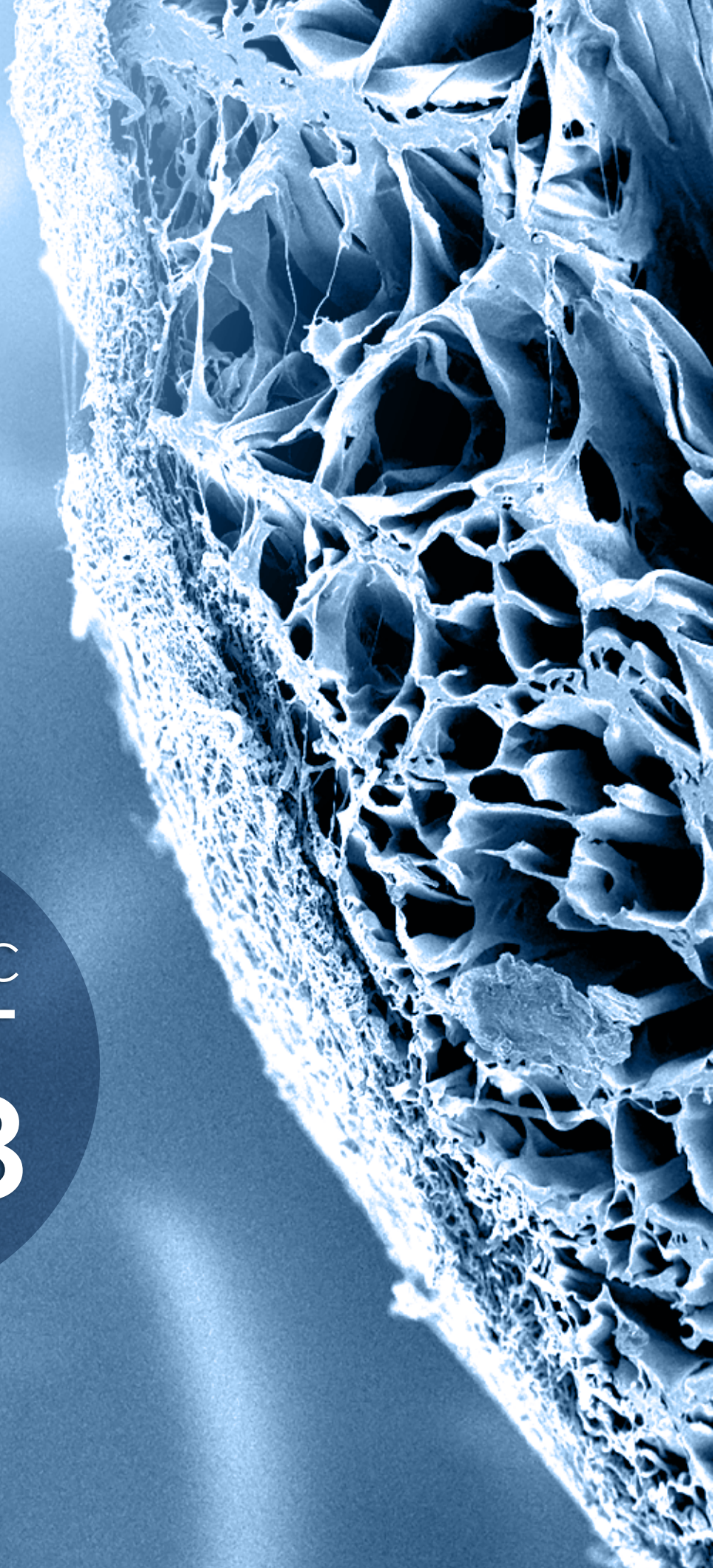
Fondazione Ri.MED



Scientific REPORT 2018

Fondazione Ri.MED

Scientific REPORT 2018

COVER PICTURE
Engineered vascular graft cross-section analyzed with scanning electron microscopy. The inset shows the double layer scaffold wall and its different morphology which was obtained by thermo-induced phase separation (porous core) and by electrospinning (external fibrous jacket). The biodegradable polymer utilized was poly(ester urethane) urea.

FOUNDING PARTNERS

Presidenza del Consiglio dei Ministri

Consiglio Nazionale delle Ricerche

University of Pittsburgh

UPMC LIFE CHANGING MEDICINE

Regione Siciliana

PARTNER

ISMETT Istituto di Ricovero e Cura a Carattere Scientifico

Alessandro Padova

DIRECTOR GENERAL

In 2018 Ri.MED achieved positive results both in terms of science, training and dissemination in the Life Science sector; thanks to the foresight of the Presidency of the Council of Ministers and the Sicilian Region, during the year the **Cluster Ri.MED-IRCCS ISMETT** gained visibility and was further strengthened, thanks to the concept of **research and translational medicine**: from patient to the laboratory, with results that return to the patient.

During 2018, as a result of the interaction with CNR and to the recognition of the grant **CheMiSt** funded by the Sicilian Region, Ri.MED started to implement a high content and high throughput screening technology platforms and enhanced the labs of of biophysics and structural biology, computational chemistry and bioinformatics. Regenerative medicine and biomedical engineering activities were focused on organ failure pathologies, as a consequence of strategic partnerships with the University of Pittsburgh, UPMC and IRCCS-ISMETT.

Tangible outcomes are the over 20 patents, developed according to a networking model aiming to integrate complementary skills, to increase the critical mass of scientific results, and to compete for research fundings.

The year 2018 also marked the beginning of the second and final phase of the tender for the construction of the **Biomedical Research and Biotechnology Center (BRBC)**: the objective is to break in 2019, a fundamental step towards the creation of the BRBC, the research and health hub in the heart of the Mediterranean. The project foresees the future integration with the ISMETT2 hospital, that will be constructed adjacent to the Ri.MED Research Center.

A recognition to all the people in Ri.MED who, with **competence, passion and team spirit**, contribute day after day to the development of innovative solutions for health and to a positive socio-economic impact in Sicily and in the South of Italy.

Dario Vignali

SCIENTIFIC DIRECTOR

Welcome to the new edition of the annual Scientific Report, which offers an overview of our activities, researchers 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.

In 2018, thanks to the commitment and effort of the Scientific Committee (Prof. Francesco Dieli - appointed by CNR- Prof. Ivet Bahar- appointed by UPMC - Prof. George Fadi Lakkis - appointed by UPMC- and Prof. Claudio Bordignon, appointed by Ri.MED BOD), a strategic review of Ri.MED's research and therapeutic focus was conducted. From now on Ri.MED will concentrate 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.

It is therefore no coincidence that the theme chosen for the 2018 edition of the annual Ri.MED Scientific Symposium was "**Cancer Immunotherapy: Recent Progress and Future Challenges**".
The event, organized in Palermo, brought together a panel of international experts and met with considerable public success, confirming the role that Ri.MED can and intends to play in the international scientific community and in the territory.

In conjunction with the Symposium, the **1st Ri.MED Research Retreat** was held during which about twenty of our researchers presented their work: a choral moment of sharing the results and objectives of the research, as well as an important occasion to explore possibilities for new scientific collaborations.

The Scientific Committee also reviewed plans to reinitiating the Ri.MED post-doctoral fellowship program. During 2019, we will develop plans to further research strategy 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.

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Ri.MED OVERVIEW UP TO 31.12.2018

Intellectual property

about **300** Scientific publications

23 Registered patents

2 Patent proposals

Training & employment

52 Employees in 2018

58% 42%

23 Scholarships

18 Post- Doc Fellowships c/o University of Pittsburgh

13 PhD Fellowships

16 Internships

Fundings for research

14.773.643€ Won through national and international GRANTS

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

Networking

3 Agreements for the laboratories management

47 Active scientific collaborations in 2018

19 Signed scientific collaborations in 2018

Scientific dissemination

35 Ri.MED international scientific events

8 Institutional events

16 Participations in scientific events or local development activities

Building the BRBC

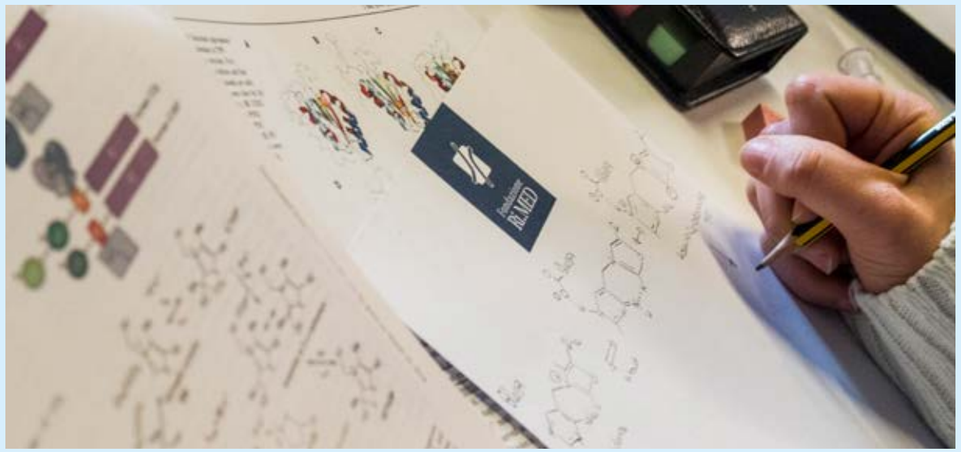
28.000 mq of laboratories

196.350.000€ Value of the investment

600 Planned occupancy opportunities

TRAINING

The training of highly qualified personnel is not only one of Ri.MED's statutory mission, but also an activity that the Foundation carries out with particular enthusiasm, being aware that the offer of a high-level training can contribute to enriching the future of young people, the competitiveness and development of the whole territory. In recent years, Ri.MED has achieved important milestones in training of professionals with specific skills in the field of biotechnology, including 41 scholarships granted, 16 internships carried out and 13 PhDs.

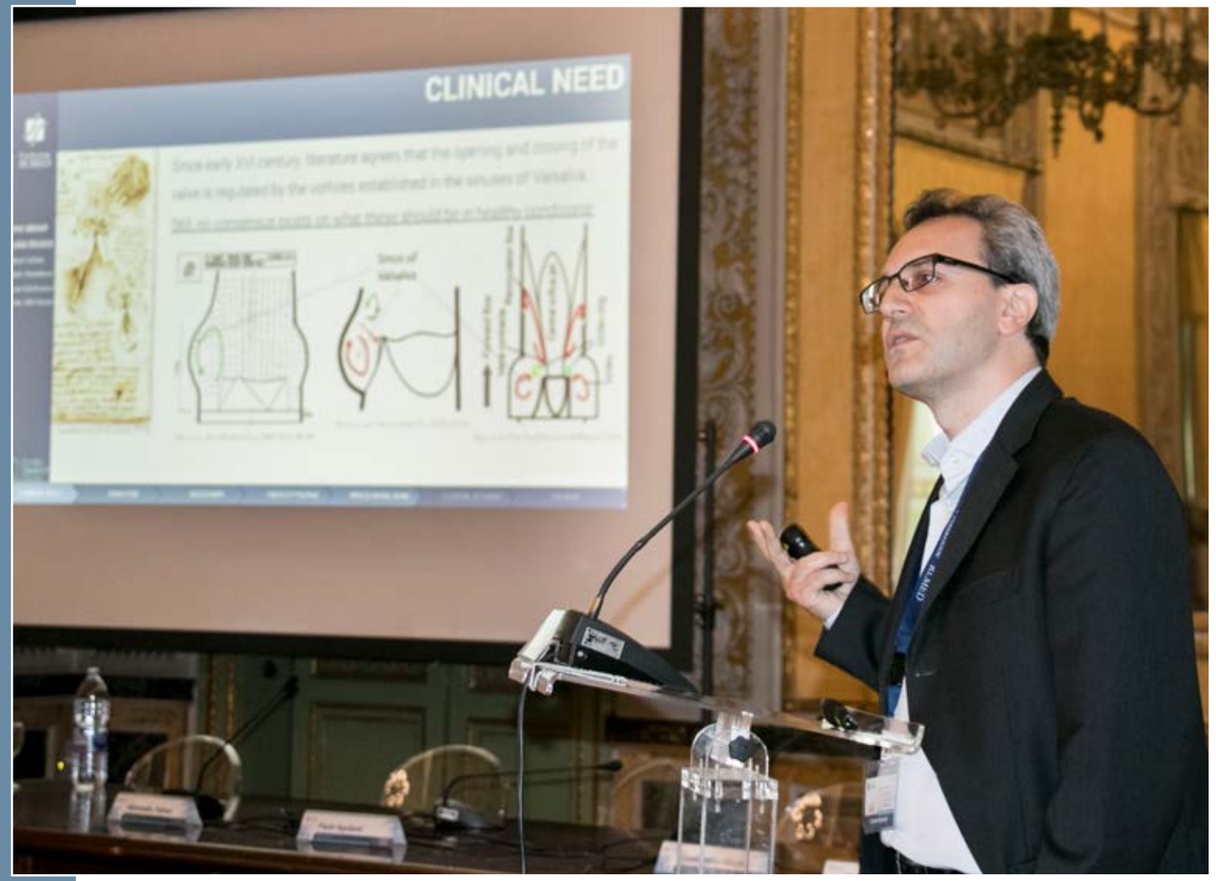


From 2007 to 2015, the Foundation sponsored 18 Post - doctoral Fellowships at the University of Pittsburgh, and from 2014 onwards has activated training programs as part of project funded by European, national and regional grants: thanks to these funded projects, in the last few years, dozens of multidisciplinary and highly qualified figures have been trained: bioengineers, biologists, computational and medicinal chemist scientific managers, stabularists, laboratory technicians and others.

DISSEMINATION OF SCIENTIFIC KNOWLEDGE

Activities related to scientific dissemination and sharing of research results are part of the Foundation's mission.

In addition to the annual Scientific Symposium, Ri.MED organizes, in collaboration with its scientific partners, lectures and workshops.



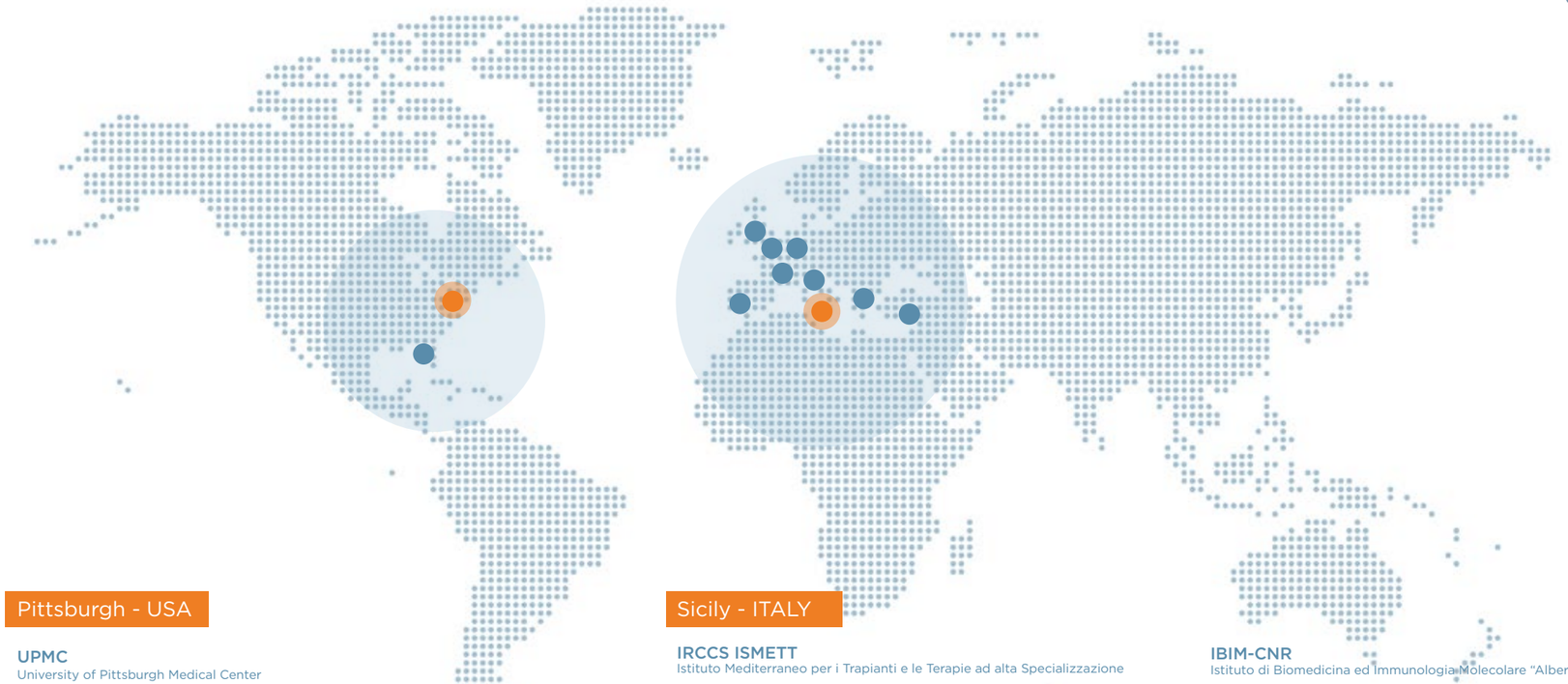
To date, our researchers have published more than 300 scientific articles on peer review journals with relevant impact factors, contributing to the scientific dissemination of the sector, as well as the generation of intellectual property, while the press office works to transfer the main results to a wider audience.

NETWORKING

Translational research is based on the development of a wide network of collaborations and scientific agreements that Ri.MED is forging with bodies and institutions working in Ri.MED's strategic areas: today 33 agreements are in place for the development of technological innovation, the promotion of research activities and the sharing of laboratories and resources with European and American institutions.

Ri.MED has signed collaboration agreements for lab hosting at the following Research Centers: the Regenerative Medicine and Biomedical Technologies laboratories at the IRCCS-ISMETT, of strategic importance for the integration of basic and clinical research; the Structural and Computational Biology laboratories at the ATeN Center of the University of Palermo and the IBIM CNR laboratories in Palermo for HTS Screening.

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



Pittsburgh - USA

UPMC
University of Pittsburgh Medical Center

UPMC
School of Medicine

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

McGowan Institute for Regenerative Medicine

Sicily - ITALY

IRCCS ISMETT
Istituto Mediterraneo per i Trapianti e le Terapie ad alta Specializzazione

ATeN Center
Advanced Technologies Network Center

Fondazione Istituto Giglio di Cefalù

IAMC-CNR
Istituto per l'Ambiente Marino Costiero del Consiglio Nazionale delle Ricerche

IBF- CNR
Istituto di Biofisica del Consiglio Nazionale delle Ricerche

IBIM-CNR
Istituto di Biomedicina ed Immunologia Molecolare "Alberto Monroy" del CNR

IZS
Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri"

Qwince LTD

Università degli Studi di Palermo

Università degli Studi di Messina

Università degli Studi di Catania

BELGIUM

Universiteit Antwerpen

Université de Louvain

FRANCE

CNRS
Centre National de la Recherche Scientifique

Sorbonne Universite

UTC
Université de Technologie de Compiègne

Institute de La Vision

INSERM
Institut National de la Santé Et de la Recherche Médicale

GERMANY

Ludwig-Maximilians-Universität München

DZNE
Deutsches Zentrum für Neurodegenerative Erkrankungen

GREECE

CERTH
Center for Research & Technology Hellas

University of Patras

MALTA

EDEX Educational Excellence Corporation Ltd
University of Nicosia

CETRI
Center for Technology Research&Innovation

SPAIN

LaFe
Hospital Universitari i Politècnic La Fe

UK

Liverpool John Moores University

ITALY

AurorA-TT
Translating Research into care

Epi-C Epigenetic Compounds

UPMC
Institute for Health - Chianciano Terme

Sterling SpA

TES PHARMA
Drug Discovery Innovation

Università degli Studi Roma Tre

BRBC

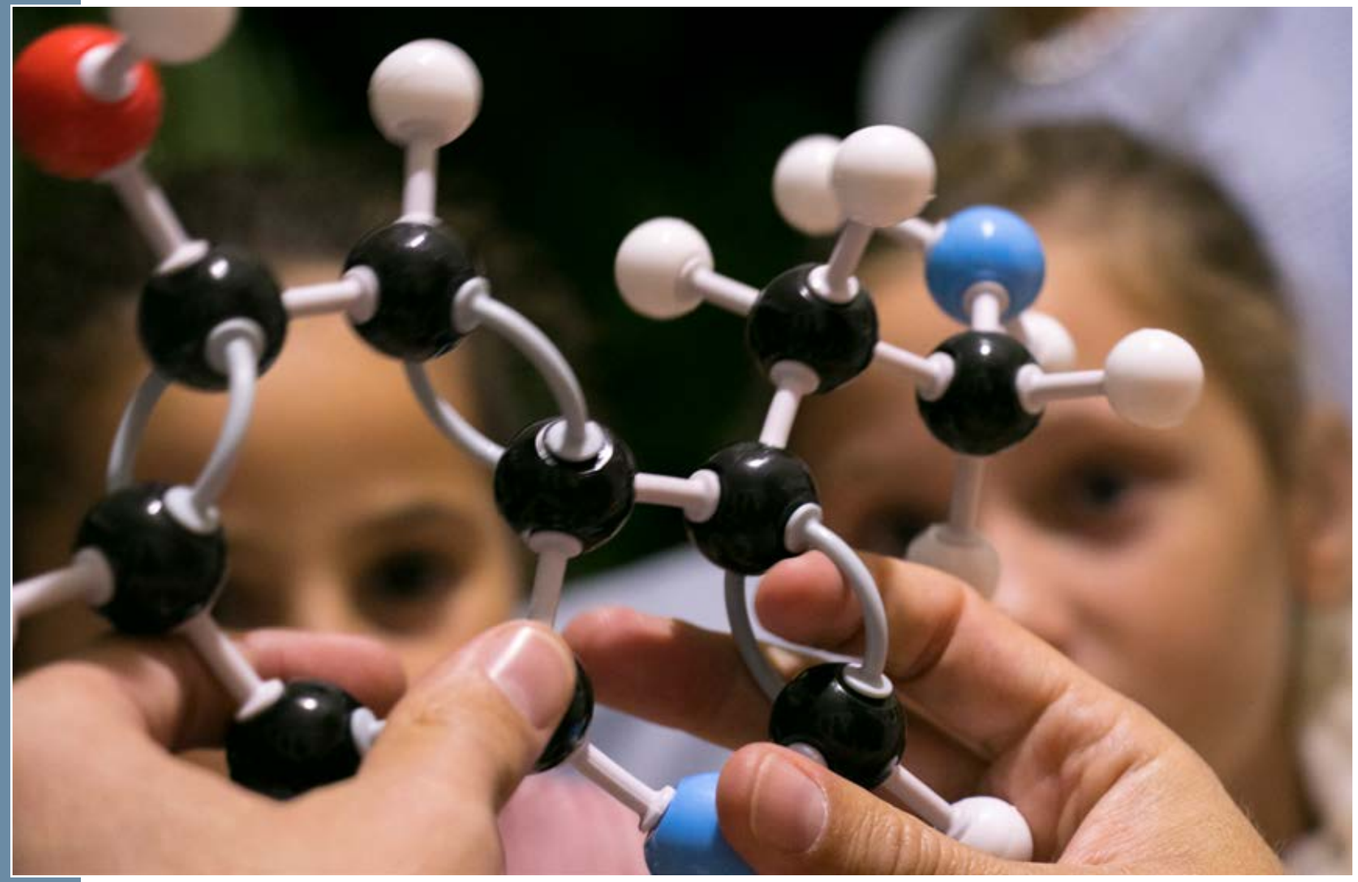
BIOMEDICAL RESEARCH AND BIOTECHNOLOGY CENTER

Simultaneously to the research activity, Ri.MED is engaged in the creation near Palermo of the Biomedical Research and Biotechnology Center. The joint venture of companies, winner of the design competition and contract for the executive design and safety coordination, is led by Hellmuth, Obata & Kassabaum Inc.. In September 2018, at the end of 91 sessions, the ranking for the first phase of the tender to appoint the constructors was approved and the second phase started in October, with the invitation of the selected economic operators. The call for tenders for construction will end in the first half of 2019.

The BRBC, which will also host a business incubator, represents a management model of public-private partnerships, able to dialogue with universities and research organizations on one hand and with pharma and biotechnological companies on the other, and to develop strategic alliances and attract financing and investments for research, with positive impact on the economy of the South. The BRBC will bring to Palermo members of the international scientific community, retaining in Italy the best Italian doctors and scientists, also thanks to the collaboration with UPMC and the University of Pittsburgh.

PUBLIC ENGAGEMENT

Involving and inspiring a heterogeneous public is one of our priorities, so we are working on a public engagement program to inspire and entertain citizens of all ages, in collaboration with the main players on the territory. Dialoguing in the local community must lead to the development of activities that facilitate and promote knowledge, from science and health, to investment and employment opportunities, up to legality and meritocracy.



The size and profile of the BRBC will make Ri.MED highly visible and able to play an important role in generating enthusiasm and interest in science, in particular through activities with schools. To achieve these goals and increase its presence on the territory, Ri.MED has several educational initiatives with schools and local community.

Ri.MED Research groups

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Isabella Mendolia
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Ri.MED Lab c/o IBIM CNR, Palermo

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Rossella Alduino
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PhD student in Technologies and Health Sciences

Sofia Di Leonardo
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Ri.MED Supported Scientist
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Courtesy of Giuseppe Peritore

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Roberto Di Gesù, PhD
POST DOCTORAL RESEARCHER
OACTIVE

DRUG DISCOVERY

Ri.MED scientists are dedicated to elucidate the pathophysiological mechanisms of incurable diseases.

Through the study of the biomolecular pathways integrated with genomic, proteomic, metabolic and secretome data, our researchers have come to the functional validation of new therapeutic targets for neurodegenerative diseases such as Parkinson's disease, tumor diseases and inflammatory diseases.
Some of these projects are now transitioning from discovery to pre-clinical phase. This process starts with the study of target proteins through biophysics and computational chemistry approaches and with the setting up of biophysical, biochemical or cellular screening assays.

Thanks to the integrated drug design and virtual screening platform developed during 2017, hundreds of molecules of synthetic and natural origin have been selected as potential leads via structure-based techniques (docking) and ligand-based (pharmacophore).
These molecules, selected *in silico*, are then synthesized or acquired to be biologically tested and validated with primary and secondary screening. In the next phase, the biologically active hit molecules will be optimized through medicinal chemistry to reach a preclinical proof of concept, thus study *in vivo* efficacy and pharmacokinetic and toxicological profiles suitable for preclinical development and subsequent clinical trials on patients.
In parallel, predictive methods are being developed to monitor the efficacy of potential drugs and stratify patients responding to therapy.

Molecular mechanisms of protein misfolding diseases: normal function versus aberrant aggregation
Caterina Alfano, PhD

Infiltrating macrophages promote over-expression of ST6GALNAC1 in IBD and colitis-associated cancer
Sandra Cascio, PhD

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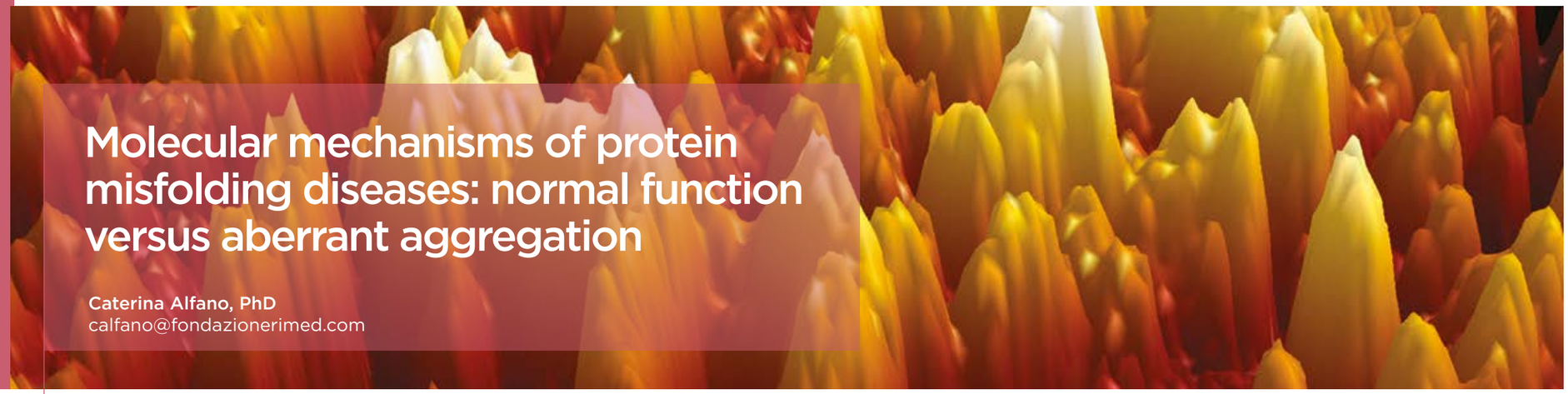
PRODUCTS: **DRUGS**



Molecular mechanisms of protein misfolding diseases: normal function versus aberrant aggregation

Caterina Alfano, PhD
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COLLABORATION

Department of Basic and Clinical Neuroscience - King's College London, London, UK

Department of Physics and Chemistry, University of Palermo, Palermo, Italy

THERAPEUTIC AREA

Aging Diseases

PIPELINE

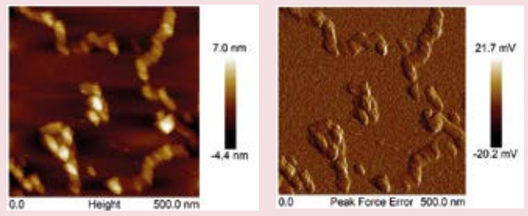
CLINICAL NEED — DISEASES ANALYSIS — DISCOVERY — PRECLINICAL VALIDATION — PRECLINICAL DEVELOPMENT — CLINICAL STUDIES

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 these diseases is still lacking.
We aim to understand the molecular mechanisms of protein misfolding diseases and rely on the concept that knowledge of the normal function and of the interaction network of aggregogenic proteins is a key tool to design molecules which can specifically compete out aggregation.
Native protein-protein interactions could indeed provide important means of altering and controlling the function and assembly of those proteins involved in neurodegenerative diseases and whether they can fulfil a protective role against aberrant aggregation. Preliminary data provide strong support to this hypothesis and encourage further studies along this line, opening new possibilities to approach the development of treatments of misfolding diseases. We selected ataxin-3, the protein responsible for the inherited Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3, as a model system in this project. 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.

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 basic knowledge provided may eventually help to approach the design of specific therapies. Furthermore, amyloid formation is not only related to neurodegenerative disease but, perhaps even more important, can hold clues to the very fundamental phenomenon of protein-folding and assembly such as making amyloid fibrils good candidates for the development of new materials. Therefore, the present project could contribute to enhance research excellence not only in medicine, but also in the biomaterials field adding a socio-economic benefit as well.



Ataxin-3 fibrils by atomic force microscopy

RESULTS ACHIEVED IN 2018

During this year we have focused on the analysis of aggregation processes of both wild-type ataxin-3 (i.e. with 18 Gln repeats) and pathological ataxin-3 (i.e. with 50 Gln repeats) and on how those processes are influenced by the presence of polyUb chains, natural binding partners of ataxin-3. We used: I) spectroscopic techniques, such as absorption and fluorescence spectroscopy, to study the conformational variations of both proteins; II) Circular dichroism to determine the secondary structure and its possible variations; III) light scattering for the analysis of the growth of the aggregates. These analyses confirmed that the expanded form of ataxin-3 has a higher propensity to form aggregates and its secondary structure changes more dramatically than the wild-type form. Our results also confirmed that the formation of ataxin-3 aggregates is strongly inhibited by the presence of polyUb chains.
Furthermore, we have been working on the creation of stable inducible cell lines able to express high levels of the two forms of ataxin-3 in controlling conditions. These cell lines will allow us to have an in-cell system suitable to study the aggregation and to test potential therapeutic molecules, that could modify the ataxin-3 aggregation process.

GOALS FOR 2019

Start a drug design approach on ataxin-3 in order to identify molecules that mimicking the natural interactors of ataxin-3 are able to act as disease modifiers. The binding properties of those molecules to ataxin-3 and their effect as modulators of the aggregation will be screened *in vitro* using biophysical techniques. Molecules resulted positive in the *in vitro* studies will then be tested in-cell using the stable inducible cell lines created *ad hoc* for this purpose.

MEETINGS

UK-Israel Synergy Symposium - Protein misfolding in ageing and neurodegeneration: from basic biology to drug development, March, 2018, London, UK

PUBLICATIONS

Martínez-Lumbreras S, **Alfano C**, Evans NJ, Collins KM, Flanagan KA, Atkinson RA, Krysztofinska EM, Vydyanath A, Jackter J, Fixon-Owoo S, Camp AH, Isaacson RL. (2018) Structural and Functional Insights into Bacillus subtilis Sigma Factor Inhibitor, CsfB. Structure, 26(4):640-648. doi: 10.1016/j.str.2018.02.007

Pecci A, Ragab I, Bozzi V, De Rocco D, Barozzi S, Giangregorio T, Ali H, Melazzini F, Sallam M, **Alfano C**, Pastore A, Balduini CL, Savoia A. (2018) Thrombopoietin mutation in congenital amegakaryocytic thrombocytopenia treatable with romiplostim. EMBO Mol Med. 10(1):63-75. doi: 10.15252/emmm.201708168

PRODUCTS: **BIOMARKERS**

Infiltrating macrophages promote over-expression of ST6GALNAC1 in IBD 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

Macrophages constitute a heterogeneous population that differentiates into distinct types, schematically categorized as M1, which secrete high levels of pro-inflammatory cytokines and have tumoricidal activity, and M2 that are considered to be involved in promotion of tissue remodeling and tumor progression. Tumor-associated macrophages (TAM) display a mixed-polarization phenotype that depends on the balance of inflammatory mediators released within the tissue environment. In this project, we assess whether tissue-infiltrating macrophages induce the expression of cancer associated glyco-antigens which in turn activate inflammatory and oncogenic signaling pathway resulting in the perpetuation of colonic inflammation and tumorigenesis.

We are currently testing the hypothesis that infiltrating macrophages secrete inflammatory factors that alter expression of glycosyltransferase genes, causing the induction of the tumor antigen MUC1. MUC1 is heavily glycosylated and expressed at low levels of normal cells whereas is hypoglycosylated and highly expressed on tumor cells. Examples of tumor-associated MUC1 glycoforms are T- and Tn- as well as their sialylated forms sT- and sTn-MUC1.

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 polarized macrophage-secreted factors induced aberrant MUC1 glycoforms in colon cancer cells. MUC1 is known to be a tumor-associated antigen and oncoprotein that is 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 specific aberrant MUC1 glycoforms and/or altered glycosyltransferases as drivers of tumor development and progression will result in novel disease-relevant targets for prevention and treatment of CAC.

RESULTS ACHIEVED IN 2018

The mechanisms that regulate MUC1 glycosylation during intestinal chronic inflammation and colitis-associated cancer are still unclear. Our data suggest that inflamed colonic tissues of MUC1.Tg mice 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 intestinal cancer cells for 3 days. We found that polarized M1 and M2 macrophages induced over-expression of hypoglycosylated form of MUC1 compared to unstimulated cells. In addition, RT2 profiler glycosylation-related gene expression array revealed that M1 or M2 macrophages modulate glycosyltrasferase enzymes, such as ST6GalNAc1 and ST3GAL1, which are involved in O-glycan biosynthesis. Using *in vivo* and *in vitro* models, we have dissected the molecular mechanisms by which tumor associated macrophages modulate the expression of glycosyltransferase enzymes and subsequent MUC1 tumor antigens.

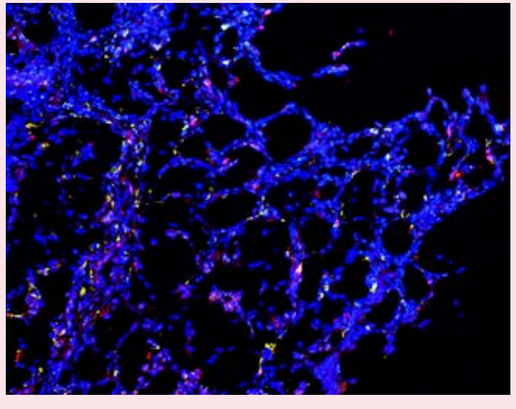
GOALS FOR 2019

We will determine the interplay between macrophages and aberrant MUC1 glycosforms as a novel model of communication of the inflammatory microenvironment and colon cancer cells. To investigate whether altered MUC1 glycoforms directly interact with macrophages, we will perform pull-down assays of protein lysates extracted from tissue-infiltrating macrophages and biotinylated synthetic MUC1 peptides differently glycosylated. 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 absence of murine St6galnac1 gene will prevent the formation of MUC1-sTn structure and its subsequent interaction with tissue- infiltrating macrophages. We expect that the prevention of this interaction might inhibit macrophage polarization toward a pro-tumor associated phenotype and will result in a delay progression to cancer.

MEETINGS

American Association of Immunologists (AAI), May, 2018, Austin (TX), USA.

4th Annual Immuno-Oncology Young Investigator Forum, April, 2018, Houston (TX), USA.



iNOS, CD206, F4/80
Tumor-associated macrophages (TAMs) in AOM/DSS-treated colon tissues of MUC1.Tg mice
Confocal Immunofluorescence. Staining was performed using an antibody against F4/80, a known marker of TAMs. Anti-CD206 and anti-iNOS antibodies were used to detect the infiltration of M1 and M2 macrophages, respectively, into the tumor microenvironment.

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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THERAPEUTIC AREA

Aging Diseases

PIPELINE

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

BRIEF DESCRIPTION

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the world. It is a disease mainly associated with aging that is characterized by a progressive and irreversible reduction of the respiratory flow. To date there is no therapy able to block the development and progression of COPD, and new drugs are urgently needed.
Airway remodeling, cellular senescence, bronchial epithelial activation 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).

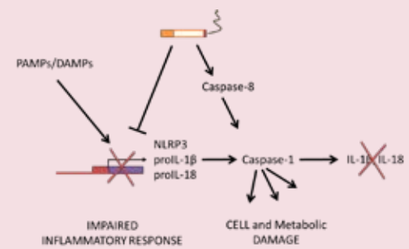
In recent years, we have investigated the innate immunity in experimental models of COPD, analyzing the inflammatory response of macrophages and bronchial epithelial cells. In particular, we have focused on the role of the NLRP3 inflammasome and on cell metabolism aiming at understanding how their crosstalk may contribute to the pathogenesis of COPD. The study of molecular mechanisms underlying this pathology will contribute to identify new targets for the development of innovative therapies.
In parallel, since NLRP3 is a target validated for many chronic inflammatory disorders, we are working on the development of selective NLRP3 inhibitors in collaboration with the Drug Design team.

IMPACT

The project will contribute to the discovery and characterization of new molecular mechanisms involved in the pathogenesis of COPD. This, in turn, will allow to identify new potential targets for the development of new therapies.
In addition, the work done in collaboration with the Drug Design team will contribute to the development of selective NLRP3 inhibitors to be used for the development of new drugs. The impact of this work will be broad and will go beyond the specific context of COPD. In fact, activation of the NLRP3 receptor contributes to the pathogenesis of several chronic diseases including atherosclerosis, metabolic disease, and neurodegenerative diseases.

RESULTS ACHIEVED IN 2018

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. Using bronchial epithelial cells and macrophages (both cell lines and monocyte-derived macrophages, MDM) exposed to cigarette smoke we observed an opposite response in the two cell types with respect to the NLRP3 inflammasome. Our data suggest that exposure to smoke causes activation of the inflammasome in the bronchial epithelium and, on the contrary, inhibits NLRP3 inflammasome in macrophages.
At the same time, smoking appears to activate caspase-1 leading to significant metabolic damage. These data help to explain the typical scenario of the COPD lung where the epithelium generates uncontrolled inflammation and macrophages have a reduced activity (immune dysfunction). Our data suggest that the activation of caspase-1 associated with NLRP3 reduction may be a new pathogenic mechanism of COPD and opens up new possibilities for intervention in order to block disease progression.



Proposed model: cigarette smokes inhibits the NLRP3 inflammasome in macrophages thus impairing immune responses; at the same time cigarette smoke activates Caspase-1 leading to cell and metabolic damage. PAMP, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns.

GOALS FOR 2019

One of the main objectives will be the validation and expansion of the data so far obtained using cells and samples isolated from COPD patients. We will also work to understand the mechanisms that correlate the NLRP3 inflammasome with cellular metabolism and inflammatory response and how the joint regulation of these events modulates the immune system and the secretory phenotype.
Regarding the development of selective NLRP3 inhibitors, the aim will be the development of robust assays for high throughput screening in order to test commercial libraries of small compounds as well as compounds selected via computer aided drug design and virtual screening.

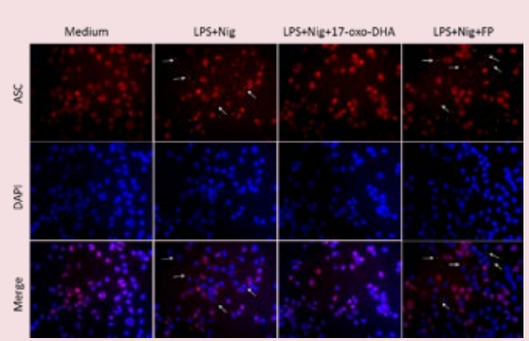
MEETINGS

The inflammasomes-EMBO Workshop, 25-28 September, 2018, Martinsried (Germany)

PUBLICATIONS

Siena L*, **Cipollina C***, Di Vincenzo S, Ferraro M, Bruno A, Gjomarkaj M, Pace E. (2018) Electrophilic derivatives of omega-3 fatty acids counteract lung cancer cell growth. Cancer Chemother Pharmacol, 81, 705-716. doi: 10.1007/s00280-018-3538-3.

Di Vincenzo S, Heijink IH, Noordhoek JA, **Cipollina C**, Siena L, Bruno A, Ferraro M, Postma DS, Gjomarkaj M, Pace E. (2018) SIRT1/FoxO3 axis alteration leads to aberrant immune responses in bronchial epithelial cells. J Cell Mol Med, 22:2272-2282. doi: 10.1111/jcmm.13509.



PRODUCTS: **BIOMARKERS**

Modeling microRNA-target interaction network

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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

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 tissues behavior. MicroRNA and mRNA expression profiles necessary to model the tissue specific interaction network can be obtained with high throughput data analysis techniques, based on microarray or Next Generation Sequencing technologies. These technologies provide quantitative information about all microRNAs and mRNAs endogenously expressed in the analyzed tissue. It is our aim to develop algorithms to model and compare the microRNA-target interaction network of tissues in different conditions.

IMPACT

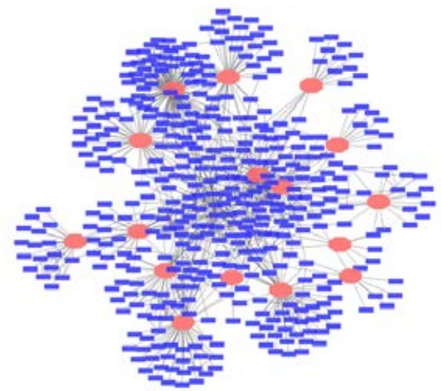
Biological Big Data repositories are rapidly growing, partly due to the fact that in order to publish results in the most important journals, it is mandatory to make available to the public the original data useful to obtain the results described on the paper. When data accounts for gene expression profiles,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

RESULTS ACHIEVED IN 2018

In 2018 we focused on the development of an algorithm able to compute the microRNA – RNA interaction network specific of a tumoral tissue. The algorithm is based on the comparison of microRNA and messenger RNA expression profiles in tumoral and normal tissues, and it is aimed to associate every feasible interaction between microRNA and messenger RNA with a score computed through a statistical validation. We analyzed expression data from the data archive The Cancer Genome Atlas (TCGA), in particular the expression data of breast and kidney normal and tumoral tissues. The algorithm we developed is more selective with respect to the other algorithms based on correlation among the expression profiles, and the selected interactions involve lists of genes functionally more focused, i.e. a gene ontology analysis reveals more statistically significant classes of genes.

GOALS FOR 2019

In 2019 we aim to test and validate the algorithm developed so far. The main obstacle in pursuing this goal is the absence of any experimental procedure able to take a picture of the whole interaction network among microRNAs and mRNAs. It is then impossible to perform a direct validation of the computed interaction networks. 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.



Example of bipartite network of microRNA and mRNA. The red nodes represent the microRNA, each of them regulates hundreds of genes, represented by the blue nodes. Our algorithm calculates which relationships between microRNA and genes are statistically significant.

PRODUCTS: **BIOMARKERS - DRUGS**

NOX2/mitochondrial interaction in the pathogenesis of Parkinson's disease

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COLLABORATIONS

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THERAPEUTIC AREA

Neurodegenerative diseases

PIPELINE

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

BRIEF DESCRIPTION

There is an increasing consensus that multifactorial events responsible for the pathogenesis of PD, correlate oxidative stress in the substantia nigra pars compacta (SNpc) affecting the physiology of dopaminergic nigrostriatal neurons. While it is generally assumed that the oxidative stress and damage seen in PD derive from mitochondria, recent evidences report that NADPH oxidase 2 (NOX2), one of the major sources of reactive oxygen species (ROS) in brain cells, may be important. Indeed, mitochondrial function and NOX2 are intimately related in a redox regulatory pathway recently termed "ROS-induced ROS production" (RIRP). Although there is very little information on the role of NOX2 activity in PD, previous evidence has provided data addressing the contribution of NOX2 to the development of the disease. Our latest results suggest that NOX2 activity plays a role in mitochondrial dysfunction, LRRK2 activation and neurotoxic modifications of alpha-synuclein, crucial events strongly related to PD pathogenesis. This study aims to provide evidences of neuroprotection against NOX2-related PD pathogenesis using novel compounds that specifically target NOX2. Pre-clinical studies conducted last year provide promising results that will lead us to further investigations on the topic. We hope to apply these molecules in clinical studies soon.

IMPACT

The exact mechanisms of the progressive dopamine neurons loss in Parkinson's disease (PD) are still underexplored. For this reason. current treatments for PD have shown low effectiveness to slow down or reverse the progression of the disease. Furthermore, the constant increase of PD cases in the world population points to the urgency of providing new and effective therapeutical approaches in the immediate future. In this context, we are testing novel highly specific NOX2 inhibitors may have a multitarget efficacy given the involvement of NOX2 in several crucial events related to PD pathogenesis, including LRRK2 activation, alpha-synuclein accumulation mitochondrial dysfunction and neuroinflammation. Positive outcomes of this study may provide novel and effective tools to the clinical approach to PD patients.

RESULTS ACHIEVED IN 2018

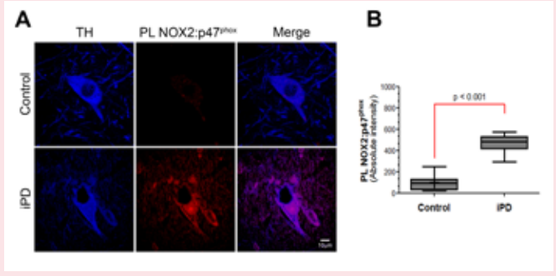
Completed (published) *in vitro/in vivo* studies on the relevance of LRRK2 kinase activity in idiopathic PD, addressing NOX2 as its possible modulator. Completed (unpublished) in vitro/*in vivo* studies on the NOX2 downstream mechanisms of PD pathogenesis.

Research Fundings

NIH/NINDS: 1R01 NS095387 (RDM Co-I) α-Synuclein inhibition of mitochondrial protein import.
NIH/NINDS: 1R01NS100744-01 (RDM Co-I) Role of LRRK2 in idiopathic Parkinson's diseases.

Awards

Research Award: "A central role for LRRK2 in idiopathic Parkinson disease"; PSG - Meeting, Portland - OR



A NOX2 hyperactivity in idiopathic Parkinson's disease. Proximity Ligation Assay (PLA) for NOX2:p47phox interaction - a crucial event for NOX2 activation - in post-mortem human brain tissue reveals a significant increased NOX2 activity in specimen derived from PD, addressing the relevance of this enzyme in the human disease.
B Quantitative analysis of NOX2 activity in dopaminergic neurons.

Teaching activity

University of Pittsburgh - Pittsburgh, PA
Guest Lecturer: Neuropharmacology; – Undergraduate Neuroscience Program spring 2018
Lecturer: Graduate Course of Neuropharmacology; spring 2018

Mentoring activity

1 PhD student in Pharmacology, University of Pittsburgh

GOALS FOR 2019

The main future objective is to complete the study in preclinical models to define the pharmacokinetics (PK) and the efficacy of NOX2 inhibitors so to block the pathogenic events of Parkinson's disease. The long-term goal is to propose the new NOX2 inhibitors for clinical trials as new therapies against Parkinson's.

MEETINGS

"A central role of LRRK2 in idiopathic Parkinson's disease". Neuroscience 2018, San Diego, CA Nov. 11-16, 2018

"NOX2/mitochondria interplay in Parkinson's disease". Neuroscience 2018, San Diego, CA – Nov. 11-16, 2018

"NOX2/mitochondria interplay in Parkinson's disease". Gordon Research Conference, May, 2018

PUBLICATIONS

R. Di Maio, E. K. Hoffman, E. M. Rocha, M. T. Keeney, L. H. Sanders, B. R. De Miranda, A. Zharikov, A. Van Laar, A. F. Stepan, T. A. Lanz, J. K. Kofler, E. A. Burton, D. R. Alessi, T. G. Hastings, J. Timothy Greenamyre, LRRK2 activation in idiopathic Parkinson's disease. Sci. Transl. Med. 10, eaar5429 (2018).

D'Aiuto L, Naciri J, Radio N, Tekur S, Clayton D, Apodaca G, Di Maio R, Zhi Y, Dimitrion P, Piazza P, Demers M, Wood J, Chu C, Callio J, McClain L, Yolken R, McNulty J, Kinchington P, Bloom D, Nimgaonkar V. (2018) Generation of three-dimensional human neuronal cultures: application to modeling CNS viral infections. Stem Cell Res Ther. 2018 May 11;9(1):134. doi: 10.1186/s13287-018-0881-6. PMID: 29751846.

Delicata F, Bombardi C, Pierucci M, Di Maio R, De Deurwaerdère P, Di Giovanni G. Preferential modulation of the lateral habenula activity by serotonin-2A rather than -2C receptors: Electrophysiological and neuroanatomical evidence. CNS Neurosci Ther. 2018 Feb 25. doi: 10.1111/cns.12830. PMID: 29479825.

PRODUCTS: **DRUGS**

Pharmacokinetics of electrophilic nitro fatty acids (NO_2-FA)

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COLLABORATIONS

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THERAPEUTIC AREA

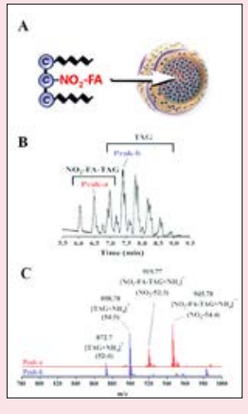
Organ Insufficiencies

PIPELINE

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

BRIEF DESCRIPTION

The electrophilic 10-nitro-oleic acid (10-NO_2-OA) showed beneficial signaling responses in preclinical studies, has successfully passed Phase 1 toxicity tests and it is now undergoing Phase II clinical trials for the treatment of chronic pulmonary and renal diseases. Detailed understanding of the pharmacokinetics (PK) of 10-NO_2-OA is complicated by the capability to alkylate thiols reversibly, become esterified in various complex lipids, and the instability of the nitroalkene moiety during enzymatic and base hydrolysis. This research project was designed to improve our understanding of the PK of this signaling mediator and new drug candidate. We showed: 1) esterification into triglycerides as the primary mechanism of systemic 10-NO_2-OA distribution and 2) the detection and characterization of *in vivo* 10-NO_2-OA isomers.



A) Esterification of NO_2-FA into triglycerides and systemic distribution by plasma lipoproteins. B) HPLC-high resolution-mass spectrometry characterization of plasma lipoprotein triglycerides (TAG) and NO_2-FA-containing-TAG (NO_2-FA-TAG). C) MS spectra analysis of peak (a) in red and peak (b) in blue show that NO2-FA-TAG have odd masses while normal TAG have even masses respectively.

IMPACT

We showed that electrophilic nitro fatty acids (NO_2-FA) display unique pharmacokinetics, with the principal mechanism of tissue distribution involving esterification into triglycerides (TAG). This compartmentalization can limit electrophilic reactions with nucleophiles and metabolic enzymes, such as prostaglandin reductase-1 (PtGR-1), and may ensure better PK and safety of lipid electrophiles, species that have typically been viewed as toxic. Furthermore, the preferential distribution of plasma 10-NO_2-OA in TAG, suggest an incorporation into chylomicron TAG and lymphatic delivery into the systemic circulation via the subclavian vein, which prevents hepatic inactivation of the electrophilic character of this mediator and thus permits efficient distribution to target organs.
Finally, the detection, characterization, new analytical methods, and profiling of 10-NO_2-OA isomers *in vivo* reveals new directions for better understanding fatty acid nitration during metabolism and inflammation and the development of synthetic homologs lipid signaling mediators having improved PK characteristics.

RESULTS ACHIEVED IN 2018

We reported the mechanism and kinetics of absorption, metabolism, and distribution of 10-nitro-oleic acid (10-NO_2-OA) in dogs after oral administration. Supported by liquid chromatography-high resolution-mass spectrometry (HPLC-HR-MS) analysis of synthetic and plasma-derived 10-NO_2-OA-containing-triacylglycerides, we showed that a key mechanism of NO_2-FA distribution is an initial esterification into complex lipids. Quantitative analysis of plasma free and esterified lipid fractions confirmed time-dependent preferential incorporation of 10-NO_2-OA into triglycerides when compared to its principal metabolite 10-nitro-stearic acid (10-NO_2-SA). Finally, we identified new isomers of 10-NO_2-OA *in vivo*, and we characterized their electrophilic reactivity and metabolism.

GOALS FOR 2019

We will define the mechanism of intestinal absorption and plasma distribution of 10-NO_2-OA in rodents. We will establish the role of chylomicrons and other lipoproteins on 10-NO_2-OA uptake and transport. A lymph-fistula rat model with cannulated portal vein will be used and human plasma lipoproteins will be separated and analyzed. In this regard, we have developed an HPLC-MS/MS analysis to detect 10-NO_2-OA-containing triglycerides and an acidic hydrolysis method to quantitate free and esterified 10-NO_2-OA in mesenteric lymph and portal plasma and lipoprotein fractions. We will also determine the effect of 10-NO_2-OA and nitro-nitrate lipids in intestinal water regulation. The modulatory action and targets of 10-NO_2-OA in intestinal water homeostasis will elucidate the watery stools found in orally supplemented preclinical animal models. The comparison between 10-NO_2-OA and nitro-nitrate lipids could potentiate the value of the latter as potential prodrugs without this unpleasant gastrointestinal effect.

MEETINGS

Invited Speaker

M. Fazzari. Esterification of electrophilic 10-nitro-oleic acid is the principal mechanism of systemic transport and distribution. 1st RI.MED Research Retreat, October 11-2018, Palermo, Italy.

Poster

M. Fazzari, S.R. Salvatore, D.A. Vitturi, S.R. Woodcock, B.A. Freeman and F.J. Schopfer. Distribuzione dell'acido nitro-oleico nei trigliceridi delle lipoproteine. ASBMB Deuel Conference on Lipids, 6-9 March 2018, San Diego, California, U.S.A.

PUBLICATIONS

Fazzari M., Vitturi D.A., Woodcock R.S., Salvatore S., Freeman B.A. & Schopfer F.J. Esterification of electrophilic 10-nitro-oleic acid is the principal mechanism of systemic transport and distribution. Journal of Lipid Research – doi:10.1194/jlr.M088815.

Buchan G.R.*, Bonacci G.*, **Fazzari M.**, Salvatore S., Wendell S.G. Nitro-fatty acid formation and metabolism. Nitric Oxide, 2018, 79, 38-44 – doi:10.1016/j.niox.2018.07.003.

Chartoumpekis D.V., Yagishita Y., **Fazzari M.**, Palliyaguru D.L., Rao U.N.M., Zaravinos A., Khoo N.K.H., Schopfer F.J., Weiss K.R., Michalopoulos G.K., Sipula I., O'Doherty R.M., Kensler T.W., Wakabayashi N. Nrf2 prevents Notch-induced severe insulin resistance and tumorigenesis in mice. JCI Insight, 2018, 3 (5), e97735 – doi:10.1172/jci.insight.97735.

Chartoumpekis D.V., Palliyaguru D.L., Wakabayashi N, **Fazzari M.**, Khoo N.K.H., Schopfer F.J., Sipula I., Yagishita Y., Michalopoulos G.K., O'Doherty R.M., Kensler T.W. Dissecting the effects of Nrf2 deletion in adipocytes and hepatocytes during high-fat diet-induced obesity in mice. American Journal of Physiology - Endocrinology and Metabolism, 2018, 315: E180–E195 – doi:10.1152/ajpendo.00311.2017.

D'Amore A., **Fazzari M.**, Jiang H.B., Luketich S.K., Luketich M.E., Hoff R.F., Jacob D.E., Gu X., Badylak S.F., Freeman B.A., and Wagner W.R. Nitro-oleic acid (NO_2-OA) release enhances regional angiogenesis in a rat abdominal wall defect model. Tissue Engineering Part A, 2018, Vol. 24, No. 11-12, pp-889-904 – doi:10.1089/ten.TEA.2017.0349.

INTELLECTUAL PROPERTY

PCT Application No. PCT/US2018/061862. Title: Nitro-oleic acid (NO_2-OA) controlled release platform to induce regional angiogenesis in abdominal wall repair. Filed: November-19-2018. Inventors: D'Amore A., Fazzari M., Freeman B., Wagner W.R.

PRODUCTS: **DRUGS**

Protective actions of the electrophilic nitro fatty acids (NO_2-FA) in Parkinson's diseases

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COLLABORATIONS

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Pittsburgh Institute for Neurodegenerative Diseases (PIND), Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, U.S.A.

THERAPEUTIC AREA

Aging Diseases

PIPELINE

BRIEF DESCRIPTION

An effective cure for Parkinson's disease (PD) is an unmet need, since current treatments have failed to slow down or reverse the progression of the disease. The exact mechanisms of the selective nigrostriatal cell loss in PD are still poorly understood, but there is an increasing consensus that oxidative stress and neuro-inflammation play a critical role. In this regard, new pharmacological strategies, based on the activation of Nrf2-dependent antioxidant, tissue-repair responses, the inhibition of pro-inflammatory NF-kB-dependent cytokine and adhesion molecule expression, and NADPH oxidase-2 (NOX-2) activity, have shown promising results in PD therapy.

Electrophilic nitro-fatty acids (NO_2-FA) have displayed tissue-protective and anti-inflammatory actions in multiple preclinical models, have cleared 5 Phase 1 human trials in 107 subjects, and are now in Phase II studies related to the treatment of chronic pulmonary and renal diseases. Notably, we have just discovered that NO_2-FA readily cross the blood-brain barrier. From this insight, it is now hypothesized that NO_2-FA access the nigrostriatal region of the brain and will inhibit oxidative stress and neurodegeneration in PD through modulation of Nrf2- and NF-kB-dependent gene expression and inhibition of both mitochondrial impairment and pro-inflammatory NADPH oxidase-2 (Nox-2) activity. Then, we propose to study preclinical models to define the efficacy of NO_2-FA in limiting the pathogenesis of PD.

IMPACT

The activation of pleiotropic pharmacologic responses by the electrophilic NO_2-FA represents a safe and effective drug strategy for treating diseases such as PD, that have a complex pathogenesis. Of note, NO_2-FA have cleared multiple Phase 1 human trials and they are being evaluated in Phase II studies related to chronic inflammatory diseases.

Our present research goals are designed to elucidate the brain bio-distribution, target engagement, anti-inflammatory actions and cytoprotective responses of NO_2-FA in well-established preclinical models of PD. The successful completion of the proposed research project will offer new insights into the metabolism, distribution, and concentrations of NO_2-FA in nigrostriatal areas of both male and female rats. In addition, our research plan will evaluate the neuroprotection of NO_2-FA, through a reduction of oxidative stress, neuroinflammation, and improvement of motor symptoms. Since PD is an incurable disease, our contribution is significant because NO_2-FA could provide a novel pharmacological approach to reverse or slow down the progression of nigrostriatal degeneration and it could be rapidly translated into human trials. This project also creates a foundation of knowledge for the potential development of even more efficacious lipid electrophiles as drugs for treating PD, improving the quality of life in patients and reducing the already staggering healthcare costs.

RESULTS ACHIEVED IN 2018

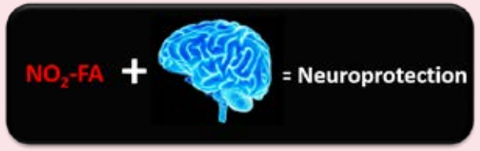
We showed that NO_2-FA cross the blood-brain barrier (BBB) in rats after oral administration. Furthermore, we assessed the mitochondrial distribution of a homolog of NO_2-FA in macrophages after optically-oriented click chemistry.

This specific cellular localization was achieved synthetizing an alkynyl-NO_2-FA, which could react with cysteine protein residues and undergoes "click reaction" with a fluorophore-azide probe in fixed cells respectively. Furthermore, we developed and validated a novel proximity ligation (PL) assay that can rapidly provide information regarding activation state and cellular localization of NOx2. Of note, the involvement in Parkinson's disease of NADPH oxidases (Noxs), which generate ROS in neurons and microglial cells, has been recently suggested. Then, using this novel assay, we observed that NO_2-FA consistently inhibit Nox2 activity in rotenone-treated N27 dopamine neural cells.

GOALS FOR 2019

The goals of this proposal are: 1) Define the neuroprotective potential of NO_2-FA through the modulation of Nrf2- and NF-kB-dependent gene expression and inhibition of Nox-2 activity in an *in vitro* model of PD, 2) Evaluate the pharmacokinetics (PK) of NO_2-FA in the midbrain and its neuroprotective effects in a rotenone model of PD in rat.

These results will shed new lights on the protection mechanisms of NO_2-FA in PD and will establish a strong scientific premise for an R01 application aimed at translating these findings into highly relevant preclinical animal models including rat model of adeno-associated virus 2 (AAV2)-mediated human α-synuclein overexpression in substantia nigra.



PRODUCTS: **DRUGS**

Pharmacology of nitro-nitrate lipids

Marco Fazzari, PhD
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COLLABORATIONS

Department of Pharmacology & Chemical Biology – University of Pittsburgh Medical Center, Pittsburgh, U.S.A.

THERAPEUTIC AREA

Aging Diseases

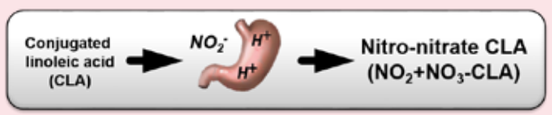
PIPELINE

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

BRIEF DESCRIPTION

The acidic gastric environment promotes efficient nitration of unsaturated fatty acids and the endogenous formation of nitro fatty acids (NO_2-FA). These species mediate salutary effects in numerous inflammatory diseases via post-translational protein modifications that modulate significant cell signaling pathways. The conjugated linoleic acid (CLA) is a preferential substrate for fatty acid nitration and in presence of both nitrite (NO_2^-) and low gastric pH is converted into electrophilic nitro-conjugated linoleic acid (NO_2-CLA). Under the same conditions we have characterized newly-discovered endogenous nitro-nitrate CLA (NO_2+NO_3-CLA).

These are prodrugs that become activated at physiological pH generating nitro-conjugated linoleic acid (NO_2-CLA) and releasing nitric oxide (NO)-like nitrosative species, with potential modulation of cGMP-independent/dependent pathways respectively. Then, the decomposition of NO_2+NO_3-CLA could induce not only the antioxidant and cytoprotective actions of NO_2-FA but also nitric oxide (NO)-mediated vasodilation.
The proposed research will define dietary and inflammatory-mediated mechanisms of generation, optimal routes for organic synthesis and the signaling actions of this structurally-unique class of lipids.



IMPACT

The proposed research will provide insight into the generation and protective biological functions of nitro-nitrate lipid derivatives, a new class of signaling mediators already covered with a recent international PCT provisional patent application.
The over-arching significance of the research project is the characterization of an endogenous metabolic and inflammatory product as both a signaling mediator and a potential drug strategy for limiting the vascular and cardiopulmonary injury that stems from metabolic and inflammatory stress.
This project creates a foundation of knowledge for potential development of this novel product of nitro-oxidative reactions as a new drug candidate.

RESULTS ACHIEVED IN 2018

The NO_2+NO_3-CLA derivatives have been characterized using state of the art biochemical, isotopic labeling and high performance liquid chromatography-coupled mass spectrometric analysis. Of note, the NO_2+NO_3-CLA-incorporated into triglycerides (NO_2+-NO_3-CLA-TAG) has been isolated by TLC and also analyzed by UV-Vis and infrared spectroscopy (IR) analysis. The kinetics and mechanisms of decomposition into NO_2-CLA have been studied under organic and acidic and alkaline buffer solutions. In addition, we described the kinetics which lead to the formation of nitrosative species by "free" NO_2+NO_3-CLA, as compared to those stemming after lipase hydrolysis of NO_2+NO_3-CLA-TAG to yield NO_2-CLA. Finally, a synthetic chemical route for NO_2+NO_3-CLA derivatives have been formulated.

GOALS FOR 2019

After devising and executing organic synthesis strategies for gram amounts of pure free and esterified NO_2+NO_3-CLA standards, we will define the digestive and inflammatory-mediated mechanisms that lead to the concerted placement of both nitro (R-NO_2) and nitrate ester (R-ONO_2) substituents on an unsaturated free fatty acid and TAG. The Research Plan will then a) evaluate the metabolism of NO_2+NO_3-CLA derivatives and b) the *in vitro* and *in vivo* responses to NO_2+NO_3-CLA, with focus on to the potential for this class of mediators to mediate both cGMP-dependent and cGMP-independent signaling.
Current preclinical and clinical data supports that oral NO_2-FA administration induces salutary gene expression responses and can mitigate pathogenic events induced by obesity and inflammation. We envision that NO_2+NO_3-CLA derivatives may also be precursors of the NO_2-FA already detected in rodents, humans and plants.

INTELLECTUAL PROPERTY

PCT Application No. PCT/US2017/055154. Title: Novel reversible nitroxide derivatives of nitroalkenes that mediate nitrosating and alkylating reactions. Filed: Oct-04, 2017. Inventors: **Fazzari M.**, Schopfer F. and Freeman B.

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) at the base of tumour pathologies. The rational design of the molecules provides for different approaches including the creation of *In Silico* models created on the target proteins, object of our study, and their validation in a retrospective way.
These models are used for virtual screening and molecular modeling in order to identify potential Hit compounds, through computational techniques and to guide chemical synthesis towards compounds that go from a hit compound profile to a lead compound profile.

IMPACT

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

RESULTS ACHIEVED IN 2018

During 2018 several computational models were developed that could serve for the rational design of molecules with inhibitory activity on the target. In particular, we focused on the use of molecular docking algorithms, Structure- and Ligand-based pharmacophores and cheminformatics approaches based on the use of molecular fingerprints.
The retrospective validation of the computational models and their application in the virtual screening campaigns on commercial libraries allowed the identification of 3 molecules hit that on the preliminary biochemical assays showed uM activity on KDM4. The same molecules have also produced interesting results from the point of view of the inhibition of cell proliferation on HCT-116 cells. Based on the results obtained from screening, we have moved on to design structural analogues of the hit series with the aim of improving their activity profile.

GOALS FOR 2019

For 2019 the goal of the project is the design and synthesis of in-house molecules from which a hit will be chosen with activity in the low range uM with good toxicity profile and selectivity towards the KDM4A compared to the other KDMs to be further developed optimizing its profile as lead compound.
Particular attention will be focused on the modulation activity of the two main forms of demethylases involved in the tumour pathology under study (KDM4A and KDM4C).
The activity of the molecule will be evaluated both from the biological point of view with enzymatic assays (on the isoforms A and C of the enzyme) and cellular assays and biophysical techniques (ITC, NMR) that will elucidate the portions mostly involved in the host interaction guest. This will also allow the further elucidation of the binding mode and the subsequent validation of the *Silico* models used for the prediction of the molecular binding mode.

X-ray of QC6352 Inhibitor bound to the binding site of KDM4A

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

The regenerative medicine and research & development of biological therapies laboratories are focused on the development of new cellular therapies for terminal organ diseases and post-transplant complications, as well as on the validation of new vaccine strategies for infectious diseases. The laboratory is strategically located at the IRCCS ISMETT and the team is made up of researchers and technical staff specialized in research and development activities (*in vitro*, *in vivo* and *first-in-man* studies) and in the manipulation of biological samples of human origin. The team has been trained to operate according to Good Manufacturing Practice (GMP) and Good Clinical Practice (GCP) for the designing and execution of preclinical / clinical trials and the production of advanced therapies.

The projects undergoing preclinical phase are aimed at developing cellular products for tissue repair and / or regeneration and for the development of organotypic cultures to be used both 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. The new generation of vaccines, made up of re-combined proteins, is aimed at the treatment of hospital-acquired infections of different etiology.

Nk cell mediated therapy in cancer and chronic infection
Ester Badami, PhD

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

Secretome of mesenchymal stromal cells (MSCs): toward a cell-free option for Regenerative Medicine
Cinzia Chinnici, PhD

Function of globins and effect of nitrite on zebrafish heart regeneration and embryonic development
Paola Corti, PhD

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

Development of a vaccine against HIV 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

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

Effects of the cellular components of ascites on the phenotype of cells derived from human placenta
Mariangela Pampalone

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

Nk cell mediated therapy in cancer and chronic infection

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COLLABORATIONS

Istituto Zooprofilattico Sicilia IZS, Palermo, Italy

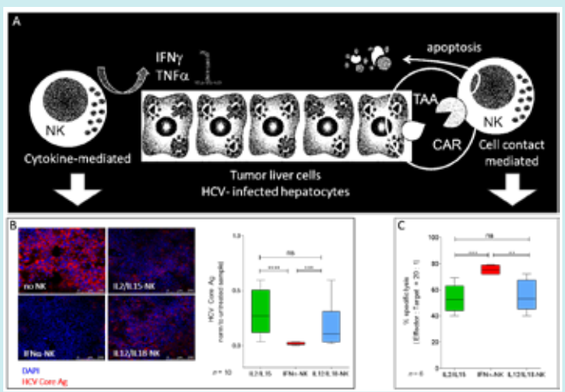
THERAPEUTIC AREA

Cancer

Organ Insufficiencies

PIPELINE

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



BRIEF DESCRIPTION

This study concerns a method for the isolation of a large number of readily available activated CD3-CD56+ NK cells for the treatment of Hepatitis C infection (HCV) and/or prevention of post-liver transplant HCV reinfection, or for use for the treatment and/or prevention of post-liver transplant Hepatocellular Carcinoma (HCC) recurrence.

Natural Killer (NK) cells perceive the presence of somatic cells altered by infections and tumors by adopting two mechanisms: cytokine release or apoptosis induction through cellular contact (A). It is possible to increase the function of NK cells with cytokines (e.g. IFNa rather than IL2 / IL15 or IL12 / IL18), either mediated by soluble factors (B) or by cellular contact (C). NKs can be engineered to express receptors for tumor antigens, thereby increasing the specificity for target cells (CARs).

IMPACT

Hepatocellular carcinoma (HCC) is currently the fifth most common malignancy and the second cause of mortality in male adult cancer population. In approximately 80% of cases, HCC is associated with an hepatitis C (HCV) chronic infection. HCV mainly infects human hepatocytes, determining a chronic liver infection that causes cirrhosis, liver decompensation, hepatocarcinoma, and liver dysfunction. HCV causes chronic infection in 60-80% of patients as the virus evades immune defenses impairing the function of the cells involved in the innate and adaptive immune response.
To date, an anti-HCV vaccine is not available. Important goals for the treatment of HCV infection were achieved thanks to new direct-acting antiviral drugs. However, there is currently no information on the side effects of long-term treatment such as, for example, the onset of drug resistance-associated substitutions (RASs), the effects on the liver tumor in HCC patients with associated HCV infection or the extra-hepatic side effects. Finally, the costs of the treatment are still extremely high, and therefore not affordable by all categories of infected patients. HCC and HCV are indications to liver transplant. However, the recurrence of post-transplant HCV occurs in almost all recipients who are not treated pharmaceutically, whereas HCC recurrence occurs in 10-60% of patients 1-2 years after the transplant.

Timely treatment upon the liver procurement could improve the prevention of post-transplant short- and long-term HCC recurrence. Natural Killer (NK) cells are key players in the initial response to cancer and viral infections. For dozens of years NK cells have been used in anti-tumor adoptive immunotherapy both in auto- and allo-transplantation. The possibility of using NK cells to prevent post-transplant HCC and HCV recurrence is currently the subject of research.

The underlying hypothesis of this study is that the infusion at the time of the transplant of NK cells isolated in the healthy donors in the liver transplant recipient can boost his/her immune system compromised by the anti-rejection therapy. Moreover, it is known that patients with chronic HCV infection and/or liver tumor have an impaired immune system. Therefore, restoring the recipient's immune defenses with a healthy donor's NK cells becomes interesting. Recently it has been shown that HCV patients treated with DAAs develop HCC. Combination therapy of DAAs with NK cells should prevent development of HCC.

RESULTS ACHIEVED IN 2018

Preliminary *in vitro* data have demonstrated that it is possible to efficiently condition NK cells to increase their anti-tumor and anti-viral function. NK cell activation with cytokines [IL2 + IL15] e [IFNα] rather than the mix of cytokines [IL2 + IL15] and [IL12 + IL18] or just the combo [IL2 + IL15], activates NK cells consistently and significantly enhancing NK anti-HCV and anti-HCC immune response. To address the mechanisms of cellular activation responsible for the enhanced NK cell immune response driven by IFNα, we identified the following pathways:

1) by studying the miRNome signature, we found two miRNAs differently expressed by NK cells activated with [IFNα]: hsa-miR-1304-3p and hsa-miR-181a-3p
2) soluble factors majorly released by IFNα-NK were identified in the protein soluble TRAIL
3) by antibody blocking assay, we could exclude that the soluble factors responsible for the enhanced anti-tumor and anti-viral function of NK cells after IFNα activation were type I Interferons.

GOALS FOR 2019

In vivo proof-of-concept of the anti-tumor function of NK cells activated with either IFNα or Il12-IL18. A mouse model of hepatocellular carcinoma will be established. Immunocompromised NOG mice will be orthotopically engrafted with a human cell line of liver tumor, then will be treated with either IFNα- or IL12-IL18-activated human NK cells and tumor growth will be traced on live animals by Magnetic Resonance Imaging (MRI) using the 7Tesla Bruker instrument available at the *Istituto Zooprofilattico Sicilia* (IZS).
Functional validation of the miRNAs of interest hsa-miR-1304-3p and hsa-miR-181a-3p.
Identification of the soluble factors involved in the anti-tumor and anti-viral function of IFNα-activated NK cells.

MEETINGS

The Society for natural Immunity annual meeting "NK2018", May, 2018, San Antonio, Texas, USA .

European Congress of Immunology ECI2018, September, 2018, Amsterdam, Netherlands .

PUBLICATIONS

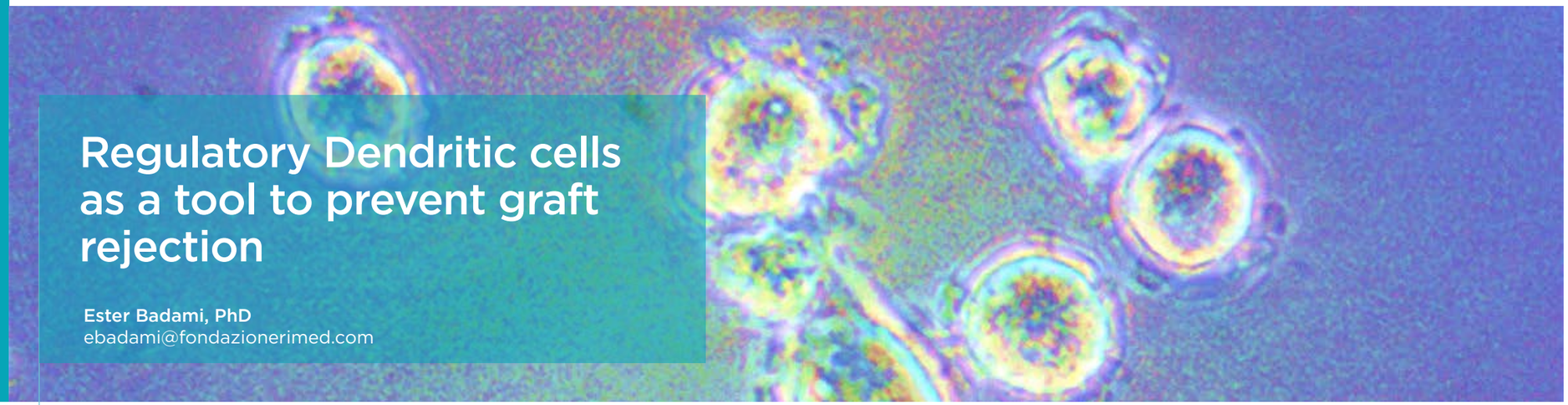
Gallo A, Miele M, **Badami E**, Conaldi PG.(2018) Molecular and cellular interplay in virus-induced tumors in solid organ recipients. Cell Immunol., DOI: 10.1016/j.cellimm.2018.02.010.

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. (2018) Liver Perfusate Natural Killer Cells from Deceased Brain Donors and Association with Acute Cellular Rejection after Liver Transplantation: a Time-To-Rejection Analysis. Transplantation. DOI: 10.1097/TP.0000000000002322.

INTELLECTUAL PROPERTY

International patent number WO 2018/099988, 15 February 2018, "NK cell 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

Starzl Institute, University of Pittsburgh, U.S.A.

THERAPEUTIC AREA

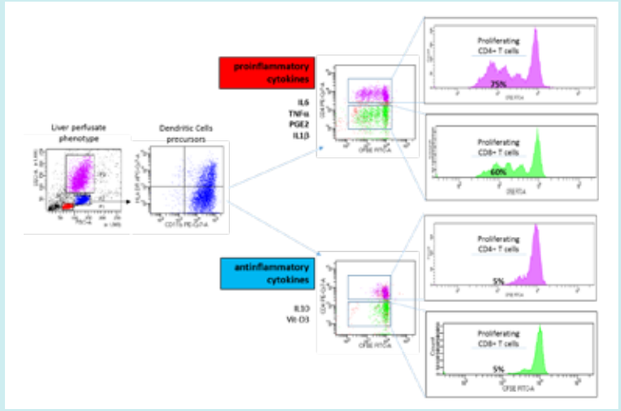
Organ Insufficiencies

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.



Liver perfusate is rich in Dendritic cell precursors (DCs), which can be manipulated *in vitro* to obtain the desired immune response: culture with inflammatory cytokines induces a proliferative response of T lymphoctytes CD4+ and CD8+ (upper righ). On the contrary, maturation of DCs in the presence of IL10 and VitD3 has the opposite effect and lymphocytic stasis (lower righ).

IMPACT

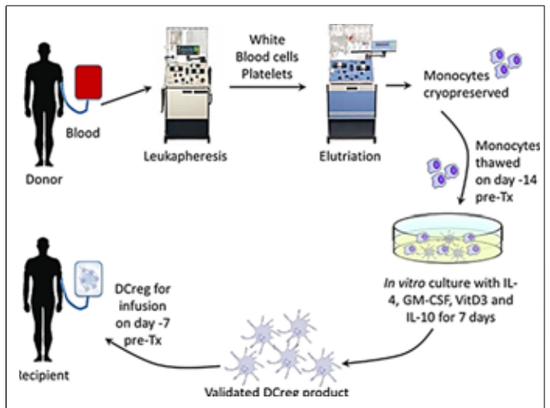
Patients who undergo solid organ transplant require lifelong immunosuppression to prevent organ rejection. Immunosuppressive therapy are associated with life-threatening side effects such as infection, malignancy, diabetes, cardiovascular disease and renal failure. In organ transplantation, the ideal 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 2018

This project has been recently started. First of all, it was necessary to demonstrate the liver perfusate was a good alternative starting biologic material for the isolation of tolerogenic dendritic cells or their precursors. The phenotype analysis therefore was conducted on the liver perfusate isolated from 20 deceased donors. This study demonstrated that there was a discrete population of CD14+ monocytes, being the precursors of DCs. More difficult was to demonstrate the presence of differentiated dendritic cells with a tolerogenic function as these cells appeared to be very rare in the liver perfusate. Ideally the possibility to isolate ready-made tolerogenic DCs already differentiated in the perfusate at the transplant time point, would have been of benefit as would have offered the opportunity to treat liver recipient few hours post-transplant. However, the presence of DCs precursors is of interest as the Pittsburgh lab has demonstrated in pre-clinical trials the efficacy of Tol-DCs treatment even after days post-transplant.

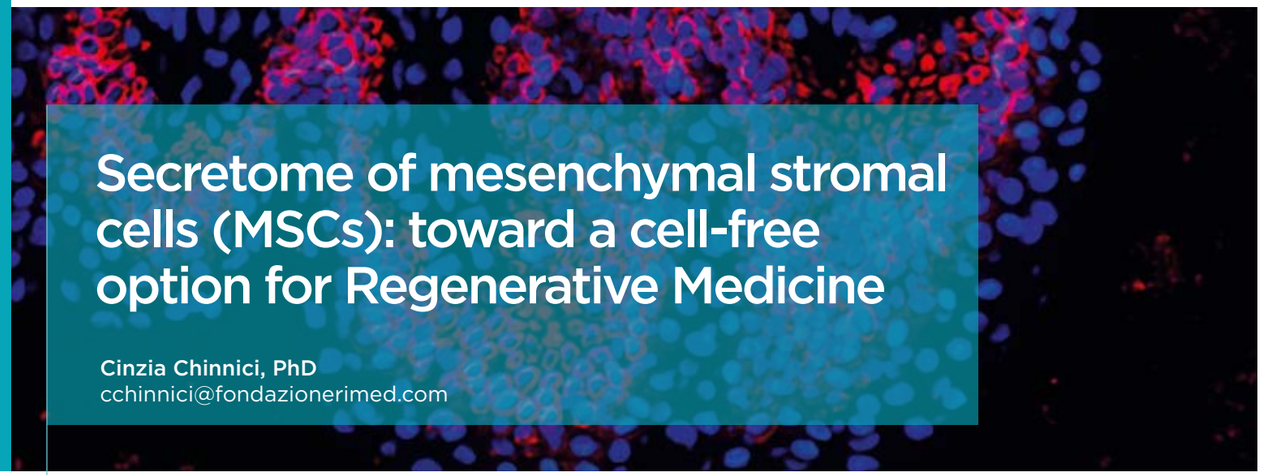
GOALS FOR 2019

Standardization of the protocol of Tol-DCs production starting from CD14+ monocyte precursors from the liver perfusate. Functional characterization of monocyte-derived tolerogenic DCs in *in vitro* assay wot hallo-reactive T lymphocytes. Optimization of the procedure of production of Tol-DCs according to ATMP standards for downstream clinical application. Application of the Pittsburgh protocol using as starting cellular source leukapheresis products such as peripheral blood from living donors with the aim of starting a synergic multicentric study between the United States and Italy.



Example of DCs mediated therapy for the prevention of rejection after solid organ transplantation from living donor. Precursors of DCs cells are obtained from the blood donor 2 weeks before transplantation. During the first week, we obtain the Tolerogenic DCs cells that are infused into the recipient a week before transplantation. This replaces the pharmacological immuno-suppressive therapy.

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



Secretome of mesenchymal stromal cells (MSCs): toward a cell-free option for Regenerative Medicine

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COLLABORATIONS

IRCCS-ISMETT, Palermo, ITA (Leading Partner)

Lab of Biocompatible Polymers, STEBICEF – University of Palermo, Palermo, Italy

THERAPEUTIC AREA

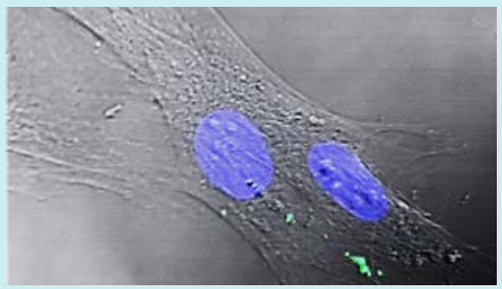
Organ Insufficiencies

PIPELINE

BRIEF DESCRIPTION

Secretome-based therapy holds the promise of "cell-free" advanced therapy for Regenerative Medicine to restore organ function. MSC secretome contains bioactive factors (including growth factors, chemokines, exosomes) with a role in tissue repair/regeneration. For its remarkable proangiogenic action, secretome of MSCs from umbilical cord seems particularly indicated for the treatment of diseases where angiogenesis is crucial, such as cardiovascular diseases, tissue repair/regeneration, and wound healing.

We are interested in evaluating the benefits of secretome-based therapy to restore function of liver and skin following tissue injury.
The goal is to compare two different administration routes of secretome (systemic administration in case of internal organs; topical administration in case of exposed organs). Whit respect to this, a central aspect of our studies is the use of biocompatible/biodegradable materials for a controlled release of secretome *in vivo*.



Bright field image merged with green (CSFE) and blue (DAPI) showing cytoplasmic localization of MSC-exosomes in target cells.

IMPACT

The use of "cell-free" products in Regenerative Medicine pose several advantages compared to conventional cell therapy, as could limit the potential risks associated with cell transplant, such as immunoreactions, tumorigenicity, or transmission of infections. Moreover, secretome can be prepared as a "ready-to-go" biological product, and produced as a drug for clinical applications.

RESULTS ACHIEVED IN 2018

A comparative analysis of secretome from MSCs of different origin (fetal dermis, fetal liver and umbilical cord) was conducted. The obtained conditioned medium (CM) was characterized for the presence of growth factors and chemokines with a role in angiogenesis/tissue repair/regeneration and wound healing. *In vitro* functional assays showed a remarkable proangiogenic effect of CM from umbilical cord-derived MSCs. Moreover, the analysis of extracellular vesicles isolated from CM showed presence of exosomes (70-100 nm size) which were active in inducing biological responses in target cells with high efficiency and in a dose-dependent manner. In addition, exosomes of fetal dermis-derived MSCs were analyzed for the presence of microRNAs (miRNAs) regulators of angiogenesis, which were significantly upregulated in fetal vs adult dermal MSCs (manuscript in preparation).
Concerning the studies to establish a suitable system for a controlled release of secretome, in collaboration with the lab of Biocompatible Polymers at STEBICEF, we found that hydrogels of hyaluronic acid derivatives allow a controlled release of our secretome (manuscript in preparation). These *in vitro* release kinetics studies will allow us to set the conditions for *in vivo* administration of secretome (topical application of hydrogel/secretome in animal models of non-healing skin wounds).
Concerning the recently approved preclinical study ("Safety and efficacy of human MSCs from fetal liver and their conditioned medium to restore organ function in a murine model of fulminant liver failure"), a pilot study was carried at the Istituto Zooprofilattico (July 2018) in which we reproduced a murine model of acute liver failure D-Gal/LPS-induced. The liver damage was evaluated through serum levels of transaminases AST and ALT, and histological examinations. In addition, a more extensive characterization of fetal liver-MSCs was conducted including *in vitro* functions of CM and analysis of exosomes (manuscript accepted).

GOALS FOR 2019

1) Conduct the preclinical study "Safety and efficacy of human MSCs from fetal liver and their conditioned medium to restore organ function in a murine model of fulminant liver failure". 2) Establish whether a method to obtain a functional secretome directly in "clinical grade" reagents is patentable or not. 3) Elaborate the study of biocompatible/biodegradable materials for a controlled release of secretome (e.g., microparticles loaded with secretome). 4) Finalizing a protocol of the preclinical study on topical application of hydrogel/secretome to treat non-healing skin wounds.

MEETINGS

Global Summit on Stem Cell &Tissue Engineering. Barcelona, 23, 24 July 2018.

Poster: Collection and preliminary *in vitro* characterization of a functional secretome from human multipotent fetal dermal cells (CM Chinnici, S Vella, G Amico, and PG Conaldi).

PUBLICATIONS

Chinnici CM, Pietrosi G, Iannolo G, Amico G, Cuscino N, Pagano V, Conaldi PG. Mesenchymal stromal cells from human fetal liver release growth factors and chemokines with a potential role in liver tissue repair. 2018 Dec 5;105:14-26. doi: 10.1016/j.diff.2018.12.001.

PRODUCTS: **BIOLOGICS**

Function of globins and effect of nitrite on zebrafish heart regeneration and embryonic development

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COLLABORATIONS

Institute of Biosciences and BioResources (IBBR) – CNR, Naples, Italy

Department of BioSciences - University of Milan, Italy

THERAPEUTIC AREA

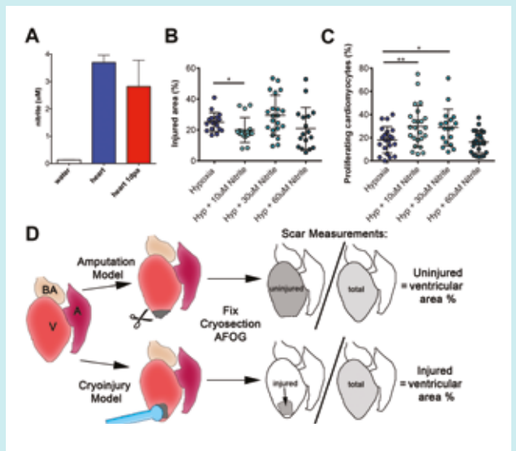
Organ Insufficiencies

PIPELINE

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

BRIEF DESCRIPTION

Recently new members of the vertebrate globin family, such as cytoglobin (Cygb) and globin X (Xgb), have been identified in vertebrates. While highly conserved, the function of these intracellular globins remains largely unknown. One described property of these globins is the ability to reduce nitrite to nitric oxide (NO) in conditions of low oxygen. Nitrite gained increased attention as important molecule for metabolic functions in humans due to its convertibility to nitric oxide (NO). This reaction is shown to be physiologically relevant during ischemic or hypoxic events and administration of nitrite has remarkable cytoprotective and anti-apoptotic effects. In the current proposal, I plan to use the zebrafish model to define the role of these globins and the effect of nitrite during zebrafish heart regeneration and embryonic development. The zebrafish is an established and increasingly important vertebrate model for studying embryonic development, organogenesis and adult physiology. Moreover, zebrafish can regenerate cardiac tissue after injury and therefore offer the unique opportunity to uncover the molecular mechanisms involved in heart repair. The goal of my research is to characterize how the heme proteins function in the heart and possibly mediate the effect of nitrite during cardiac regeneration.



Nitrite dose response effects on scar size and cardiomyocyte proliferation following cryoinjury. A. Levels of nitrite were measured using a nitric oxide analyzer (NOA). While there is almost no detectable nitrite in the water (0.14 µM), the endogenous nitrite concentration in the uninjured heart and in the amputated heart is 3.69 ± 0.43 µM and 2.82 ± 1.32 µM respectively. Following cryoinjury the injury size (B) and number of proliferating cardiomyocytes (C) were measured comparing hypoxia, hypoxia (hyp) + 10 µM nitrite, hyp + 30 µM nitrite, and hyp + 60 µM nitrite. The 10 µM nitrite dose significantly decreased both the scar size and increased the number of proliferating cardiomyocytes at 5 days post cryoinjury (dpc). Student's t-test: *P<0.05, **P<0.01. Data are expressed as mean ± SEM. D Schematic of amputation and cryoinjury heart regeneration models. Following fixation, cryosection and AFOG staining, scar measurements were calculated differently for each model. In amputated hearts the total uninjured area per the total ventricular area was calculated. In cryoinjured hearts the total injured area over the total ventricular area was calculated. V: ventricle, A: atrium, BA: bulbous arteriosus.

IMPACT

This research investigates the chemical biology, signaling and biological function of the globins and the effect of nitrite *in vivo*. For the first time this function and the nitrite effect will be studied in a model of zebrafish heart regeneration. I propose that these investigations will define the currently unknown function of the cellular heme globins, and reveal important insights into the control of the cardiac regenerative events.

The role of nitrite in zebrafish regeneration processes is unknown. Our work shows that under hypoxia, physiological levels of nitrite are able to improve the heart's ability to repair following injury in zebrafish amputation and cryoinjury models of heart regeneration. Nitrite enhances regeneration by modulating the immune response through NO signaling; faster recruitment of thrombocytes, neutrophils and macrophages, accelerates cardiomyocyte proliferation, angiogenesis and cardiac repair. Interventions that activate NO signaling pathways via nitrite or other mechanisms may provide an opportunity to improve outcomes in hypoxic conditions such as intrauterine fetal or neonatal cardiac surgery.

RESULTS ACHIEVED IN 2018

Our preliminary results show that nitrite exposure improved heart regeneration and increased cardiomyocyte proliferation. Globin X (Xgb) and cytoglobin 1 (Cygb1) are two globins candidate to utilize the nitrite and play a fundamental role in NO homeostasis in the fish blood and in the cardiac muscle. In order to understand the role of Xgb and Cygb1 in cardiac regeneration, using CRISPR/Cas9, we generated zebrafish knock-out mutants. We have discovered that Xgb, the most ancestral globin, is a potent nitrite reductase and displays anticoagulant properties on human platelets in the presence of nitrite. The nitrite reductase role of Xgb in blood during the zebrafish heart regeneration is now under investigation. Zebrafish Cygb1 is a globin that may mediate the cardiac regenerative response to nitrite.

We have studied cytoglobin expression in zebrafish heart by *in situ* hybridization and immunohistochemistry. Our data show that Cygb1 is expressed in the epicardium and its expression is increased following cardiac injury. Cygb1 may be mediating this response via its fast nitrite reductase rate (28.6 ± 3.1 M-1s-1) in its deoxy state or oxy-Cygb1 (oxygen P50 is 0.277 torr) may react with nitrite to produce reactive oxygen and nitrogen species (ROS/RNS), modulating the immune response via ROS/RNS signaling.

This project was awarded the **"AHA Career Development Award " – PI: Paola Corti. (2018-2021)**.

GOALS FOR 2019

Other globins such as neuroglobin, myoglobin and cytoglobin 2 are expressed in various tissues and have unidentified functions. Other than their ability to bind oxygen and act as oxygen storage or delivery proteins, their physiological function became uncertain based on new evidences recently described in literature.

Our goal is to understand the function of globins *in vivo*, including neuroglobin, cytoglobin 1, cytoglobin 2, globin X, myoglobin.

We will generate stable mutant lines using CRISPr/Cas9 for all of these globins and will carry out experiments on heart regeneration and embryonic development.

Using a combination of tools including transgenic lines and cardiomyocyte cultures we will test the globins ability to reduce nitrite to nitric oxide and we will determine the consequences on the regenerating process and on embryonic development.

We will also test the possible contribution of nitrite to ROS/RNS generation by the action of oxidized globins and we will study the effect on immune system response. Nitrite is a non-toxic compound at low dose, easily available and recently found to be cytoprotective in ipoxic/ischemic environment.

Understanding how we can modulate these protective mechanisms and possibly enhance cardiac regeneration based on globins function in presence of nitrite will be of great importance for therapeutic applications.

MEETINGS

Rochon E, Xue J, Tejero J, Gladwin M, and **Corti P**. Nitrite improves zebrafish cardiac regeneration potentially mediated by Cytoglobin 1. 13th International Zebrafish Conference, Madison WI (USA), June, 2018.

Rochon E, Xue J, **Corti P** and Gladwin M. Nitrite treatment in hypoxia improves zebrafish cardiac and fin regeneration. American Heart Association Conference, Chicago IL (USA), November, 2018.

Invited lecture. O2BiP Oxygen Binding Protein Conference, Barcelona (Spain), September 3-6, 2018.

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
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Azienda Ospedaliera Universitaria Careggi, Firenze, 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 compete with *K. pneumoniae* for adhesion to cells and mucosal surfaces, 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.

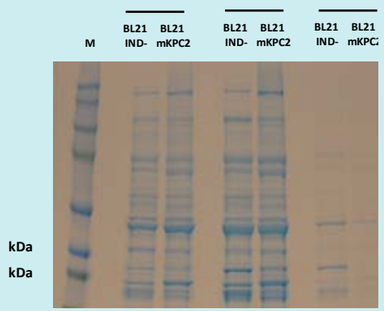
To date, no vaccine is available against *K. pneumoniae*. We believe that probiotic strains of *S. cerevisiae* may be an ideal immunization strategy against pathogens that require an immune response at the mucosal level to prevent infection, for example, gastrointestinal bacteria or HIV. In the specific case of *K. pneumoniae*, the use of genetically engineered probiotic strains to express proteins involved in the adhesion processes of this bacterium would prevent the colonization of this pathogen, through competition for the binding with the cellular receptors used by *K. pneumoniae* to form biofilms on intestinal cells 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*.

RESULTS ACHIEVED IN 2018

During this year, we have focused on the cloning, expression and purification of recombinant proteins in *Escherichia coli*, which would be used to assess and to compare the efficacy of the probiotic yeasts approach regarding a vaccine against *K. pneumoniae*. We cloned eight bacterial proteins, most of them surface proteins, which makes the best antigens. Cloned proteins were fimbrial proteins MrkA, MrkD, fimA and fimH, KPC-2, 01507, 01508 and cuxinase. However, all proteins but for KPC-2 were insoluble, even when co-expressed with GST-tag, which usually renders proteins soluble. KPC-2 purification, on the other hand, led always to a dirty extract, i.e., we were never able to obtain a pure, clean protein free of other cellular debris. Therefore, we abandoned the recombinant protein strategy and started focusing on the recombinant yeast protocol instead.

GOALS FOR 2019

Our goals to 2019 are to clone the aforementioned proteins in *S. cerevisiae*, thus obtaining genetically-engineered fungal strains which express, on their surface, those proteins. Once they are available, we will assess their expression levels by routine immunogenicity techniques, such as flow cytometry and Western blot, to see if the yeast-produced proteins are recognized by the antibodies present in the sera of patients who suffered a *K. pneumoniae* infection. We will also observe if the recombinant yeasts have the potential to interfere with common bacterial traits, such as formation of abiotic biofilms and cellular adhesion and adhesion competition assays. These assays will allow us to have an idea of the potential of the recombinant *S. cerevisiae* expressing *K. pneumoniae* to mimic bacterial functions, which might reflect in an immunogenic profile.



Purification of carbapenemase KPC-2 expressed in E. coli

MEETINGS

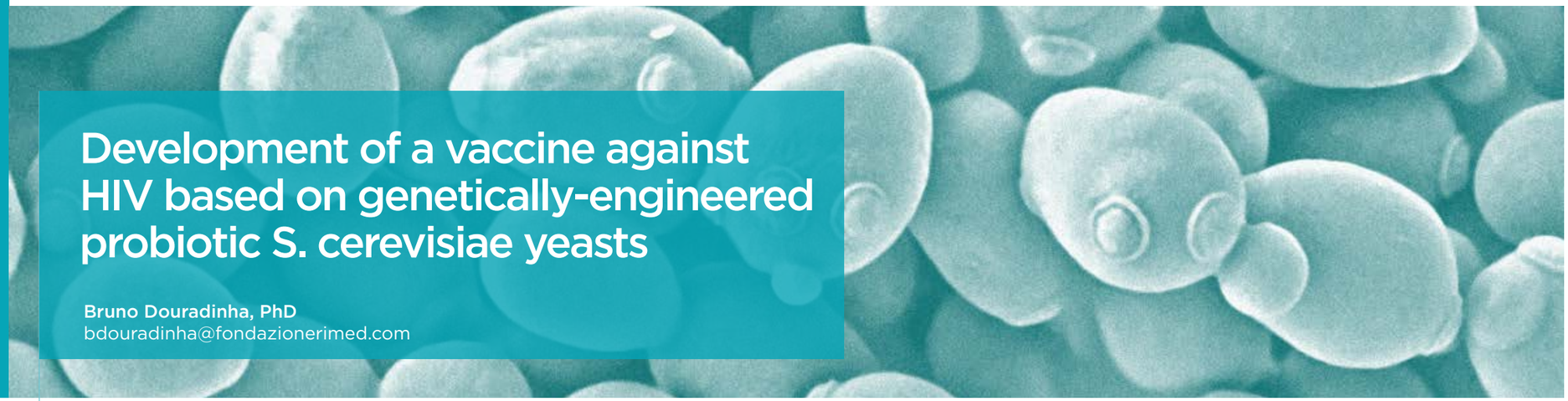
28th European Congress of Clinical Microbiology and Infectious Diseases, April, 2018, Madrid (Spain)

Ri.MED Research Retreat, October, 2018, Palermo (Italy)

PUBLICATIONS

Di Mento G, Cuscino N, Carcione C, Cardinale F, Conaldi PG e **Douradinha B**. (2018). Emergence of a *Klebsiella pneumoniae* ST392 clone harbouring KPC-3 in an Italian transplantation hospital. Journal of Hospital Infection, 98(3), 313-314. doi: 10.1016/j.jhin.2017.11.019

PRODUCTS: **BIOLOGICS**



Development of a vaccine against HIV based on genetically-engineered probiotic S. cerevisiae yeasts

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COLLABORATIONS

University of Pittsburgh (Pitt), Pittsburgh, PA, USA

THERAPEUTIC AREA

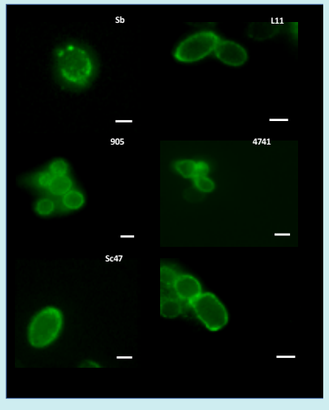
Infectious diseases

PIPELINE

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

BRIEF DESCRIPTION

So far, no vaccine is available against HIV. The use of genetically engineered *S. cerevisiae* strains expressing HIV antigen HIV GAG induced a potent cellular immune response in mice. However, a response against HIV must elicit both cellular and humoral arms of the immune system and also a mucosal immune response, since this virus can be transmitted sexually through mucosal surfaces. While laboratorial *S. cerevisiae* strains confer a weak mucosal response, their probiotic counterparts induce a potent sIgA secretion in the colon and have immunomodulatory properties. Interestingly, different probiotic *S. cerevisiae* strains were shown to induce diverse types of immune response. In this work, we genetically modified probiotic strains of *S. cerevisiae* to express in their surface the HIV Gag antigen to be further used as a vaccination approach against this viral pathogen.



Genetically engineered *S. cerevisiae* strains express HIV Gag antigen.
Expression of HIV Gag was determined by immunofluorescence and it was observed only in pJRP-transformed strains.
Bar 10 µm.

IMPACT

Novel approaches are still needed for an HIV vaccine. Since the main route of infection for HIV-1 is through mucosal surfaces, proper elimination of HIV-1 requires immune activation of both humoral and mucosal responses, in addition to cellular immunity. While laboratorial *S. cerevisiae* strains confer a weak mucosal response, their probiotic counterparts induce a potent secretory Immunoglobulin A (sIgA) release into the gut lumen and have immunomodulatory properties.
Interestingly, different probiotic *S. cerevisiae* strains were shown to induce diverse types of immune responses in the gut. Our results showed that yeasts were eagerly phagocytosed by human dendritic cells (DCs) and were able to induce maturation of these immune cells and to induce their polarization into a type 1 immune response.
Only HIV Gag-expressing probiotic strains were able to induce specific T cell immune response against HIV Gag *in vitro*, using DCs and T cells derived from an HIV-1+ donor. We strongly believe that our results reveal the potential of genetically engineered probiotic *S. cerevisiae* strains as novel vaccination vectors against HIV. This approach has also the advantage of using these yeasts in their lyophilized form, which would be an excellent alternative in developing countries lacking efficient health facilities or with unreliable access to electricity and/or refrigerated storage equipment.

RESULTS ACHIEVED IN 2018

During this year, we confirmed that genetically-engineered yeasts expressing HIV Gag viral antigen were definitely polarizing human DCs into a type 1 immune response, as shown by the surface expression of Siglec-1. Activation and maturation of yeasts was shown both by the expression of the surface molecules CD86, CD83 and CCR7, and by an increase in the levels of IFN-γ, IL-10, IL-12p70, IL-1β, IL-6, IL-8 and TNF-α was observed in the growth medium. This data strengthens the hypothesis that these genetically-engineered probiotic *S. cerevisiae* yeasts are a potential vaccination strategy against HIV due to their proven immunomodulatory abilities, coupled with the results obtained earlier *in vitro* and *ex vivo* using the DCs of an HIV+ patient.

GOALS FOR 2019

Our goals to 2019 are, according to the available funding, to repeat those experiments using DCs of more HIV+ patients to confirm the previous results. We also plan to use humanized mice which allow replication of HIV and thus would be a good model to study not only the immunogenic potential of this vaccination strategy but also to assess its protection levels, by immunizing mice with the yeasts and later challenging them with viable HIV viral particles. With this model, we can study which cell populations are activated during immunization with the probiotic *S. cerevisiae*, as well as induce a mucosal immunity and assess its effects on a mucosal challenge with HIV. This animal model will provide useful information that can be crucial to decide whether or not we should move to clinical studies involving human volunteers.

MEETINGS

12th Vaccine Elsevier Congress, September, 2018, Budapest (Ungheria)

INTELLECTUAL PROPERTY

n. WO/2018/091637, 24/05/2018, Probiotic yeasts as novel vaccination vectors.

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 2018

During this year, we assessed and characterized retrospectively the epidemiological scenery from 2011 to 2017 of carbapenem-resistant *Klebsiella pneumoniae* strains at IRCCS-ISMETT. One hundred sixty-one carbapenem-resistant strains have been isolated from patients and genetic material submitted to next generation sequencing to determine their origin, ST and detect the presence of potential resistance and virulence genes. We observed that the vast majority of the resistant strains were *K. pneumoniae* (86,3%), almost all of them KPC-producers (99,3%). In agreement with the current Italian epidemiological scenery, we have identified strains from ST512 clade II, ST258, ST101 and ST307, but also of STs not previously observed in other Italian health settings, such as ST392. The presence of carbapenemase genes involved in resistance, such as blaKPC-3, blaOXA-1, among others, have been also detected. Our work highlights that resistance genes are rapidly disseminating between different and novel *K. pneumoniae* clones which, combined with resistance to multiple antibiotics, can derive into more aggressive multidrug-resistant strains of clinical importance. Our results stresses the importance of continuous surveillance of carbapenem-resistant Enterobacteriaceae in health facilities so that novel STs carrying resistance genes that may become increasingly pathogenic can be rapidly identified and adequate therapies promptly adopted.

***K. pneumoniae* isolates with a particular carbapenemase gene**

genes[a]	2011	2012	2013	2014	2015	2016	2017	Total
$bla_{CTX\text{-}M\text{-}15}$	-	3	8	6	5	10	4	36
$bla_{KPC\text{-}3}$	4	16	18	15	22	22	28	**125**
$bla_{OXA\text{-}1}$	-	-	-	9	4	8	10	31
$bla_{OXA\text{-}48}$	-	-	-	-	-	-	1	1
$bla_{SHV\text{-}1}$	-	-	1	1	-	1	1	4
$bla_{SHV\text{-}11}$	4	13	9	6	15	13	26	**86**
$bla_{SHV\text{-}27}$	-	-	1	-	1	-	-	2
$bla_{SHV\text{-}28}$	-	2	7	2	3	9	2	25
$bla_{SHV\text{-}38}$	-	-	-	-	-	1	-	1
$bla_{SHV\text{-}76}$	-	-	-	3	-	-	-	3
$bla_{TEM\text{-}1}$	-	3	10	11	9	14	4	**51**

GOALS FOR 2019

Our goals to 2019 are to finish and publish the retrospective surveillance data described above, by sequencing more clinical isolates from both colonization and infection cases from 2008 to 2011, since 2008 was the year that a first *Klebsiella pneumoniae* carbapenemase-resistant was identified at IRCCS-ISMETT. We intend to give a full panoramic view of the KPC strains that were present in our clinical partner from 2008 to 2017 and to understand which ones followed the national and international epidemiological trends and which ones, such as ST392, were particular cases identified just at IRCCS-ISMETT.
In parallel, we will also, in strict collaboration with IRCCS-ISMETT Diagnostics unit, characterize any potential novel infection cases that might occur, such as a recent identification of Mycobacterium saskatchewanense, a non-tuberculous mycobacterium so far only isolated in North America.

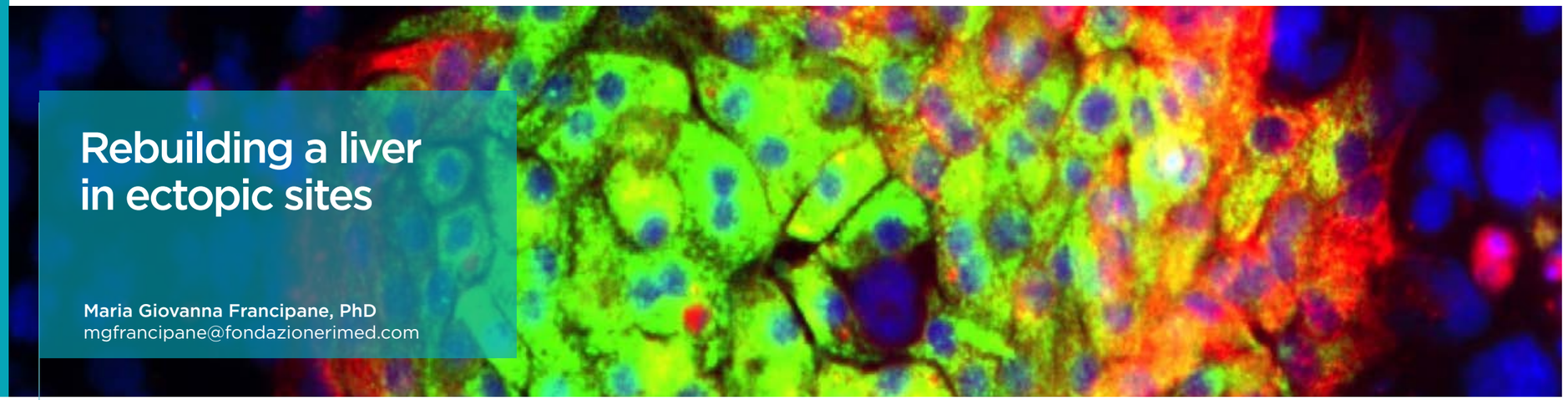
MEETINGS

- 28th European Congress of Clinical Microbiology and Infectious Diseases, April, 2018, Madrid, Spain.
- Ri.MED Research Retreat, October, 2018, Palermo, Italy.

PUBLICATIONS

Di Mento G, Cuscino N, Carcione C, Cardinale F, Conaldi PG e **Douradinha B**. (2018). Emergence of a *Klebsiella pneumoniae* ST392 clone harbouring KPC-3 in an Italian transplantation hospital. Journal of Hospital Infection, 98(3), 313-314. doi: 10.1016/j.jhin.2017.11.019.

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



Rebuilding a liver in ectopic sites

Maria Giovanna Francipane, PhD
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COLLABORATIONS

LyGenesis, Inc., Pittsburgh, U.S.A.

Mayo Clinic, Rochester, Minnesota, U.S.A.

THERAPEUTIC AREA

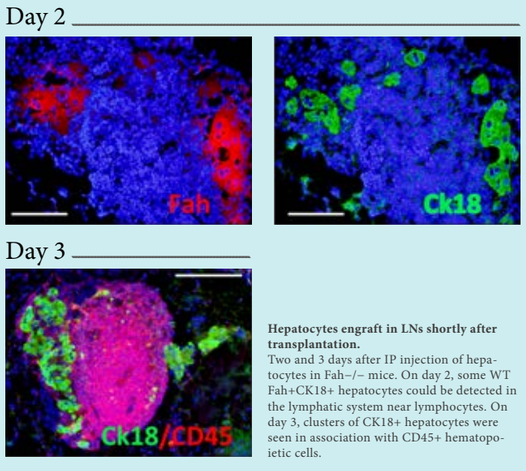
Organ Insufficiencies

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 for patients with end stage liver disease. 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 have been encouraging and LyGenesis is in discussion with the FDA (US Food and Drug Administration) to complete a final IND (Investigational New Drug)-enabling preclinical study, which is required to initiate the human clinical trial. Other therapeutic targets for organ regeneration include the thymus, pancreas, and kidney.



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 worldwide 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 transplantation of corrected cells could provide a healthier milieu to enable hepatocyte engraftment and proliferation. LNs are one such alternative site due to several defining characteristics. Depending on the stage of the disease, the rebuilding of liver inside the LNs could serve as a bridge to transplant, 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 for transplantation of donor cells, which grow and become a functioning ectopic organ.

RESULTS ACHIEVED IN 2018

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 showed 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 and we are currently identifying the molecular and cellular mechanisms permitting ectopic 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: The dog was chosen as a preclinical large animal model to explore the safety and tolerability of the LN-based therapeutic approach. In the laboratory, we were all trained for good manufacturing practice (GMP) and clinical-grade dog hepatocytes isolation. The first dog received hepatocyte transplantation in LNs through endoscopy mid-December. The dog survived the transplantation, demonstrating the safety of the procedure. Moreover, when the LNs were isolated and analyzed, engrafted hepatocytes could be identified.

GOALS FOR 2019

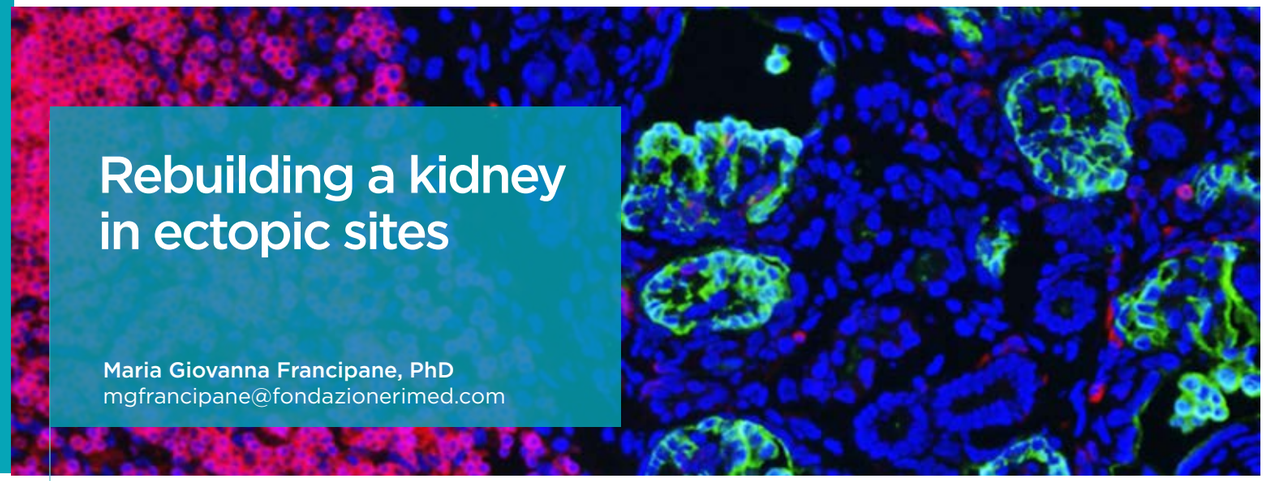
The Lagasse lab and LyGenesis will complete the dog study and submit an IND to the FDA. Programs for the thymus will also start. Preliminary data in our lab supports the idea that thymus transplantation into a LN could rejuvenate the immune system, and thus prevent aging related diseases.

PUBLICATIONS

Nicolas CT, Hickey RD, Allen KL, Du Z, Guthman RM, VanLith CJ, 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. *Under review.*

Han B, **Francipane MG**, Cheikhi A, Lagasse E. 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

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THERAPEUTIC AREA

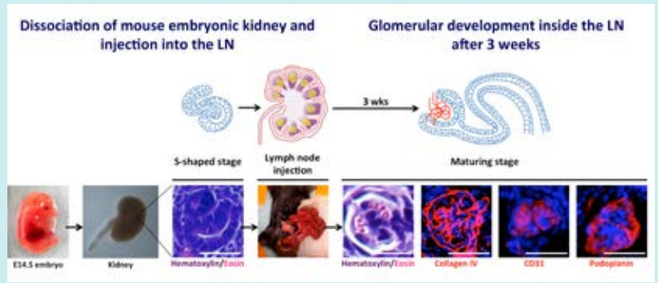
Organ Insufficiencies

PIPELINE

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

BRIEF DESCRIPTION

Chronic and end stage kidney disease impose a large burden on human health worldwide and projected prevalence trend lines are not comforting, suggesting an urgent need to explore new approaches overcoming limitations associated with traditional interventions. Dialysis —the most common method used to treat advanced kidney failure— can only replace 10-20% of kidney function. Orthotopic kidney transplantation offers better quality of life and longer survivals, yet is limited by organ shortage. Repopulation of decellularized kidney scaffolds to create bioengineered organs for transplantation have yet to prove effective for the treatment of patients with kidney failure.



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.

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 unpublished 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 2018

As LN development and homeostasis involve signaling through the lymphotoxin-β receptor (LTβR) in the stromal cell compartment, we hypothesized that host LTβR signaling also modulated angiogenesis in LN kidney grafts. To test our hypothesis, we treated graft-bearing mice with a recombinant LTβR-Fc fusion protein, which antagonizes LTβR-mediated effects by engaging LTβR ligands. LTβR knockout (LTβR-/-) mice were also used as transplant recipients. As LTβR-/- mice do not have LNs, we used omentum containing milky spots as an alternative transplantation site to LNs. In both lymphoid sites, defective LTβR signaling impaired angiogenesis, and expansion of the kidney implants. Surprisingly, the use of mice deficient for the Nuclear Factor kappa-B (NF-κB)-inducing kinase (NIK), which mediates non-canonical NF-κB signaling downstream of LTβR, indicated that NIK activation is not necessary for LTβR-mediated effects during ectopic kidney development. Furthermore, we showed that LTβR signaling participates also in adaptive responses to renal mass reduction, indicating a broader role for LTβR signaling in kidney regeneration. Collectively, our study indicated that in both the LN and omentum the LTβR pathway generates angiogenic cues to support kidney organogenesis.

Cell therapy using intra- or extrarenal stem cells has shown effectiveness in animal models, but this approach does not appear to be feasible in the clinical setting, due to the inflammation, fibrosis, and scar tissue that obstruct blood flow in the failing kidneys, creating an adverse environment for renal cell engraftment and function.
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.

GOALS FOR 2019

The LTβR-dependent modulation of ectopic kidney organogenesis is likely a result of multicellular processes. LTβR signals on LN fibroblastic reticular cells regulate LN Vascular Endothelial Growth Factor (VEGF) levels and, in turn, LN endothelial cell proliferation. Likewise, LTβR triggering on LN endothelial cells impacts vasculature development.
Cell type–specific deletion of LTβR will be pursued to unravel the contribution of each stromal cell population to kidney organogenesis.
Moreover, transplantation of renal organoids, which are devoid of immune and endothelial cells will facilitate our understanding of the fine-tuned symphony modulating kidney organogenesis in secondary lymphoid organs.

MEETINGS

- 2nd Annual Summit on Stem Cell Research, Cell & Gene Therapy: Bridging the Gap from Basic Cell Science to Advanced Cellular Therapies for a Better Life, November, 2018, Atlanta, GA, USA.
- ISSCR Annual Meeting, June, 2018, Melbourne, Australia
- Ri.MED Research Retreat, October, 2018, Palermo, Italy.

PUBLICATIONS

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. Under review.

M. G. Francipane, B. Han and E. Lagasse. Host lymphotoxin-beta receptor signaling is crucial for angiogenesis of metanephric tissue transplanted into lymph nodes and omenta. *Under review.*

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 Insufficiencies

PIPELINE

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

BRIEF DESCRIPTION

Infusion of specific T-cell lymphocytes is a valid and alternative therapeutic strategy to conventional anti-viral drugs for the treatment of virus-related complications in organ transplant recipients.
In order to increase the clinical potential of this cell therapy, we are developing innovative approaches in our research laboratories to generate and select specific multi-virus T clones. The clones of T lymphocytes, generated from blood samples from healthy donors, are activated *in vitro* against the Epstein-Barr virus (EBV), the Cytomegalovirus (CMV), Adenovirus (ADV), BK Poliomavirus (BKV) and Herpesvirus-8 (HHV-8) through the use of immunodominant viral peptide mixtures and interleukins. The creation of a bank of heterologous specific multi-virus T cells represents our final goal because it would guarantee the availability of a "ready to use" product. Therefore, T cells derived from a healthy donor to be administered to a patient, HLA compatible, at the time of diagnosis of virus-related post-transplant complications.

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) which 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 potentially lethal for the patient.

RESULTS ACHIEVED IN 2018

The first step of our study, in 2018, was the collection and evaluation of the most suitable cellular sources for the creation of the bank of specific multi-virus T lymphocytes. To this end, the yield and viability of cells from healthy donor blood and cord blood were analyzed. In order to optimize the production protocols of multi-virus-specific T clones, in the second step we evaluated different experimental approaches of production: a) different types of viral antigens presenting cells (dendritic cells, B cells and monocytes); b) the addition in the culture medium of different interleukins combinations to promote priming and cell proliferation. To evaluate the different protocols, the T lymphocytes produced were characterized *in vitro* at different time points in terms of immunophenotype and specific cytotoxic activity against viral antigens. We analyzed the release in the culture medium of some essential molecules for maintaining the cell-mediated response *in vivo* (eg. IFN-Y) and cell proliferation. The encouraging preliminary experimental data as how the feasibility to generate specific multi-virus T lymphocytes from both cell sources tested, but more efficiently from adult donor blood cells than from cord blood cells. More in detail, the protocol defined as "rapid", i.e. without the aid of dendritic cells, is the one that has shown the best efficacy *in vitro* in terms of potency and specificity.

GOALS FOR 2019

The main purpose of our study is to produce specific multivirus T lymphocytes for clinical use, following the Good Manufacturing Practices (GMP) guidelines. Activities for the next future are:
- to define the most suitable cellular source and production protocol, increasing the number of batches produced to confirm the experimental data obtained so far.
- To perform process validation and quality control methods validation to define purity and potency/specificity.

PUBLICATIONS

Gallo A, **Miele M**, Badami E, Conaldi PG. "Molecular and cellular interplay in virus-induced tumors in solid organ recipients." Cell Immunol. 2018 Feb 16. Https://doi.org/10.1016/j.cellimm.2018.02.010

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

Effects of the cellular components of ascites on the phenotype of cells derived from human placenta

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COLLABORATIONS

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THERAPEUTIC AREA

Organ Insufficiencies

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 O_2 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 TNF-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.

RESULTS ACHIEVED IN 2018

Preliminary experiments were conducted to evaluate the effect of ascitic fluid on mesenchymal and epithelial cells at different times. The results obtained have shown that both cell types in contact with the ascitic fluid do not show morphological variations, proliferation inhibitions, phenotypic variations or significant necrosis / apoptosis values. The leukocyte component of ascites from 12 patients was also determined. The dominant component of the fluid represented by monocytes (with a lower percentage of B lymphocytes and NK cells) has been characterized in terms of expression of activation markers (HLADR, TLR4) in absence or in co-culture with mesenchymal and epithelial cells from amnion placental to different times.

GOALS FOR 2019

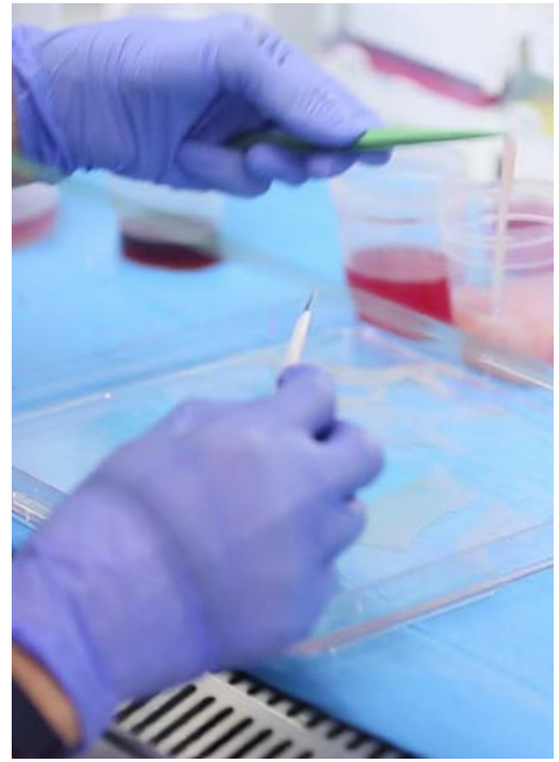
Study of the conditioned medium produced by the cells in co-culture with the monocytic component isolated from the ascitic fluid of cirrhotic patients for the evaluation of the expression of pro and anti-inflammatory secret cytokines.

Evaluation of bacterial load of ascitic fluid before and after co-culture of samples with hMSCs / hAECs with analysis of expression markers of activation and phagocytic capacity of macrophages.

Optimization of the mesenchymal and epithelial cell extraction protocol by human placenta amnion for ATMP development.

PUBLICATIONS

Miceli V, **Pampalone M**, Frazziano G, Grasso G, Rizzarelli E, Ricordi C, Casu A, Iannolo G, Conaldi PG
"Carnosine protects pancreatic beta cells and islets against oxidative stress damage" Mol Cell Endocrinol. 474: 105-118 (2018)



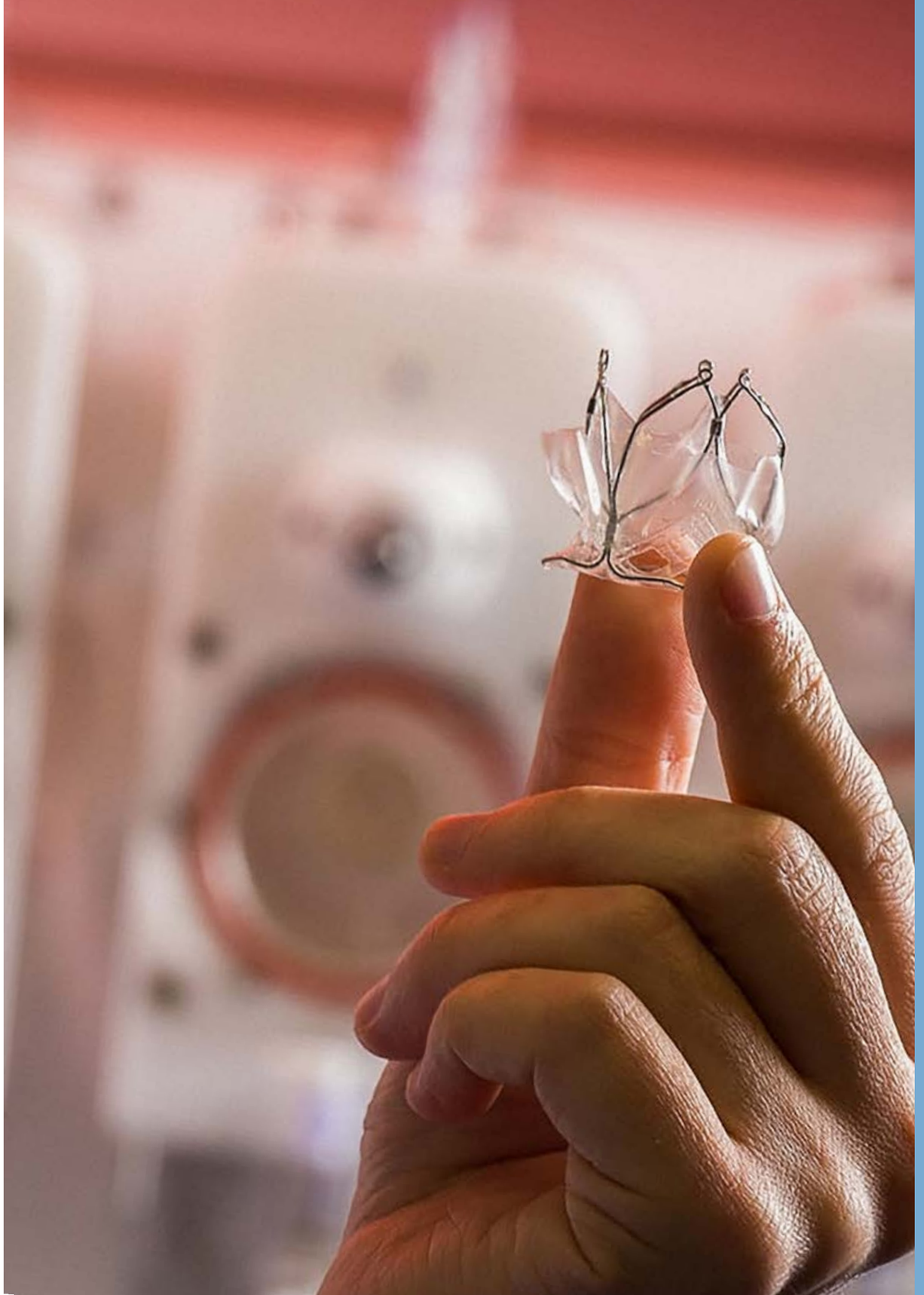
TISSUE ENGINEERING AND BIOMEDICAL DEVICES

The Bioengineering team of the Fondazione Ri.MED is composed of engineers, biologists, chemists and pharmacologists who work in close collaboration with doctors and surgeons. The research focus is on the study of biomaterials and engineered tissue, their rheological-mechanical characterization and the development of their clinical devices.

Ri.MED has created a platform based on instruments (both physical and computational) and equipment for conducting numerical simulations and experimental tests for the verification and qualification of structural and fluid-dynamic performance of the clinical solutions developed, in accordance with the regulations required for CE certification and FDA approval.

This platform allows the optimization of tissue engineered for different applications, particularly in the cardiovascular field, thanks to a better understanding of the effect of fluid-mechanical and structural stimuli on cellular differentiation and proliferation. The platform also allows the use of engineered tissue in design, development and preclinical validation of implantable cardiovascular organs and components of new generation.

The possibility of in-house development and validation of clinical solutions, together with collaborations with major clinical centers in the area, facilitate the introduction of patient-specific treatments while offering new support instruments in therapeutic planning and in pre-operative decision process.



Development of non-toxic 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

Prediction of the ischaemic lesions potential after heart valve therapy
Gaetano Burriesci, PhD

In vitro simulation of mitral valve therapies
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 Cardiac Patch (TECP)
Antonio D'Amore, PhD

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

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

3D osteochondral models to study degenerative disorders and therapies in microgravity
Riccardo Gottardi, PhD

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

In-silico modeling for clinical risk stratification of cardiovascular pathologies
Salvatore Pasta, PhD

PRODUCTS: **MEDICAL DEVICES**

Development of nontoxic bio-adhesives for wet environments

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COLLABORATIONS

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Department of Basic and Clinical Neuroscience – King's College London, London, UK

Department of Pharmacy – University of Salerno, Salerno, 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 adhesive biomaterials 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 regenerative medicine, biotechnology and material science. In particular, mussels have received significant attention especially because of their ability to adhere so 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 are synthesised in the mussel foot. Six mussel foot proteins (mfp) have been identified in the Mytilus genus (mfp-2, -3S, -3F, -4, -5 and -6). Mfp are thought to acquire their adhesive properties mainly through a post-translational modification that consists in the enzymatic hydroxylation of tyrosine to 3,4-dihydroxyphenyl-L-alanine (DOPA).
However, firm confirmation of these hypothesis is still lacking because of the insufficient characterization of the proteins especially in their non-DOPA bound form.

IMPACT

In the last years, there is a growing interest focusing on the development of novel naturally-derived glues in several areas of clinical applications such as tissue engineering, implantation of medical devices and wound closure. In fact, there are situations where other 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.

RESULTS ACHIEVED IN 2018

Three mfp were recently identified in the Asian green mussel *Perna viridis* foot by RNA-sequencing integrated with proteomic analysis: Pvfp-3α, Pvfp-5β and Pvfp-6. They have molecular weights ranging between 8-16.9 kDa. Saline-induced adhesive secretions from mussel foot of *P. viridis* showed that, among them, Pvfp-5β variant is secreted first and it was then hypothesized that this protein is the first protein to initiate interaction with the substrate. We performed the first implementation of the successful production of recombinant *Perna viridis* foot protein type 5β (Pvfp-5β) and its characterization by Circular Dichroism (CD), nuclear magnetic resonance (NMR), and Dynamic Light Scattering (DLS). We showed that the protein can be refolded in a semi-native form. We prove for the first time that Pvfp-5β, despite features which are not typical of a beta-sheet, folds as beta-rich protein. We also analysed in detail the aggregation and adhesion properties of Pvfp-5β as part of a longer term involvement aiming at the characterization of mfp-based biomaterial. Overall, our results show that Pvfp-5β has intrinsic adhesive properties also in the absence of DOPA attachment. These properties make the protein an excellent and efficient surface coating material which could be used in several biomedical applications including the regeneration of damaged tissues.

GOALS FOR 2019

Future studies want to address a detailed comparison of the properties of the native and the post-translationally modified *Perna viridis* proteins. The successful set up of a scheme for the recombinant production of Pvfp-5β obtained this year, gives us the possibility to optimize quickly the expression and purification conditions also for the other two members of the family: Pvfp-3α and Pvfp-6. This will allow us in the future to produce suitable quantities of the three proteins without the bottleneck of having to purify them directly from mussel. Recombinant production also provides higher flexibility to obtain the protein with and without post-translational modifications allowing us the biophysical characterization and the determination of the three-dimensional structure of the three recombinant proteins from *Perna viridis* mussels with and without DOPA modification.

MEETINGS

UK-Israel Synergy Symposium - Protein misfolding in ageing and neurodegeneration: from basic biology to drug development, March, 2018, London (UK).

PUBLICATIONS

Martínez-Lumbreras S, **Alfano C**, Evans NJ, Collins KM, Flanagan KA, Atkinson RA, Krysztofinska EM, Vydyanath A, Jackter J, Fixon-Owoo S, Camp AH, Isaacson RL. (2018) Structural and Functional Insights into Bacillus subtilis Sigma Factor Inhibitor, CsfB. Structure, 26(4):640-648. doi: 10.1016/j.str.2018.02.007.

Pecci A, Ragab I, Bozzi V, De Rocco D, Barozzi S, Giangregorio T, Ali H, Melazzini F, Sallam M, **Alfano C**, Pastore A, Balduini CL, Savoia A. (2018) Thrombopoietin mutation in congenital amegakaryocytic thrombocytopenia treatable with romiplostim. EMBO Mol Med. 10(1):63-75. doi: 10.15252/emmm.201708168.

Structural model of *Perna viridis* foot protein 5β (Pvfp5b) obtained by sequence homology with Notch ligand delta-like 1 protein (PDB ID: 4xbm).

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

Development of a Novel Transcatheter Heart Valve

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COLLABORATIONS

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THERAPEUTIC AREA

Organ Insufficiencies

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 with a self-expanding nitinol wireframe, polymeric leaflets and a sealing cuff, which 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 2018

The research previously performed had led to the design optimisation and manufacturing of a novel TAVI device. In the last year, an alternative polymer, already assessed in terms of biocompatibility and biostability, was considered for use in the prosthetic leaflets and sealing cuff. Mechanical characterisation of these polymers has confirmed its potential suitability for the application.

The manufacturing process for the leaflets and cuff creation was substantially revised and optimised, and a set of valve prototypes of average size (nominal size 26 mm) was successfully produced. *In vitro* tests have confirmed that the hydrodynamic performance of the device meets the requirements recommended in the international standard ISO 5840-3:2013. Durability *in vitro* tests have demonstrated that the proposed technology is suitable to guarantee the durability requirement for flexible leaflets prosthetic valves (200 million cycles). Funding to initiate the in animal chronic evaluation have now been successfully sourced. These will allow to perform essential tests to confirm the ability of the device to successfully operate in an *in vivo* environment for a period of 3 months.

GOALS FOR 2019

A set of valve prototypes and delivery systems, based on the developed technology, will be manufactured for the study. Hydrodynamic assessment will be performed in a hydro-mechanical cardiovascular pulse duplicator system to ensure that the valve prototypes meet the ISO 5840-3:2015 performance requirements.

Finally, preclinical *in vivo* evaluation will be performed by means of 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.

MEETINGS

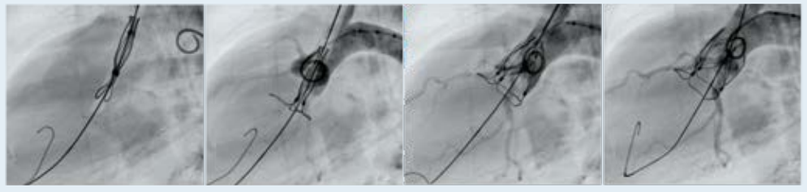
Ri.MED Research Retreat, October 2018, Palermo (Italy).

INTELLECTUAL PROPERTY

Burriesci, G., Bozkurt, S., Rahmani, B., Mullen, M.J. (2018) Prosthesis heart valve. Patent EP3310301 (A1).

Burriesci, G., Bozkurt, S., Rahmani, B., Mullen, M.J. (2018) Prosthesis heart valve. Patent EP3310301 (A1).

Picture of the transcatheter device



Sequence of implantation of the device

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

Development of a Novel Alfa-Gal Free Xenograft Heart Valve

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COLLABORATIONS

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University of Alabama, Birmingham, USA

THERAPEUTIC AREA

Organ Insufficiencies

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 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 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 (>60 years), where they are more durable. Patients under 60 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.

Valve prototype

RESULTS ACHIEVED IN 2018

All planned α1,3-galactosyltransferase gene-knockout pig pregnancies were successfully obtained by Prof McGregor's team. Sets of valves based on a manufacturing approach developed in house were manufactured, using wildtype and gene-knockout porcine pericardium. Prototypes were validated *in vitro* in terms of hydrodynamic performance and three samples per each valve group were selected for testing in an *in vitro* accelerated wear system.
All study valves met the hydrodynamic requirements from the ISO5840 and passed 30 million cycles of durability, meeting and exceeding the project milestone.

GOALS FOR 2019

The valves previously developed will be used to perform mitral valve replacement in juvenile sheep with 90-day survival. The study will aim at detecting the presence of unexpected risks associated with gene-knockout valves by comparing their performance with wild type valves after 90 day implantation in the ovine model.
Effective acceptable performance will be judged by comparison of mortality, haemodynamic valve function (effective orifice area, leaflet motion, regurgitation and transvalvular gradient pressure) measured by echocardiography cardiac thrombus deposition, pannus formation, inflammation, calcification and structural valve deterioration (determined by means of histological analysis).

MEETINGS

Rahmani, B., Salmonsmith, J., McGregor, C.G.A., Byrne, G.W., **Burriesci, G.** (2018) In Pursuit of a Durable Porcine Pericardial Bioprosthesis: A Proof of Concept Study. 8th Biennial Heart Valve Biology & Tissue Engineering Meeting, London, UK.

McGregor, C.G.A., Rahmani, B., Byrne, G.W., **Burriesci, G.** (2018) Physical and Hydrodynamic Equivalency of Wild Type and alpha-Gal Free GTKO Porcine Pericardium; A New Source Material For Bioprosthetic Heart Valves. Annual Scientific Meeting of the Heart Valve Society, New York, USA.

INTELLECTUAL PROPERTY

Burriesci, G., Rahmani, B., Byrne, G., Mc Gregor, C. (2018) Bioprosthetic heart valve. Patent n. WO2018011592 A1.

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

Analysis of the left atrial appendage to predict Thrombosis risk

Gaetano Burriesci, PhD
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COLLABORATIONS

University College London (UCL), London, UK

Great Ormond Street Hospital (GOSH), London, UK

Università degli Studi di Palermo, Palermo, Italy

THERAPEUTIC AREA

Organ Insufficiencies

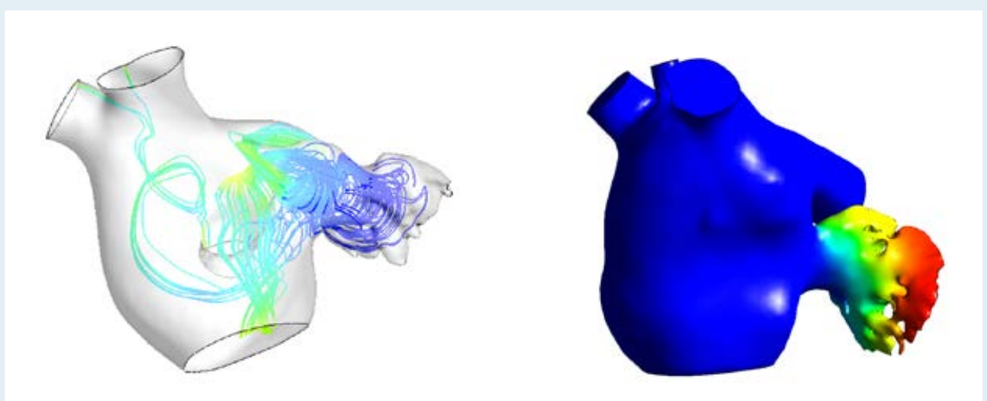
Aging Diseases

PIPELINE

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

BRIEF DESCRIPTION

Thromboembolic events, mainly caused by atrial fibrillation (AF), affect 1-2% of the population. More than 90% of the left atrial thrombi responsible for these originate in the left atrial appendage, a trabeculated finger-like projection about 2-4 cm long departing from the main body of the left atrium. Current treatment to prevent thromboembolic event is oral anticoagulation, surgical left atrial appendage exclusion or percutaneous left atrial appendage occlusion. However, the role played by the appendage morphology in the clotting mechanism is still poorly understood.



Map of the risk of thrombosis predicted for the different regions of a patient specific atrial appendage

IMPACT

The study aims to identify the morphological characteristics of the left atrial appendage morphology that promote the pathology, so as to classify the patients at higher risk. The presented approach can become a powerful tool to quantitatively analyse parameters otherwise impossible to measure in clinics and to study the geometrical factors influencing thrombus formation.
Lastly, supporting clinical stratification of patients under high risk of thrombus formation, this methodology could support the selection of individualised therapies, improving the patient's safety and standard of care.

RESULTS ACHIEVED IN 2018

In collaboration with the Great Ormond Street Hospital, London, UK, the patient specific anatomies of the left atrium and atrial appendage were reconstructed for over fifty subjects.
These were used to perform a preliminary shape analysis, with the aim of identifying objective parameters characterising the different morphologies. Few representative anatomies were used to create optically transparent physical models by rapid prototyping (3D printing). These can be integrated in the available setup for the simulation of the cardiac haemodynamics. Moreover, as they are made from transparent materials, they are suitable to be analysed with the common techniques of experimental fluid-dynamics.

GOALS FOR 2019

The shape analysis of the appendix anatomical features will be further implemented, with the aim of obtaining new approaches to determine an objective classification of the different anatomies in the clinical practice. In parallel, the physical models previously created will be used to validate the computational platform already implemented by our research group.
The latter will then be used to perform numerical simulations aimed at identifying the morphological parameters of the appendix that are more directly responsible for the onset of the hemodynamic conditions associated with thrombogenic risk, under normal conditions and atrial fibrillation.

MEETINGS

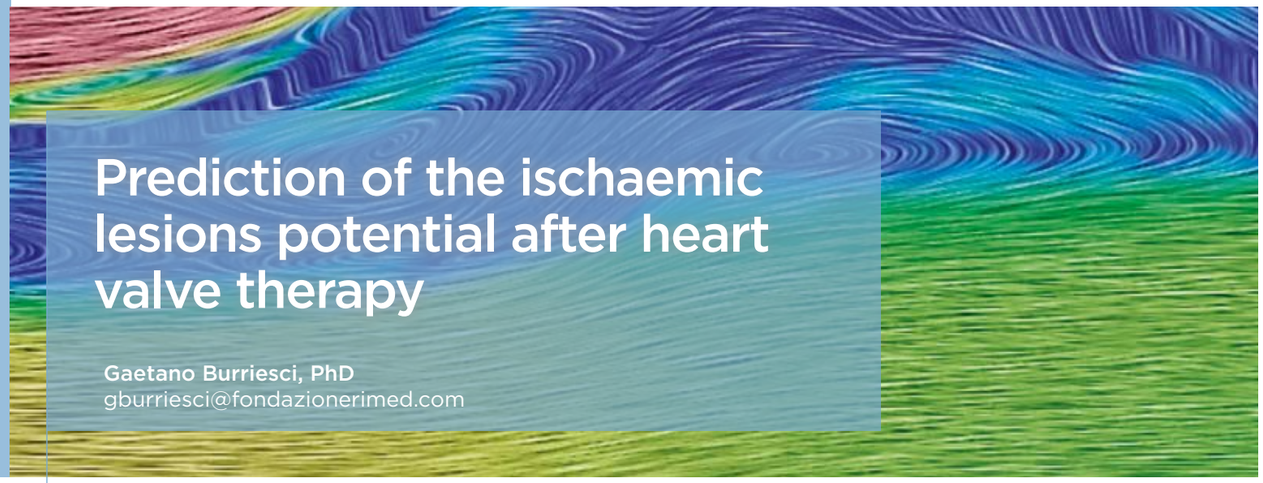
Bosi, G.M., Cook, A., Rai, R., Menezes, L., Schievano, S., Torii, R., **Burriesci, G.** (2018) Prediction of thrombosis risk by computational fluid dynamic simulations in the left atrial appendage. VPH-CaSE Young Researchers' Conference: Frontiers of Simulation and Experimentation for Personalised Cardiovascular Management and Treatment, London, UK.

Bosi, G.M., Cook, A., Rai, R., Menezes, L., Torii, R., **Burriesci, G.** (2018) On the relation between different Left Atrial Appendage morphologies and thrombosis risk: a Computational Fluid Dynamic analysis. 8th World Congress of Biomechanics, Dublin, Ireland. (P2043).

PUBLICATIONS

Bosi, G.M., Cook, A., Menezes, L., Schievano, S., Torii, R., **Burriesci, G.** (2018) Computational Fluid Dynamic Analysis of the Left Atrial Appendage to Predict Thrombosis Risk. Frontiers in Cardiovascular Medicine 5:34. doi: 10.3389/fcvm.2018.00034.

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



Prediction of the ischaemic lesions potential after heart valve therapy

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COLLABORATIONS

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THERAPEUTIC AREA

Organ Insufficiencies

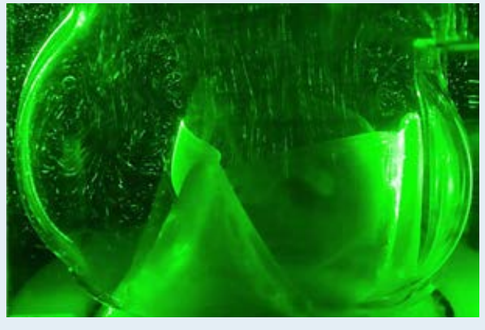
Aging Diseases

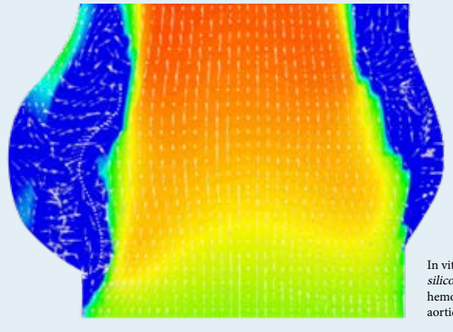
PIPELINE

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.





In vitro and *in silico* analysis of the hemodynamics in the aortic root

IMPACT

The aim of this study is to develop a new platform integrating experimental and numerical approaches, able to enhance the current insights into the flow dynamics that establishes in the heart valves districts in healthy, diseased and treated conditions.This platform will assist in the assessment of the haemodynamic performance for different anatomical phenotypes, pathological situations and valve devices. This step is essential to enhance the safety and efficacy of valve treatments, and provide information to support the selection of the best therapy for a specific patient.
Moreover, the implemented technologies can result important tools for the development of next generation valve treatments and medical devices.

RESULTS ACHIEVED IN 2018

A study of the fluid dynamics in the aortic root was performed by adopting a combined approach involving both experimental and numerical methodologies. The experimental study was performed on a porcine bioprosthetic valve, tested in a pulse duplicator and analysed by means of particle image velocimetry (PIV).
The numerical model was based on a fluid structure interaction (FSI) approach, thus allowing the simulation of both the mechanical behaviour of the soft tissues and the hemodynamics.
The latter was adjusted so as to replicate the experimental results, thus achieving a sound validation of the approach. Hence, the numerical model was modified to overcome the main limitations of the experimental setup and describe more accurately ideal physiological conditions.
This synergistic approach has led to a more complete understanding of the physiological mechanisms that determine the function of the healthy aortic valve. The study has identified, for the first time, critical phenomena previously unknown.

GOALS FOR 2019

The study performed so far suggests a new operating mechanism for the healthy aortic valve which is considerably different from what reported in the literature to date and largely more efficient in terms of hydrodynamic performance. It will be essential to find further confirmation for this finding, as its implication may be major in terms of bioprosthetic valve design. Also, the platform previously developed will be completed by including the presence of the coronaries and studying their interaction with the main flow. The model will serve as a benchmark for the analysis of the clinical effect of flow alterations induced by pathologies and therapeutic interventions, such as the implant of surgical or transcatheter heart valves.

MEETINGS

Tango, A.M., Salmon, J.A., Ducci, A., **Burriesci, G**. (2018) Fluid-structure-interaction model of a Prosthetic Aortic Valve Implantation configuration: comparison with an *in-vitro* study. VPH-CaSE Young Researchers' Conference: Frontiers of Simulation and Experimentation for Personalised Cardiovascular Management and Treatment, London, UK.

Salmon, J., Tango, A.M., Ducci, **A., Burriesci, G**. (2018) Validation of Fluid Structure Interaction Models of the Aortic Valve with *In-Vitro* Testing. 8th World Congress of Biomechanics, Dublin, Ireland. (O1034).

PUBLICATIONS

Tango, A.M, Salmonsmith, J, Ducci, A., **Burriesci, G.** (2018) Validation and Extension of a Fluid–Structure Interaction Model of the Healthy Aortic Valve. Cardiovascular Engineering and Technology 9(4): 739-751.

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

In vitro simulation of mitral valve therapies

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COLLABORATIONS

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Great Ormond Street Hospital (GOSH), London, UK

THERAPEUTIC AREA

Organ Insufficiencies

Aging Diseases

PIPELINE

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

BRIEF DESCRIPTION

Mitral regurgitation, the reverse blood flow through the mitral valve, is the most prevalent valvular heart disease and, if untreated, often leads to life threatening complications. Various repair and replacement treatments are available to restore the valve function, but it is often difficult to evaluate and compare the risk and result of the alternative approaches, due to the great anatomical heterogeneity in mitral pathologies. In order to enhance the safety and efficacy of the mitral valve therapies, it is essential to develop new models that reflect how a pathological mitral valve malfunctions under different physiological conditions, allowing a clearer understanding of the contributory role of each component of the mitral apparatus.

A swine mitral valve sample used in the investigation

IMPACT

This project aims to build a physical model of an *ex vivo* mitral valve in a hydro-mechanical closed circulatory system which approximates *in vivo* function.
The model will allow full control of the anatomical parameters that determine its correct of dysfunctional valve behaviour (such as the position of the papillary muscle and the annulus shape). This *in vitro* setup would provide an essential tool to understand the mitral valve function and aetiology, and serve as a benchmark to predict the effect of current treatments and support the development of new therapeutic solutions. Moreover, the physical model of a dysfunctional mitral valve is essential to validate future *in silico* models of the mitral valve.

RESULTS ACHIEVED IN 2018

A novel test rig has been developed, which incorporates the ability to house an *ex vivo* mitral valve in a hydromechanical pulse duplicator.
The system allows the fine adjustment of the main anatomical parameters that control the mitral valve function, such as the position of the papillary muscles and the annulus shape, and incorporates a contractile left ventricular mock chamber.
The test rig has been successfully used to replicate the performance of a set of porcine mitral valves under various operatic conditions, evaluating the effect of the position of the papillary muscles on the valve efficiency and on the mitral regurgitation.
Results were consistent with data from *in vivo* observation in swine models, and provide the relationship between the mitral valve geometric parameters and the risk of regurgitation.

GOALS FOR 2019

Based on the experience that is being acquired on *ex vivo* mitral valves with the implemented test system a standardised classification of the clinical geometric parameters essential to define the mitral valve function will be proposed. The system will be used to support the development, validation and design revision of new treatments for mitral pathology, including a range of percutaneous solutions for mitral valve repair and replacement. Moreover, the effect of the mitral valve model on the assessment of the aortic valve hemodynamic performance and efficiency will be investigated, in order to identify the potential limitations of current regulatory requirements and, where needed, suggest corrections.

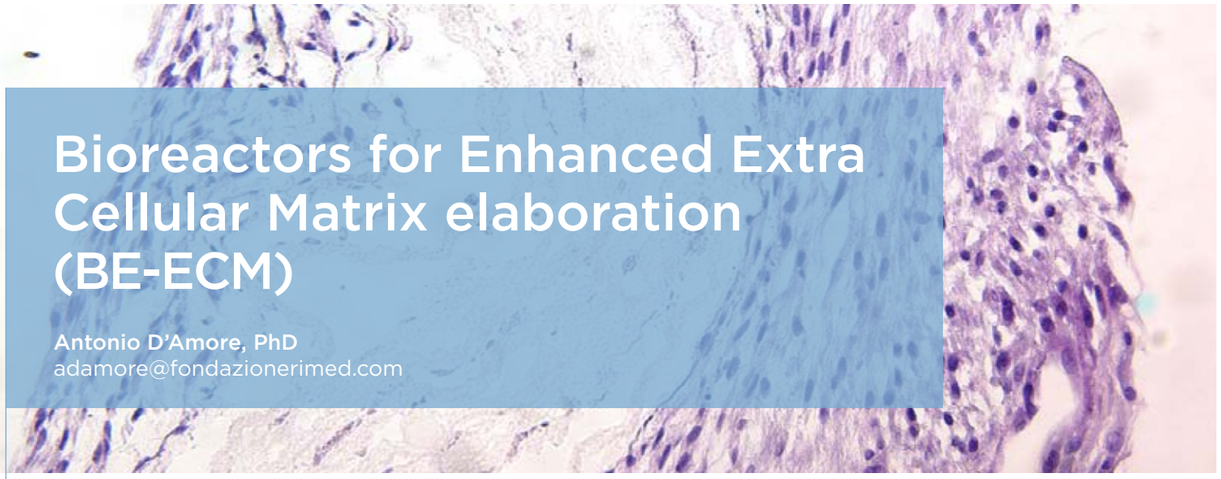
MEETINGS

Zhou, W., Bozkurt, S., Cheang, M.H., Schievano, S., **Burriesci, G.** (2018) *In-Vitro* Modelling of Mitral Valve Therapies. BioMedEng18 Conference, London, UK (ISBN 978-1-9996465-0-9)

Zhou, W., Bozkurt, S., Cheang, M.H., Schievano, S., **Burriesci, G.** (2018) An *in-vitro* model for the assessment of mitral valve therapies. Cardiovascular Innovations 2018, Denver, USA.

In vitro **simulation of the mitral valve function in healthy and dysfunctional operating conditions**

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



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

Antonio D'Amore, PhD
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COLLABORATIONS

Politecnico di Milano, Italy
University of Pittsburgh, USA
University of Zaragoza, Spain

THERAPEUTIC AREA

Organ Insufficiencies
Aging Diseases

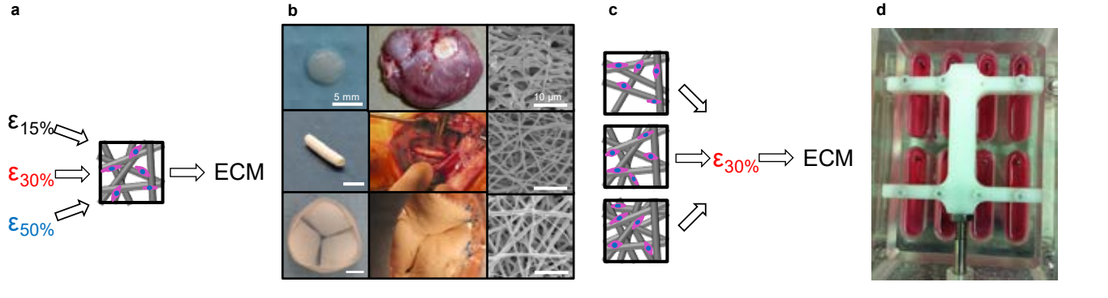
PIPELINE

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

BRIEF DESCRIPTION

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.

BE-ECM. Meso scale topological cues affect extracellular matrix production in an elastomeric scaffold model for cardiac tissue engineering applications. A) Previous hypothesis: different strain magnitudes induce different levels of ECM elaboration on vascular smooth muscle cell (VSMC) seeded polyurethane, electrospun scaffold model, mechanical load acting on the scaffold is imposed by the bioreactor. B) Examples of cardiac tissue engineering applications: pre-implants (left), in situ (center) and fiber based micro-structure (right) of polyurethane cardiac patch, vascular graft and heart valve processed by electrospinning, mechanical load acting in these setting are imposed by the physiology. C) Hypothesis for this study: given a specific mechanical load acting on a cell seeded polyurethane scaffold, different levels of ECM elaboration can be achieved by altering material topology at the meso scale. D) Custom made stretch bioreactor1 utilized in the study, top view shows the eight well plate chamber, the actuator arm connected with steel rod to the linear motor and the vertical pins holding the ring shape samples



IMPACT

Potential impact of this research might involve improved capacity 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 allow to expand the understanding of biomaterials mechanobiology and might allow to assess, using simplified tissue surrogates, the efficacy of novel tissue engineering strategies. Examples of these strategies include: mechanisms to accelerate tissue growth, solutions to modulate material degradation characteristics, topological cues to dictate cell differentiation and lineage.

RESULTS ACHIEVED IN 2018

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.

Teaching activity
(2017-2018) Mentor for BIOENG 1095 - Special projects. Individual research project under the guidance of a faculty member. Department of bioengineering, Univ. of Pittsburgh. Trainees: David Jacob Li-Ming, Armaan A. Fazal.

Mentoring activity
A. Adamo, 2018-2020, University of Palermo Italy, engineered chordae development;
H. Dagan 2017-2018, ORT Braude College Israel, engineered chordae mechanical conditioning;
D. Jacob Li-Ming, 2016-present, Univ. of Pittsburgh USA, quantitative methods for ECM mass detection.

Invited speech
(09/2018 - present) Chair of the wound healing and repair seminar, University of Pittsburgh, Pittsburgh, USA

GOALS FOR 2019

To perfect and promote the BE-ECM experimental platform, in particular:
- To submit first author publication on the topic: effects of degradation on mechanics and structure of polymeric heart valves;
- 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.

PUBLICATIONS

[J. A. 1] **A. D'Amore**, G. Nasello, S. Luketich, D. Denisenko, D. Jacob-Li Ming, R. Hoff, G. Gibson, A. Bruno, M. T. Raimondi, and W. R. Wagner. Meso-scale topological cues influence extracellular matrix production in an elastomeric scaffold model. *In press* on Soft Matter, IF 3.89

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

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

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COLLABORATIONS

University of Pittsburgh Medical Center, U.S.A.

Politecnico di Milano, Italy

THERAPEUTIC AREA

Organ Insufficiencies

Cancer

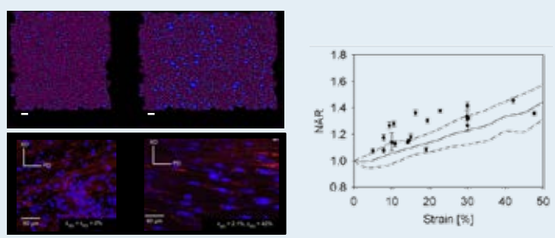
Aging Diseases

PIPELINE

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

BRIEF DESCRIPTION

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: Meso scale level validation and prediction. Model prediction for NAR vs. strain are provided in (a-c), and compared quantitatively (c) and qualitatively (a-b) with the experimental data[inter fibers voids are represented in dark purple at εPD=εXD= 0% (starting configuration g-h left) and εPD= 40%, εXD= 0% (final stretched configuration g-h right) whereas the fibers are represented in magenta.

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 2018

- 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 funded NIH-R01 project in collaboration with Dr F. Ambrosio.

- Abiltiy to reproduce fibrous materials and tissue topologies.

Research grants obtained and/or managed

(2016-2021) 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 (8% efforts), W. Wagner, D. Stolz, University of Pittsburgh.

Awards

(06/2018) Aging Cell Best Paper Prize 2017' for: "K. Stearns-Reider, A. D'Amore, K. Beezhold, B. Rothrauff, L. Cavalli, W. Wagner, D. Vorp, A. Tsamis, Changqing Zhang, A. Barchowsky, T. A. Rando, R. Tuan, F. Ambrosio. Aging of the skeletal muscle extracellular matrix drives a stem cell fibrogenic conversion', Aging Cell 2017 16 (3),518-528". The paper was judged to be the most outstanding one published during 2017. The prize is awarded by the Anatomical Society.

Teaching activity

(11/2015-11/2018) Guest lecturer for the biomedical engineering PhD program, MSCMP 3735. Department of Bioengineering, University of Pittsburgh. Title: "Cardiac ECM: structure - function, damage mechanism, and tissue engineering approaches to facilitate constructive remodelling".

Mentoring activity

C. T. Rhoades 2018 - present, School of Medicine, University of Pittsburgh USA, FEM of the chordal apparatus.

Invited speech

(09/2018 - present) Chair of the wound healing and repair seminar, University of Pittsburgh, Pittsburgh, USA.

GOALS FOR 2019

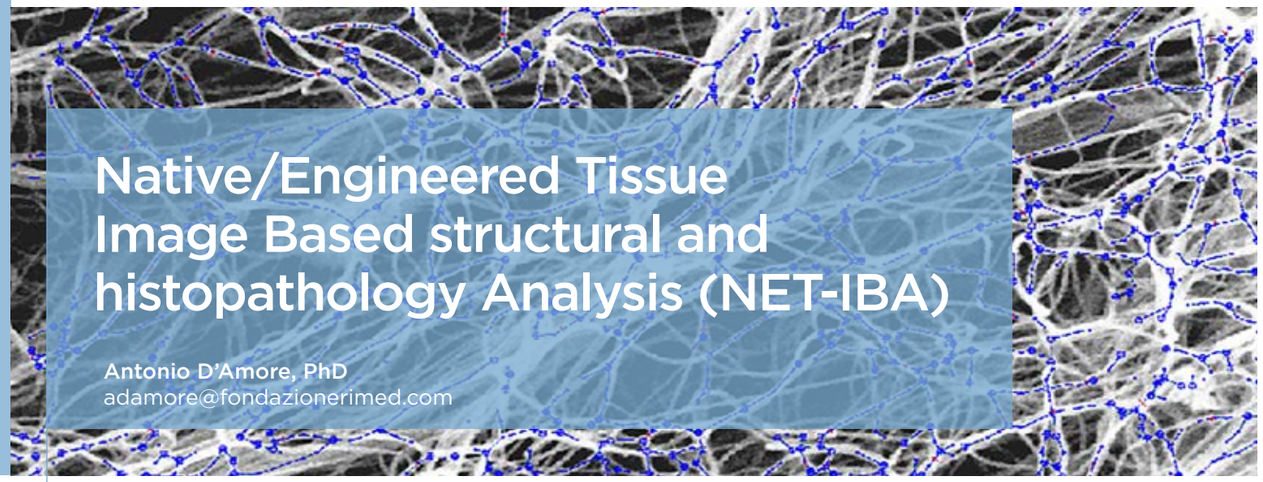
Goals set for the 2019 reflect the ancillary nature of this research line within the more broad scheme the PI envision for the cardiac tissue engineering program at Ri.MED and the collaborations with our clinical partners, more specifically:

- 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.

PUBLICATIONS

[J. A. 1] **A. D'Amore,** G. Nasello, S. Luketich, D. Denisenko, D. Jacob-Li Ming, R. Hoff, G. Gibson, A. Bruno, M. T. Raimondi, and W. R. Wagner. Meso-scale topological cues influence extracellular matrix production in an elastomeric scaffold model. *In press*, Soft Matter, IF 3.89

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



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

Antonio D'Amore, PhD
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COLLABORATIONS

University of Pittsburgh

PECA lab (Carnegie Mellon University Spin-off), Pittsburgh, USA

Università degli Studi di Palermo, Palermo, Italy

University of Nagoya, Japan

THERAPEUTIC AREA

Organ Insufficiencies

Aging Diseases

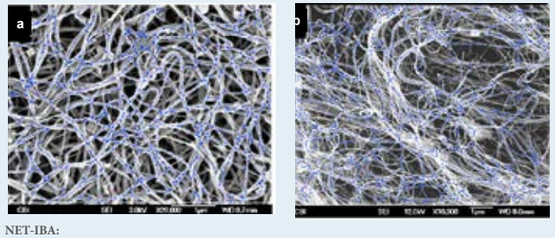
Cancer

PIPELINE

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

BRIEF DESCRIPTION

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.



NET-IBA:
A) Rabbit MSC seeded collagen gel analysis, detected fiber network and fiber diameters.
B) Decellularized rat carotid arteries analysis, detected fiber network and fiber diameters.

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 2018

- 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 includes: 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, Dr Ardizzone and Dr Pirrone Univ. Palermo.

Research grant obtained and/or managed
(2016-2021) NIH R01, Dysfunctional muscle remodeling and regeneration in environmental disease, $ 2.6 million. PIs: F. Ambrosio, A. Barchowski, University of Pittsburgh. Co-Investigators: A. D'Amore (8% efforts), W. Wagner, D. Stolz, University of Pittsburgh.

Awards
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Mentoring activity
A. Adamo, 2018-2020, University of Palermo Italy, cardiac patch development;
H. Dagan 2017-2018, ORT Braude College Israel, engineered chordae tendineae mechanical conditioning;
D. Jacob Li-Ming, 2016-present, University of Pittsburgh USA, quantitative methods for ECM mass detection;
D. Pedersen, 2015-present, University of Pittsburgh USA, Whitaker scholar, PIV study of electrospun tri-leaflets valves;
S. Luketich, 2013-present, University of Pittsburgh USA, biaxial testing of cardiac and abdominal wall explants.

Invited speech
(09/2018 - present) Chair of the wound healing and repair seminar, University of Pittsburgh, Pittsburgh, USA.

GOALS FOR 2019

- 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."

PUBLICATIONS

[J. A. 4] T. K. Valencia-Rivero, J. C. Cruz, J. C. Briceño, **A. D'Amore**, S-H Ye, J. Vande Geest, W. R Wagner. Decreased platelet deposition in SIS-based vascular grafts via covalent conjugation of RAFT polymers. 2018 IX International Seminar of Biomedical Engineering (SIB), 1-6, IF NA.

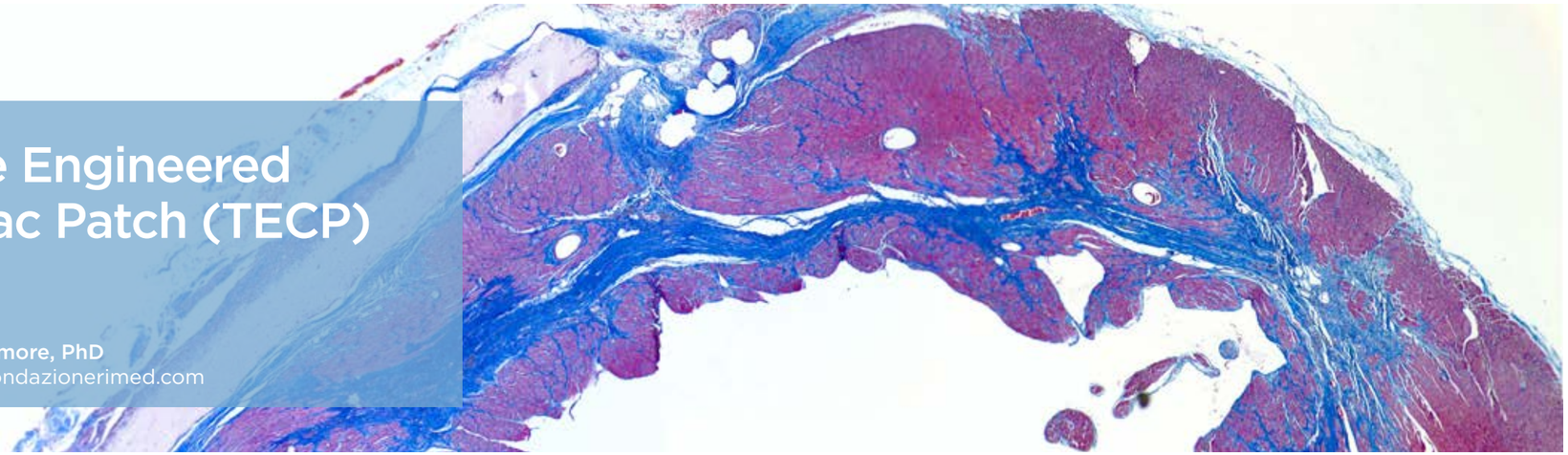
[J. A. 3] **A. D'Amore**, G. Nasello, S. Luketich, D. Denisenko, D. Jacob-Li Ming, R. Hoff, G. Gibson, A. Bruno, M. T. Raimondi, and W. R. Wagner. Meso-scale topological cues influence extracellular matrix production in an elastomeric scaffold model. In press on Soft Matter, IF 3.89.

[J. A. 2] **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. Tissue Engineering Part A, 2018 Jun;24(11-12):889-904, IF 3.58. McGowan Institute for Regenerative Medicine' paper and picture of the month (12-2017).

[J. A. 1] **A. D'Amore**, S. K. Luketich, G.M. Raffa, S. Olia, G. Menallo, A. Mazzola, F. D'Accardi, T.Grunberg, X. Gu, M. Pilato, M. V. Kameneva, V. Badhwar, W.R. Wagner. Heart valve scaffold fabrication: bioinspired control of macro-scale morphology, mechanics and micro- structure. Biomaterials 2018, 150, 25-37, 5Y-IF 8.97.

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

Tissue Engineered Cardiac Patch (TECP)



Antonio D'Amore, PhD
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COLLABORATIONS

University of Pittsburgh, Pittsburgh, U.S.A.
University of Pittsburgh Medical Center, Pittsburgh, U.S.A.
University of Cincinnati, Cincinnati, U.S.A.
IRCCS ISMETT, Palermo, Italy
Mario Negri, Milano, Italy
Universidade Estadual de Campinas, Campinas, Brasil
University of Texas, Austin, U.S.A.
Virginia Commonwealth University, Richmond, U.S.A.
ATeN Center, University of Palermo, Italy

THERAPEUTIC AREA

Organ Insufficiencies

PIPELINE

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

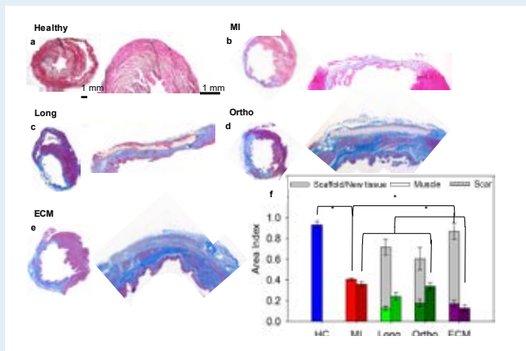
BRIEF DESCRIPTION

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.



TECP: MT staining, muscle and scar area index. (A-E) Typical MT stained whole heart sections and infarct/patch regions at the 8 wk time point. The ECM scaffold explants showed higher host cell infiltration than either of the oriented PECUU patches. (F) Quantitative comparison of muscle, scaffold/newly formed tissue and scar areas in histological sections between patch types. n=6, mean ± sem, *p<0.05.

RESULTS ACHIEVED IN 2018

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
(2018-2020) 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;
(2014-2018) Armed Forces Institute of Regenerative Medicine, AFIMRII, W81-XWH-13-2-XXXX, $900,000. Creating innervated vascularized muscle flaps from elastic, cellularized biocomposites developed *in situ* for facial muscle reconstruction. PI: W. Wagner, Project leader: A. D'Amore, University of Pittsburgh.

Awards
(9/2018) Winner of the Franco Strazzabosco award for young engineers – ISSNAF, #1 Italian engineer working in US, Washington DC 11/23/2018, early stage career award under the high patronage of the Italian president of the Republic.
(05/2018) Honorable mention for the 2018 Carnegie Science Awards in the Advanced Manufacturing and Materials category. Carnegie Science Award program recognizes outstanding science and technology achievements in western Pennsylvania.

Teaching activity
(2017-2018) Mentor for BIOENG 1095 - Special projects. Individual research project under the guidance of a faculty member. Department of bioengineering, Univ. of Pittsburgh.
(11/2015-11/2018) Guest lecturer for the biomedical engineering PhD program, MSCMP 3735. Department of bioengineering, University of Pittsburgh. Title: "Cardiac ECM: structure - function, damage mechanism, and tissue engineering approaches to facilitate constructive remodelling".

(06/2016-06/2017) Guest lecturer, lecture on: "Processing methods for polymeric biodegradable scaffolds", regenerative medicine summer school, endorsed by TERMIS and Society for Biomaterials, University of Pittsburgh, ~20 students;
(02/2016-02/2018) Guest lecturer for the biomedical engineering master of science program, BIOENG 2810. Department of bioengineering, University of Pittsburgh. Title: *"A brief overview on polymers processing methods for soft tissue engineering"*.

Mentoring activity
A. Adamo, 2018-2020, University of Palermo Italy, cardiac patch development;
D. Jacob Li-Ming, 2016-present, Univ. of Pittsburgh USA, quantitative methods for ECM mass detection;
S. Luketich, 2013-present, Univ. of Pittsburgh USA, biaxial testing of cardiac and abdominal wall explants.

Invited speech
(09/2018 - present) Chair of the wound healing and repair seminar, University of Pittsburgh, Pittsburgh, USA.

GOALS FOR 2019

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;
- Numerical study to investigate on the impact of scaffold thickness and elastic modulus on the efficacy of cardiac patch. Project in collaboration with Dr Soares and Dr Sacks;
- 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).

MEETINGS

[C. P. 1] 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. L. M. Silveira-Filho, G. Coyan, **A. D'Amore,** S. K. Luketich, G. Menallo, A. Adamo, Y. Matsumura, N. Kashiyama, W. R. Wagner. Proceedings of the Society for Biomaterials Annual Meeting (SfB 2019), 3rd -6h April 2019 Seattle, Washington.

PUBLICATIONS

[J. A. 4] N. Kashiyama, R. L Kormos, Y. Matsumura, S. Higuchi, L. M Silveira-Filho, G. Coyan, J. Hong Bin, **A. D'Amore**, W. R Wagner. Adipose derived stem cells enhance cardiac function preservation of a biodegradable cardiac patch and increase vascularization in rats with subacute myocardial infarction. Circulation. 2018;138:A12295, IF 19.3.

[J.A. 3] 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, 2018, S0022-5223(18)32289-X. IF 4.46.

[J. A. 2] Chen, S. Ye, Y. Zhu, S. Vesselin, T. Tarannum, **A. D'Amore,** S. K. Luketich, W. Gojiang, W. Wagner. Hybrid scaffolds of Mg alloy mesh reinforced polymer/extracellular matrix composite for critical-sized calvarial defect reconstruction. Journal of Tissue Engineering and Regenerative Medicine, 2018, 12(6):1374-1388, IF 4.71.

INTELLECTUAL PROPERTY

[I. D. 1] US patent application PCT/US2018/061862, filed on 11/2018, 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.**

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

Tissue Engineered Heart Valve (TEHV)

Antonio D'Amore, PhD
adamore@fondazionerimed.com

COLLABORATIONS

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

THERAPEUTIC AREA

Organ Insufficiencies

PIPELINE

BRIEF DESCRIPTION

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 2018

Successfully completed assessment of DCD pulmonary and tricuspid valves on acute, large animal model. DCD mitral valve assessment is currently ongoing 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. Initiated characterization of human samples from donors via collaboration with Core Foundation, USA. Consolidated OneValve's IP portfolio.

Research grant obtained and/or managed
(2016-2018) Coulter foundation 2016 $100,000. Co-PI: V. Badhwar, Co-PI: A. D'Amore, Co-PI: W. Wagner, University of Pittsburgh;
(2016-2018) Clinical & Translational Science Institute (CTSI), University of Pittsburgh; $50,000. Co-PI: A. D'Amore, Co-PI: W. Wagner, University of Pittsburgh;
(2018) Dean's summer research program (DSRP) Pitt, $3,000, student: C. T. Rhoades. Mentors: S. Pasta (RiMED-Palermo), A. D'Amore (Pitt-Pittsburgh);
(2017-2018) Industrial collaboration with Peca lab, start-up company created by Carnegie Mellon University, $5,000 renewable consultancy service. PI: A. D'Amore.

Awards
(9/2018) Winner of the Franco Strazzabosco award for young engineers – ISSNAF, #1 Italian engineer working in US, Washington DC 11/23/2018, early stage career award under the high patronage of the Italian president of the Republic;
(05/2018) Honorable mention for the 2018 Carnegie Science Awards in the Advanced Manufacturing and Materials category. Carnegie Science Award program recognizes outstanding science and technology achievements in western Pennsylvania.

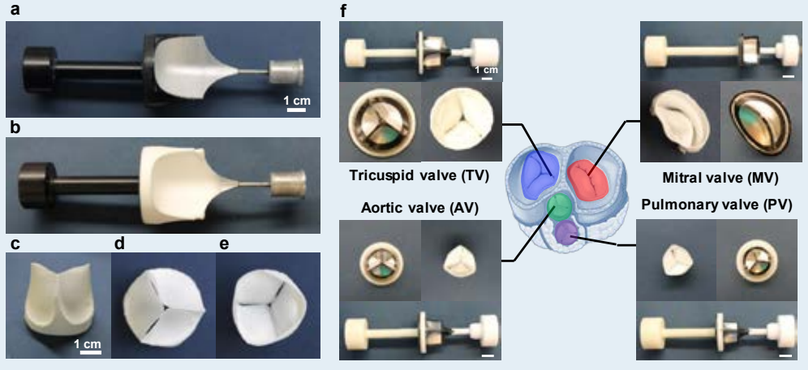
Teaching activity
(06/2018) Guest lecturer, lecture on "Heart valve diseases and tissue engineering approach to valve replacement", regenerative medicine summer school, endorsed by TERMIS and Society for Biomaterials, University of Pittsburgh, ~20 students.

Mentoring activity
A. Adamo, 2018-2020, University of Palermo Italy, engineered chordae development;
C. T. Rhoades 2018 - present, School of Medicine, University of Pittsburgh USA, engineered chordae FEM;
H. Dagan 2017-2018, ORT Braude College Israel, engineered chordae mechanical conditioning;
M. Luketich, 2016-2018, University of Pittsburgh USA, electrospun aortic valve fabrication optimization.

Invited speech
(03/2018) "Heart valve structure, functional heterogeneity and mechanics ". McGowan Institute for Regenerative Medicine scientific retreat. Cardiovascular session, Pittsburgh, PA.

GOALS FOR 2019

The long term goal is the translation of the technology (class III FDA), obejectives for the 2019 are defined as follows:
- Completion of the ongoing DCD mitral valve *in-vivo* acute study;
- Submit manuscript describing the completed DCD tricuspid valve *in-vivo* acute study;
- Preparation and submission of research proposal to fund chronic studies for atrio-ventricular engineered valves;
- Completion of *in-vitro* study on the mechano-biology of engineered chordae;
- Completion of FEM study of the chordal apparatus;
- Assessment of new stategies for selective fiber deposition via DCD;
- Protect and consolidate IPs;
- Perfect OneValve business plan and financial modeling;
- Promote and present the technology to VCs;
- Higher education and training: seven MSc' degree students, two PhD students;
- Consolidate the McGowan-UNIPA internship program.



TEHV: Double component fiber deposition (DCD) process control of engineered heart valve morphology. a) Double component mandrel full assembly before fiber deposition. Mandrel component 1: non-conductive shield of acrylonitrile butadiene styrene (in black). Component 2: main collecting target of aluminum alloy. b) Double component mandrel after polymer fiber deposition. c) Tri-leaflet valve removed from the mandrel and trimmed, showing leaflet coaptation at rest. d) View from the ventricular side and e) view from the atrial side of the tri-leaflet valve. f) DCD processing method applied to the four valve types, prototypes shown in the picture demonstrate the capacity of DCD to generate valves with variable macro-scale morphology and size.

MEETINGS

- [C. P. 2] 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**. American Association for Thoracic Surgery (AATS 2019) 2019 meeting:, May 4-7, 2019 Toronto, Canada.
- [C. P. 1] G. N. Coyan, MD, **A. D'Amore,** Y. Matsumura, D. Pederson, S. K. Luketich, B. Kandala, V. Shanov, T.E. David, W. R. Wagner, V. Badhwar. *In Vivo* Functional Assessment of a Novel Bioinspired Scaffold-Based Tissue Engineered Heart Valve. American Association for Thoracic Surgery (AATS 2018) 2018 meeting: focus aortic symposium, April 27-28, 2018 New York NY.

PUBLICATIONS

- [J.A.3] 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. In press on J. of Thoracic and Cardiovascular Surgery, IF 4.46.
- [J.A.2] 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, 2018, S0022-5223(18)32289-X. IF 4.46.
- [J.A.1] **A. D'Amore**, S. K. Luketich, G.M. Raffa, S. Olia, G. Menallo, A. Mazzola, F. D'Accardi, T.Grunberg, X. Gu, M. Pilato, M. V. Kameneva, V. Badhwar, W.R. Wagner. Heart valve scaffold fabrication: bioinspired control of macro-scale morphology, mechanics and micro- structure. Biomaterials 2018, 150, 25-37, 5Y-IF 8.97.

INTELLECTUAL PROPERTY

- [I. D. 6] Pitt invention disclosure filed on 09/2018, Pitt#04752, topic: biomedical device, title: "Valved stent for the treatment of severe tricuspid regurgitation."
- [I. D. 5] Pitt invention disclosure filed on 07/2018, Pitt#7715, topic: biomedical device, title: "Processing method and apparatus for micro-structured rope-like material. ". Lead innovator/developer: **A. D'Amore**
- [I. D. 4] US provisional patent application 62/663,721 filed on 04/2018, topic: biomedical device, title: "Biodegradable metallic stent for heart valve tissue engineering". Lead innovator/developer: **A. D'Amore**
- [I. D. 3] US patent application PCT/US2018/022863 with WO (International publication number WO) published in 08/2018, topic: biomedical device, title: "Mandrel-less Electrospinning Processing Method and System, and Uses Therefor". Lead innovator/developer: **A. D'Amore**.
- [I. D. 2] US patent application PCT/US2018/017795 with WO (International publication number WO/20118/148646) published in 06/2018, topic: biomedical device, title: "Expandable percutaneous cannula". Lead innovator/developer: **A. D'Amore.**
- [I. D. 1] US patent application PCT/US2018/019358 with WO (International publication number WO/2018/156856) published in 08/2018, topic: biomedical device, title: "A stentless biopolymer heart valve replacement capable of living tissue regeneration". Lead innovator/developer: **A. D'Amore.**

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

Tissue Engineered Vascular Graft (TEVG)

Antonio D'Amore, PhD
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COLLABORATIONS

Ospedale Cervello – Villa Sofia, Palermo, Italy
University of Pittsburgh, U.S.A.
University of Pittsburgh Medical Center, U.S.A.
ATeN Center, University of Palermo, Italy

THERAPEUTIC AREA

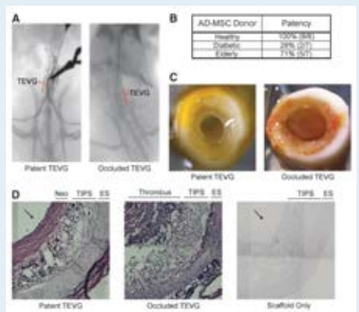
Organ Insufficiencies

PIPELINE

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

BRIEF DESCRIPTION

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 and structure.



TEVG: Diabetic AD-MSCs do not produce patent vessels, whereas healthy and elderly do. (A) Angiograms were performed to assess the patency of TEVGs at the 8-week endpoint with a patent vessel showing clear flow past the graft to the hindquarters. (B) Total patency rate was calculated based on the number of patent versus total TEVGs; note that TEVGs created using cells from diabetic donors show a marked reduction in patency. (C) Gross inspection of the explants revealed that the primary reason for nonpatent TEVGs was occlusive thrombosis. (D) Hematoxylin and eosin-stained sections of a patent 8-week TEVG, a nonpatent 8-week TEVG, and a nonimplanted PEUU scaffold. Significant remodeling of patent TEVGs included newly developed neotissue (Neo) lumenally and breakdown of the original PEUU scaffolding material (inner layer: TIPS, outer layer: ES). Occluded grafts displayed the presence of a thrombus and no remodeling. Arrows indicate lumen in Figure 2D. ES, electrospun; PEUU, poly(ester urethane)urea; TEVGs, tissue-engineered vascular grafts; TIPS, thermally induced phase separation.

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 2018

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. Pipeline of scaffold fabrication maintained to supply the Vorp' laboratory with TEVGs necessary to execute ongoing NIH funded R01 project.

Research grants obtained and/or managed

NIH 1R01 HL130077-01, Artificial Stem Cells for Vascular Tissue Engineering. PI: D. Vorp. University of Pittsburgh. Role: A. D'Amore collaborator, vascular graft fabrication.

Awards

(05/2018) Honorable mention for the 2018 Carnegie Science Awards in the Advanced Manufacturing and Materials category. Carnegie Science Award program recognizes outstanding science and technology achievements in western Pennsylvania.
(9/2018) Winner of the Franco Strazzabosco award for young engineers – ISSNAF, #1 Italian engineer working in US, Washington DC 11/23/2018, early stage career award under the high patronage of the Italian president of the Republic;
(9/2018) Winner of the "Franco Maria Montevecchi" award for the best MSc thesis: "Three-layered, bio-inspired, small-diameter vascular graft for tissue engineering applications ",awarded by the Italian national group of bioengineering, mentors Drs A. D'Amore, M. Raimondi.

Teaching activity

(2017-2018) Mentor for BIOENG 1095 - Special projects. Individual research project under the guidance of a faculty member. Department of bioengineering, Univ. of Pittsburgh. Trainees: David Jacob Li-Ming, Armaan A. Fazal.

Mentoring activity

P. I. Gonzalez, 2017-present, University of Pittsburgh USA, biomimetic three-layers vascular graft;
A. Fazal, 2017-present, University of Pittsburgh USA, biomimetic three-layers vascular graft;
D. Jacob Li-Ming, 2016-present, University of Pittsburgh USA, biomimetic three-layers vascular graft;
S. Luketich, 2013-present, University of Pittsburgh USA, support to the collaboration with Dr Vorp's Lab.

GOALS FOR 2019

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.

MEETINGS

- [C. P. 4] D. Haskett, S. Madala, E. M. Cunnane, K. L. Lorentz, C. Zhang, S. K. Luketich, J. S. Weinbaum, **A. D'Amore,** L E Kokai, K. G Marra, W. R Wagner, J Peter Rubin, D. A Vorp. Development of a Seeding Device for Bulk-Seeding of Cells into a Long "Human-Sized" Scaffold for Tissue Engineered Vascular Grafting. 8th World congress on biomechanics, July 8-12, 2018 Dublin Ireland.
- [C. P. 3] K.Lorentz, J. Krawiec, D. Haskett, J.Weinbaum, M. Fedorchak, L. Bruk, A. Acharya, **A. D'Amore**, W. R. Wagner, S. Little, D. Vorp. Validating Microspheres for use in porous scaffolds. 8th World congress on biomechanics, July 8-12, 2018 Dublin Ireland.
- [C. P. 2] K. Lorentz, J. Krawiec, D. Haskett, J. Weinbaum, M. Fedorchak, **A. D'Amore**, W. R. Wagner, S. Little, D. Vorp. Validating Microspheres for Use in Porous Scaffolds. 8th World congress on biomechanics, July 8-12, 2018 Dublin Ireland.
- [C. P. 1] M. Sedlak, K. Lorentz, S. Luketich, S-H Ye, **A. D'Amore**, W. Wagner, J. Weinbaum, D. Vorp. Comparison of cell seeding quality of porous, biomimetic, tubular scaffolds for vascular tissue engineering fabricated by two different methods. Biomedical engineering society (BMES 2018), October 17-20, 2018, Atlanta Georgia.

PUBLICATIONS

- [J. A. 3] T. K. Valencia-Rivero, J. C. Cruz, J. C. Briceño, **A. D'Amore**, S-H Ye, J. Vande Geest, W. R Wagner. Decreased platelet deposition in SIS-based vascular grafts via covalent conjugation of RAFT polymers. 2018 IX International Seminar of Biomedical Engineering (SIB), 1-6, IF NA.
- [J. A. 2] D. G Haskett, K. Saleh, J. T Krawiec, J. S. Weinbaum, **A. D'Amore,** W. R. Wagner, L. E Kokai, K. G Marra, J P. Rubin, D. A Vorp. An Exploratory Study into the Preparation and Evaluation of a "Same Day" Adipose Stem Cell-Based TEVG. Journal of Thoracic and Cardiovascular Surgery, 2018;156:1814-22, IF 4.46.
- [J. A. 1] 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, 2018, S0022-5223(18)32289-X. IF 4.46.

INTELLECTUAL PROPERTY

[I. D. 1] US patent application PCT/US2018/043889, filed on 07/2018, topic: biomedical device, title: "Multi-Layered Graft for Tissue Engineering Applications". Lead innovator/developer: A D'Amore.

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

3D osteochondral models to study degenerative disorders and therapies in microgravity

Riccardo Gottardi, PhD
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COLLABORATIONS

Vanderbilt University, Nashville, Tennessee, USA

Bioserve, Boulder, Colorado, USA

THERAPEUTIC AREA

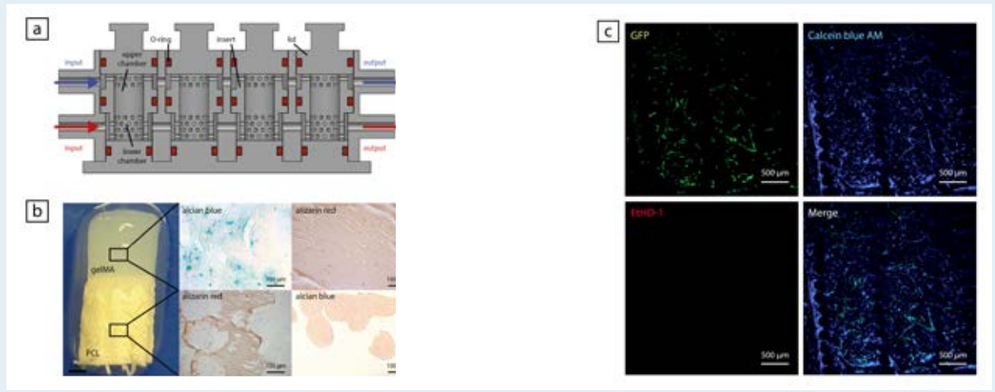
Aging Diseases

PIPELINE

CLINICAL NEED — TECHNOLOGY FORMULATION — PROTOTYPE — VALIDATION — PRODUCTION — COMMERCIAL APPLICATION

BRIEF DESCRIPTION

Skeletal disorders present significant disease burden for the general population and are specifically associated with space flight under microgravity. In this project, we aimed at establishing a 3D organoid model to research the effects of microgravity on osteochondral tissues, test drugs and develop therapies against bone and cartilage damage in space as well as on the ground. We leveraged our established bioreactor combined with advanced microfluidic to examine the response to stress of native as well as engineered osteochondral tissues. We plan on using the extreme environment of the Space Station to model disorders such as osteoporosis and osteoarthritis and identify potential treatments that could be effective in space, but most importantly on Earth.



a Cross-sectional bioreactor schematic. b Macroscopic and histological analysis of engineered osteochondral interface. c Live/dead staining of capillary-like network formed by HUVECs in bone compartment (GFP, green = HUVEC; Calcein Blue AM, blue = live cells; EthD-1, red = dead cells). gelMA gelatin methacrylate, PCL poly(ε-caprolactone), GFP green fluorescent protein, EthD-1 ethidium homodimer-1

IMPACT

Our long-term goal is to develop new capabilities in studying biology, medicine, pharmacology, physiology, and related toxicology of skeletal tissues by combining tissue engineered organotypic models with human stem cells and non-invasive, real-time analytical techniques. A human stem cell-based organ-on-chip can accelerate drug development by reducing the number of compounds that reach Phase I and II clinical trials while more precisely predicting outcomes of those that are clinically tested, which will also dramatically reduce the cost of drug development.
This research will serve the space community and our approach can be used to analyze the effects of the multiple environmental stresses experienced during space travel, including but not limited to weightlessness and radiation, as well as therapies to counteract them. In this way large cohorts can be generated and tested with low weight, volume, cost, and maintenance requirements, without jeopardizing the health of astronauts and the success of missions. Uniquely, we aim at using the extreme environment of space to generate in a few weeks a disease phenotype that would take years to develop on Earth. This will offer a unique window into disease development and an exceptional opportunity to study the effect of candidate therapeutics.

RESULTS ACHIEVED IN 2018

We established the use of our biphasic bioreactor to culture osteochondral tissue and to develop vascularized osteochondral constructs. In particular, we established the relevance of our system in studying the crosstalk between the cartilage and bone compartments and its relevance in studying disorders and the response to treatments. The bioreactor is fabricated by additive manufacturing allowing tremendous flexibility in meeting experimental requirements and accommodating other multiphasic tissues for testing and even engineering of implants.
The native tissue can be cultured in the bioreactor for over a month and the engineered constructs uniquely comprise up to three different tissue types, such as engineered cartilage, bone, and vasculature, allowing to study the interplay between them in health, development, and disease.

GOALS FOR 2019

This project was funded by CASIS and the on-ground preliminary development of the organoid system within the bioreactor, including its microfluidic controls, was concluded by 2018. The next phase of this research will include pre-flight validation and optimization of the system along with all the necessary testing to adapt operation for use on the International Space Station. We are currently exploring funding opportunities to support this next phase of the project that is necessary prior to deployment on the ISS. In the meantime, we are identifying potential disease-modifying compounds that could be tested within our *in vitro* system, assessing possible cross-tissue interactions. This will offer further validation of the system and support its commercialization.

MEETINGS

American Institute of Chemical Engineers Annual Meeting, Ottobre 2018, Pittsburgh, PA, USA.

Biomedical Engineering Society Annual Meeting, October 2018, Atlanta, GA, USA.

Tissue Engineering and Regenerative Medicine International Society – World Annual Meeting, September 2018, Kyoto, Japan.

Society for Biomaterials, April 2018, Atlanta, GA, USA.

Orthopaedic Research Society Annual Meeting, March 2018, New Orleans, LA,USA.

European Orthopaedic Research Society Annual Meeting, September 2018, Galway, Ireland.

PUBLICATIONS

Pirosa A., **Gottardi R.**, Alexander P.G., Tuan R.S., (2018), Engineering *in vitro* vascularized bone models for drug screening and predictive toxicology. Stem Cell Research & Therapy. 9: 112. DOI: 10.1186/s13287-018-0847-8.

Nichols D.A., Sondh I.S., Little S.R., Zunino P., **Gottardi R.**, (2018), Design and validation of an osteochondral bioreactor for the screening of treatments for osteoarthritis. Biomedical Microdevices. 2018, 20: 18. DOI: 10.1007/s10544-018-0264-x.

INTELLECTUAL PROPERTY

US20160201037A1, August 22, 2014. Modular, microfluidic, mechanically active bioreactor for 3d, multi-tissue, tissue culture.

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

R-CaRe - Rehabilitation for Cartilage Regeneration

Riccardo Gottardi, PhD
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COLLABORATIONS

Orthopaedic Robotics Laboratory,
University of Pittsburgh, PA, USA

THERAPEUTIC AREA

Aging Diseases

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.

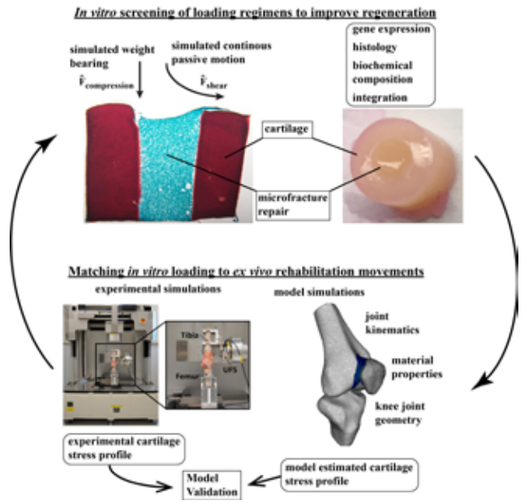
IMPACT

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 2018

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 2019

In 2019 we aim at identifying if/how pro-regenerative mechano-activation 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

Biomedical Engineering Society Annual Meeting, October 2018, Atlanta, GA, USA.

European Orthopaedic Research Society Annual Meeting, September 2018, Galway, Ireland.

Tissue Engineering and Regenerative Medicine International Society – World Annual Meeting, September 2018, Kyoto, Japan.

Gordon Research Conference, – Musculoskeletal Biology and Engineering, August 2018, Andover, NH, USA.

Orthopaedic Research Society Annual Meeting, March 2018, New Orleans, LA,USA.

PUBLICATIONS

Gottardi R., Stoddart M., (2018). Regenerative rehabilitation of the musculoskeletal system. Journal of the American Academy of Orthopaedic Surgeons. 26(15): e321-e323, DOI: 10.5435/JAAOS-D-18-00220.

PRODUCTS: **BIOMARKERS - MEDICAL DEVICES & ARTIFICIAL TISSUES AND ORGANS**

In-silico modeling for clinical risk stratification of cardiovascular pathologies

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COLLABORATIONS

ISMETT – IRCCS, Italy

McGowan Institute for Regenerative Medicine, University of Pittsburgh, U.S.A.

CIS Centre for Biomedical and Heathcare Engineering, Ecole des Mines de Saint-Étienne. France

Mayo Clinic, Rochester, USA

University of Palermo, Italy

THERAPEUTIC AREA

Aging Diseases

PIPELINE

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

BRIEF DESCRIPTION

Cardiovascular diseases are the largest cause of death in Italy and Europe and cause of the population aging and the increasing prevalence of risk factors. There is therefore a pressing need to couple biomechanical principles to the advances in medical knowledge in order to improve our understanding in the pathophysiology of cardiovascular diseases. This has the potential to revolutionize the way we diagnose, treat, and prevent cardiovascular diseases to benefit both patient and society. *In-silico* modeling can be therefore useful to support physicians in their clinical-decision support system related to the interventions of cardiovascular diseases such as the aortic aneurysms and heart failure.

IMPACT

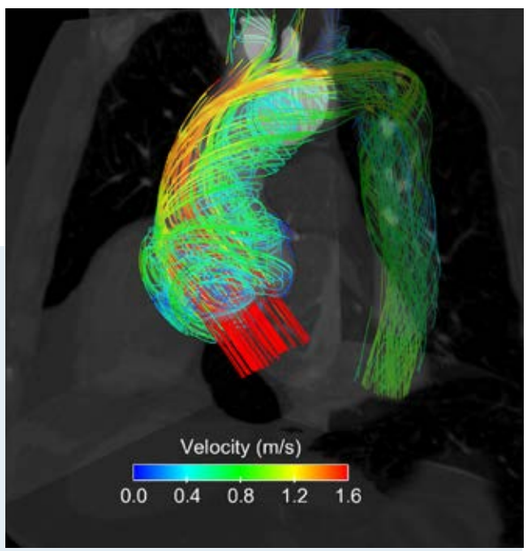
In-silico modeling can be adopted to non-invasively predict organ physiology and provide further information on the progress of cardiovascular diseases. This can determine not only a benefit for the patient undergoing serial invasive imaging but also a refinement of surveillance imaging regimens, medical management and decision regarding early intervention for cardiovascular diseases.

RESULTS ACHIEVED IN 2018

We developed a database of computational models and bio-markers (ie miRNA) on cohort of >200 patients with aortic aneurysms. Specifically, we found that miR-34a is associated to the stiffness of ascending aortic aneurysm. In the field of LVAD, we developed a numerical model to assess the risk of right heart failure in patients waiting for heart transplant. We are working on a PPG sensor to assess vital signals including blood pressure.

GOALS FOR 2019

The goal will be to develop clinical-decision support system based on computational modeling for an improved clinical risk stratification of cardiovascular pathologies and to provide new predictive tools and metrics that cannot be obtained by current clinical gold-standard.



MEETINGS

- Di Giuseppe, M., **S. Pasta**, E. Bologna and M. Zingales (2018). Hereditariness of aortic tissue: *In-vitro* time-dependent failure of human and porcine specimens. RTSI 2018. Palermo.
- **Pasta, S.**, M. Condipodero, V. Mendez, V. Agnese, D. Bellavia, G. Gentile, G. M. Raffa and G. Pilato (2018). Comparison of Hemodynamic and Structural Indices of Ascending Thoracic Aortic Aneurysm as predicted by 2-way FSI, CFD Rigid Wall Simulation and Displacement-Based FEA. WCB 2018. Palermo.
- **Pasta, S.**, F. Scardulla, D. Bellavia, G. Gentile, G. M. Raffa and G. Pilato (2018). Hemodynamic of the celiac trunk in patients with a continuous-flow left ventricular assist device: *in-silico* and *in-vitro* flow analyses. WCB 2018. Palermo.
- Bellavia, D., V. Agnese, **S. Pasta**, R. GM, A. Iacovoni, M. Caputo, C. Fino, G. Romano, C. Minà, J. Maalouf, H. Michelena, F. Clemenza and G. Pilato (2018). Is Bicuspid Aortic Valve a Predictor of Faster Ascending Aorta Dilatation or Earlier Cardiac Surgery? A Longitudinal Long-Term Follow-Up Study Euro ECHO 2018. Palermo.
- **Pasta, S.**, D. Bellavia, G. Gentile, G. M. Raffa and G. Pilato (2018). *in silico* computational modeling and circulating microrna signature of ascending thoracic aortic aneurysms. SICCH 2018. Roma.

PUBLICATIONS

- Gallo, A., Agnese, V., Coronnello, C., Raffa, G.M., Bellavia, D., Conaldi, P.G., Pilato, M., **Pasta, S**. On the prospect of serum exosomal miRNA profiling and protein biomarkers for the diagnosis of ascending aortic dilatation in patients with bicuspid and tricuspid aortic valve (2018) 273, pp. 230-236.
- Scardulla, F., Agnese, V., Romano, G., Di Gesaro, G., Sciacca, S., Bellavia, D., Clemenza, F., Pilato, M., **Pasta, S**. Modeling Right Ventricle Failure After Continuous Flow Left Ventricular Assist Device: A Biventricular Finite-Element and Lumped-Parameter Analysis. (2018) 9 (3), pp. 427-437.
- Mendez, V., Di Giuseppe, M., **Pasta, S.** Comparison of hemodynamic and structural indices of ascending thoracic aortic aneurysm as predicted by 2-way FSI, CFD rigid wall simulation and patient-specific displacement-based FEA (2018) 100, pp. 221-229.
- Falletta, C., **Pasta, S.**, Raffa, G.M., Crinò, F., Sciacca, S., Clemenza, F. Peripheral Artery Disease and Continuous Flow Left Ventricle Assist Device: An Engaging Complement Analysis May Help to Guide Treatment (2018) 42 (7), pp. 756-759.
- Scardulla, F., Hu, S., D'Acquisto, L., **Pasta, S.**, Barrett, L., Blanos, P., Yan, L. A novel multi-wavelength procedure for blood pressure estimation using opto-physiological sensor at peripheral arteries and capillaries

INTELLECTUAL PROPERTY

Pasta S. Scardulla C. Method and system for the evaluation of the risk of aortic rupture or dissection in an individual with an ascending thoracic aortic aneurysm. WO/2018/220573

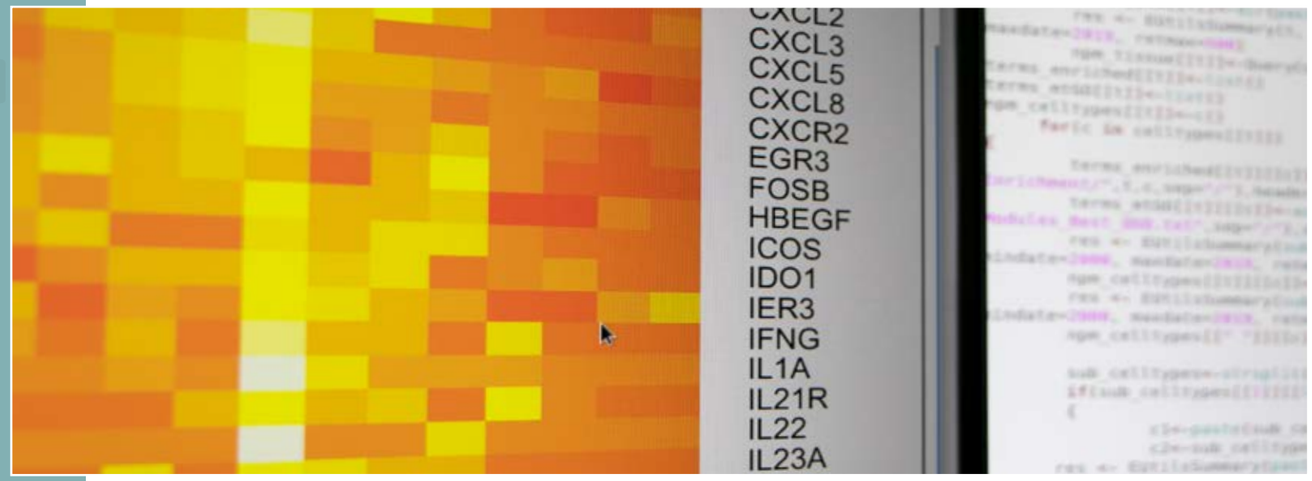
TECHNOLOGY PLATFORMS

The translational research engine of the Fondazione Ri.MED envisages the development of competence and technology platforms to support discovery and preclinical development projects, both for traditional drug discovery as well as for regenerative medicine and immunotherapy approaches.

The **Computational Chemistry platform** allows to perform molecular dynamics studies and to select potential drugs through the silico screening of millions of molecules on the therapeutic targets of interest; the chemo-informatic infrastructure has been implemented for the structured analysis of the chemical-physical properties and for the commercial availability of the molecules.
Of great relevance for the identification and validation of new therapeutic targets, as well as for the analysis of biological matrix data, it is the **Bioinformatics platform** that uses open source software and proprietary applications.
The **Biophysics and Structural Biology platform** is dedicated to the production and purification and to the three-dimensional study of proteins of interest, and plays a fundamental role both in screening and in identification of hit and lead, and in the functional and pathophysiological study of proteins of therapeutic interest. The **Computational Bioengineering platform** aims at the development of predictive models based on biomechanical models integrated with clinical and preclinical data.
The **Proteomics platform** supports the identification of new pharmacological targets and biomarkers, and the study of potential side effects of particular therapeutic molecules.
During 2018 the new **Magnetic Resonance Imaging platform** was defined, with two 3T and 7T spectrometers, and expertise for the analysis of multimodal data and images for the predictive diagnosis of pathologies and relapses.
The platforms will be further strengthened during 2019 both in terms of human resources and instrumentation, also thanks to CheMISt, regional funding for the creation of an integrated laboratory of Drug Design, Biophysics and Screening.

- Bioinformatics
- Bioengineering
- Structural Biology and Biophysics
- Computer Aided Drug Design
- Magnetic Resonance Imaging
- Proteomics

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. 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. To this purpose, the group developed new algorithms useful to analyze gene expression profiles and microRNA interaction networks, and it is going to make them available through web-tools.

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 aim to realize user friendly pipelines for data analysis by using the software Knime. In order to better satisfy the collaborators needs we are able to enrich our analysis by comparing them with the results obtained with the software Ingenuity Pathway Analysis.

Hardware
3 Workstations
Server in HPC mode: 248 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 AGO2 and GW182 RIP-Chip experiments. In the next future, we will extend the application to other RISC proteins, for example AGO1.

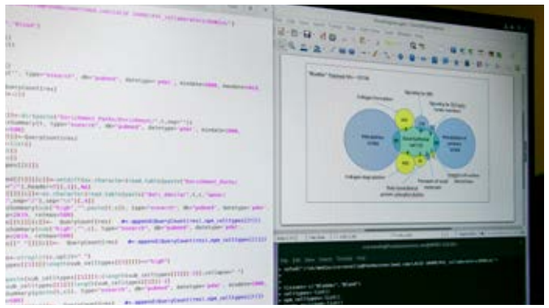
DE.SSA (Differential expression by Single Sample Analysis)
We are developing an algorithm useful for identifying differentially expressed genes by comparing two single samples, avoiding the necessity of experimental replicates. Currently, the algorithm is optimized to identify differentially expressed genes in RIP-Chip experiments, but we aim to extend the application to the comparison of two different tissue conditions, i.e. tumor vs normal tissue.

PUBLICATIONS

Perconti G, Contino F, Rubino P, Bivona S, Feo S, Giallongo A, Coronnello C. (2018) AGO2 and GW182 IP show different characteristics in co-immunoprecipitated RNA features, BMC Bioinformatics, *in press.*

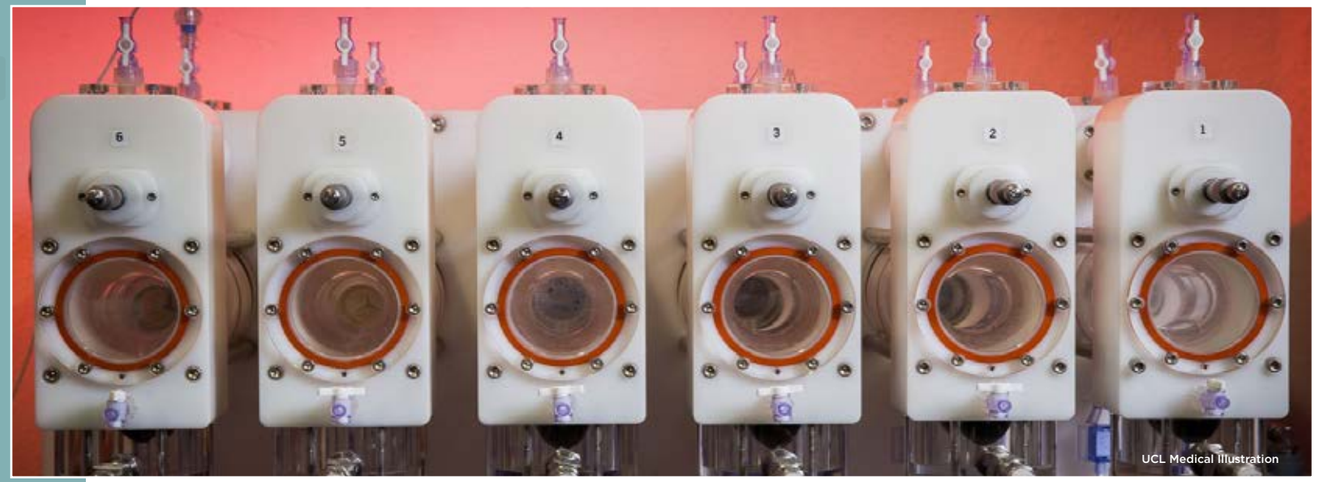
Iacovoni A, Bellavia D, Coronnello C, Simon M, Link C, Falletta C, Romano G, Sciacca S, Di Gesaro G, Maalouf J, Pilato M, Gorcsan III J, Terzi A, Clemenza F (2018) Predicting Acute and Chronic Right Ventricular Failure in Patients Undergoing Left Ventricular Assist Device Implant: The Importance of Right Atrial Strain and Regional Deformation of the Right Ventricular Free Wall, The Journal of Hearth and Lung Transplantation, 37 (4) Supplement https://doi.org/10.1016/j.healun.2018.01.982.

Gallo A, Agnese V, Coronnello C, Raffa GM, Bellavia D, Conaldi PG, Pilato M, Pasta S (2018) On the prospect of serum exosomal miRNA profiling and protein biomarkers for the diagnosis of ascending aortic dilatation in patients with bicuspid and tricuspid aortic valve, International Journal of Cardiology 273, 230-236, https://doi.org/10.1016/j.ijcard.2018.10.005.



COLLABORATIONS:
IBIM-CNR, Palermo, Italy
University of Palermo, Italy
University of Pittsburgh, U.S.A.

Bioengineering
Platform



CONTACTS:
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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, Lausanne, Switzerland
Great Ormond Street Hospital for Children, London, UK
IRCCS - ISMETT, Palermo, Italy
Mines Saint Etienne, France
Politecnico di Milano, Italy
IRCCS Policlinico San Donato, Italy
University of Padova, Italy
University of Palermo, Italy
Université de Technologie de Compiègne, France
University of Pittsburgh, U.S.A
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

The Bioengineering division is currently undertaking a major expansion of its technology platform, integrating the following facilities and equipment:

- 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 pathologies

PUBLICATIONS

Provaggi, E., Capelli, C., Rahmani, B., Burriesci, G., Kalaskar, D. M. (2018) 3D printing assisted finite element analysis for optimising the manufacturing parameters of a lumbar fusion cage. Materials and Design, https://doi.org/10.1016/j.matdes.2018.107540.

Gallo, A., Agnese, V., Coronnello, C., Raffa, G.M., Bellavia, D., Conaldi, P.G., Pilato, M., Pasta, S. (2018) On the prospect of serum exosomal miRNA profiling and protein biomarkers for the diagnosis of ascending aortic dilatation in patients with bicuspid and tricuspid aortic valve. International Journal of Cardiology 273:230-236.

Tango, A.M, Salmonsmith, J, Ducci, A., Burriesci, G. (2018) Validation and Extension of a Fluid–Structure Interaction Model of the Healthy Aortic Valve. Cardiovascular Engineering and Technology 9(4): 739-751.

Mendez, V., Di Giuseppe, M., Pasta, S. (2018) Comparison of hemodynamic and structural indices of ascending thoracic aortic aneurysm as predicted by 2-way FSI, CFD rigid wall simulation and patient-specific displacement-based FEA. Computers in Biology and Medicine 63:221-229.

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Bosi, G.M., Cook, A., Menezes, L., Schievano, S., Torii, R., Burriesci, G. (2018) Computational Fluid Dynamic Analysis of the Left Atrial Appendage to Predict Thrombosis Risk. Frontiers in Cardiovascular Medicine 5:34. doi: 10.3389/fcvm.2018.00034.

Falletta, G., Pasta, S., Raffa, G.M., Crino, F., Sciacca, S., Clemenza, F. (2018) Peripheral Artery Disease and Continuous Flow Left Ventricle Assist Device: An Engaging Complement Analysis May Help to Guide Treatment. Artificial Organs 42(7):756-759.

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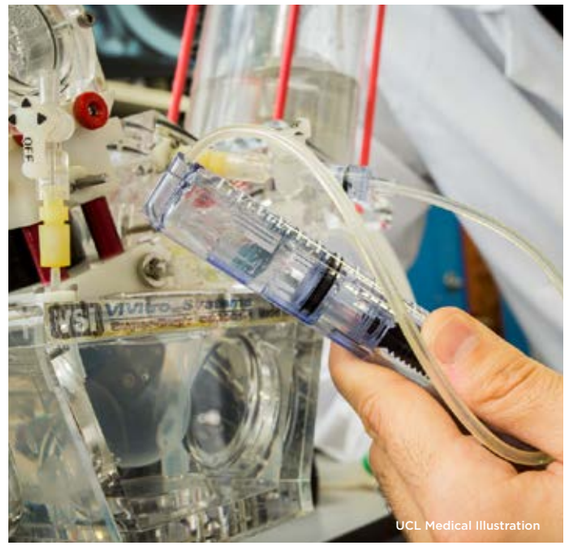
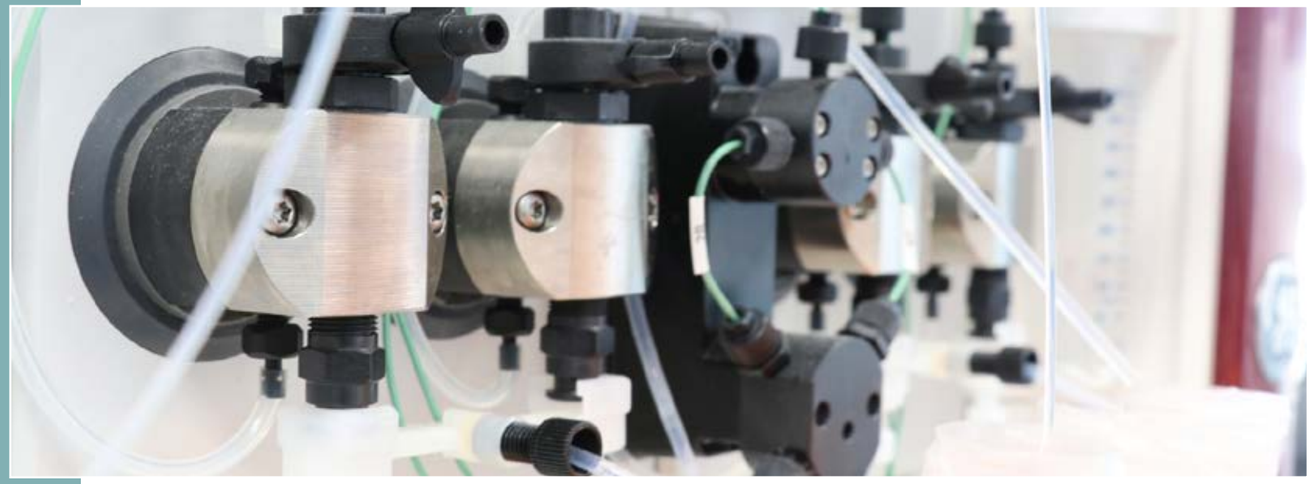


Structural Biology and Biophysics
Platform



CONTACTS:
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The Group of Structural Biology and Biophysics aims to provide 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 certain pathologies. 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.

Expertise

- Development of methods for expression and purification of recombinant proteins;
- Determination of size, shape, folding and thermodynamic stability of macromolecules;
- Identification of ligands by screening and structure-guided approaches, including fragment-base screening by NMR;
- Kinetic and thermodynamic properties of interactions;
- Biochemical activity assays.

Technology platform

- Wet lab for cloning, expression and purification of recombinant proteins;
- Octet Red96 - Bio-Layer Interferometry technology;
- MicroCal PEAQ-ITC Isothermal Titration Calorimeter;
- J-1500 Circular Dichroism Spectrophotometer;
- 800 MHz NMR spectrometer with cryogenically-cooled probe for 1H/13C/15N multiple-resonance experiments;

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
- Impact of molecular crowding on protein folding
- Structural and biophysical studies on proteins involved in the epigenetic regulation of tumor pathologies (SIRT1, KDM4)

COLLABORATIONS:

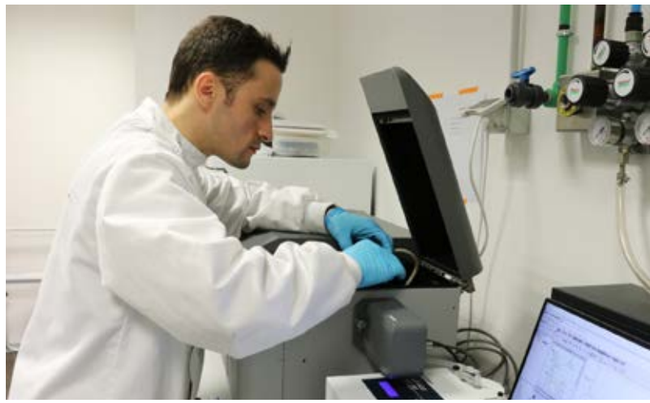
King's College London, UK
Scuola Normale Superiore di Pisa, Italy
Università degli Studi di Perugia, Italy
Università degli studi della Campania Luigi Vanvitelli, Italy
Università degli Studi di Palermo, Italy
Biophysics Institute - CNR, Palermo, Italy

PUBLICATIONS

Martínez-Lumbreras S.*, Alfano C.*, Kelly G., Atkinson R.A., Krysztofinska E.M., Flanagan K.A., Camp A.H. and Isaacson R.L. (2018) Solution structure of B. subtilis Sigma G inhibitor CsfB reveals a new fold. Structure, 26(4):640-648.

Pecci A., Ragab I., Bozzi V., De Rocco D., Barozzi S., Giangregorio T., Ali H., Melazzini F., Sallam M., Alfano C., Pastore A., Balduini C. and Savoia A. (2018) Thrombopoietin mutation in congenital amegakaryocytic thrombocytopenia treatable with romiplostim. EMBO Molecular Medicine, 10:63-75.

Zacco E., Graña-Montes R., Martin S.R., Sanchezde Groot N., Alfano C., Tartaglia G., Pastore A. (2019) RNA as a key factor in driving or preventing self-assembly of the TAR DNA-binding protein 43. JMB, doi: 10.1016/j.jmb.2019.01.028.



Computer Aided Drug Design
Platform

CONTACTS:
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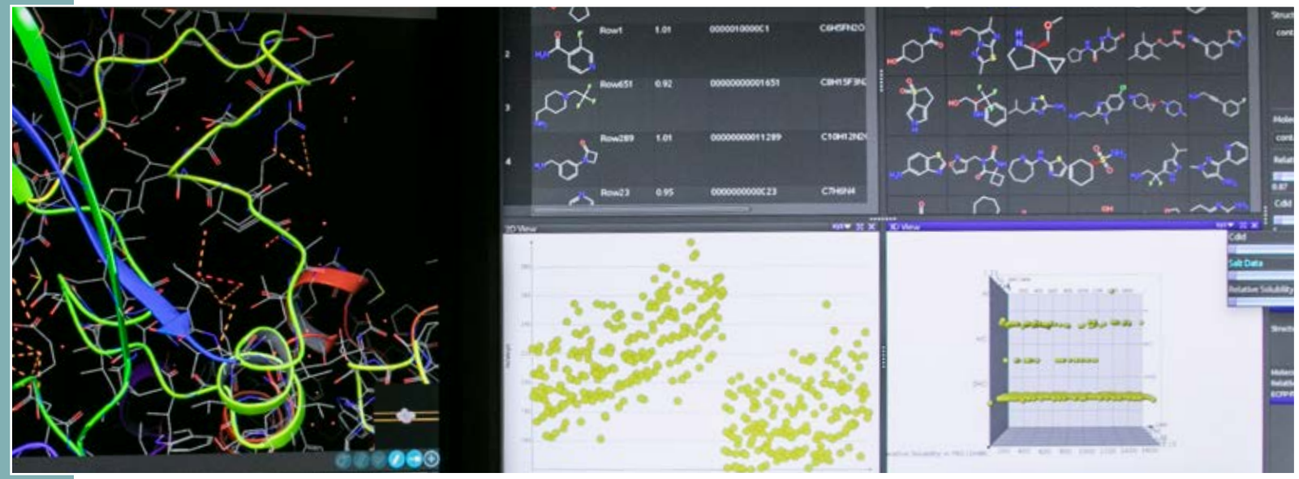
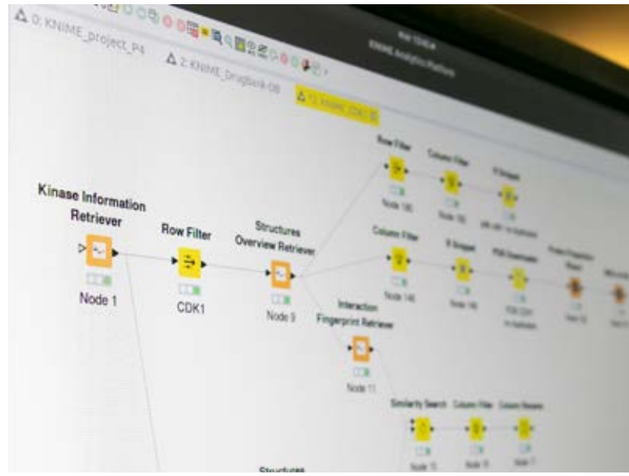
The CADD group at Ri.MED Foundation is mainly focused on the identification and optimisation of biologically active molecules through the use of in silico techniques both in the field of virtual screening and chemoinformatics. The team has matured different experiences in the field of medicinal chemistry and computational chemistry. All these expertise is used to design chemical libraries and create and validate reliable models to be used for virtual ligand screening (VLS), prior to experimentally validate results through biological or biophysical assays. The computational chemistry group is also involved in the chemical space exploration and enrichment optimisation of available virtual molecular libraries to be used for high throughput screening (HTS) campaigns. Another important goal of the group is the use of mixed techniques such as virtual screening techniques/Molecular dynamics in order to deepen understanding of bi-molecular interactions and the binding mode for small molecules targeting a specific protein. Recently the CADD group moved also into the field of protein-protein interactions and protein-nucleic acids interactions.

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 Philadelphia, USA
University of Naples Federico II, Italy
Italian National Council of Research (CNR), Italy
University of Palermo, Italy





Expertise

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

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)
- Gromacs
- RDKit
- KNIME

Hardware
- 4 Workstation
- Server in HPC mode: 248 CPUs and 2 x NVIDIA Tesla K80

Calculation capability:
Library optimisation: ~ 6,000 molecules/min
Virtual Screening HTVS: ~ 5,000 molecules/min
Virtual screening SP: ~ 1,500 molecules/min
Molecular Dynamics: ~ 150 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

The group is currently involved in several projects focused on the following targets:

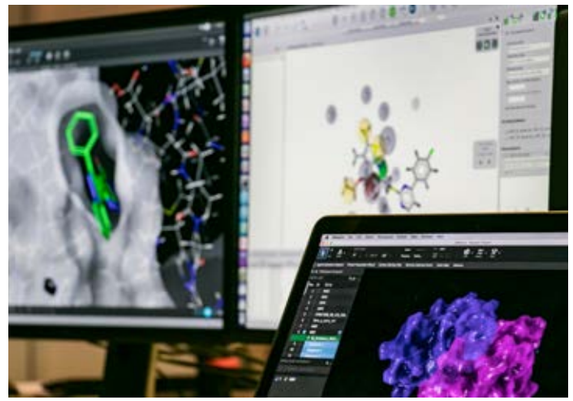
- Design of selective inhibitors of the **CD14** target involved in age-related degenerative maculopathy.
- Design of inhibitors of **NLRP3** as targets of inflammatory pathology.
- Research of protein modulators involved in the epigenetic regulation of cancer (**SIRT1, KDM4, PDH1, EZH2**).
- Design and development of **CDK1** inhibitors involved in cancer.
- Research of 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 and TOM20 / alpha-synuclein complexes**.
- Study of selective modulators of **mitochondrial CB1** receptor.
- Use of chemogenomic approaches for the research of active molecules in the **Huntington** disease.
- Study of chondrocytic differentiation pathways involving **SMAD 2 and 3** and study of potential-active molecules inducing this process.

PUBLICATIONS

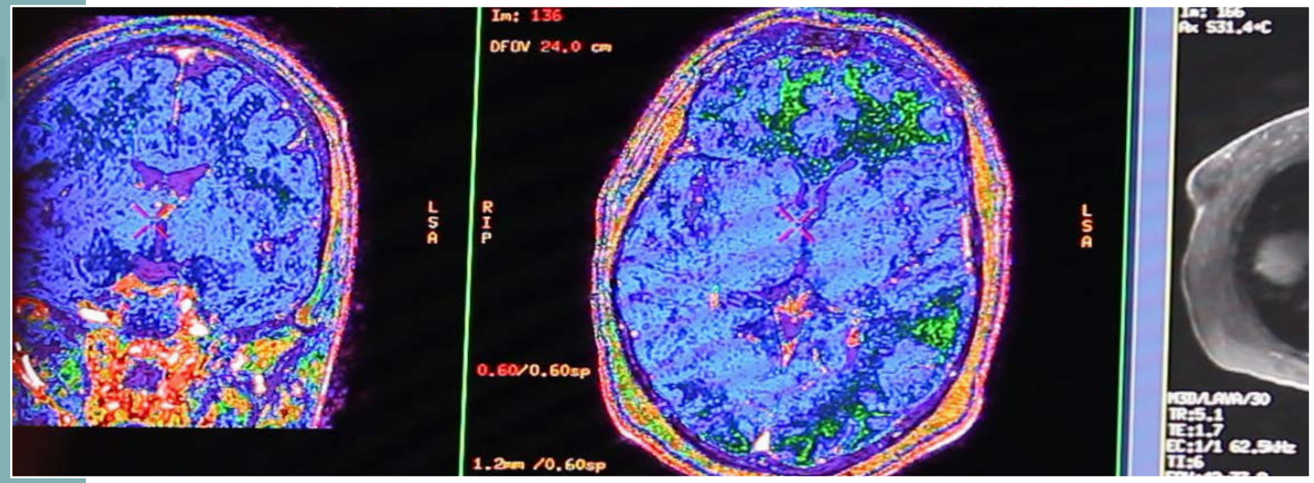
Perricone U., Gulotta M.R., Lobino J., Parrino B., Cascioferro S.M., Diana P., Cirrincione G., Padova A., An overview of recent Molecular Dynamics applications as medicinal chemistry tool for undruggable sites challenge, MedChemComm, 2018, DOI: 10.1039/C8MD00166A.

Perricone U., Wieder M., Seidel T., Langer T., Padova A., The use of Dynamic Pharmacophore in Computer Aided Hit Discovery: a case study, Rational Drug Design: Methods and Protocols, Springer (Book Chapter), 2018, DOI: 10.1007/978-1-4939-8630-9_19.

Gorska-Ponikowska M., Kuban-Jankowska A., Eisler S., **Perricone U.**, Lo Bosco G., Barone G., Nussberger S., Mitochondrial biogenesis as a target for 2-methoxyestradiol in osteosarcoma cancer cells, Cancer Genomics Proteomics, 2018, DOI:10.21873/cgp.20067.



Magnetic Resonance Imaging
Platform



CONTACTS:
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The Magnetic Resonance Imaging (MRI) 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 veterinary, a physics and an informatician expert in analysis and elaboration of bio-medical and imaging data, and it was competently formed to acquire and analyze MRI data in small animals and patients, in collaboration with the institutions hosting the instrumental facilities. The MRI platform has access on two NMR spectrometers (3T and 7T), acquired by IspeMi project, and based at IRCCS ISMETT and Istituto Zooprofilattico Sperimentale, respectively. In 2019, we expect to enrich the platform with instruments based on alternative (Bioluminescence) or integrative (PET) technologies, in order to offer more options for *in vivo* imaging data analysis.

COLLABORATIONS:
IRCCS - ISMETT, Palermo, Italy
Istituto Zooprofilattico Sicilia (IZS), Palermo, Italy

Expertise

- Magnetic Resonance Imaging (T1, T2, DP, DWI, ADC and DCE)
- Spectroscopy on phantoms, *in-vivo* and *ex-vivo* samples
- Independent operator system implementation of automatic and semi-automatic analysis, registration and segmentation of multimodal images (MR / PET / CT and histology)
- Segmentation and 3D modeling of biomedical images for localization, predictive diagnosis and volumetric monitoring of tumor pathologies
- Intelligent techniques for the analysis of multimodal data and images for predictive diagnosis of pathologies and relapse
- Phantom design and creation of phantoms for spectroscopy and morphovolumetric studies

Technology platform

At Istituto Zooprofilattico Sperimentale:

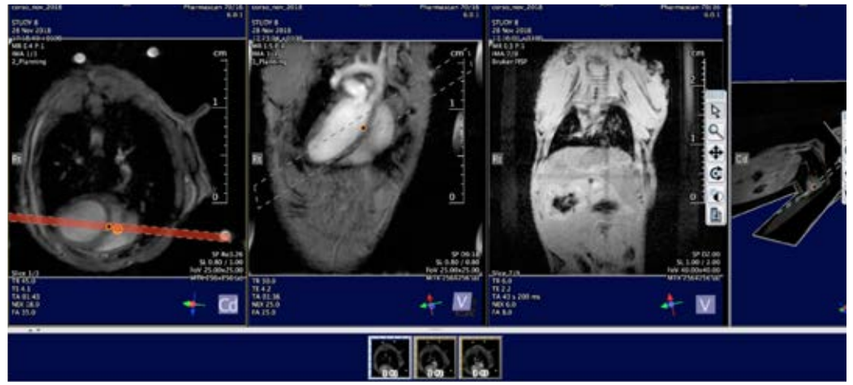
- Bruker Pharmascan 70/16 (7 Tesla). Available coils:
  - Mouse and rat brain 2x2 receive surface array coils
  - Mouse and rat transmit-receive volume coil (40 mm inner diameter and 75 mm outer diameter)
  - Rat body 8x2 transmit volume array coil (72 mm inner diameter and 89 mm outer diameter)
- Software: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

At IRCSS ISMETT

GE DISCOVERY MR 750 W 3 Tesla
New diagnostic system for High-Field Magnetic Resonance (3.0 T), addressed to the analysis of the whole body with top clinical efficiency. It allows, thanks to an enhanced hardware and a new user inteface, to explore new frontiers on all the advanced clinical application fields (neuro, body, breast, angio, osteoarticular, cardio, etc.).

ACTIVE RESEARCH PROJECTS

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



Proteomics
Platform

CONTACTS:
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The proteome is the entire set of proteins that is expressed by a cell, tissue or organism. The systematic high-throughput analysis of proteomes, known as proteomics, enables the identification of proteins and their relative content within biological samples. Furthermore, proteomics allows quantification of differentially regulated proteins across multiple conditions. Nowadays the "one gene-one protein hypothesis" is clearly outdated, with more than 90,000 different proteins having been identified in the human proteome compared to 20,000 entries of the human genome.
Thus, compared to the most advanced methods in genomics or transcriptomics, proteomics can be preferential for analysis of proteins that can be post-translationally regulated.
Proteomics has broken through over the past decade with the evolution of several approaches, mainly mass spectrometry-based technologies for large-scale study of proteins. Proteomic applications in preclinical and clinical research are numerous.
They span from identification of novel potential drug targets, to discovery of disease-associated biomarkers and prediction of drug-dependent side-effects.

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
- Phosphopeptide enrichment (PTM)
- Metabolic labelling, SILAC
- Isotopic labelling, TMT
- Label free quantitative proteomics
- Western Blot
- SDS-PAGE
- Quantitative and qualitative analysis of predicted and/or annotated proteins by liquid chromatography tandem mass spectrometry (LC-MS/MS) with Bottom Up and Shot-gun approaches.

Technology platform

Hardware

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

Software:

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

Sistema Nano LC UltiMate 3000 utilizzato per la separazione dei peptidi, connesso con lo spettrometro di massa Q-Exactive per l'analisi LC-MS / MS.

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.

ACTIVE RESEARCH PROJECTS

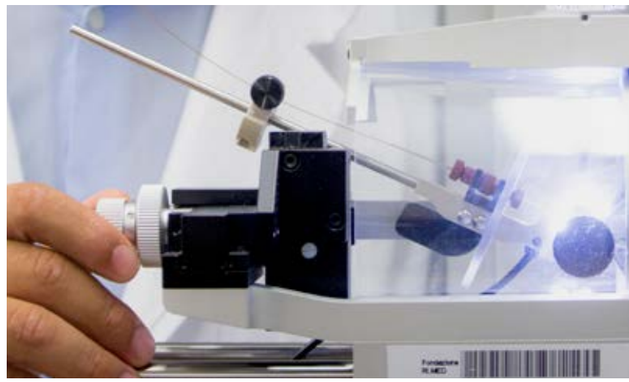
Identification of novel ADAM17 substrates: ADAM17 is a member of disintegrin and metalloproteinases with crucial roles in development and inflammation. Cutting-edge methods in proteomics will be used to identify novel ADAM17 substrates, which may link function of this protease to undiscovered patho-physiological processes.

iRhom-dependent substrate selectivity of ADAM17: the inactive rhomboids, namely iRhom1 and iRhom2, have emerged as major regulators of ADAM17 activity, being able to direct ADAM17 towards the cleavage of specific groups of proteins. Secretome analysis will be used to identify ADAM17 substrates whose cleavage is specifically regulated by either iRhom.

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, *in press*



GRANTS

Ri.MED supports the realization of its scientific activity through funding opportunities offered by public and private bodies, regional, national and international institutions. The work aimed at obtaining research funding is therefore a strategic activity for the Foundation. For this reason, a Grants office has been set up, which is responsible for:

- **Selecting financial programs to support biomedical research**
- **Specialized training and international cooperation**
- **Submitting proposals - also in collaboration with other bodies**
- **Managing relations with the administrations holding the financing programs, as well as coordinating and supervising the approved projects.**

During this year the activities related to the five projects funded in 2017 continued; in 2018 three new projects, two of which relate to the National Operational Program for research funded by MIUR and one financed by the Sicilian Region within the Transfer Activities Plan for innovative methodologies in the field of biotechnology, were funded Among the activities in 2018, it is worth mentioning the submission of three projects for the "Patto per il SUD" funding, three European projects, two for the Innovative Medicine Initiative and one for Marie Curie Sklodowska for the Innovative Training Networks - and twelve proposals for PhD with innovative industrial characterization.

Active projects in 2018

CheMISt
Computational Molecular Design e Screening
Funding: Sicilian Department of Productive Activities - Patto per il Sud

The goal of the project is the development of an integrated laboratory and a team of scientists with interdisciplinary skills able to support the Ri.MED research and become a reference point with high added value at regional, national and international level for public research institutions and private companies. Using the “lab hosting” format, the project involves the implementation of 4 operating units: 1) Structural Biology and Biophysics; 2) Computational and Medicinal Chemistry; 3) High Throughput Screening; 4) Bio-engineering.

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
Funding: Italian Ministry of Health -Ricerca Finalizzata 2013.

A prospective, double-blinded, randomized phase II study of two parallel groups designed to include 106 consecutive subjects who will undergo Liver Transplantation for the first time. The aim of the project is to evaluate the efficacy of the administration of simvastatin during liver procurement, in preventing ischemia/reperfusion injuries and to study whether the simvastatin, which is a very cheap vasoprotective compound, might be a rapid and useful pharmacological support to donor surgeons for increasing the viability of the harvested organ from deceased donors.

GMP Facility
Laboratori di Ricerca e Servizi Diagnostici e Terapeutici dell’Istituto Mediterraneo per i Trapianti e le Terapie ad Alta Specializzazione
Funding: Sicilian Department of Productive Activities PO FERS Sicilia 2014-2020

The aim is to strengthen Ri.MED-ISMETT research infrastructure through the acquisition of new equipment and technologies able to support of clinical and research activities and to accelerate the translation of the results to the patients.

OACTIVE
Advanced personalised, multi-scale computer models preventing OsteoArthritis
Funding: European Commission Research and Innovation Action Horizon 2020 SC1-PM-17

The project aims to elaborate models for better osteoarthritis diagnosis and cure, adopting a multi-scale holistic approach, where patient-specific information from various levels, including cell, tissue, organ and whole body will be integrated and combined with behavior modelling and social/ environmental risk factors.

Scientific projects approved for funding in 2018

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

The objective is to implement an innovation of a process on the vegetation waters of the oil production chain that allows: the extraction and repurposing of pharmacologically active organic compounds, the abatement of their polluting power and the possible reuse in the production processes of the treated water.

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

The goal is to create a computational tool to simulate the sensorial platform as a whole of sensors and therefore of vital and environmental parameters that will be collected during the clinical work-up phase. The simplicity and versatility of the computational implementation will allow to quickly simulate different virtual scenarios of the possible alterations of the vital and environmental signals associated with a pathological condition.

PLAN OF ACTIVITIES "TRANSFERS FOR INNOVATIVE METHODOLOGIES IN THE FIELD OF BIOTECHNOLOGIES 2018”
Funding: Stability Law of Sicilian Region- Health Department-DASOE

The objective is to support the research activities of the Ri.MED-ISMETT cluster in the fields of regenerative medicine, immunotherapy, bio-engineering and precision medicine.

Scientific projects submitted in 2018

AIM
Artificial Intelligence for Medicine- AIM
Call: Sicilian Department of Productive Activities - Patto per il Sud

The project has two objectives:
- to set up and develop laboratories of computer artificial intelligence for the research and development of translational and personalized medicine (AIM Laboratory);
- to set up a Center of Intelligence, Valorization of Patents and Technology Transfer for the Life Science Sector of the Sicily Region, including a Business Incubator and Open Science Lab for the training of primary and secondary school students and Sicilian university graduates.

DEVELOP
Call: Sicilian Department of Productive Activities - Patto per il Sud

The project has two objectives:
- to set up a Center for Synthesis and Preclinical Research of Life Science Products (innovative drugs, biomarkers and biomedical products) for the toxicology and pharmaceutical characterization including laboratories with GLP (Good Laboratory Practice) and GMP certifications (Good manufacturing practice);
- to strength ISPeMI Consortium, maintenance and enhancement of the Preclinical Research Laboratory based on the structures of the ISPeMI Consortium at the Zootechnical Institute and the Zooprofilattico Institute, for preclinical proof of concept studies using in vivo imaging, bioluminescence, toxicology, bio-analytics, etc.

SICILY INSTITUTE FOR HEALTH
Call: Sicilian Department of Productive Activities - Patto per il Sud

The strategic objective of the project is the development in Sicily, in the province of Palermo and Trapani, of two advanced and integrated centers of preventive medicine. The Center for the Prevention and Treatment of Non-Communicable Chronic Diseases will offer research and diagnostic services for the prevention and treatment of chronic non-communicable diseases (hepato-bilio-pancreatic and gastrointestinal, cardiovascular system, diabetes and metabolic disease, oncological diseases).

DEVENET
Development Of Technologies For The Design Of Light-Responsive Molecular Systems For Retinal Diseases
Call: European Commission H2020-MSCA-ITN-2018

European training project for the development of combined pharmacological/genetic approaches to improve and restore vision in retina diseases and in particular in *retinitis pigmentosa.*

INNODRUG
Federated and privacy preserving for machine learning for INNovative DRUG discovery.
Call: Innovative Medicine Initiative (IMI2) TOPIC 3

The goal of the project is to develop a cutting-edge, powerful and flexible strategy and a technology platform for sharing federated machine learning models that protect privacy.
Principal Investigator: U. Perricone

Generation and immunoregulatory activity of keto-polyunsaturated fatty acids (PUFA) produced by the enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in malignant pleural effusions of patients with lung cancer
Call: Roche for Research 2018

The main objective of the project is to test the hypothesis that alterations of the enzymatic axis Cox-2/15-PGDH/PTGR2 and its metabolic profile in malignant pleural effusions of patients with lung cancer contribute to immune dysregulation and escape.
Principal Investigator: C. Cipollina

Innovative PhDs activated in 2018

Networks from Biological Big Data: analysis of the sample specific miRNome
Promoter University: Università degli Studi di Palermo
Foreign University: University of Pittsburgh
Industrial Partner: UPMC Italy
Tutor: Claudia Coronnello

Development of active drugs in molecular target therapy
Promoter University: Università degli Studi di Messina
Foreign University: University of Vienna
Industrial Partner: Sterling
Tutor: Alessandro Padova

Design and synthesis of molecules active in the epigenetic control of tumors
Università promotrice: Università degli Studi di Palermo
Promoter University: Università degli Studi di Palermo
Foreign University: University of Vienna
Industrial Partner: UPMC Italy
Tutor: Alessandro Padova

Knoweldge management techniques for the federation and interrogation of large distributed databases to support pharmaceutical design
Promoter University: Università degli Studi di Palermo
Foreign University: University of Vienna
Industrial Partner: QWINCE
Tutor: Ugo Perricone

Micromechanical modeling of aortic diseases
Promoter University: Università degli Studi di Palermo
Foreign University: Ecole Mines Saint Etienne
Industrial Partner: UPMC Italy
Tutor: Salvatore Pasta

Innovative PhDs submitted in 2018

Design of inhibitors of the CDK1/CiclinB1 protein complex as therapeutic chemotherapeutic agents
Promoter University: Università degli Studi di Palermo, Italy
Foreign University: University of Amsterdam
Industrial Partner: UPMC Italy
Tutor: Ugo Perricone

Design of tyrosine kinase inhibitors as therapeutic chemotherapeutic agents
Promoter University: Università degli Studi di Palermo
Foreign University: University of Amsterdam
Industrial Partner: UPMC Italy
Tutor: Ugo Perricone

Use of Federated Machine Learning techniques in the drug discovery process
Promoter University: Università degli Studi di Palermo, Italy
Foreign University: University of Vienna
Industrial Partner: Italtel
Tutor: Ugo Perricone

Models with concentrated parameters for cardiac pathologies
Promoter University: Università degli Studi di Palermo, Italy
Foreign University: École polytechnique fédérale de Lausanne
Industrial Partner: UPMC Italy
Tutor: Salvatore Pasta

Role of the interaction between alpha-synuclein and cell membranes in Parkinson's disease: *in-vitro* biophysical analysis and in cell molecular mechanisms
Promoter University: Università degli Studi di Palermo, Italy
Foreign University: Institute for Neurodegenerative Diseases - University of Pittsburgh – USA
Industrial Partner: UPMC Italy
Tutor: Caterina Alfano

Biomechanical analysis of the left atrial appendix for the prevention of thromboembolic events
Promoter University: Università degli Studi di Palermo, Italy
Foreign University: University College London, UK
Industrial Partner: UPMC Italy
Tutor: Gaetano Burriesci

Computational models for the analysis and classification of the morphological and hemodynamic parameters of the left atrial appendix
Promoter University: Università degli Studi di Palermo, Italy
Foreign University: UCL - University College London, UK
Industrial Partner: UPMC Italy
Tutor: Claudia Coronnello

Development of High Throughput Screening cell assays for the development of NLRP3 inhibitors and tests on experimental models of chronic inflammatory diseases
Promoter University: Università degli Studi di Palermo, Italy
Foreign University: University College London, UK
Industrial Partner: Institut de la Vision, Paris
Tutor: Chiara Cipollina

Study of peptides and peptidomimetics as modulators of protein-protein interactions in oncological and neurodegenerative diseases
Promoter University: Università degli Studi di Palermo, Italy
Foreign University: University of Dundee
Industrial Partner: Sterling
Tutor Alessandro Padova

Tolerogenic dendritic cells derived from bone marrow and residents for promoting operative tolerance in liver transplantation
Promoter University: Università degli Studi di Messina, Italy
Foreign University: CEMIR, Trondheim, Norway
Industrial Partner: UPMC Italy
Tutor: Ester Badami

Development of a new immunotherapy against Klebsiella pneumoniae based on probiotic yeasts Saccharomyces cerevisiae genetically engineered
Promoter University: Università degli Studi di Messina, Italy
Foreign University: CEMIR, Trondheim, Norway
Industrial Partner: UPMC Italy
Tutor: Bruno Douradinha

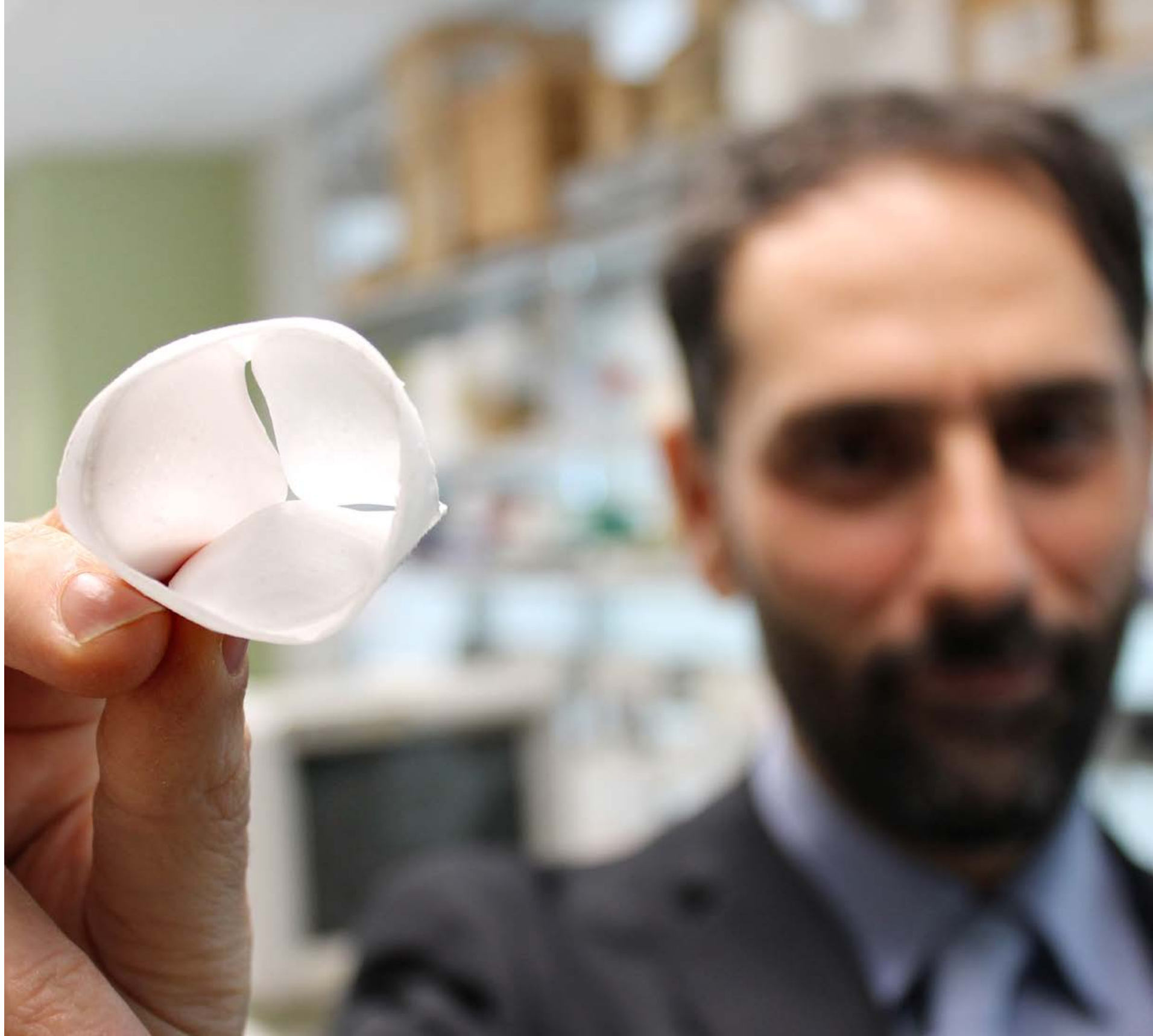
Interaction between mitochondrial dysfunction and reprogramming of the neuronal cell cycle: relevance in degenerative processes related to Alzheimer's dementia and Parkinson's disease
Promoter University: Università degli Studi di Catania, Italy
Foreign University: Institute for Neurodegenerative Diseases - University of Pittsburgh
Industrial Partner: UPMC Italy
Tutor: Roberto Di Maio

INTELLECTUAL PROPERTY AND TECHNOLOGY TRANSFER

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

The protection of intellectual property is a fundamental value for the Fondazione Ri.MED so as to developing an innovative model of research sustainability. For this reason, in 2017 the Intellectual Property and Technology Transfer area (IP & TT) was set up in order to support, promote and foster the progress of translational research through the enhancement of its application implications: patenting, patent license, industrial sponsorship and creation of technological spin-offs.

Thanks to the ability to communicate with universities and research institutions on one hand and with pharmaceutical and biotechnology companies on the other, the IP & TT area works to develop collaboration programs aimed at developing enabling technologies and new solutions for improving health and the patients' quality of life.



Patent portfolio up to 31.12.2018

DRUG DISCOVERY

Nitro-oleic acid (NO_2-OA) controlled release platform to induce regional angiogenesis in abdominal wall repair.
Fondazione Ri.MED - University of Pittsburgh

Novel nitro-nitrate-lipid intermediates that mediate nitrosating and alkylating reactions.
Fondazione Ri.MED - University of Pittsburgh

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

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

Probiotic yeasts as novel vaccination vectors.
Fondazione Ri.MED - IRCCS ISMETT

Mandrel-less electrospinning processing method and electrodes for bio-mimetic tendinous tissue engineering.
Fondazione Ri.MED - University of Pittsburgh

TISSUE ENGINEERING AND BIOMEDICAL DEVICES

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

Trans-atrial access for transcatheter valve repair or replacement.
Fondazione Ri.MED - University of Pittsburgh

Bi-layer Polyurethane - Extra Cellular Matrix Scaffolds for Improved Ischemic Ventricular Wall Remodeling.
Fondazione Ri.MED - University of Pittsburgh

A double components mandrel for electrospun stentless, multi-leaflet valves fabrication.
Fondazione Ri.MED - University of Pittsburgh

A Retrievable Self-expanding Non-thrombogenic Low-profile Percutaneous Tricuspid Valve Prosthesis.
Fondazione Ri.MED - University of Pittsburgh

A Method to Characterize the Complete Fiber Network Topology of Planar Fibrous Tissues and Scaffolds.
Fondazione Ri.MED - University of Pittsburgh

Three-layered, bio-inspired, small-diameter vascular graft for tissue engineering applications.
Fondazione Ri.MED - University of Pittsburgh

A microfluidic device for the optical monitoring of high throughput 3D single and multi-tissue microsystems during development, response to stress and to treatments.
Fondazione Ri.MED - University of Pittsburgh

Prevention of soft tissue ossification by controlled release.
Fondazione Ri.MED - University of Pittsburgh

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

Recruitment of mesencymal stem cells using controlled release systems.
Fondazione Ri.MED - University of Pittsburgh

Polyvinylpyrrolidone and Stearic acid coated Cranberry Extract for the Prevention of Dental Biofilm.
Fondazione Ri.MED - University of Pittsburgh

An organ chip to model mammalian joint.
Fondazione Ri.MED - University of Pittsburgh

High throughput mechanical activation device.
Fondazione Ri.MED - University of Pittsburgh

Ethyl lauroyl arginate and Stearic acid coated Cranberry Extract for the Prevention of Dental Biofilm.
Fondazione Ri.MED - University of Pittsburgh

Enhancing cranberry extract residence time on biofilm and pellicle-2.
Fondazione Ri.MED - University of Pittsburgh

A Stentless Biopolymer Heart Valve Replacement Capable of Living Tissue Regeneration.
Fondazione Ri.MED - University of Pittsburgh

An Expandable Percutaneous Venous Cannula for Use in Extracorporeal Cardiopulmonary Support.
Fondazione Ri.MED - University of Pittsburgh

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

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