MED start-up) completed, 4 Pitt-Ri.MED shared IPs have been licensed. Completed 4 more surgeries to evaluate the engineered mitral valve with chordal apparatus prototype. *In vitro* evaluation and mechano-biology study of isolated engineered chordae was continued. Consolidated and extended IP for the fabrication of scaffolds with integrated micro-topology capable of manipulating cellular activity. Established a research unit in Tissue Engineering Ri.MED in Palermo (ITA) in collaboration with ATeN Center and UniPA. Re-enforced the international collaboration Italy/Ri.MED- USA/McGowan Institute.

Funded grant proposals

- (2020-2025) European Research Council (ERC) "Consolidator Award 2020": "BIOMITRAL", $ 2 milion for 5 years. Sole Principal-Investigator: A. D'Amore, Fondazione Ri.MED, Italy;
- 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;
- Randall family big idea competition, University of Pittsburgh. Team: A. Adamo, G. Coyan, D Pedersen. Second place presentation winner - $15000, principal investigator: A. D'Amore.

Teaching activity

- Mentor for the Institute for Clinical Research Education (ICRE). Career development program training the next generation of clinical and translational scientists, Univ. of Pittsburgh. Trainee: Casey, Tompkins-Rhoades 2019-2021;
- Guest lecturer for the biomedical engineering master of science and PhD program, BIOENG 2810 – Biomaterials & biocompatibility. Department of bioengineering, University of Pittsburgh. Title: "A brief overview on polymers processing methods for soft tissue engineering";
- Guest lecturer for the biomedical engineering master of science and PhD program, MSCMP 3735 – Extracellular matrix in tissue biology and bioengineering. Department of bioengineering, University of Pittsburgh. Title: "Cardiac ECM: structure - function, damage mechanism, and tissue engineering approaches to facilitate constructive remodeling".

Mentoring activity

- A. Adamo, PhD candidate, University of Palermo Italy, engineering chordae tendineae, bioprocessing and cell seeding;
- P. Terranova, University of Palermo Italy, topological cues for enhanced endothelial cell proliferation, bioprocessing;
- C. T. Rhoades, School of Medicine, University of Pittsburgh USA, engineered mitral valve optimization via FEM.

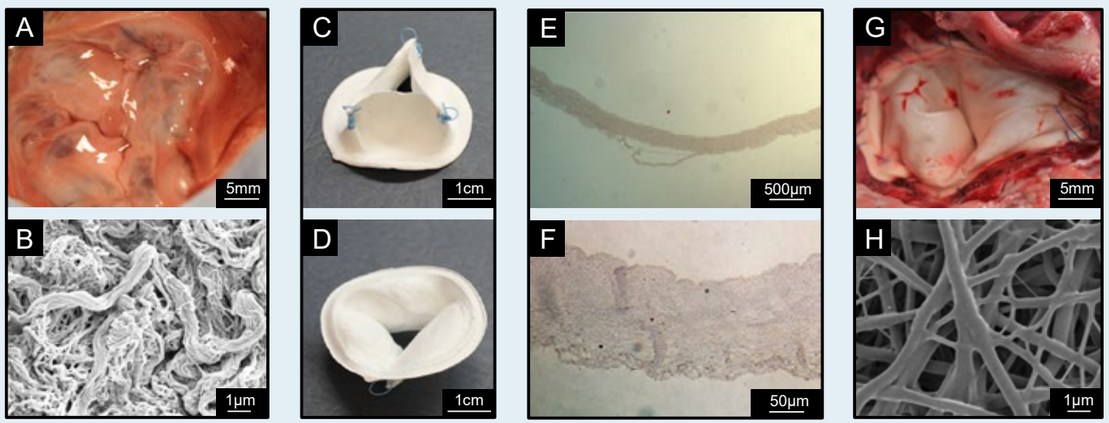
Invited speech

- A. D'Amore. "Advancing Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", school of engineering Polytechnic University of Turin, Turin, December 17th 2020;
- A. D'Amore. "Advancing electrodeposition technologies to enhance control of polymeric heart valve structure and function", Pathway to Market for TEMP Companies Workshop - a BioFab and Mayo Clinic Collaboration, Cleveland, December 7th 2020;
- A. D'Amore. "Ri.MED cardiac tissue engineering program, an overview to foster joint proposal applications", Monzino Cardiology Center, Milan, December 1st 2020;
- A. D'Amore. "Does bioinspired control of structure – function matter for tissue engineering heart valves? ", Division of pediatric cardiothoracic surgery, Children Hospital, University of Pittsburgh, Pittsburgh, March 6-2020.

GOALS FOR 2021

The long-term goal is the translation of technology (class III FDA), specific objectives for 2021 are defined as follows:

- Execution of Pittsburgh Innovation Challenge (PInCh) funded study of stented DCD pulmonary valve + degradable magnesium;
- Establishing a pipeline for polymer synthesis, ECM extraction, scaffold processing, histology, immunofluorescence, biomechanics and advanced microscopy at Aten center/Ri.MED Labs-Palermo;
- Formulation of new NIH R01project proposal to further support chronic study on mitral prosthesis DCD;
- Review publication on bioprocessing models for TEHV;
- Final round at the Betty Moore Foundation Innovators award;
- FEM modeling of the chordal apparatus;
- Evaluation of new selective fiber deposition strategies for DCD;
- Initiate *in-vivo* acute study for engineered mitral valve with chordal apparatus.
- Further IP protection;
- Further development of Neoolife targeting round A funding.
- Training of personnel: four master's degree students, three PhD students;
- McGowan Institute-UNIPA internship program continues.



TEHV: Acute *In Vivo* Functional Assessment of a Biodegradable Stentless Elastomeric Tricuspid Valve.
A) Organ level view of porcine native valve. **B)** Fiber level view of the native tricuspid valve via scanning electron microscopy. Appearance of ventricular **(C)** and atrial **(D)** surface of an elastomeric, biodegradable, stentless tricuspid valve processed with double-component deposition (DCD) electrospinning prepared for the implantation. Hematoxylin and eosin (H&E) staining of poly(carbonate urethane)urea leaflet cross sections prior to implant under low-**(E)** and high **(F)**-power magnification demonstrated porous and non-delaminated leaflet architecture. **G)** In situ view of engineered stentless tricuspid valve. Note how the shape of the engineered valve mimics the configuration observed in the native valve structure in (A). **H)** Fiber scale structure of the engineered valve via scanning electron microscopy showing comparable fiber intersection density, fiber diameter, and pore size to those observed in (B). After 24 h of implantation, H&E demonstrated that leaflets retained their initial structure with a proteinaceous deposit detected on lower power magnification **(I)**,and no evidence of thrombi and cellular infiltration on higher power examination **(J)**.

MEETINGS

- Y. Kawakami, K. Nonaka, N. Fukase, **A. D'Amore**, J. Cummins, R. Kuroda, W. Wagner, J. Huard. A New Cell-free Biodegradable Synthetic Artificial Ligament for The Reconstruction Of Anterior Cruciate Ligament (ACL) In A Rat Model. Orthopedic Research Society (ORC) 2020 Annual Meeting. February 8-11, 2020 Phoenix, Arizona.

PUBBLICAZION

- Elastomeric tissue engineered template based tricuspid valve. Y. Matsumura, L. M. Silveira-Filho, G. Coyan, **A. D'Amore**, W. R. Wagner. To be submitted to J. of Thoracic and Cardiovascular Surgery, IF 4.88.
- Controlling in-plane mechanics of electrospun polyurethane scaffolds for cardiac tissue engineering applications. S. K. Luketich, G. Menallo, G. Nasello, M. Maneschi, F. Gulizzi, P. Livreri, W. R. Wagner, and **A D'Amore**. To be submitted to Journal of Mechanical Behavior of Biomedical Materials, IF: 3.23.
- 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.
- Can a biohybrid acellular patch salvage ventricular function at late time point post-infarction remodeling process? L. M. Silveira-Filho, G. Coyan, A. Adamo, S. K. Luketich, G. Menallo, **A. D'Amore**, W. R. Wagner. In press on Journal of American College of Cardiology Basic Trans. Science, IF 3.7.
- P. Mela, **A. D'Amore**. In situ heart valve tissue engineering: Is scaffold structural biomimicry overrated? In press on Journal of American College of Cardiology Basic Trans. Science, IF 3.7.

- G. Coyan, L. Silveira Filho, Y. Matsumura, S. Luketich, W. Katz, V. Badhwar, W. Wagner, **A. D'Amore**. Acute in vivo functional assessment of a stentless elastomeric biodegradable tricuspid valve. In press on J. of Cardiovascular Translational Research, DOI: 10.1007/s12265-020-09960-z, IF 2.75.
- Y. Matsumura, Y. Zhu, H. Jiang, **A. D'Amore**, S. K. Luketich, V. Charwat, T. Yoshizumi, H. Sato, B. Yang, T. Uchibori, K. E. Healy, W. R. Wagner. Intramyocardial injection of a fully synthetic hydrogel attenuates left ventricular remodeling post myocardial infarction. Biomaterials 2019, 217, 119289, 5Y-IF 8.97.
- Y. Kawakami, K. Nonaka, N. Fukase, **A. D'Amore**, J. Cummins, R. Kuroda, W. Wagner, J. Huard. A Cell-free Biodegradable Synthetic Artificial Ligament For The Reconstruction Of Anterior Cruciate Ligament (ACL) In A Rat Model. Orthopedic Research Society (ORC) 2020 Annual Meeting. February 8-11, 2020 Phoenix, Arizona.

INTELLECTUAL PROPERTY

Patent issued

- US patent application PCT/US2016/019849 with WO (International publication number WO 2016/138423) published on 09/2016, topic: biomedical device, title: "Retrievable self-expanding non-thrombogenic low-profile percutaneous atrioventricular valve prosthesis". Nationalization phase in USA and EU. US Patent issued in 06-04-2020.

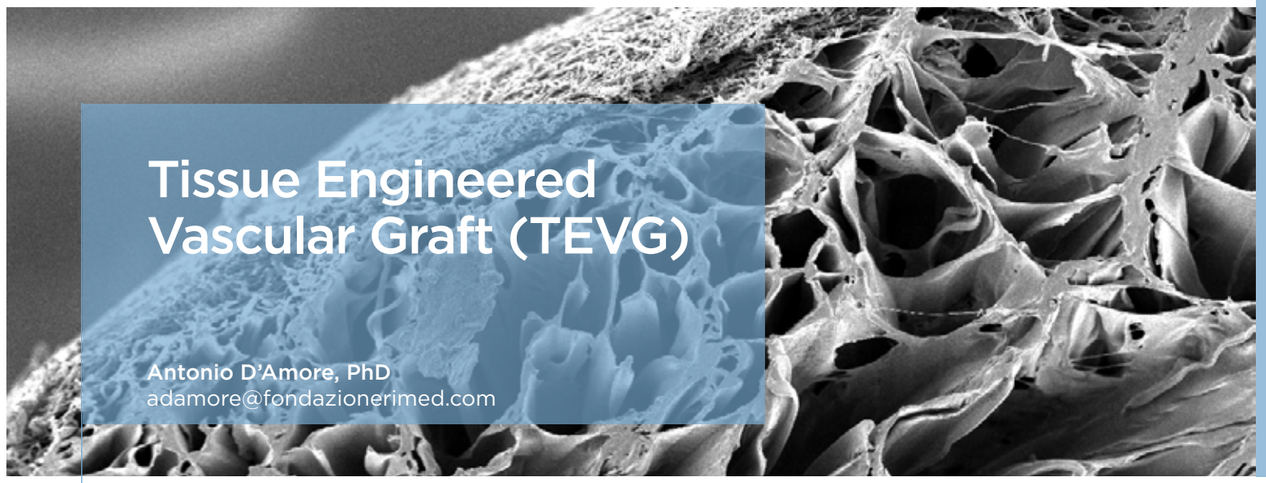
New patent applications

- US provisional patent application. Pitt invention disclosure ID#05453 filed on 07/2020, topic: bioprocessing methods, title: "Hybrid micro molding-fiber deposition substrate processing for cell biology manipulation and local anisotropy". Lead innovator/developer: **A D'Amore**.
- 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**.

Licensed patents and patent applications

- US patent application PCT/US2019/029121 with WO (International publication number WO/2019/210059) published in 11/2019, topic: biomedical device, title: "Biodegradable metallic stent for heart valve tissue engineering". Lead innovator/developer: **A D'Amore**. Licensed to Neoolife Inc. in 10-2020.
- US patent application PCT/US2018/019358 with WO (International publication number WO/2018/156856) published in 08/2018, topic: biomedical device, title: "A stentless biopolymer heart valve replacement capable of living tissue regeneration". Lead innovator/developer: **A D'Amore**. Nationalization phase in USA and EU. Licensed to Neoolife Inc. in 10-2020.
- US patent application PCT/US2016/019837 with WO (International publication number WO 2016/138416) published in 09/2016, topic: biomedical device, title: "Double component mandrel for electrospun stentless multi-leaflet valve fabrication". Lead innovator/developer: **A D'Amore**. Nationalization phase in USA, EU, Canada and Japan. Licensed to Neoolife Inc. in 10-2020.
- US patent application PCT/US2016/019849 with WO (International publication number WO 2016/138423) published on 09/2016, topic: biomedical device, title: "Retrievable self-expanding non-thrombogenic low-profile percutaneous atrioventricular valve prosthesis". Nationalization phase in USA and EU.
- US Patent issued in 06-04-2020, publication number: US-2020-0170791-A1. **Licensed to Neoolife** Inc. in 10-2020.

PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**



Tissue Engineered Vascular Graft (TEVG)

Antonio D'Amore, PhD
adamore@fondazionerimed.com

COLLABORATIONS

- Ospedale Cervello – Villa Sofia, Palermo, Italy
- University of Pittsburgh Medical Center, Pittsburgh, USA
- University of Pittsburgh, USA
- ATeN Center, University of Palermo, Italy
- LivaNova PLC, London, United Kingdom

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

BRIEF DESCRIPTION

Main topic: TEVG, development of engineered vascular graft for coronary bypass.

Solutions that are 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 to harvest sufficient viable autologous tissue. The second category utilizes 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 ad 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 2020

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 (Provisional patent: "Hybrid micro molding-fiber deposition substrate processing for cell biology manipulation and local anisotropy disclosure in progress"). Preliminary in vitro study, thanks to a custom-made bioreactor, to evaluate cellular infiltration into three layers graft.

Research funds

Fraction of start-up package as Head of the Cardiac Tissue Engineering Program, $1.75 million for 3 years. Sole Principal-Investigator: A. D'Amore, Fondazione Ri.MED, Italy (2020-2023).

Award

Best poster presentation at The Annual Biomedical Research Conference for Minority Students (ABRCMS 2020). Title: "The Design, Fabrication, and Analysis of the *In-vitro* Efficacy of a Novel, Three-Layered, Small Diameter Vascular Graft". Author: M. Haghkar. Mentor: A. D'Amore, 2020.

Teaching activity

- Guest lecturer for the biomedical engineering master of science and PhD program, BIOENG 2810 – Biomaterials & biocompatibility. Department of bioengineering, University of Pittsburgh. Title: "A brief overview on polymers processing methods for soft tissue engineering".
- Mentor for BIOENG 1095 - Special projects. Individual research project under the guidance of a faculty member. Department of bioengineering, University of Pittsburgh. Trainee: Mahdi Haghkar 2020.
- Mentor for ENGR1000 - Lab experiences. Individual research project under the guidance of a faculty member. Department of chemical and petroleum engineering, University of Pittsburgh. Trainee: Mahdi Haghkar 2020.

Mentoring Activity

- M. Barbuto, University of Palermo and University of Pittsburgh, development of three-layers small diameter vascular graft for reduced intima hyperplasia and enhanced endothelialization.
- M. A. Rodriguez Soto, 2020, Universidad de los Andes, Colombia, rational design and additive manufacture of regenerative vascular grafts: Understanding the interaction between blood cells-surface.
- M. Haghkar, University of Pittsburgh USA, topological cues for enhanced endothelial cell proliferation and structural organization in engineered vascular graft.
- P. Terranova 2019-2020, University of Palermo Italy, bioprocessing of polymeric fibrous substrates at the meso scale for cell manipulation.
- G. C. Miceli 2019-2020, Milan Polytechnic Italy, biomimetic three-layers vascular graft, cell proliferation and de-novo collagen synthesis.

GOALS FOR 2021

The final goal of this research line is the translation of the technology (FDA class III).

Targets set for the year 2021 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 optimize the design of the pattern and to investigate cell adhesion and proliferation induced by superficial pattern;
- To establish an 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.

MEETINGS

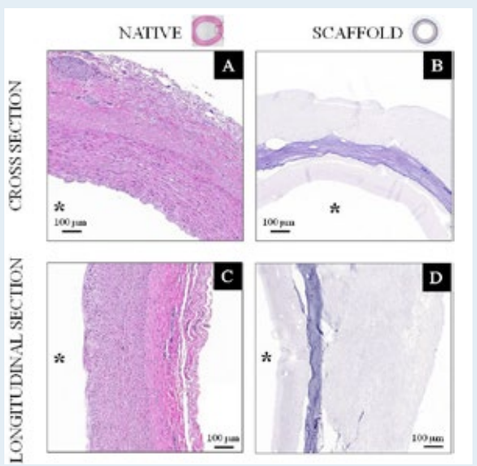
- "Advancing Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", Dicembre, 2020, Scuola di Ingegneria, Politecnico di Torino, Torino, Italy. Speaker: **A. D'Amore.**
- "Ri.MED cardiac tissue engineering program, an overview to foster joint proposal applications", Dicembre, 2020, Centro Cardiologico Monzino, Milano, Italy. Speaker: **A.D'Amore.**

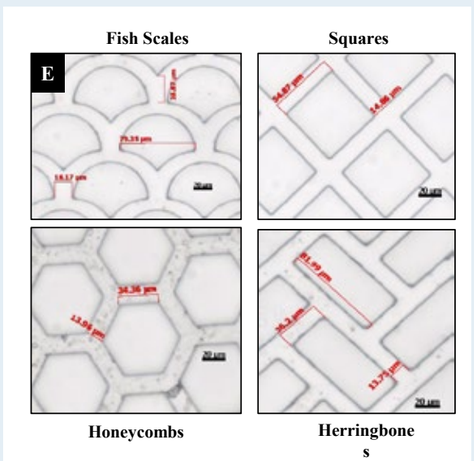
PUBLICATIONS

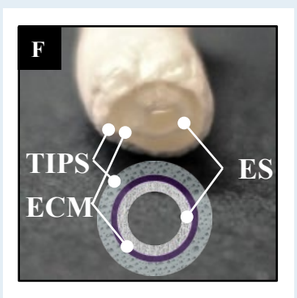
- T. K. Valencia-Rivero, J. Cruz, A. Castillo-Martínez, L. Lina, **A. D'Amore**, W. Wagner, J. C. Briceño. "SIS-Based regenerative vascular grafts: PEGylation and carboxylic acid conjugation to improve key attributes". Submitted to Journal of Materials Science and Engineering: C, IF 3.42.
- E. M. Cunnane, K. L. Lorentz, L. Soletti, A. K. Ramaswamy, T. K. Chung, D. G. Haskett, S. K. Luketich, E. T. Tzeng, **A. D'Amore,** W. R. Wagner, J. S. Weinbaum, D. A Vorp., "Development of a semi-automated, bulk seeding device for large animal model implantation of tissue engineered vascular grafts", Frontiers in Bioengineering and Biotechnology, 8, 2020, doi.org/10.3389/fbioe.2020.597847.

INTELLECTUAL PROPERTY

- US provisional patent application. Pitt invention disclosure ID#05453 filed on 07/2020, topic: bioprocessing methods, title: "Hybrid micro molding-fiber deposition substrate processing for cell biology manipulation and local anisotropy". Lead innovator/developer: **A. D'Amore.**
- 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.**
- US patent application PCT/US2018/043889, with WO (International publication number WO/2019/023447) published in 08/2019, topic: biomedical device, title: "Multi-Layered Graft for Tissue Engineering Applications". Lead innovator/developer: **A D'Amore.**
- US patent application PCT/US2018/061862 with WO (International publication number WO/2019/100021) published in 05/2019, topic: controlled release system/drug for angiogenesis, title: "Nitro-oleic acid (NO2-OA) controlled release platform to induce regional angiogenesis in abdominal wall repair". Lead innovator/developer: **A D'Amore.**







TEVG: Three-layer vascular graft duplicating 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)** Patterned PDMS transferred via eelctrodeposition on scaffold for enhanced cell adhesion and proliferation. **(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).

PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**

R-CaRe - Regenerative Cartilage Reheabilitation

Riccardo Gottardi, PhD
rgottardi@fondazionerimed.com

COLLABORATIONS

- Bioengineering and Biomaterials Laboratory, Children's Hospital of Philadelphia (CHOP), Philadelphia, USA
- Dept. of Pediatrics, Perelman School of Medicine, University of Pennsylvania (UPenn), Philadelphia, USA
- Dept. of Bioengineering, School of Engineering and Applied Sciences, University of Pennsylvania (UPenn), Philadelphia, USA
- Center for Cellular and Molecular Engineering, Dept. of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, USA

THERAPEUTIC AREA

Aging diseases

PIPELINE

CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

BRIEF DESCRIPTION

Focal cartilage lesions represent an important problem that mainly affects the active population at a young age. Currently, the traditional first-line treatment employs the use of the microfracture technique, used in over 100,000 cases / year in the United States. However, this technique still has limitations in terms of long-term efficacy and does not always ensure a rapid return to the pre-injury level. In fact, about 25% of microfractures require re-surgery within 2 years, with a high probability of treatment failure and the development of arthritic pathologies within 5-10 years. Furthermore, rehabilitation after microfracture treatment takes up to 6 months and includes initial immobilization followed by continuous passive movement and progressive loading. However, there is no consensus on the timing and extent of joint loads that can lead to optimal rehabilitation. Since mechanical forces significantly affect cellular behavior (mechanobiology), anticipating the time when the joint is loaded could improve tissue healing, accelerate extracellular matrix deposition, and promote a more hyaline rather than fibrocartilaginous phenotype of regenerated tissue. as suggested by the results of animal and *in vitro* studies. We expect that mechanical loading during rehabilitation can be exploited to direct the formation of regenerated tissue. With this project, we aim to improve cartilage repair outcomes by identifying loading regimens that can be applied during rehabilitation to promote cartilage regeneration, improve tissue repair and extend its longevity.

IMPACT

Currently there are no reference values regarding the timing and extent of joint load to be applied for optimal rehabilitation after microfracture, since the mechanisms by which controlled mobilization promotes cartilage repair are still unknown. The results obtained after the completion of the project activities will provide a link between physiotherapy-induced mechanotransduction and regeneration and integration of repair cartilage. This study will provide indications for modifying current rehabilitation protocols and, in the long term, postponing the development of osteoarthritis. As a further added value, the technological platforms developed in this project could be applied in addition to the field of cartilage repair, also to other orthopedic injuries, as well as to the development of preventive measures on rehabilitation. Our platforms have in fact a precise control of the various load parameters to identify the regenerative load patterns *in vitro* that can be replicated *in vivo* through rehabilitation protocols for both treatment and prevention.

RESULTS ACHIEVED IN 2020

Working on the Regenerative-Cartilage Rehabilitation (R-CaRe) project we have designed and built an automated device (High Throughput Mechanical Activator for Cartilage Engineering - HiT-MACE) capable of applying mechanical stress on a large number of tissues (native or engineered) simultaneously and under sterile conditions. HiT-MACE is designed to quantify accurately the amount of load applied, keeping this value constant over time and thus reducing experimental variability. In the context of the project we have applied compressive loading on native cartilage using regimens mimicking physiological and supra-physiological loads. Then, we thoroughly studied the tissue response to these regimes to determine the optimal conditions promoting cartilage regeneration. Interestingly, we found that a physiological loading regime promotes the activation of important pro-anabolic intracellular pathways (TGF-β SMAD 2/3) that bias cartilage toward a pro-regenerative metabolism. This experimental approach allowed to determine the optimal loading regimen that can potentially maximize the clinical rehabilitation outcomes.

GOALS FOR 2021

The results obtained in 2020 has been employed to develop an advanced pre-clinical validation model that faithfully replicates the response of cartilage *in vivo*. However, it is well note as cartilage is spatially and metabolically interconnected with the underneath bone layer in joints. The objectives for 2021 in the context of the R-CaRe project aim to further improve our experimental model by developing a more advanced pre-clinical platform including the contribution of subchondral bone to the mechanical stimulation response. To this end, we will perform further studies using native osteochondral (OC) units that faithfully reproduce the joint microenvironment. The expected results will provide even more detailed information about the duration and intensity of the load cycles to be applied to maximize the effects of rehabilitation therapy.

PUBLICATIONS

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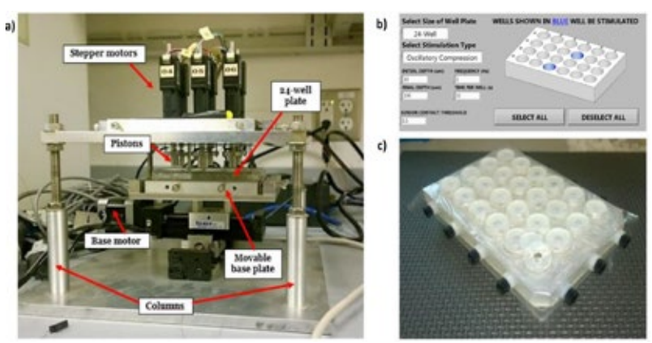


Figure 1: Hit-MACE device used to apply compressive load over cartilage plugs **(a)**, screenshot acquired during a compressive loading experimental set-up **(b)**, customized lid used to maintain sterile conditions during compressive cycles **(c)**.

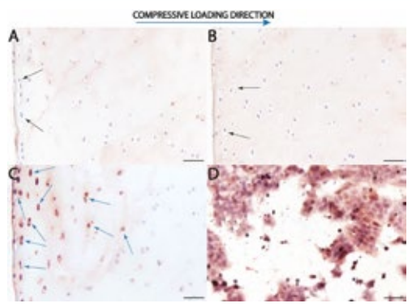
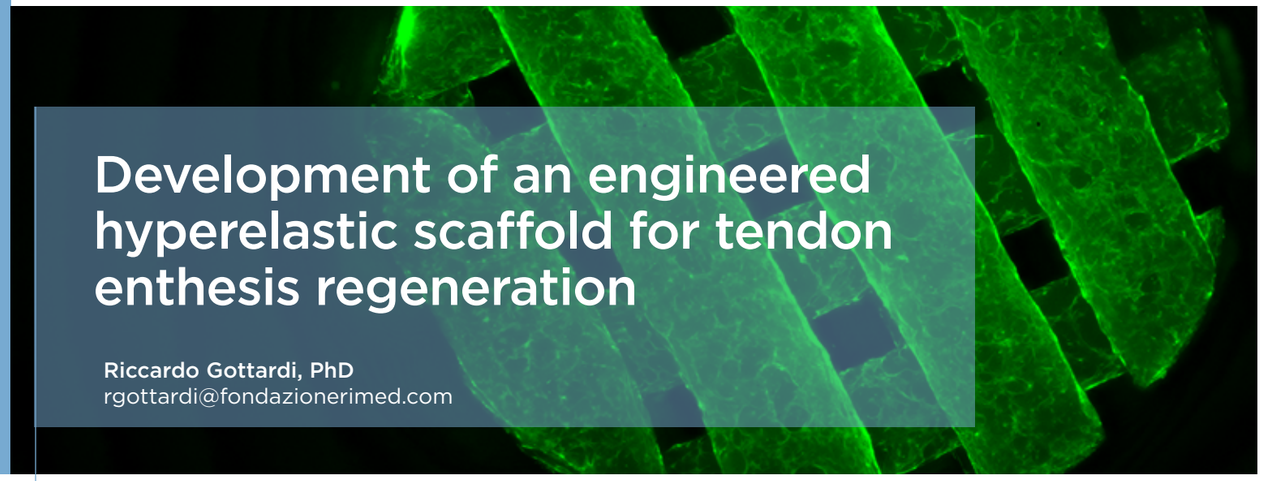


Figure 1: Immunohistochemistry on SMAD3 on **(a)** T=0 group, before loading, **(b)** unloaded group, **(c)** loaded group, and **(d)** positive immunostaining control for SMAD3 (MCF-7 cells). Blue arrows show SMAD3 overexpression in loaded samples compared to unloaded and T=0 samples (black arrows) Scale bar = 50 μm.

PRODUCTS: **MEDICAL DEVICES & TISSUE ENGINEERING**



Development of an engineered hyperelastic scaffold for tendon enthesis regeneration

Riccardo Gottardi, PhD
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COLLABORATIONS

- University of Pennsylvania (UPenn), United States
- Bioengineering and Biomaterials Laboratory, Children's Hospital of Philadelphia (CHOP), United States
- Center for Cellular and Molecular Engineering (CCME), University of Pittsburgh, United States
- Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, United States
- Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, United States
- The Chinese University of Hong Kong, China
- Cell Biology Inspired Tissue Engineering, Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Netherlands
- MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Netherlands

THERAPEUTIC AREA

Aging diseases

PIPELINE

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

BRIEF DESCRIPTION

The tendon-to-bone enthesis (TTBE) is a specialized connective tissue structure essential to guarantee a smooth transition between tendons and bones. Injuries affecting the TTBE have high clinical incidence especially in the elderly and in the more active populations that play sports, at both the professional and non-professional level. It is estimated that each year in the EU and the USA about 30 million people undergo tendon/ligament repair procedures, causing an annual expense of over €150 billion. Despite several innovative techniques that have been developed, surgical repair of massive enthesis injuries is still inadequate, with up to 79% failure rate in the most severe cases. In this project we fabricated a cellularized graft mimicking the tendon-fibrocartilaginous biphasic transition tissue of the TTBE. The construct is composed of a tendon-like side and a cartilage-like side, cellularized with adult human mesenchymal stem cells (hMSCs) The structural core of our construct is a scaffold composed of medical grade poly(L-lactide-co-glycolide) (PLGA), with high internal porosity and elastic properties sufficient to form a self-supporting mesh, and able to support cell proliferation.

IMPACT

With this project we aimed to develop a construct mimicking the tendon-to-bone enthesis to satisfy the clinical need of high-performance constructs to be used in reconstructive tendons surgery. Our engineered construct promoted the differentiation of stem cells toward cartilaginous and tendinous phenotypes. To achieve this we used an innovative, biphasic bioreactor that is part of the Intellectual Property portfolio of Ri.MED Foundation and that we have previously employed to engineer other biphasic tissues. In terms of materials, the high tensile properties of hyperelastic PLGA scaffold confer mechanical resistance to our construct. Hence, our construct may support the surgical repair of tendons injuries, promoting a fast healing and improving the post-surgery outcomes.

RESULTS ACHIEVED IN 2020

In this study, we have realized an engineered construct that recapitulates some aspects of the tendon fibrocartilage transition tissue of a tendon-to-bone enthesis, and that can support cell viability, proliferation, and differentiation. Our differentiation system was based on a 3D printed, highly biocompatible scaffold of hyperelastic PLGA, characterized by a microporous architecture with aligned fiber orientation on the tendon side, and dishomogeneous fiber organization on the cartilaginous side, mimicking some anatomical features of the native enthesis. Engineered enthesis cellularized with hMSCs, the scaffolds were cultured within an advanced biphasic bioreactor system that allowed simultaneous exposure of each side of the biphasic construct to a specific differentiation medium. Results from gene expression analysis, biochemical analysis, and histological examination suggest that we have successfully obtained an engineered construct composed of a tendon-like side and a cartilage like side, that is potentially applicable for the surgical tendon repair.

GOALS FOR 2021

The construct developed with this work, showed interesting properties in directing the hMSCs toward chondrogenic and tenogenic lineage differentiation as well as their correct spatial orientation along the scaffold. In future studies we plan to further improve the biomimetic properties of our construct, inducing a more effective hMCSs spatial distribution within the scaffold's meshes. The main goal is to fabricate a construct characterized from a more defined tendon-cartilage transition to increase their biomimetic properties.

PUBLICATIONS

- **Gottardi**, R., Möeller, K., Di Gesù, R., Tuan, R.S., van Griensven, M., Balmayor, E.R.. Application of a Hyperelastic 3D Printed Scaffold for Mesenchymal Stem Cell-based Fabrication of a Bizonal Tendon Enthesis-like Construct. Frontiers in materials - biomaterials, (in press). doi: 10.3389/fmats.2021.613212

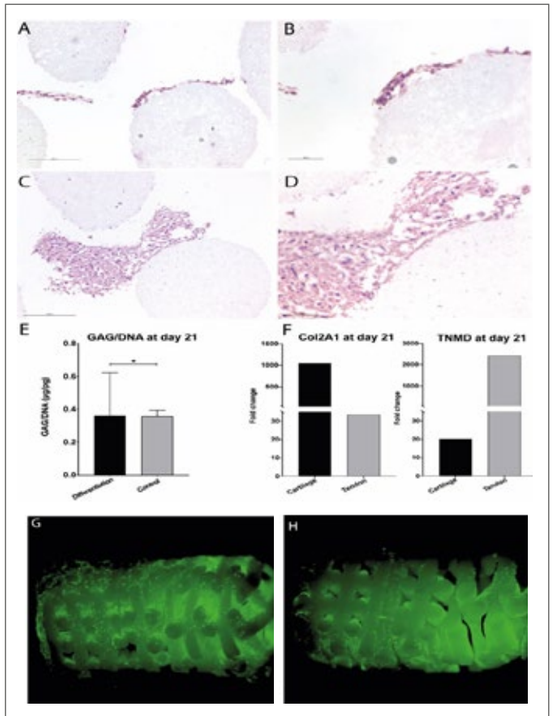

Figura 1:H&E staining of the biphasic construct (cartilaginous side) after 7 days (A, B) and 21 days (C, D) of culture at two different magnifications. Black arrows indicate newly produced extracellular matrix. Scale bar = 150 µm (A, C) and 50 µm (B, D). GAG content of the constructs expressed as GAG/DNA ratio at day 21. Data were analyzed using an unpaired t test with Welch's corrections, *$p < 0.05$ (E). Relative gene expression of COL2A1, and TNMD at day 21 measured by qRT-PCR (F,) normalized to control at the same time point. Calcein AM staining selective for living cells performed over the entire construct (G), and a longitudinal section of the construct (H) after 7 days of culture in bioreactor.

TECHNOLOGY PLATFORMS

In recent years, thanks to the funding provided by the Sicilian Region, 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 the analysis of chemical-physical properties.

An automated system was also supplied for the storage and manipulation of molecule libraries for the **High Throughput Screening** laboratory, as well as a cardiac simulator and instrumentation 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 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; there is also a **Cell factory** for the production of ATMp.

In 2020, the **Medicinal Chemistry** Platform was set up, 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 the compounds that will enter the development phase.

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

Bioengineering

Platform

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The Bioengineering Platform is equipped with state of the art facilities for 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.

COLLABORATIONS:

- IRCCS - ISMETT, Palermo, Italy
- Policlinico Giaccone, Palermo, Italy
- University of Palermo, Italy
- University of 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

Expertise

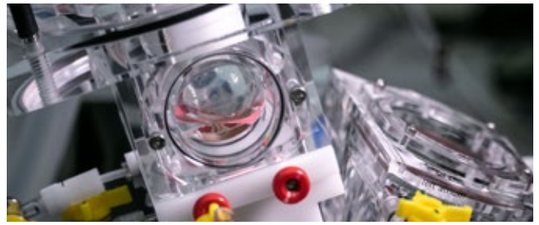
- Development of cardiovascular medical implants;
- Mechanical and thermo-mechanical characterisation of biomaterials;
- Numerical simulation of physiological systems and their interaction with medical devices (by means of structural, fluid dynamic and fluid-structure interaction analyses);
- Development 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.

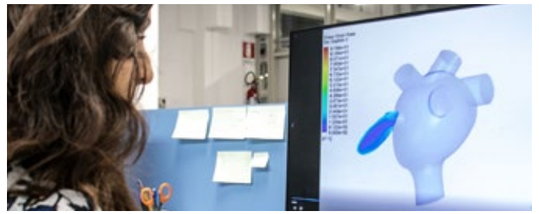
Technology platform

- 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.

ACTIVE RESEARCH PROJECTS

- Development of a novel transcatheter heart valve
- Development of a novel Alfa-Gal Free Xenograft heart valve
- Analysis of the left atrial appendage to predict the risk of thrombosis
- Smoothed Particle Hydrodynamics computational analysis for cardiovascular bioengineering applications
- Thermo-mechanical characterisation of super-elastic Ni-Ti biomaterials





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- Vella D, Monteleone A, Musotto G, Bosi GM, **Burriesci G**. *Effect of the alterations in contractility and morphology produced by atrial fibrillation on the thrombosis potential of the left atrial appendage*. Front. Bioeng. Biotechnol. doi: 10.3389/fbioe.2021.58604

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- Rahmani B, **Burriesci G**. *Polymeric Heart Valves*. In: Roberts G., Watts A., European Biophysical Societies (eds) Encyclopedia of Biophysics. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-35943-9_702-1

- **Burriesci G**, Seifalian AM, Zervides C. *Heart Valve Prosthesis* US10357358B2

- Salsac AV, Fiore A, **Burriesci G.** *Implant pour valve cardiaque* FR1858233

- Tseng, YT, Latif N, El-Nashar H, Salmon J, Sarathchandra P, **Burriesci G**, Sohier J, Chester A, Yacoub M. *Design and pre-clinical testing of a tissue engineered semilunar root*. 2nd International Conference of Tissue-Engineered Heart Valves, 14 February 2020, Abu Dhabi, United Arab Emirates

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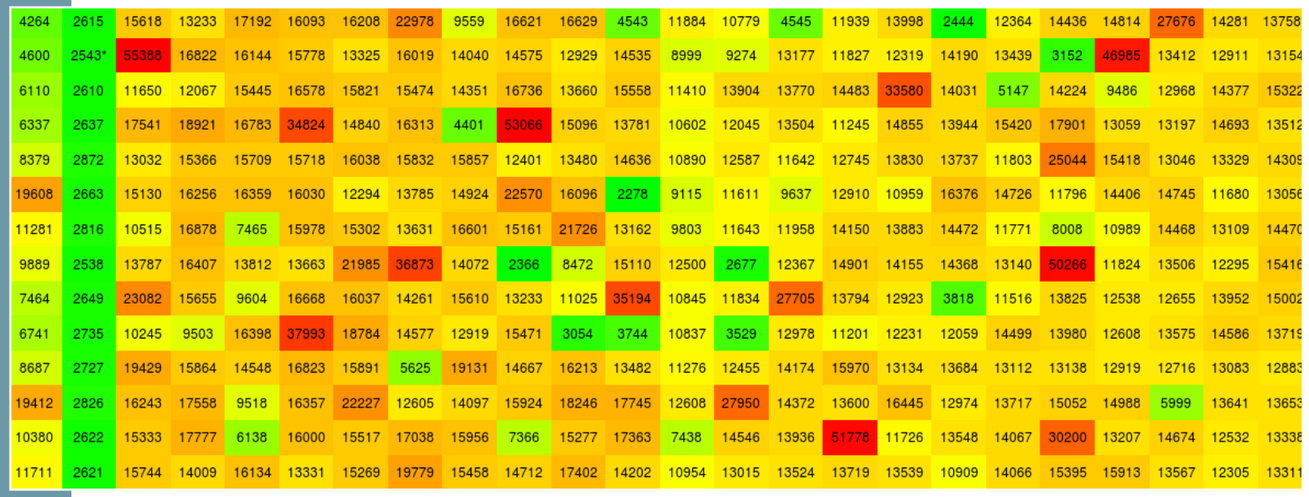
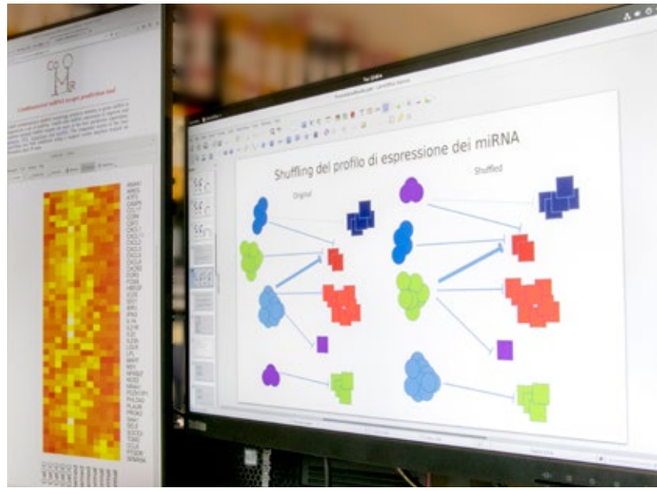
Bioinformatics
Platform





CONTACTS:
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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, we support the Drug Discovery Unit in high throughput screening experimental design and data analysis. We perform 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, we use our 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, we are able to describe the regulatory interaction network of the endogenous microRNA in a specific tissue of interest, by analyzing its microRNA and gene expression profiles.

Expertise

- 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: 80 CPUs e 2 x NVIDIA Tesla K80

COLLABORATIONS:

- IRIB-CNR, Palermo, Italy
- University of Palermo, Italy
- University of Pittsburgh, USA
- IRCCS ISMETT, Palermo, Italy

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.

PUBLICATIONS

Bellavia, D, Iacovoni, A, Agnese, V, Falletta, **C, Coronnello,** C, Pasta, S, Novo, G, di Gesaro, G, Senni, M, Maalouf, J, Sciacca, S, Pilato, M, Simon, M, Clemenza, F, Gorcsan III, SJ. (2020) *Usefulness of regional right ventricular and right atrial strain for prediction of early and late right ventricular failure following a left ventricular assist device implant: A machine learning approach*, The International Journal of Artificial Organs 43 (5), 297-314. doi.org/10.1177/0391398819884941

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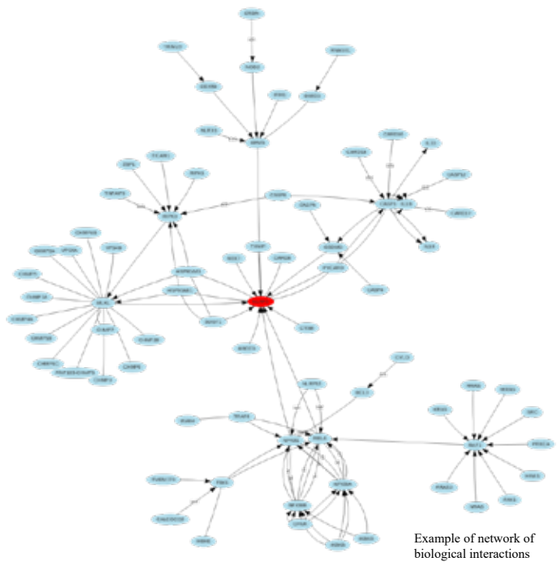
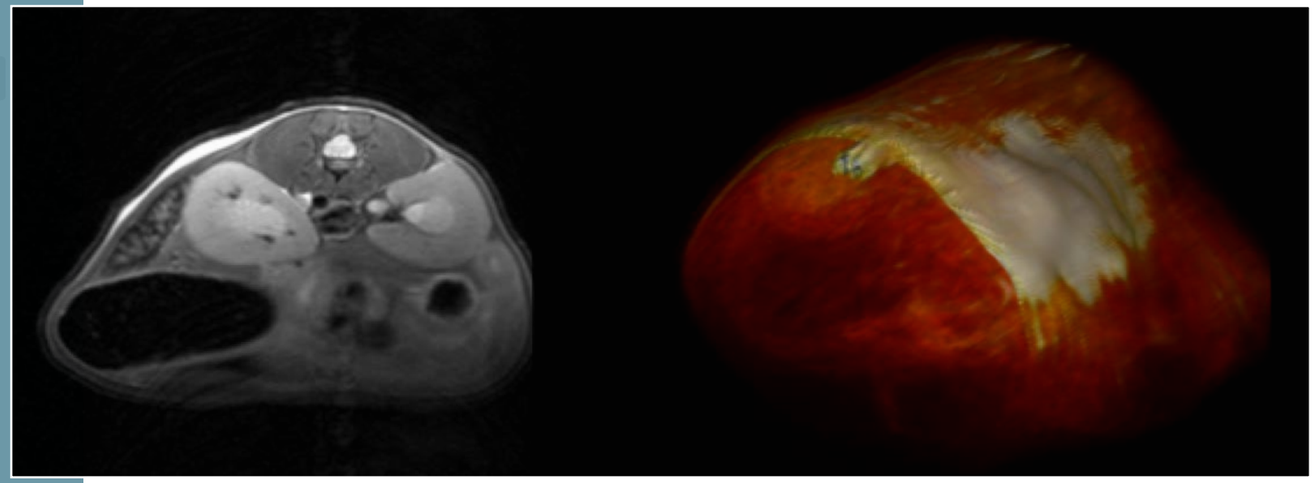
Cicero, L, Cirincione, R, Comelli, A, **Coronnello, C**, Cassata, G, *Residue analysis of a synthetic glucocorticoid in liver samples by a 1HMR spectroscopy approach: An exploratory study on animal model*, Food Additives & Contaminants. Part A 37 (10), 1640-1650, doi.org/10.1080/19440049.2020.1787528



Example of network of biological interactions

Biomedical Imaging and Radiomics

Platform



CONTACTS:
Albert Comelli, PhD
acomelli@fondazionerimed.com

During 2020, 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 will be enriched with two Workstation with GPU and two monitor 4k for Deep Learning and Machine Learning Algoritms in order to offer more options for in vivo imaging and data analysis.

COLLABORATIONS:

- Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT- IRCCS), Palermo, Italy
- Institute of Molecular Bioimaging and Physiology, (IBFM-CNR), Cefalù, Italy
- Georgia Institute of Technology, (GIT), Atlanta, USA
- Nuclear Medicine Unit, University of Messina, Italy
- Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, (PROMISE), University of Palermo, Italy
- Department of Biomedicine, Neuroscience and Advanced Diagnostics, (BIND) University of Palermo, Palermo, Italy
- Department of Engineering, University of Palermo, Italy
- Department of Agricultural, Food and Forests Sciences University of Palermo
- Medical Physics Unit, Cannizzaro Hospital, Catania, Italy
- Nuclear Medicine Department, Cannizzaro Hospital, 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;
- Phantoms design and creation for spectroscopy, contrast agents and morphovolumetric studies.

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 Risonanza Magnetica ad alto campo 3.0 T (neuro, body, mammella, angio, osteoarticolare, cardio, etc.).

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

- PET/CT Clinica e Preclinica.

ACTIVE RESEARCH PROJECTS

- I*n 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.

- *Diagnostic classification of microbleeds/calcifications using radiomics features and artificial intelligent algorithms on susceptibility weighted imaging (SWI) brain MRI sequences*. ISMETT side: Dr. Roberto Miraglia and Dr. Gianvincenzo Sparacia. Fondazione Ri.MED side: Dr. Albert Comelli and Dr.ssa Claudia Coronello.

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

- *Phantom spectroscopy study of metabolites on MRI 3 tesla clinical and MRI 7 Tesla preclinical.* ISMETT side: Dr. Roberto Miraglia and Dr. Fabio Calogero Caruso. Fondazione Ri.MED side: Dr. Albert Comelli and Dr.ssa Claudia Coronello.

PUBLICATIONS

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- Alongi P, Stefano A, **Comelli A**, Laudicella R, Barone S and Russo G. (2020). *New Artificial intelligence model for 18F-Choline PET/CT in evaluation of high-risk prostate cancer outcome: texture analysis and radiomics features classification for prediction of disease progression*. Journal of Nuclear Medicine, 61(supplement 1), 1303-1303.
- Cannella R, Vernuccio F, **Comelli A**, Salvaggio G and Midiri M. (2020). *Texture analysis for noninvasive assessment of hepatic fibrosis: current knowledge and future perspectives*. European Congress of Radiology 2020.
- Cicero L, Cirincione R, **Comelli A**, Coronnello C, Cassata G (2020). *Residue analysis of a synthetic glucocorticoid in liver samples by a 1HMR spectroscopy approach: An exploratory study on animal model.* FOOD ADDITIVES & CONTAMINANTS. PART A. CHEMISTRY, ANALYSIS, CONTROL, EXPOSURE & RISK ASSESSMENT, vol. 37, p. 1640-1650, ISSN: 1944-0049, doi: 10.1080/19440049.2020.1787528.

CELL FACTORY

Platform

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The Cell Factory Group, including Production, Quality Assurance, Quality Control staff and a Qualified Person for the release of Advanced Therapy Medical Products for clinical use, provides through the platform the necessary skills to support the definition of Good Manufacturing Practice (GMP) compliant production processes and quality control tests.

The new Cell Factory at IRCCS ISMETT hospital, in which Ri.MED Foundation and ISMETT staff is involved in Production, Quality Assurance and Quality Control, will allow to produce advanced therapies developed by Ri.MED Foundation and ISMETT researchers, and to use these therapies in clinical trials or for hospital patients' individual use.
Moreover, thanks to specific 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 for local use at ISMETT or to be provided to other hospitals.

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;
- 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;

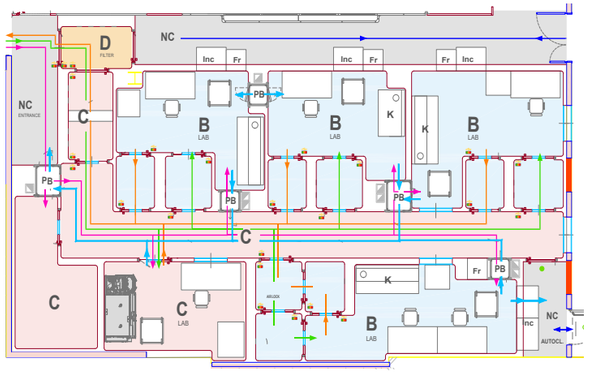
Facility Description

The new cell factory, completed in 2020, guarantees flexibility in the type of production and functionality of the environment. Based on specific User Requirement Specification, production and quality control layouts for the three types of advanced therapies (Gene Therapy, Cell Therapy, Tissue Engineering) were designed, and were approved by AIFA during a Scientific Advice meeting.

There are 4 class B laboratories, one of which allows for a higher containment (with an autoclave for waste treatment). The other 3 class B laboratories can be used in a totally independent way, allowing 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 (eg. organ cleaning) and other manipulations (eg. cell isolation and culture) can be performed in the second lab, after passing the intermediate product through a passbox.

Closed cell preparation systems will be installed in a class C Lab. Technical compartments, on which the engines of the instrumentation protrude, allow maintenance without access to the production areas.

The quality control laboratories are equipped to conduct all the tests on raw materials, intermediates and final products required for product release, as well as to receive and adequately store reagents, materials and products. Both the production area and the QC labs are equipped with a remote monitoring system.



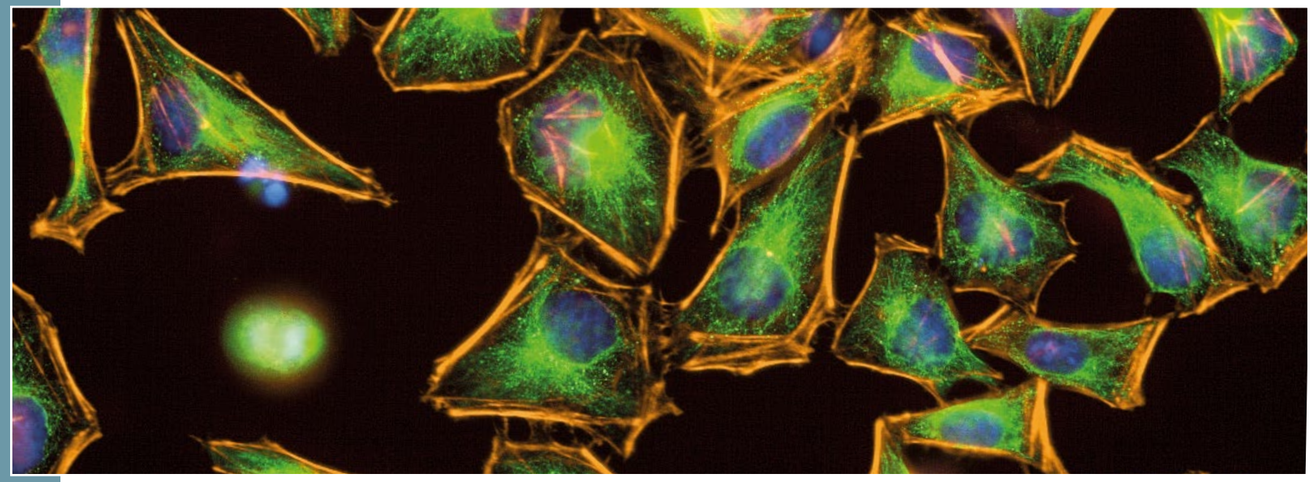
GMP facility layout, with personnel and material flows.

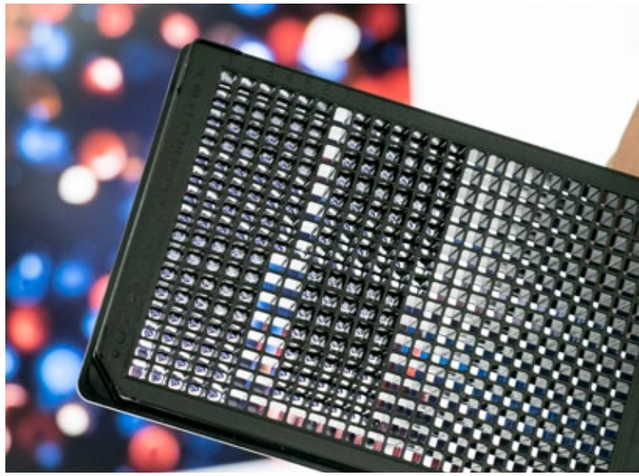
ACTIVITIES

The renovation works of the new Cell Factory and of the Quality Control laboratories were completed in December 2020. Most of the equipment was installed in the aforementioned areas and the furnishings are being finalized. The qualification of the instrument monitoring system was carried out. In 2021, the qualifications of the air filtration system (HVAC) and all standard equipment (incubators, hoods, refrigerators, freezers, centrifuges and thermostats) will be completed.
The Cell Factory staff will carry out in 2021 the validations of fundamental general processe (gowning validation, sanitization and clean hold time, passage of materials, etc.). Once the necessary development/technology transfer data of the first advanced therapy products (in the context of adoptive immune therapies) are available, specific validations 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.
The emission of the quality documentation and the periodic training of internal and external staff who will access the Cell Factory are fundamental activities that will continue in 2021.

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 for 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 validation assays, orthogonal and secondary assays.

Expertise

- Set-up and validation of primary assay (cell-free and cell-based);
- Different readout 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 actives 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.

COLLABORATIONS:

- Istituto per la Ricerca e l'Innovazione Biomedica (IRIB) - CNR, Palermo, Italy
- Institut de la Vision, Parigi, Francia
- Luigi Vanvitelli University of Campania - Naples, Italy
- University of Palermo, Palermo, Italy.

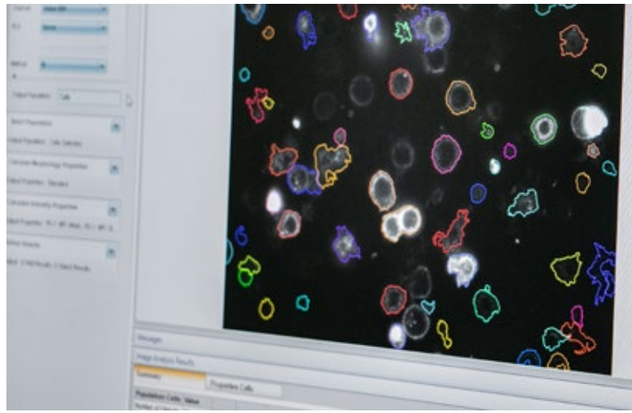
ACTIVE RESEARCH PROJECTS

- *Development of inhibitors of the Lysine-specific demethylase 4A (Kdm4A) for new anticancer therapies.* The project aims to discover new molecules able to effectively and selectively inhibit the Kdm4A enzyme. To this purpose, the screening will be performed using an enzymatic primary assay to screen libraries of molecules selected through a virtual screening approach. After successfully completing primary assay optimization, miniaturization and validation, the first screening campaign has been launched in December 2020;

- *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 of selectively inhibiting the activation of NLRP3. The primary assay that will be used for this screening campaign is a phenotypic assay in which the release of the cytokine IL-1β from human macrophages is measured following selective activation of the NLRP3 inflammasome. During 2020, the primary assay has been optimized and miniaturized and the validation phase has started. An orthogonal assay that measures the release of lactate dehydrogenase (LDH) has been developed and will be used for the confirmation of primary actives.

PUBLICATIONS

Cellular Models and Assays to Study NLRP3 Inflammasome Biology. Zito G, Buscetta M, Cimino M, Dino P, Bucchieri F, **Cipollina C**. Int J Mol Sci. 2020 Jun 16;21(12):4294. doi: 10.3390/ijms21124294.



Medicinal Chemistry

Platform

CONTACTS:
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The Medicinal Chemistry group is focused on the design and chemical synthesis of novel compounds (small molecules, peptides and peptidomimetics), together with the creation of compound libraries and building blocks collections. The ultimate goal is the discovery of new active ingredients towards the biological target of interest for therapeutic treatment. Moreover, the Medicinal Chemistry platform supports the Drug Discovery Unit in the early phases of screening campaigns, with hit structures confirmation, hit re-synthesis and validation, hit series expansion; and in the later stages of hit optimization, and hit-to-lead. Main tasks of the group are the design, the organic synthesis, the structural elucidation, and the analytical characterization of newly synthesized compounds, as well as the structure-activity-relationship (SAR) exploration studies, aiming at improving the hits pharmacokinetic profile (potency, efficacy, toxicity, selectivity, solubility and permeability). The platform uses classical manual techniques for a restricted number of samples, and automated systems for bigger collections size, with the advantage of saving time and resources, during the steps of the entire work-flow, and including: a) reactions set-up; b) reaction mixtures purification; c) isolation of compounds of interest; d) structure elucidation and characterizations; e) standard purity grade assessment for testing compouds.

COLLABORATIONS:

Luigi Vanvitelli University of Campania - Naples, Italy

Expertise

- Drug design;
- Organic synthesis;
- Microwave-assisted chemistry;
- Solid-phase synthesis;
- Peptide chemistry;
- Combinatorial chemistry;
- Purification of complex reaction mixtures in normal and reverse phase;
- Structural elucidation and analytical characterization;
- Quality control 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.

Implementation:

- Microwave reactor: Homeogenous and heterogenous catalytic transformations;
- Freeze dryer: Water content removal;
- Solvents purification system: Safe and rapid production of anhydrous solvents, required in air-free reactions;
- Peptide synthesizer: Preparation of dozens of peptides at the same time.

ACTIVE RESEARCH PROJECTS

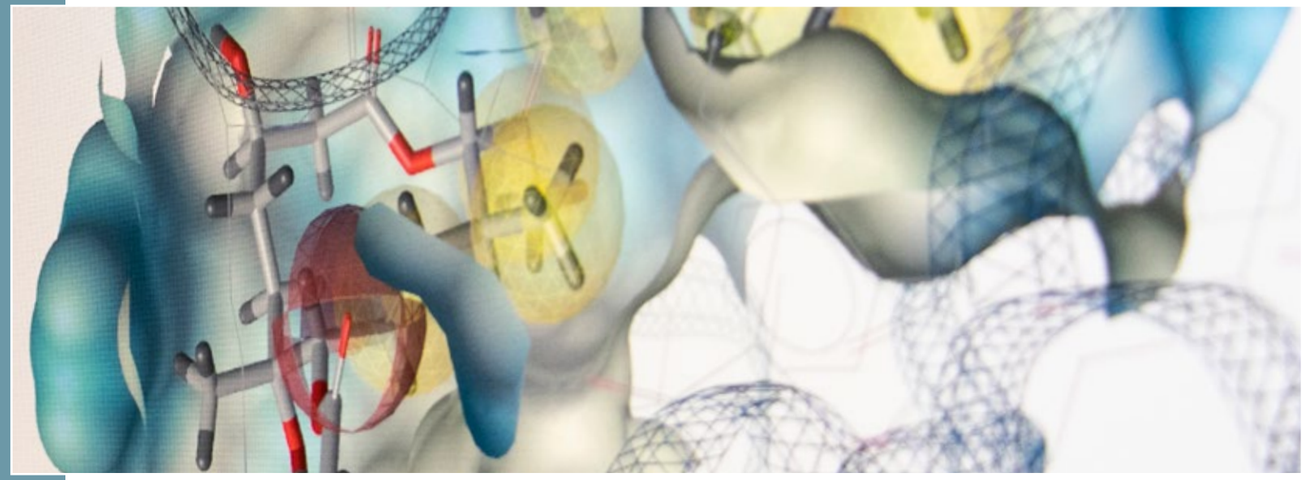
- Design and synthesis of new selective allosteric inhibitors of NLRP3 inflammasome, validated target for age-related diseases
- Design and synthesis of new KDM4A selective inhibitors, as potential anti-cancer drugs

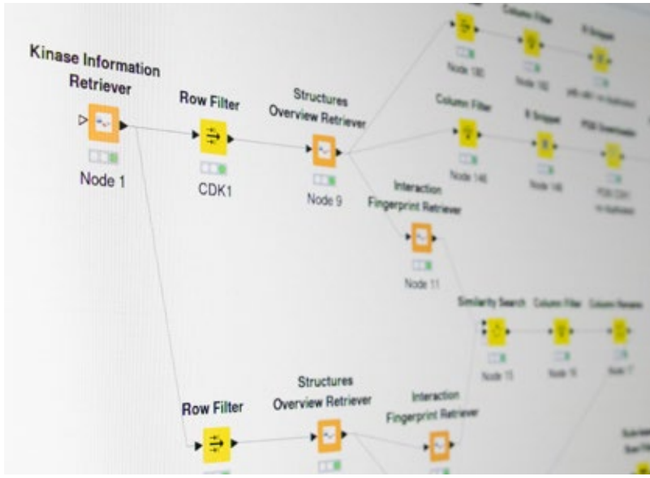
PUBLICATIONS

- *Dynamic-shared pharmacophore approach as tool to design new allosteric PRC2 inhibitors, targeting EED binding pocket*. Lombino, J.; Gulotta, M. R.; De Simone, G.; Mekni, N.; **De Rosa, M**.; Carbone, D.; Parrino, B.; Cascioferro, S. M.; Diana, P.; Padova, A.; Perricone, U. Mol. Inf. 2020, 39, 1-10.
- T*argeting SARS-CoV-2 RBD Interface: A Supervised Computational Data-Driven Approach to Identify Potential Modulator*s. Gulotta, M. R.; Lombino, J.; Perricone, U.; De Simone, G.; Mekni, N.; **De Rosa, M**.; Diana, P.; Padova, A. ChemMedChem 2020, 15, 1–12.
- *In silico insights towards the identification of NLRP3 druggable hot spots*. Mekni, N.; **De Rosa, M**.; Cipollina, C.; Gulotta, M. R.; De Simone, G.; Lombino, J.; Padova, A.; Perricone, U. Inter. J. Mol. Sci. 2019, 20, 4974 (1-13).

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-Troughput (HTS) mode. In the last year, the collaboration with the computer engineering group 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.

COLLABORATIONS:

- Institut de La Vision, Paris, France
- University of Vienna Department of Pharmaceutical Sciences, Austria
- University of Pittsburgh, USA
- University of Naple Federico II, Italy
- Consiglio Nazionale delle Ricerche IRIB-CNR, Palermo, Italy
- University of 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;
- Neural Network in Drug Design.

Technology platform

Software

- Schrodinger suite for small molecule drug discovery;
- LigandScout expert suite;
- Autodock and Autodock Vina;
- Desmond (OPLS3 and OPLS3e);
- AMBER;
- NAMD;
- VMD;
- Gromacs;
- RDKit;
- KNIME.

Hardware

- 6 Workstations;
- Server in HPC mode: 80 CPUs and 2 x NVIDIA Tesla K80.

Calculation capability:

- Library optimisation → ~ 6,000 molecules/min
- *Virtual Screening* HTVS → ~ 5,000 molecules/min
- *Virtual Screening* SP → ~ 1,500 molecules/min
- Molecular Dynamics → ~ 2200 ns/day/Card (on 40,000 atoms system)

Integrated in Silico Platform

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

ACTIVE RESEARCH PROJECTS

- Design of selective inhibitors of the **CD14** target involved in age-related degenerative maculopathy.
- Design of inhibitors of **NLRP3** as targets of inflammatory pathology .
- Research of protein modulators involved in the epigenetic regulation of tumor pathology (**KDM4, EZH2**).
- 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.
- Progettazione di inibitori della **Main protease (Mpro)** SARS- CoV-2.
- Deep learning and machine learning approaches for *in silico* profiling development.

PUBLICATIONS

- Jessica Lombino, Maria Rita Gulotta, Giada De Simone, Nedra Mekni, Maria De Rosa, Daniela Carbone, Barbara Parrino, Stella Maria Cascioferro, Patrizia Diana, Alessandro Padova, and **Ugo Perricone**, *Dynamic-shared pharmacophore approach as tool to design new allosteric PRC2 inhibitors, targeting EED binding pocket*, 2020, Molecular Informatics, doi: 10.1002/minf.202000148.
- Giulia Culletta, Maria Rita Gulotta. **Ugo Perricone**, Maria Zappalà, Anna Maria Almerico, Marco Tutone, *Exploring the SARS-CoV-2 Proteome in the Search of Potential Inhibitors via Structure-Based Pharmacophore Modeling/Docking Approach*, Computation 2020, 8, 77, doi: 10.3390/computation8030077.
- Maria Rita Gulotta, Jessica Lombino, **Ugo Perricone**, Giada De Simone, Nedra Mekni, Maria De Rosa, Patrizia Diana, Alessandro Padova, *Targeting SARS-CoV-2 RBD interface: a supervised computational data-driven approach to identify potential modulators*, 2020, ChemMedChem 2020 Jul 23. doi: 10.1002/cmdc.202000259.
- Jose A Souto, Federica Sarno, Angela Nebbioso, Chiara Papulino, Rosana Álvarez, Lucia Altucci, Jesica Lombino, **Ugo Perricone**, Alessandro Padova, Ángel R. De Lera, *A new family of JmjC-containing domain KDM inhibitors inspired on natural product purpurogallin,* 2020 , Front Chem May 25;8:312. doi: 10.3389/fchem.2020.00312.
- Isabella Mendolia, M.D; Salvatore Contino; **Ugo Perricone**; Edoardo Ardizzone; Roberto Pirrone, *Convolutional Architectures for Virtual Screening*, 2020, BMC Bioinformatics, doi https://doi.org/10.1186/s12859-020-03645-9.
- Cascioferro S., Li Petri G., Parrino B., El Hassouni B., Carbone D., Arizza V., **Perricone U.**, Padova A., Funel N., Peters G.J., Cirrincione G., Giovannetti E., and Diana P., *3-(6-Phenylimidazo [2,1-b][1,3,4]thiadiazol-2-yl)-1HIndole Derivatives as New Anticancer Agents in the Treatment of Pancreatic Ductal Adenocarcinoma*, Molecules 2020, 25, 329; doi:10.3390/molecules25020329.

Proteomics

Platform

CONTACTS:
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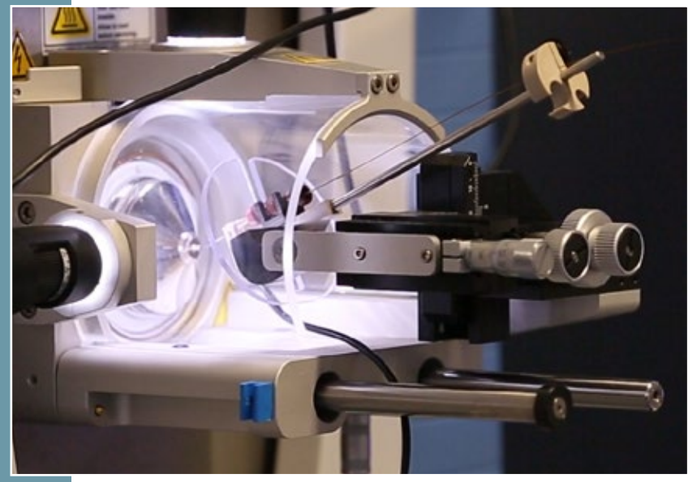
sdscilabra@fondazionerimed.com

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

COLLABORATIONS:

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

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

Software:

- Chromeleon;
- Xcalibur;
- Proteome Discoverer;
- MAX QUANT;
- Perseus for statistical analysis;

ACTIVE RESEARCH PROJECTS

iRhom2: a new therapeutic target for osteoarthritis

Osteoarthitis (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.

Generalized mass spectrometry based proteomic workflow

iRhom2 regulates surface levels of MHC class I molecules and immune responses

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. Investigating the molecular mechanism by which iRhom2 controls levels of class I molecules and functional consequences in vivo of this regulatory pathway are major aims of this project.

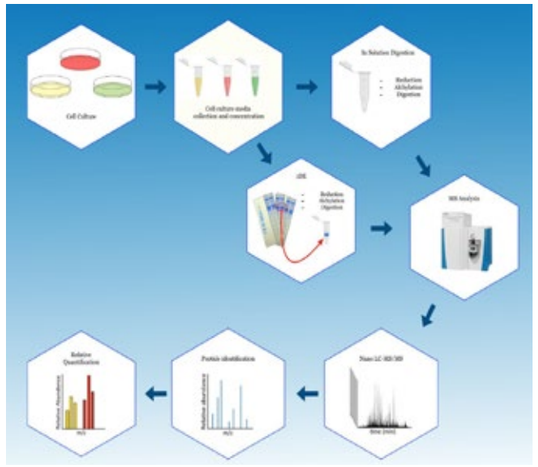
PUBLICATIONS

- 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. doi: 10.3390/molecules26040944. PMID: 33579029.

- Yang CY, Chanalaris A, Bonelli S, McClurg O, Hiles GL, Cates AL, Zarebska JM, Vincent TL, Day ML, Müller SA, Lichtenthaler SF, Nagase H, **Scilabra SD**, *Troeberg L. Interleukin 13 (IL-13)-regulated expression of the chondroprotective metalloproteinase ADAM15 is reduced in aging cartilage*. Osteoarthr Cartil Open. 2020 Dec;2(4):100128. doi: 10.1016/j.ocarto.2020.100128. PMID: 33381768; PMCID: PMC7762825.

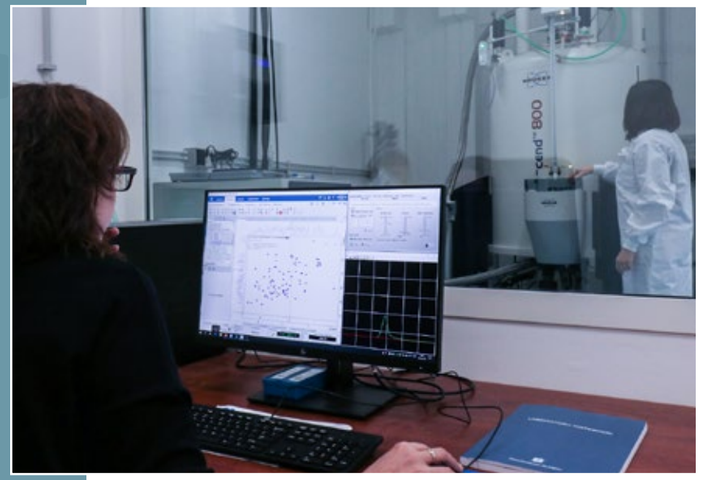
- Carreca AP, Pravatà VM, Markham M, Bonelli S, Murphy G, Nagase H, Troeberg L, **Scilabra SD**. *TIMP-3 facilitates binding of target metalloproteinases to the endocytic receptor LRP-1 and promotes scavenging of MMP-1*. Sci Rep. 2020 Jul 21;10(1):12067. doi: 10.1038/s41598-020-69008-9. PMID: 32694578; PMCID: PMC7374751.

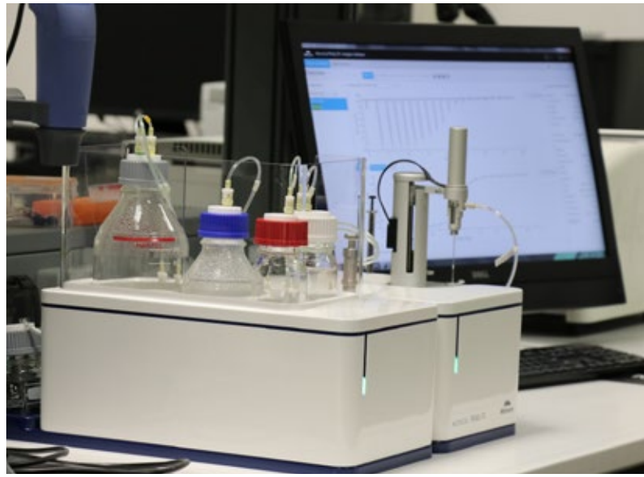
- *Quantitative mass spectrometrybased secretome analysis as a tool to investigate metalloprotease and TIMP activity*. Methods in Molecular Biology, 2020, 2043:265-273



Structural Biology and Biophysics

Platform





CONTACTS:
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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.

COLLABORATIONS:

- King's College London, London, United Kingdom
- TES Pharma, Perugia, Italy
- Scuola Normale Superiore of Pisa, Italy
- University of Perugia, Italy
- Luigi Vanvitelli University of Campania - Naples, Italy
- University of Palermo, Italy
- Institute of Biophysics (IBF) – (CNR), Palermo, Italy
- 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

- Investigation of the molecular mechanisms of protein misfolding diseases.
- Structural studies to elucidate the oligomerization mechanism of nucleophosmin protein NPM1.
- Generation of mussel-inspired bio-adhesives molecules able to work in wet environment.
- Structural and biophysical studies on KDM4a, a protein involved in the epigenetic regulation of tumor pathologies.
- Role of the interaction alfa-synuclein/membranes in Parkinson disease.
- Klebsiella pneumoniae proteins production for vaccine development.
- BLI- and NMR-based fragment screening.
- Structure/function relationship studies of SARS-CoV-2 proteins.

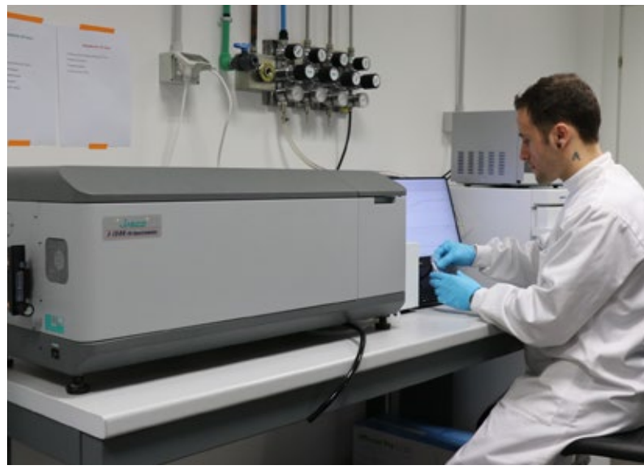
PUBLICATIONS

Astoricchio E., **Alfano C**., Rajendran L., Temussi P., Pastore A. (2020) *The Wide World of Coacervates: From the Sea to Neurodegeneration*. Trends Biochem Sci, 45(8):706-717.

Puglisi R., Brylski O., **Alfano C**., Martin S.R., Pastore A., Temussi P. (2020) *Quantifying the thermodynamics of protein unfolding using 2D NMR spectroscopy*. Comm Chemistry, doi: 10.1038/s42004-020-00358-1.

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, Accepted.

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, Accepted.



GRANTS

The Ri.MED Foundation supports its scientific activity through funding opportunities offered by public and private bodies, regional, national and international institutions. Accessing research funds is a strategic activity for the Foundation. For this reason, a Grants Office was established to select financial programs supporting biomedical research, specialized training and dissemination activities, submitting proposals (also in collaboration with other bodies) and managing relationships with administrations managing financing programs, as well as coordinating and supervising the approved projects.

During 2020, activities continued on the nine multi-year projects financed in 2018 and 2019 and currently in progress.
In 2020, three new R&D projects received funding under regional (PO FERS 2014-2020) and national (PON MIUR and PON MISE) funds. The Ri.MED Foundation is also host institution for the prestigious ERC Consolidator Grant of the European Commission (HORIZON 2020) obtained by a Ri.MED scientist. This very positive result recognizes the commitment and skills of our researchers.

Among the 2020 activities aimed at obtaining research funds, two projects were submitted for the HORIZON 2020 EU calls, four under national funds (MUR) and one under the scientific culture dissemination call (MUR).
In addition, ten new innovative industrial research doctorate proposals were submitted of which seven were funded. Moreover, one fellowship was funded by INPS.

Ongoing scientific project in 2020

CheMISt
Computational Molecular Design e Screening
Funded by: Assessorato alle Attività Produttive of the Region of Sicily within the scope of the "Patto per il Sud" (Interministerial Committee for Economic Planning – CIPE funds)

CheMISt aims at developing integrated laboratories and interdisciplinary research teams to support Ri.MED's research, in order to become a point of reference for scientific research on a regional, national and international level for public and private entities. The project involves the realization of four units according to the "lab hosting" formula: Structural Biology and Biophysics; Computational and Medicinal Chemistry; High Throughput Screening; Bioengineering.

RESEARCH INFRASTRUCTURES - "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 of the Region of Sicily

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.

OACTIVE
Advanced personalised, multi-scale computer models preventing OsteoArthritis
Funded by: HORIZON 2020 Action SC1-PM-17 European Commission

The goal of the project is to develop models to improve osteoarthritis diagnosis and treatment using a holistic approach, providing for the integration of patient-specific information from different sources (cells, tissues, organs) with behavioral patterns and socio-environmental risk factors.

The addition of simvastatin portal venous infusion to cold storage solution of explanted whole liver grafts for facing ischemia/reperfusion injury in an area with low rate of deceased donation
Funded by: Italian Ministry of Health within the scope of the 2013 Finalized Research Program

The goal of the project is to test the clinical efficacy of simvastatin administration by gavage three hours prior to whole liver cross-clamp in the deceased donor. This monocentric, prospective, randomized, double-blind study provides for the enrollment of 106 patients and comparison with standard procedure on placebo sample.

PROGEMA
Green processes for the extraction of active ingredients and the purification of waste and non-waste matrices
Funded by: Italian Ministry of Education, University and Research, 2014-2020 PON Research and Innovation

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

PROMETEO
Advanced Medicinal Products placenta-derived for liver and endometrial diseases
Funded by: Region of Sicily - PO FERS Action 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 by-products will be tested on *in vivo* models of acute and chronic liver injury and on *in vitro* models for endometrial reactivation. All tasks will be optimized according to the principles of Good Manufacturing Practices (GMP) to develop cellular therapies.

Piano delle attività "TRASFERIMENTI PER METODOLOGIE INNOVATIVE NEL CAMPO DELLE BIOTECNOLOGIE 2018"
Funded by: Legge di stabilità Regione Siciliana Assessorato Salute Dipartimento DASOE

The goal is to support and improve Ri.MED - ISMETT research activities on regenerative medicine, immunotherapy, bioengineering and precision medicine.

OBIND
Oncological therapies through Biological Interaction Network Discovery
Funded by: Region of Sicily - PO FERS Action 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.

Progetto iRhom2
"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.

Projects eligible for funding in 2020

SENSO
Development of a miniaturized device for monitoring oxidative stress in cellular systems
Funded by: Region of Sicily - PO FERS Action 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.

4FRAILTY
Intelligent sensors, infrastructures and management models for the safety of fragile subjetcs
Funded by: Italian Ministry of Education, University and Research, 2014-2020 PON Research and Innovation

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.

NABUCCO
New Drugs and Biomarkes of pharmacological response and resistance in colorectal cancer
Funded by: Italian Ministry of Economic Development (MISE) Innovation Agreement Call

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.

BIOMITRAL
Engineering the mitral valve: bioinspired control of structure and function for enhanced *in vivo* performance
Funded by: European Commission HORIZON 2020 – ERC Consolidator 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.

Scientific projects submitted in 2020

PORTRAIT
Personalized healthcare data storage and analysis platform to support non-invasive diagnosis of liver steatosis, fibrosis and inflammation in NAFLD by artificial intelligence
Call: H2020-SC1-2020- HORIZON 2020 – European Commission

The aim of the project is to create new tools for an accurate, non-invasive and personalized assessment of the progression of non-alcoholic steatopatitis (NASH) by the engineering of a framework able to collect and aggregate clinical data from several European hospitals and by using machine learning and deep learning techniques.

JEDI GRANDCHALLENGE – BILLION MOLECULES AGAINST COVID-19
Call: Joint European Disruptive Initiative (JEDI)

The aim of the project is to identify through *in silico* screening potential compounds to inhibit three different therapeutic targets associated with coronavirus.
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Meet Ri.MED
An experience inside the research laboratories

The Foundation participated as consultant in the 2020 Special Integrative Fund for Research FISR-MIUR COVID 19 Call with the following projects:

- **SAAB: Targeting of genes that cause SARS-CoV-2 disease**
- **DROPS: Development of an advanced platform for the systemic assessment of personal protective equipment**
- **SEMAR: Nano-structured sensor for oxidative stress integrated in a mask for the remote evaluation**
- **SOAR-MIA: Early warning operational strategy through statistical monitoring of epidemiological data and Artificial Intelligence**

INTELLECTUAL PROPERTY AND TECHNOLOGY TRANSFER

Research activity of Ri.MED is strongly patient-oriented, but in order to ensure 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 license, industrial sponsorship and creation of technological spin-offs.

In addition to filing new patent applications and managing the existing portfolio, in 2020 Ri.MED completed the acquisition of the patent family "Triskele" from UCL Business, whose inventors include Gaetano Burriesci, Bioengineering group leader of the Foundation. The patent family consists of a heart valve prosthesis prosthetic, a heart valve and their related delivery system. A license and collaboration agreement for this patent family is currently under negotiation between Ri.MED and a Chinese tech company.

Patent portfolio up to 31.12.2020

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

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

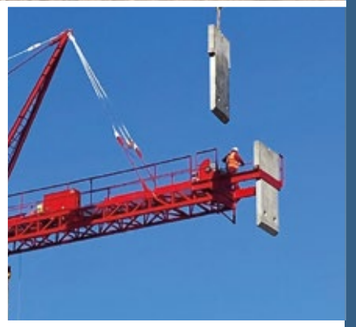
Semi-rigid annuloplasty ring and method of manufacturing
WO2019220365
Fondazione Ri.MED

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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