Fondazione Ri.MED

Scientific REPORT 2019

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FOUNDING PARTNERS

Presidenza del Consiglio dei Ministri

Regione Siciliana

Consiglio Nazionale delle Ricerche

University of Pittsburgh

UPMC LIFE CHANGING MEDICINE

PARTNER

ISMETT Istituto di Ricovero e Cura a Carattere Scientifico

COVER PICTURE:
Polymeric transcatheter heart valve (non-surgical)

A MEDITERRANEAN **HUB** FOR TRANSLATIONAL RESEARCH

Alessandro Padova

DIRECTOR GENERAL

Fondazione Ri.MED closed successfully the year 2019 completing all the formal steps towards the ground-breaking milestone for the **BRBC** construction thanks to a dedicated team committed to address technical, administrative and legal aspects with the instrumental support of the Italian partners, from the Presidency of the Council of Ministers, the Region of Sicily and the Italian National Research Center (CNR) and the leadership of the US partners, the University of Pittsburgh and University of Pittsburgh Medical Center (UPMC). It is important to underline that Fondazione Ri.MED has also executed legality and anticorruption protocol agreements with ANAC (Italian Anti Corruption Agency) and Palermo Prefecture respectively.

Important steps were moved towards the realization of the Ri.MED institutional mission. In terms of **translational research**, several research agreements were signed at regional, national and international level. The portfolio was consolidated with target-based projects entering into the drug discovery phase and others moving towards preclinical proof-of-concept. Several **grants** were awarded at regional and national level. There was also a strong effort from the science teams, together with the Grants and Administrative Offices, to submit several novel grant applications. **Technology platforms** and research areas were consolidated thus ensuring high level of productivity thanks to the continuous support of the Purchasing Office.

New profiles were appointed according to the 2019 approved **human resources** plan in order to potentiate admin offices, such as the technical office, as well as specific technology platforms and projects. **Custom training programs** were organized covering key competencies such as leaderships or relevant topics as intellectual property.

The 2019 Ri.MED International Symposium “Organ Insufficiency Change It or Fix It” was a successful event intended to highlight the strength of the IRCCS ISMETT-Ri.MED Cluster, with the underlying contribution of UPMC and UP.

I would like to acknowledge Ri.MED Scientific Director Dario Vignali and the Scientific Committee Members for the constant leadership in developing a vision for Ri.MED translational research. A special thanks to Ri.MED Board of Directors, and in particular to our President Paolo Aquilanti and to Vice President Bruno Gridelli and to our legal advisor Giuseppe Mazzarella that constantly work, behind the scene, to fulfill 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 2019 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 three research areas: **cancer,** with an emphasis on immunotherapy, organ insufficiency, which includes organ transplantation and **regenerative medicine**, and **diseases of aging**, with an emphasis on neurodegeneration.

In 2019, thanks to the commitment and effort of our Scientific Committee (Prof. Francesco Dieli, Prof. Ivet Bahar, Prof. George Fadi Lakkis, Prof. Claudio Bordignon, Prof. Antonino Cattaneo), multiple topics were discussed, with two particularly impactful. First, we conducted an extensive scientific review of all the research programs at Ri.MED. Second, we established a new Ri.MED Postdoctoral fellowship program and conducted the first round of recruitment, with three fellowships awarded.

The 2019 edition of our annual Ri.MED Scientific Symposium was entitled "**Organ Insufficiency: change it or fix it**", co-organized by Profs. Fadi Lakkis and Riccardo Gottardi. This event was again held in Palermo and brought together a panel of international experts. It met with considerable public success, with a sizeable attendance, confirming the role that Ri.MED can and intends to play in the international scientific community and in the region.

In conjunction with the Symposium, we held our **2nd Ri.MED Research Retreat** during which there were nine team presentations, a poster session and team building events. This proved to be a terrific event that allowed all Ri.MED investigators and trainees to share their results and served as an important opportunity to explore new scientific collaborations.

A major focus of 2020 for the Scientific Committee will be to develop a research strategic plan in order to build a path towards the opening of the Ri.MED Biomedical Research and Biotechnology Center in Sicily.
A commitment to excellence and growth of our researchers, the 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.

TABLE OF CONTENTS

Ri.MED OVERVIEW UP TO 31.12.2019

Intellectual property

About **300** Scientific publications

25 Patents

1 Patent proposal

Training & employment

54 Employees in 2019

57% 43%

30 Scholarships

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

14 Ph.D. Fellowships

21 Internships

Fundings for Research

16.849.193€ Won through national and international GRANTS

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

Networking

3 Agreements for labs management

48 Active scientific collaborations in 2019

19 Scientific collaborations signed in 2019

Scientific knowledge dissemination

9 Ri.MED institutional events

37 Ri.MED scientific meetings

7 Ri.MED internal meetings

30 Participations in scientific events or local development activities

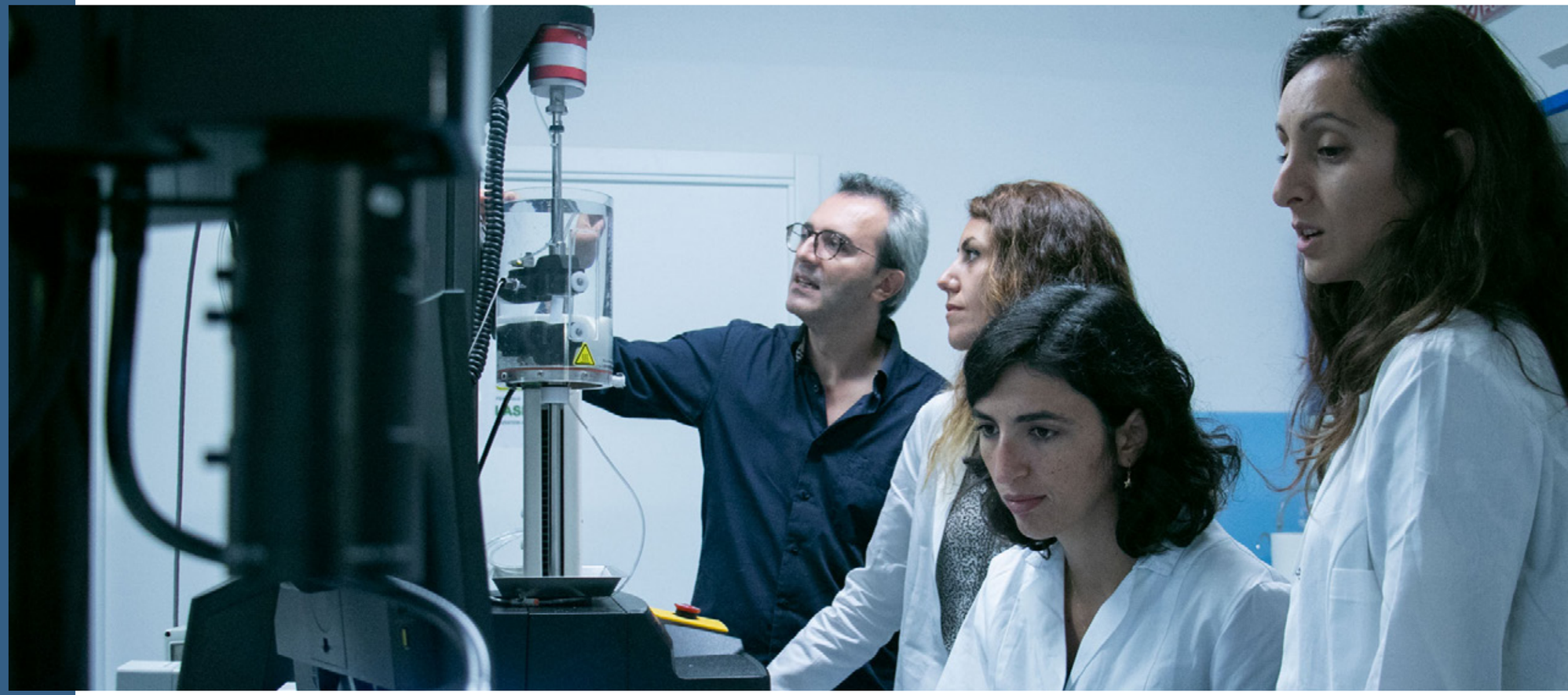
Building the BRBC

28.000 sq m of laboratories

196.350.000 € Value of the investment

600 Planned occupancy opportunities

TRAINING
Nurturing new talents



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

To date, Ri.MED has activated training programs including 21 traineeships, 30 scholarships, 14 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 post-docs as part of the **Ri.MED Fellowship**.

During 2019, three new brilliant researchers were selected to travel to Pittsburgh and Philadelphia in 2020.

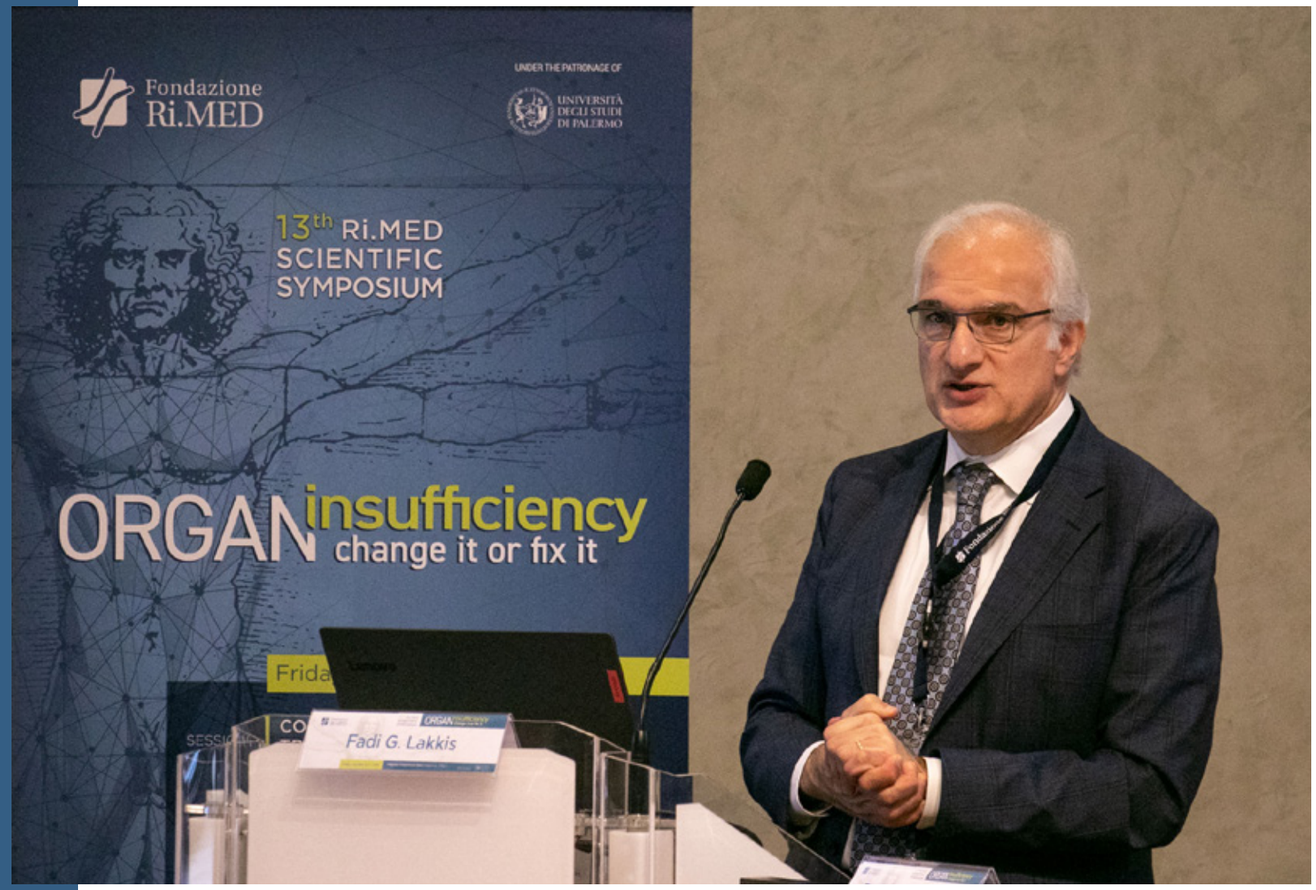
Numerous **training programs** were activated in the scope of projects funded by European, Italian and regional grants: thanks to the co-financing of these scientific projects, dozens of highly-qualified professions were trained in 2019.

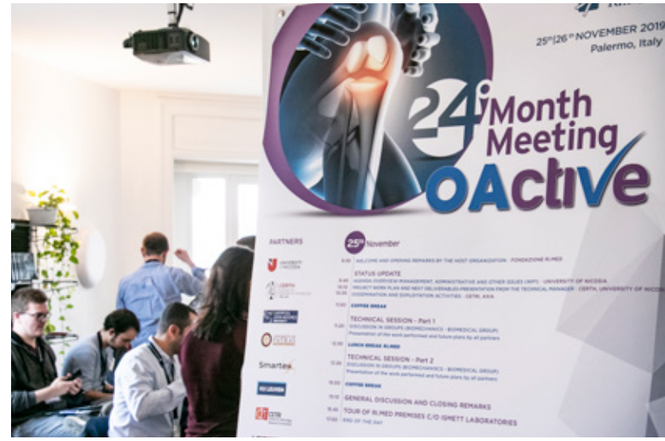
Last autumn Ri.MED also obtained from FonARCom the approval of the "**Innovation and Development" training plan** for the provision of corporate training courses for its employees.

DISSEMINATION OF SCIENTIFIC KNOWLEDGE

Activities linked to scientific dissemination and sharing of research results are part of the Foundation's own missions.

The 2019 edition of the annual RI.MED Scientific Symposium was met with great interest. Co-chairs of the symposium were Fadi G. Lakkis, scientific director of the Thomas E. Starzl Transplantation Institute and member of the Ri.MED Scientific Committee, and Riccardo L. Gottardi, director of the Bioengineering and Biomaterials Laboratory, Children's Hospital of Philadelphia, and Ri.MED Principal Investigator.





lunedì Siciliano

La Storia

La biologa Ester Badami in Ri.Med e Ismett studia come potenziare le difese immunitarie dei pazienti trapiantati di fegato

Tornata a fare ricerca a Palermo

Dr. Lakkis and Dr. Gottardi assembled a panel of top international experts in Palermo to discuss the topic of "**Organ Insufficiency: change it or fix it**".

In 2019, the Foundation hosted the meeting of the **Horizon 2020 project OActive**, of which Ri.MED is one of the 13 European partners: a project focused on early diagnosis and the development of rehabilitation approaches to prevent osteoarthritis.

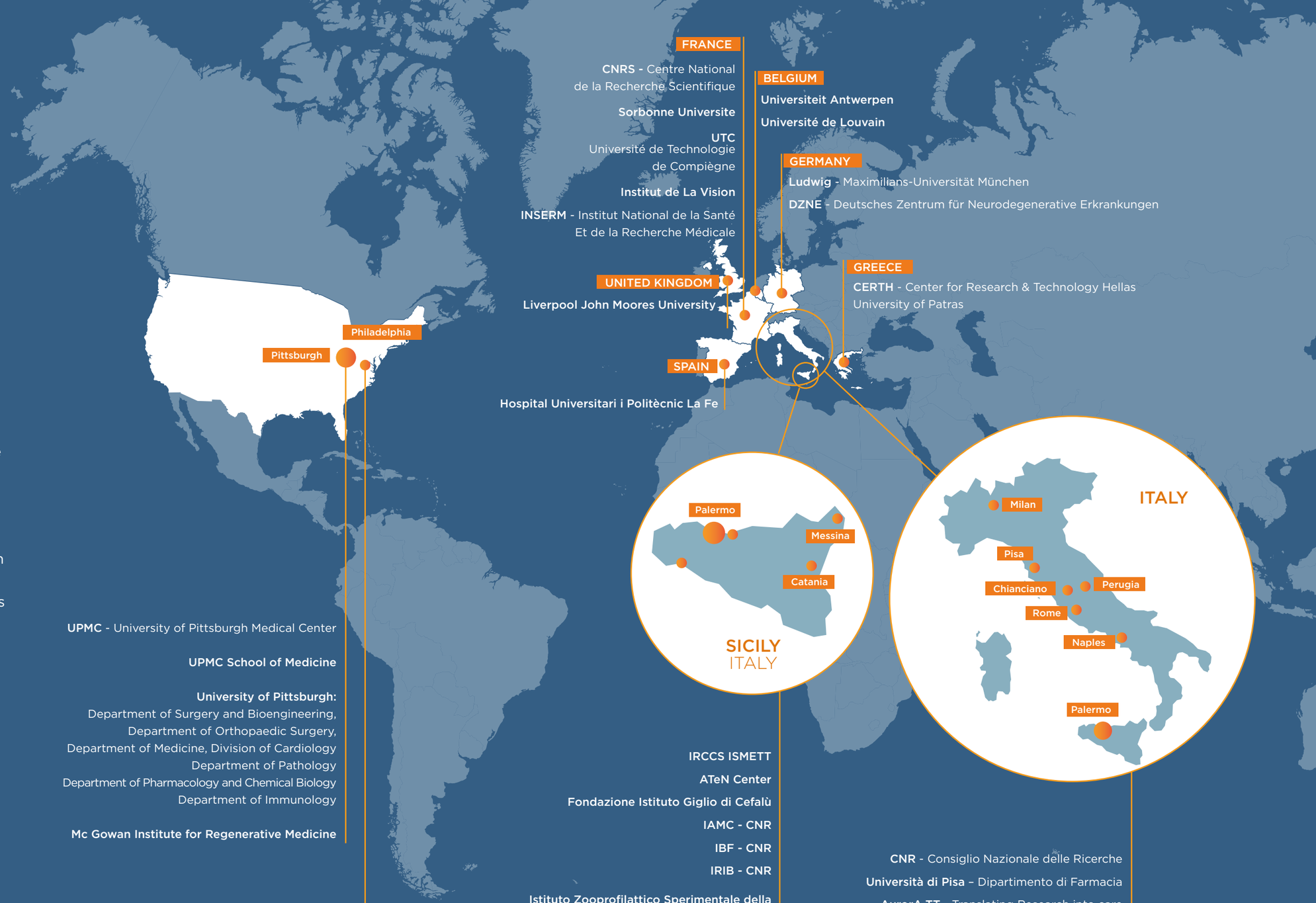
In the meantime, our researchers continue to publish scientific articles on peer review journals with relevant impact factor, contributing to the scientific dissemination of the sector, and generating intellectual property, while our press office works to transfer the most important results to a wider audience.

NETWORKING

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

Ri.MED focuses on the continuous development of its network of scientific collaborations and scientific agreements with bodies and institutions operating in its areas of interest: there are currently 48 agreements in place to develop technological innovation, promote research activities and sharing laboratories and resources with European and U.S. institutions. Nineteen agreements were signed during 2019.

Ri.MED signed agreements for lab hosting: the management of the Regenerative Medicine and Immunology laboratories at IRCCS ISMETT, of strategic importance for integrating basic and clinical research, has already been operating for years.
Last year was characterized by the strong expansion of the Structural Biology and Biophysics laboratories at ATeN Center, of the Bioengineering and Medical Devices Lab at the University of Palermo and of the High Throughput Screening Lab at the CNR IRIB.



UPMC - University of Pittsburgh Medical Center

UPMC School of Medicine

University of Pittsburgh:
Department of Surgery and Bioengineering,
Department of Orthopaedic Surgery,
Department of Medicine, Division of Cardiology
Department of Pathology
Department of Pharmacology and Chemical Biology
Department of Immunology

Mc Gowan Institute for Regenerative Medicine

CHOP - Children's Hospital of Philadelphia

IRCCS ISMETT

ATeN Center

Fondazione Istituto Giglio di Cefalù

IAMC - CNR

IBF - CNR

IRIB - CNR

Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri"

Università degli Studi di Palermo

Università degli Studi di Messina

Università degli Studi di Catania

LNS-INFN
Laboratori Nazionali del Sud dell'Istituto Nazionale di Fisica Nucleare

CNR - Consiglio Nazionale delle Ricerche

Università di Pisa – Dipartimento di Farmacia

AurorA-TT - Translating Research into care

Università degli Studi della Campania Luigi Vanvitelli
Dipartimento di Medicina di Precisione

Epi-C Epigenetic Compounds

UPMC Institute for Health - Chianciano Terme

TES PHARMA

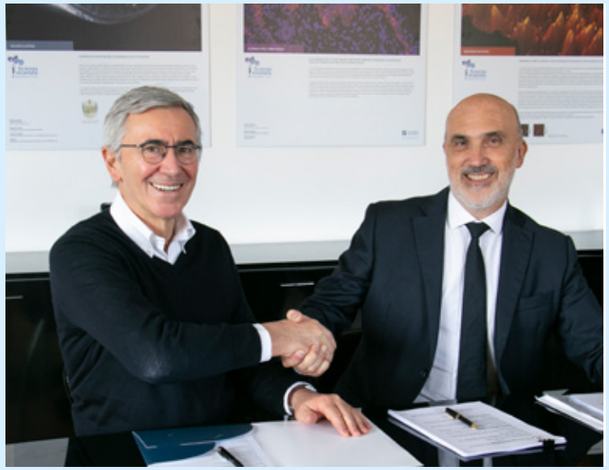
Università degli Studi Roma Tre

BRBC

Socio-economic impact on Sicily and Southern Italy

Alongside research activity, 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 entering partnerships with universities and research centers, and with pharmaceutical and biotechnological companies, developing strategic alliances and attracting funds and investments for research, with a positive impact on the economy of southern Italy.



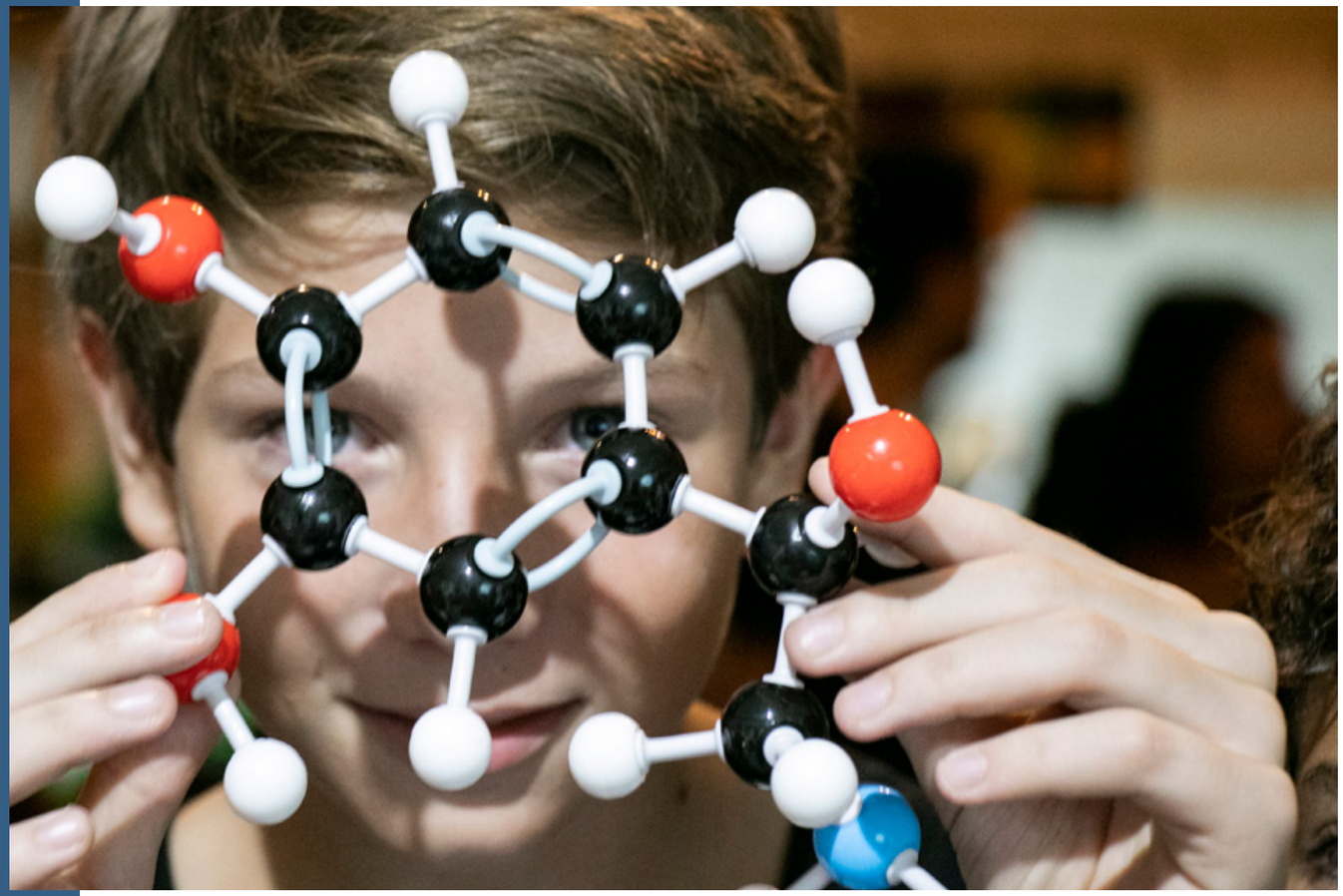
During 2019 the contract for the construction of the BRBC was awarded to the temporary association of enterprises (ATI) led by Italiana Costruzioni.

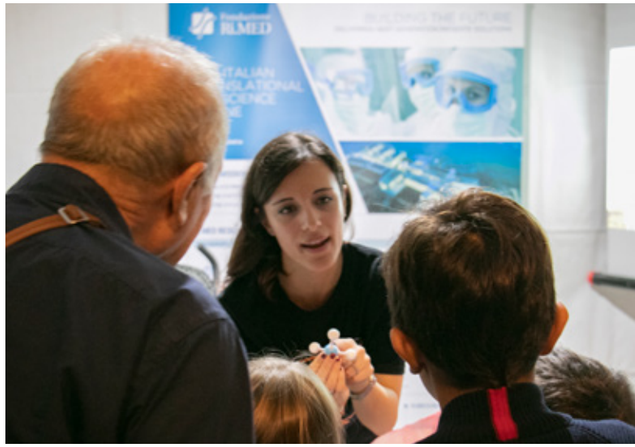
This was the final chapter of a process that had begun with the publication of the international design competition won by a joint venture led by Hellmuth, Obata & Kassabaum Inc., followed by the approval of the preliminary, definitive and executive projects, and, finally, with the publication of the restricted procedure for the awarding of the construction works, ended in June 2019. The construction site will open in the first months of 2020 and construction works are expected to end within two years.

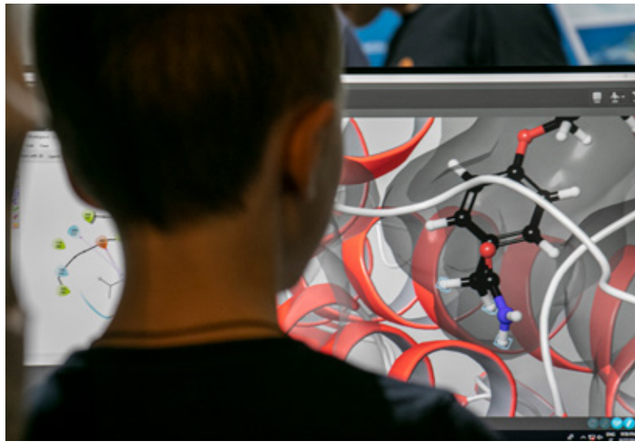
The BRBC will attract members of the international scientific community in Palermo, retaining the best Italian doctors and scientists in our country, also thanks to the collaboration with UPMC and the University of Pittsburgh (UP).

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.

In 2019 Ri.MED took part in the **European Researchers' Night** with the initiative "From molecules to the heart", organized the "**Research Friday**" laboratories of applied sciences at the STEM High School of CEI in Palermo, continued its collaboration with the Institute of Carini “Mursia”, and started a new project with the Industrial Technical Institute “Volta” of Palermo to train technicians specialized in the use of diagnostic and biomedical devices.

The first edition of "**Incontra Ri.MED**" (Meet Ri.MED), a series of five information seminars on specific research areas (from *Big Data* to ATMPs) designed for university students' orientation on tasks, challenges and job opportunities in *life sciences*, was a great success.

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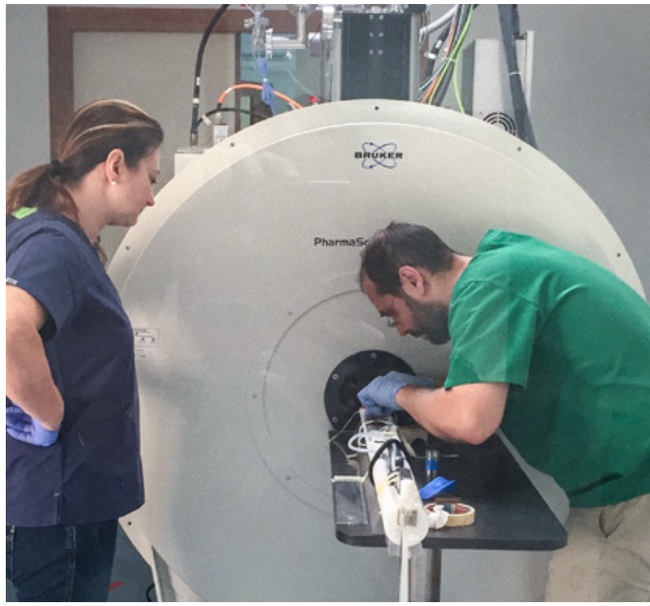

MOLECULAR INFORMATICS

Computer Aided Drug Design

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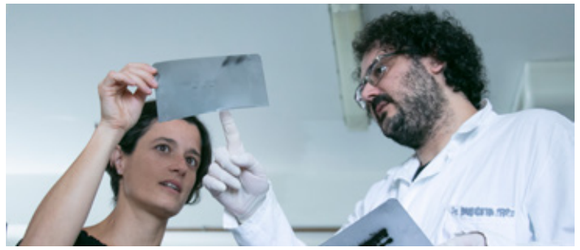

IDENTIFICATION OF THERAPEUTIC TARGETS AND SCREENING

Inflammatory diseases

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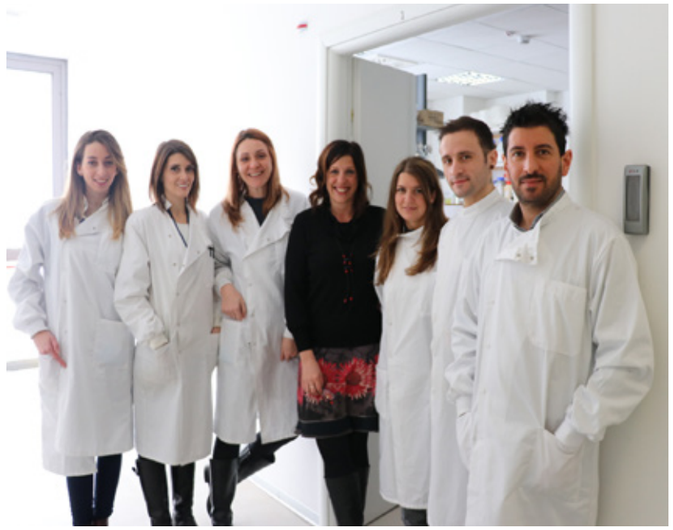

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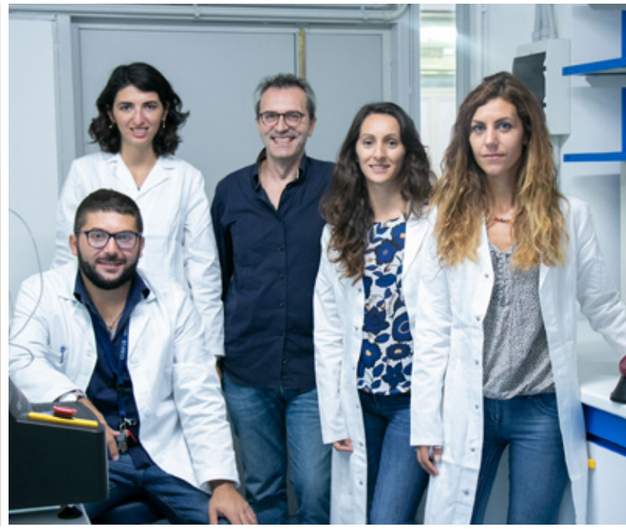

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Ri.MED Lab c/o Università di Palermo

Ri.MED SUPPORTED SCIENTIST

Courtesy of Giuseppe Peritore

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DRUG DISCOVERY

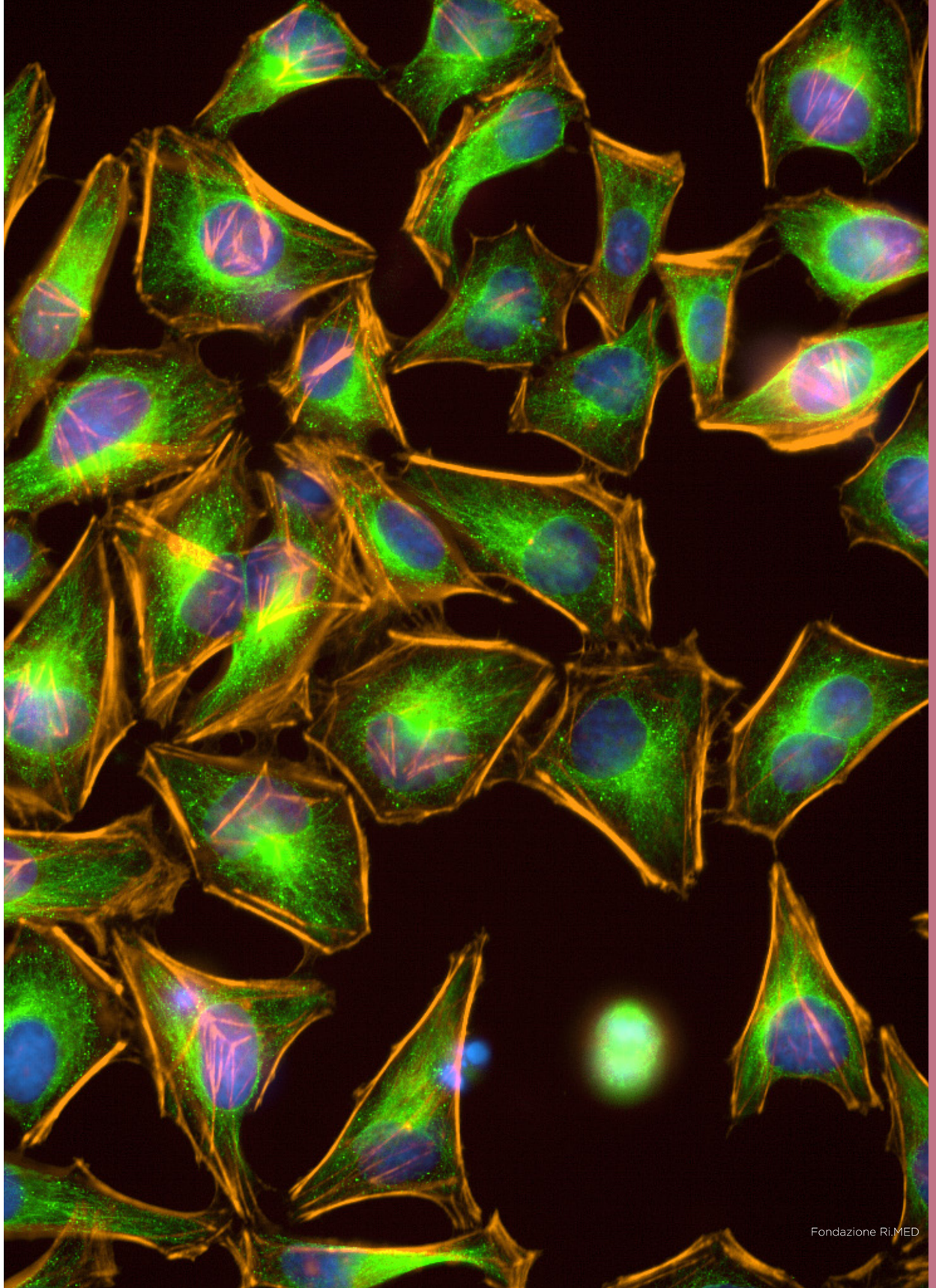
Ri.MED Foundation researchers are involved in drug discovery projects that aim to identify new biologically-active molecules.

Studying biomolecular pathways integrated with genomics, proteomics, metabolic and secretomics data, allowed our researchers to reach 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 the 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. Also, 2019 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.



Molecular mechanisms of protein misfolding diseases
Caterina Alfano, PhD

Elucidation of the binding mode of molecules able to interfere with the oligomerization process of NPM1
Caterina Alfano, PhD

Novel crosstalk between epithelial colon cells and macrophages in ulcerative colitis and colitis associated cancer
Sandra Cascio, PhD

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

Modeling microRNA-target interaction network
Claudia Coronnello, PhD

Pharmacology of nitro-nitrate lipids
Marco Fazzari, PhD

Design of CD14 modulators in aging-related macula degeneration
Ugo Perricone, PhD

Design of non-covalent inhibitors of NLRP3 as potential novel anti-inflammatory drugs
Ugo Perricone, PhD

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

PRODUCTS: **DRUGS - BIOLOGICS**

Molecular mechanisms of protein misfolding diseases

Caterina Alfano, PhD
calfano@fondazionerimed.com

COLLABORATIONS

- UK Dementia Research Institute (UK DRI) - King's College London, London, UK

- Department of Physics and Chemistry (DiFC) – University of Palermo, Italy

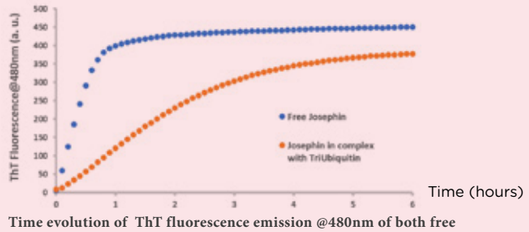
THERAPEUTIC AREA

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 relies 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.



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

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 project has several aspects of interest and originality.
The cellular importance of the protein chosen as model system, ataxin-3, is testified by its involvement in diseases which are 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 2019

We focused on the effect of polyubiquitin chains in the growth of fibrils of the N-terminal catalytic domain of ataxin-3, named *Josephin*. The latter, although not containing the polyglutamine tract and being spatially far from it, has been involved in protein misfolding and it is the element that determines the aggregation properties and morphologies in non-expanded ataxin-3. Our results confirmed our hypothesis that the formation of ThT-positive aggregates is strongly inhibited by the presence of polyubiquitin chains, natural binding partners of ataxin-3.
This is in agreement with the known aggregogenic role of *Josephin* and with the hypothesis that when ataxin-3 is in its free state exposes the Ubiquitin-interacting surfaces sited on *Josephin* domain. The hydrophobic nature of those surfaces triggers the aggregation and explains the role of *Josephin* as nucleation core in the aggregation pathway also of the full-length 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.

GOALS FOR 2020

We aim at a better understanding of the role of protein-protein interactions in ataxin-3 aggregation in order to use this information to design drugs specific against ataxin-3 aggregation. We plan to use an interdisciplinary approach that combines virtual screening approaches complemented by state-of-the-art biophysical techniques such as nuclear magnetic resonance, calorimetry, and interferometry in order to develop molecules able to target specific interactions and conformational states of ataxin-3. To be successful, the designed molecules must mimic the non-pathologic interactions in which ataxin-3 is involved. This will be obtained using peptidomimetics molecules library in the virtual screening approaches. The possible hits will be then confirmed by biophysical experiments.

MEETINGS

- XLVIII National Congress on Magnetic Resonance, september, 2019, L'Aquila (Italy)

- Resonance in Biology, september, 2019, Pavia (Italy)

PUBLICATIONS

- Santonocito R., Venturella F., Dal Piaz F., Morando M.A., Provenzano A., Rao E., Costa M., Bulone D., San Biagio P.L., Giacomazza D., Sicorello A., **Alfano C.***, Passantino R.*, Pastore A. (2019) Recombinant mussel protein Pvfp-5β: a potential tissue bioadhesive. J Biol Chem. 294(34):12826-12835. *Co-corresponding authors.

- Fricano A., Librizzi F., Rao E., **Alfano C.**, Vetri V. (2019) Blue autofluorescence in protein aggregates "lighted on" by UV induced oxidation. BBA - Proteins and Proteomics 1867(11):140258.

- Zacco E., Graña-Montes R., Martin S.R., de Groot N.S., **Alfano C.**, Tartaglia G.G., Pastore A. (2019) RNA as a key factor in driving or preventing self-assembly of the TAR DNA-binding protein 43. J Mol Biol. 431(8):1671-1688.

PRODUCTS: **DRUGS - BIOLOGICS**

Elucidation of the binding mode of molecules able to interfere with the oligomerization process of NPM1

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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

Cancer

PIPELINE

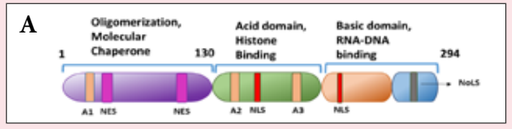
CLINICAL NEED | DISEASES ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

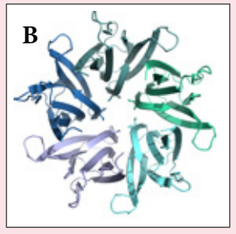
BRIEF DESCRIPTION

About one-third of patients affected by Acute myeloid leukemia (AML), an aggressive cancer of the myeloid cells, have an aberrant cytoplasmic expression of nucleophosmin protein (NPM1).
This is a highly abundant nucleolar phosphoprotein with functions associated with ribosome biogenesis, maintenance of genome stability, nucleolar stress response, modulation of the p53 tumor suppressor pathway, and regulation of apoptosis. It was found that about 35% of adult AML patients have heterozygous mutations at the C-terminus domain of NPM1 which leads to an aberrant expression of the protein in the cytoplasm.
The heterozygous nature of the mutations allows that a cert amount of the wild-type protein is retained by the nucleolus and it is able to fulfill its function and keep the AML cells alive. We aim at structurally characterize NPM1 and perform a biophysical screening to identify molecules able to block the physiological role of NPM1 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 resulting in a strong impact on health care, both because AML is the most common acute leukemia in adults and in about one-third of cases the disease is associated to NPM1 mutations.





A) Domain organizzation of Nucleophosmin NPM1. A1-A3: acidic regions; NES: nuclear export signal; NLS: nuclear localization signal; NoLS: nucleolar localization signal.

B) X-Ray Structure of the N-terminal oligomerization domain of NPM1, PDB ID 4N8M.

RESULTS ACHIEVED IN 2019

We performed nuclear magnetic resonance (NMR), circular dichroism (CD) and cross-linking experiments on three different constructs of the N-terminal domain of NPM1 (1-130, 1-130 S88E and 1-182) in order to find a stable monomeric form to be used in the screening of molecules able to interfere with the oligomerization state of the protein. Currently, all our results consistently highlight the presence of several oligomeric forms in all the tested conditions for all the three constructs. A deep understanding of the molecular bases which guide NPM1 oligomerization process remains far, but our results are crucial to understand that a classical drug discovery approach has high chance to result unsuccessful.

GOALS FOR 2020

The inhibition of the NPM1 oligomerization leads to the inhibition of important processes also in the healthy cell.
Together with the difficulties in expressing and purifying a homogeneous, folded and monomeric sample for a classical drug discovery approach, we propose to move towards the development of biologics drugs (nanobodies) to target NPM1.
Our goal for the next year is therefore the creation and selection of synthetic antibodies able to target mutated-NPM1 and characterize the resulting interactions both structurally and biophysically using nuclear magnetic resonance (NMR), isothermal calorimetry (ITC) and BioLayer Interferometry technique (BLI). The obtained information will then be used to generate single domain antibodies, also known as nanobodies.

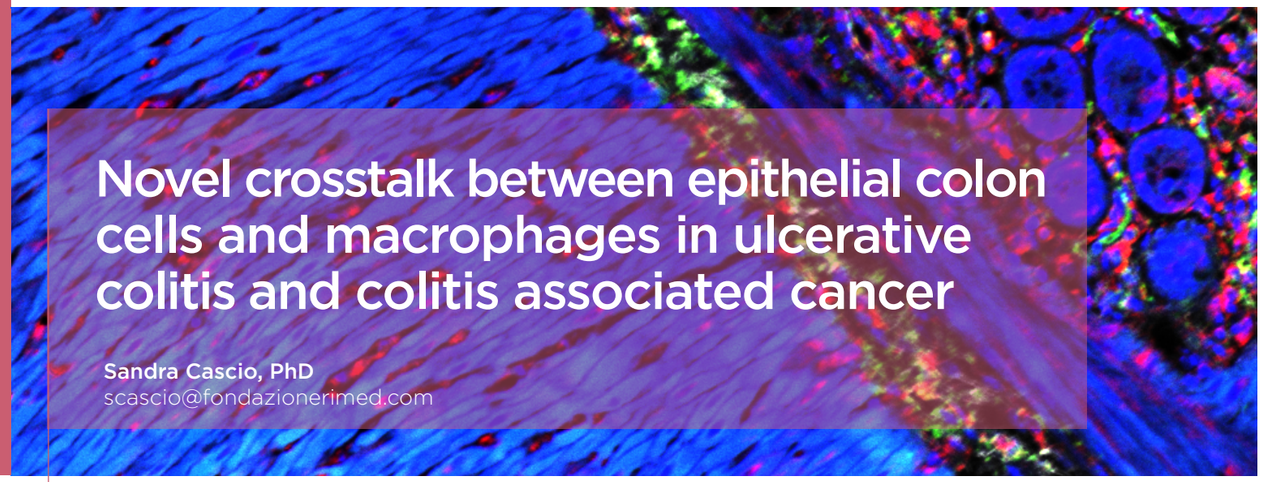
MEETINGS

- XLVIII National Congress on Magnetic Resonance, september, 2019, L'Aquila (Italy)
- Resonance in Biology, september, 2019, Pavia (Italy)

PUBLICATIONS

- Santonocito R., Venturella F., Dal Piaz F., Morando M.A., Provenzano A., Rao E., Costa M., Bulone D., San Biagio P.L., Giacomazza D., Sicorello A., **Alfano C.***, Passantino R.*, Pastore A. (2019) Recombinant mussel protein Pvfp-5β: a potential tissue bioadhesive. J Biol Chem. 294(34):12826-12835. *Co-corresponding authors.
- Fricano A., Librizzi F., Rao E., **Alfano C.**, Vetri V. (2019) Blue autofluorescence in protein aggregates "lighted on" by UV induced oxidation. BBA - Proteins and Proteomics 1867(11):140258.
- Zacco E., Graña-Montes R., Martin S.R., de Groot N.S., **Alfano C.**, Tartaglia G.G., Pastore A. (2019) RNA as a key factor in driving or preventing self-assembly of the TAR DNA-binding protein 43. J Mol Biol. 431(8):1671-1688.

PRODUCTS: **BIOMARKERS**



Novel crosstalk between epithelial colon cells and macrophages in ulcerative colitis and colitis associated cancer

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COLLABORATIONS

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THERAPEUTIC AREA

Cancer

PIPELINE

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

BRIEF DESCRIPTION

Patients with ulcerative colitis (UC) have an increased risk of developing colitis-associated colon cancer (CACC). Changes in glycosylation of the oncoprotein MUC1 commonly occur in chronic inflammation, including UC, and this abnormally glycosylated MUC1 has been shown to promote cancer development and progression. What causes changes in glycosylation of MUC1 is not known. Gene expression profiling of myeloid cells in inflamed and malignant colon tissues showed increased expression levels of inflammatory macrophage-associated cytokines compared to normal tissues. We analyzed the involvement of these cytokines and macrophages that produced them in the induction of aberrant MUC1 glycoforms. A co-culture system was used first to examine the effects of M1 and M2 macrophages on glycosylation-related enzymes in colon cancer cells. M2 macrophages induced the expression of the glycosyltransferase ST6GALNAC1, an enzyme that adds sialic acid to O-linked GalNAc residues, promoting the formation of tumor-associated sialyl-Tn (sTn) O-glycans. Immunostaining of UC and CACC tissue samples confirmed the elevated number of M2-like macrophages as well as high expression of ST6GALNAC1 and the altered MUC1-sTn glycoform on colon cells. Cytokine arrays and blocking antibody experiments indicated that the macrophage-dependent ST6GALNAC1 activation was mediated by IL-13 and CCL17. We demonstrated that IL-13 promoted phosphorylation of STAT6 to activate transcription of ST6GALNAC1. A computational model of signaling pathways was assembled and used to test IL-13 inhibition as a possible therapy. Our findings reveal a novel cellular cross-talk between colon cells and macrophages within the inflamed and malignant colon that contributes to the pathogenesis of UC and CACC.

IMPACT

The tumor microenvironment (TME) plays a pivotal role in disease progression. Tissue-infiltrating macrophages represent the most abundant immune cell type in the colon tumor microenvironment and play several roles in promoting tumor progression. A hallmark of macrophages is their plasticity and ability to change phenotype and function according to the immediate environment. Many open questions remain with regard to the specifics of the involvement of tissue-infiltrating macrophages in the pathogenesis and the chronicity of UC and progression to CAC. We have found that macrophage type 2 (M2) secrete inflammatory cytokines including IL-13 and CCL17 that induced overexpression of the glycosyltrasferase ST6GALNAC1 and the aberrant glycoform MUC1-sTn in inflamed and tumor colon cells. Both ST6GALNAC1 and MUC1-sTn are known to be over-expressed and hypoglycosylated in colon cancer. In addition, hypoglycosylated MUC1 establishes a positive feedback circuit of inflammatory cytokines aggravating chronic inflammation and promoting tumorigenesis. The identification of this specific aberrant MUC1-sTn glycoform and glycosyltransferases as drivers of tumor development and progression will result in novel disease-relevant targets for prevention and treatment of CACC.

RESULTS ACHIEVED IN 2019

We have elucidated a novel mechanism that regulate MUC1 glycosylation during intestinal chronic inflammation and colitis-associated cancer. Our data suggest that inflamed colonic tissues of patients with ulcerative colitis and colon cancer display high abundance of infiltrating macrophages. To test whether macrophages are directly involved in the control of aberrant glycosylation of MUC1, we used a trans-well coculture system. Briefly, human monocytes were differentiated in M1 or M2 and then co-cultured with colon cancer cells HT-29 and SW-640 for 3 days. We found that polarized M2 macrophages induced over-expression of the altered glycoform MUC1-sTn compared to unstimulated cells. In addition, RT2 profiler glycosylation-related gene expression array revealed that M2 macrophages modulate the glycosyltrasferase enzymes ST6GALNAC1, which is involved in O-glycan biosynthesis.

GOALS FOR 2020

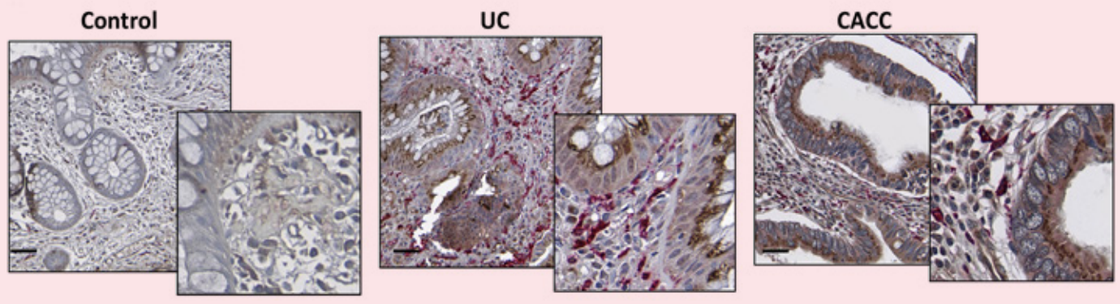
We will determine the interplay between tumor-associated myeloid cells and epithelial cells of solid tumor including colon, breast and ovarian cancer. We will also investigate whether the inhibition of specific cytokines, including CCL17 and IL-13, secreted by tumor associated macrophages, can result in the reduction of inflammation and inhibition of initiation and progression of tumors. To test this hypothesis, we will use CCL17 and/or IL-13 neutralizing antibodies. ST6GALNAC1 is the enzyme that by catalyzing sialylation of GalNAc residues, forms the specific tumor antigen MUC1-sTn that resulted over-expressed both in murine and human IBD and CAC diseases. We will test if the blockade of CCL17 and/or IL-13-dependent signaling will inhibit the expression of ST6GALNAC1 and prevent the formation of MUC1-sTn structure. We do expect that the inhibition of the network between M2 macrophage and epithelial cells will result in a delay progression to cancer.

MEETINGS

American Association of Immunologists (AAI), May, 2019, San Diego, USA

PUBLICATIONS

Crosstalk between colon cells and macrophages increases ST6GALNAC1 and MUC1-sTn expression in ulcerative colitis and colitis-associated colon cancer. Kvorjak M, Ahmed Y, Miller ML, Sriram R, Coronnello C, Hashash JG, Hartman DJ, Telmer CA, Miskov-Zivanov N, Finn OJ, **Cascio S.** Cancer Immunol Res. 2019 Dec 12. pii: canimm.0514.2019. doi: 10.1158/2326-6066.CIR-19-0514.



Representative image of ST6GALNAC1 (brown) and CD163 (magenta) double staining in paraffin-embedded human colon tissues sections from non-inflamed (Control), ulcerative colitis (UC), and colitis- associated colon cancer (CACC). Scale bars, 20 mm.

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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- Istituto Mediterraneo per i Trapianti (IRCCS ISMETT), Palermo, Itay
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- Ospedale Civico Di Cristina Benfratelli, Palermo, Italy
- Department of Engineering - Università degli Studi di Palermo, 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 one of the leading causes of death in the world. It is a disease associated with aging and exposure to 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 focused our research on innate immunity in experimental models of COPD, analyzing the inflammatory response associated with macrophages and bronchial epithelium activation. The work carried out over the last year has led to the discovery of a new regulatory axis leading to the activation of caspase-1 independent of NLRP3 in macrophages exposed to cigarette smoke. This mechanism could be at the basis of the immuno-metabolic dysfunctions observed in smoking subjects and COPD patients and that are associated with increased risk of infection. The study of these molecular mechanisms is necessary in order to identify new targets for the development of innovative therapies.

In parallel, since NLRP3 is a validated target for several chronic inflammatory diseases, in collaboration with the Drug Discovery Unit at Ri.MED, we are working on the development of new selective inhibitors of NLRP3.

IMPACT

The project will contribute to the discovery and characterization of new molecular mechanisms involved in the pathogenesis of COPD. This, in turn, will lead to the identification of new potential targets for the development of new therapies.

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 numerous chronic diseases including atherosclerosis, type II diabetes, and neurodegenerative diseases.

RESULTS ACHIEVED IN 2019

Cigarette smoking is one of the major risk factors for COPD. The use of *in vitro/ex vivo* experimental models of inflammation associated with cigarette smoke is able to mimic the inflammatory context typical of COPD airways. In 2019 our work focused on the study of the effects of cigarette smoke exposure in human monocyte-derived macrophages (hMDM).

The work has led to the discovery of a new regulatory axis involving TLR4, TRIF and caspase-8 and leading to activation of caspase-1 independently of NLRP3. In turn, caspase-1 caused a reduction of the glycolytic flow. We hypothesize that the activation of this pathway may contribute to the immuno-metabolic dysfunction observed in COPD lung.

GOALS FOR 2020

One of the main objectives for 2020 will be the validation of data obtained using samples isolated from COPD patients and control groups. This will be possible thanks to the development of advanced experimental models based on the use of primary cultures and biological samples derived from patients. The achievement of this objective will be possible thanks to the collaboration with ISMETT IRCSS and the University of Palermo - Department of Biomedicine, Neuroscience and Advanced Diagnostics. We will also work to understand what are the molecular mechanisms that lead to the activation of caspase-1 independent of NLRP3.

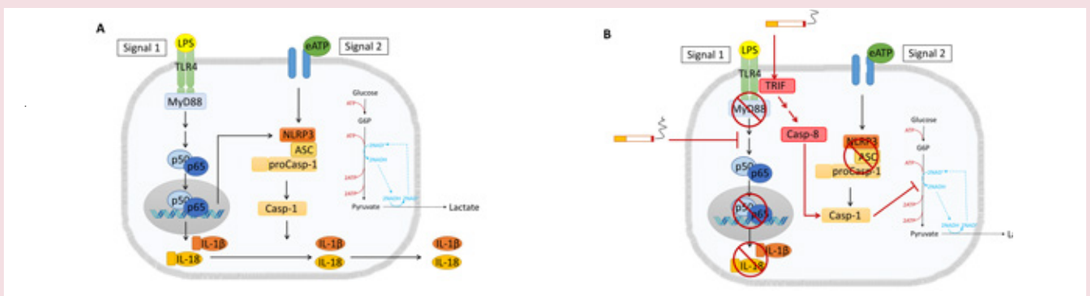
With regard to our involvement in Drug Discovery projects, our main objective will be the development and validation of the primary assay for screening of NLRP3 inhibitors, which will be followed by the primary screening, the identification of primary hits and their validation through secondary assays.

MEETINGS

- Buscetta M, Di Vincenzo S, Pace E, Cipollina C "Cigarette smoke activates the TLR4/caspase-8/caspase-1 axis leading to glycolysis impairment in human macrophages". EMBO Workshop - Cell death in immunity and inflammation, 06 – 09 October 2019, Crete (Greece).
- Buscetta M, Di Vincenzo S, Pace E, Cipollina C "Cigarette smoke extract inhibits the NLRP3 inflammasome in human macrophages and impairs cell metabolism via NLRP3-independent activation of Caspase-1". European Respiratory Society 2019 International Congress, Madrid (Spagna), Set 28- Oct 02, European Respiratory Journal 54 (suppl 63) PA2409; DOI: 10.1183/13993003.congress-2019.PA2409

PUBLICATIONS

- Buscetta M, Di Vincenzo S, Miele M, Badami E, Pace E, **Cipollina C**. "Cigarette smoke inhibits the NLRP3 inflammasome and leads to caspase-1 activation via the TLR4-TRIF-caspase-8 axis in human macrophages". Faseb J, 2019.
- Mekni N, De Rosa M, **Cipollina** C, Gulotta MR, De Simone G, Lombino J, Padova A, Perricone U. "In Silico Insights towards the Identification of NLRP3 Druggable Hot Spots." Int J Mol Sci, 2019, 20(20). doi: 10.3390/ijms20204974.
- Ferraro M, Di Vincenzo S, Dino P, Bucchieri S, **Cipollina C**, Gjomarkaj M, Pace E. "Budesonide, Aclidinium and Formoterol in combination limit inflammaging processes in bronchial epithelial cells exposed to cigarette smoke." Exp Gerontol, 2019, 118:78-87. doi: 10.1016/j.exger.2019.01.016.



Proposed model. (A) Following LPS transcriptional priming (Signal 1) and treatment with extracellular ATP (Signal 2) the NLRP3 inflammasome becomes active, leading to activation of caspase-1 and cleavage and release of mature IL-1β and IL-18. (B) When macrophages are exposed to cigarette smoke, the response to LPS is altered: transcription of NLRP3, pro-IL-1β and pro-IL-18 is inhibited and no NLRP3 inflammasome complex can be formed. Also, the release of IL-1β and IL-18 is reduced due to the lack of precursors. Cigarette smoke leads to activation of caspase-1 via the TLR4-TRIF-caspase-8 axis. Active caspase-1 in turn inhibits glycolysis.

PRODUCTS: **DRUGS - BIOMARKERS**

Modeling microRNA-target interaction network

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COLLABORATIONS

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

THERAPEUTIC AREA

Cancer

PIPELINE

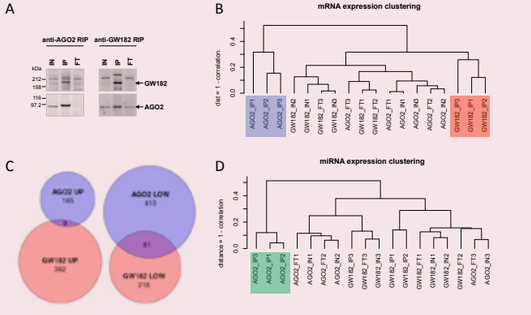
CLINICAL NEED | DISEASES ANALYSIS | DISCOVERY | PRECLINICAL VALIDATION | PRECLINICAL DEVELOPMENT | CLINICAL STUDIES

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. 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 tissue 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 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".



RIP-Chip experiments overview (extracted from [1]). A) Western blot of co-immunoprecipitated proteins. B) Cluster analysis of mRNA expression profiles. C) Venn diagrams of differentially expressed genes. D) Cluster analysis of miRNA expression profiles.

RESULTS ACHIEVED IN 2019

In 2019 we focused on the update of the web-tool ComiR, a tool useful 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.

GOALS FOR 2020

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.

PRODUCTS: **DRUGS**

Pharmacology of nitro-nitrate lipids

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COLLABORATIONS

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THERAPEUTIC AREA

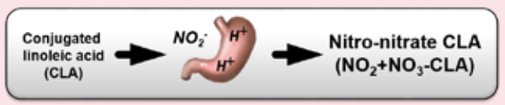
Aging diseases

PIPELINE

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

BRIEF DESCRIPTION

Among unsaturated fatty acids, the conjugated linoleic acid (CLA) has shown to be the preferential substrate for the generation of electrophilic nitro-fatty acids (NO_2-FA) under acidic gastric conditions in presence of nitrite (NO_2^-). NO_2-FA modulate enzymatic functions and cell signaling via post-translational protein modifications and induce beneficial effects in numerous inflammatory diseases.
In this study we showed that gastric nitration of both free and esterified CLA proceeds through the formation of novel unstable non-electrophilic nitro-nitrate fatty acids, which decompose at physiological pH yielding electrophilic nitro-conjugated linoleic acid (NO_2-CLA) derivatives and nitric oxide (NO)-like nitrosative species.
These nitro-nitrate lipids were detected in rat gastric fluid after oral gavage of physiological levels of CLA-containing triglycerides and NO_2^-. Once generated, nitro-nitrate lipids get absorbed at the intestinal level leading to the formation of NO_2-CLA that was subsequently detected in circulating plasma triglycerides.
Then, the decomposition of nitro-nitrate lipids could induce not only the antioxidant and cytoprotective actions of NO_2-FA but also nitric oxide (NO)-mediated vasodilation. In conclusion, the proposed research identifies a new class of nitro-nitrate lipids as important intermediates in the formation of electrophilic NO_2-FA, and will define signaling actions of this structurally-unique class of lipids.



IMPACT

The proposed research will provide insight into the formation and actions of a newly discovered byproduct of fatty acid nitro-oxidation, with the long-range vision of advancing this novel class of mediators into a potentially transformative new drug strategy for limiting the vascular and cardiopulmonary injury that stems from metabolic and inflammatory stress.
Of relevance, nitro-nitrate lipid derivatives have been already covered with a recent international PCT provisional patent application published in April 2018 (WO2018/067709)

RESULTS ACHIEVED IN 2019

The novel nitro-nitrate species have been characterized using multiple analytical techniques such as thin layer chromatography (TLC), high performance liquid chromatography-coupled Uv-Vis and mass spectrometric analysis (HPLC-Uv-Vis, HPLC-MS/MS) and infrared spectroscopy (IR) analysis.
The formation and decomposition of nitro-nitrate derivatives were studied in rats and *in vitro* under different pH, buffer and oxygen tension conditions, with oxygen promoting their formation and basic pH catalyzing their decomposition. Finally, organic synthesis of nitro-nitrate lipid standards further confirmed the assigned structures of these endogenous species.

GOALS FOR 2020

The nitro-nitrate lipid standard will be synthetized in large amounts to study the absorption, distribution, metabolism and excretion in rodents of this newly discovered class of lipids. Furthermore, we will study the *in vitro* and *in vivo* responses to nitro-nitrate lipids, with focus on to the potential for this class of mediators to mediate both cGMP-dependent and cGMP-independent signaling.

MEETINGS

(Presentazione orale) Society for Redox Biology and Medicine's (SfRBM) 26th Annual Conference, November 20-23, Las Vegas.

INTELLECTUAL PROPERTY

Domanda provvisoria di brevetto internazionale (PCT) No. PCT/US2017/055154. Titolo: Novel reversible nitroxide derivatives of nitroalkenes that mediate nitrosating and alkylating reactions. Registrata: Oct-04, 2017. Inventori: **Fazzari M.**, Schopfer F. and Freeman B.

PRODUCTS: **DRUGS**

Design of CD14 modulators in aging-related macula degeneration

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COLLABORATIONS

Institut de La Vision, Paris, France

THERAPEUTIC AREA

Aging diseases

PIPELINE

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

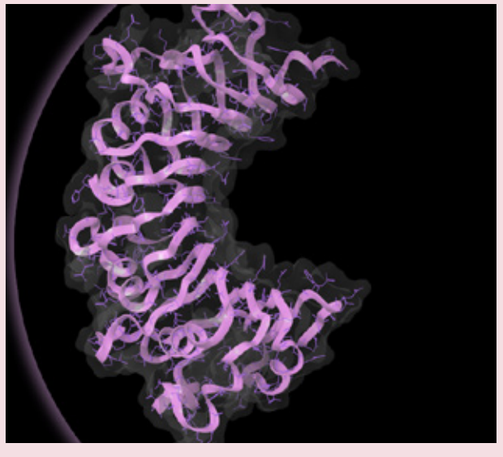
BRIEF DESCRIPTION

Age-related macular degeneration (AMD) is a multifactorial, heterogeneous, degenerative disorder of the human eye that affects patients over the age of 50 and can lead to severe vision loss. Clinically, two forms of AMD are recognized: the dry form, which affects 80% of patients and the more In the pathological picture, the CD14 protein plays a crucial role in mediating the inflammatory process underlying tissue degeneration. CD14 acts as a co-receptor (together with the Toll-like TLR 4 and MD-2 receptor) for the detection of bacterial lipopolysaccharide (LPS). CD14 can bind LPS only in the presence of lipopolysaccharide-binding proteins (LBP). Although LPS is considered to be its main ligand, CD14 also recognizes other molecular ligands associated with pathogens such as lipoteic acid.
The aim of the project is to design selective CD14 inhibitors that by binding to the protein can prevent the recognition of the LPS or other inflammatory mediators thus allowing the restoration of the physiological tissue condition at the macula level.

IMPACT

Currently age-related degenerative maculopathy is considered the main cause of irreversible blindness in Western societies and, given that life expectancy is constantly increasing, the prevalence of the disease is expected to increase significantly. At the moment, however, there are no real preventations for maculopathy since the etiology is still unclear today: the genetic component has a lot on macular degeneration but the type and number of genes involved are difficult to define. Furthermore, to date, patients with macular degeneration are forced to resort to surgical practice. The registered drugs are mainly biological in nature (e.g. antibodies, aptamers) and are not always effective and safe in their use.

Together with biological drugs, antioxidant therapy is always supported, because free radicals have a great impact on the progression of the disease.
The aging process is in fact likely to trigger oxidative reactions which subsequently affect the retina and synthesize free radicals. The discovery of small molecules selectively capable of modulating the activity of CD14 would therefore allow an effective arrest of the inflammatory degenerative process and the restoration of the physiological tissue condition of the macula by excluding all the secondary effects and any bioavailability problems typical of biological drugs.



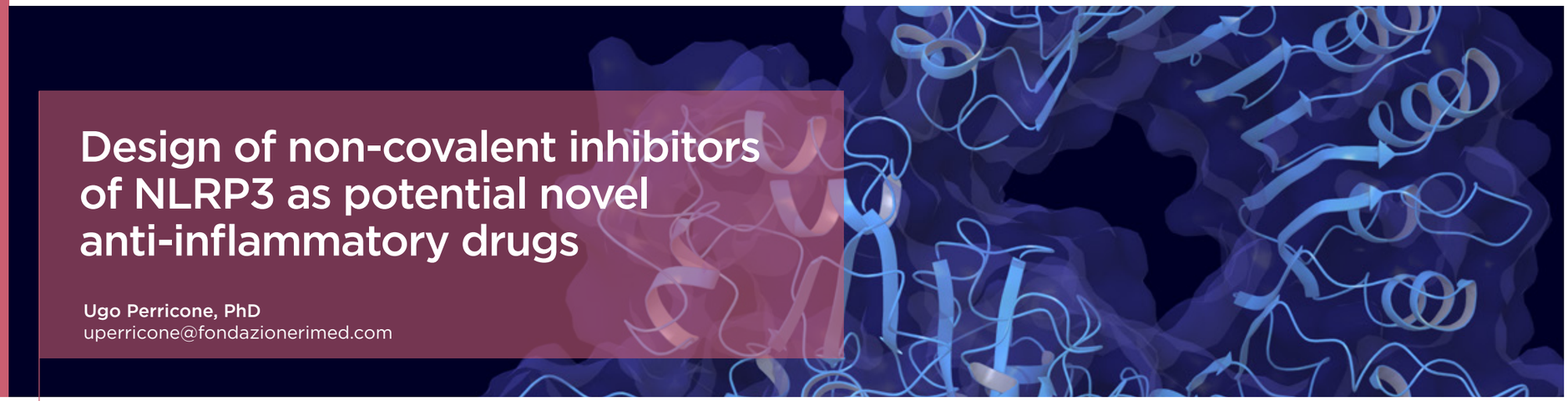
Representation of the human horseshoe-like CD14 protein, a host protection agent.

RESULTS ACHIEVED IN 2019

In 2019, the Computer-aided drug design group developed computational models mainly based on the single resolved structure of CD14. One of the limitations of this project is in fact the almost total absence of structures resolved with co-crystallized ligands or known molecules and with significant chemotypes that allow the creation of a robust and reliable model. However, thanks to the models created by our computational chemistry laboratory, three active molecules have been identified which have been confirmed on both the primary and orthogonal assays.
These molecules belong to a set of 65 molecules initially selected from different type libraries and subjected to virtual screening through molecular docking and ligand based pharmacophore created on the mold of only three LPS-like molecules known in the literature as CD14 modulators.
The newly discovered molecules showed activity data (IC50) around 20μM responding positively to all orthogonal assays performed. One of these is also similar to a fragment, therefore it has a good ligand efficiency and lends itself to further structural optimizations that improve the activity profile.

GOALS FOR 2020

2020 will begin with the search and selection of structural analogues of the assets previously found so that they can confirm their activity as a series. In addition, the computational study will be further deepened in order to strengthen the predictive capacity of the models in use thanks to the data obtained in 2019.
The goal for 2020 will therefore be the optimization of chemical structures, thanks to the collaboration with the medicinal chemistry group of the Foundation, in order to obtain at least one family of hits confirmed with activities below 10 μM and which maintains a selectivity for CD14. In fact, starting from the most promising molecular scaffolds and having validated the computational model in use, we will proceed with the design and synthesis of new proprietary compounds with good activity profile and low toxicity.



Design of non-covalent inhibitors of NLRP3 as potential novel anti-inflammatory drugs

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COLLABORATIONS

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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 pyroptosis. Greater activation of NLRP3 is recognized to be related to several chronic conditions, including neurodegenerative diseases, atherosclerosis, type-II diabetes, fibrosis and rheumatoid arthritis. Preclinical evidences support 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 target for the development of potential novel anti-inflammatory drugs. Recently, many research groups have focused on the development of selective modulators of the NLRP3 inflammasome (NLRC4, AIM2) which do not interfere with the protective activity of other types of inflammasome. However, the lack of structural information of the protein and its molecular mechanism, and an unclear mechanism of action of the few known inhibitors hinders the task of designing new selective modulators.

IMPACT

In the last decade, the chronic inflammation-related diseases have been rising in the western countries, and, to date, the molecular mechanisms responsible for the diseases remain unclear. Anti-inflammatory drugs commercially available seem to dampen the immune response involved in the onset of chronic inflammation, suppressing the symptoms of inflammation. Unfortunately, a complete remission of the disease rarely happens.
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-competitor selective inhibitors, able to act on the process causing the inflammatory pathology mediated by NLRP3.

RESULTS ACHIEVED IN 2019

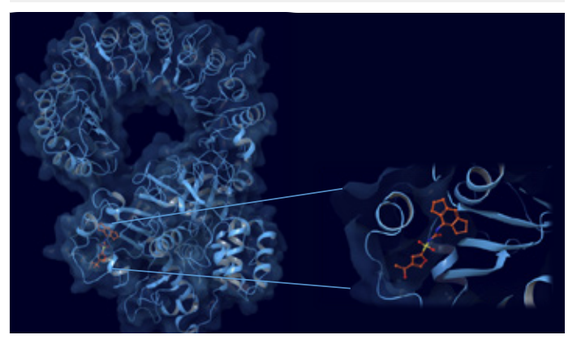
During the year 2019, the Computer-Aided Drug Design group developed two computational approaches aimed at the rational design of modulators of the NLRP3 inflammasome. In particular, the first approach concerns a non-covalent inhibition of the NACHT domain of the NLRP3 protein through a non-competitive mechanism for the ATP binding site.
The second approach, on the other hand, concerns the design of protein-protein inhibitors (PPIs) targeting the PYD domain of NLRP3, and preventing its binding to PYD domain of the ASC protein partner, limiting the recruitment of procaspase 1 and the consequent activation of the inflammasome complex responsible for the inflammation cascade.
The lack of a crystal structure of NLRP3 makes the design of new inhibitors a highly challenging task. We decided to build a homology model of the protein, with the aim of gaining more structural information, to date provided only by a cryo-EM solved structure available in literature.
Virtual screening and computational studies on our homology models, allowed us to select 1000 compounds (500 structures for each approach).
During the year 2020, these molecules will be purchased and tested on a primary assay and the biological activity will be confirmed on a panel of orthogonal assays by the HTS group.

GOALS FOR 2020

During the year 2020, the main goal for this project will be the discovery of at least two hit families (one for each approach considered) with a biological activity lower than 10 μM on the primary phenotypic assay. The activity will be next confirmed in a panel of orthogonal assays. Based on the structure of primary hits, the group of medicinal chemistry will start the synthesis program for the hit expansion/optimization phase.
An extensive SAR investigation is a good premise for the discovery of a potential lead compound with the best biological activity *in vitro* and a very promising pharmacokinetic profile (ADMET properties).
The computational chemistry group will focus the screening on small molecules and peptidomimetic compounds as well as on fragment libraries, with the aim of deeply exploring all the hot spots available on the protein surface.

PUBLICATIONS

Mekni N., De Rosa M., Cipollina C., Gulotta M.R., De Simone G., Lombino J., Padova A., **Perricone U.**, In silico insights towards the identification of NLRP3 druggable hot spots, Int. J. Mol. Sci. 2019, 20, 4974



A focus insight the NLRP3 protein putative binding site.

PRODUCTS: **DRUGS**

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

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COLLABORATIONS

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THERAPEUTIC AREA

Cancer

PIPELINE

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

BRIEF DESCRIPTION

Epigenetic processes are essential mechanisms in the development and physiological functioning of cellular gene expression patterns. Global changes in the epigenetic scenario are distinctive signs of cancer initiation and progression. N-Methylation of lysine and arginine residues is one of the most frequent mechanisms of transcriptional epigenetic regulation in eukaryotes. In humans, there are two families of enzymes that catalyse the demethylation of lysine residues (KDMs). The KDM2-7 family is the largest class of demethylases, consisting of 20 enzymes. In particular, KDM4A is frequently amplified and over-expressed in various types of human cancers, for example in ovarian cancer, colon or squamous cell carcinoma. The main objective of the research project is the rational design and the synthesis of small molecules able to modulate the epigenetic mechanisms regulated by Histone lysine demethylase 4 (KDM4). The ultimate goal is the development of novel drugs against tumour pathologies. The rational design of the molecules provides for the use of *in silico* models created on the target proteins of interest and their validation in a retrospective way. Virtual screening and molecular modelling studies on the computational models allow to identify potential Hit compounds, and are necessary for guiding the chemical synthesis during the hit expansion/optimization and hit-to lead phases.

IMPACT

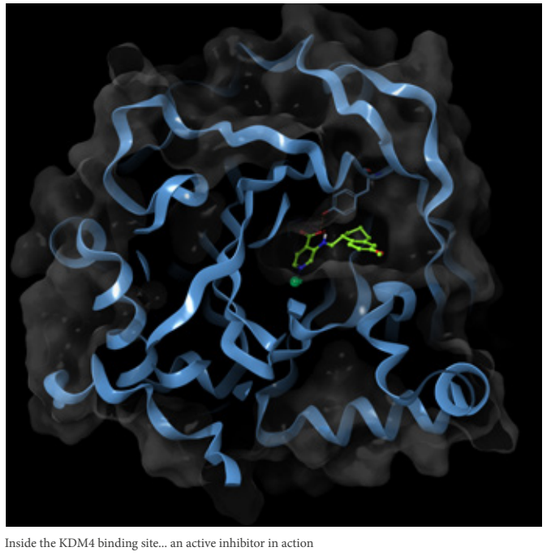
Recent advances in the field of cancer epigenetics have highlighted the importance of epigenetic mechanisms in the development of tumour pathology. Particular importance has been given to DNA methylation, histone modifications, and microRNA expression modifications. The reversible nature of epigenetic 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. In recent years, several drugs in the epigenetic area have been approved by the public institutions responsible for regulatory activity, and many clinical trials are currently underway. Therefore, there are numerous possibilities for developing new drugs that act at the level of epigenetic mechanisms. In this scenario, KDM4 represents an appealing and innovative target for developing novel anti-cancer entities, especially considering that to date no drugs known to be active on it and available in the market.

RESULTS ACHIEVED IN 2019

During the year 2019, the computational models used for the rational design of new 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 libraries allowed the identification of 8 hit molecules that showed μM activity on KDM4 on the primary biochemical assay. The same molecules were tested with biophysical techniques (BLI) and showed dissociation constant (Kd) values in the μM range. Based on these results, we focussed on the rational design of structural analogues of the hit series with the aim of improving their biological activity profile.

GOALS FOR 2020

During the year 2020, the goal of the computational chemistry group together with the medicinal chemistry laboratory is the design and synthesis of compounds structurally related to the preliminary hits found in 2019, in order to improve their potency, and investigate on their toxicity profile and specificity towards the KDM4A isoform. Furthermore, binding assays will be performed on other KDM isoforms with the aim of defining the selectivity profile of the compounds. In detail, the medicinal chemistry group will focus on the synthesis of two hit families. An extensive structure-activity-relationship (SAR) analysis will be carried out with the aim of exploring the chemical space for the structural optimization and development of potential lead compounds, with the best pharmacokinetic profile (good potency and efficacy, optimal binding, low toxicity).
The newly synthesized molecules will be tested *in vitro* on two main forms of demethylase involved in the tumor pathology of our interest (KDM4A and KDM4C). The biological activity will be evaluated both on enzymatic and cellular assays, and using biophysical techniques (BLI, NMR), in order to gain information on the binding mode and to validate the computational models.



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

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

The regenerative medicine and research and development of biological therapies 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 at 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.

CAR-NK cell engineering for the treatment of hepatocellular carcinoma
Ester Badami, PhD

Regulatory Dendritic cells as a tool to prevent graft rejection
Ester Badami, PhD

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

Immuno-therapy against *K. pneumoniae* based on genetically-engineered probiotic *S. cerevisiae* yeasts
Bruno Douradinha, PhD

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

Rebuilding a liver in ectopic sites
Maria Giovanna Francipane, PhD

Rebuilding a kidney in ectopic sites
Maria Giovanna Francipane, PhD

OActive - Advanced personalised, multi-scale computer models preventing OsteoArthritis
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 liver 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

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

CAR-NK cell engineering for the treatment of hepatocellular carcinoma

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THERAPEUTIC AREA

Cancer

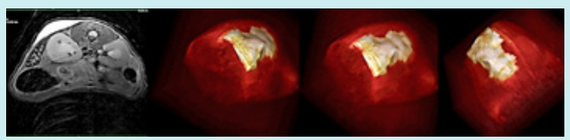
PIPELINE

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

BRIEF DESCRIPTION

The most common liver cancer in adults is hepatocellular carcinoma (HCC) which originates from hepatocytes, the cells that constitute the liver. Hepatocellular carcinoma patients can be treated with surgery to remove the area affected by cancer. However in advanced liver cancer patients, the most adopted clinical practice is transplant. Unfortunately, post-transplant liver cancer has still high incidence and more advanced and efficacious therapies are therefore necessary.

Natural Killer (NK) cells of the innate immune system are capable of recognizing and destroying tumoral cells. The possibility to engineer NK cells to confer specificity to a specific Tumor Associated Antigen (TAA) is finally possible through the induction of expression of what are known as Chimeric Antigen Receptor o CAR. This is a membrane-bound receptor specific for a given TAA highly expressed by HCC and not by normal hepatocytes. NK cells engineered to express the CAR destroy tumoral cells sparing non cancerous hepatocytes. Moreover, binding of the CAR to its ligand on the cancer cell will induce NK cell activation and release of pro-inflammatory factors important to enhance the immune response against cancer.



The image on the left show a cross segment of the tomographic acquisition at time 0 of a mouse injected on its back subcutaneously with a murine cell line of hepatocarcinoma. The three images on the right are the relative 3D reconstruction of the injected area in the three spatial axes. Images were acquired with a 7Ts MRI instrument (Bruker) available at IZS. Images were acquired and edited by Dr Albert Comelli.

IMPACT

Natural Killer (NK) destroy anything they recognize as non-self such as cancer and infected cells. Compared to T lymphocytes, NK cells do not give rise to Graft versus Host Disease (GvHD) a common and lethal side effect of T cell mediated cell therapy.
NK cells are therefore perfect for an “off-the-shelf” therapy that is capable of treating a wide range of cancer patients starting from a single batch obtained from one single healthy donor. In oncology, CAR-NK cells represent the most promising platform for cell-mediated immuno-therapies, given their ability to overcome the limitations bound to personalized autologous therapies, with significant technical and economic benefits. Genetically engineered NK cells can be therefore considered one of the most interesting and innovative area of pre-clinical research and cellular immunotherapy.

RESULTS ACHIEVED IN 2019

The first step for the generation of an innovative CAR-NK cells design the CAR itself starting from the TAA of interest. We picked a HCC TAA expressed at high frequency not only locally (Mediterrenean basin) but also more globally.
The vector was synthesized accordingly, to induce expression of a membrane-bound receptor specific for the target tumor antigen. Alongside, engineered NK cells express pro-inflammatory soluble factor necessary to mount an efficacious anti-tumor response.
For these reasons, the vector is considered a third generation construct because it contains all the information for the expression of the target, the machinery to trigger the cytotoxic immune response and cytokines to corroborate the anti-tumor NK cells function. In order to prevent unwanted responses, the vector is equipped with a suicide gene, which expresses an inert membrane protein recognized by the antibody Cetuximab. In case of outburst of dangerous side effects, NK cells can be arbitrarily killed by infusing the patients with Cetuximab which binds to CAR-NK cells and kills them by opsonization and e activation of the complement cascade. The vector has been used to engineer both human primary NK cells obtained from healthy donors and the NK92 cell line. However the efficiency of CAR expression was very low, as also observed by others when using lentiviral vectors.

GOALS FOR 2020

In the light of the very low efficiency of CAR expression by using lentiviral vecotrs, we will attempt the use of an alternative methodology such as nucleofection or electroporation.
Our lab are equipped with the nucleofection instrument. Interestingly, nucleofection work by creating pores in the cell membrane which would let nucleic acid material (DNA plasmid/mRNA containing the CAR sequence) in the cell and the nucleus itself. Being clearly virus-free, this alternative method would better fit into a future clinical application.
Once reached this milestone, we will investigate the cytotoxic function of CAR-NK cells and evaluate the desired augmented immune response *in vitro* by using target cells that expressed the TAA.
The CAR-NK function will be investigated *in vivo* too on a humanized mouse model where in immunocompromised NSG mice will be induced a human liver tumor expressing the TAA of choice. Mice will be treated with NK cells or with CAR-NK cells are the anti-tumor response will be monitored on live animals by Magnetic Resonance Imaging (MRI). A 7 tesla MRI instrument is available at the IZS.

MEETINGS

- 5th Conference on Translational Medicine on Pathogenesis and Therapy of Immune-mediated Diseases - May 2019 Milano (Italy)
- International Society for Cancer and Gene Therapy Annual Meeting – ISCT 2019, May 2019, Melbourne (AUSTRALIA)
- Dendritic Cells Immunotherapy Days (DCID) & Next Generation Vaccines - October 2019, sede centrale Miltenyi Biotec Bergisch Gladbach (GERMANIA)

PUBLICATIONS

- Gallo A, Miele M, **Badami E**, Conaldi PG. 2019 "Molecular and cellular interplay in virus-induced tumors in solid organ recipients" Cell Immunol. Sep;343:103770. doi:10.1016/j.cellimm.2018.02.010.
- Pagano D, **Badami E**, Conaldi PG, Seidita A, Tuzzolino F, Barbàra M, di Francesco F, Tropea A, Liotta R, Chiarello G, Luca A, Gruttadauria S. 2019 “Liver Perfusate Natural Killer Cells From Deceased Brain Donors and Association With Acute Cellular Rejection After Liver Transplantation: A Time-to-Rejection Analysis” Transplantation. 2019 Feb;103(2):371-380. doi:0.1097/TP.0000000000002322.
- **Badami E**, Cexus ONF, Quaratino S. “Activation-induced cell death of self-reactive regulatory T cells drives autoimmunity” 2019 Proc Natl Acad Sci U S A. Dec 9. pii: 201910281. doi:10.1073/pnas.1910281116
- Marco Buscetta, Serena Di Vincenzo, Monica Miele, **Ester Badami**, Elisabetta Pace, Chiara Cipollina 2019”Cigarette smoke inhibits the NLRP3 inflammasome and leads to caspase-1 activation via the TLR4-TRIF-caspase-8 axis in human macrophages” FASEB Journal First published: 08 December 2019 https://doi.org/10.1096/fj.201901239R

INTELLECTUAL PROPERTY

Brevetto internazionale PCT/EP2017/080848
NK-mediated immunotherapy and uses thereof

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

Regulatory Dendritic cells as a tool to prevent graft rejection

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COLLABORATIONS

- Prof Angus W. Thomson, Starzl Institute of Transplantation, University of Pittsburgh, Pittsburgh, USA
- Prof Fadi G. Lakkis, Starzl Institute of Transplantation, University of Pittsburgh, Pittsburgh, USA

THERAPEUTIC AREA

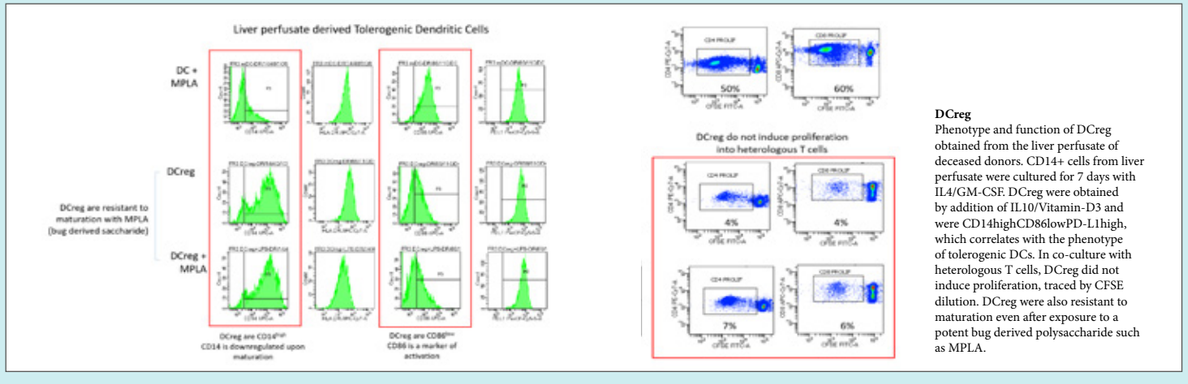
Organ insufficiences

PIPELINE

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

BRIEF DESCRIPTION

This study aims at the production of regulatory Dendritic Cells (DCreg) to be used in therapy in patients transplanted with solid organs in order to fasten weaning from immunosuppressive therapy. Liver is a tolerogenic organ per se. In this study, patients transplanted with liver sill be considered. We will assess if from the liver it is possible to isolate readily available DCreg to be used in cellular therapy in the liver recipient. DCreg are a population of DCs capable of inducing hypo-responsiveness into recipients T cells specific for donors alloantigen and establishing a long term immunological memory.
The source of cells will be the liver perfusate consisting in the lavage buffer of the solid organ before transplant.



DCreg
Phenotype and function of DCreg obtained from the liver perfusate of deceased donors. CD14+ cells from liver perfusate were cultured for 7 days with IL4/GM-CSF. DCreg were obtained by addition of IL10/Vitamin-D3 and were CD14highCD86lowPD-L1high, which correlates with the phenotype of tolerogenic DCs. In co-culture with heterologous T cells, DCreg did not induce proliferation, traced by CFSE dilution. DCreg were also resistant to maturation even after exposure to a potent bug derived polysaccharide such as MPLA.

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 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 use of an alternative source of tolerogenic DCs obtainable from the liver perfusate of liver deceased donors would greatly increase the group size of patients eligible for this therapy.

RESULTS ACHIEVED IN 2019

In 2019, I achieved very encouraging results, mainly thanks to the strong collaboration with Profs F Lakkis and AW Thomson laboratories. They helped us through many conference calls and of crucial impact was the period of training that I spent in Pittsburgh in their labs. During this period, I learnt in details the protocol of small-scale DCreg production starting from monocytes derived from the peripheral blood of healthy donors. Moreover, I increased my scientific knowledge in the field through the interesting talks with the members of Thomsons' and Lakkis' labs and understood the mistakes I was making when trying to generate DCreg before my visit in Pittsburgh. So this training was extremely useful and important. Thanks to this, I am now able to reproduce the protocol in ISMETT's labs and I started the first cultures of DCreg starting from the monocytes isolated from both peripheral blood of healthy donors and the liver perfusate of deceased donors. I am finally obtaining the wished results as liver perfusate DCreg express the expected phenotype, with high concentrations of a tolerogenic marker PD-L1 and low concentrations of the activation marker CD86. Functionally, these DC induce reduced proliferation into allogenic T lymphocytes if compared to the same monocytes cultured without the polarizing growth factors Interleukin-10 and Vitamid-D3 (Fig 1).
To conclude, we are able to apply the protocol of generation of DCreg, already in use and optimized in Pittsburgh, starting from the monocytes derived from liver perfusate.

GOALS FOR 2020

For 2020, we want to optimize the large-scale production of DCreg starting from CD14+ monocytes from the liver perfusate of deceased donors. In ISMETT, we have access to the elutriation platform ELUTRA, an instrument that can separate individual blood-derived cellular fractions such as T lymphocytes and monocytes in fraction with very high purity (>85%). This allows to skip the further expensive step of purification of individual cell populations using magnetic beads, with a consequent loss in cell number. DCreg will be tested *in vitro* for phenotype (PD-L1high/CD86low) and function (reduction of proliferation of allogeneic T cells). Further, DCreg will be tested in a small animal model of transplantation in order to ascertain the *in vivo* proof-of-concept, a step necessary for clinical application of the protocol and to test the real induction of operational tolerance in the host T cells.

VISITING

Training period in the laboratories of Profs F Lakkis and AW Thomson, Starzl Institute of Transplantation, October 2019.

PUBLICATIONS

- Gallo A, Miele M, **Badami E**, Conaldi PG. 2019 "Molecular and cellular interplay in virus-induced tumors in solid organ recipients" Cell Immunol. Sep;343:103770. doi:10.1016/j.cellimm.2018.02.010.
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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

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- Laboratorio Polimeri Biocompatibili, STEBICEF – Università di Palermo, Palermo, Italy
- Dipartimento di Farmacia e Biotecnologie - Università di 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 phases 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.

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 2019

In collaboration with the lab of Biocompatible Polymers at STEBICEF we have identified two different formulations of HA-EDA hydrogels, functionalized or not with heparin, integrated with secretome of MSC isolated from human fetal dermis. Thanks to a collaboration with the Department of Farmacia e Biotecnologie at the University of Bologna, we will soon test these two formulations in a murine model of diabetic ulcer (preclinical proof of concept).
Two manuscripts are ready for submission (Chinnici CM et al. "Exosomes of human mesenchymal stromal cells from fetal dermis and their microRNA cargo: putative target genes related to angiogenesis and wound healing"; Chinnici CM et al. "Hyaluronic acid/heparin-based hydrogels as delivery solutions for mesenchymal stromal cells and their secretome").
The results obtained so far have been used for drafting and submitting the project of the same name on the MIUR Fondo integrativo speciale per la ricerca (FISR) in November 2019 (please refer to "Grants" section in this volume).

GOALS FOR 2020

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

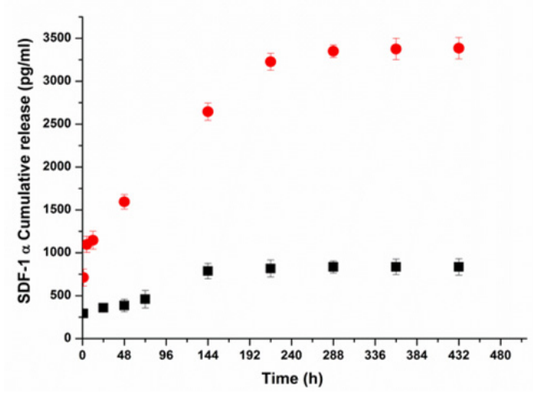
MEETINGS

7th International Stem Cell Meeting (12 e 13 november 2019, Tel Aviv)

PUBLICATIONS

- Miceli V, **Chinnici CM**, Bulati M, Pampalone M, Amico G, Schmelzer E, Gerlach JC, Conaldi PG Comparative study of the production of soluble factors in human placenta-derived mesenchymal stromal/stem cells grown in adherent conditions or as aggregates in a catheter-like device. Biochem Biophys Res Commun. 2019 Nov 19. pii: S0006-291X(19)32189-8. doi: 10.1016/j.bbrc.2019.11.06

- Mesenchymal stromal cells isolated from human fetal liver release soluble factors with a potential role in liver tissue repair. **Chinnici CM**, Pietrosi G, Iannolo G, Amico G, Cuscino N, Pagano V, Conaldi PG. Differentiation. 2019 Jan - Feb;105:14-26. doi: 10.1016/j.diff.2018.12.00



Sustained release of SDF1-alpha from HA-EDA hydrogel

HA-EDA hydrogel sponge

PRODUCTS: **BIOLOGICS**

Immuno-therapy against *K. pneumoniae* based on genetically-engineered probiotic *S. cerevisiae* yeasts

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COLLABORATIONS

- **Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (IRCSS-ISMETT), Palermo, Italy**
- **Università degli Studi di Siena, Siena, Italy**
- **Azienda Ospedaliera Universitaria Careggi, Florence, Italy**
- **Università degli Studi di Messina, Messina, Italy**
- **GSK Vaccines, Siena, Italy**

THERAPEUTIC AREA

Infectious diseases

PIPELINE

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

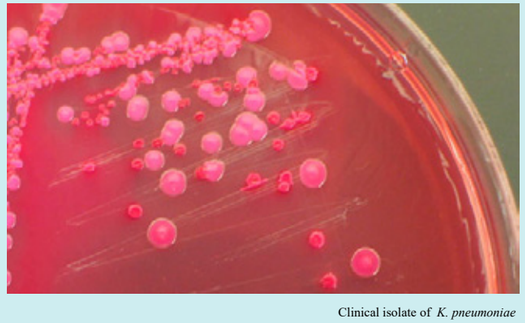
BRIEF DESCRIPTION

In this project, we propose the use of probiotic *Saccharomyces cerevisiae* strains as a novel immune-therapy strategy to both prevent and treat *Klebsiella pneumoniae* infections. These yeasts will be genetically modified to express in their surface proteins involved in the adhesion of *K. pneumoniae* to human cells. It is expected that these recombinant yeasts, once administered, are able to induce an immune response against the antigens of this pathogenic bacterium, thus acting as powerful mucosal vaccines.

At the same time, these probiotic yeasts would also induce a mucosal immune response at the colon level, thus preventing bacterial colonization and subsequent systemic infections.
The proposed immunotherapy would also work for the multidrug resistant *K. pneumoniae* strains, as the mechanisms leading to resistance cannot avoid an immune response directed against these bacteria. We are convinced that this new approach will be effective against *K. pneumoniae* and will help combat these multidrug resistant pathogens.

IMPACT

K. pneumoniae is a Gram-negative bacterium of clinical importance, which readily colonizes mucosal surfaces and, from there, gain access to other tissues and establish severe infections. Resistance to several antibiotics has been reported, reducing the number of effective treatments. *K. pneumoniae* is increasingly becoming a public health concern. In fact, the World Health Organization has issued a list of antibiotic resistant bacteria in which the need of novel interventions against *K. pneumoniae* is considered crucial.
To date, no vaccine is available against *K. pneumoniae*. We believe that probiotic strains of *S. cerevisiae* may be an ideal immunization strategy against pathogens that require an immune response at the mucosal level to prevent infection, for example, gastrointestinal bacteria or HIV. In the specific case of *K. pneumoniae*, the use of genetically engineered probiotic strains would prevent the colonization of this pathogen, through induction of an immune response at intestinal level and, consequently, would decrease the probability of further systemic infections that could occur a posteriori. At the same time, it would work as a vaccination approach: once the genetically modified yeasts are phagocytized by dendritic cells and macrophages, they will induce an immune response against the antigens of *K. pneumoniae*.



Clinical isolate of *K. pneumoniae*

RESULTS ACHIEVED IN 2019

During this year, we focused on the cloning, expression and purification of recombinant proteins in *Escherichia coli*, which would be used to evaluate and compare the effectiveness of the probiotic yeast method with a vaccine against *K. pneumoniae*.
Through reverse vaccinology, we analyzed sequenced genomes of *K. pneumoniae* available on online databases and found 528 conserved proteins.
Of these, we selected proteins with a size greater than 250 kDa and located in the outer or extracellular membrane, downsizing the number to 7.
We confirmed the presence of these proteins in the sequenced genomes of clinical isolates of the Careggi University Hospital and of the IRCCS ISMETT, in a total of 606 isolates. Of the 7 potential antigens, 4 were present in more than 95% of clinical isolates.
These 4 antigens have been cloned and expressed and we are currently starting the protein purification optimization procedure in collaboration with Caterina Alfano, Ri.MED/ATeN Center, in Palermo. In parallel, we have cloned reference antigens of *K. pneumoniae,* MrkA and MrkD, in the surface of probiotic yeast *Saccharomyces boulardii* and non-probiotic yeast *Saccharomyces cerevisiae* BY4743. The success of cloning was confirmed by PCR and RT-PCR screening.

GOALS FOR 2020

Our goals for 2020 are to purify the potential antigens identified by reverse vaccinology. Once the recombinant antigens are obtained, we will perform ELISA tests using sera from patients who have suffered an infection of *K. pneumoniae* 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. pneumonia*e natural antibodies would develop against these antigens.
We are also starting the procedure to obtain the authorization from the Ministry to perform immunization animal studies.
This will allow us to obtain serum containing antibodies against the chosen antigens and study the immunogenicity of these antigens, for example, their potential to induce a humoral immune response. The antibodies obtained will also be used in other assays, for example, to assess the expression of antigens in the surface of transformed probiotic yeasts or in a passive immunization approach.

PRODUCTS: **BIOLOGICS**

Surveillance and characterization of multidrug resistant bacterial strains of clinical relevance

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COLLABORATIONS

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- Università degli Studi di Siena, Siena, Italy
- Azienda Ospedaliera Universitaria Careggi, Florence, Italy

THERAPEUTIC AREA

Infectious diseases

PIPELINE

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

BRIEF DESCRIPTION

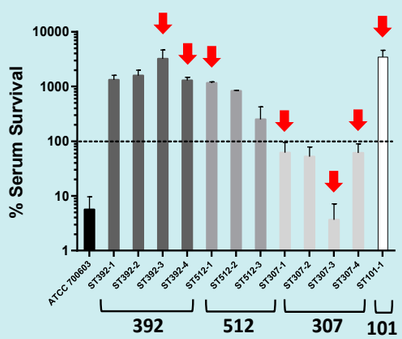
Within this project, we intend to characterize particular and/or multidrug resistant bacterial strains that arise from continuous microbiological surveillance at our clinical partner, IRCSS-ISMETT. Their pathogenic potential will be assessed *in vitro*, to understand if these bacterial isolates can become 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 2019

We characterized the virulence potential of *K. pneumoniae* ST392 isolates that produce the KPC-3 enzyme, responsible for antibiotic resistance.
As far as we know, we have isolated the first *K. pneumoniae* ST392 strain that produces KPC in Europe. We assessed their potential for virulence, to see if this particular ST can become endemic at the clinical level. ST392 strains and representatives of other clinically relevant KPC-producing STs added for comparison were studied to assess their virulence potential, i.e. resistance to human sera, abiotic and biotic biofilm formation, and *in vivo* pathogenesis for infection of the larvae of the wax moth *Galleria mellonella*.
The strains belonging to ST392 were highly resistant to human sera. These strains have a high capacity to form abiotic biofilms and adhere to a human epithelial adenocarcinoma cell line.
An increase in the transcriptional levels of genes involved in serum resistance (*aroE* and *traT*) and adhesion (pgaA) has been observed. Infection of *G. mellonella larvae* with ST392 clinical isolates showed that the latter were not highly pathogenic in this model. Together, our results indicate that ST392 isolates have the potential to become a clinically relevant strain, especially in health contexts where patients are immunosuppressed, such as transplant patients.



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

GOALS FOR 2020

Our goals to 2020 are to finish and publish the data described above. Literature has shown that the *K. pneumoniae* ST392 KPC-3 producing clinical isolates are fast spreading in Europe and we believe our data is important and corroborates this endemic trend of these particular isolates and should be made available. This will allow other researchers to have more information about the the *K. pneumoniae* ST392 KPC-3 producing clinical isolates, which will give them more tools to know how to deal with these nosocomial bacterial isolates.
We will also continue the surveillance program to understand if more strains of this particular ST are spreading among the patients of our clinical partner, the IRCCS ISMETT.

MEETINGS

8° Congress of European Microbiologists FEMS, July , 2019, Glasgow (UK)

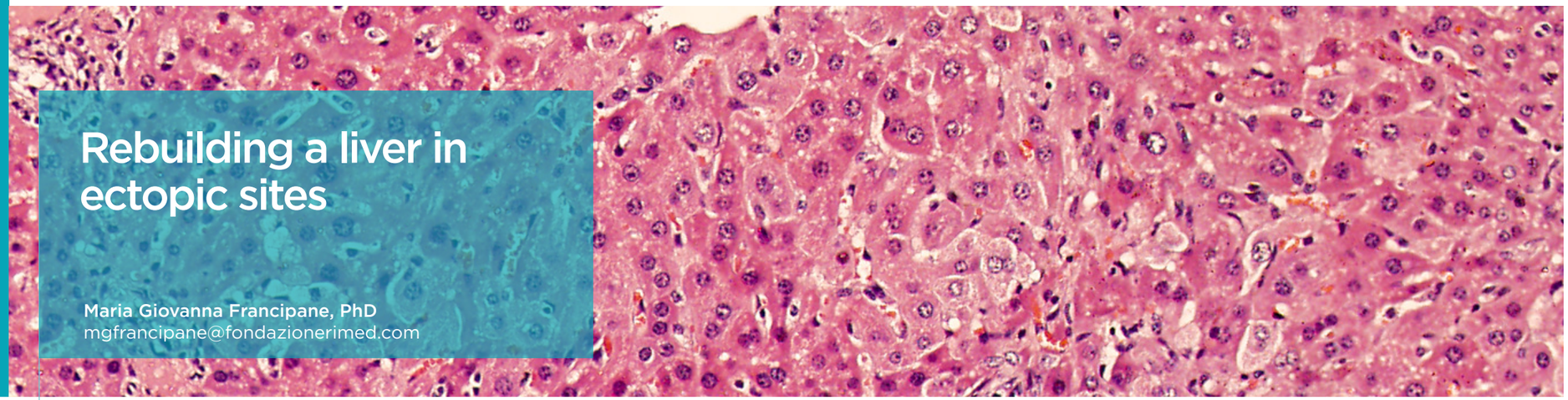
Ri.MED Research Retreat, October, 2019, Palermo (Italy)

PUBLICATIONS

- Mularoni A, Martucci G, **Douradinha B**, Campanella O, Hazen B, Medaglia A, Arena G, Gruttadauria S, Tuzzolino F, Arcadipane A, Gioè S, Luca A, Conaldi PG, Grossi P, Gridelli B. (2019) Epidemiology and successful containment of a carbapenem-resistant Enterobacteriaceae outbreak in a Southern Italian Transplant Institute. Transpl Infect Dis, 21(4), e13119. doi: 10.1111/tid.13119

- Di Mento G, Carreca AP, Monaco F, Cuscino N, Cardinale F, Conaldi PG, **Douradinha B**. (2019) Mycobacterium saskatchewanense strain associated with a chronic kidney disease patient in an Italian transplantation hospital and almost misdiagnosed as Mycobacterium tuberculosis. Infect Control Hosp Epidemio, 40(4), 496-497. doi: 10.1017/ice.2019.6

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



Rebuilding a liver in ectopic sites

Maria Giovanna Francipane, PhD
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COLLABORATIONS

- **LyGenesis, Inc, Pittsburgh, USA**
- **Mayo Clinic, Rochester, Minnesota, USA**

THERAPEUTIC AREA

Organ insufficiences

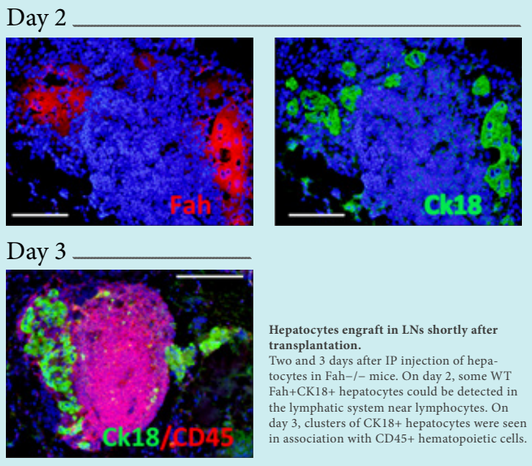
PIPELINE

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

BRIEF DESCRIPTION

Groundbreaking academic research by Prof. Eric Lagasse and his lab members recently lead to the establishment of LyGenesis, Inc., a biotechnology company with an organ regeneration technology platform enabling a patient's lymph nodes (LNs) to be used as bioreactors to regrow functioning ectopic organs.

LyGenesis' lead preclinical program is focused on liver regeneration. Liver cell injection into LNs worked in mice, where the surrogate mini-livers made up for the missing function of a diseased liver. Preclinical studies in pigs and dogs have been encouraging too, and LyGenesis will soon initiate a human clinical trial for patients with end stage liver disease.



Hepatocytes engraft in LNs shortly after transplantation.
Two and 3 days after IP injection of hepatocytes in Fah−/− mice. On day 2, some WT Fah+CK18+ hepatocytes could be detected in the lymphatic system near lymphocytes. On day 3, clusters of CK18+ hepatocytes were seen in association with CD45+ hematopoietic cells.

IMPACT

Nearly 14,000 patients wait annually for liver transplantation in the U.S. alone. The problem is considerably worse world-wide and represents one of the most challenging hurdles in medicine. With a universal shortage of organs and limited resources, alternatives to whole organ transplantation are required to address this pandemic. The effectiveness of cell-based therapies to treat liver failure is limited by the inflamed and fibrotic environment of the diseased liver. Alternative anatomical sites for hepatocyte transplantation could provide a healthier milieu to enable their engraftment and function. LNs and additional lymphoid clusters are alternative sites due to several defining characteristics. Depending on the stage of the disease, the rebuilding of liver in these sites could serve as a bridge to transplantation, or if done early enough in the disease progression, eliminate the eventual need for organ transplant. Importantly, instead of one donor organ treating one patient, LyGenesis enables one donor organ to treat dozens of patients. Instead of major surgery, LyGenesis uses outpatient endoscopy.

RESULTS ACHIEVED IN 2019

MOUSE: Abdominal fat-associated lymphoid clusters (aFALCs) are highly vascularized lymphoid aggregates similar to LNs and located in the adipose tissue throughout the abdominal cavity. We found that, following intraperitoneal injection, hepatocytes engrafted and proliferated in aFALCs, eventually forming functional liver nodules that rescued FAH deficient mice. Minimal growth of hepatocytes in abdominal adipose tissue was observed in FAH deficient mice lacking aFALCs. By inducing inflammation in the abdomen which increases the number of aFALCs, more ectopic liver nodules were formed and more FAH deficient mice were rescued. Collectively, aFALCs are unique structures in the peritoneal cavity that can facilitate functional liver development.

PIG: FAH-deficient pigs received autologous hepatocyte transplantation into LNs after *ex vivo* transduction with a lentiviral vector carrying the pig Fah gene. Transplanted Fah positive hepatocytes showed early (6 hour) and durable (8 month) engraftment in LNs. In addition, transplanted hepatocytes migrated to and repopulated the diseased native liver. The corrected cells generated enough liver mass to clinically restore liver function in this metabolic disease as early as 97 days post-transplantation, with complete normalization of tyrosine levels. Integration site analyses indicated that the population of corrected hepatocytes in the liver were a subpopulation of the cells present in the LNs, demonstrating that the LNs can serve as a source for healthy hepatocytes to repopulate a diseased liver.

DOG: During 2019, the Lagasse lab and LyGenesis completed additional preclinical experiments in dogs. GMP-like isolated canine hepatocytes were directly or indirectly (via an endoscope) transplanted into dog mesenteric LNs. Recipient dogs were subjected to partial hepatotectomy or portacaval shunt (diversion of portal blood from liver) before transplantation to mimic hepatic injury. Both autologous and allogenic hepatocyte transplantation were performed. These additional studies established safety and tolerability of the LN-based therapeutic approach.

GOALS FOR 2020

Molecular and cellular mechanisms allowing ectopic liver development in aFALCs will be investigated. A Phase 2a clinical trial utilizing the LN technology will soon start for patients with end stage liver disease.

MEETINGS

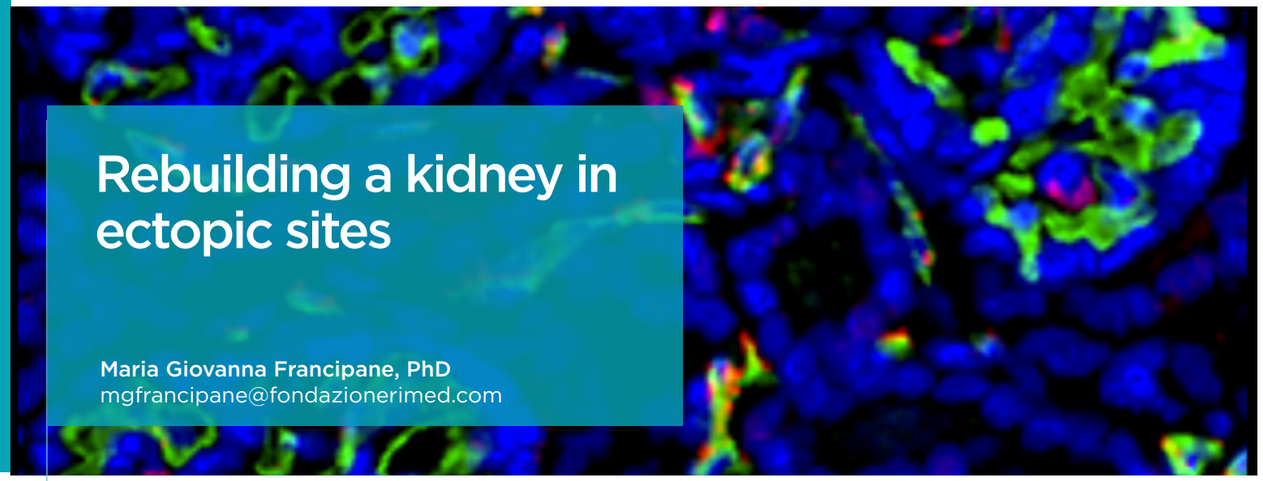
POSTER

C.T. Nicolas, R.D. Hickey, K.L. Allen, Z. Du, R.M. Guthman, C.J. VanLith, B. Amiot, L. Suksanpaisan, B. Han, **M.G. Francipane,** A. Cheikhi, H. Jiang, A. Bansal, M.K. Pandey, I. Garg, V. Lowe, A. Bhagwate, D. O'Brien, J.A. Kocher, T.R. DeGrado, S.L. Nyberg, R.A. Kaiser, E. Lagasse, J.B. Lillegard. ASGCT 22nd Annual Meeting. April 29 – May 2. Washington, DC, USA. Abstract published in Molecular Therapy. April 2019. Vol 27, No 4S1.

PUBLICATIONS

- Nicolas CT, 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 JPA, DeGrado TR, Nyberg SL, Kaiser RA, Lagasse E, Lillegard JB. Ectopic hepatocyte transplantation cures the pig model of tyrosinemia. bioRxiv 648493. 2019 May.
- Ectopic Liver Development in Abdominal Fat-associated Lymphoid Clusters (aFALCs). *In preparation.*

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



Rebuilding a kidney in ectopic sites

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COLLABORATIONS

- Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA
- Maine Medical Center Research Institute, Scarborough, ME, USA
- University of Southern California, Los Angeles, CA, USA

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

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

BRIEF DESCRIPTION

850 million people worldwide have some form of kidney disease. Kidney diseases cover a wide spectrum of diseases with diverse etiologies, clinical courses, and functional severity, from acute kidney failure (AKI) to the various chronic kidney disease (CKD) stages (1 to 5), and include end-stage renal disease (ESRD), which demands chronic dialysis and renal transplantation. ESRD causes at least 2.4 million deaths per year and is now the sixth main cause of death. Thus, kidney diseases are now an unquestionable global public priority.
Transplantation is the only definitive treatment method that restores kidney function, but has its own challenges with rejection and life-long immunosuppression. Moreover, kidney transplantation is often limited by organ shortage.

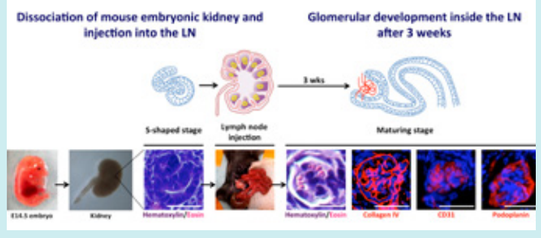
A potential solution to kidney organ shortage problem may be tissue-engineering, for example, through cell seeding on a scaffold with specific physical and mechanical properties. However, this approach has yet to prove effective for the treatment of patients with kidney failure.
In the laboratory, we have pioneered an *in vivo* vascularized tissue-engineering model in which target cells and tissues are implanted into a lymph node (LN). The LN - a secondary lymphoid organ, which acts as filter for foreign particles and cancer cells - rapidly responds to accommodate increases in cellularity and has immediate vascular access, prerequisites for successful development of functional organs.

IMPACT

Mouse and human kidney rudiments mature in renal structures with excretory, homeostatic, and endocrine functions inside the LN, thanks to an excellent host-derived vascularization. Such kidney-in-a-LN technology is opening up unprecedented opportunities to model renal development and test the fate of newly emerging cell sources in kidney tissue engineering including induced pluripotent stem cells. While organoids containing renal structures have been generated from pluripotent stem cells, there are still critical unanswered questions that are difficult to attain via *in vitro* systems, including whether these nonvascularized organoids have a stable and physiologically relevant phenotype. Our data show that the LN lends itself well as a niche to also grow renal organoid cultures derived from both mouse nephron progenitors and human induced pluripotent stem cells that were directed toward a renal fate *in vitro*. Indeed, we observed that host vasculature connects to the engrafted organoid tissues, strongly suggesting the feasibility of obtaining functional nephrons. Thus, the LN might help understand how emerging differentiation protocols can get closer to obtaining a functional kidney tissue, while reducing the risks of unwanted tissue formation. Moreover, elucidating the molecular mechanisms regulating the formation of new vessels in transplanted kidney tissues and cultures might help define new strategies to increase vascularization of tissue engineering constructs.

RESULTS ACHIEVED IN 2019

We showed that host lymphotoxin-beta receptor (LTβR) pathway generates angiogenic cues to support kidney organogenesis in both LNs and omenta. Our hypothesis that LTβR could impact kidney organogenesis in these sites sprang from LTβR's central role in the development and homeostasis of lymphoid organs, as well as from our finding that glomeruli maturing in both LNs and omenta contained, during the early stages of engraftment, endothelial cells expressing the nuclear factor κB-inducing kinase (NIK), a downstream target, among others, of LTβR. Given an established role for NIK in neovascularization, and the presence of host endothelial cells in ectopic kidney grafts, it was speculated that a host LTβR/NIK axis could allow successful kidney organogenesis. In accordance, defective LTβR signaling was found to be associated with decreased numbers of glomerular NIK-expressing endothelial cells, decreased absolute vascularization/angiogenesis as well as structural alterations of the kidney grafts. However, kidney organogenesis was not impacted in the absence of NIK. This result might suggest either the existence of different targets downstream of LTβR leading to angiogenesis or the activation of compensatory angiogenic pathways in the absence of NIK.
Finally, the absence of LTβR signals impaired compensatory glomerular adaptation to renal mass reduction, indicating that kidney regeneration approaches, besides whole kidney reconstruction, might benefit from the presence of LTβR signals.



LNs are permissive sites for kidney organogenesis.
Schematic view of transplantation of mouse embryonic kidney at the S-shaped body stage of nephrogenesis into the jejunal LN. Renal tissues were harvested from embryos, minced, and injected directly into a single LN of adult mice. Following 3 weeks, recipient mice were sacrificed, LN were collected, and histologically examined. Maturing glomeruli inside the LN are shown following staining with hematoxylin and eosin, anti-Collagen IV (constituent of mesangial matrix), anti-CD31 (blood vessel marker) or anti-Podoplanin (podocyte-specific marker) antibodies. Blue=Hoechst. Scale bar=50μm.

GOALS FOR 2020

La modulazione dell'organogenesi renale all'interno del LN mediata da LTβR è probabilmente il risultato di processi multicellulari. I segnali mediati da LTβR nelle cellule fibroblastiche reticolari del LN regolano i livelli del fattore di crescita dell'endotelio vascolare (VEGF) e a sua volta, la proliferazione delle cellule endoteliali linfonodali. In maniera simile, l'attivazione di LTβR nelle cellule endoteliali linfonodali controlla lo sviluppo vascolare. Attraverso la delezione cellulo-specifica di LTβR comprenderemo il contributo di ciascuna popolazione stromale all'organogenesi renale. Inoltre, attraverso il trapianto di organoidi renali, che sono privi di cellule immuni ed endoteliali, ci proponiamo di elucidare ulteriormente i meccanismi che modulano l'organogenesi renale nei tessuti linfoidi secondari.

MEETINGS

POSTER
M. G. Francipane, B. Han, L. Oxburgh, S. Sims-Lucas, Z. Li, E. Lagasse. Bioartificial kidneys: a translational journey. Ri.MED Research Retreat. Palermo, Italy. Oct 23, 2019.

PUBLICATIONS

- M. G. Francipane, B. Han and E. Lagasse. Host lymphotoxin-beta receptor signaling is crucial for angiogenesis of metanephric tissue transplanted into lymphoid sites. The American Journal of Pathology. 2020 Jan.
- M. G. Francipane, B. Han, L. Oxburgh, S. Sims-Lucas, Z. Li and E. Lagasse. Kidney-in-a-lymph node: a novel organogenesis assay to model human renal development and test nephron progenitor cell fates. Journal of Tissue Engineering and Regenerative Medicine. 2019 Sep;13(9):1724-1731.

PRODUCTS: **MEDICAL DEVICES & TISSUES AND ORGANS ENGINEERING**

OActive - Advanced personalised, multi-scale computer models preventing OsteoArthritis

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COLLABORATIONS

- **Partner of the European project OActive - H2020: Lead partner: University of Nicosia; Cyprus**
  other partners on https://www.oactive.eu/partners/
- **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, Philadelphia, USA**

THERAPEUTIC AREA

Aging diseases

PIPELINE

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

BRIEF DESCRIPTION

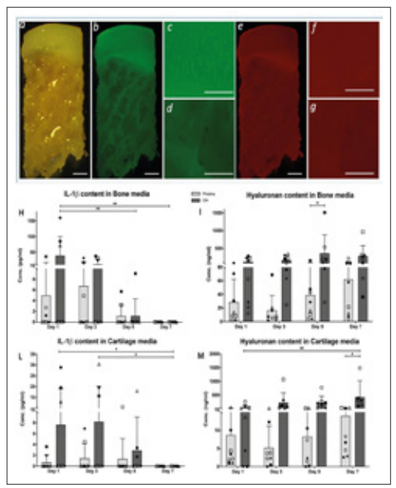
Osteoarthritis (OA) is an inflammatory frequent joint disease, mainly affecting middle-aged and elderly population. A systematic epidemiologic study, estimate that about 27% of people ≥ 65 years old suffer a symptomatic osteoarthritis worldwide, and it has been predicted that up to 25 million of people 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 2019

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-1δ and IL-6 in subchondral bone, which suggest an active role of this tissue in osteoarthritis development. 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.



Brightfield microscopy of an osteochondral plug's longitudinal section (a). Images captured by fluorescence microscopy after Live/Dead staining of an osteochondral plugs at day 7 of culturing showing living cells (b-d) and dead cells (e-g). Bone (H, I) and cartilage (L, M) content of a proinflammatory cytokine (IL-1δ) and a degradative marker (Hyaluronan) detected by ELISA assays.

GOALS FOR 2020

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

- Tissue Engineering and Regenerative Medicine – Americas (TERMIS AM), December 2019, Orlando (FL), USA
- Biofabrication, October 2019, Columbus (OH), USA
- American Orthopaedic Society for Sports Medicine, AOSSM Annual Meeting, July 2019, Boston (MA), USA
- Tissue Engineering and Regenerative Medicine – EU (TERMIS EU), May 2019, Rodi, Greece
- CWRU Center for Multimodal Evaluation of Engineered Cartilage, Investigator's Meeting, May 2019, Cleveland (OH), USA
- Orthopaedic Research Society Annual Meeting, February 2019, Austin (TX), USA

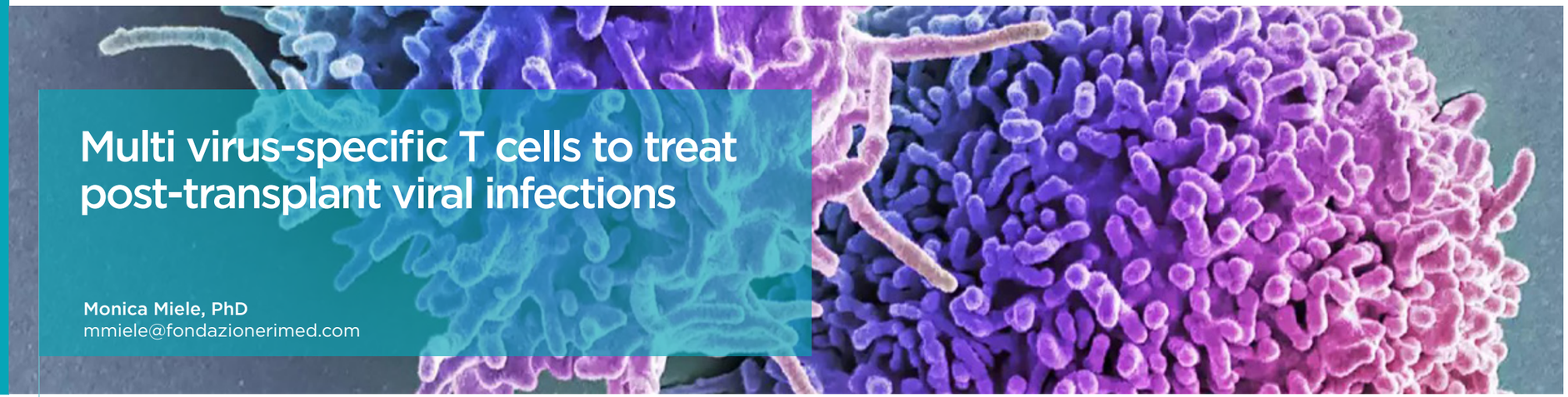
PUBLICATIONS

- Iseki T., Rothrauff B.B., Kihara S., Sasakin H., Yoshiya S., Fu H.F., Tuan R.S., **Gottardi R**. 2019. Dynamic Compressive Loading Improves Cartilage Repair in an In Vitro Model of Microfracture: Comparison of 2 Mechanical Loading Regimens on Simulated Microfracture Based on Fibrin Gel Scaffolds Encapsulating Connective Tissue Progenitor Cells. The American Journal of Sports Medicine, 47, 2188-2199 https://doi.org/10.1177/0363546519855645
- Chiesa I., De Maria C., Lapomarda A., Fortunato G.M., Montemurro F., Di Gesù R., Tuan R.S., Vozzi G., **Gottardi R**. 2019. Endothelial cells support osteogenesis in an in vitro vascularized bone model developed by 3D bioprinting. Biofabrication. https://doi.org/10.1088/1758-5090/ab6a1d. IN PRESS

INTELLECTUAL PROPERTY

- US20160201037A1, 22-08-2013, Modular, microfluidic, mechanically active bioreactor for 3d, multi-tissue, tissue culture
- US20190276784A1, 17-11-2017, Organ chip to model mammalian joint

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



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

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COLLABORATIONS

IRCCS ISMETT, Palermo, Italy

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

CLINICAL NEED — PRECLINICAL DEVELOPMENT — GMP PROCESS VALIDATION — CLINICAL STUDIES

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

IMPACT

Infectious disease, particularly those caused by viral agents, are the main cause of post-transplant morbidity and mortality. Up to 75% of transplanted patients develop infections during the first year after transplantation.

The primary cause of this phenomenon is the inhibition of the cell-mediated virus-specific immune response induced by the immunosuppressant drugs used to prevent rejection. Since T cells play a key role in the control and clearance of viral infections, the state of immunodepression promotes primary infection, reinfection or reactivation of viral agents with high prevalence, such as herpes viruses (eg EBV, CMV and HHV-8), with possible development of systemic or organ diseases.

The treatment of these infections is a significant challenge because of the scarcity of antiviral drugs and their associated toxicity. An alternative treatment, now clinically validated, is the infusion of virus-specific T lymphocytes, an advanced Therapy Medicinal Product (ATMP) that enables the patient to develop *in vivo* a cytotoxic response against infected cells: an effective therapy both as a prophylaxis and as cure of virus-induced pathological manifestations which could be lethal for the patient.

RESULTS ACHIEVED IN 2019

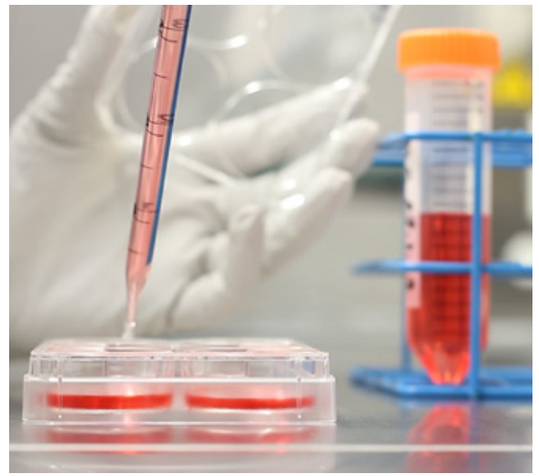
The activities carried out in 2019 included the optimization of the protocol for the production of multivirus specific T lymphocytes, with focus on the development of specific HHV-8 T cells. We increased the number of T cell lines produced by both cord blood donors and adult healthy blood donors. The final protocol of production will not require the use of dendritic cells, professional antigen presenting cells, since it has been demonstrated that monocytes and B lymphocytes contained in the cells (PBMCs) isolated from the blood of healthy adult donors are equally capable to stimulate proliferation and activation of *in vitro* virus-specific cytotoxic of T lymphocytes against viral peptide pulsed target cells.

The preparation of HHV-8 specific T lines was more challenging. Despite the testing of different culture conditions, the final cell product did not meet the required activity/specificity tests.

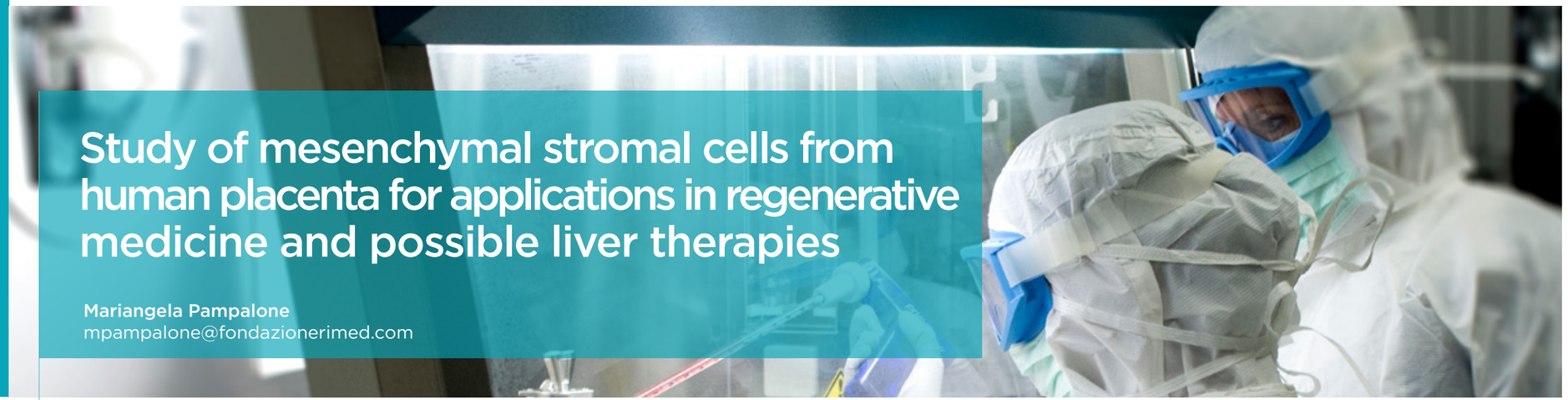
An *in silico* analysis of HHV-8 proteins was conducted in order to identify other peptides combination of viral antigens, smaller than those previously used (9-11 aa), and restricted for HLA-E and for the most frequent Class I HLA in our population.

GOALS FOR 2020

During 2020 the method of a production of multivirus T lymphocytes will be tested using innovative"devices" for suspension cell culture, allowing to increase the number of cells produced by each donor while minimizing their manipulation. In addition, experiments will be carried out with a new mix of HHV-8 virus peptides in order to obtain specific HHV-8 T lymphocytes that will be characterized, at different time points, from an immunophenotypic point of view, for the specific *in vitro* cytotoxic activity against virus and for the release into the culture medium of some fundamental molecules for the maintenance of the *in vivo* cell-mediated response (e.g. IFN-γ).



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



Study of mesenchymal stromal cells from human placenta for applications in regenerative medicine and possible liver 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 cirrhosis of the liver have an increased risk of developing multi-organ failure due to infections caused by bacterial translocation, that is passage of bacteria and their products, such as endotoxins, from the lumen to the intestinal wall and from here to the mesenteric lymph nodes and the stream circulatory. Bacteria and their products are able to activate the immune system with increased release of mediators able to induce systemic inflammatory response syndrome (SIRS) whose progression culminates in multi-organ failure (MOF).
Both endotossine and cytokines can cause hepatocyte necrosis through changes in hepatic microcirculation. A key role in this type of liver injury is coated by Kupffer cells, which are a source of highly reactive O2 and NO species that can determine lipid peroxidation and hepatic damage.
The impairment of antibacterial defenses, such as functional alterations of non-specific and cell-mediated humoral immunity, facilitates the engraftment of infections in the various sites, including the ascitic fluid.

IMPACT

In patients with advanced cirrhosis, DNA-bacterial translocation induces the activation of the complement system in both plasma and ascitic fluid and activates cell-mediated immune response and nitric oxide hyperproduction by peritoneal macrophages with a higher production of pro-inflammatory cytokines (IL-6 and TN-F-α). Macrophages, cells of innate immunity, represent the first line of defense against microbes and could be used as targets for the treatment of ascites in basal conditions and in the presence of over-infection. It is possible to evaluate the activity of macrophages in terms of activation and secretion of pro and anti-inflammatory cytokines in the absence or presence of mesenchymal or epithelial cells in the ascitic fluid and in the peripheral blood of cirrhotic patients (Child-Turcotte-Pugh B and C) in the presence of different degrees of bacterial infection.

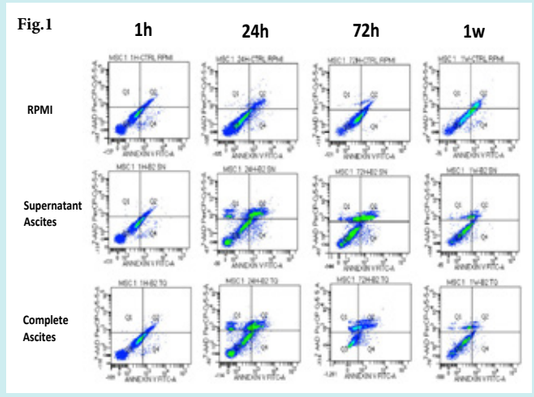


Fig.1: Representative panel of FC analyses showing how hA-MSCs were able to respond to AF exposure reducing apoptosis and necrosis at 1w suggesting a resistance to the stress (revealed at 24 and 72h), also compared with cells grown in RPMI

RESULTS ACHIEVED IN 2019

In vitro studies were conducted to evaluate the effect of ascitic fluid from 3 cirrhotic patients (Child-Turcotte-Pugh B) who underwent paracentesis on 3 different lots of amniotic mesenchymal cells (hA-MSCs) at different times.
The results obtained showed that the cells in contact with the ascitic fluid do not show morphological variations, inhibitions to proliferation, phenotypic variations nor significant necrosis / apoptosis values.

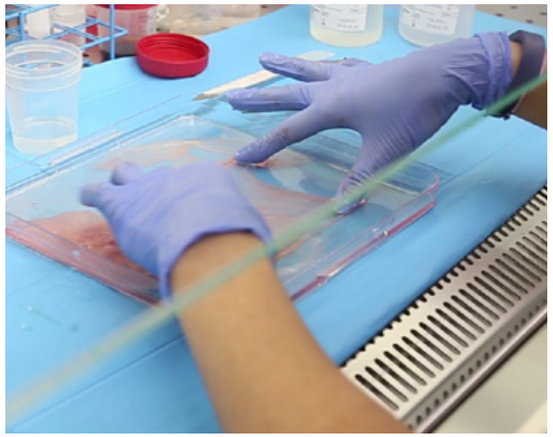
GOALS FOR 2020

Study of the conditioned medium 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.

PUBLICATIONS

- Comparison of Immunosuppressive and Angiogenic Properties of Human Amnion-derived Mesenchymal Stem Cells between 2D and 3D Culture Systems. Vitale Miceli; **Mariangela Pampalone**; Serena Vella; Anna Paola Carreca; Giandomenico Amico; and Pier Giulio Conaldi." Stem Cells Int. 2019 Feb 18;2019:7486279.

- Comparative study ofthe production of soluble factors in human placentaderived mesenchymal stromal/stem cells grown in adherent conditions or as aggregates in a catheter-like device. Miceli V., Chinnici CM., Bulati M., **Pampalone M.**, Amico G., Schmelzer E., Gerlach JC., Conaldi PG. Biochem Biophys Res Commun. 2019 Nov 19. pii: S0006-291X(19)32189-8



PRODUCTS: **DRUGS - BIOLOGICS**

iRhom2: a new therapeutic target in osteoarthritis

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COLLABORATIONS

- Institute of Aging and Chronic Diseases, University of Liverpool, Liverpool, UK
- The William Harvey Research Institute, Queen Mary University of London, London UK
- Pharmacy Department, University of Pisa, Pisa, Italy
- German Center for Neurodegenerative Diseases (DZNE), Munich, Germany

THERAPEUTIC AREA

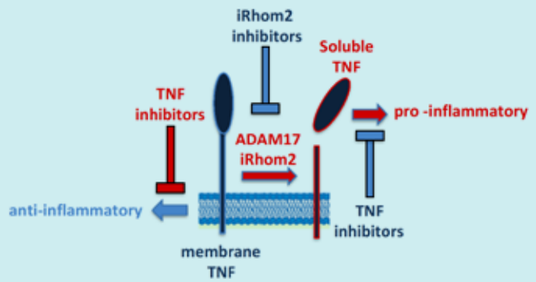
Aging diseases

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.



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.

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 2019

Although the project has just begun, a number of crucial results has been already accomplished. First, we set up all necessary collaborations to carry out this project successfully. This comprises a collaboration with George Bou-Gharios group at University of Liverpool, who will analyse OA progression in iRhom2 knockout mice, with Ahuva Nissim, Queen Mary University of London, who has developed one of the biggest phage-display libraries available in the world and will aid us with the development of an iRhom2 antibody that can be potentially used in the therapy of OA. Secondly, we employed Matteo Calligaris, a bright and enthusiastic student from University of Udine, who will carry out the project. In addition, we set up a collaboration with the University of Pisa, in order to allow Matteo Calligaris to enter its PhD program, and being tutored by Armando Rossello and Elisa Nuti, two of the most prominent Italian scientists in the field of metalloproteinases. Very importantly, we completed the MTA agreement with the University Health Network (UHN, Toronto, Canada) for the use of the iRhom2 knockout transgenic mouse.

GOALS FOR 2020

iRhom2 knockout mice have been recently transferred to University of Liverpool where OA progression will be assessed in surgically-induced murine model of the disease. We expect that ablation of iRhom2 will ameliorate the progression of the disease by reducing LRP-1 shedding and extracellular levels of MMP-13, ADAMTS-4 and ADAMTS-5. Proteomics will reveal protein changes in the cartilage of OA mouse, providing interesting new information about shifts in the catabolic/anabolic balance of ECM factors upon induction of OA. Potentially, this will identify novel mechanisms, in addition to metalloproteinase turnover, that may be involved in the pathogenesis of OA. If so, we plan to patent iRhom2 as a therapeutical target for OA and diffuse our discoveries through high impact pubblications.

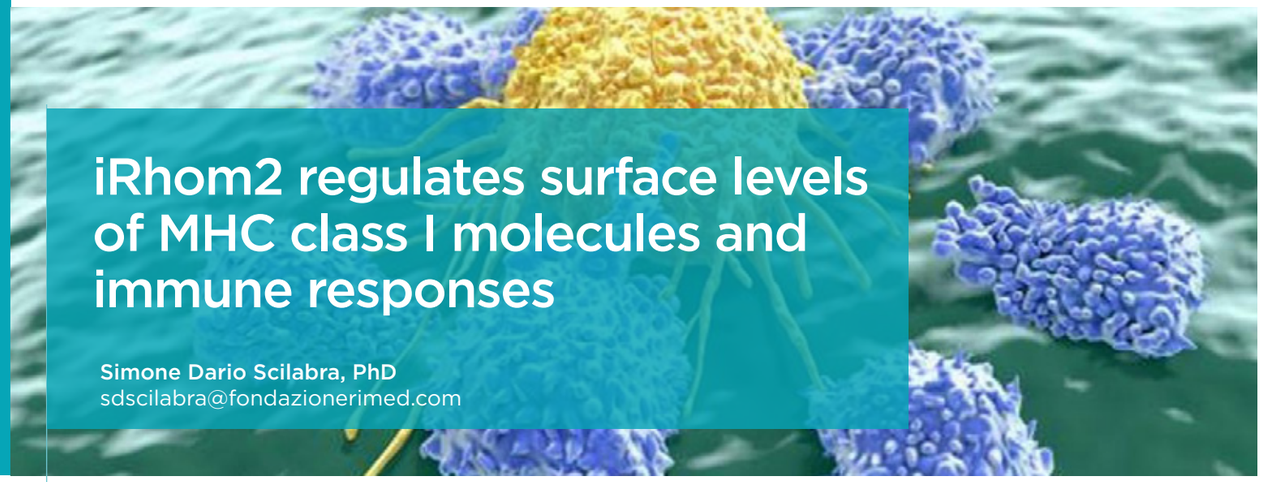
MEETINGS

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

PUBLICATIONS

- Anna P. Carreca, Veronica Pravatà, Gillian Murphy, Hideaki Nagase, Linda Troeberg, **Simone D. Scilabra**. TIMP-3 facilitates binding of target metalloproteinases to the endocytic receptor LRP-1 and promotes scavenging of MMP-1. BiorXiv doi: https://doi.org/10.1101/2019.12.23.886762
- 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.

PRODUCTS: **DRUGS - BIOLOGICS**



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

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COLLABORATIONS

- German Center for Neurodegenerative Diseases (DZNE), Monaco, Germania
- Weill Cornell Medicine Graduate School of Medical Sciences, New York, Stati Uniti

THERAPEUTIC AREA

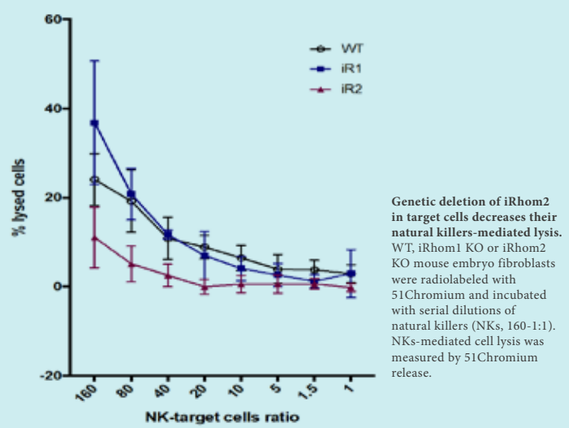
Cancer

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 2019

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 2020

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.

MEETINGS

British Society for Matrix Biology (BSMB) Autumn Meeting, University of East Anglia, Norwich, UK (Presentazione Orale).

Gordon Research Conference (GRC) in metalloproteinases, Il Ciocco Resort, Lucca (Italy) (Presentazione Poster).

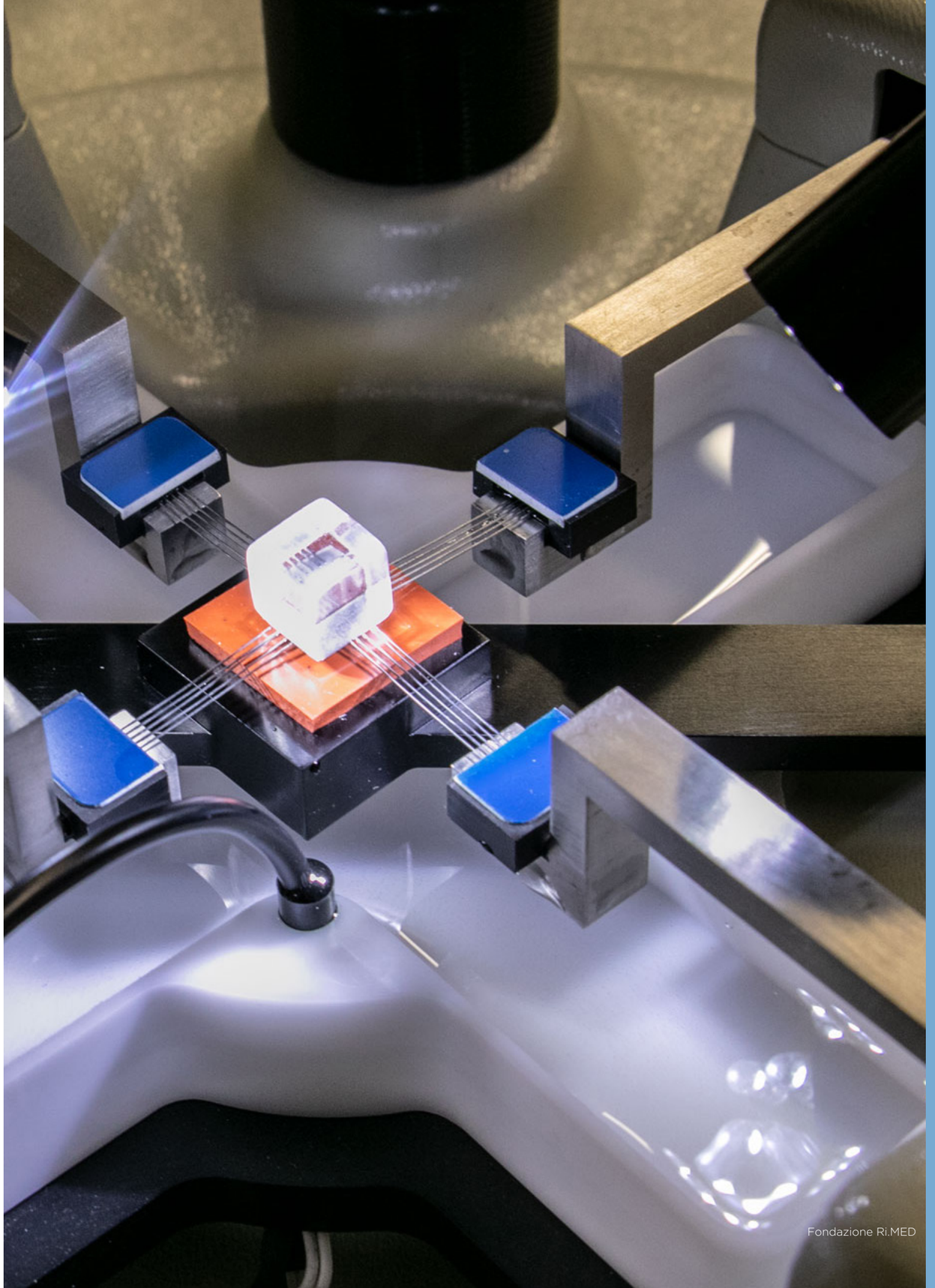
TISSUE ENGINEERING AND BIOMEDICAL DEVICES

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 based on the latter.

The devices provided to our researchers allow to optimize biomaterials and engineered tissues for multiple applications. Also, it allows for their use to design, develop and for preclinical validation of new generation implantable organs and devices, particularly in the cardiovascular field.

During 2019, our research in these areas produced significant results in terms of basic research and applications for patients.
New approaches were studied for the characterization of biological tissues and biomaterials, and scaffold fabrication. A greater understanding was acquired of the biological and physiological mechanisms responsible for adhesion in humid environments, cell proliferation and thromboembolic events. In addition, 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 or genetic engineering applications.

The possibility of in-house development and validation of clinical solutions, and collaborations with the most important clinical centers in the area, will help introduce these treatments while offering new support tools in therapeutic planning and in the pre-intervention decision-making process.



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
Gaetano Burriesci, PhD

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

Hydrodynamic analysis of the aortic valve in optimum and altered conditions
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

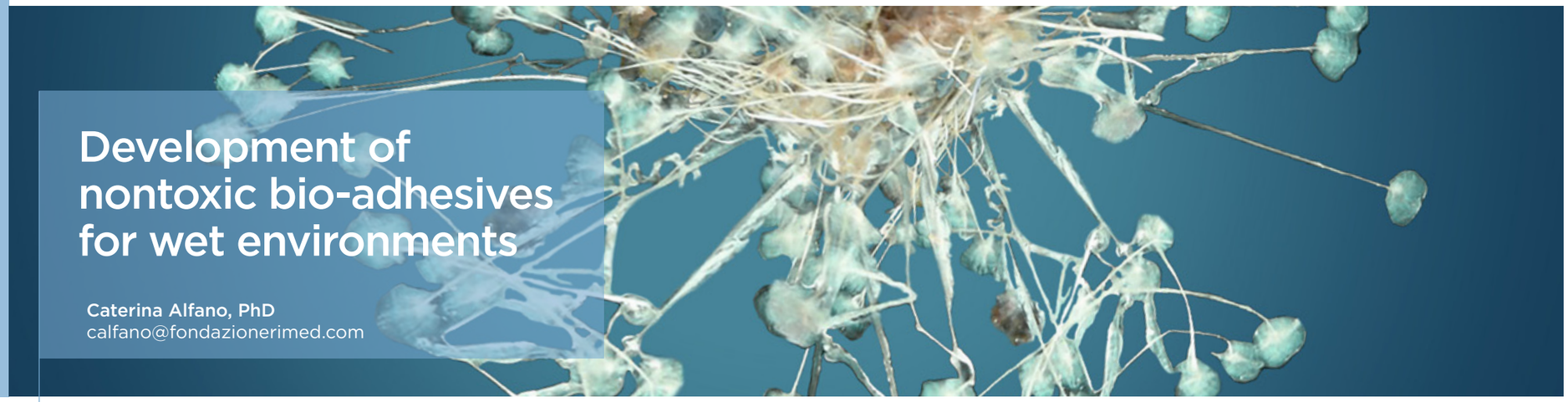
Tissue engineered cardic patch (TECP)
Antonio D'Amore, PhD

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

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

R-CaRe - Rehabilitation for Cartilage Regeneration
Riccardo Gottardi, PhD

PRODUCTS: **MEDICAL DEVICES**



Development of nontoxic bio-adhesives for wet environments

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COLLABORATIONS

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- Istituto di Biofisica (IBF) - CNR, Palermo, Italy

PIPELINE

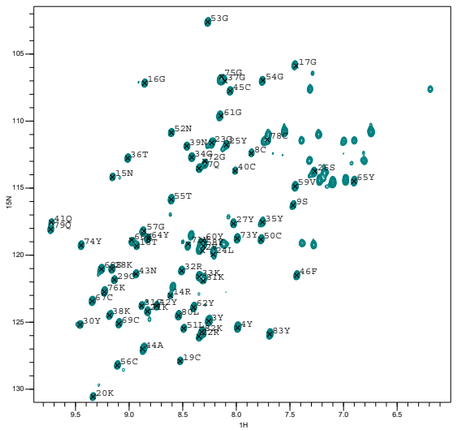
CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

BRIEF DESCRIPTION

Marine sessile animals have developed adaptive strategies to overcome obstacles that inhibit their adhesion in water (pH, hydration layers and dielectric properties). This made these animals great potential sources of nontoxic bio-compatible adhesives which have the additional advantage to be suited for wet environments. These strong and water-insoluble adhesion properties have attracted increasing interest for potential applications in surgery, regenerative medicine, biotechnology and material science. In particular, mussels have received significant attention especially because of their ability to adhere tightly to their substrates to resist also 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, mfp). Three mfps were recently identified in the Asian green mussel *Perna viridis* foot: Pvfp-3α, Pvfp-5β and Pvfp-6. Our aim is to structural characterize those proteins and derive bio-compatible recombinant proteins with adhesive properties.

IMPACT

The development of novel naturally-derived glues has a great impact in areas such as tissue engineering, implantation of medical devices and wound closure. In fact, 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.



Assigned ^{15}N-HSQC NMR spectrum of Pvfp5β The peaks result well dispersed as expected for a folded and non-aggregated protein.

RESULTS ACHIEVED IN 2019

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 interaction with the marine substrate. We performed the first implementation of the successful production of recombinant Pvfp-5β and its characterization by Circular Dichroism (CD), nuclear magnetic resonance (NMR), and Dynamic Light Scattering (DLS). Our biophysical studies confirm that we are dealing with a folded protein that presents β-sheet and random coil structures. We also demonstrate the adhesive properties of recombinant Pvfp-5β in its non-Dopa bound form and show that it does not exhibit cytotoxicity to cells as requested for a biomaterial. Our results prove for the first time that recombinant Pvfp-5β could be a good candidate as a bioadhesive.

GOALS FOR 2020

Thanks to the successful set up of a scheme for the recombinant production of Pvfp-5β, we are able to produce suitable quantities of the protein without the bottleneck of having to purify it directly from the natural source. This also allow us to obtain the protein with and without post-translational modifications and compare the adhesive properties of the two forms. We are now in process of resolving the three-dimensional NMR structure of Pvfp-5β in its no DOPA form. Future studies want to address a detailed comparison of the properties of the native and the post-translationally DOPA modified protein allowing a better understanding of the chemistry behind the interaction of Pvfps with marine surface. Biodegradability and mechanical properties of Pvfp-5β will be also tested.

MEETINGS

- XLVIII National Congress on Magnetic Resonance, september, 2019, L'Aquila (Italy)

- Resonance in Biology, september, 2019, Pavia (Italy)

PUBLICATIONS

- Santonocito R., Venturella F., Dal Piaz F., Morando M.A., Provenzano A., Rao E., Costa M., Bulone D., San Biagio P.L., Giacomazza D., Sicorello A., **Alfano C.***, Passantino R.*, Pastore A. (2019) Recombinant mussel protein Pvfp-5β: a potential tissue bioadhesive. J Biol Chem. 294(34):12826-12835. *Co-corresponding authors.

- Fricano A., Librizzi F., Rao E., **Alfano C.**, Vetri V. (2019) Blue autofluorescence in protein aggregates "lighted on" by UV induced oxidation. BBA - Proteins and Proteomics 1867(11):140258.

- Zacco E., Graña-Montes R., Martin S.R., de Groot N.S., **Alfano C.**, Tartaglia G.G., Pastore A. (2019) RNA as a key factor in driving or preventing self-assembly of the TAR DNA-binding protein 43. J Mol Biol. 431(8):1671-1688.

PRODUCTS: **MEDICAL DEVICES**

Development of a Novel Transcatheter Heart Valve

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THERAPEUTIC AREA

Organ insufficiences
Aging diseases

PIPELINE

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

BRIEF DESCRIPTION

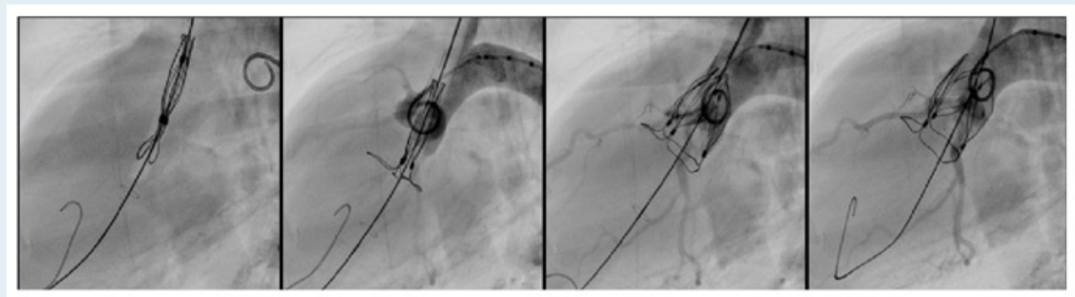
Though standard open heart surgical aortic valve replacement has represented an effective treatment in the past, it is not ideal for the new patients' population. In fact, degenerative aortic stenosis due to senile valve calcification has now become the most common valvular disease, affecting more than 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.

IMPACT

The work performed as part of this project demonstrated the feasibility of a new transcatheter heart valve concept, the TRI-SKELE 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 achievable without calcification, as confirmed in the animal models, reveals an important potential to expand the therapeutic advantages of transcatheter valve implantation to the class of patients suffering from aortic insufficiency, for which first generation TAVI devices are unsuitable.

RESULTS ACHIEVED IN 2019

The research previously performed had led to the design optimisation and manufacturing of a novel TAVI device. *In vitro* tests have confirmed that the hydrodynamic performance and predicted durability of the device meets the requirements recommended in the international standard ISO 5840-3:2013. A surgical version of the valve with identical polymeric leaflets as the TAVI device was implanted in juvenile sheep for 90 days chronic *in vivo* evaluation, as required by European regulations. The valve maintained functionality for the entire duration of the test, and histological analyses are now being performed. In 2019, Ri.MED Foundation was assigned the intellectual property for the device (whose patent is granted both in Europe and USA) and is now the sole assignee of this technology.



Sequence of implantation of the devices.

GOALS FOR 2020

A set of valve prototypes and delivery systems based on the developed technology will be manufactured and used for pre-clinical *in vivo* evaluation by means of percutaneous in animal implants in juvenile ovine models (50-70 kg).
The *in vivo* evaluation will demonstrate the implantation easy to handle, haemodynamic performance comparable or superior to equivalent predicate valves and the absence of device related pathologies counterindicating the use of the developed valve.

PUBLICATIONS

- Gallarello A., Palombi A., Annio G., Homer-Vanniasinkam S., De Momi E., Maritati G., Torii R., **Burriesci G.**, Wurdemann H.A. (2019) Patient-specific aortic phantom with tunable compliance. ASME J of Medical Diagnostics 2(4):041005.

- Palombi A., Bosi G., Di Giuseppe S., De Momi E., Homer-Vanniasinkam S., **Burriesci G.**, Wurdemann, H. (2019) Sizing the aortic annulus with a robotised, commercially available soft balloon catheter: in vitro study on idealised phantoms. Proceedings of the IEEE International Conference on Robotics and Automation 2019, TuCT1-12.5.

INTELLECTUAL PROPERTY

- Burriesci G., Seifalian, A.M., Zervides, C. (2019) Heart valve prosthesis. Patent US10357358 (B2)

- Burriesci G., Seifalian, A. M., Zervides, C. (2017) Heart Valve Prosthesis. Patent EP2413842 B1

PRODUCTS: **MEDICAL DEVICES**

Development of a Novel Alfa-Gal free Xenograft Heart Valve

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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 block the opening of the valve. Scientist 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. So far, the calcification blocking treatments which have been developed 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 their immune system reacts with a substance called Gal, not made by people, but commonly made in animals 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.

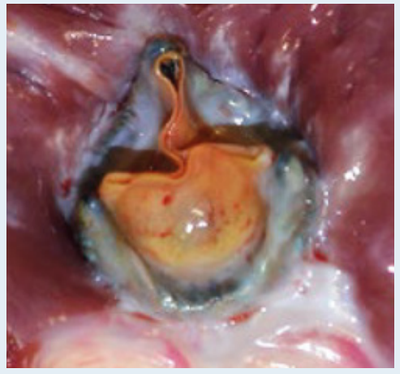
IMPACT

Approximately 300,000 valve replacements are performed annually worldwide. Two types of replacement valves are available, mechanical heart valves (MHVs) 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 (SVD) occurs within 5 years. More durable BHVs would advance the standard of care by eliminating the need for anticoagulation in younger patients and extending access to this therapy to more patients.

RESULTS ACHIEVED IN 2019

Sets of valves based on a manufacturing approach developed in house were built, using wildtype and α1,3-galactosyltransferase gene-knockout porcine pericardium. Prototypes were validated *in vitro* in terms of hydrodynamic performance and durability up to 200 million cycles. 6 prototypes per each group of tissue were used to perform mitral valve replacement in juvenile sheep with 90-day survival.
All valves implanted maintained functionality for the entire durability of the test, and histological analysis is currently ongoing.

Prototype of the valve made from transgenic porcine pericardium.



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

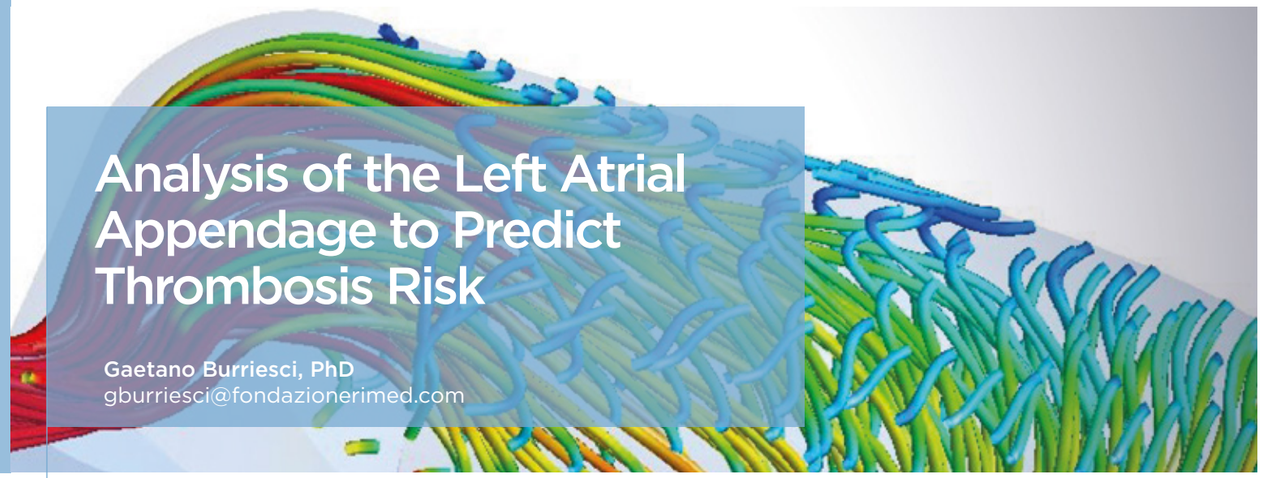
GOALS FOR 2020

Si esaminerà in futuro la possibilità di utilizzare tessuti di altre specie animali geneticamente modificate, come il pericardio bovino, che offre un'esperienza clinica ben più estesa rispetto quello suino. Si verificherà inoltre la possibilità di passare alla fase preclinica con i dispositivi precedentemente sviluppati.

MEETINGS

Rahmani B., McGregor C., Byrne G., **Burriesci G.** (2019) A Durable Porcine Pericardial Surgical Bioprosthetic Heart Valve: a Proof of Concept. Journal of Cardiovascular Translational Research, 12(4):331-337. https://doi.org/10.1007/s12265-019-09868-3.

PRODUCTS: **BIOMARKERS - MEDICAL DEVICES**



Analysis of the Left Atrial Appendage to Predict Thrombosis Risk

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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 study, 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.
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IMPACT

The study demonstrates that the LA and LAA wall motion, neglected in the available literature, play a leading role in promoting hydrodynamic alterations typically associated with thromboembolism. The proposed model lays the foundation for developing new computational studies to better investigate the AF pathology.

RESULTS ACHIEVED IN 2019

Two different geometries were created to represent the healthy (control) and AF cases, founded on previous morphological studies of LA and LAA based on MR images and CT measures of AF and healthy subjects. Three CFD simulations were run, replicating healthy, AF and an intermediate case. Boundary conditions included the application of the atrial wall velocity, the LAA lateral and medial motion, and the pressures at the mitral valve orifice and pulmonary veins, obtained from mathematical elaborations of clinical Echocardiographic and CT measurements.
CFD analyses were compared in terms of Shear Strain Rate (SSR), a quantity directly linked to the rheological response and the thrombogenicity of blood. Results indicate that in AF condition SSR are substantially lower than for the healthy control case. This is typically associated with higher blood stagnation and potential thrombogenicity.

GOALS FOR 2020

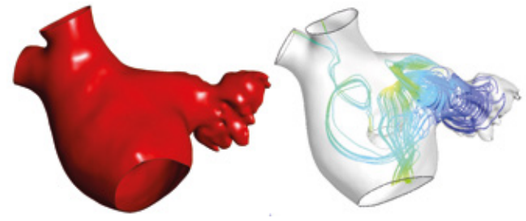
The described model is based on a number of assumptions, such as the laminar Newtonian description of blood. New models will be implemented to overcome these simplifications. Moreover, as the role of the morphology is relevant, its contribution will be investigated aside the wall motion. For this purpose, the geometry of the model will be modified by integrating more complex morphological parameters, allowing a population specific study.

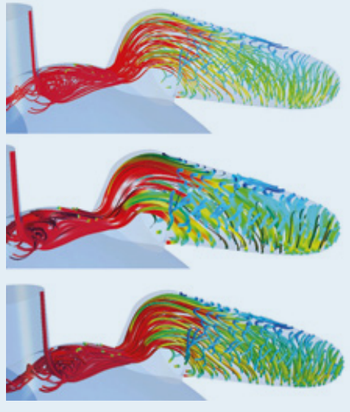
MEETINGS

Vella D., Musotto G., Bosi G., Zuccarello B., **Burriesci G.** (2019) Influenza Della Contrattilità Delle Camera Cardiache Sul Rischio Tromboembolico. 48° Convegno Nazionale AIAS

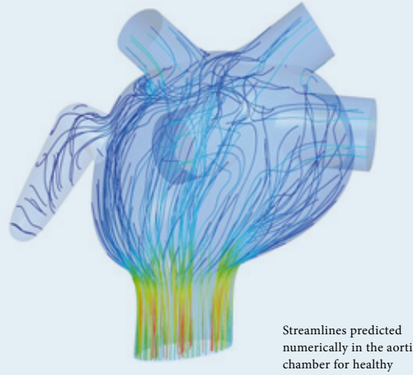
PUBLICATIONS

Annio G., Franzetti G., Bonfanti M., Gallarello A., Palombi A., De Momi E., Homer-Vanniasinkam S., Wurdemann H.A., Tsang V., Diaz-Zuccarini V., Torii R., Balabani S., **Burriesci G.** (2019) Low cost fabrication of PVA based personalized vascular phantoms for in vitro haemodynamic studies: three applications. ASME Journal of Engineering and Science in Medical Diagnostics and Therapy, n. JESMDT-19-1037. https://doi.org/10.1115/1.4045760





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



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

PRODUCTS: **MEDICAL DEVICES**

Hydrodynamic analysis of the aortic valve in optimum and altered conditions

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THERAPEUTIC AREA

Organ insufficiences

Aging diseases

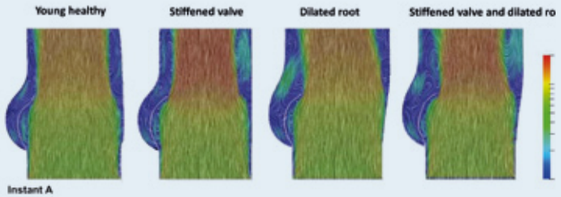
PIPELINE

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

BRIEF DESCRIPTION

Optimal valve function, limitation of blood damage, and frequency of thromboembolic events are all dependent upon the haemodynamics in the valve region. Improved understanding of the healthy physiological state via investigation of the fluid dynamics around and through the aortic valve is essential to identify detrimental changes leading to pathologies. It is also necessary in order to develop novel therapeutic procedures, improved cardiovascular implants, and clinical strategies based on patient specific treatments.

The combination of the latest computational engineering methodologies, *in vitro* approaches and medical imaging advances can significantly contribute to gain an adequate insight in the phenomenon, by overcoming the limitations in time and space resolution of the individual techniques.



Maps of the systolic blood velocity, predicted numerically for the young and aged healthy, and for pathological models.

IMPACT

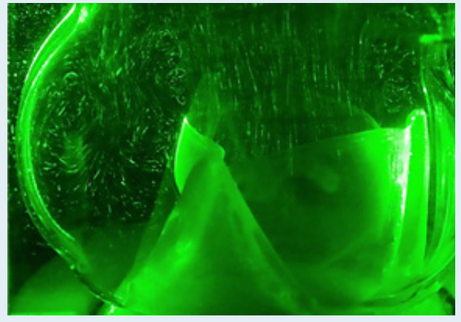
The aim of the project is the development of an innovative platform, based on the integration of numerical and experimental techniques, capable of analysing the hemodynamics of the aortic valve in healthy and pathological conditions. This can be used to predict the physiological hemodynamic changes that occur with aging, as well as the effect of potential treatments, to evaluate their safety and efficacy. Furthermore, the technologies implemented can serve as important tools for the development of novel clinical solutions and next generation medical devices.

RESULTS ACHIEVED IN 2019

A numerical model previously implemented on the basis of a combined experimental and numerical study of the fluid dynamics in the aortic root was parametrically modified to examine the hydrodynamic variations expected with normal ageing (tissue stiffening and expansion of the aortic root) and following the different replacement therapies currently available. The approach has led to a more complete understanding of the mechanisms that determine and alter the aortic valve function during physiological ageing, suggesting parameters for the estimation of the effectiveness of the different treatments.

GOALS FOR 2020

In the next phase, we will seek experimental confirmations of the results obtained so far, by means of *in vitro* methods such as particle image velocimetry. The platform previously developed will be further refined to provide a reference for the analysis of the effect of the flow alterations induced by other pathologies and therapeutic interventions.



In vitro PIV analysis of the blood flow exiting the aortic valve.

MEETINGS

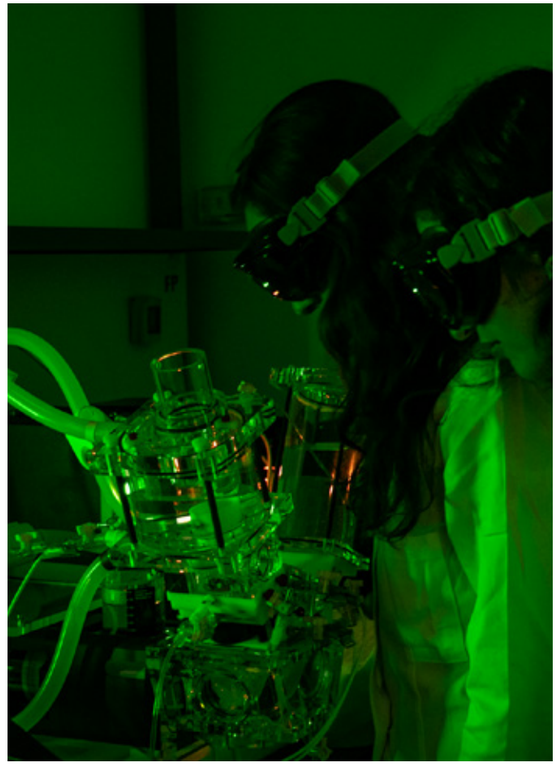
Salmonsmith J., Tango A. M., Ducci A., **Burriesci G.** (2019) Healthy Aortic Valve Dynamics - Extension after PIV Validation of a Fluid Structure Interaction. ICIAM 2019 (International Congress on Industrial and Applied Mathematics), Valencia, Spagna.

PUBLICATIONS

- Salmonsmith J., Ducci A., **Burriesci G.** (2019) Does Transcatheter Aortic Valve Alignment Matter Open Heart 6:e001132. doi:10.1136/openhrt-2019-001132

- D'Ascenzo F., Salizzoni S., Saglietto A., Cortese M., Latib A., Franzone A., Barbanti M., Nietlispach F., Holy E.W., **Burriesci G.**, De Paoli A, Fonio P., Atzeni F., Moretti C., D'Amico M., Rinaldi M., Conrotto, F. (2019) Incidence, predictors and cerebrovascular consequences of leaflet thrombosis (LT) after transcatheter aortic valve implantation (TAVI): a systematic review and meta-analysis. European Journal of Cardio-Thoracic Surgery, ezz099.

- Salmonsmith J., Tango A.M., Ducci A., **Burriesci, G.** (2019) Haemodynamic Issues with Transcatheter Aortic Valve Implantation. In: Giordano A., Biondi-Zoccai G., Frati G. (eds) Transcatheter Aortic Valve Implantation. Springer, Cham, pp 47-59.



PRODUCTS: **MEDICAL DEVICES**

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

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COLLABORATIONS

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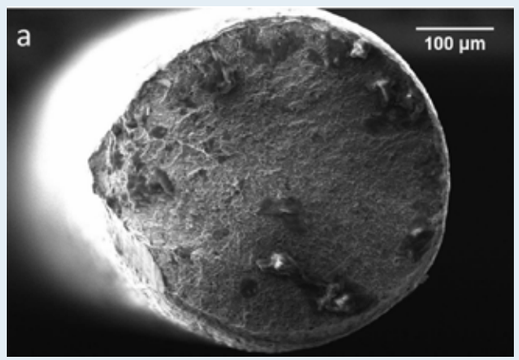
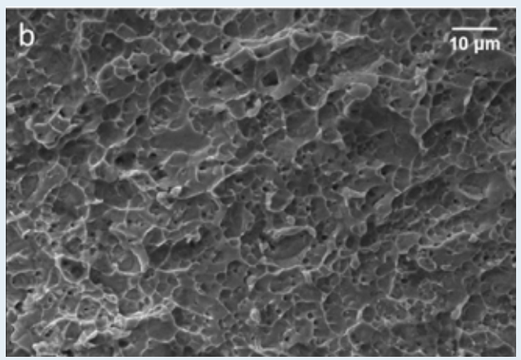
PIPELINE

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 study we exploit these thermal effects to gain a better understanding of the phenomenon and a more accurate characterisation of nitinol.





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

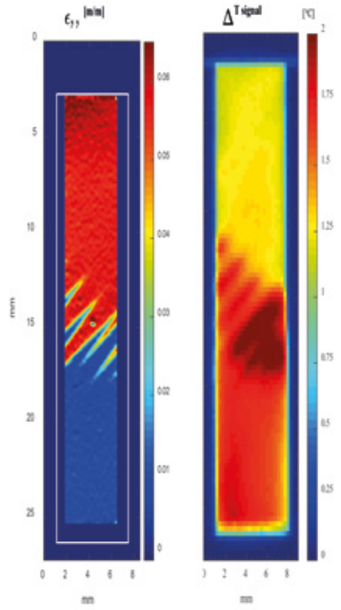
A more accurate understanding and characterisation of nitinol's behavior would contribute to increase the safety of medical devices based on this material.
The combined implementation of Digital Image Correlation and Infrared Thermography optical techniques, adopted in this study, can support the evaluation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

RESULTS ACHIEVED IN 2019

This study has identified a first approach enabling the application of Thermoelastic Stress Analysis to nitinol.
This technique, already well established for the study of conventional materials, allows the full field determination of the stresses in specimen and components, opening new opportunities for the structural characterisation of critical prosthetic components.

GOALS FOR 2020

In order to enhance the developed methodology, the phase signal of the Thermo-elastic effect will be further studied. This will then be adopted to perform the thermo-mechanical characterisation of complex medical devices, obtaining the full-field stress map from temperature and deformation maps.

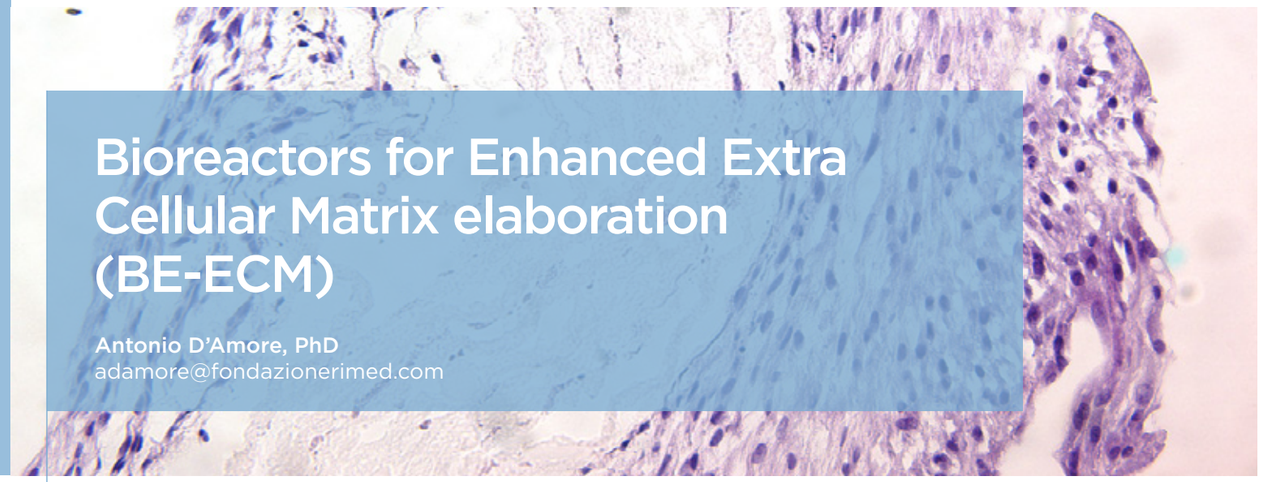


Deformation (left) and reversible temperature variation (right) maps acquired from a nitinol specimen subjected to cyclic tensile load.

MEETINGS

Di Leonardo S., Pitarresi, G., **Burriesci G.** (2019) Evaluation of the transformation domains of super-elastic NiTi shape memory alloy sheets with DIC and Thermoelastic Stress Analysis. 48a Conferenza Nazionale AIAS, Assisi, Italy

PRODUCTS: **MEDICAL DEVICES & TISSUES AND ORGANS ENGINEERING**



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

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- Universidad de Zaragoza, Zaragoza, Spain

THERAPEUTIC AREA

Organ insufficiences

Aging diseases

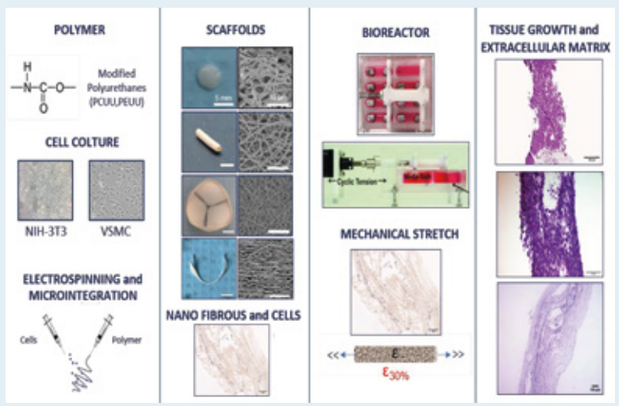
Cancer

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 which are recognized as relevant for the tissue engineering approach, still need more effective numerical and *in vitro* 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 models able to correlate mass loss with mechanical loads and deformations. This research line, integrated by NET-IB and NET-MTG, tries to address these three critical topics by introducing and perfecting physical, *in-vitro* models able to study tissue growth and biomaterials degradation.



IMPACT

Potential impact of this research might involve improved capacity to:
- to simulate endogenous tissue growth on engineered scaffolds under mechanical load and deformation;
- to simulate *in vivo* degradation of engineered scaffolds;
- to investigate the impact of material topological and mechanical cues on ECM elaboration.

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

RESULTS ACHIEVED IN 2019

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, this research direction was designed to assist the development of tissue engineered heart valve (TEHV), engineered vascular graft (TEVG)and cardiac patch (TECP). 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 2018 was to address fundamental questions regarding mechano-transduction mechanism *in vivo* by utilizing simplified systems *in silico* able to simulate 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 and TEVG design;
- to implement a novel apparatus for chordae tendineae mechanical conditioning;
- to implement a novel apparatus to induce accelerated degradation conditions on polymeric heart valves.

Awards

University of Pittsburgh 43rd annual Honors Convocation as outstanding faculty member.

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.

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;
- Mentor for BIOENG 1095 - Special projects. Individual research project under the guidance of a faculty member. Department of bioengineering, Univ. of Pittsburgh. Trainees: Daniel Jacobs Li-Ming 2017-2019.

Mentoring activity
- A. Adamo, PhD candidate, University of Palermo Italy, engineering chordae tendineae, mechanical conditioning and mechano-biology;
- D. Jacobs Li-Ming, University of Pittsburgh USA, quantitative methods for ECM mass detection 2016-2019, modelling cell motility on fibrous substrate 2019.

Invited speech
- "Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", Chemical and petroleum engineering department, graduate seminar, Pittsburgh, July 29, 2019;
- "Bioengineered cardiac tissue: how improved control over biomaterial structure-function can facilitate translation", bioengineering department, graduate seminar, Pittsburgh, April 26, 2019;
- "How to improve control over biomaterials structure-function to design better performing tissue surrogates". Ecole Polytechnique, Paris, France.

GOALS FOR 2020

To perfect and promote the BE-ECM experimental platform, in particular:
- To identify conditioning regimen for artificial chordae tendineae able to duplicate mass and mechanical properties of native chordae, submit one manuscript as senior author, 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.

MEETINGS

- H. Mamiya, A. Sahu, A. Cheikhi, S. Shinde, S. Sivakumar, S. Luketich, G. Nasello, B. Van Houten, A. Wise, P. LeDuc, **A. D'Amore**, A. Barchowsky, F. Ambrosio. Exposure of muscle stem cells to a stiff microenvironment drives an "aged" mitochondrial phenotype. Biomedical engineering society annual meeting, (BMES) October 16- 19 2019, Philadelphia PA, USA.
- H. Mamiya, A. Sahu, A. Cheikhi, S. Shinde, S. Sivakumar, S. Luketich, G. Nasello, B. Van Houten, A. Wise, P. LeDuc, **A. D'Amore**, A. Barchowsky, F. Ambrosio. Exposure of muscle stem cells to a stiff microenvironment drives an "aged" mitochondrial phenotype. June 9 - 14 2019, Lucca, Italy Myogenesis, Gordon Research Conference.

PUBLICATIONS

- **A. D'Amore**, S. Luketich, R. Hoff , S-H. Ye, and W. Wagner. Blending polymer labile elements at differing scales to affect degradation profiles in heart valve scaffolds. Biomacromolecules, 2019, 20, 7, 2494-2505, IF 5.73.
- D. Pedersen, F. Madonia, C.Tompkins-Rhoades, **A. D'Amore**, W. R. Wagner. Tissue to Organ Level Evaluation of Heart Valve Scaffold Performance under Dynamic Loading Conditions. American Heart Association (AHA) 2019 Annual meeting November 16–18, Philadelphia PA, USA. Circulation 140 (Suppl. 1), A16249-A16249.

PRODUCTS: **MEDICAL DEVICES & TISSUES AND ORGANS 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
- UPMC, Pittsburgh, USA
- Politecnico di Milano, Milan, Italy

THERAPEUTIC AREA

Organ insufficiences

Cancer

Aging diseases

PIPELINE

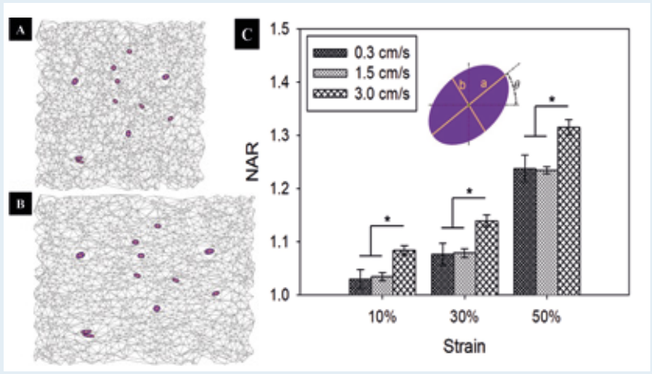
CLINICAL NEED → DISEASES ANALYSIS → DISCOVERY → PRECLINICAL VALIDATION → PRECLINICAL DEVELOPMENT → CLINICAL STUDIES

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.



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.

IMPACT

This research line has potential implications on a number o 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 multi-scala 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 2019

- Utilized algorithm and predictive methods developed in 2010-2014 to support the research line BE-ECM. This involved correlating cell nuclear deformation with scaffold meso-architecture and its impact on de-novo collagen formation. *In vitro* system utilized to test the numerical model: polyurethane scaffold seeded with vascular smooth muscle cells.
- Prediction capacity for: macro-scale mechanics (e.g. biaxial response), meso-scale mechanics (e.g. nuclear aspect ratio changes, single fiber deformation histogram), micro-scale mechanics (e.g. single fiber characteristics). Activity conducted to support two funded NIH-R01 projects in collaboration with Dr F. Ambrosio.
- Ability to reproduce fibrous materials and tissue topologies.
- Developed multi-physics models for voltage distribution in double component deposition material processing.

Research grants obtained and/or managed

- NIH R01, Aging of the myomatrix and its effect on skeletal muscle regeneration, $ 2 million for 2019-2023. PI: F. Ambrosio, University of Pittsburgh. Co-Investigator: A. D'Amore (15% efforts), University of Pittsburgh;
- NIH R01, Dysfunctional muscle remodeling and regeneration in environmental disease, $ 2.6 million for 2016-2021. PIs: F. Ambrosio, A. Barchowski, University of Pittsburgh. Co-Investigators: A. D'Amore (10% efforts), W. Wagner, D. Stolz, University of Pittsburgh;

Awards

- University of Pittsburgh 43rd annual Honors Convocation as outstanding faculty member.

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;
- Mentor for BIOENG 1095 - Special projects. Individual research project under the guidance of a faculty member. Department of bioengineering, Univ. of Pittsburgh. Trainees: Daniel Jacobs Li-Ming 2017-2019.

Mentoring activity

- Terranova, University of Palermo Italy, predictive models for electric voltage distribution in double component deposition;
- Traina, University of Palermo Italy, novel fiber deposition technique for biodegradable suture material;
- C. T. Rhoades, School of Medicine, University of Pittsburgh USA, engineered mitral valve optimization via FEM;
- D. Jacobs Li-Ming, University of Pittsburgh USA, quantitative methods for ECM mass detection 2016-2019, modelling cell motility on fibrous substrate 2019.

Invited speech

- "Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", Chemical and petroleum engineering department, graduate seminar, Pittsburgh, July 29, 2019;
- Bioengineered cardiac tissue: how improved control over biomaterial structure-function can facilitate translation", bioengineering department, graduate seminar, Pittsburgh, April 26, 2019;
- "How to improve control over biomaterials structure-function to design better performing tissue surrogates". Ecole Polytechnique, Paris, France.

GOALS FOR 2020

Goals set for the 2020 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 (2018-2020) 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. Accepted on Tissue Engineering Part A, IF 3.58;
- development of (2018-2020) numerical models to simulate *in vivo* scaffold degradation.
- development of (2018-2020) numerical models to simulate influence of biomaterials topology on cellular migration.

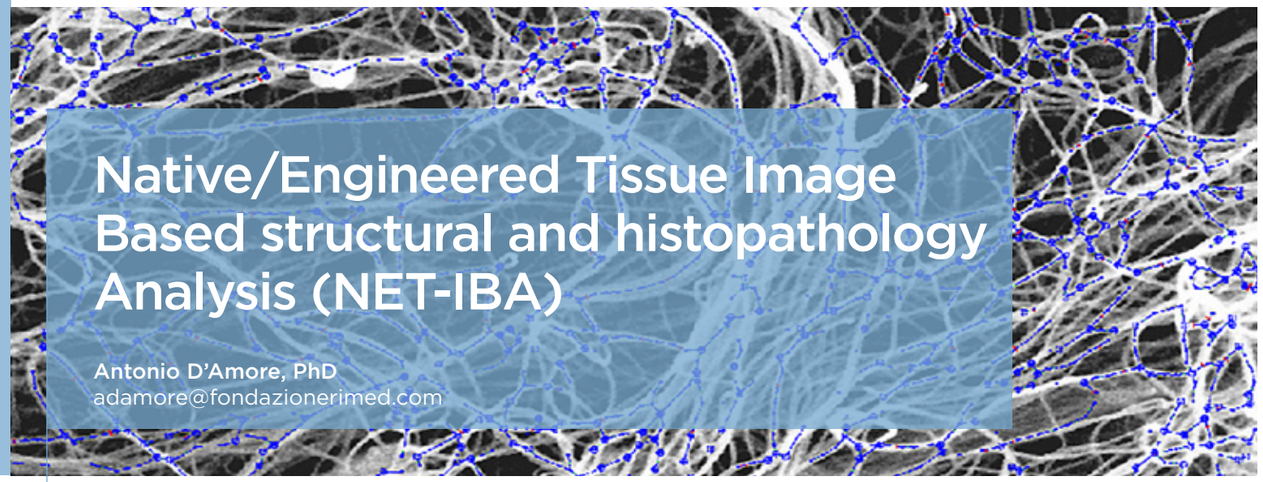
MEETINGS

- H. Mamiya, A. Sahu, A. Cheikhi, S. Shinde, S. Sivakumar, S. Luketich, G. Nasello, B. Van Houten, A. Wise, P. LeDuc, A. D'Amore, A. Barchowsky, F. Ambrosio. Exposure of muscle stem cells to a stiff microenvironment drives an "aged" mitochondrial phenotype. Biomedical engineering society annual meeting, (BMES) October 16- 19 2019, Philadelphia PA, USA.
- H. Mamiya, A. Sahu, A. Cheikhi, S. Shinde, S. Sivakumar, S. Luketich, G. Nasello, B. Van Houten, A. Wise, P. LeDuc, A. D'Amore, A. Barchowsky, F. Ambrosio. Exposure of muscle stem cells to a stiff microenvironment drives an "aged" mitochondrial phenotype. June 9 - 14 2019, Lucca, Italy Myogenesis, Gordon Research Conference.

PUBLICATIONS

- A. D'Amore, S. Luketich, R. Hoff , S-H. Ye, and W. Wagner. Blending polymer labile elements at differing scales to affect degradation profiles in heart valve scaffolds. Biomacromolecules, 2019, 20, 7, 2494-2505, IF 5.73.
- D. Pedersen, F. Madonia, C.Tompkins-Rhoades, A. D'Amore, W. R. Wagner. Tissue to Organ Level Evaluation of Heart Valve Scaffold Performance under Dynamic Loading Conditions. American Heart Association (AHA) 2019 Annual meeting Nov. 16–18, Philadelphia PA, USA. Circulation 140 (Suppl. 1), A16249-A16249.

PRODUCTS: **MEDICAL DEVICES & TISSUES AND ORGANS 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
- Università degli Studi di Palermo, Italy
- University of Nagoya, Japan

THERAPEUTIC AREA

Organ insufficiences

Aging diseases

Cancer

PIPELINE

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

BRIEF DESCRIPTION

Main topic: NET-IBA, development of algorithm 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 evaluation are still conducted with qualitative or semi-qualitative assessment. Similarly, digital image analysis tools developed for material science applications or process engineering are not design with a potential clinical focus. This research line acts at the interface between these two disciplines and tries to fill this gap in knowledge. More specifically, our group aims to define novel software analysis tools and methods which can be utilized to solve common problems currently faced in both clinical practice and material science. .

Fig. 1 - NET-IBA: Algorithm for quantitative angiogenesis evaluation. Accurate identification and quantification of blood vessels can be labour intensive, time consuming and heavly dependet on the operator experience. An automated, objective method has been developed and validated, the block diagram illustrates the structure of the algorithm. (from left to right): a) input image, b) filtering and thresholding on red or green color channels, c1) detection of connected components , c2) morphological segmentation based on size and shape, c3) additional detection of connected components, d) segmentation criteria in c1,c2,c3 are combined together using morphological operators, e) labeling of connected components , f) algorithm' result including vessel area quantification and spatial distribution (right).

IMPACT

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

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

RESULTS ACHIEVED IN 2019

- Leadership on methods for topological analysis of native tissue ECM and scaffolds;
- Re-enforced expertise and leadership in image based structural analysis of native tissue and scaffolds. Available software include: micro-structural analysis of fibrous tissue, porosity analysis, collagen and elastin fiber analysis, topological analysis of cellular infiltration, specific markers spatial distribution, macrophages polarization;
- Continued industrial collaboration with start-up company PECA Lab, topic: structural characterization of FDA class III medical device;
- Initiated new collaboration with Dr Takanari, Dep. Plastic Surgery, University of Nagoya, topic: morphological evolution of collagen structure following surgical procedure, human sample data;
- Completed software for the automatic detection of blood vessels on histological sections. Active collaboration with Dr Bruno and Dr Ardizzone from CNE and Univ. Palermo.

GOALS FOR 2020

- 3D upgrade of 2D analysis methods developed for micro and nano materials. Current version of the software developed "Gordium" relies on scanning electron microscopy 2D data, the research planned for years 2018-2019 includes the upgrade of this methodology to 3D confocal microscopy data.

- Complete validation of techniques to assess angiogenesis. The current version of the algorithm developed in collaboration with Dr Bruno is based on routine able to process fluorescence microscopy data, the same approach will be adapted to histological data (e.g. H&E and Masson' staining). In second step of the project we plan to implement an automatic code to segment detected blood vessels based on their morphology and to categorize them according to the method documented in : "A. 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. Accepted on Tissue Engineering Part A."

MEETINGS

- H. Mamiya, A. Sahu, A. Cheikhi, S. Shinde, S. Sivakumar, S. Luketich, G. Nasello, B. Van Houten, A. Wise, P. LeDuc, **A. D'Amore**, A. Barchowsky, F. Ambrosio. Exposure of muscle stem cells to a stiff microenvironment drives an "aged" mitochondrial phenotype. Biomedical engineering society annual meeting, (BMES) October 16- 19 2019, Philadelphia PA, USA.
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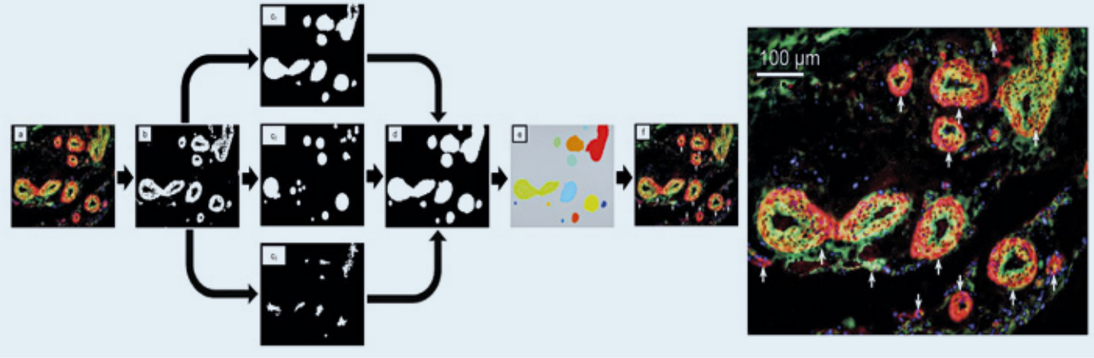
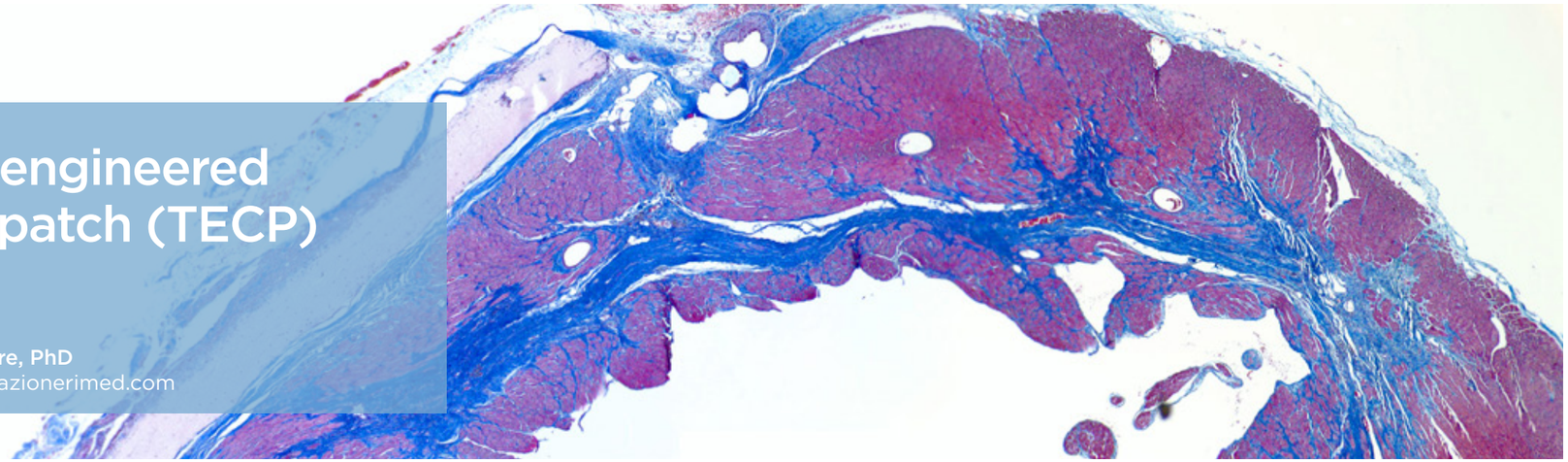
PUBLICATIONS

- **A. D'Amore**, S. Luketich, R. Hoff , S-H. Ye, and W. Wagner. Blending polymer labile elements at differing scales to affect degradation profiles in heart valve scaffolds. Biomacromolecules, 2019, 20, 7, 2494-2505, IF 5.73.
- D. Pedersen, F. Madonia, C.Tompkins-Rhoades, **A. D'Amore**, W. R. Wagner. Tissue to Organ Level Evaluation of Heart Valve Scaffold Performance under Dynamic Loading Conditions. American Heart Association (AHA) 2019 Annual meeting November 16–18, Philadelphia PA, USA. Circulation 140 (Suppl. 1), A16249-A16249.



PRODUCTS: **MEDICAL DEVICES & TISSUES AND ORGANS ENGINEERING**

Tissue engineered cardic patch (TECP)



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COLLABORATIONS

- University of Pittsburgh, Pittsburgh, USA
- UPMC, Pittsburgh, USA
- University of Cincinnati, Cincinnati, USA
- IRCCS ISMETT, Palermo, Italy
- Mario Negri, Milan, Italy
- Universidade Estadual de Campinas, Campinas, Brazil
- University of Texas, Austin, USA
- Virginia Commonwealth University, Richmond, USA
- ATeN Center, Università di Palermo, Italy

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

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

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 treatments or to the adoption of ventricular aided devices (VAD). The general notion of cardiac patching is to provide mechanical support to the ventricle by surgically implanting engineered patch on the infarcted epicardium (local approach) or around the entire ventricle (global). The path 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.

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 2019

Completed *in vivo* study on rat model, the experiment evaluated the effects of the intervention timing for bi-layered cardiac patch. Main results of patching: mitigates wall thinning, facilitates angiogenesis, reduces fibrotic tissue, sustains ventricle function up to 10 weeks from the infarction, M1-M2 macrophage switch. Successfully completed autologous muscle flap transplant, bi-layered patch, project funded by AFIRM – DoD, animal model: rat and rabbit.

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;

Awards

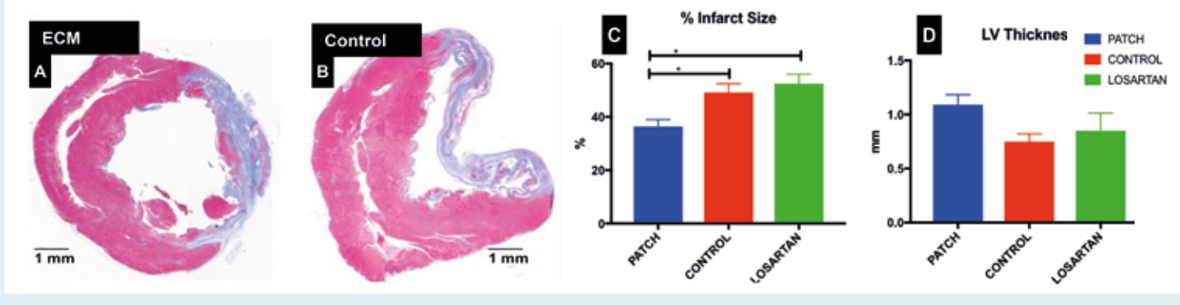
- University of Pittsburgh 43rd annual Honors Convocation as outstanding faculty member.

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;
- P. I. Gonzalez, University of Pittsburgh USA, biomimetic three-layers vascular graft;



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.

- A. Fazal, University of Pittsburgh USA, engineered chordae tendineae;
- D. Jacobs Li-Ming, University of Pittsburgh USA, quantitative methods for ECM mass detection;

Invited speech

- " Bioinspired polymer processing: how improved control over biomaterial structure-function can facilitate translation", Chemical and petroleum engineering department, graduate seminar, Pittsburgh, July 29, 2019;
- "Bioengineered cardiac tissue: how improved control over biomaterial structure-function can facilitate translation", bioengineering department, graduate seminar, Pittsburgh, April 26, 2019;
- "How to improve control over biomaterials structure-function to design better performing tissue surrogates". Ecole Polytechnique, Paris, France;

GOALS FOR 2020

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 2019 include:

- Assessment of cardiac patch scaffold on large animal model, primary goals: (I) sustain ventricula function; (II) induce endogenous tissue growth\ reduce fibrotic tissue; (III) mitigate wall thinning;
- To assess the impact of nitro-fatty acid controlled release for myocardium regeneration, project in collaboration with Dr Fazzari;
- To explore and test techniques for minimally invasive deployment of cardiac patch. Projec in collaboration with Drs Pilato, Morsolini, Raffa (ISMETT) and Drs Coyan, Silveira Filho (UPMC and Univ. of Campinas Brazil);
- Effetcts, methods and therapuetical potetnial of right ventricle patching, project in collaboration with Drs Coyan, Silveira-Filho and Sciortino (UPMC).

MEETINGS

L. M. Silveira-Filho, G. Coyan, **A. D'Amore**, S. K. Luketich, G. Menallo, A. Adamo, Y. Matsumura, N. Kashiyama, W. R. Wagner. Bio-Hybrid Cardiac Patch Combining Poly(ester carbonate urethane)urea and Porcine Cardiac Extracellular Matrix Digest Induces Improved Ventricular Remodeling in a Model of Chronic Ischemia. Proceedings of the Society for Biomaterials Annual Meeting (SfB 2019), 3rd -6h April 2019 Seattle, Washington.

PUBLICATIONS

- 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, IF 10.23.

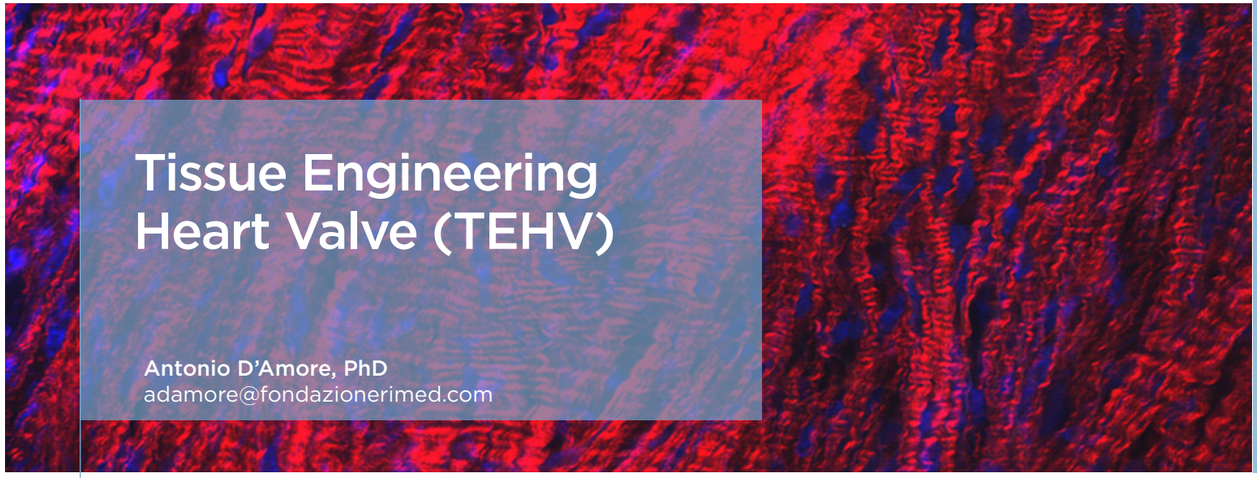
- M. Murdock, J. Chang, S. Luketich, D. Pedersen, G. Hussey, **A. D'Amore,** S. Badylak. Cytocompatibility and mechanical properties of surgical sealants for cardiovascular applications. Journal of Thoracic and Cardiovascular Surgery, 2019, S0022-5223(18)32289-X. IF 4.88.

INTELLECTUAL PROPERTY

- US provisional patent application 62/874114, filed on 07/2018, additional data filed in 10/2019, topic: biomedical device, title: "Processing method and apparatus for micro-structured rope-like material. ". Lead innovator/developer: **A. D'Amore**.

- US patent application PCT/US2018/061862 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**.

MEDICAL DEVICES & TISSUES AND ORGANS ENGINEERING



Tissue Engineering Heart Valve (TEHV)

Antonio D'Amore, PhD
adamore@fondazionerimed.com

COLLABORATIONS

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

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

BRIEF DESCRIPTION

Main topic: TEHV, to develop 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 bioprostheses. 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's 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 2019

Successfully completed assessment of DCD pulmonary and tricuspid valves on acute, large animal model. Continued the DCD mitral valve assessment utilizing the same animal model. Consolidated and extended IP for chordae tendineae biofabrication. Extended cardiac tissue characterization including: heart valve, myocardium, coronary arteries, chordae tendineae. Continued the characterization of human samples from donors via collaboration with Core Foundation, USA. Initiated study on chordae mechano-biology. Consolidated OneValve's IP portfolio.

Funded grant proposals

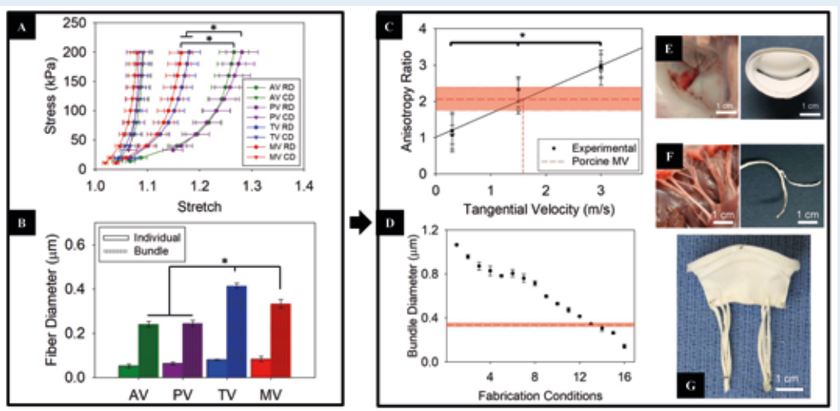
- Pitt Innovation Challenge (PInCh) program. OneValve: The Self Generating Heart Valve, the team (Drs Coyan, D'Amore, Wagner) was ranked #1st and was awarded for $ 100,000. PI: A. D'Amore, Univ. of Pittsburgh;
- Pitt Ventures First Gear Program (NSF grant number IIP1734751). OneValve: The Self Generating Heart Valve, $ 3000. PI: A. D'Amore, University 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.

Awards

- University of Pittsburgh 43rd annual Honors Convocation as outstanding faculty member;
- Acute *In Vivo* Functional Assessment of a Biodegradable Stentless Elastomeric Tricuspid Valve. G. Coyan, L. Silveira Filho, Y. Matsumura, S. Luketich, W. Katz, V. Badhwar, W. Wagner, A. D'Amore. First place presentation winner of the 27th American Heart Association's Fellows research day, Pittsburgh 01-2019, senior investigator: A. D'Amore;
- Top 2 proposte progettuali presso Pitt e UPMC per la Betty Moore Foundation Innovators award 2020.

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;
- Mentor for BIOENG 1095 - Special projects. Individual research project under the guidance of a faculty member. Department of bioengineering, Univ. of Pittsburgh. Trainee: Mahdi Haghkar 2019;
- Mentor for ENGR1000 - Lab experiences. Individual research project under the guidance of a faculty member. Department of chemical and petroleum engineering, Univ. of Pittsburgh. Trainee: Mahdi Haghkar 2019.



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

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;
- F. Madonia, University of Palermo Italy, functional assessment of engineered mitral valve and chordal apparatus via pulse duplicator test;
- M. Traina, University of Palermo Italy, novel fiber deposition technique for biodegradable suture material;
- G. Miceli, University of Palermo Italy, micro-groove patterns for enhanced engineered valve anisotropy and fiber geometry control;
- C. T. Rhoades, School of Medicine, University of Pittsburgh USA, engineered mitral valve optimization via FEM.

Invited speech

- "How scaffold processing variables affect engineered heart valve morphology and mechanics", The Heart Institute annual meeting, Children Hospital, University of Pittsburgh Pittsburgh, September 18-2019;
- "Bioengineered cardiac tissue: how improved control over biomaterial structure-function can facilitate translation", bioengineering department, graduate seminar, Pittsburgh, April 26, 2019;
- "How scaffold processing variables affect engineered heart valve morphology and mechanics", session: valve tissue engineering: do we understand variability in outcomes? The Heart Valve Society annual meeting, Sitges, Barcelona, Spain, April 11-13, 2019.

GOALS FOR 2020

The long term goal is the translation of the technology (class III FDA), objectives for the 2020 are defined as follows:

- Implement PinCh funded study on pulmonary valve with biodegradable magnesium stent;
- Completion of the ongoing DCD mitral valve in-vivo acute study;
- Preparation and submission of research proposal to fund chronic studies for atrio-ventricular engineered valves: NIH-R01, ERC consolidator, Betty Moore Foundation Innovators awaes;
- Publish in-vitro study on the mechano-biology of engineered chordae;
- Completion of FEM study of the chordal apparatus;
- Assessment of new strategies for selective fiber deposition via DCD;
- Protect and consolidate IPs;
- Perfect OneValve business plan and financial modeling;
- Start-up company formation;
- Promote and present the technology to VCs;
- Higher education and training: seven MSc' degree students, two PhD students;
- Consolidate the McGowan-UNIPA internship program.

MEETINGS

- Valve. 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 Biodegradable Stentless Elastomeric Tricuspid American Association for Thoracic Surgery (AATS 2019) 2019 meeting: May 4-7, 2019 Toronto, Canada.
- C. Tompkins-Rhoades, S. Pasta, G. Coyan, L.M. Silveira Fihlo, **A. D'Amore**. Optimizing the development of a tissue engineered mitral valve using a computational model. The Society of Black Academic Surgeons (SBAS 2019) annual meeting. April 25th - 27th New York, USA.
- A. Adamo, G. Spiaggia, W. Wagner, **A. D'Amore.** A new mandrel-less electrospinning method for generating Biomimetic Engineered Chordae Tendineae. 8th International Conference on Mechanics of biomaterials and tissues 2019 – Waikoloa Beach, Hawaii, USA.

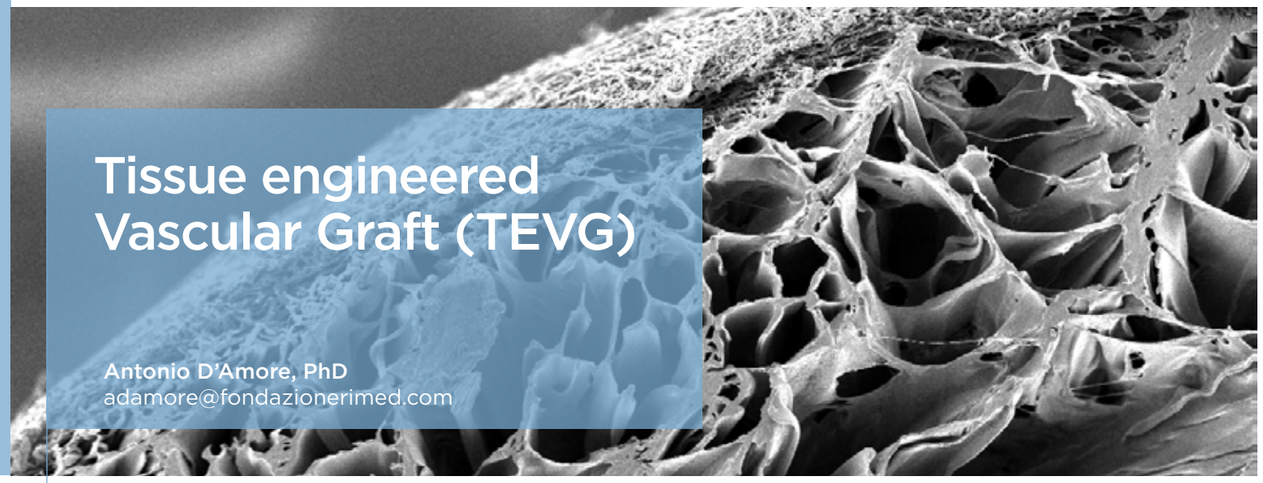
PUBLICATIONS

- **A. D'Amore**, S. Luketich, R. Hoff , S-H. Ye, and W. Wagner. Blending polymer labile elements at differing scales to affect degradation profiles in heart valve scaffolds. Biomacromolecules, 2019, 20, 7, 2494-2505, IF 5.73.
- G. N. Coyan, **A. D'Amore**, Y. Matsumura, D. D. Pedersen, S. K. Luketich, V.Shanov, T. E. David, W. R. Wagner, V. Badhwar. In vivo functional assessment of a novel degradable metal and elastomeric scaffold-based tissue engineered heart valve. J. of Thoracic and Cardiovascular Surgery, 2019; 157: 1809-1816, IF 4.88.
- D. Pedersen, F. Madonia, C.Tompkins-Rhoades, **A. D'Amore**, W. R. Wagner. Tissue to Organ Level Evaluation of Heart Valve Scaffold Performance under Dynamic Loading Conditions. American Heart Association (AHA) 2019 Annual meeting November 16–18, Philadelphia PA, USA. Circulation 140 (Suppl. 1), A16249-A16249.
- G. Spiaggia, A. Adamo, G. Coyan, W. Wagner, **A. D'Amore.** Biomimetic engineered chordae tendineae produced via novel mandrel-less electrospinning. Annual Scientific Meeting of the Heart Valve Society, April 11-13, 2019, Sitges, Spain. Structural Heart. Volume 3, 2019 - Issue 1, IF NA.

INTELLECTUAL PROPERTY

- US provisional patent application 62/868,275 filed on 07/2019, topic: biomedical device, title: "Valved stent for the treatment of severe tricuspid regurgitation.".
- US provisional patent application 62/874114, filed on 07/2018, additional data filed in 10/2019, topic: biomedical device, title: "Processing method and apparatus for micro-structured rope-like material. ". Lead innovator/developer: **A D'Amore.**
- 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**.

PRODUCTS: **MEDICAL DEVICES & TISSUES AND ORGANS ENGINEERING**



Tissue engineered Vascular Graft (TEVG)

Antonio D'Amore, PhD
adamore@fondazionerimed.com

COLLABORATIONS

- **Ospedale Cervello - Villa Sofia, Palermo, Italy**
- **University of Pittsburgh, Pittsburgh, USA**
- **UPMC, Pittsburgh, USA**
- **ATeN Center, Università di Palermo, Italy**

THERAPEUTIC AREA

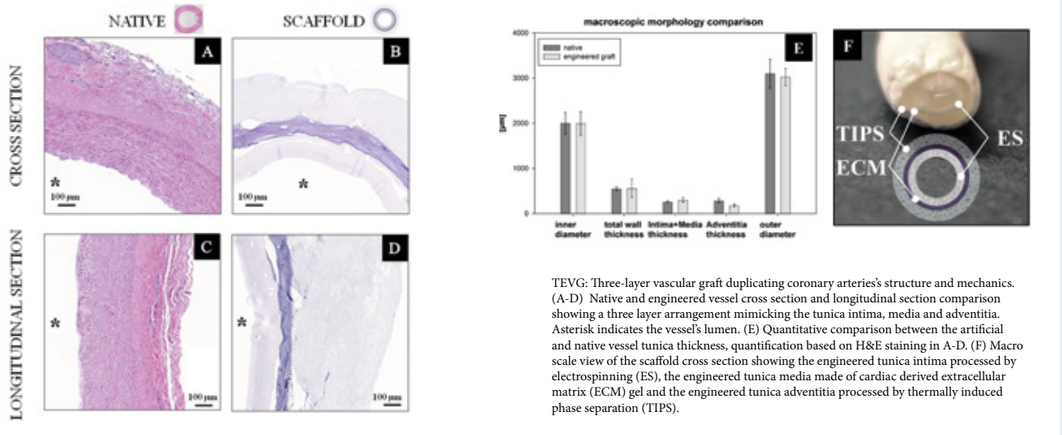
Organ insufficiences

PIPELINE

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

BRIEF DESCRIPTION

Main topic: TEVG, development of engineered vascular graft for coronary bypass.
Solutions clinically available to replace or treat a stenotic blood vessel include the auto-transplant, for example utilizing a section of the saphenus vein, or the adoption of synthetic materials such as Dracron or Teflon.
The first class of intervention is limited by the availability of sufficient, viable autologous tissue. The second category utilized synthetic materials that induce re-stenosis of the vessel up to the 50% of the treated cases.
These issues could be potentially addressed by the tissue engineering approach. The tissue engineering paradigm proposes the use of biodegradable scaffolds able to induce in-situ regeneration and lead to the formation of autologous, functional, non thrombogenic tissue. In this research line our group has identified two main targets: to design grafts able to reproduce structure and mechanics of the native tissue, to reduce the tunica intima hyperplasia by the adoption of ad-hoc scaffold surface morphology ad structure.



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) Quantitative comparison between the artificial and native vessel tunica thickness, quantification based on H&E staining in A-D. (F) Macro scale view of the scaffold cross section showing the engineered tunica intima processed by electrospinning (ES), the engineered tunica media made of cardiac derived extracellular matrix (ECM) gel and the engineered tunica adventitia processed by thermally induced phase separation (TIPS).

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 2019

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 (related provisional patent filed). Hypothesized a novel mechanism to mitigate tunica intima hyperplasia. Characterized native, porcine coronary arteries. Introduced novel technique for polymer surface modification at the micro and meso scale. Patterns prescribed will be utilized to manipulate cells attached/seeded into the scaffold, IP disclosure in progress.

Awards

University of Pittsburgh 43rd annual Honors Convocation as outstanding faculty member.

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".

Mentoring activity

- G. C. Miceli, Milan Polytechnic Italy, biomimetic three-layers vascular graft, cell proliferation and de-novo collagen synthesis;
- A. Kelly, University of Pittsburgh, USA, topological cues for enhanced endothelial cell proliferation, cell vitality tests;
- H. Hashim, University of Palermo Italy, topological cues for enhanced endothelial cell proliferation and structural organization in engineered vascular graft;
- M. Haghkar, University of Pittsburgh USA, topological cues for enhanced endothelial cell proliferation and structural organization in engineered vascular graft;
- P. I. Gonzalez, University of Pittsburgh USA, biomimetic three-layers vascular graft.

GOALS FOR 2020

The final goal of this research line is the translation of the technology (FDA class III), targets set for the year 2019 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;
- 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.

PUBLICATIONS

- T. K.Valencia-Rivero, J. C. Cruz, N. Wagner-Gutierrez, **A. D'Amore**, M. Miranda, R. López, A. Guerrero, W. Wagner, N. Sandoval, J. C. Briceño. Evaluation of Microscopic Structure-Function Relationships of PEGylated Small Intestinal Submucosa Vascular Grafts for Arteriovenous Connection. ACS Appl. Bio Mater. 2019, 2, 9, 3706-3721, IF 3.23.

INTELLECTUAL PROPERTY

- 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.**

- 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.**

PRODUCTS: **MEDICAL DEVICES & TISSUES AND ORGANS ENGINEERING**

R-CaRe - Rehabilitation for Cartilage Regeneration

Riccardo Gottardi, PhD
rgottardi@fondazionerimed.com

COLLABORATIONS

- Orthopaedic Robotics Laboratory, University of Pittsburgh, PA, USA
- Center for Cellular and Molecular Engineering, Dept. of Orthopaedic Surgery, University of Pittsburgh, Phialdelphia, USA
- AO Research Institute, Davos, Switzerland

THERAPEUTIC AREA

Organ insufficiences

PIPELINE

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

BRIEF DESCRIPTION

Focal cartilage injuries are a major challenge especially in the younger, active population and the traditional first-line treatment is microfracture, estimated at 100,000/year in the US. However, microfracture still presents limitations in terms of longevity and expeditious return to preinjury level. In fact, ~25% of microfractures require re-operation within 2 years, with near universal treatment failure and development of osteoarthritis expected within 5-10 years. Furthermore, rehabilitation after microfracture lasts up to 6 months and includes initial immobilization followed by continuous passive motion and progressive weight-bearing. However, there is no consensus on the timing and magnitude of joint loading for optimal rehabilitation.

Since mechanical forces direct cell behavior (i.e., mechanobiology), early weight bearing may improve tissue healing, accelerate extracellular matrix deposition, and promote a more hyaline rather than a fibrocartilaginous phenotype of the repaired tissue, as suggested by animal and *in vitro* studies.

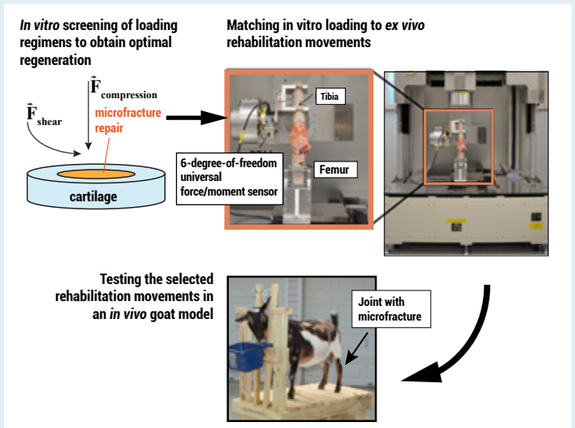
We expect that mechanical loading during rehabilitation can be exploited to direct repair tissue formation. With this project, we aim to improve outcomes of cartilage repair by identifying loading regimens that can be delivered during rehabilitation to promote cartilage regeneration, improve tissue repair, and extend its longevity.

IMPACT

There is no consensus on the timing and magnitude of joint loading for optimal rehabilitation after microfracture since the mechanisms by which controlled mobilization promotes cartilage repair is yet unknown. Successful completion of this project will provide a link between physical therapy induced mechanotransduction and repair cartilage regeneration and integration. Most importantly, the successful outcomes of this project will provide evidence to modify current rehabilitation protocols to extend the time before additional treatments for the patients is require and, in the long-term, postpone the development of osteoarthritis.
As a further added value, the combined platform technologies established in this project could be applied beyond the field of cartilage repair to other orthopaedic injuries, as well as to the development of rehabilitation-based preventive measures. In fact, our platforms allow a fine control of various loading parameters to identify *in vitro* regenerative loading patterns that can be replicated through rehabilitation protocols *in vivo* for both treatment and prevention.

RESULTS ACHIEVED IN 2019

We have developed a realistic *in vitro* model of microfracture to study the chondrogenic effects on the microfracture repair tissue of continuous passive motion and progressive weight bearing simulated through specific bioreactors.
Our *in vitro* studies in a microfracture model suggest that compressive loading (which models progressive weight bearing) promotes better repair tissue, a more hyaline phenotype, and better integration with the surrounding cartilage, than shear loading (which models continuous passive motion). Furthermore, shear loading induced the upregulation of catabolic markers, suggesting more significant remodeling. Our findings on the positive effect of compressive loading are further supported by observations in large animal that initiate full weight bearing immediately after microfracture. This research was supported by pilot funding by the Alliance for Regenerative Rehabilitation Research and Training.



GOALS FOR 2020

In 2020 we aim at securing funding to identify if/how pro-regenerative mechanoactivation can be delivered locally by whole joint rehabilitation exercise. We aim at established a multiscale framework for the design of improved rehabilitation regimens that could then be applied to the study of regenerative rehabilitation in large animal models and in clinical trials.
We will specifically focus on identifying the combination of compressive and shear loading that promotes more hyaline and better integrated repair cartilage, modelling different rehabilitation regimens. Then we will match the simulated exercise to the equivalent rehabilitation movements in human knees using a six-axis robotic testing system with position and force feedback allowing for realistic loading conditions. Arthroscopically placed force sensors and advanced finite element modeling will serve to identify the local load on cartilage as a function of location in the joint. The analysis will serve to identify in human knees the rehabilitation protocol that would match *in vivo* those screened *in vitro*.

MEETINGS

- Biomeical Engineering Society – BMES, October 2019, Philadelphia (PA), USA
- American Orthopaedic Society for Sports Medicine, AOSSM Annual Meeting, July 2019, Boston (MA), USA
- Tissue Engineering and Regenerative Medicine – EU (TERMIS EU), May 2019, Rodi, Greece
- CWRU Center for Multimodal Evaluation of Engineered Cartilage, Investigator's Meeting, May 2019, Cleveland (OH), USA
- Orthopaedic Research Society Annual Meeting, February 2019, Austin (TX), USA

PUBLICATIONS

- Iseki T., Rothrauff B.B., Kihara S., Sasakin H., Yoshiya S., Fu H.F., Tuan R.S. Gottardi R. 2019. Dynamic Compressive Loading Improves Cartilage Repair in an In Vitro Model of Microfracture: Comparison of 2 Mechanical Loading Regimens on Simulated Microfracture Based on Fibrin Gel Scaffolds Encapsulating Connective Tissue Progenitor Cells. The American Journal of Sports Medicine, 47, 2188-2199 https://doi.org/10.1177/0363546519855645
- Gottardi R. Load-induced osteoarthritis on a chip. Nature Biomedical Engineering. 2019, 3(7):502-503, DOI: 10.1038/s41551-019-0427-y

TECHNOLOGY PLATFORMS

With its strongly oriented translational approach, this strategy provides for the development of skills and technological platforms supporting discovery and preclinical development projects.

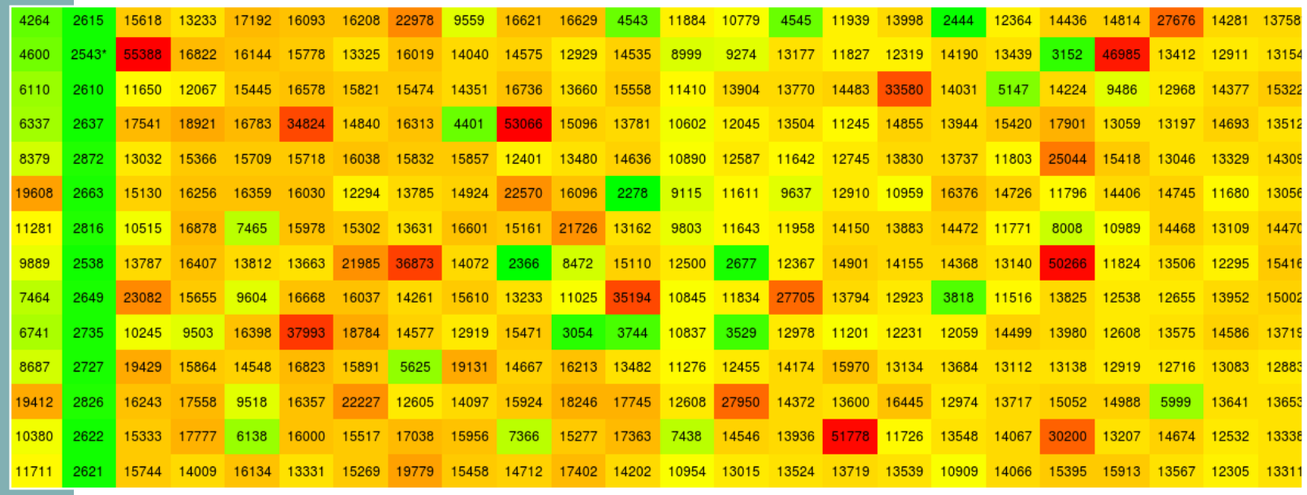
In 2019, the platforms were strengthened thanks to **CheMISt**, a regional funding that allowed the **Bioinformatics and Computational Chemistry** teams to increase computing performance with hardware and software that reach virtual screening of 5,000 molecules per minute, integrated with the proprietary algorithms for the study of molecular interactions at the cellular level and with the infrastructure for the analysis of chemical-physical properties. CheMISt also enabled the purchasing of an automated system for the storage and manipulation of molecule libraries for the **High Throughput Screening** Lab, a cardiac simulator and instruments for the characterization of biomaterials and medical devices that the **Bioengineering** team uses to develop new solutions for the 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, unique in southern Italy, acquired as part of the Sicilian Region Operational Program (POR) "**Infrastruttura di Ricerca**" and installed in 2019.

Some Ri.MED platforms are already operating at IRCCS ISMETT following the bench-to-bedside approach at the basis of the ISMETT-Ri.MED cluster. The **MRI platform** uses 3T and 7T spectrometers for the analysis of multimodal data and images, predictive diagnosis of pathologies and recurrences. The **Proteomics** team supports the identification of new pharmacological targets and biomarkers, as well as the study of potential side effects of particular therapeutic molecules. Also, the **GMP Facility** for the production of ATMp, renewed in 2019, is currently under assessment to become the first AIFA [Italian Medicines Agency] authorized facility of its kind in southern Italy.

- Bioinformatics
- Bioengineering
- Structural Biology and Biophisics
- Computer Aided Drug Design
- High-throughput Screening
- Magnetic Resonance Imaging
- Proteomics
- Cell Factory

Bioinformatics
Platform



CONTACTS:
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Bioinformatics group is devoted to help Ri.MED researchers and collaborators to retrieve the most amount of information from their data, with a particular interest on Big Data. For instance, we support the Drug Discovery Unit with the development of softwares for compound management and high throughput screaning 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.

COLLABORATIONS:

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

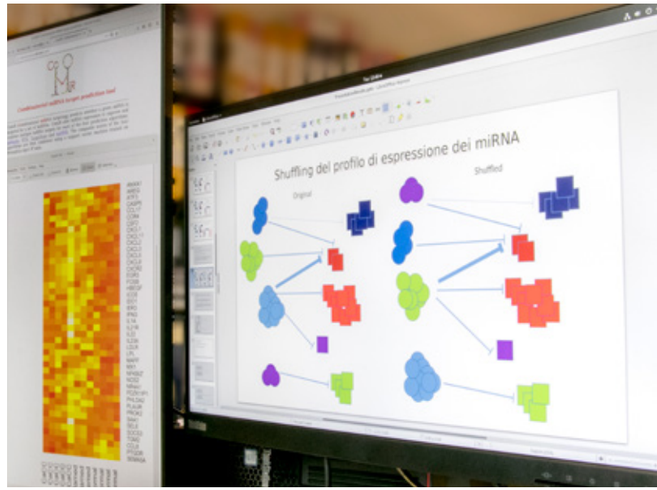
Expertise

- 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



ACTIVE RESEARCH PROJECTS

The Bioinformatics group supports other Ri.MED research projects in computational biology, bioinformatics and statistical topics. In addition, it is currently involved in scientific projects with the aim of realizing new algorithm useful for the analysis of the microRNA regulatory network

microRNA-mRNA interaction network.
It is our aim to develop algorithms to model, visualize and compare the microRNA interaction networks from different tissues. We are training and testing algorithms by using microRNA and mRNA expression profiles from the The Cancer Genome Atlas (TCGA) database, which includes omics data from thousands of oncological patients.

RISC proteins RIP-Chip prediction
We developed a machine learning based algorithm useful to predict the differentially expressed genes in the RIP-Chip data. The algorithm is currently optimized to predict the outcome of AGO1, AGO2 and GW182 RIP-Chip experiments.

PUBLICATIONS

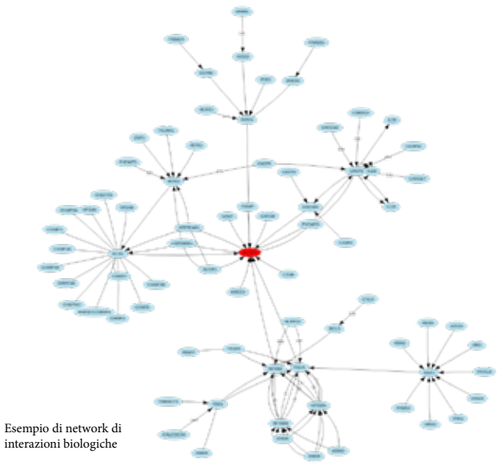
Nazzicari N, Vella D, Coronnello C, Di Silvestre D, Bellazzi R, Marini S (2019) **MTGO-SC, A Tool to Explore Gene Modules in Single-Cell RNA Sequencing Data**, Frontiers in genetics 10

Badami E, Coronnello C, Barbera F, Paini DS, Conaldi P (2019) **NK cell anti-tumor and anti-viral function can be enhanced *in vitro* and exploited for successful cell mediated therapy**, Cytotherapy 21(5)

Perconti G, Contino F, Rubino P, Bivona S, G Bertolazzi, M Tumminello, Feo S, Giallongo A, Coronnello C. (2019) **AGO2 and GW182 IP show different characteristics in co-immunoprecipitated RNA features**, BMC Bioinformatics, 20 (4).

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 J (2019) **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, https://doi.org/10.1177/0391398819884941

Kvorjak M, Ahmed Y, Miller ML, Sriram R, Coronnello C, Hashash JG, Hartman DJ, Telmer CA, Miskov-Zivanov N, Finn OJ and Cascio S (2019) **Crosstalk between colon cells and macrophages increases ST6GALNAC1 and MUC1-sTn expression in ulcerative colitis and colitis-associated colon cancer**, Cancer Immunol Res, DOI:10.1158/2326-6066.CIR-19-0514



Esempio di network di interazioni biologiche

Bioengineering

Platform

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The activity of the Bioengineering division is focused on the development and clinical implementation of innovative biomedical solutions, aimed at improving the efficacy and sustainability of the health service, with immediate impact to patients' quality of life. These innovations include medical devices based on the application of the latest advances in materials science and regenerative medicine; patient-specific holistic decision-making processes and diagnostic tools; minimally invasive and personalized therapeutic approaches.
The division is rapidly expanding, and is now implementing an appropriate technology platform enabling 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:

- **Barts Heart Centre at St Bartholomew's Hospital, London, UK**
- **École Polytechnique Fédérale de Lausanne, Losanna CH**
- **Great Ormond Street Hospital for Children, London, UK**
- **RCCS ISMETT, Palermo, Italy**
- **Mines Saint Etienne, France**
- **Politecnico di Milano, Italy**
- **IRCCS Policlinico San Donato, Italy**
- **Università degli studi di Padova, Italy**
- **Università degli Studi di Palermo, Italy**
- **Université de Technologie de Compiègne, France**
- **University of Pittsburgh, USA**
- **University College London, London, 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 characterization 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

- Risk prediction of right ventricular failure in patients with pulmonary hypertension and ventricular assist device (VAD)
- 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 Thrombosis Risk
- Prediction of Ischaemic Lesions Potential after Heart Valve Therapy
- *In vitro* simulation of mitral valve therapies
- In-silico modeling for clinical risk stratification of cardiovascular pahtologies

PUBLICATIONS

- Annio G., Franzetti G., Bonfanti M., Gallarello A., Palombi A., De Momi E., Homer-Vanniasinkam S., Wurdemann H.A., Tsang V., Diaz-Zuccarini V., Torii R., Balabani S., Burriesci G. (2019) **Low cost fabrication of PVA based personalized vascular phantoms for *in vitro* haemodynamic studies: three applications.** ASME Journal of Engineering and Science in Medical Diagnostics and Therapy, n. JESMDT-19-1037. https://doi.org/10.1115/1.4045760
- Nazzicari N., Vella D., Coronnello C., Di Silvestre D., Bellazzi R., Marini S. (2019) **MTGO-SC, a tool to explore gene modules in single cell RNA-seq data.** Front. Genet. doi:10.3389/fgene.2019.00953
- Annio G., Torii R., Ariff B., O'Regan3 D.P., Muthurangu V., Ducci A., Tsang, V., Burriesci G. (2019) **Enhancing Magnetic Resonance Imaging with Computational Fluid Dynamics**. ASME Journal of Engineering and Science in Medical Diagnostics and Therapy. doi: https://doi.org/10.1115/1.4045493
- Salmonsmith J., Ducci A., Burriesci G. (2019) **Does Transcatheter Aortic Valve Alignment Matter?** Open Heart 6:e001132. doi:10.1136/openhrt-2019-001132
- Agnese V., Pasta S., Michelena H.I., Minà C., Romano G.M., Carerj S., Zito C., Maalouf J.F., Foley T.A., Raffa G., Clemenza F., Pilato M., Bellavia D. (2019) **Patterns of ascending aortic dilatation and predictors of surgical replacement of the aorta: A comparison of bicuspid and tricuspid aortic valve patients over eight years of follow-up.** Journal of Molecular and Cellular Cardiology 135:31-39
- McGregor C., Rahmani B., Chisari E., Kyriakopoulou K., Burriesci G., Byrne G. (2019) **Initial validation of Gal knockout pig tissues as materials for biological heart valves**. XENOTRANSPLANTATION 26(5):401.2.
- Di Micco L., Peruzzo P., Colli A., Burriesci G., Boso D., Besola L., Gerosa G., Susin F.M. (2019) **The Neochord Mitral Valve Repair Procedure: Numerical Simulation of Different Neochords Tensioning Protocols.** Medical Engineering & Physics 74:121-128. doi: https://doi.org/10.1016/j.medengphy.2019.09.014
- D'Acquisto L., Scardulla F., Montinaro N., Pasta S., Zangla D., Bellavia, D. (2019) **A preliminary investigation of the effect of contact pressure on the accuracy of heart rate monitoring by wearable PPG wrist band**. 2019 IEEE International Workshop on Metrology for Industry 4.0 and IoT, MetroInd 4.0 and IoT 2019 - Proceedings 8792834:334-338
- Peruzzo P., Burriesci G., Susin M.F., Colli A. (2019) **In vitro and ex-vivo Hemodynamic Testing of an Innovative Occluder for Paravalvular Leak after Transcather Aortic Valve Implantation.** Journal of Cardiovascular Translational Research 12(6):551-559. doi: https://doi.org/10.1007/s12265-019-09902-4.
- Gallarello A., Palombi A., Annio G., Homer-Vanniasinkam S., De Momi E., Maritati G., Torii R., Burriesci G., Wurdemann H.A. (2019) **Patient-specific aortic phantom with tunable compliance**. ASME J of Medical Diagnostics 2(4):041005.
- Cosentino F., Agnese V., Raffa G.M., Gentile G., Bellavia D., Zingales M., Pilato M., Pasta S. (2019) **On the role of material properties in ascending thoracic aortic aneurysms**. Computers in Biology and Medicine 109:70-78.
- Palombi A., Bosi G., Di Giuseppe S., De Momi E., Homer-Vanniasinkam S., Burriesci G., Wurdemann H. (2019) **Sizing the aortic annulus with a robotised, commercially available soft balloon catheter: in vitro study on idealised phantoms**. Proceedings of the IEEE International Conference on Robotics and Automation 2019, TuCT1-12.5.
- Peruzzo P., Susin F.M., Colli A., Burriesci G. (2019) **In-vitro assessment of pacing as therapy for aortic regurgitation.** Open Heart, 6:e000976. doi: 10.1136/openhrt-2018-000976.
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- Rahmani B., McGregor C., Byrne G., Burriesci G. (2019) **A Durable Porcine Pericardial Surgical Bioprosthetic Heart Valve: a Proof of Concept.** Journal of Cardiovascular Translational Research, 12(4):331-337. https://doi.org/10.1007/s12265-019-09868-3.
- Di Giuseppe M., Alotta G., Agnese V., Bellavia D., Raffa G.M., Vetri V., Zingales M., Pasta S., Pilato M. (2019) **Identification of circumferential regional heterogeneity of ascending thoracic aneurysmal aorta by biaxial mechanical testing.** Journal of Molecular and Cellular Cardiology 130:205-215.
- Provaggi E., Capelli C., Rahmani B., Burriesci G., Kalaskar D. M. (2019) **3D printing assisted finite element analysis for optimising the manufacturing parameters of a lumbar fusion cage.** Materials and Design, 163:107540.

Structural Biology and Biophysics

Platform

CONTACTS:
Caterina Alfano, PhD
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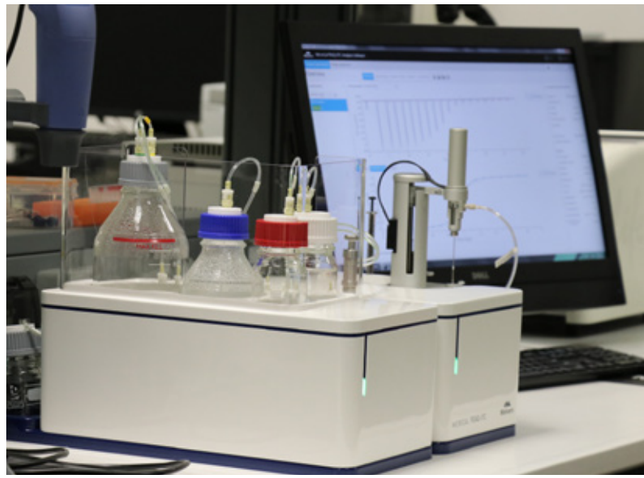
The Group of Structural Biology and Biophysics aims at providing biophysical and structural information of biological phenomena guided by the folding, aggregation and interaction of proteins with the ultimate goal of understanding the molecular mechanisms underlying serious pathologies. Furthermore, the platform supports the Drug Discovery Unit for the screening and the identification of hits and leads.
The group uses an interdisciplinary approach that combines cutting-edge biophysical techniques - such as nuclear magnetic resonance, calorimetry, interferometry and X-ray crystallography - complemented by consolidated technical and methodological expertise in molecular biology and protein science. The integrated use of this variety of biophysical and biochemical techniques allows us to characterize the intrinsic properties of target proteins, their complexes and the interactions in which they are involved, thus guiding us in the understanding of molecular mechanisms underlying serious pathologies and in the conception of possible intervention strategies.
The Unit carries on 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, UK**
- **TES Pharma, Perugia, Italy**
- **Università degli Studi di Perugia, Italy**
- **Università degli studi della Campania Luigi Vanvitelli, Naple, Italy**
- **Università degli Studi di Palermo, Italy**
- **Istituto di Biofisica - CNR**



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 in E.coli 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

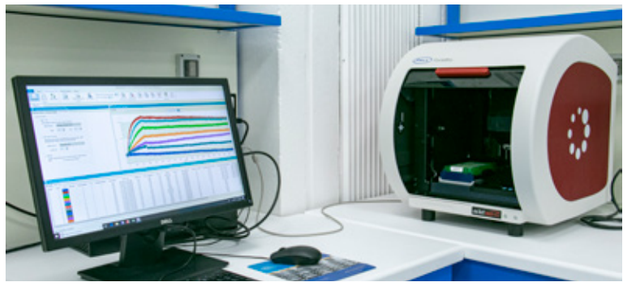
- Study of the molecular mechanisms of protein misfolding diseases.
- Elucidation of the binding mode of molecules able to interfere with the oligomerization process of NPM1.
- Generation of mussel-inspired bio-adhesives molecules able to work in wet environment
- Structural and biophysical studies on proteins involved in the epigenetic regulation of tumor pathologies (SIRT1, KDM4)
- Impact of molecular crowding on protein folding
- Role of the interaction alfa-synuclein/membranes in Parkinson disease.
- *Klebsiella pneumoniae* proteins production for vaccine development.
- BLI- and NMR-based fragment screening.

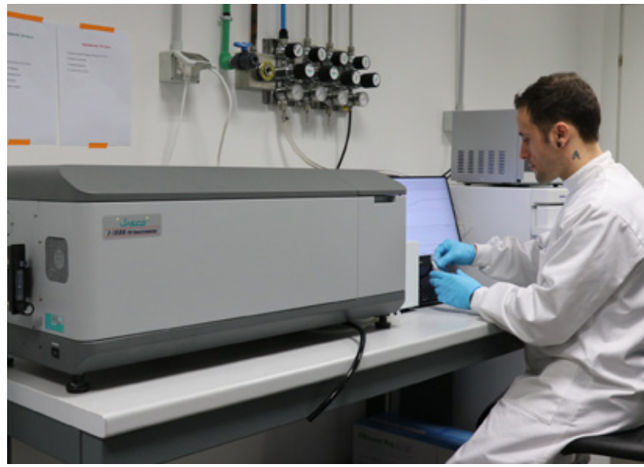
PUBLICATIONS

Santonocito R., Venturella F., Dal Piaz F., Morando M.A., Provenzano A., Rao E., Costa M., Bulone D., San Biagio P.L., Giacomazza D., Sicorello A., Alfano C.*, Passantino R.*, Pastore A. (2019) Recombinant mussel protein Pvfp-5β: a potential tissue bioadhesive. J Biol Chem. 294(34):12826-12835. *Co-corresponding authors.

Fricano A., Librizzi F., Rao E., Alfano C., Vetri V. (2019) Blue autofluorescence in protein aggregates "lighted on" by UV induced oxidation. BBA - Proteins and Proteomics 1867(11):140258.

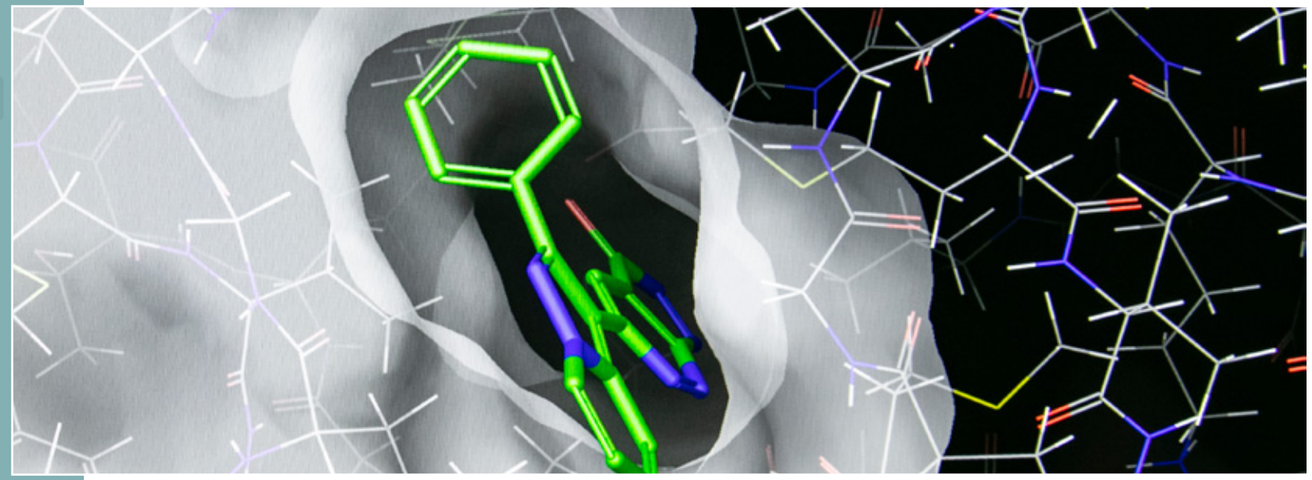
Zacco E., Graña-Montes R., Martin S.R., de Groot N.S., Alfano C., Tartaglia G.G., Pastore A. (2019) RNA as a key factor in driving or preventing self-assembly of the TAR DNA-binding protein 43. J Mol Biol. 431(8):1671-1688.

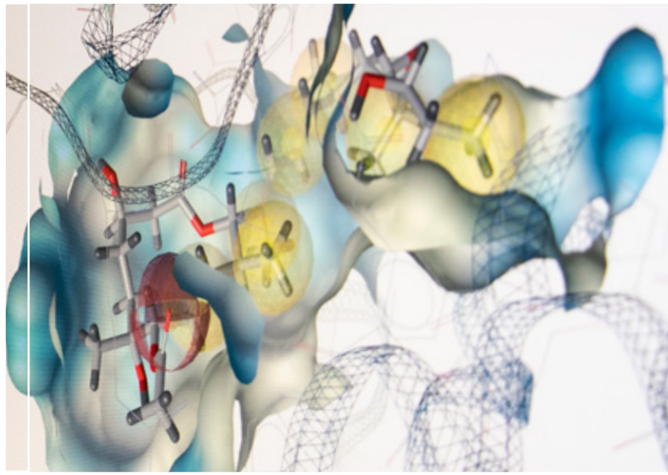




Computer Aided Drug Design

Platform





CONTACTS:
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The Computer-Aided Drug Design (CADD) 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 (VS). 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**
- **Institute of Oncology Research (IOR) Switzerland**
- **University of Vienna (Pharmaceutical chemistry department), Austria**
- **University of Pittsburgh, USA**
- **University of Naples Federico I, Naples, Italy**
- **Italian National Council of Research (CNR), Italy**
- **University of Palermo, 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
- Cambridge Crystallographic Data Centre suite (CCDC)
- LigandScout expert suite
- Autodock and Autodock Vina
- Desmond (OPLS3 and OPLS3e)
- AMBER
- NAMD
- VMD
- Gromacs
- RDKit
- KNIME

Hardware

- 6 Workstations
- Server in HPC mode: 200 CPUs e 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 → ~ 200 ns/day/Card (on 40,000 atoms system)

Integrated *in Silico* Platform

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

ACTIVE RESEARCH PROJECTS

- 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.

- Search for targets and modulators of antiproliferative activity in prostate tumor cells in "**Pten-null" cells**

- Design of modulators of **EPHB4** in tumor pathology

- Modulation of protein-protein interaction modulators with particular reference to **MUC1-CIN85** complexe

PUBLICATIONS

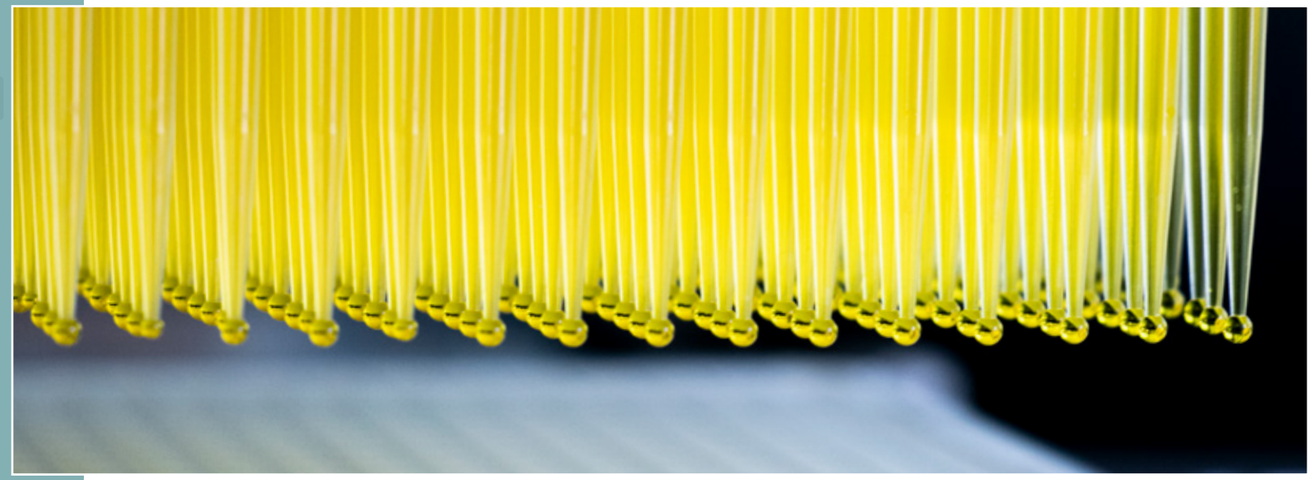
Mekni N., De Rosa M., Cipollina C., Gulotta M.R.., De Simone G., Lombino J., Padova A., Perricone U., *In silico* insights towards the identification of NLRP3 druggable hot spots, Int. J. Mol. Sci. 2019, DOI: 10.3390/ijms20204974.

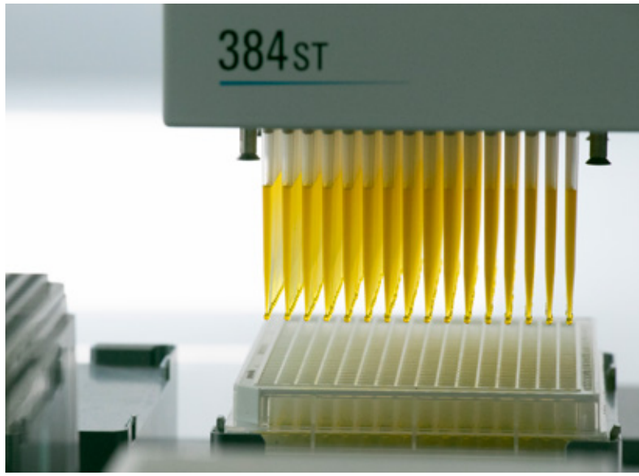
Mendolia I., Contino S., Perricone U., Pirrone R., Ardizzone E., A Convolutional Neural Network for Virtual Screening of Molecular Fingerprints, ICIAP 2019, (Book Chapter), DOI: 10.1007/978-3-030-30642-7_36

Perricone U., Gulotta M.R., Lobino J., Parrino B., Cascioferro S.M., Diana P., Cirrincione G., Padova A., An overview of recent Molecular Dynamics applications as medicinal chemistry tool for undruggable sites challenge, MedChemComm, 2018, DOI: 10.1039/C8MD00166A

High-throughput Screening

Platform





CONTACTS:
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ccipollina@fondazionerimed.com

The high-throughput screening (HTS) platform has developed expertise for the development, validation and miniaturization of biochemical and cell-based assays for small molecule screening projects. Our instrumentation allows the set-up of flexible, partially automated assay protocols, using several assay readouts including absorbance, luminescence, fluorescence, TR-FRET, imaging. The lab is equipped with a system for high-content analysis coupled with software tools for image analysis and data evaluation. At present, our platform supports the activities of the Drug Discovery Unit at Ri.MED by performing primary screening, dose-response validation as well as specific secondary assays.

Expertise

- Assay set-up and validation (biochemical and cell-based);
- TR-FRET- based assays;
- Miniaturization of the assay into 384-well plates;
- High-content Imaging;
- Screening;
- Data analysis and selection of primary hits;
- Hit picking of primary hits for hit validation and preparation of secondary screening plates to perform dose-response experiments (IC50 determination);
- Counter-assays, orthogonal assays and toxicity testing.

Technology Platform

- Wet lab;
- Operetta-CLS (Perkin Elmer);
- Spark (Tecan) - Multimode microplate reader;
- In-Hood-Bravo (Agilent) - Liquid handling system.

ACTIVE RESEARCH PROJECTS

- **Development of selective inhibitors of Histone lysine demethylase 4 (KDM4) for cancer treatment**: the project aims at discovering novel small molecules able to effectively and selectively inhibit the enzyme KDM4. Within this project we will use a primary enzymatic assay to screen focused small molecule libraries.

- **Development of selective inhibitors of the intracellular receptor NLRP3 for the treatment of chronic, age-related diseases**: the project aims at discovering novel small molecules able to inhibit the activation of NLRP3. We are currently focusing on two targeted-approaches aiming at inhibiting the ATPase activity of NLRP3 (by targeting the NACHT domain) as well as the formation of the complex with ASC by inhibiting PYD-PYD interaction.

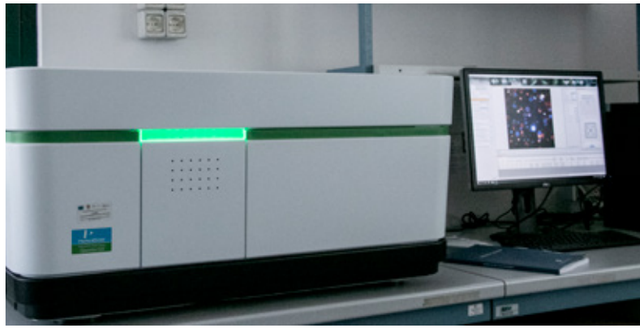
- **Discovery of small molecules able to revert $T_{exhausted}$ (T_{ex})phenotype and to reactivate immune response in the tumor microenvironment:** in collaboration with IRIB-CNR we are currently working to set-up and validate an experimental model of T_{ex} to be used for the high-content screening campaign.

PUBLICATIONS

Mekni N, De Rosa M, Cipollina C, Gulotta MR, De Simone G, Lombino J, Padova A, Perricone U. "*In Silico* Insights towards the Identification of NLRP3 Druggable Hot Spots." Int J Mol Sci, 2019, 20(20). doi: 10.3390/ijms20204974.

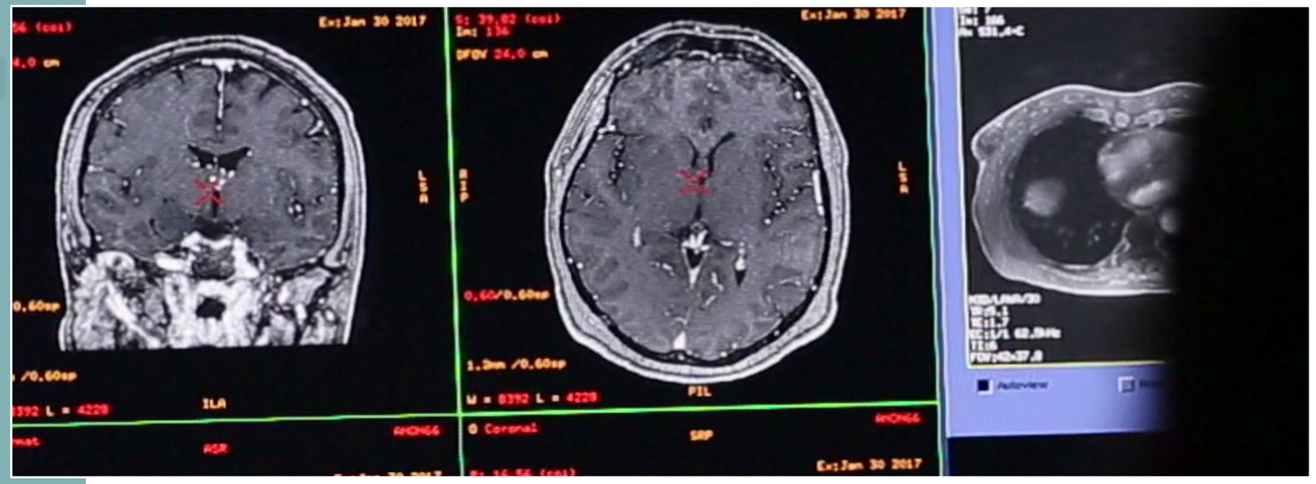
COLLABORATIONS:

- Istituto per la Ricerca e l'Innovazione Biomedica (IRIB) - CNR, Palermo, Italy
- Institut de la Vision, Paris, France
- Università degli Studi della Campania "Luigi Vanvitelli", Caserta, italy
- Università degli Studi di Palermo, Palermo, Italy



Magnetic Resonance Imaging

Platform



CONTACTS:
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During 2019, the Biomedical Imaging platform made use of two magnetic resonances (3T and 7T), other imaging methods made available by partecipant institutions, and segmentation, extraction and statistical features/radiomics analysis skills from images through machine learning techniques for the predictive diagnosis of pathologies and relapses, and medical decision support.
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. In particular, the platform uses two MRIs, 3T and 7T, acquired through the ISpeMi project, and located respectively at the IRCCS ISMETT and Istituto Zooprofilattico Sperimentale, and clinical and preclinical PET/CT available at the IBFM-CNR. During 2020, the platform will be enriched with instruments based on alternative technologies (Bioluminescence), (PET/RM) and (CT) in order to offer more options for *in vivo* imaging data analysis.

COLLABORATIONS:

- IRCCS ISMETT, Palermo, Italy
- Istituto Zooprofilattico Sicilia (IZS), Palermo, Italy
- Institute of Molecular Bioimaging and Physiology, (IBFM-CNR), Cefalù, Italy
- Georgia Institute of Technology, Atlanta, USA
- University of Palermo, Italy
- Medical Physics Unit, Cannizzaro Hospital, Catania, Italy
- Nuclear Medicine Department, Cannizzaro Hospital, Catania, Italy
- Nuclear Medicine Unit, University of Messina, Italy

Expertise

- 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
- Image Processing Models (MR/PET/CT and histological), 3D Segmentation, Deep Learning and Machine Learning to Extract, Classify and Delineate Tumor Volumes and Radiomics Features for Predictive Diagnosis of Pathologies and Relapses and Medical Decisions Support
- Phantoms design and creation for spectroscopy, contrast agents and morphovolumetric studies

Technology platform

At Istituto Zooprofilattico Sperimentale:

- Bruker Pharmascan 70/16 (7 Tesla). Available coils:
  -Mouse and rat brain 2x2 receive surface array coils
  - Mouse and rat transmit-receive volume coil (40 mm inner diameter and 75 mm outer diameter)
  - Rat body 8x2 transmit volume array coil (72 mm inner diameter and 89 mm outer diameter)
- Software: TopSpin, Paravision 6.1, Jmrui, Tarquin, Horos

At IRCCS ISMETT:

GE DISCOVERY MR 750 W 3 Tesla High-Field Magnetic Resonance 3.0 T (neuro, body, breast, angio, osteoarticular, cardio, etc.)

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

- PET/CT Clinica e Preclinica

ACTIVE RESEARCH PROJECTS

In vivo small animals imaging supporting the Project Immunoterapia NK-mediata per il trattamento e/o la prevenzione della recidiva HCC e/o HCV post-trapianto, supervised by Dr. Ester Badami.

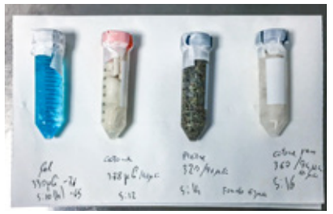
PUBLICATIONS

D. Giambelluca, R. Cannella, F. Vernuccio, A. Comelli, A. Pavone, L. Salvaggio, M. Galia, M. Midiri, R. Lagalla and G. Salvaggio. PI-RADS 3 Lesions: Role of Prostate MRI Texture Analysis in the Identification of Prostate Cancer. Current Problems in Diagnostic Radiology. Accepted October 2019. DOI: 10.1067/j.cpradiol.2019.10.009

Alongi, P., A. Stefano, A. Comelli, S. Bignardi, M. Sabini, A. Yezzi, M. Ippolito, and G. Russo. A Machine Learning Segmentation Approach For The Extraction Of Radiomic Features In PET Studies. in european journal of nuclear medicine and molecular imaging, vol. 46, no. suppl 1, pp. s764-s764. 233 spring st, new york, ny 10013 usa: springer, 2019.

A. Comelli, A. Stefano, S. Bignardi, C. Coronnello, G.Russo, M.G.Sabini, M.Ippolito, and A.Yezzi. Tissue Classification to Support Local Active Delineation of Brain Tumors. In Annual Conference on Medical Image Understanding and Analysis. MIUA 2019, CCIS 1065, pp. 3–14, 2020. DOI: 10.1007/978-3-030-39343-4_1

A. Comelli, and A. Stefano. A fully automatic system of Positron Emission Tomography Study segmentation. In Annual Conference on Medical Image Understanding and Analysis. MIUA 2019, CCIS 1065, pp. 353–363, 2020. DOI: 10.1007/978-3-030-39343-4_30



Proteomics

Platform

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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, UK**
- **Dipartimento di Scienze Farmacologiche, Universita di Pisa, Italy**
- **STEBICEF, Università di Palermo., Italy**

Expertise

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

Technology platform

Hardware devices:

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

Software devices:

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

ACTIVE RESEARCH PROJECTS

iRhom2: a new therapeutic target for osteoarthritis

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.

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

Scilabra S.D., Pigoni M., Pravata V., Schatzl T., Muller S.A., Troeberg L., and Lichtenthaler S.F. (2018). Increased TIMP-3 expression alters the cellular secretome through dual inhibition of the metalloprotease ADAM10 and ligand-binding of the LRP-1 receptor. Sci Rep 8, 14697.

Yang C.Y., Troeberg L., Scilabra S. D., Quantitative mass spectrometry-based secretome analysis as a tool to investigate metalloprotease and TIMP activity. Methods in Molecular Biology, 2020, 2043:265-273

Carreca A.P., Pravatà V.M., Murphy G., Nagase H., Troeberg L., Scilabra S.D. (2019) TIMP-3 facilitates binding of target metalloproteinases to the endocytic receptor LRP-1 and promotes scavenging of MMP-1 bioRxiv doi: https://doi.org/10.1101/2019.12.23.886762

Nano LC System UltiMate 3000 used for peptide separation interfaced directly to the Q-Exactive Mass Spectrometer for LC-MS/MS analysis.

CELL FACTORY

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The Cell Factory Group, including Production, Quality Assurance and Quality Control staff, as well as 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 staff is involved in Production, Quality Assurance and Quality Control, will allow to produce advanced therapies developed by ISMETT and Ri.MED Foundation researchers, and to use them 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.

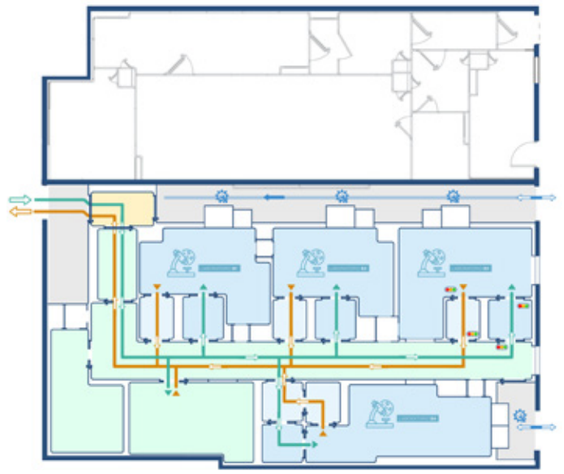
COLLABORATIONS:
- IRCCS ISMETT, Palermo, Italy
- University of Pittsburgh, USA
- UPMC, Pittsburgh, USA

Competenze

- 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 will guarantee flexibility in the type of production and functionality of the environments. 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 the possibility of being put in negative pressure and 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, so that part of the operations can be performed in one lab (eg. organ cleaning) and others -passing the intermediate product through a passbox- in the second lab (eg. cell isolation and culture). In a class C room, closed cell preparation systems will be installed. Technical compartments on which the engines of the instrumentation protrude will 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 which are necessary 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.



ACTIVITIES

The progress status of the new cell factory and Quality Control laboratories structural works is 90% at the time of writing this report (Dec 2019)

Fundamental formal documentation for the future authorization of the structure was prepared. The new Quality Assurance system, consisting of general/ quality control/ instrumentation/ production Standard Operating Procedures and QC Methods, is being optimized.

Development of adoptive immunotherapy products for future production is on going
The main activities for 2020, once the construction of the new Cell Factory and Quality Control laboratories are completed, will consist in the validation of the environments and all the equipment to make the new areas operational, as well as in training and validation of all the operators. At the same time, the validation of the new productions and the related quality control tests will be started.

Examples of activities for the Cell Factory activation are: Installation, Operation and Performance Qualification of the equipment, validation of the Heating, Ventilation and Air Conditioning system, validation of environmental classes, validation of sterile dressing (for personnel involved in production, quality control, sanitization, maintenance), specific and complete GMP training of all operators based on their job description, validation of the sanitization and clean hold time, material transfer validation, supplier validation. Production-related activities will include: Media Fill, QC methods validation.

GRANTS

The Ri.MED Foundation supports the realization of 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 international cooperation, 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 2019, activities continued on the six multi-year projects financed in 2017 and 2018 and currently in progress. In addition, three new innovative industrial research doctorates were started.

In 2019 four new R&D projects received funding under the 2013/2020 Sicilian Region POR. This very positive result recognizes the commitment and skills of our researchers. The Ri.MED Foundation was also selected as host institution for a research project aimed at defining a new therapeutic target for osteoarthritis using cutting-edge proteomics methods and *in vivo* models, funded by Fondazione con il Sud.

Among the 2019 activities aimed at obtaining research funds, three projects were submitted for the Horizon 2020 EU calls and three R&D projects on national funding (PON MIUR, PON MISE, "Ricerca Finalizzata" by the Ministry of Health). One Ri.MED researcher also applied for an EU ERC Starting grant.

Ongoing scientific projects in 2019

CheMISt
Computational Molecular Design e Screening
Founded 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 an integrated laboratory and interdisciplinary research team to support Ri.MED's research, becoming a point of reference for scientific research of high added value 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.

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
Founded 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.

GMP Facility
Laboratori di Ricerca e Servizi Diagnostici e Terapeutici dell'Istituto Mediterraneo per i Trapianti e le Terapie ad Alta Specializzazione
Founded by: Assessorato alle Attività Produttive of the Region of Sicily, 2014-2020 PO FERS Sicilia

In compliance with the goal set by Action 1.5.1 "Support to research infrastructure deemed strategic for regional systems for purpose of enforcing S3", the project aims at upgrading the IRCCS ISMETT- Ri.MED research infrastructure purchasing new devices and technology to support research and clinical activity, expediting bench-to-bedside transition.

OACTIVE
Advanced personalised, multi-scale computer models preventing OsteoArthritis
Founded 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.

Research infrastructures - "GMP Facility, Laboratori di Ricerca e Servizi Diagnostici e Terapeutici dell'Istituto Mediterraneo per i Trapianti e le Terapie ad Alta Specializzazione" project.
Founded by: Assessorato alle Attività Produttive of the Region of Sicily

The goal of the project is to upgrade research, cell production, and diagnostic laboratories of the Ri.MED - ISMETT cluster purchasing state-of-the-art devices and equipment, and upgrading the laboratories.

4FRAILTY
Sensoristica intelligente, infrastrutture e modelli gestionali per la sicurezza di soggetti fragili
Founded 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.

TRASFERIMENTI PER METODOLOGIE INNOVATIVE NEL CAMPO DELLE BIOTECNOLOGIE 2018
Founded by: Stability Law, Region of Sicily, Assessorato della Salute, Dipartimento per le attività sanitarie e osservatorio epidemiologico (DASOE).

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

Projects eligible for funding in 2019

PROGEMA
Processi green per l'estrazione di principi attivi e la depurazione di matrici di scarto e non
Founded 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 reuse treated waters in the production processes.

OBIND
Oncological therapies through Biological Interaction Network Discovery
Founded by: Region of Sicily within the scope of 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.

SENSO
Sviluppo di un dispositivo miniaturizzato per il monitoraggio di stress ossidativo in sistemi cellulari
Founded by: Region of Sicily within the scope of 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.

Scientific projects submitted in 2019

Prometeo
Prodotti medicinali derivati da placenta per terapie avanzate per patologie epatiche ed endometriali
Founded by: Region of Sicily within the scope of 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.

Progetto iRhom2
"iRhom2a new therapeutic target in osteoarthritis"
Founded 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.

Discovery of novel selective inhibitors of the NLRP3 inflammasome
Call: UE Openscreen – European High-capacity screening network

The project aims at finding selective inhibitors of the NLRP3 receptors screening a library of 100,000 small molecules using a primary sample developed by the Foundation's high-throughput screening laboratory.

Ottimizzazione di approcci di tipo cell-based nel riparo delle ferite cutanee croniche
Call: Special supplementary research fund (FISR) - Italian Ministry of Education, University, and Research (MIUR)

The project aims at addressing critical aspects related to the use of mesenchymal stem cells and of their byproducts in wound healing and tissue regeneration, such as: donor source and variables relating to specific clinical applications and personalized medicine; comparison between cells and cell-based products from different sources; cell and secretion release solutions in view of two potential clinical applications: chronic skin lesions and trauma-induced spinal cord lesions.

Liver and cardiovascular damage in non-alcoholic fatty liver disease: a new non-invasive MR imaging approach
Call: 2019 Finalized Research, Italian Ministry of Health

The goal of the project is to create a robust, non-invasive protocol for nonalcoholic steatohepatitis (NASH) diagnosis and staging of fibrosis, which will significantly reduce the need for biopsies in non-alcoholic fatty liver disease (NAFLD).

iRhomMunity - iRhom2: treating multiple diseases by hitting the major regulatory hub of immune responses
Call: ERC - Starting Grant 2019

The goal of the project is to generate drugs targeting iRhom2 using different approaches ranging from developing specific antibodies with phage display to high throughput screening of small molecules.

Role of iRhom2 in the regulation of MHC class I molecules
Call: American Department of Defence - Cancer Research Program

The scope of the project is to investigate the molecular mechanisms used by iRhom2 to control the major histocompatibility complex, class I, using proteomics. Furthermore the project aims at studying the functional impact of these regulatory mechanisms in cancer.

Rete integrata per il potenziamento della ricerca clinica traslazionale per una medicina personalizzata per il cancro alla mammella
Call: National Health Plan 2019

The goal of the project is to strengthen the network of research centers (CNR, INFN, Ri.MED, universities) and hospitals (Villa Sofia-Cervello and AUOP Giaccone of Palermo) to improve clinical and diagnostic research in oncology and senology.

Placenta4Liver
Placenta Derived Stem Cells to treat Chronic Liver Disease
Bando: H2020-SC1-BHC-2018-2020- HORIZON 2020 – European Commission

NABUCCO
Nuovi Farmaci e Biomarkes di risposta e resistenza farmacologica nel cancro del colon retto
Call: Innovation Agreement - Italian Ministry of Economic Development (MISE)

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

Plat4BioReg
Biology centred bioink and bioprinting platform development for musculoskeletal repair and regeneration
Call: H2020-NMBP-TR-IND-2018-2020- HORIZON 2020- European Commission

The project aims at developing new biomaterials based on natural products and on a decellularized extracellular matrix, which once combined with innovative cellular products can repair and replace damaged cartilage, ligaments and tendons.

AMADEUS
A modular multi-orgAn-on-chip platforM for investigating the role of the crosstAlk between gut, gut microbiota, white aDipose tissue and ostEochondral Unit in the onset of osteoarthritiS
Call: H2020-NMBP-TR-IND-2018-2020- HORIZON 2020- European Commission

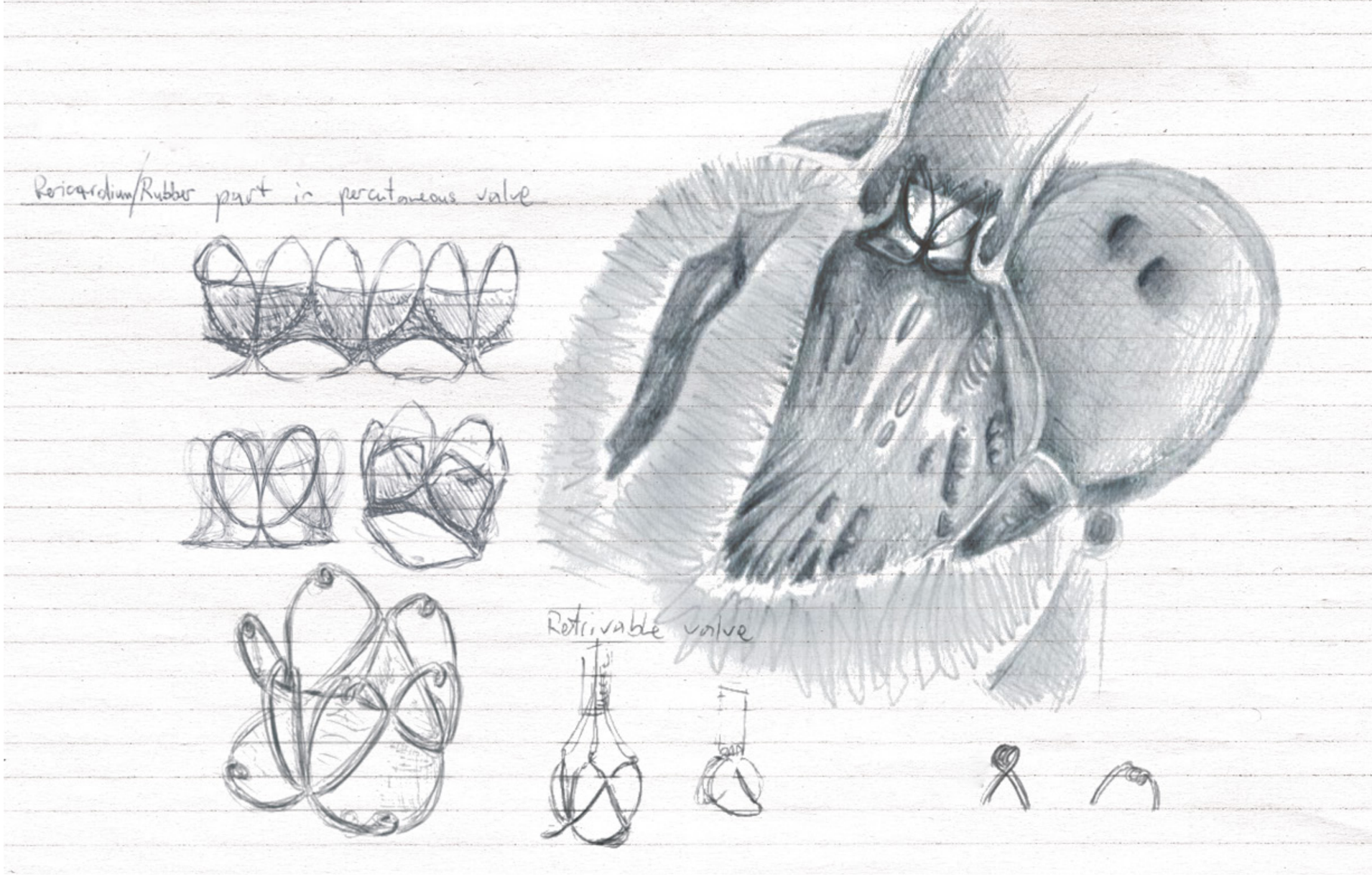
The project aims at creating an in vitro organ-on-chip model that includes joints, adipose tissue, microbiota and gut for drug screening and animal test reduction.

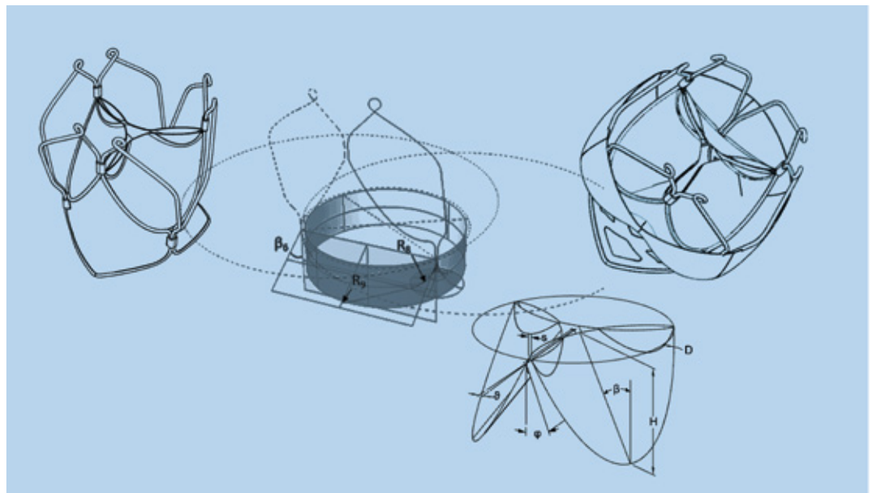
INTELLECTUAL PROPERTY AND TECHNOLOGY TRANSFER

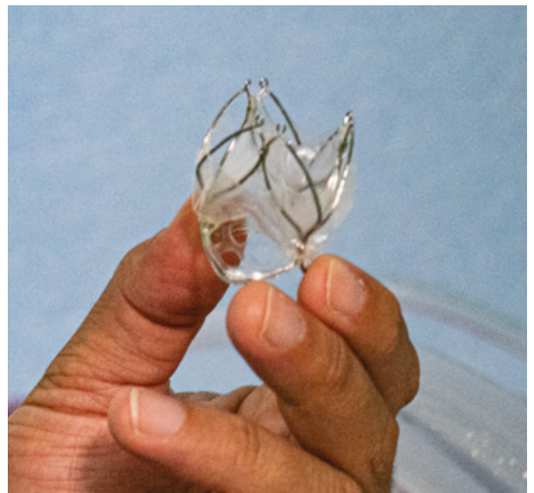
Research activity of Ri.MED is strongly patient-oriented, but in order to ensure that scientific results reach clinical needs, it is necessary to correctly manage the intellectual property generated by our researchers as well as the process of technology transfer which derives from it. From the laboratories,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

The protection of intellectual property is a fundamental value for Ri.MED to develop an innovative model of research sustainability. For this reason, Ri.MED has set up an 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 2019 Ri.MED acquired full ownership of the "Triskele" patent from UCL Business, whose inventors include Gaetano Burriesci, Bioengineering group leader of the Foundation. This is an invention of strong interest concerning a highly innovative endovascular implantable cardiac valve already validated *in vitro*, which would allow to acquire visibility in an area of great clinical interest.







Patent portfolio up to 31.12.2019

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 therefor
WO2018099988
Fondazione Ri.MED - IRCCS ISMETT

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

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

TISSUE ENGINEERING AND BIOMEDICAL DEVICES

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

Transatrial access for intracardiac therapy
WO2017127682
Fondazione Ri.MED - University of Pittsburgh

Bi-layer extra cellular matrix scaffolds and uses therefor
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 mesenchymal 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

Ri.MED Research Retreat
October 2019, Teatro Garibaldi - Palermo

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