Fondazione Ri.MED

SCIENTIFIC REPORT 2017

- DRUG DISCOVERY
- REGENERATIVE MEDICINE AND IMMUNOTHERAPY
- TISSUE ENGINEERING AND BIOMEDICAL DEVICES
- TECHNOLOGY PLATFORMS

ENG

Fondazione
Ri.MED

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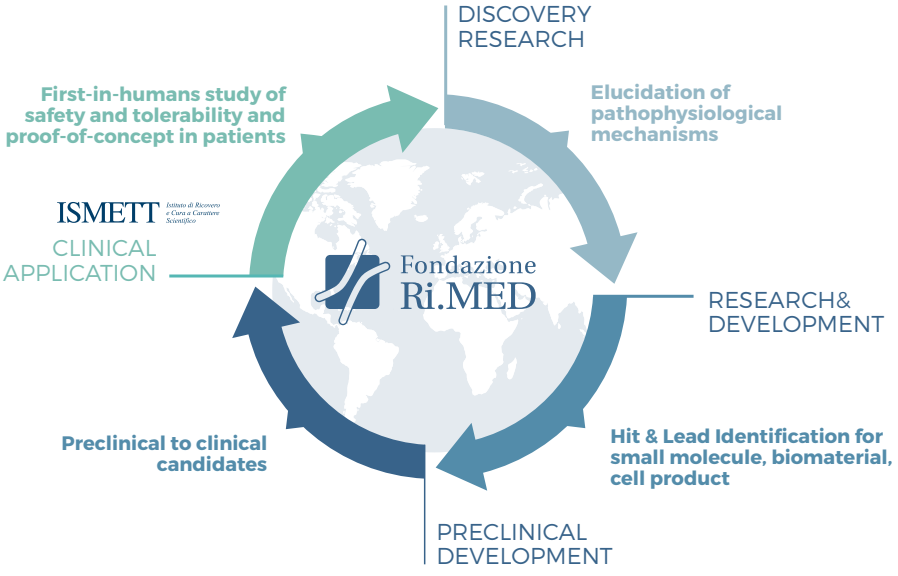
Alessandro Padova
DIRECTOR GENERAL

Statutory missions of the Foundation are the development of biotechnological and biomedical research approaches, aimed at transferring innovative therapies to patients, the dissemination of scientific knowledge and the training of highly qualified professionals in the Life Science *sector.*

Ri.MED translational research approaches are based on three main matrices: tissue engineering and bioengineering, drug discovery and immunotherapies and regenerative medicine.

The drug discovery and preclinical research activities are potentiated thanks to the wide network of collaborations and scientific alliances that Ri.MED develops with research institutions: research and preclinical development agreements are already operative, promoting the research and sharing of laboratories and resources with institutions at regional, national and international level.

TRANSLATIONAL RESEARCH



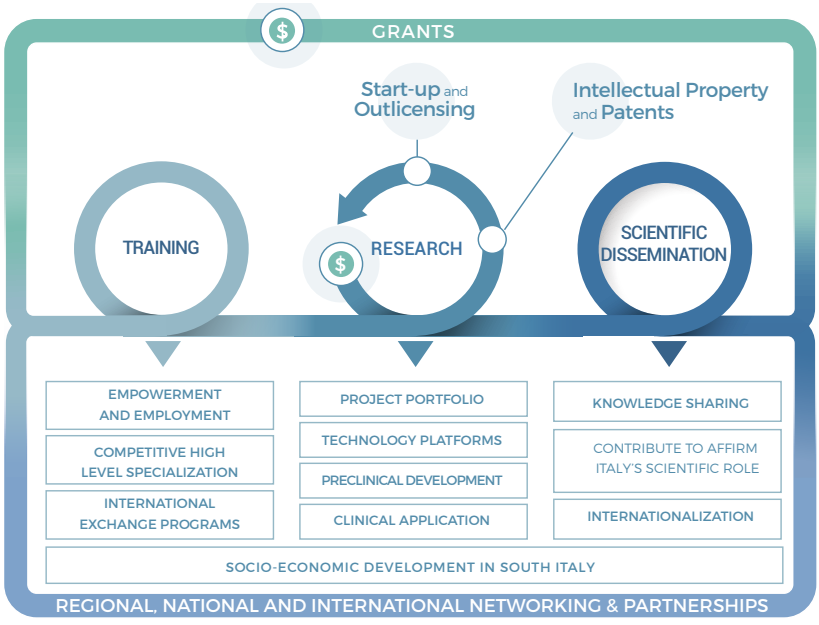
The aim of these collaborations is to integrate complementary skills to joint translational research projects, increasing the critical mass and the potential for success. Crucial it is the ability to create networks able to compete at regional, national and international level for funding research lines/areas.

In preclinical and clinical research, strategic are the partnerships with the University of Pittsburgh, UPMC and IRCCS-ISMETT, which have yielded proprietary intellectual property resulting in several joint patent applications. The generation of intellectual property represents a fundamental intangible asset in the valorization of the Fondazione Ri.MED, with the perspective of developing an innovative model of sustainable research. Some of these inventions, now in the preclinical development phase, are currently under evaluation for the creation of start-ups, for investments by venture funds and for the in-licensing by biotech and pharma companies.

Ri.MED now has a diversified and balanced project portfolio, led by a multidisciplinary team with clear milestones related to product development objectives from a bench to bedside perspective. The integration with the IRCCS ISMETT partner, allows to do truly translational research, thanks to complementary skills of discovery, preclinical and clinical research.

A recognition to all the people that work every day to achieve, with passion and resilience, the tangible objectives of our mission, improving patients' life, training new generations of researchers, and contributing to the socio-economic rebirth of Sicily and of Southern Italy.

MISSION AND BUSINESS MODEL



Dario Vignali
SCIENTIFIC DIRECTOR

Ri.MED Foundation's mission is to translate biotechnology and biomedical research into improved therapies for patients, and to deliver a positive socio-economic impact, especially in Sicily and Southern Italy. Ri.MED's mission is also to facilitate the recruitment, education and training of the next generation of Italian biomedical scientists and physician-scientists. Current translational research projects aim to address therapeutic needs in areas such as oncology, cardiovascular, neurodegenerative and metabolic disease, and organ-failure complications.

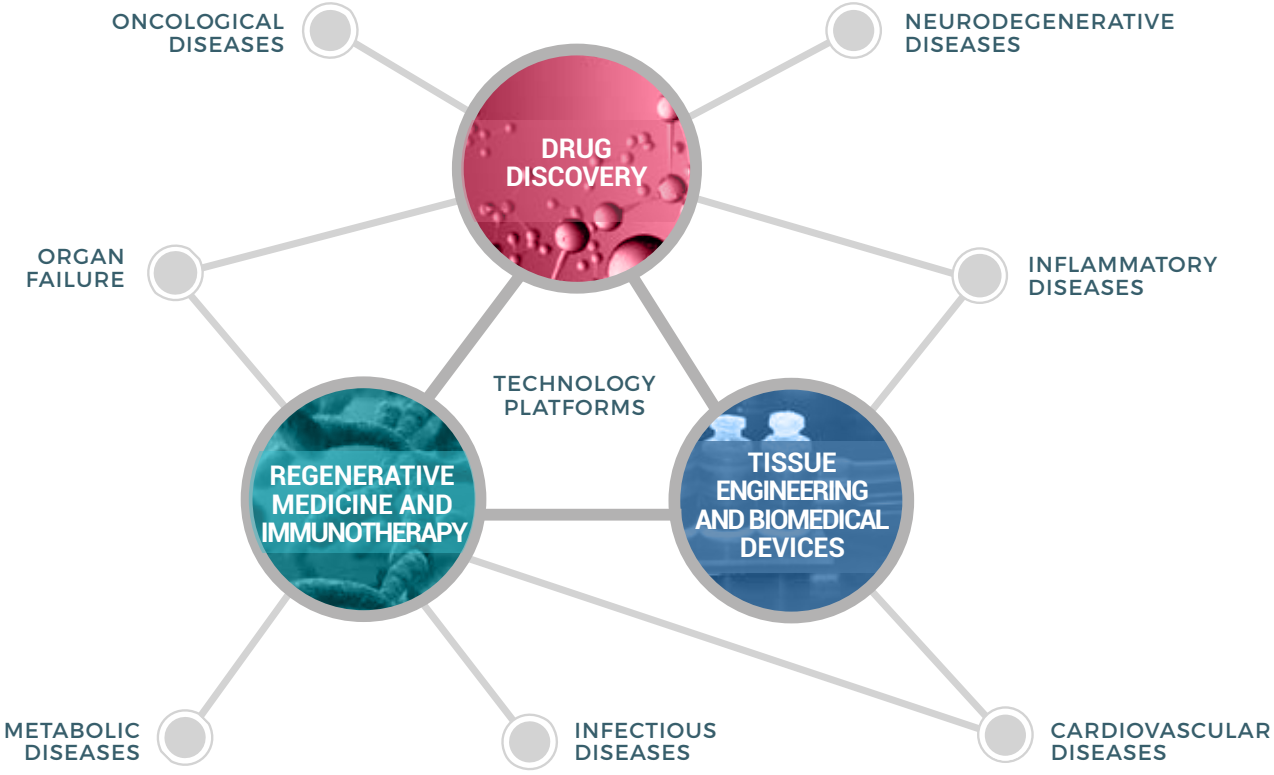
Translational research is focused on the complementary integration of resources and skills of different matrices: basic research, preclinical research and development of new therapies, medical devices and biomarkers, and finally clinical trials. It is thanks to the commitment of the founding partners, especially the Italian National Research Council (CNR), the University of Pittsburgh and the University of Pittsburgh Medical Centre (UPMC), that the Foundation has been able to develop truly translational research programs and facilitate the training of young Italian biomedical scientists.

Thanks to the strategic partnership with the Palermo-based IRCCS ISMETT, Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, it has been possible to foster multidisciplinary projects in the field of regenerative medicine and cellular therapies including the creation of a GMP cell factory to support clinical trials. The goal of the Foundation is to facilitate the exchange of know-how between physicians and researchers, and translation for new discoveries and novel therapies from bench to bedside. The Foundation is also committed to the development of novel immunotherapies for the treatment of cancer and other diseases, and to limit rejection in patients receiving organ transplants.

The Scientific Committee initiated a strategic review of Ri.MED's research and therapeutic focus in the last quarter of 2017 with the objective of developing a strategy to ensure competitiveness at the national and international level, to maximize the effective and efficient translation of research into clinical trials, and to deliver a meaningful socio-economic impact at the regional and national level. The Scientific Committee is also reviewing its recruitment and training strategy for young Italian biomedical scientists.

During 2018, our research strategy will be finalized and a medium-term research plan drafted in order to build a path towards the opening of the Ri.MED Center for Biotechnology and Biomedical Research (CBBR) in Sicily.

RESEARCH APPROACHES AND THERAPEUTIC AREAS



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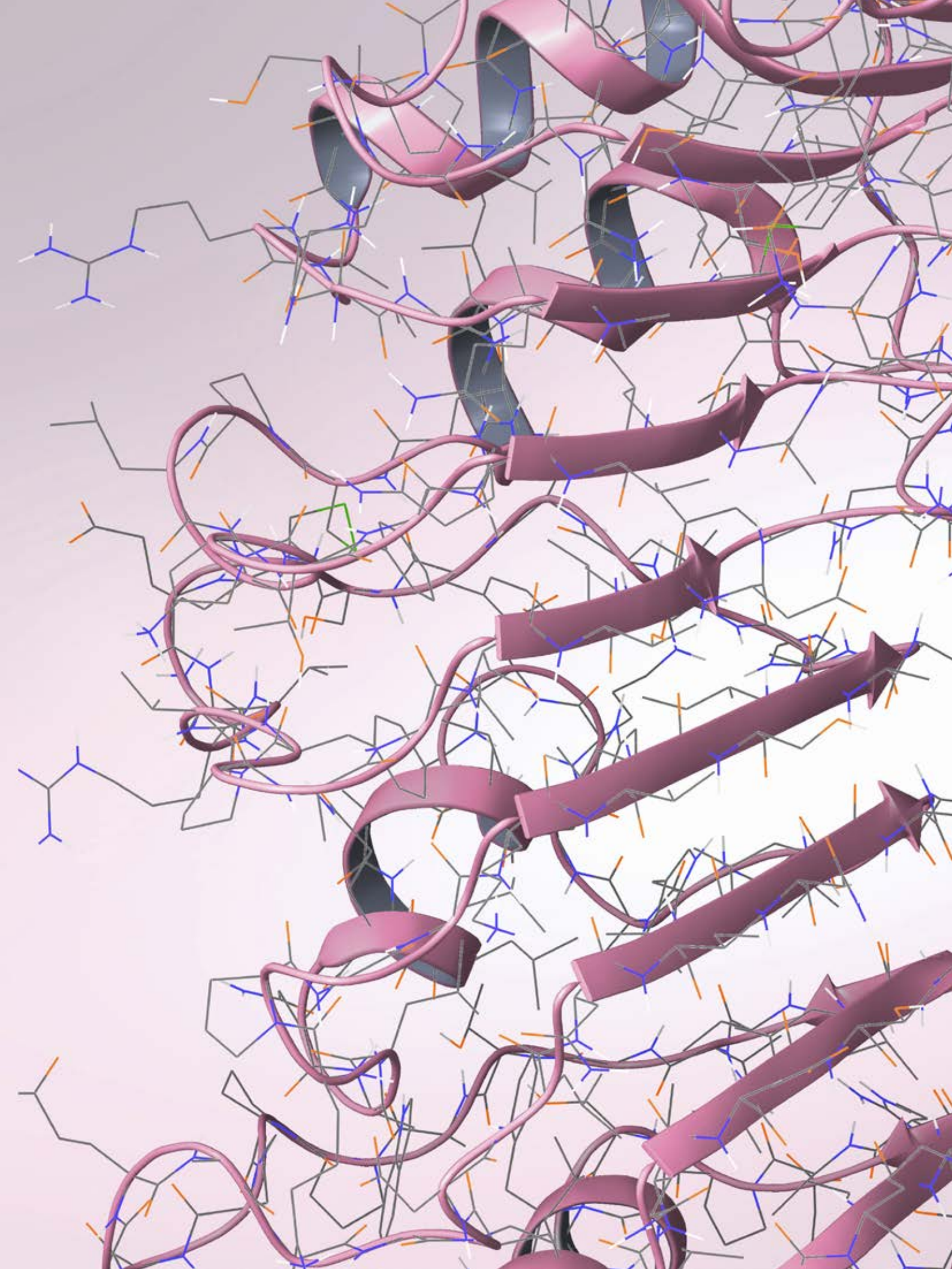

DRUG DISCOVERY

The researchers of the Fondazione Ri.MED are committed to elucidate pathophysiological mechanisms with own projects and in collaboration with research centers.

The study of biomolecular pathways integrated with genomic, proteomic, metabolic and secretomic data has led 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 currently in the drug discovery 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 virtual screening platform developed during 2017, the selection of hundreds of molecules of synthetic and natural origin has begun through structure-based techniques (docking) and ligand-based (pharmacophore).

Selected molecules are then synthesized or acquired to be biologically tested and validated with biophysical screening. The next phase, the biologically active molecules will be optimized through medicinal chemistry and then preclinical experimentation, the study of effectiveness through *in vivo* studies integrated with molecular imaging and the characterization of the pharmacokinetic and toxicological profile suitable for clinical trials on patients.

In parallel, predictive methods are being developed to monitor the efficacy of potential medicines and to stratify patients responding to therapy.

Therapeutic Area: **NEURODEGENERATIVE DISEASES**

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

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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. This is well distinct from approaches which have focused on identification of generic and non-specific antiaggregating agents such as methylene blue, polyols or other small molecules. 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. The assumption is nevertheless supported by increasing evidence: preliminary data both on ataxin-1 and ataxin-3 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. 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.

Results achieved in 2017

Our studies performed on ataxin-3, the protein responsible for the inherited spinocerebellar ataxia type 3 - the most common autosomal dominant spinocerebellar ataxia worldwide - have shown that both, non-expanded ataxin-3 (i.e. with 18 Gln repeats) and expanded ataxin-3 (i.e. with 50 Gln repeats), self-assemble into fibrils with markedly similar features over a large temperature range. The fibrils have an irregular structure, which can be interpreted either as a string of spheroidal beads or as a twisted, planar sheet. Notably, our results show that the formation

of these fibrils is strongly inhibited by the presence of polyUb chains, natural binding partners of ataxin-3, confirming our hypothesis that natural interactors could alter and control the function and assembly of those proteins involved in neurodegenerative diseases and fulfil a protective role against aberrant aggregation.

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, accepted.

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.

Wang A.F., Deighan P., Chen S., Barrasso K., Garcia C., Martínez-Lumbreras S., Alfano C., Krysztofinska E.M., Thapaliya A., Camp A.H., Isaacson R.L., Hochschild A. and Losick R. (2017) A Novel RNA Polymerase-binding Protein that interacts with a Sigma-Factor Docking Site. *Molecular Microbiology*, 105(4):652-662.

Alfano C., Sanfelice D., Martin S., Pastore A. and Temussi P. (2017) An optimized strategy to measure protein stability highlights differences between cold and hot unfolded states. *Nature Comm*, 8:15428.

Watts N., Zhuang X., Kaufman J., Palmer I., Dearborn A., Coscia S., Blech-Hermoni Y., Alfano C., Pastore A. Mankodi A. and Wingfield P. (2017) The Expression and Purification of ZASP Subdomains and Clinically Important Isoforms: High-affinity Binding to G-actin. *Biochemistry*, 56(14):2061-2070.

Bottega R., Nicchia E., Alfano C., Glembotsky A.C., Pastore A., Bertaggia-Calderara D., Bisig B., Duchosal M.A., Arbesú G., Alberio L., Heller P.G. and Savoia A. (2017) Gray platelet syndrome: Novel mutations of the NBEAL2 gene. *American Journal of Hematology* 92(2):E20-E22.

Goals for 2018

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.

Therapeutic Area: **NEURODEGENERATIVE DISEASES**

Generation and validation of a Zebrafish Model of Inherited Cerebellar Ataxia

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Brief description

Members of the calmodulin-binding transcription activator (CAMTA) family of proteins function as calcium-sensitive regulators of gene expression in multicellular organisms ranging from plants to humans. In humans, intragenic CAMTA1 rearrangements have been associated with nonprogressive congenital cerebellar ataxia (NPCA) and gait instability in several unrelated families. Common variants within CAMTA1 have also been reported to be associated with variation in human episodic memory and, more recently, CAMTA1 has been associated with survival in patients with sporadic amyotrophic lateral sclerosis (ALS). Studies in mouse demonstrated that CAMTA1 is an essential regulator of Purkinje cell function and survival, but the mechanistic basis of its function has not been defined. To explore the functions of CAMTA1 in vivo, we performed knock down experiments with camta1a antisense morpholino in zebrafish larvae and generated zebrafish CRISPR/Cas9 mutant lines for CAMTA1 zebrafish orthologues camta1a and camta1b. Knock down and knock out of camta1a in zebrafish resulted in neuronal degeneration, reduction in Purkinje cells numbers and motor abnormalities, approximating the phenotype observed in humans and mice.

Impact

Our results so far indicate that camta1-mutant zebrafish provide an ideal model organism for understanding the mechanistic basis of ataxias and cerebellar function. Further studies of the CAMTA-signaling pathway in the nervous system may contribute to the identification of therapeutic targets for intervention. The relevance of this project extends to the understanding of other forms of neuronal degeneration diseases in humans where mutations in molecular motors have been implicated.

Results achieved in 2017

- Identification of zebrafish camta1 orthologues and expression analyses (*in situ* hybridization).
- Knock down experiment with camta1a antisense morpholino, morphological and behavioral analyses.
- Generation of camta1a, camta1b CRISPR/Cas 9 mutant lines (camta1a-/-, camta1b-/- and double mutants camta1a-/-;camta1b-/- fish lines).
- camta1a-/-; camta1b-/- and camta1a-/-;camta1b-/- double mutants fish screening, morphological, functional and behavioral analyses.
- Cerebellar Purkinje neurons isolation, FACS sorting and transcriptome analyses of control and Camta1 KO mice.

Meetings

From fish to men: Zebrafish as a model system to study human CAMTA1-related ataxia. Seminar at *The zebrafish model in Biotechnology and Health Sciences*, December 2017, Palermo, ITA

Publications

Cianciolo Cosentino C. (2017). Paradigms for the Quantification of Behavioral Responses in zebrafish. In A. Celik, M. F. Wernet (Eds.) Decoding Neural Circuit Structure and Function (pp 223-239). *Springer.*

Mansouri M, Bellon-Echeverria I, Rizk A, Ehsaei Z, Cianciolo Cosentino C, Silva CS, Xie Y, Boyce FM, Davis MW, Neuhauss SC, Taylor V, Ballmer-Hofer K, Berger I, Berger P. (2016) Highly efficient baculovirus-mediated multigene delivery in primary cells. *Nat Commun*. (2016) 4;7:11529. PMID: 27143231.

Goals for 2018

Comparing the gene-expression profiles of Purkinje cells in control and Camta1-deficient mice we have identified a series of dysregulated genes in Camta1 mutants. Our next goal is to analyze selected genes in detail by morpholino-based knockdown and/or CRISR/Cas9 mutagenesis in zebrafish larvae in order to gain insights into the role of CAMTA1 in the cerebellum. The cerebellum is implicated in the acquisition, coordination, and calibration of motor activity. In zebrafish, the electrical activity of Purkinje and granule cells in the cerebellum can be readily recorded *in situ* in live organisms with intact sensory modalities and motor output. Our next goal is to investigate the impact of camta1a mutations in the electrical property and functional maturation of cerebellar Purkinje and granule cells. This will allow a better understanding of the mechanistic basis of ataxias and cerebellar function.

Therapeutic Area: **NEURODEGENERATIVE DISEASES**

Endocannabinoid stimulation in prevention of Temporal Lobe Epilepsy

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Brief description

During the last few years, the use of cannabinoids in the treatment of epilepsy has produced promising evidence of efficacy, but, to date, the possibility of the application in clinical practice remains highly debated due to the high potential for side effects related to a "Biphasic" and dose-dependent response to cannabinoids impact. It is still necessary to provide evidence on the mechanisms of action of cannabinoids. The understanding of the exact biochemical responses induced by cannabinoids in the central nervous system will help to optimize the therapeutic through the synthesis of new molecules with beneficial action (anti-epileptic in this case) similar to cannabinoids but with low potential in inducing undesirable effects. The creation of these new drugs will contribute to the treatment and prevention of epileptic disease, reducing its social and economic impact.

Impact

It is still necessary to provide evidence on the mechanisms of action of cannabinoids. The understanding of the exact biochemical responses induced by cannabinoids in the central nervous system will help to optimize the therapeutic through the synthesis of new molecules with beneficial action (anti-epileptic in this case) similar to cannabinoids but with low potential in inducing undesirable effects. The creation of these new drugs will contribute to the treatment and prevention of epileptic disease, reducing its social and economic impact.

Results achieved in 2017

Studies conducted in our laboratory highlight the importance of cellular oxidative phenomena during the progression of epileptic disease. Furthermore, we obtained experimental evidence on the use of cannabinoids in preventing the onset of temporal lobe epilepsy in experimental models. This prevention also extends through the modulation of the oxidative responses induced by the excitatory paroxysm typical of the disease. However, the results suggest that the action of cannabinoids on brain receptors could have opposite effects related to the treatment dose, thus posing the problem of unpredicted effects.

Goals for 2018

Our research will be directed towards the synthesis of new molecules able to not interact with the canonical receptors for cannabinoids but directly and specifically with the cannabinoid receptors expressed in mitochondria, cellular organelles that modulate the bioenergetic and oxidative cellular response, whose dysfunction represents a critical factor in the progression of epileptic disease.

Therapeutic Area: **NEURODEGENERATIVE DISEASES**

Pathogenic mechanisms in Parkinson's disease

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Brief description

The aim of the research is to elucidate the crucial cellular events leading to neurodegeneration of the brain areas involved in Parkinson's disease.

Impact

The definition of early pathogenic events in the Parkinson's disease supports the potential development of new therapeutic approaches aimed at preventing the progression of the disease. Therefore, these studies aim to cure a disease with a high social and economic burden.

Results achieved in 2017

The results obtained reveal interesting evidences on the importance of some molecules or enzymes, such as alpha-synuclein and LRRK2 in the initiation of Parkinson's disease through the induction of mitochondrial dysfunction and the consequent oxidative damage, a phenomenon common to many neurodegenerative diseases.

Meetings

A central role of LRRK2 in idiopathic Parkinson's disease. Gordon research Conference, June 2017 Portland, USA

Pubblications

Di Maio R., Barrett P.J., Hoffman E.K., Barrett C., Zharikov A., Borah A., Hu X., McCoy J., Chu C.T., Burton E.A., Hastings T.G. and Greenamyre J.T., (2016) α-Synuclein binds TOM20 and inhibits mitochondrial protein import in Parkinson's disease. *Sci Transl Med.* 2016 Jun 8;8(342):342ra78. doi: 0.1126/scitranslmed.aaf3634. PMID:27280685

Goals for 2018

The future objectives of this project concern the study of the enzyme NADPH oxidase isoform 2 (Nox2), one of the major enzymes responsible for the production of free radicals in neurons. We already have preliminary evidence that the enzyme can modulate, through a functional interaction with mitochondria, the formation of toxic forms of alpha-sinuclein and the activation of the LRRK2 enzyme, two critical events in the initiation of Parkinson's disease. In the next future, we will test the neuroprotective efficacy of novel highly specific Nox2 inhibitors in experimental Parkinson's models. Positive results have the great potential to identify therapeutic agents able to intervene on different pathogenic aspects of Parkinson's and therefore to prevent the disease in its complexity.

Therapeutic Area: **INFLAMMATORY DISEASES**

Steroid-unresponsive inflammation in chronic obstructive pulmonary disease (COPD): mechanisms and development of new drugs

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Brief description

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the world. It is a progressive lung disease characterized by not fully reversible airflow obstruction and is associated with abnormal inflammatory responses of the lung to noxious particles and gases, including cigarette smoke and pollutants. Inflammation in COPD does not respond to steroid treatment and current therapy is mainly symptomatic.

New effective drugs are very much required. Abnormal activation of innate immune responses in the lung epithelium is a hallmark of COPD. Our project aims at investigating the involvement of both bronchial epithelium and alveolar macrophages, as well as their interaction, in the pathogenesis of steroid-resistant inflammation.

We are currently focusing on oxidative stress, mitochondrial damage and cell aging. Furthermore, we are testing the hypothesis that the NLRP3 inflammasome, as well as other inflammasome complexes, may be activated in the lung of COPD patients and contribute to tissue damage, chronic inflammation and steroid-resistance.

Finally, we are evaluating whether endogenous generated electrophilic derivatives of omega-3 fatty acids (including 17-oxo-DHA) may be effective for the treatment of steroid-unresponsive inflammation as well as lung cancer.

Impact

This research project will contribute to the discovery of new mechanisms leading to steroid-unresponsive inflammation in chronic lung diseases such as COPD. Identification of new pathological mechanisms will reveal previously unknown targets that may be used for the development of novel, more effective therapies. Furthermore, we will generate new knowledge regarding the potential use of 17-oxo-DHA for the treatment of steroid-unresponsive inflammation and lung cancer.

Results achieved in 2017

We have discovered that 17-oxo-DHA displays additive anti-inflammatory effects with the steroid fluticasone propionate (FP) in peripheral blood mononuclear cells (PBMC) isolated from COPD patients and healthy individuals. Compared to FP, 17-oxo-DHA acts through different and complementary mechanisms, both transcriptional and post-transcriptional. More specifically, we have reported that 17-oxo-DHA strongly inhibits the activation of the NLRP3 inflammasome thus reducing caspase-1-dependent IL-1β release and glucocorticoid receptor degradation. Inhibition of the NLRP3 inflammasome by 17-oxo-DHA occurs downstream of ERK pathway and mitochondrial

ROS and does not depend on AKT. We have also reported that the steroid FP is not able to reduce inflammasome activation. We have also investigated the anticancer properties of 17-oxo-DHA and found that this electrophilic compound counteracts lung cancer cell growth and enhances the efficacy of the conventional anticancer drug gemcitabine.

Meetings

17-oxo-DHA inhibits the NLRP3 inflammasome downstream of mitochondrial ROS and ERK pathway. Presentazione orale a *ERS Congress 2017*, September 2017, Milano, ITA: Cipollina C, Di Vincenzo S, Lo Piparo D, Pace E., European Respiratory Journal Sep 2017, 50 (suppl 61) OA284; DOI: 10.1183/1393003.congress-2017.OA284.

Pubblications

17-oxo-DHA counteracts lung cancer cell growth and enhances the efficacy of gemcitabine, Siena L , Cipollina C , Di Vincenzo S, Ferraro M, Bruno A, Gjomarkaj M, Pace E. *Cancer Chemotherapy and Pharmacology.* equal contribution. *Request of revision.*

SIRT1 / FoxO3 axis alteration leads to aberrant immune responses in bronchial epithelial cells, Di Vincenzo S, Heijink IH, Noordhoek JA, Cipollina C, Siena L, Bruno A, Ferraro M, Postma DS, Gjomarkaj M, and Pace E, J *Cell Mol Med*, 2018. *In press.*

Ceftaroline modulates the innate immune and host defense responses of immunocompetent cells exposed to cigarette smoke, Bruno A, Cipollina C, Di Vincenzo S, Siena L, Dino P, Di Gaudio F, Gjomarkaj M, Pace E. *Toxicol Lett* , 2017, *279*: 9-15.

Goals for 2018

- Evaluate the impact of cigarette smoke on inflammasome activation.
- Investigate the involvement of inflammasome activation in COPD pathogenesis.
- Elucidate the mechanisms of inflammasome inhibition by 17-oxo-DHA.
- Discover new selective inhibitors of the NLRP3 inflammasome.

Therapeutic Area: **INFLAMMATORY DISEASES**

Gastric formation, intestinal absorption and lipoprotein-dependent systemic distribution of nitro fatty acids (NO2-FA)

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Brief description

Based on discoveries made during the execution of my project last year, I found that triglycerides are a main component of NO2-FA bio-distribution, entailing absorption, transport and targeted delivery. Through [14C] labeling studies and mass spectrometry based investigation in clinical and preclinical samples, I was able to establish esterification as a main yet unexplored mechanism of NO2-FA delivery.

Impact

This proposed research plan will provide important information about:

- the potential absorption of nitro-oleic acid (NO2-OA) via the intestinal lymphatic system, which will essentially protect NO2-OA from hepatic first-pass metabolism.
- the pharmacology of newly discovered endogenous nitrated fatty acid that could be used as prodrugs to reduce the limited side effects observed with NO2-OA.

Results achieved in 2017

I have been studyng the pharmacokinetic of NO2-OA in supplemented dogs after oral and i.v. administration.

Meetings

Proceedings of conference:

M. Fazzari, D. Chartoumpekis, L. Li, D.A. Guimaraes, S. Shiva, B.A. Freeman, N. Khoo. *Nitro-oleic acid protects mice from diet-induced hepatic steatosis and insulin resistance without the adverse side effects of thiazolidinediones*. Free Radical Biology and Medicine 2017, 112: S1–doi:10.1016/j.freeradbiomed.2017.10.232.

Pubblications

M. Fazzari, N. Khoo, R.S. Woodcock, D.K. Jorkasky, L. Li, F.J. Schopfer, B.A. Freeman. Nitro-fatty acid pharmacokinetics in the adipose tissue compartment. *Journal of Lipid Research*, 2017, 58:(2), 375-385 – doi:10.1194/jlr.M072058.

S.R. Salvatore, D.A. Vitturi, M. Fazzari, D.K. Jorkasky and F.J. Schopfer. Evaluation of 10-nitro oleic acid bio-elimination in rats and humans. *Nature Scientific Reports*, 2017, 7**:** 39900 – doi:10.1038/srep39900.

Goals for 2018

Define the mechanism of intestinal absorption of nitro-oleic acid (NO2-OA) in rodents. We will establish the role of chylomicron on NO2-OA uptake and transport. A lymph-fistula rat model with cannulated portal vein will be used. In this regard, we have developed an HPLC-MS/MS analysis to detect NO2-OA-containing triglycerides and an acidic hydrolysis method to quantitate free and esterified NO2-OA in mesenteric lymph and portal plasma.

Determine the effect of NO2-CLA and nitro-nitrates in intestinal water regulation. Aquaporins play a major role in intestinal water homeostasis and may be involved in the watery stools found in preclinical animal models supplemented with NO2-OA. The comparison between the different NO2-FA will elucidate the ability of nitro-nitrates to reduce this unpleasant side effect. This will potentiate the value of nitro-nitrate lipids as potential prodrugs without gastrointestinal effect.

Therapeutic Area: **INFLAMMATORY DISEASES**

Gastric generation and signaling actions of nitro-nitrates: novel lipid intermediates that form nitrosating and electrophilic species

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Brief description

The acidic gastric environment is a seminal site for nitrite (NO_2^-)-mediated generation of electrophilic nitro-fatty acids. These species demonstrate salutary effects in numerous inflammatory disease processes by modulating cell signaling events via post-translational protein modification.
The present research proposes that gastric nitration of both free and esterified conjugated linoleic acid (CLA) proceeds through the formation of unstable nitro-nitrate intermediates, which were characterized by HPLC-MS/MS. Decomposition of these species, which was spontaneous at physiological pH for free CLA-derived products and catalyzed by lipase-mediated hydrolysis for nitro-nitrate triglycerides, yielded both nitro-conjugated linoleic acid (NO_2-CLA) and active nitrogen oxides with concomitant *in vitro* activation of guanylate cyclase and nitrosation of glutathione and 2,3-diaminonaphtalene. Importantly, these nitro-nitrate derivatives were detected in rat gastric fluid after oral administration of CLA and NO_2^-, highlighting the physiological relevance of our findings.
In aggregate, our results shed new light on the complex reactions and processes involved in the gastric generation of nitrated fatty acids and identify a new class of nitro-nitrate lipid mediators with the potential to modulate cGMP-dependent pathways, participate on nitrosation of critical protein targets and promote NO_2-CLA formation.

Impact

Successful completion of the proposed research plan will provide insight into the generation and protective biological functions of nitro-nitrate lipid derivatives, a class of signaling mediators already covered in IP generated during the last year. This new approach, based on species recently discovered to be formed during digestion, is foreseen to decrease side effects reported for nitro-oleic acid, a drug currently being evaluated in three different clinical trials. A successful completion would provide a new generation of molecules with an improved pharmacological profile and added value.

Results achieved in 2017

I have identified and characterized novel nitro-nitrate lipid intermediates after *in vitro* gastric acidification of synthetic CLA-containing lipid standards. Furthermore, I have studied the decomposition of nitro-nitrate derivatives under physiological conditions.

Intellectual Property

Patent U.S.A. application N. 62/404,354 titled: *Novel reversible nitroxide derivatives of nitroalkenes that mediate nitrosating and alkylating reactions.* Inventors: Marco Fazzari, Francisco J. Schopfer and Bruce Freeman.

Goals for 2018

My current and near term research objectives will address the biochemistry and pharmacology of newly-discovered nitro-nitrate lipid signaling mediators.
The following aims will be pursued:

- characterize the release of NO and nitrosating species from nitro-nitrate lipids *in vitro* and *in vivo* using state of the art biochemical, isotopic labeling and mass spectrometric analysis. Impact on lipid absorption and nitro-nitrate CLA pharmacokinetics will be evaluated;

- evaluate the formation of nitro-nitrate at sites of inflammation and their role as immune-modulators. The lipid matrix of triglycerides and phospholipids provides a platform for *in vivo* formation of these species. New data shows that the release from membranes and lipid droplets may play an important role in nitro-fatty acid physiology and pharmacology. This will be addressed using animal models of inflammation and will provide a strong platform to request external funding.

Therapeutic Area: **ONCOLOGICAL DISEASES**

Cancer and Inflammation

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Brief description

In this project we tested the hypothesis that the tumor and hypoglycosylated form of Mucin 1 (MUC1) served as a link between inflammation and cancer. To study the pro-inflammatory and pro-tumorigenic role of the hypoglycosylated form of MUC1, we used mice transgenic for the human MUC1 (MUC1.Tg). Recently, we showed that the presence of human MUC1 aggravates colonic inflammation and increases tumor initiation and progression in an *in vivo* AOM/DSS mouse model of colitis-associated cancer (CAC). Higher levels of pro-inflammatory cytokines, such as TNF-α, and IL-6, were detected in inflamed colon tissues of MUC1.Tg mice compared to WT mice and we decided to assess their the source. After AOM/DSS treatment, our results revealed that IL-6 and TNF-α cytokines (Figure 1) are produced only by macrophages in WT mice whereas they are produced by both macrophages and intestinal epithelial cells (IECs) in MUC1.Tg mice. Chronic inflammation promoted the expression of hypoglycosylated MUC1 in IECs whereas no or very low expression of this form was detected in colon tissues of healthy mice. Also, hypoglycosylated MUC1, by association with p65 and EzH2, up-regulated the expression of inflammatory cytokines in IECs. In summary, our findings provided a mechanistic basis for the tumorigenic role of the hypoglycosylated MUC1 in CAC, involving a transcriptional positive feedback loop of pro-inflammatory cytokines.

Impact

MUC1 is a heavily O-glycosylated protein and represents one of the major components in colonic mucus that functions as a lubricant and a physiologic barrier between luminal contents and mucosal surfaces. The degree of glycosylation of the mucins is central to their role in the mucus barrier. Altered MUC1 glycosylation reduces the effectiveness of its supramucosal layer function and makes the epithelium more susceptible to bacterial degradation. Restoration of barrier is therefore important to prevent the continuous activation of a pro-inflammatory immune-response in the lamina propria and the perpetuation of chronic inflammation in inflammatory bowel disease (IBD) and CAC. We will highlight new mechanisms that will help us to determine novel and appropriate MUC1 glycoform biomarkers that could also serve as early colon cancer prognostic factors and be used for new therapeutic approaches for IBD patients.

Results achieved in 2017

Inflamed and cancer tissue cells express mucins with immature O-glycosylation, but how and why epithelial cells express short glycans remains not well understood. Our preliminary data suggest that infiltrating macrophages control the expression of aberrant MUC1 glycoforms in IECs and consequently activate specific MUC1-associated oncogenic pathways. In particular, co-culture model system indicates that differentially polarized M1 and M2 macrophages induced over-expression of hypoglycosylated forms of MUC1, compared to unstimulated cells. In addition, gene expression array of glycosylation-related genes revealed that macrophages modulate glycosyltrasferase enzymes involved in O-glycan biosynthesis.

Meetings

AACR Annual Meeting 2017, April 2017, Washington, USA

Pubblications

Cascio S, Faylo J.L., Sciurba J.C., Xue J, Ranganathan S, Lohmueller J.J., Beatty P.L. and Finn O.J. Abnormally glycosylated MUC1 establishes a positive feedback circuit of inflammatory cytokines, mediated by NF-kB and EzH2, in colitis-associated cancer. *Oncotarget*. 2017; 8:105284-105298. https://doi.org/10.18632/oncotarget.22168

Goals for 2018

The goal of this project is to dissect the mechanisms by which macrophage induce changes of MUC1 glycans and to determine specific MUC1 glycoforms involved in the transition from IBD and CAC. To find novel MUC1 structure glycans that drive and perpetuates chronic inflammation we will perform mass spectrometry and magnetic resonance. The data will be then confirmed in human colon tissues of IBD and CAC patients.

Therapeutic Area: **ONCOLOGICAL DISEASES**

MUC1-CIN85 interaction as a new therapeutic target to inhibit ovarian tumor growth and metastasis

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Brief description

We have identified CIN85 (Cbl-interacting protein 85 KDa), as a binding partner of MUC1 in tumors. MUC1/CIN85 complex is found in early as well as advanced clinical stages of breast, ovarian, colon and prostate cancers among others. Co-localization of MUC1 and CIN85 on invadopodia structures enhances invasion and migration of cancer cells.
Our hypothesis is that preventing the formation or dissociating existing CIN85/MUC1 complexes may result in a less aggressive tumor by decreasing local invasion and preventing distant metastases. To test this hypothesis, we have already identified and tested two novel compounds that significantly reduce the association between hypoglycosylated form of MUC1 and CIN85, and drastically inhibit the migratory activity of mouse and human epithelial cancer cells. Importantly, an initial set of experiments in an *in vivo* mouse model confirmed the ability of these drug compounds to decrease tumor growth and metastasis. We are currently investigating the MUC1/CIN85-dependent signaling pathways implicated in the control of invasive activities of cancer cells. Thus, this project will explore the CIN85/MUC1complex as a viable therapeutic target and will develop and test *in vitro* and *in vivo* novel potent antagonists of epithelial cancer metastasis.

Impact

Metastatic spread of cancer is responsible for most cancer deaths. Critical steps of cancer cells leaving a solid tumor are the loss of epithelial polarity and acquisition of migratory and invasive capabilities. In this project our goal is to develop and test in a preclinical ovarian and breast cancer mouse model several drug compounds designed to reduce cancer invasion and metastasis. The target of these drugs is a CIN85/MUC1 complex on cancer cells that we identified.
We have already identified and tested two novel drug compounds that in our *in vitro* experiments significantly inhibited the association between MUC1 and CIN85 and drastically reduced the migratory activity of mouse and human ovarian cancer cells. The validation of CIN85/MUC1 complex as a novel therapeutic target will support the development and testing of more potent antagonists. If the *in vivo* results replicate the promising data already obtained, we expect to see decrease tumor growth and metastasis. We also expect to come up with the best drug candidate that could be promptly tested in a small Phase I trial for safety, leading to larger trials further exploring safety and also efficacy

Results achieved in 2017

We have previously discovered that overexpressed and hypoglycosylated MUC1 in tumor cells associates with CIN85 in mouse and human epithelial cancer cells. CIN85 is a multifunctional scaffold protein implicated in many protein-protein interactions. Interestingly, this complex was

found on cell protrusions that we confirmed to be the invadopodia, important for invasion and metastasis of cancer cells. We have identified two drug compounds that inhibit:

- the interaction between MUC1 and CIN85
- the migratory activity of human ovarian cancer cells.

A similar experiment was performed on primary culture of normal human ovarian cell line wherein, as expected, no association between tumor form of MUC1 and CIN85 was detected. We also tested the effect of these drug compounds on cancer cell migration. In a trans-well invasion assay, their treatments resulted in 69-75% reduction of ovarian and breast cancer cells migration. Treated and untreated normal ovarian cells did not show migratory activity. *In vivo* preliminary data suggested that these compounds are reducing tumor metastasis in mice.

Meetings

Ovarian Spore Retreat RPCI/UPCI, 2017, Buffalo, USA. *Invited Speaker*

Immunology/Comp Biology Retreat, 2017, Pittsburgh, USA. *Invited Speaker*

Pubblications

Sandra Cascio, Michael Kvorjak, Raahul Sriram, Anda Vlad, Carlos Camacho and Olivera Finn. Identification of two drug compounds that by targeting the interaction between MUC1 and CIN85 inhibit migratory activities of breast and ovarian cancer cells. *In preparation*

Goals for 2018

One aim of this project is to confirm the therapeutic efficacy *in vivo* of the two novel compounds that inhibit the interaction between MUC1 and CIN85. We will use tumor cell lines derived from mouse ovarian tumors that arose spontaneously in MUC1.Tg mice. We selected MUC1 positive cell lines with two different phenotypes: one derived from a primary tumor site, grow as single tumors and are not invasive, the second one derived from a secondary metastatic site is highly invasive. Tumor cell lines and the selected drugs will be injected intraperitoneally (i.p.) and tumor growth and metastasis measured using *in vivo* imaging (mCherry).

Based on the structure of our active compounds, in collaboration with the Department of Medicinal Chemistry we will use computational, biochemical and cell-based screening technologies to identify natural and synthetic compounds with pharmacological activity.

Therapeutic Area: **ONCOLOGICAL DISEASES**

Modeling microRNA-target interaction network

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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 count 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 specific cellular tissue, it is highly probable that such data repositories contain a huge collection of suitable set of gene expression profiles.
This kind of data contains the information of the expression of the entire genome in the tissues of interest and it is generally useful to perform the initial screening to decide on which features focus the research. In the face of a huge amount of available data, what is missing is data analysis algorithms useful to integrate many sources of biological big data. While it is common practice to detect differentially expressed microRNAs or mRNAs among two different tissue conditions in order to detect anomalies in the expression profiles, it does not exist 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 2017

In 2017 we focused on the analysis of gene expression profiles of samples derived from the breast cancer cell line MCF-7. We used RIP-Chip technology to identify the RNA associated to the proteins of the RISC complex. Specifically, we studied two proteins: AGO2 and GW182. With microarray

technology we analyzed the RNA extracted from total cell lysate and from the immunoprecipitated fraction, in order to quantify respectively the gene expression profiles of the total RNA and the RNA enriched of targets of the set of microRNA endogenously expressed in the MCF-7 cell line. These data were used to train and test two algorithms:

- RIP-Chip prediction, useful to predict the genes that are differentially expressed in the RIP-Chip sample. It is based on machine learning algorithms and uses only features available by analyzing the total cell lysate only, avoiding to actually perform the RIP-Chip experiment. In the process of developing the algorithm, we discovered that the RNA associated with the two proteins AGO2 and GW182 show different characteristics, suggesting that they participate to the RISC complex with different roles.
- DE.SSA, useful to detect differentially expressed genes by comparing two single samples. This algorithm allows to identify differentially expressed genes without the use of experimental replicates, and open a new path to personalized analysis of gene expression profiles.

Meetings

Methods, tools & platforms for Personalized Medicine in the Big Data Era, NETTAB 2017 Workshop, October 2017, Palermo, ITA

Pubblications

Coronnello C., Perconti G., Rubino P., Contino F., Bivona S., Feo S., Giallongo A. (2017) Detecting significant features in modeling microRNA-target interactions. *PeerJ Preprints*, 5:e3337v1

Perconti G., Contino F., Rubino P., Bivona S., Feo S., Giallongo A., Coronnello C. (2017) AGO2 and GW182 IP show different characteristics in co-immunoprecipitated RNA features. To be submitted to *BMC Bioinformatics*

Goals for 2018

Up to now, our studies have been focalized on the selection of the actors of the biological interaction network among microRNA and mRNA and on the selection of the relevant features of these actors. Next year we will use the obtained information to model the actual interaction network. 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 only possible to perturb the system, for example by over-expressing or inhibiting one microRNA, and detect how the gene expression profile is modified. We will use data sets selected from public data repositories or appositely generated by our laboratories to test the predictions obtained by using our interaction network models. Specifically, we will use our models to predict the effects of the perturbation of the expression profile, starting with simple variations, i.e. the overexpression of one single microRNA, and continuing with changes involving the entire microRNA system, similarly to what happens in tumor samples. In addition, in order to test more the developed algorithms and better describe the activity of the RISC complex, we will analyze the RNA associated with another RISC complex protein, i.e. AGO1.

Therapeutic Area: **ORGAN FAILURE**

Nephrotic Syndrome and Zebrafish Model

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Brief description

Project started in 2016 in collaboration with Prof. Valérie Doye and her team, in the Institut Jacques Monod, Univ. Paris Diderot. Nucleoporins (Nups) are highly conserved proteins that form the nuclear pore complexes (NPCs), huge macromolecular assemblies embedded in the nuclear envelope, important in mediating transport of macromolecules between the nucleus and the cytoplasm. Besides nucleocytoplasmic transport, Nups are involved in many fundamental cellular processes and genetic alterations in some Nup genes have been linked to different human diseases. Recently, mutations in some nucleoporins have been linked to nephrotic syndrome (NS) in humans, a renal disease caused by a dysfunction of glomerular podocytes leading to proteinuria, hypoalbuminemia and edema. In the present study, we have identified a new nucleoporin involved in the development of nephrotic syndrome (NS). The major goal of the project is to study the role of nucleoporins in NS *in vivo* using zebrafish as model system.

Impact

We demonstrated that mutations in NUP genes can cause a renal disease. The goal of this study is to investigate the possible molecular mechanisms leading to the development of the nephrotic syndrome, thereby potentially opening new approaches to therapy.

Results achieved in 2017

- Identification of zebrafish orthologues of nucleoporins potentially involved in nephrotic syndrome (NS)
- Expression analyses (*in situ* hybridization)
- Knock-down experiments with antisense morpholino technology
- Rescue of the knock-down phenotype with full length mouse mRNA
- Functional analyses of the glomerulus and the kidney proximal tubules after Nups knock down
- CRISPR/Cas9 fish mutant lines generation (F0 generation; F1 generation)

Goals for 2018

- Podocyte morphology analyses in morphants and mutant lines (TEM)
- *In vivo* glomerular functional analyses in control and morphant larvae
- Rescue of the knock down phenotype with different mutated forms of nups mRNA to shed light on possible interactions with other glomerular proteins
- CRISPR/cas9 mutant lines screening, morphological and functional analyses
- Identification the underlying mechanisms of how nucleoporins play cell type or tissue specific roles in the glomerulus.

Therapeutic Area: **ORGAN FAILURE**

Identification and characterization of novel candidate genes controlling epithelial sodium transport in the distal convolutet tubule (DCT)

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Brief description

The thiazide-sensitive NaCl cotransporter (NCC) of the renal distal convoluted tubule (DCT) plays a critical role in renal control of ion homeostasis and blood pressure, and mutations in this channel lead to human diseases including electrolyte disorders and arterial hypertension. In this study, through a comparative transcriptomic approach comparing zebrafish distal tubule transcriptome with mouse DCT transcriptome, we aim at identify novel regulators of renal function.

Impact

The goal of this research project is to get insights into mechanism controlling DCT growth and function by using experimental approaches in wildtype and gene-modified zebrafish and mice. Knowledge about these processes will not only help to better understand the unique structural and functional plasticity of the DCT but also help to prevent or treat its pathophysiological consequences. Furthermore, these studies may set the basis for novel approaches to identify signaling mechanism in the DCT and to study them further in mouse and zebrafish models.

Results achieved in 2017

Through a comparative transcriptomic approach, we identified a subset of genes enriched in both teleost DL and mammalian DCT. Briefly, we generated a transgenic zebrafish line with expression of the red fluorescent mCherry protein under the control of the zebrafish DCT-specific promoter of the thiazide-sensitive NaCl cotransporter (NCC). The mCherry expression was then used to isolate from the zebrafish mesonephric kidneys the distal late (DL) segments, the equivalent of the mammalian DCT, for subsequent RNA-seq analysis.
We next compared this zebrafish DL transcriptome to the previously established mouse DCT transcriptome and identified a subset of gene products significantly enriched in both the teleost DL and the mammalian DCT, including SLCs and nuclear transcription factors. The present work is the first report on global gene expression profiling in a specific nephron portion of the zebrafish kidney, an increasingly used model system for kidney research. Our study suggests that comparative analysis of gene expression between phylogenetically distant species may be an effective approach to identify novel regulators of renal function. Based on the results of this study, we selected and tested the functional significance of two candidate gene by morpholino-based knockdown in the zebrafish larval pronephros using custom-made antibodies for zebrafish NCC. The two candidate genes are the transcription factor AP-2 beta (TFAP2B) and the estrogen related receptor, beta (ESRRB).
We performed expression analyses and knock down experiments with morpholinos for both

genes in the tg (ncc:mcherry) transgenic line and looked at ncc phosphorylation with IHC. Functional knockdown of one of these genes induced kidney damage and reduced the phosphorylation of NCC in the zebrafish pronephros tubules in zebrafish larvae
For both genes we generated CRISPR/cas9 mutant lines.

Meetings

10th Annual Swiss Zebrafish Meeting. January 2017, Bern, CH

6th International Kidney.CH Symposium. June 2016 J, Zurich, CH

Pubblications

Sugano Y, Cianciolo Cosentino C, Loffing-Cueni D, Neuhauss SCF, Loffing J. Comparative transcriptomic analysis identifies evolutionarily conserved gene products in the vertebrate renal distal convoluted tubule. Pflugers Arch. 2017 Aug; 469 (7-8):859-867. doi: 10.1007/s00424-017-2009-8. Epub 2017 Jun 27. PMID: 28656378

Goals for 2018

We are waiting to cross the F0 generation of tfap2b-/- and esrrb -/- to obtain homozygous mutant lines.
Screening, morphological and general functional analyses of the kidney, and in particular of the distal late segment (equivalent of the mammalian DCT) of tfap2b-/- and esrrb -/- will be performed.
These experiments will be complemented with in depth analyses of mouse mutant lines for the same genes performed in the Laboratory of Johannes Loffing, Institute of Anatomy, CH.

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

The laboratories for regenerative medicine and research & development of biological therapies are focusing 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 personnel specialised in the activity of research and development (*in vitro* and *in vivo* studies, *first-in-man* studies) and in the manipulation of biological samples of human origin. The personnel have been trained to operate according to rules of Good Manufacturing Practice (GMP) and Good Clinical Practice (GCP) for the designing and carrying out of pre-clinical/ clinical experimentation and the production of advanced therapies. Projects in the pre-clinical development stage are aimed at the development of cellular products for the repairing and/or regeneration of tissue and the development of organotypic cultures to be used both for regenerative objectives and as models for pharmacological screening. Another important point of focus for research is the study and development of cellular therapies for the prevention of disease recurrence and the treatment of post-transplant infections. The new generation of vaccines, made up of re-combined proteins, is aimed at the treatment of the more important hospital-acquired infections of varying etiology.

Therapeutic Area: **ORGAN FAILURE**

Regulatory Dendritic cells as a tool to prevent graft rejection

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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 immunosuppresisve therapy. Liver is a tolerogenic organ per se. In this study, patients transplanetd 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 hyporesponsiveness into recipients T cells specific for donors alloantigens and establishing a long term immunological memory. The source of cells will be the liver perfusate, that is the lavage buffer of the solid organ before transplant.

Impact

Patients who undergo solid organ transplant require lifelong immunosuppression to prevent organrejection. 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 defences 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 alloantigens by inducing donor-specific T cell hyporesponsiveness and memory to donor alloantigens. DCreg functionally prevent organ rejection and early weaning from immunosuppresisve therpay in transplanted patients.

Results achieved in 2017

I have demonstrated that the liver perfusate is enriched in DCs expressing a phenotype compatible with regualtory DCs, expressing low levels of HLA-II molecules and no co-stimulatory markers such as CD80, CD83 and CD40. This cell population is variably represented in the liver perfusate of different healthy donors, being the 5-30% of total cell isolate and between 30 -150 million of cells readily available.

Goals for 2018

Isolation of regulatory DCs from the liver perfusate using CD14 antibodies conjugated to magnetic microbeads.Functional characterization of freshly isolated liver derived CD14+ DCreg in Mixed Lymphocytes reactions (MLR) where donors DCreg will be co-cultured with recipients CD3+ T cells derived from peripheral blood. Proliferation and acquired phenotype fo T cells will be assessed to determine induced hyporesponsiveness to donors allo-antigens. Optimization of the protocol of *in vitro* culture of liver-derived CD14+ DC to detemine if a stronger tolerogenic fucntion can be induced using specifici growth factrs such as IL10 and Vitamin-D3.

Therapeutic Area: **ORGAN FAILURE**

Nk cell mediated therapy in cancer and chronic infection

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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.

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 a 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 2017

This study showed that the NK cell function can be addressed towards a more efficient antiviral and antitumor phenotype. The activation of NK cells with cytokines [IL2 + IL15] and [IFNα] or with the cytokine cocktail [IL2 + IL15] and [IL12 + IL18] or with the only combination of [IL2 + IL15] most commonly used in the current cell therapy, activates the most efficient NK cells granting a superior anti-HCV and anti-HCC activity.
IFNa-NK cells have an increased cytotoxicity and also release soluble factors that mediated viral and tumor eradication to a greater extent. Data were demonstrated both *in vitro* and *in vivo* in a small animal model of HCV infection. In this study I have optimized a protocol of GMP-compliant large scale isolation and expansion of NK cells from the liver perfusate for clinical application.
I have written the protocol to be presented to the IACUC of the Istituto Zooprofilattico Sicilia (IZS) for the animal study. The protocol has been accepted by the IZS and submitted to the Italian ministry of Health (IMH) and, after minor revision, accepted.
The higher anti-viral function of IFNa-NK cells has been demonstrated in an *in vitro* model of Zika virus infection where we used as target cells Huh7.5 cells (a cell line of hepatocellular carcinoma) and on a cell line of neuroblastoma.

Meetings

4th Conference of Translational Medicine on Pathogenesis and Therapy of Immune-mediated diseases, March 2017, Palermo, ITA

Third CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference, September 2017, Mainz , DE

MACS Day New Frontiers in Cellular Therapies, October 2017 Rome, ITA

ImmunoTherapy Bridge 2017, November 2017, Naples, ITA

Pubblications

Giovanna Russelli; Paola Pizzillo; Gioacchin Iannolo; Floriana Barbera; Fabio Tuzzolino; Rosa Liotta; Mario Traina; Giovanni Vizzini; Bruno Gridelli; Ester Badami and Pier Giulio Conaldi "HCV replication in Gastrointestinal mucosa: potential extra-hepatic viral reservoir and possible role in HCV infection recurrence after liver transplantation". *PLoS One* (2017 July) 27;12(7):e0181683. Cited in 1 Impact factor 3.54

Alessia Gallo, Monica Miele, Ester Badami, Pier Giulio Conaldi. "Molecular and cellular interplay in virus-induced tumors affecting solid organ recipients" *Clinical immunology* (2018) – accepted

Duilio Pagano*, Ester Badami*, Pier Giulio Conaldi, Aurelio Seidita, Marco Barbàra, Fabrizio di Francesco, Alessandro Tropea, Rosa Liotta, Gaia Chiarello, Angelo Luca, Salvatore Gruttadauria. "Liver Perfusate Natural Killer Cells from Deceased Brain Donors and Prediction of Recurrence-free Survival after Liver Transplantation" to *Transplantation* - Submitted. Impact factor: 3,678

Badami E., Cexus O., Quaratino S. "Naturally arising antigen-specific Foxp3+CD4+CD25+ regulatory T cells fail to protect from spontaneous autoimmunity". *Nat Immunol* - Submitted. Impact factor: 20

Badami E., Barbera F., Vella S., Gallo, Coronello C., Carcione C., Conaldi P.G. "NK cell miRNOme signature in cancer and infection" (in preparation)

Intellectual property

Patent n. PCT/EP2017/080848 "NK- mediated immunotherapy and uses thereof"

Goals for 2018

- MiRNOme characterization of NK activated with IFNa or IL2/IL15 or IL12/IL18.
- Metabolomic characterization of NK cells activated with IFNa or IL2/IL15 or IL12/IL18.
- Investigation of NK cell anti-viral function in other viral system such as Zika virus
- Proof-of-concept *in vivo* of the anti-HCV function of NK cells in a small animal model of HCV infection.
- Proof-of-concept *in vivo* of the anti-tumor function of NK cells in a small animal model of HCC.

Therapeutic Area: **ORGAN FAILURE**

Clinically relevant secretome from human fetal dermal cells

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Brief description

Secretome from mesenchymal stromal cells (MSC) holds a great potential as a cell-free based therapy. The vast array of immunomodulatory and tissue-protective molecules and the presence of extracellular vesicles (exosome/microvesicles) detected in secretome may allow the treatment of various clinical conditions such as restoration of cardiovascular functions or neurodegenerative diseases. Moreover, due to its proangiogenic action, secretome from human fetal dermal cells (MSC-like cells) may be particularly suitable to treat diseases with impaired or insufficient angiogenesis, such as chronic wounds.

Impact

The use of cell-free products holds several advantages over traditional cell therapy. Secretome-based therapy could potentially avoid or limit issues related to conventional cell therapy such as immune rejections, tumorigenicity, trasmission of infections associated to cell admininstration. In addition, large quantity of secretome can be prepared in advance and be ready-to-use in acute conditions.

Results achieved in 2017

We collected secretome in a form of conditioned medium from cultured cells. We established optimal collection procedures in terms of concentration of soluble factors and functional activity of secretome. A quantitative analysis of the secreted molecules revealed the presence of proangiogenic and wound healing-related molecules with high concentrations (> 1 ng/ml). *In vitro* functional characterization revealed the ability of secretome to induce biological responses in target cells with high efficiency and in a dose-dependent manner. We also evidenced secretome stability following two-year storage at -80°C.

Meetings

9th annual conference on Stem cells and Regenerative Medicine, September 2017, Berlin, DE

Pubblications

Chinnici C.M., Iannolo G., Amico G., Pagano V., Pietrosi G., Conaldi P.G., Mesenchymal stromal cells from human fetal liver reelase growth factors and chemokines with a potential role in liver tissue repair. Submitted and under revision *Stem Cells Dev.*

Chinnici C.M., Miceli V., Pampalone M., Lo Nigro A., Amico G., Conaldi P.G. *In vitro* evidences for epithelial to mesenchymal transition in low cell-density cultured human fetal hepatocytes, (2017), *Biochem. Biophys Res Comm*. 490: 472-479.

Gaetani M., Chinnici C.M., Carreca A.P., Di Pasquale C., Amico G., Conaldi P.G. Unbiased and quantitative proteomics reveals highly increased angiogenesis induction by the secretome of mesenchymal stromal cells isolated from fetal rather than adult skin, (2017), *J Tissue Eng Regen Med.* doi 10.10012/term.2417.

Pietrosi G. and Chinnici C. Report on liver cell transplantation by using human fetal liver cells. *Methods Mol Biol* 2017; 1506: 283-294

Goals for 2018

Conditioned media are often collected using reagents not intendent for a human use. We are currently working to produce a clinical-grade conditioned medium. We are testing different conditions of concentration/dialysis and lyophilization, and verify the effects of these procedures on conditioned medium stability, protein contents and functionality.
Conditioned medium produced as lyophilized powder could be later on solubilized in clinical-grade buffers (e.g., Ringer's Lactate solution or others) in order to speed up the regulation process for a future Advanced Therapy Medicinal Products (ATMP) certification. The lyophilized powder will also allow to prepare conditioned media with different volumes and concentrations according to the specific needs.

Additonal aims are:
- establishment of a delivery system for *in vivo* topical application of secretome;
- isolation and characterization of extracellular vesicles from conditioned medium (exosomes) to demonstrate their role in tissue repair;
- production of cells, conditioned media, and exosome in large scale and according to GMP guidelines in ISMETT Cell Factory.

Therapeutic Area: **ORGAN FAILURE**

Bioengineering a kidney in secondary lymphoid organs

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Brief description

Two million people worldwide suffer from end-stage renal disease (ESRD), and the number of patients diagnosed with the disease continues to increase at a rate of 5-7% per year. ESRD patients must go on dialysis or have a kidney transplant to survive. While life-saving in the short-term, dialysis is not a long-term substitute for a human kidney, performing only about 10% of kidney function. Kidney transplantation is the only definitive treatment option for ESRD patients. However, despite efforts to promote the practice of organ-donation, the resources available in many countries are currently insufficient to meet medical needs.

Tissue engineering has the potential to produce alternative therapies which circumvent the obstacles posed by organ shortage. When the sophisticated structure of the kidneys is totally disrupted by ESRD, traditional stem cell-based therapy is unable to completely regenerate the damaged tissue. Conversely, whole organ regeneration may be a promising therapeutic approach to alleviate ESRD patients. Different strategies have been employed to engineer a functional whole kidney de novo, including the re-population of decellularized kidney scaffolds with renal primary cell cultures or immortalized renal cell lines, and more recently, with stem/progenitor cells. Unfortunately, these approaches failed to generate vascularized and perfused renal tissues that have sufficient renal function to produce urine and erythropoietin.

In our lab, we have pioneered an *in vivo* vascularized tissue-engineering model, in which target cells/tissues are implanted into a mouse lymph node (LN). Upon transplantation into the LN, mouse metanephroi acquired markers of mature renal structures, had functional glomeruli, tubules, and erythropoietin-secreting cells, and produced entrapped fluid that concentrated urea. Driven by these results, we next asked whether: 1) a cellular/ molecular mechanism in the LN supports the developing kidney; and 2) we could regenerate a kidney in the LN using new candidate cells for tissue engineering.

Impact

Controversies regarding the existence of a true adult kidney stem cell highlight the importance of studying tissue engineering therapies using pluripotent cells, progenitor cells from fetal kidney, or de-differentiated/reprogrammed adult kidney cells.

Several methods have been established to generate 3D organ-buds termed "organoids" from these cells. There are many challenges to using organoids to generate functional bioengineered kidney tissues. One of the challenges relates to vascularization. Paradoxically, the mouse kidney subcapsular space - a routinely used transplant site - does not provide an environment conducive to vascularization and functional differentiation of organoid cultures. Conversely, the application of the LN technology has the potential to allow incorporating a vascular system into transplanted renal organoids, an important step towards modeling human kidney development and injury.

Results achieved in 2017

We previously showed the generation of a functional mouse kidney tissue by grafting kidney rudiments in the LN. Interestingly, our most recent data also show rapid re-vascularization and function of human fetal kidney grafts in this site. Signaling through the lymphotoxin beta receptor (LTβR) in LN-resident stromal cells is critical for LN development and homeostasis, and our further studies - exploiting the use of several knockout mouse models - suggest that a similar molecular mechanism is also critical to the promotion of kidney graft vascular integration, both in the LN and in another secondary lymphoid organ, the greater omentum. This information could prove valuable in future endeavors to create a "niche" for human kidney cells.

Meetings

Oral presentations

Bioengineering a kidney in secondary lymphoid tissues: A LTβR dependent pathway for ectopic organogenesis, M. G. Francipane, B. Han, L. Oxburgh, S. Sims-Lucas, C. Bates and E. Lagasse. *ASN Kidney Week 2017*, November 2017, New Orleans, USA.

Poster

B*ioengineering a kidney in secondary lymphoid tissues: the importance of the Lymphotoxin beta receptor pathway in tuning host-derived vascularization,* M. G. Francipane, B. Han, L. Oxburgh, S. Sims-Lucas, C. Bates and E. Lagasse.

University of Pittsburgh 17th Annual Celebration of Science and Technology, October 2017, Pittsburgh, USA.

Polycystic Kidney Disease: Challenges, Differing Viewpoints and Ways Forward, FASEB Conference. June 2017, Big Sky, USA.

Omental Milky Spots as Sites for Ectopic Liver Development, B. Han, J. Komori, M.G. Francipane, F. Chen and E. Lagasse. *McGowan Institute for Regenerative Medicine – 16th Annual Scientific Retreat*. March 2017, Farmington, USA.

Rebuilding Renal Functions for Chronic Kidney Disease in Children. Pediatric Innovation Retreat, M. G. Francipane, B. Han, L. Oxburgh, S. Sims-Lucas, C. Bates and E. Lagasse. January 2017, Pittsburgh, USA.

Seminars

Bioengineering a kidney in secondary lymphoid tissues: the importance of the Lymphotoxin beta receptor pathway in tuning host-derived vascularization. Bridgeside Research Forum Seminar Series, October 2017, Pittsburgh, USA.

Awards

Travel award for the best presentation. *Bridgeside Research Forum Seminar Series*. December 2017, University of Pittsburgh, USA.

Pubblications

M. G. Francipane, and E. Lagasse. Regenerating a kidney in a lymph node. Pediatric Nephrology. 2016 Oct; 31(10):1553-60.

M. G. Francipane, and E. Lagasse. Towards organs on demand: breakthroughs and challenges in models of organogenesis. Current Pathobiology Reports. Springer. 2016 Sept; 4(3):77-85.

M. G. Francipane, and E. Lagasse. Pluripotent stem cells to rebuild a kidney: the lymph node as a possible developmental niche. Cell Transplantation. 2016 Jun; 25(6):1007-23.

M. G. Francipane, and E. Lagasse. The lymph node as a new site for kidney organogenesis. Stem Cells Translational Medicine. 2015 Mar; 4(3):295-307.

M. G. Francipane, and E. Lagasse. Maturation of embryonic tissue in a lymph node: a new approach for bioengineering complex organs. Organogenesis. 2014 December; 10(3):323-31.

in preparation
M. G. Francipane, et.al. Bioengineering a kidney in secondary lymphoid organs: the importance of host lymphotoxin-beta receptor signaling for graft vascular integration.

Goals for 2018

My current work on rebuilding a functional kidney is driving efforts toward the reconstitution of compartmentalized and vascularized nephrons from progenitor cells. This goal entails defining the culture requirements for a scalable and reproducible process for expansion and differentiation of the nephron progenitors cells. Preliminary data show that nephron progenitor-derived organoids can engraft and become vascularized in the LN or omentum.
A further challenge to generate functional bioengineered kidney tissues involves the egress of formative urine. This challenge can be resolved by inducing the formation of collecting duct cells in organoids. Collecting ducts originate from the ureteric bud progenitor cell population. By transplanting organoids consisting of specific combinations of nephron progenitor cells and ureteric bud progenitor cells in the LN or omentum, we hope we can get closer to making a fully functioning artificial kidney.

Therapeutic Area: **ORGAN FAILURE**

Characterization Mesenchymal and Epithelial cells from placenta

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Leading Partner
IRCCS ISMETT, Palermo, ITA

Brief description

The project involves the production and study of cells and their derivatives obtained from the amniotic membrane of the human placenta to evaluate their potential in the field of regenerative medicine.

Impact

Mesenchymal cells can be used in regenerative medicine, in cell therapy and in tissue engineering alone or in association with biomaterials that function as scaffolds. The high proliferative potential *in vitro*, the tropism, the anti-inflammatory capacity able to restore tissue homeostasis and in particular the possibility of differentiating and transdifferentiating towards specialized cells, if implanted in the right context of the emicro-environment, mean that MSCs can be a tool for the regeneration and repair of tissues.

Results achieved in 2017

Optimization of the extraction protocol of mesenchymal and epithelial cells to obtain a high degree of purity and vitality of the final preparation. Production of mesenchymal cells in 2D and 3D and evaluation of the immunomodulatory capacity and angiogenesis of both cells grown in 2D and 3D and of the respective Conditional Means and Exosomes. Evaluation of pro and anti-inflammatory and pro-anti-angiogenic cytokine production by Luminex. Application of mesenchymal and epithelial cells for the study of liver diseases (in collaboration with ISMETT and Barcelona) and for the reconditioning of the lung.

Publications

Chinnici CM., Miceli V., Pampalone M., Lo Nigro A., Amico G., Conaldi PG., *In vitro* evidences of epithelial to mesenchymal transition in low cell-density cultured human fetal hepatocytes (2017) *Biochem Biophys Res Commun.* June 15.

Goals for 2018

Characterization in terms of identity, purity, potency and tumorigenicity for the production and development of CBMP (Cell-based medicinal products).

Therapeutic Area: **INFECTIOUS DISEASES**

Development of a vaccination strategy against *Klebsiella pneumoniae*

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Brief description

In this project, we propose the development of a vaccine against *K. pneumoniae*. Potential membrane and secreted antigens will be chosen using a reverse vaccinology approach. Our aim is to select proteins that can be used either in a strategy to prevent bacterial colonization and consequent systemic infection or directed specifically against the former. The antigens will be cloned in suitable bacterial strains, expressed and purified. Recombinant antigens will be thoroughly characterized at functional, biophysical and immunologic levels. We will evaluate their opsonophagocytic potential, the level of protection they can induce in mouse models of *K. pneumoniae* infection and immunological biomarkers related to control of bacterial infection. We hope to identify suitable antigens that can be included in a prophylactic strategy against this multidrug-resistant bacterial pathogen.

Impact

Currently, in Italy, the mortality rate for infection of multi-resistant *K. pneumoniae* strains (KPC type carbapenemase producers) in patients who have undergone allogeneic stem cell transplantation (SCT) is 64.4%; The current trend is not to perform this type of transplantation in patients who are positive for *K. pneumoniae*, due to the high rate of failure. Infections with *K. pneumoniae* are also a serious problem in patients who are candidates for solid organ transplantation. Our laboratories are located in the Mediterranean Institute for Transplantation and High Specialization Therapies (ISMETT), in Palermo, Sicily, a leading health facility for transplantation procedures. At ISMETT, patients are regularly monitored for infection and / or intestinal colonization by *K. pneumoniae*, due to its impact on the success of the transplant procedure and potential further infections of other organs. We have successfully identified and followed cases of *K. pneumoniae* infection, distinguishing between colonization and infection. We also observed the *in vivo* transfer of the blaKPC-3 gene conferring resistance to carbapenems between *K. pneumoniae* and E. coli in patients who had to undergo surgery, which emphasizes the need for constant surveillance to prevent the spread of multi-organisms resistant. In fact, we have been part of national multicentric networks both for nosocomial infections and for pathogens that can compromise the success of transplants.

Results achieved in 2017

We have identified and cloned six potential adhesive proteins of *K. pneumoniae* that play an important role in the adhesion processes, for example, those that compose the fimbriae of this pathogen (FimH, FimA, MrkD and MrkA) or are involved in its colonization, as KPN_01507 and KPN_01508, from an isolated clinical *K. pneumoniae* ST512 belonging to the bank of patient isolates of IRCCS ISMETT.

We are optimizing protein purification protocols and future functional assays for the characterization of these antigens. These recombinant proteins will be used as controls in experiments where the potential of genetically engineered yeasts which express the same antigens will be assessed.

Publications

Di Mento G, Cuscino N, Carcione C, Cardinale F, Conaldi PG, Douradinha B, Emergence of a *Klebsiella pneumoniae* ST392 clone harbouring KPC-3 in an Italian transplantation hospital, *Journal of Hospital Infection. In press.*

Goals for 2018

We expect to identify potential antigens which can integrate a future vaccine against *K. pneumoniae*. A vaccine against this multidrug resistant bacterium is required, as more and more resistance to different treatments is reported. Patients who must endure medium to long term recovery periods in health facilities risk infection with multidrug resistant bacteria, including *K. pneumoniae*, which can result into serious complications due to their already debilitated health conditions.
The identification of both multidrug resistant and hypervirulent strains is worrisome, since it implies that this pathogen can infect also healthy individuals, prompting it to be considered a public health concern and no longer restricted to hospital patients or immunosuppressed individuals. Thus, it is peremptory that novel prophylactic strategies be developed to help fighting *K. pneumoniae* and to avoid it becomes a public health issue. This project is in line with the proposed goals of the future Fondazione Ri.MED Research Center which will be built near Palermo, in Italy. One of the main activities planned for this Center is Vaccine Development, thus the suggested vaccination strategy falls within its scopes.

Therapeutic Area: **INFECTIOUS DISEASES**

Development of a novel immunotherapy against *Klebsiella pneumoniae*

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Brief description

In this project, we propose the use of probiotic strains of *S. cerevisiae* as part of a strategy to prevent and treat *K. pneumoniae* infections. The yeasts will be genetically modified to express proteins involved in the adhesion of *K. pneumoniae* to human cells on their surface. It is expected that these recombinant yeasts, once administered, will be able to induce an immune response against the antigens of the 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 consequent systemic infections. Genetically modified yeasts will be tested *in vitro* on eukaryotic cells and *in vivo* in mice, to determine both the ability to compete with *K. pneumoniae* for binding to cells, and the level of immune response against these bacteria. The proposed immunotherapy would also work for the multidrug resistant *K. pneumoniae* strains, as the mechanisms leading to resistance would not allow the immune response to be avoided. This approach will be potentially effective against *K. pneumoniae* and will build a useful defense to combat this drug-resistant pathogen.

Impact

Currently, in Italy, the mortality rate for infection of multi-resistant *K. pneumoniae* strains (KPC type carbapenemase producers) in patients who have undergone allogeneic stem cell transplantation (SCT) is 64.4%; The current trend is not to perform this type of transplantation in patients who are positive for *K. pneumoniae*, due to the high rate of failure. Infections with *K. pneumoniae* are also a serious problem in patients who are candidates for solid organ transplantation. Our laboratories are located in the Mediterranean Institute for Transplantation and High Specialization Therapies (ISMETT), in Palermo, Sicily, a leading health facility for transplantation procedures. At ISMETT, patients are regularly monitored for infection and / or intestinal colonization by *K. pneumoniae*, due to its impact on the success of the transplant procedure and potential further infections of other organs. We have successfully identified and followed cases of *K. pneumoniae* infection, distinguishing between colonization and infection. We also observed the *in vivo* transfer of the blaKPC-3 gene conferring resistance to carbapenems between *K. pneumoniae* and E. coli in patients who had to undergo surgery, which emphasizes the need for constant surveillance to prevent the spread of multi-organisms resistant. In fact, we have been part of national multicentric networks both for nosocomial infections and for pathogens that can compromise the success of transplants.

Results achieved in 2017

We have identified and cloned 6 potential adhesive proteins of *K. pneumoniae* that play an important role in the adhesion processes, for example, those that compose the fimbriae of this pathogen (FimH,

FimA, MrkD and MrkA) or are involved in its colonization, as KPN_01507 and KPN_01508, from an isolated clinical *K. pneumoniae* ST512 belonging to the bank of patient isolates of IRCCS ISMETT. We are optimizing protein purification protocols and future functional assays for the characterization of these antigens. These recombinant proteins will be used as controls in experiments where the potential of genetically engineered yeasts which express the same antigens will be assessed.

Publications

Di Mento G, Cuscino N, Carcione C, Cardinale F, Conaldi PG, Douradinha B, Emergence of a Klebsiella pneumoniae ST392 clone harbouring KPC-3 in an Italian transplantation hospital, *Journal of Hospital Infection* (2018)

Goals for 2018

With this project we plan to develop a new immunotherapy against *K. pneumoniae* based on genetically modified probiotic yeasts to express proteins with a role in the adhesion of this bacterial pathogen and able to reduce its colonization. The probiotic strains will be characterized, not only, for the ability to induce a specific humoral immune response against this bacterium but for that of stimulating the secretion of important chemokines able to keep the bacterial infection under control. The immuno-therapy we proposed would also work for the multidrug-resistant strains of *K. pneumoniae*, since the mechanisms that lead to resistance cannot evade the immune response. We are convinced that this new approach will be effective against *K. pneumoniae* and that it will help fight this drug resistant pathogen. In further experiments, the ability of experimental vaccines to prevent infections caused by hypervirulent pathogens will be explored, especially meningitis, which is the most severe manifestation. Also, our approach will also be tested on other colonization models of *K. pneumoniae*, such as the urinary and respiratory tract. Furthermore, given the clinical importance of this pathogen in immunosuppressed persons, for example, those who need to undergo a transplant, we will study the impact of our vaccination strategy on a mouse model of immunosuppression.

Therapeutic Area: **INFECTIOUS DISEASES**

Genetically engineered probiotic *Saccharomyces cerevisiae* strains as potent inducers of cellular and mucosal immune responses against HIV

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Brief description

With this project, we expect to obtain a novel vaccination strategy against HIV based on transformed probiotic *S. cerevisiae* strains. We assume that a synergistic effect between the probiotic modulation of the immune system and mucosal immunity and the immune response elicited by the expression of HIV Gag will provide a broad immunogenicity and protection against this viral pathogen.

Impact

The vaccination strategy we are proposing would prevent novel infections, thus reducing the costs of future and expensive antiretroviral therapies. Also, production and maintenance of genetically-engineered probiotic yeasts is very economic, since the inherent production and storage costs are very low. Due to the low costs associated with probiotic yeasts production and potential storage in lyophilized form without losing their efficacy, this vaccination strategy would be ideal to be used in areas which lack medical and health infrastructures able to efficiently maintain vaccine and other drugs batches that need to be kept in a refrigerated environment. Plus, it will also benefit the general population, including the most affected areas worldwide. Likewise, our proposed vaccination approach could be extended to all individuals at risk of being infected by HIV, either in the developing or in the developed world, which would contribute to the eradication of this disease. Finally, use of genetically-engineered probiotic yeasts as a vaccination strategy could be applied synergically with another similar approach currently under development, which might result into a faster development of a more effective vaccine against HIV.

Results achieved in 2017

We have genetically-engineered several *S. cerevisiae* strains, both probiotic and non-probiotic with a bicistronic plasmid carrying the sequence of the HIV Gag antigen optimized for expression in this yeast. Using the AGA1p/AGA2p system, we successfully expressed this viral antigen in the surface of all the tested yeasts, as confirmed by indirect immunofluorescence and flow cytometry. Also, genetically-engineered strains were still resistant to GIT simulated stresses, indicating that the genetic manipulation did not impair their ability to resist to these harsh conditions. For the remaining assays, we selected the two probiotic yeasts with higher level of expression of HIV Gag, S. boulardii and the veterinary *S. cerevisiae* Sc47, and *S. cerevisiae* BY4743 as a negative, non-probiotic control. Transformed yeasts were eagerly phagocytosed by DCs derived from healthy donors and matured these immune cells into an immune response of type 1 (Th1), according to the cell surface markers and secreted cytokines analyzed. Interestingly, only the probiotic strains transformed with the Gag-expressing plasmid, but not with the empty plasmid,

were able to induce a specific Gag memory T cell immune response using immune cells derived from an HIV+ patient from the Multicenter AIDS Cohort Study (MACS) from the University of Pittsburgh, PA, USA.

Meetings

FEMS Microbiology Congress 2017, July 2017, Valencia, ESP

Goals for 2018

We will mature DCs from both healthy and MACS HIV+ patients with the transformed yeasts and assess the levels of secreted cytokines to characterize the type of immune response induced by our vaccination strategy. The ability to induce a T cell response will be measured by co-cultivating matured DCs with autologous T cells and quantifying the number of IFN-γ secreting CD4+ and CD8+ T cells. While DCs of naïve donors will determine if the maturation with yeasts induces a specific response against Gag, maturation of DCs from HIV+ patients will confirm if the yeasts can stimulate a memory response. We will also test the ability of the transformed yeasts to induce an immune response *in vivo* using a BLT-derived humanized mouse model. The peripheral blood mononuclear cells will be collected and DCs and T cells will be purified and characterized as described above, to confirm if immunization with the Gag-expressing yeasts stimulates a specific cellular immune response against Gag. Mice will be immunized orally with the transformed yeasts using a pre-established regimen and characterized likewise. Intestinal fluids from orally immunized mice will also be collected and the levels of Gag specific IgG and sIgA assessed. Finally, immunized mice, either intraperitoneally or orally, will be challenged with infectious HIV viral particules and confronted with non-immunized controls to assess the level of protection mediated by Gag-expressing probiotic *S. cerevisiae* strains.

Therapeutic Area: **METABOLIC DISEASES**

Hsp10/EPF in human pancreas: possible role in islets of Langerhans function

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Brief description

Type 1 diabetes (T1D) is a disease of autoimmune origin, in which insulin-producing cells (β cells) present in endocrine pancreatic tissue (islets of Langerhans) are drastically destroyed by the immune system. The most recent approach to cure diabetes is based on the transplantation of human pancreatic islets isolated from pancreases of healthy deceased donors. However, the use of immunosuppressive drugs might affect patients by one or more of side effects. Heat Shock Protein of 10 kDa (Hsp10) is a molecular chaperone involved in intracellular activities such as protein folding, and in materno-fetal immune tolerance when secreted as a soluble factor (called Early Pregnancy Factor - EPF). Mesenchymal stem cells (MSCs) are known to modulate immune responses, thus emerging as prominent candidates for cell-based therapies to control conditions in which the immune system is hyperactivated. The goals of this project are: first, to examine the expression levels and physiological roles of Hsp10/EPF in both human pancreas and human MSCs (hMSCs) from different sources (bone marrow- and pancreatic islet-derived hMSCs); and second, to engineer hMSCs to overexpress Hsp10/EPF aiming at generating a cell-based immunosuppressive therapy to potentially be used during islet transplantation.

Impact

This project has a significant impact on the advancement of T1D therapeutic approaches, specifically islet transplantation, as it is part of a novel strategy to reduce or eliminate the dependence on the deleterious immunosuppressive regimes post-transplantation. The use of "engineered" MSCs (Hsp10-overexpressing MSCs) may have a strong impact on the immune control after islet transplantation by inducing a definitive state of "immune tolerance". This may improve and prolong islet function and life, avoiding the patients to be affected by drastic side effects due to immunosuppressive therapy.

Results achieved in 2017

As described in the Scientific Report 2016, Hsp10, which shares structural homologies with some virus-derived proteins involved in the T1D onset, showed for the first time a correspondence with insulin, in terms of both localization and intensity, and a marked expression in non-diabetic seropositive-CMV/EBV human pancreases, compared to seronegative ones. Therefore, Hsp10 could also have a role in modulating β cell functions, such as insulin trafficking and release. To ascertain the co-localization of the Hsp10 and insulin, and possible involvement of Hsp10 in β cell functions, other samples of human pancreas were used for further analyses by immunofluorescence. In particular, the Hsp10/insulin co-localization was analyzed in human pancreases from different healthy and T2D donors. Hsp10 is localized inside the islets in all of

the three different non-diabetic donors and, specifically, co-localized in β cells together with insulin. However, positive staining was also detected in non-endocrine tissue, suggesting that Hsp10 could be also expressed by exocrine cells. In order to confirm the expression of Hsp10 by different pancreatic cell populations, the tissue derived from digested pancreases was stained for flow cytometry analyses. Both endocrine and exocrine cells express Hsp10, confirming the immunofluorescence data. Hsp10 was also analyzed in pancreases from three deceased donors who were diagnosed of T2D. Hsp10 was localized in both endocrine and exocrine tissue and, interestingly, was found to decrease along the duration of the pathology. This may suggest that Hsp10 could be involved in insulin release. To support this hypothesis, Hsp10 was also analyzed in human pancreatic islets transplanted under the kidney capsule of STZ-induced diabetic nude mice. The outcome after transplantation was followed and it was seen that not all the preparations had the same effect in reversing diabetes and this seemed to correlate with the expression of Hsp10 in β cells. The results obtained could support the hypothesis of a possible role of Hsp10 in islets function, metabolism and insulin release. Since the correct function of the islets is related to a proper metabolic support and oxygenation, the importance of the vasculature was also taken into account. Moreover, one of the most challenging approach is the therapeutic use of MSCs during islet transplantation. Therefore, the immunomodulatory features are crucial. In this study, the pancreases from healthy donors were used for analyzing in perivascular cells (by immunofluorescence and flow cytometry) the expression of both Hsp10 and HLA-G, because both of molecules are involved in the materno-fetal immune tolerance. Moreover, the inflammatory status may induce MSCs to express protective molecules with immune modulatory features. Therefore, a preliminary experiment on pancreatic islet-derived MSCs exposed to inflammatory cytokines, such as IFNY and TNFα, separate or combined (cytomix) was carried on in order to evaluate any change in Hsp10 expression, as immune modulating molecule. The result obtained may suggest a critical role of the balance between inflammatory cytokines in both the extracellular and intracellular space. However, a single observation is not enough and needs to be verified and statistically proved with further experiments and using different sources of MSCs, such as bone marrow and pancreatic islet.

Meetings

50th Miami 2017 Winter Symposium - Diabetes: Today's Research - Tomorrow's Therapies, January 2017, Hyatt Regency, Miami (USA)

Goals for 2018

- Establish the best hMSCs sources and the correct combination of cytokines needed for priming MSCsthem to induce the overexpression of Hsp10.
- *In vitro* analyses on the effect of "engineered" hMSCs on immune responses with and without human islets for possible application in *in vivo* animal models.

Therapeutic Area: **METABOLIC DISEASES**

Pancreatic Islet Transplantation

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Brief description

The procedure was performed to activate the clinical activity concerning the "Processing and use of human cells for transplantation purposes". In particular, the aim of the project is to transplant the Langherans Islet in patients with Type I diabetes. Type 1 diabetes mellitus falls into the category of autoimmune diseases caused by the production of autoantibodies against Beta cells involved in the production of insulin.

Impact

The removal of the whole pancreas can be associated with a series of complications, a high risk of hypoglycaemia (despite insulin treatment) and the lack of hormones important for maintaining blood glucose regulation, such as glucagon. Pancreatic islet transplantation offers advantages over total organ replacement, thus favoring a blood glucose control.

The process includes the removal of pancreas from cadaver donor and subsequent processing aimed at preparing pancreatic islets for transplantation in patients with type I diabetes mellitus. In order to achieve insulin-independence there is a need to transplant a sufficient number of insula which can also be obtained from more than one pancreas; the success of the therapy depends on the possibility of preserving the functional insular mass during all the phases that precede and follow the transplant: cold preservation, optimization of the isolation procedure, transplantation and post-transplant follow-up. The isolation procedure represents a critical phase capable of influencing the number and quality of the final preparation for transplantation.

The same intrahepatic transplant procedure contributes to the loss of a conspicuous mass of insulae. Approximately 60% of the implanted islets have been estimated to undergo cell death immediately after intra-oral infusion due to a nonspecific inflammatory response. This criticality can be remedied by carrying out, in parallel with the transplantation activity, a research activity on the pancreatic islet unsuitable for transplantation that can provide for the application of biocampable systems capable of preserving the islet from immune attack.

Results achieved in 2017

Following the revision and remodulation of the Quality Manual and of all the Standard Operating Procedures (SOPs) concerning both the optimization of the isolation protocol and the management of the CNT, the following results have been achieved: 05/05/2017: CRT authorization for procurement, isolation and transplantation of the Pancreatic Islet. 12/07/2017: Obtaining authorization by the Istituto Superiore di Sanità following the inspection of 03 / 07/2017 and start of the transplantation program. We have tried six pancreas and we did a Transplant on 03/09/2017 and another suitable for transplantation (which was not carried out due to positive cross-match between donor and recipient).

Goals for 2018

Perform at least three islet transplantations in 2018. Being able to obtain authorization for the use of Pancreatic Isles unsuitable for research.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Study of the function of globins in zebrafish heart regeneration and embryonic development

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Breve descrizione

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. The major finding to this regard was the discovery that the hemoglobin can react with nitrite and generate NO when the protein is deoxygenated and the heme iron is in the ferrous state. Myoglobin was subsequently found to protect the cardiac muscle during myocardial infarction through NO production. In the current research, I use the zebrafish model to define the role of these newly discovered globins (Cygb1, Cygb2 and Xgb) in the zebrafish heart regeneration and during 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 organism and understand their role *in vivo*. Specifically, this project is developed in the following directions:

- Study of the function of the globins and role of nitrite on zebrafish heart regeneration.
- Study of Cytoglobin 2 in embryonic development.
- Study of the role of Cytoglobin 1 in heart regeneration.

Impact

Cytoglobin was discovered in 2002 and Globin X in 2006 and yet the physiological function of these cellular globins is unknown. The current consensus is that the globins signals through redox signaling properties. We discovered novel functions for Xgb and Cygb. Xgb is a nitro-reductase with anticoagulant effects of nitrite in zebrafish heart regeneration favorable to an increase in cardiomyocyte proliferation and neovascularization. Cygb2 is important in signal transduction to establish left-right patterning in cardiac development. These effects on cardiomyocyte proliferation and cardiac development have important potential therapeutic implications for cardiovascular disease. Understanding the mechanisms that govern cardiac development and regeneration and ultimately to gain access to the pathways that allow modulation of these processes is a long-term goal that can lead to significant advances in developing new therapeutics in treating heart disease associated with cardiomyocyte loss.

Results achieved in 2017

Xgb is found in the fish red blood cells and displays anticoagulant properties on human platelets in the presence of nitrite. These findings are published in a paper in PNAS (Corti et al., 2016). We investigated the role of nitrite and Xgb in the zebrafish heart regeneration model and found that nitrite exposure stimulates the immune system and improves the heart regeneration program by increa-

sing cardiomyocyte proliferation. Specifically, neutrophils macrophages and thrombocytes accumulate in higher amounts while red blood cells are cleared faster at the site of the injury. These results in a higher neovascularization rate and improvement in heart regeneration. We present a new role for cygb in signal transduction to establish LR patterning during heart development. This discovery sets a new paradigm for globin function and establishes a central role for cygb in cardiac development. In order to understand the role of xgb and cygb in cardiac regeneration and development, using CRISPR/Cas9 technology for gene editing we generated zebrafish xgb, cygb1 and cygb2 knock-out mutants. We are in the process to establish our maternal zygotic line in order to understand the role of the globins *in vivo*.

Meetings

Nitric Oxide Gordon Research Conference. Invited Lecture *Globin X is a nitrite reductase*, February 2017, Ventura, USA.

SFRBM Annual Meeting, Poster presentations. *Interactions of zebrafish Cytoglobins with oxygen and nitric oxide* and *Nitrite improves zebrafish cardiac regeneration potentially via Cytoglobin 1*,29 November - December 2017, Baltimore, USA

Publications

Corti P., Xue J., Tejero J., Wajih N., Sun M., Stolz D.B., Tsang M., Kim-Shapiro D.B., Gladwin M.T. (2016). Globin X is a six-coordinate globin that reduces nitrite to nitric oxide in the fish red blood cells. *PNAS* 113(30):8538-43. PMID: 27407144.

Amdahl M.B., Sparacino C.E. -Watkins, Corti P., Gladwin M.T., Tejero J. (2017). Efficient Reduction of Vertebrate Cytoglobins by the Cytochrome b5/Cytochrome b5 Reductase/NADH System. *Biochemistry* 56(30):3993-4004. PMID: 28671819.

Rochon E., Corti P., Gladwin M.T. (2017). Hemoglobin lost in the lung: non-erythroid expression and putative functions of hemoglobin family members. Commentary on "Targeting pulmonary endothelial hemoglobin α improves nitric oxide signaling and reverses pulmonary artery endothelial dysfunction." *American Journal of Respiratory Cell and Molecular Biology*. In *press*.

Goals for 2018

The proposed 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 will be studied in the embryonic development and the nitrite effect will be tested on 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. It is worthwhile to speculate that a deeper knowledge of zebrafish heart regeneration will have significant impact on our understanding of mammalian heart regeneration.

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 focus of research is the study of bio-material and engineered tissue, their rheological-mechanical characterisation, and the development of relative clinical devices.

Ri.MED is creating a platform based on essential instruments (both physical and computational) and equipment for the carrying out of numerical simulations and experimental tests in order to verify and qualify the structural and fluid-dynamic performance of the clinical solutions developed, in accordance with regulatory requirements for EC certification and FDA approval.

The platform allows for the optimisation of tissue engineered for various applications, in particular in the cardiovascular field, thanks to a better understanding of the effect of fluid-mechanical and structural stimuli on cell differentiation and proliferation. The platform also allows for the use of engineered tissue in the planning, development and pre-clinical validation of organs and new-generation implantable cardiovascular components. The possibility for in-house development and validation of clinical solutions, together with collaboration with the most important clinical centres in the area, facilitates the introduction of patient-specific and population-specific treatment, while at the same time offering new instruments for support in therapeutic planning and in the pre-operative decision process.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Development of a Novel Alfa-Gal Free Xenograft Heart Valve

Gaetano Burriesci, Ph.D.
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Leading Partner
University College London, UK

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. We 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, we have genetically altered pigs so they no longer make Gal and have shown that this Gal-free tissue has a reduced rate of calcification. 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.

Results achieved in 2017

We have already verified that both genetically altered and standard pig tissue have the same general morphology and collagen content, and uniaxial stress and suture retention testing has shown that the tissues are equivalent in tensile strength. Bioprosthetic valve prototypes based on the two tissues, designed and developed in our team, have indicated that genetically altered and standard pig tissue provide similar valve durability and failure mode. *In vitro* and *in vivo*

acute assessment has shown for both tissues excellent and equivalent hydrodynamics (as per the standard ISO 5840:2015). Durability tests are ongoing, and we have already shown the potential of our devices to meet the estimated life requirements from the international regulation.

Meetings

Physical and hydrodynamic equivalency of wild type and alfa-gal free GTKO porcine pericardium; a new source material for bioprosthetic heart valves, McGregor, C., Byrne, G., Rahmani, B., Chisari, E., Kyriakopoulou, K., Burriesci, G. *Heart Valve Society Meeting*, March 2017, Montecarlo, MCO

Publications

McGregor C., Byrne G., Rahmani B., Chisari E., Kyriakopoulou K., Burriesci G. (2016) Physical equivalency of wild type and Galactose α 1,3 Galactose free porcine pericardium; a new source material for bioprosthetic heart valves. *Acta Biomateralia* 41:204–209

Intellectual property

Burriesci G., Rahmani B., Byrne G., McGregor C. (2016) Bioprosthetic heart valve. Application Number: GB1612180.8

Goals for 2018

Approximately 300,000 valve replacements are performed annually worldwide. Two types of replacement valves are available, mechanical heart valves, which require lifetime anticoagulation, and bioprosthetic heart valves, made from biological tissues, typically human or porcine heart valve leaflets or animal pericardium. Bioprosthetic heart valves are preferred in older patients (>60 years) where they are more durable. Patients under 60 generally receive mechanical heart valves, due to rapid age-dependent bioprosthetic heart valves degeneration. In patients under 35 years of age, up to 100% structural valve deterioration occurs within 5 years. More durable bioprosthetic heart valves would advance the standard of care by eliminating the need for anticoagulation in younger patients and extending access to this therapy to more patients.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Prediction of the Ischaemic Lesions Potential after Heart Valve Therapy

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Brief description

An advanced understanding of the implications of the hemodynamic alterations produced by diseases and clinical corrections (such as surgical and transcatheter bioprosthetic valves) is essential in order to improve the therapeutic planning and the outcome from the treatments.
The latest developments in computational engineering, in-vitro assessment and medical imaging can substantially contribute to gain an adequate insight in the phenomenon, by overcoming the limitations in time and space resolution of the individual techniques.

Impact

Our ultimate aim is the assessment of the flow dynamics for different valve devices, anatomical phenotypes and diseases. This step is essential to enhance the safety and effectiveness of valve treatments, and will provide information to support the selection of the best therapy for a specific patient. Moreover, the developed technologies will allow the development of next generation valve treatments and medical devices.

Results achieved in 2017

Our studies have shown that procedural approaches and specific medical devices (such as transcatheter valves) can produce significant variations of the blood flow, which could lead to suboptimal performance and/or the higher incidence of ischemic events.

Meetings

Fluid-structure-interaction model of Transcatheter Aortic Valve Implantation configuration: comparison with an in-vitro study, Tango, A.M., Ducci, A., Burriesci, G. *7th International Conference on Computational Bioengineering*, September 2017, Compiègne, FRA.

Blood flow triple-imaging, Annio, G., Ducci, A., Burriesci, G., Torii, R. *7th International Conference on Computational Bioengineering*, September 2017, Compiègne, FRA.

Publications

Maneas, E., Xia, W., Nikitichev, D., Daher, B., Manimaran, M., Wong, R.Y., Chang, C.W., Rahmani, B., Capelli, C., Schievano, S., Burriesci, G., Ourselin, S., David, A., Finlay, M., West, S., Vercauteren, T., Desjardins, A. (2017) Anatomically realistic ultrasound phantoms using gel wax with 3D printed moulds. *Physics in Medicine and Biology* DOI: https://doi.org/10.1088/1361-6560/aa9e2c

Capelli, C., Corsini, C., Biscarini, D., Ruffini, F., Migliavacca, F., Kocher, A., Laufer, G., Taylor, A.M., Schievano, S., Andreas, M., Burriesci, G., Rath, C. (2017) Pledget-armed sutures affect the hemodynamic performance of biologic aortic valve substitutes: a preliminary experimental and computational study. *Cardiovascular Engineering and Technology* 8(1):17-29.

Goals for 2018

The main objective of the project is to understand the clinical implications of the alteration of the blood flow produced by surgical and transcatheter bioprosthetic valves, in order to improve the therapeutic planning and the outcome from the treatments. This involves experimental techniques to identify the phenomena, coupled with computer simulations to expand the finding and simplify it potential clinical use.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Analysis of the Left Atrial Appendage to Predict Thrombosis Risk

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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.

Impact

The study supports the hypothesis that the left atrial appendage morphology is a leading factor in the pathology, thus making some atrial fibrillation patients more at risk, depending on their anatomical features. The presented approach can become a powerful tool to quantitatively analyze parameters otherwise impossible to measure in clinics and to study the geometrical factors influencing thrombus formation. Lastly, this methodology has shown a significant potential in supporting clinical stratification of patients under high risk of thrombus formation, thus supporting the selection of individualized therapies and improving the patient's safety and standard of care.

Results achieved in 2017

Computational Fluid Dynamic analyses based on patient-specific anatomies were implemented in four cases descriptive of the most common morphologies of the left atrial appendage. Transient simulations were carried out allowing the flow to fully develop, estimating the residence time in the different regions of the appendage in normal conditions and in the presence of atrial fibrillation. Our results highlight the effect of the appendage shape on the blood flow pattern and the fluid alterations produced in presence of atrial fibrillation.

Meetings

Computational Fluid Dynamic Analysis of the Left Atrial Appendage to Predict Thrombosis Risk ,Bosi, G.M., Cook, A., Rai, R., Menezes, L., Schievano, S., Torii, R., Burriesci, G. *7th International Conference on Computational Bioengineering*, September 2017, Compiègne, FRA.

Goals for 2018

A much larger number of patient specific cases is needed to clarify and generalize the relation between the morphology of the left atrial appendage and the hemodynamic behavior leading to high risk of thrombus formation. More simulations will be performed in the future, and results will be validated against purposely designed experiments.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Development of a Novel Transcatheter Aortic Valve

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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 co-morbidity 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/replacement (TAVI or TAVR) represents an ideal answer to the need 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 feasible and promising, 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 shown in the animal model, 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 2017

The research performed so far within this project has led to the design optimization and manufacturing of a novel TAVI device that meets the initial requirements.
In particular, three valve models of nominal size equal to 23, 26 and 29 mm have been developed, optimized in terms of structural performance and manufacturing.
Functional and hydrodynamic tests have confirmed that the developed solution is characterized by global hydrodynamic performance comparable or superior than the main competing devices currently in the market, thanks to a significant reduction of the paravalvular leakage. Performance of the prototypes meets the requirements recommended in the international standard ISO 2840-3:2013. Delivery systems allowing the valve loading and release have been designed and manufactured. Acute animal implants have confirmed that: the implantation of the study valve with

the developed delivery system is easy to handle; the device is retrievable and can be repositioned after full deployment; the properly sized device has good haemodynamic performance, with proper leaflets motion, minimum stenosis, reduced regurgitation and no interference with coronary blood flow. Durability *in vitro* tests have demonstrated that the proposed technology is suitable to guarantee and largely exceed the durability requirement for flexible leaflets prosthetic valves (200 million cycles).

Meetings

Barts Heart Valve Program Symposium Aortic stenosis current and future practice - *Developing new valve technology - an engineering perspective* - November 2017, London, UK

Translational Research Symposium - 28° European Society for Biomaterials Annual Conference - *Reimagining transcatheter heart valves* - September 2017, Athens, GR

Publications

Rahmani, B., Tzamtzis, S., Sheridan, R., Mullen, M.J., Yap, J., Seifalian, A.M., Burriesci, G. (2017) In-Vitro Hydrodynamic Assessment of a New Transcatheter Heart Valve Concept (The TRISKELE). *Journal of Cardiovascular Translational Research* 10(2):104-115.

Intellectual property

- Burriesci, G., Tzamtzis, S., Seifalian, A. M. (2017) Prosthesis delivery system. Patent US9597211 B2
- Burriesci, G., Tzamtzis, S., Seifalian, A. M. (2017) Prosthesis delivery system. Patent EP2629700 B1
- Burriesci, G., Tzamtzis, S., Mullen, M. J., Seifalian, A., Yap, J. (2017) Vascular implant. Patent EP3043746 B1

Goals for 2018

The funded project has been successfully completed, achieving a solid proof of concept for the proposed technology. Our main goal is now to source support to perform the in animal chronic evaluation and attract interest from industrial partners, to facilitate the route to market.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Development of a Novel Transcatheter Mitral Valve

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Brief description

Valve replacement with transcatheter implantation allows delivering a valve substitute into orthotopic position through a catheter inserted from a peripheral vessel. This technique avoids the need for open-heart surgery and eliminates the main risks associated with conventional invasive operations, such as the need for cardiac arrest/restart, or the potential damages due to bypass pumps; therefore, it is suitable for all categories of patients currently excluded from the surgery. This approach has already become the treatment of preference for high-risk patients suffering from aortic stenosis. However, percutaneous replacement of the human mitral valve is still far from established, due to the more challenging technical difficulties associated with the atrio-ventricular valve. The objective of this project is to develop, a novel prosthetic device suitable for transcatheter mitral replacement. This would represent a major contribution to the development of a more sustainable treatment.

Impact

Mitral regurgitation is one of the major mitral valve pathologies leading to heart failure. Patients with severe symptomatic Mitral regurgitation have a very low survival rate in absence of corrective interventions. However, only a small portion of patients suffering from functional mitral regurgitation and approximately half of those suffering from degenerative mitral regurgitation is currently suitable for surgery, due to the high risk of the procedure. Minimally invasive transcatheter implantation can reduce the risks in these patients and offer a more sustainable alternative therapy for mitral valve diseases.
The device developed in this project is expected to improve the device's placement and interaction with the surrounding anatomical structures, compared to competing transcatheter mitral valves under development. *In vitro* assessment has provided a sound proof-of-concept for the proposed technology, and the next step will involve completion preclinical *in vivo* evaluation, necessary before first-in-man.

Results achieved in 2017

A novel Transcatheter Mitral Valve was developed, consisting of two bovine pericardial leaflets designed to ensure proper functionality across a range of implantation configurations, and a sealing cuff, supported by a wireframe, optimized to minimize stresses whilst crimped. The device met and exceeded the minimum performance requirement from the international standard ISO 5840-3:2013, thereby proving its feasibility as a mitral valve substitute to treat mitral regurgitation. The transcatheter mitral valve prototypes have currently achieved 80 Million cycles. The developed device is expected to be a viable alternative to transcatheter repair techniques and, due to its geometric similarity to the human mitral valve anatomy, may provide a more appropriate option compared to the other circular transcatheter valves in development.

Publications

Bozkurt S., Preston-Maher G.L., Torii R., Burriesci G. (2017) Design, Analysis and Testing of a Novel Mitral Valve for Transcatheter Implantation. *Annals of Biomedical Engineering* 45(8):1852-1864.

Intellectual property

Burriesci G., Bozkurt, S., Rahmani, B., Mullen, M.J. (2016) Prosthetic heart valve. Patent WO2016203241 A1

Goals for 2018

We are currently planning to perform the in-animal evaluation with the device developed in this project. If successful, this may result in further validation of the safety and efficacy of the proposed solution, stimulating the route to the clinic.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Tissue engineered heart valve (TEHV)

Antonio D'Amore, Ph.D.
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Brief description

TEHV: to develop effective tissue surrogates and biomedical devices for heart valve replacement and repair.

Impact

Introduction of strategies and innovative technologies for heart valve repair and replacement.

Results achieved in 2017

Scientific results:
Introduced innovative technologies for biomimetic, polymeric valve fabrication reproducing native tissue structure and function. Demonstrated capacity to duplicate in-plane mechanics, flexural mechanics, geometry and size of native valves at macro and micro scale. Initiated related IP *in vivo* assessment on large animal model. Developed new IP for chordae tendineae biofabrication. Extended cardiac tissue characterization including: heart valve, myocardium, coronary arteries, chordae tendineae. Obtained authorization and access to human samples from donors for advanced characterization of heart valve physiology. Consolidated OneValve IP portfolio. Numerical simulation of mitral valve with chordae project in collaboration with Dr Pasta.

Active grants:
(2016-2017) Coulter foundation 2016 $100,000. Assessment of acute response to: polyurethane, stentless, multi-leaflet mitral valve; on porcine model. Co-PI: V. Badhwar, Co-PI: A. D'Amore, Co-PI: W. Wagner, University of Pittsburgh;

(2016-2017) Clinical & Translational Science Institute (CTSI), University of Pittsburgh; $50,000. Assessment of acute response to: self-expanding non-degradable, low-profile percutaneous tricuspid valve; "template based", electrospun, biodegradable, tricuspid valve with chordae tendineae; electrospun, biodegradable, polyurethane, stentless, multi-leaflet tricuspid valve; on porcine model. Co-PI: A. D'Amore, Co-PI: W. Wagner, University of Pittsburgh;

(2017) Industrial collaboration with Peca lab, start-up company created by Carnegie Mellon University, $5,000 renewable consultancy service. PI: A. D'Amore.

Awards:
(11/2017) Finalist for the Franco Strazzabosco award for young engineers - ISSNAF (Italian Scientists and Scholars in North America Foundation), top 3 engineers < 40 years old in Italy, Washington DC.

Invited speech:
(11/2017) "Heart valve structure, functional heterogeneity and mechanics". Mediterranean institute for transplantation and advanced specialized (ISMETT) Palermo, Italy;

(11/2017) "Bioinspired control of structure-function to design better performing tissue engineering heart valves". ETH, Zurich, Switzerland.

Meetings

In Vivo Functional Assessment of a Novel Bioinspired Scaffold-Based Tissue Engineered Heart Valve, 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. *American Association for Thoracic Surgery (AATS 2018) 2018 meeting*, April 2018, New York, USA

Publications

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

US provisional patent application 62/473,588 filed on 03/2017, topic: biomedical device, title: "Mandrel-less Electrospinning Processing Method and System, and Uses Therefor". Lead innovator/developer: A D'Amore;

US provisional patent application 62/458,234 filed on 02/2017, topic: biomedical device, title: "Expandable percutaneous cannula". Lead innovator/developer: A D'Amore;

US provisional patent application 62/462,628 filed on 02/2017, topic: biomedical device, title: "A stentless biopolymer heart valve replacement capable of living tissue regeneration". Lead innovator/developer: A D'Amore;

US patent application PCT/US2017/014341 with WO (International publication number WO 2017/127682 A1) published on 08/2017, topic: biomedical device and surgical method, title: "Trans-atrial access for intracardiac therapy".

Goals for 2018

To complete on-going pre-clinical studies with the main aims:

- to assess kinematics and function on acute large animal model (e.g. , moderate regurgitation, good leaflet motility);
- to characterize and duplicate human heart valve physiological mechanics;
- to assess endogenous tissue growth/resistance to calcification/low thrombogenicity;
- to explore percutaneous delivery strategies.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Tissue engineered cardiac patch (TECP)

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Brief description

TECP : to develop effective cardiac restraint devices for post myocardial infarction patients.

Impact

Introduction of strategies and innovative technologies to mitigate the pathological remodeling induced by miocardium infarction.

Results achieved in 2017

Scientific results:
Completed rat study assessing bilayered cardiac patch, main results: reduction of wall thinning, angiogenesis, reduced scar formation, mitigating functional deteriorating of the left ventricle at 10 weeks from infartion, M1-M2 switch. Completed *in vivo* evaluation on abdominal wall defect of scaffold for drug controlled release, project in collaboration with Dr Fazzari and Dr Freeman. Main results: enhanced angiogenesis, reduced fibrosis. Demonstrated *in vivo* concept for autologous transplant of muscle flap obtained from multi-layered patch, project financially supported by AFIRM- DoD.

Active grants/collaborations:
Armed Forces Institute of Regenerative Medicine, AFIMRII, W81-XWH-13-2-XXXX, $900,000 for 1/2014 - 12/2017. 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;

NIH 5R01 AR054940-0. Cellular remodeling of ECM scaffolds. PI: S. Badylak, University of Pittsburgh. Role: A. D'Amore collaborator, ECM-polymeric scaffolds for cardiac patch design and fabrication;

Teaching activity:
(11/2015-11/2017) 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 remodeling.

Meetings

Design of Thermoresponsive Hydrogels and Biodegradable, Thermoplastic Elastomers for Interventions in Cardiac Wall Remodeling Following Myocardial Infarction, Y. Zhu, X Gu, A. D'Amore, Y Matsumura, W. Wagner. *Society for Biomaterials Conference (SFB),* April 2017, Minneapolis, USA

Lectio magistralis:
Reducing the injury from myocardial infarction: engineered biohybrid cardiac patch. McGowan Institute for Regenerative Medicine scientific retreat. Cardiovascular session, March 2017, Nemacolin, USA.

Publications

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. McGowan Institute for Regenerative Medicine' paper and picture of the month (12-2017).

Chen, Y. Zhu, S. Ye, S. K. Luketich, A. D'Amore, W. Wagner. Hybrid scaffolds of Mg alloy mesh reinforced polymer/extracellular matrix composite for critical-sized calvarial defect reconstruction. Submitted to *Journal of Tissue Engineering and Regenerative Medicine*, IF 4.71."

Intellectual property

US patent application PCT/US2017/051005 with WO, (International publication number WO 2016/044787 A1) published on 03/2017, topic: biomedical device, title: "Bi-layer polyurethane - extra cellular matrix scaffolds for improved ischemic ventricular wall remodeling". Lead innovator/developer: A. D'Amore;

US provisional patent application 62/588,830, filed on 11/2017, 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.

Goals for 2018

- Development of scaffold for cardiac patch, primary aims:
  - help sustain left ventricle function
  - endogenous tissue growth/reduce scar formation
  - mitigate left ventricle wall thinning
- Numerical study of the impact of thickness and elastic modulus of scaffolds utilized for myocardium patching, project in collaboration with Dr Soares and Dr Sacks.
- To explore minimally invasive techniques for cardiac patching, project in collaboration with Dr Pilato, Dr Morsolini, and Dr Raffa (ISMETT).
- Effects, methods and potential of right ventricle patching, project in collaboration with Dr Coyan, Dr Silveira-Filho and Dr Sciortino (UPMC).

Therapeutic Area: **CARDIOVASCULAR DISEASES**

Tissue engineered vascular graft (TEVG)

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Brief description

TEVG: to develop effective engineered blood vessels for coronary artery bypass graft.

Impact

Introduction of strategies and innovative technologies for coronary bypass and critical limb ischemia

Results achieved in 2017

Scientific results:
Completed rat study assessing bilayer vascular graft and same day scaffold seeding. Perfected fabrication technique and prototyped new vascular graft model for 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 R21 and R01 projects.

Active grants/collborations:
NIH (NIBIB) 1R21EB016138, Autologous stem cell-based tissue engineered vascular grafts. PI: D. Vorp. University of Pittsburgh. Role: A. D'Amore collaborator, vascular graft fabrication;

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.

Lectures:
(02/2016-02/2017) 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".

Meetings

Assessment of Human Stem Cell Retention and Host Cell Invasion in an Implanted Seeded Tubular Scaffold, Snyder, K. Lorentz, D. Haskett, K. Saleh, A. D'Amore, W. R. Wagner, J. Weinbaum, D. Vorp. *Biomedical engineering society, annual meeting (BMES 2017)*, October 2017 Phoenix, USA.

Feasibility of a "same day" autologous tissue-engineered vascular graft remodeling in a seeded elastomeric scaffold, D. G Haskett, K.I 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. *Summer Biomechanics, Bioengineering and Biotransport Conference*. June 2017, Tucson, USA.

Publications

J. T. Krawiec, H.T. Liao, L. Y. Kwan, A. D'Amore, J. S.Weinbaum, J. P.Rubin, W. R.Wagner, D. A. Vorp. Evaluation of the Stromal Vascular Fraction of Adipose Tissue as the Basis for a Stem Cell-Based Tissue Engineered Vascular Graft. *Journal of Vascular Surgery 2017*, 66 (3), 833-890, IF 3.77.

Intellectual property

US provisional patent application 62/537,143, filed on 07/2017, topic: biomedical device, title: "Multi-Layered Graft for Tissue Engineering Applications". *Lead innovator/developer*: A. D'Amore.

Goals for 2018

- 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.
- 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.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

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

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Brief description

NET-IBA: development of algorithms and automatic methods for structural and morphological analysis of native tissues and scaffolds.

Impact

Innovative methods for quantitative histology;
Innovative methods for morphological analysis of micro and nano materials.

Results achieved in 2017

Scientific results:

- Leadership on methods for topological analysis of native tissue ECM and scaffolds.
  - Re-enforced expertise and leadership in image based structural analysis of native tissue and scaffolds. Available software include: micro-structural analysis of fibrous tissue, porosity analysis, collagen and elastin fiber analysis, topological analysis of cellular infiltration, specific markers spatial distribution, macrophages polarization.
- Initiated industrial collaboration with start-up company PECA Lab, topic: structural characterization of FDA class III medical device.
  - Consulting activity focusing on the development of ePTFE pediatric heart valve device.
- Validated software for the automatic detection of blood vessles on histological sections.
  - Active collaboration with Dr. Bruno, Dr. Ardizzone and Dr. Pirrone (Univ. Palermo).
- Validated software for structural characterization of ePTFE components.

Active grants/collaborations:
(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.

(2017) Industrial collaboration with Peca lab, start-up company created by Carnegie Mellon University, $5,000 renewable consultancy service. PI: A. D'Amore.

Awards/board qualifications:
(03/2017) Italian national board qualification for associate professor in biomedical engineering.

(12-2017)Nitro-oleic acid (NO_2-OA) release enhances regional angiogenesis in a rat abdominal wall defect model. Accepted on Tissue Engineering Part A, *IF 3.58*. McGowan Institute for Regenerative Medicine' paper and picture of the month (12-2017).

Meetings

Analysis of Expanded Polytetrafluoroethylene (ePTFE) Membranes for Use Within a Valved Conduit for Right Ventricular Outflow Tract Reconstruction, Loneker, A. Kalra, S.Luketich, D. Bernstein, A. D'Amore, D. Faulk. *Biomedical engineering society, annual meeting (BMES 2017)*, October 2017, Phoenix, USA

Preclinical Development of a Radially Expandable Vascular Conduit for Pediatric Cardiovascular Surgery, Loneker, A. Kalra, A. Nugent, S. Luketich, D. Bernstein, A. D'Amore, D. Faulk, *Biomedical engineering society, annual meeting (BMES 2017)*, October 2017, Phoenix, USA

Mechanical and Microstructural Analysis of an Expandable Vascular Conduit for Pediatric Cardiovascular Surgery, Loneker, S. Luketich, D. Bernstein, A. Kalra, A. D'Amore, D. Faulk. *Society for Biomaterials Conference (SFB)* April 2017, Minneapolis, USA

Publications

Loneker, S. K. Luketich, D. Bernstein, A. Kalra, A. Dees, A. D'Amore, D. M. Faulk. Mechanical and Microstructural Analysis of a Radially Expandable Vascular Conduit for Neonatal and Pediatric Cardiovascular Surgery. In press *on Journal of Biomedical Materials Research Part A*, IF 3.26.

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 (NO_2-OA) release enhances regional angiogenesis in a rat abdominal wall defect model. Accepted on Tissue Engineering Part A, *IF 3.58*. McGowan Institute for Regenerative Medicine' paper and picture of the month (12-2017).

Goals for 2018

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."

Therapeutic Area: **CARDIOVASCULAR DISEASES**

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

Antonio D'Amore, Ph.D.
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Brief description

NET-MTG: development of structural deterministic numerical models to predict mechanics, endogenous tissue formation and degradation of engineered and native tissue.

Impact

- 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.

Results achieved in 2017

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 characteristics), meso-scale mechanics (e.g. nuclear aspect ratio change, single fiber strain histogram at the meso-scale), micro-scale mechanics (e.g. single fiber flexural response).

Capacity to recapitulate fibrous materials topology.

Teaching:
(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;

Meetings

Engineering micro-architecture to enhance de novo extracellular matrix elaboration in an elastomeric scaffold model. International symposium on Nanoengineering for Mechanobiology, March 2017,Camogli, ITA

Publications

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. The aged skeletal muscle extracellular matrix promotes muscle stem cell fibrogenic conversion. *Aging Cells 2017* 16 (3), 518-528, IF 6.34

A. D'Amore, J. Soares, J. Stella, W. Zhang, N. Amoroso, J. Mayer. W. Wagner, M. Sacks. Large strain stimulation enhances extracellular matrix production and stiffness in an elastomeric scaffold model. Journal of the Mechanical Behavior of Biomedical Materials 2016 (62), 619–635, *5Y-IF 3.15.*

Goals for 2018

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.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

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

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Brief description

In vitro elastomeric models to investigate soft tissue mechanobiology.

Impact

- Capacity to simulate endogenous tissue growth on engineered scaffolds under mechanical load.
- Capacity to simulate *in vivo* degradation of engineered scaffolds.
- Capacity to investigate the impact of topological and mechanical cues on ECM elaboration.

Results achieved in 2017

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 is to address fundamental questions regarding meccano-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 desing;
  - mechanical load applied to biological tissues are imposed by physiology, the research hypothesis consists in verifying that given a specific mechanical load the meso-structure of the artificial tissue can be tailored in order to enhance endogenous tissue growth. The structural variable studied was the fiber intersection density.

- to implement a novel apparatus for chordae tendineae mechanical conditioning;
  - new IP developed by the PI and his collaborators allowed for the fabrication of micro-structured chordae tendineae, this invention complemented the engineered atrio-ventricular valves (mitral and tricuspid, TEHV reearch line) and also offered an ideal model to study fibroblasts ECM synthesis on higly aligned polymeric matrixes.

- to implement a novel apparatus to induce accelerated degradation conditions on polymeric heart valves
  - engineered tissue design aim to gradually replace scaffold material with endogenous, autologous, functional tissue. This notion, requires the calibration of the material degradation curves which in turn require costly and complex *in vivo* measurements. *In silico* systems developed aim to simulate the *in vivo* degradation mechanism reducing time, cost and number of animals utilized in the pre-clinical studies.

Meetings

Engineering micro-architecture to enhance de novo extracellular matrix elaboration in an elastomeric scaffold model. Keynote speech at *International symposium on Nanoengineering for Mechanobiology*, March 2017, Camogli, ITA.

Publications

Article in preparation, experiments fully concluded, first author publication, targeted journal: Soft Matter.

Goals for 2018

To perfect and promote the BE-ECM experimental platform, in particular:

- To submit first author publication on the topic: topological cues and mechanobiology, tentative journal: Soft Matter.
- To identify conditioning regimen for artificial chordae tendineae able to duplicate mass and mechanical properties of native chordae.
- To assess degradation curves of engineered atrioventricular valves developed in research line TEHV.

Therapeutic Area: **CARDIOVASCULAR DISEASES**

In-silico modeling for clinical risk stratification of cardiovascular pahtologies

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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 modelling 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 modelling 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 2017

We have developed a database of in-silico results and innovative metrics obtained from studies on more than n. 200 patients with vascular pathologies such as aortic aneurysms and dissections and cardiac diseases such as the right heart failure induced by pulmonary hypertension or by ventricular assist device as a bridge for heart transplant. Complications induced by off-label applications of endoprosthesis for traumatic aortic transections.

Meetings

A novel multiwavelength procedure for blood pressure estimation using PPG sensor at peripheral artery and capillaries, Scardulla F, D`Acquisto L, Barrett L, Pasta S, Hu S. *10th International Symposium on Mobile Mapping for Sustainable Development*, January 2017, Modena , ITA

Predicting Right Heart Failure in Patients with Pulmonary Hypertension, Scardulla F, Bellavia D, Vitulo P, Romano G, Minà C, Gentile G, et al. *Annual Cardiovascular Magnetic Resonance conference (EuroCMR 2017),* May 2017, Prague, CZE.

Publications

Scardulla F., Pasta S., D'Acquisto L., Sciacca S., Agnese V., Vergara C., et al. Shear stress alterations in the celiac trunk of patients with a continuous-flow left ventricular assist device as shown by in-silico and in-vitro flow analyses. *The Journal of heart and lung transplantation: the official publication of the International Society for Heart Transplantation*. 2017.

Scardulla F., Bellavia D., Vitulo P., Romano G., Mina C., Gentile G., et al. Biomechanical Determinants of Right Ventricular Failure in Pulmonary Hypertension. *ASAIO journal*. 2017.

Scardulla F., Bellavia D., D'Acquisto L., Raffa GM, Pasta S. Particle image velocimetry study of the celiac trunk hemodynamic induced by continuous-flow left ventricular assist device. *Medical engineering & physics*. 2017; 47:47-54.

Pasta S., Zingales M. Special Issue on ""Frontier Biomechanical Challenges in Cardiovascular Physiopathology"". *Medical engineering & physics*. 2017; 47:1.

Pasta S., Gentile G., Raffa G.M., Scardulla F., Bellavia D., Luca A., et al. Three-dimensional parametric modeling of bicuspid aortopathy and comparison with computational flow predictions. *Artificial organs*. 2017

Pasta S, Gentile G, Raffa GM, Bellavia D, Chiarello G, Liotta R, et al. *In Silico* Shear and Intramural Stresses are Linked to Aortic Valve Morphology in Dilated Ascending Aorta. *European journal of vascular and endovascular surgery: the official journal of the European Society for Vascular Surgery*. 2017

Pasta S, Agnese V, Di Giuseppe M, Gentile G, Raffa GM, Bellavia D, et al. *In Vivo* Strain Analysis of Dilated Ascending Thoracic Aorta by ECG-Gated CT Angiographic Imaging. *Annals of biomedical engineering*. 2017

Malvindi PG, Pasta S, Raffa GM, Livesey S. Computational fluid dynamics of the ascending aorta before the onset of type A aortic dissection. *European journal of cardio-thoracic surgery: official journal of the European Association for Cardio-thoracic Surgery*. 2017; *51*:597-9.

Intellectual property

UA2017A003931, 31/05/2017, Metodo e sistema per la valutazione del rischio di un aneurisma dell'aorta toracica ascendente

Goals for 2018

The goal will be to develop clinical-decision support system based on computational modelling 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.

Therapeutic Area: **INFLAMMATORY DISEASES**

A Microphysiological 3D Organotypic Culture System for Studying Degradation and Repair of Composite Skeletal Tissues in Microgravity Environment

Riccardo Gottardi, Ph.D.
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Leading Partner
University of Pittsburgh, USA

Brief description

In this project we are adapting our bioreactor to be used on the International Space Station to study the effects of bisphosphonates on cartilage and bone when administered to counteract microgravity-induced bone loss.

Impact

The International Space Station offers the unique opportunity to observe accelerated ageing, offering a unique, valuable model of bone loss similar to osteoporosis. Understanding the effects of bisphosphonates will have direct effects for healthcare on Earth where bisphosphonates are clinically used to counter osteoporosis. Furthermore, this work will serve as further validation of our bioreactor system to further our commercializzation efforts.

Results achieved in 2017

We are still developing the *in vitro* bone model including osteoclasts and osteoblasts. The cartilage model based on methacrylated gelatin is completed. The bioreactor design has been improved for operation on the ISS. A first model of the fluidic system and controls has been realized and is now being improved.

Meetings

Application of 3D Microtissue Chip Technology to Study Composite Skeletal Tissue Physiology: Microgravity-Induced Pathologies and Drug Screening, R.S. Tuan. Symposium Speaker *33rd Annual Meeting, American Society for Gravitational and Space Research*, October 2017, Seattle, USA

A Microphysiological 3D Organotypic Culture System for Studying Degradation and Repair of Composite Skeletal Tissues in Microgravity, R. Gottardi, P.G. Alexander, P. Simson, V. Rotolo, C. Britt, J.P. Wikswo, R.S. Tuan. *International Space Station Research&Development Conference 2017*, July 2017, Washington DC, USA

An Osteochondral Microphysiological System To Study Cartilage-Bone Interaction In Native Tissues And Engineered Model Constructs, R. Gottardi, A. Pirosa, G. Conoscenti, H. Lin, G. D'Urso, L. Iannetti, P. Zunino, T.P. Lozito, P.G. Alexander, V. La Carrubba, V. Brucato, R.S. Tuan. *Fusion Conference on Musculoskeletal Development and Regeneration*, February 2017, Cancun, MEX

Publications

E. Bayer, J. Jordan, A. Roy, R. Gottardi, M.V. Fedorchak, P. Kumta, S.R. Little. Programmed PDGF-BB and BMP-2 Delivery from a Hybrid, Calcium Phosphate/ Alginate Scaffold. *Tissue Engineering*. 2017, 23(23-24): 1382-1393. Article featured on the Journal Cover.

Goals for 2018

Complete the *in vitro* bone model. Test the response to an inflammatory signal and to bisphosphonates. Improve the fluidic system to reduce its footprint.

Therapeutic Area: **INFLAMMATORY DISEASES**

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

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Brief description

Osteoarthritis (OA) is a degenerative disease of the articular cartilage and the most common form of arthritis. Although the usual population associated with the condition is the elderly, who are mostly inactive, athletes and younger individuals are also susceptible. Whilst the available data have implicated the role of the various modifiable or nonmodifiable risk factors, no study has conclusively explored the interaction and integration of other information sets in a patient-specific manner.
OACTIVE intends to make a significant leap forward adopting a multi-scale holistic approach where patient-specific information from various levels, including cell, tissue, organ and whole body will be integrated with information from other sources such as biochemical/ inflammatory biomarkers, behaviour modeling and social/environmental risk factors to generate robust predictors for new personalised interventions for delaying onset and slowing progression of OA. OACTIVE will use a combination of mechanistic, phenomenological computational models, simulations and big data analytics to simulate and predict optimal treatments and improved patient outcomes. Furthermore, Augmented Reality empowered interventions will be developed in a personalised framework allowing patients to experience the treatment as more enjoyable, resulting in greater motivation, engagement, and training adherence.

Impact

OACTIVE's mission is to improve healthcare by transforming and accelerating the OA diagnosis and prediction based on a more comprehensive understanding of disease pathophysiology, dynamics, and patient outcomes.

Results achieved in 2017

The project was secured and funding was obtained. Fondazione Ri.MED was registered with the EU Commission as a recognized institution for future project proposals. The project kick off meeting took place in November 2017.

Goals for 2018

Implement the project objectives for year 1. Specifically, perform baseline screening of OA osteochondral units, and develop *in vitro* OA induced models of OA. Identify the necessary project personnel.

Therapeutic Area: **INFLAMMATORY DISEASES**

Development of osteochondral bioreactors and scaffold matrices for the study of the role of sex hormones in joint disease and for musculoskeletal tissue repair

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Brief description

We are studying the protective effect of sex hormones on cartilage and bone accounting for osteochondral cross-talk. To screen effects and developing therapies we are developing novel, microphysiological bioreactors. Furthermore, with the overall objective of repairing musculoskeletal tissues, we are developing scaffolds and matrix derive extracts to help treat tissue damage.

Impact

Our approach is focused on the development of new therapies for musculoskeletal tissue repair, the main cause of disability in Europe and in the U.S. The study on sex hormones will serve to understand their role in osteoporosis and osteoarthritis, and to devise new, pro-regenerative hormone replacement therapies, as well as to identify novel targets for disease treatments. For musculoskeletal repair, we are further investigating the role of extracellular matrix extracts to directing mesenchymal stem cells towards specific tissue phenotypes.

Results achieved in 2017

Four publications. Development and validation of anterior and posterio cruciate ligaments and drafting of a manuscript. Validation of the bioreactor v1.7 with porcine tissues. Validation of bioreactor v2.0 with fluorescent cells. Response of cartilage explants to estrogen when subject to an inflammatory signal.

Meetings

A 3D Printed Microfluidic Bioreactor to Engineering Biphasic Construct, G. De Riccardis, P.G. Alexander, M.T. Raimondi, R.S. Tuan, R. Gottardi. *Tissue Engineering and Regenerative Medicine International Society – Americas Annual Meeting*, December 2017, Charlotte, USA.

A 3D Printed Microfluidic Bioreactor to Engineering Biphasic Construct: Modelling and Experimental Validation, G. De Riccardis, P.G. Alexander, M.T. Raimondi, R.S. Tuan, R. Gottardi. *Biomedical Engineering Society Annual Meeting,* October 2017, Phoenix, USA.

An In Vitro Chondro-Osteo-Vascular Triphasic Model of the Osteochondral Complex, A. Pirosa, R. Gottardi P.G. Alexander, D. Puppi, F. Chiellini, R.S. Tuan. *Biomedical Engineering Society Annual Meeting,* October 2017, Phoenix, USA.

Validation of an Osteochondral Bioreactor for In Vitro Drug Screening, K. Overholt, A. Pirosa, R.S. Tuan, R. Gottardi. *Biomedical Engineering Society Annual Meeting*, October 2017, Phoenix, USA.

Validation of an osteochondral bioreactor applied to study the protective role of sex hormones, R. Gottardi, H. Lin, L. Iannetti, G. D'Urso, P. Zunino, T.P. Lozito, P.G. Alexander, P. Manner, R.S. Tuan. ***Tis-sue*** *Engineering and Regenerative Medicine International Society – EU Annual Meeting*, June 2017, Davos, CH.

An in Vitro Chondro-Osteo-Vascular Triphasic Model of the Osteochondral Complex, R. Gottardi, A. Pirosa, P.G. Alexander, D. Puppi, F. Chiellini, R.S. Tuan. *Tissue Engineering and Regenerative Medicine International Society – EU Annual Meeting*, June 2017, Davos, CH.

A continuous pore size gradient PLLA scaffold for osteochondral regeneration, R. Gottardi, G. Conoscenti, P.G. Alexander, V. La Carrubba, V. Brucato, R.S. Tuan. ***T****issue Engineering and Regenerative Medicine International Society – EU Annual Meeting,* June 2017, Davos, CH.

H. Sasaki, B.B. Rothrauff, P.G. Alexander, R. Gottardi, H. Lin, F.H. Fu, R.S. Tuan. *In Vitro* Repair of Meniscus Radial Tear Using Hydrogels Seeded With Adipose-Derived Stem Cells And TGF-ß3. 11th Biennial International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine Congress, June 2017, Shanghai, China.

Distributed and Lumped Parameter Models for The Characterization of High Throughput Bioreactors, L. Iannetti, G. D'Urso, G. Conoscenti, E. Cutrì, R.S. Tuan, M.T. Raimondi, R. Gottardi, P. Zunino. *5th International Conference on Computational and Mathematical Biomedical Engineering – CMBE2017,* April 2017, Pittsburgh, USA.

Dual Fluidic High Throughput Bioreactor Obtained by 3D Printing, for Osteochondral and Other Biphasic Tissue Constructs Targeted at High Throughput Screening: Computational Model and Experimental Validation, R. Gottardi, G. D'Urso, L. Iannetti, G. Conoscenti, E. Cutrì, M.T. Raimondi, P. Zunino, R.S. Tuan. *Orthopaedic Research Society Annual Meeting*, March 2017, San Diego, USA.

In Vitro Repair of Meniscus Radial Tear Using Hydrogels Seeded with Adipose-derived Stem Cells and TGF-β3, H. Sasaki, B.B. Rothrauff, P.G. Alexander, H. Lin, R. Gottardi, F. Fu, R.S. Tuan. ***O****rthopaedic Research Society Annual Meeting*, March 2017, San Diego, USA.

An Osteochondral Microphysiological System to Study Cartilage-Bone Interaction in Native Tissues and Engineered Model Constructs, R. Gottardi, A. Pirosa, G. Conoscenti, H. Lin, G. D'Urso, L. Iannetti, P. Zunino, T.P. Lozito, P.G. Alexander, V. La Carrubba, V. Brucato, R.S. Tuan. *Fusion Conference Development and Regeneration,* February 2017, Cancun, MEX.

Publications

M.L. Ratay. E. Bellotti, R. Gottardi, S.R. Little. Modern Therapeutic Approaches for Ocular Diseases Involving Inflammation. Advanced Healthcare Materials. 2017, 6(2):1700733. Article featured on the *Journal Cover.*

B.B. Rothrauff, L. Coluccino, R. Gottardi, L. Ceseracciu, S. Scaglione, L. Goldoni, R.S. Tuan. Efficacy of thermoresponsive, photocrosslinkable hydrogels derived from decellularized tendon and cartilage extracellular matrix for cartilage tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine*. Accepted.

B.B. Rothrauff, K. Shimomura, R. Gottardi, P.G Alexander, R.S. Tuan. Anatomical region-specific enhancement of 3-dimensional chondrogenic differentiation of human mesenchymal stem cells by soluble meniscus extracellular matrix. *Acta Biomaterialia*. 2017, 49:140-151

P.O.Numpaisal, B.B. Rothrauff, R. Gottardi, C.-L. Chien, R.S. Tuan. Rapidly dissociated autologous meniscus tissue to enhance meniscus healing: an *in vitro* study. *Connective Tissue Research*. 2017, 58(3-4):355-365

Goals for 2018

Complete validation of the new, 3D printed micro-bioreactor.

Therapeutic Area: **INFLAMMATORY DISEASES**

R-CaRe - Rehabilitation for Cartilage Regeneration

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Brief description

At present there is little mechanistic knowledge about the role of rehabilitation in determining the quality of the repair tissue after cartilage defect treatment by microfracture. Although this most common surgical procedure is effective in the short term, near universal treatment failure is expected within 5-10 years. In this project, we will apply rehabilitation regimens such as continuous passive motion and progressive weight bearing simulated through specific bioreactors on an *in vitro* model of microfracture cartilage repair. We aim at identifying the mechanistic relationship between rehabilitation regimes and repair tissue differentiation and integration.

Impact

This approach will allow the *in vitro* development and testing of loading patterns that promote improved cartilage regeneration and that could then be replicated through rehabilitation protocols *in vivo*. The proposed research in Regenerative Rehabilitation for cartilage regeneration addresses a significant gap in knowledge that once filled could significantly improve health care and revolutionize our approach to orthopedic rehabilitation.

Results achieved in 2017

The project was awarded funding. The effects of compression and shear forces (simulating progressive weight bearing and continuous passive motion rehabilitation exercises) applied independently to a model of microfracture were studied.
These preliminary data served for the submission of a further project application. A manuscript has been drafted.

Meetings

Engineered In Vitro Models for the Guidance of Rehabilitation Regimens that Promote Cartilage Regeneration after Repair Surgery, T. Iseki, S. Kihara, H. Sasaki, S. Yoshiya, F. Fu, R.S. Tuan, R. Gottardi. *Tissue Engineering and Regenerative Medicine International Society - Americas Annual Meeting*, December 2017, Charlotte, USA

In vitro models for the guidance of rehabilitation regimens that promote cartilage regeneration after repair surgery, T. Iseki, S. Kihara, H. Sasaki, S. Yoshiya, F. Fu, R.S. Tuan, R. Gottardi. *Biomedical Engineering Society Annual Meeting*, October 2017, Phoenix,USA

The Response of Bone Marrow Mesenchymal Stem Cells with Fibrin Gel Scaffolds Mimicking Microfracture to Mechanical Stress, T. Iseki, R. Gottardi, H. Sasaki, S. Kihara, S. Yoshiya, F. Fu, R.S. Tuan. *9th annual meeting of the Japanese Orthopaedic Society of Knee, Arthroscopy and Sports Medicine (JOSKAS)*, June 2017, Sapporo, JPN

Intellectual property

Provisional Patent filed on 10/30/2017: Exemplary Multi-Well Plate Lids, Mechanical Stimulation Systems, and Incubators.

Goals for 2018

Study the mechanobiological mechanism connected to the better performance of simulated weight bearing.

BIOMEDICAL APPLICATIONS

Development of nontoxic bio-adhesives for wet environments

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Brief description

Investigation performed on marine sessile animals, as mussels, indicated that these organisms have developed adaptive strategies to overcome obstacles that inhibit their adhesion in water (pH, hydration layers and dielectric properties).

Mussels are able to strongly anchor the marine surfaces through the secretion of adhesive proteins that in Mytilus are indicated as mussel foot proteins MFP-2, -3S, -3F, -4, -5 e -6. All MPFs proteins are modified post-translationally with the amino acid 3,4-dihydroxyphenyl-L-alanine (Dopa), whose catecholic structure very likely provides the adhesive properties. Furthermore, among Dopa and any adjacent lysine (Dopa-Lys) there is a synergy in the bioadhesive capacity: the lysine displaces the hydrated cations from the surface and facilitates subsequent bonding of the adjacent Dopa to the substrate.

Going beyond the 'Dopa paradigm' the efficiency of Mytilus proteins seems to be due to combined redox and hydrophobic interactions among all MPFs proteins. Despite the many efforts focused on the development of bio-inspired adhesive from MFPs, particularly for biomedical applications, no effective products have been issued so far.

We do believe that this is mainly due to the lack of information about the structure adopted by those proteins, how the Dopa post-translational modification modifies it, the dynamics and the assembling properties. We thus want to structurally characterize both model and mussel proteins to address this important topic.

Impact

In the last few 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 important. The big challenge in developing new bio-adhesive molecules is to find molecules able to work in wet and hostile environment. Engineering of proteins bio-inspired from sessile animals, which are able to secrete proteins with adhesive properties in water, could overcome these difficulties.

Results achieved in 2017

Engineering of three different recombinant proteins from *Perna viridis* mussels with potential adhesive properties.

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, accepted.

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.

Wang A.F., Deighan P., Chen S., Barrasso K., Garcia C., Martínez-Lumbreras S., Alfano C., Krysztofinska E.M., Thapaliya A., Camp A.H., Isaacson R.L., Hochschild A. and Losick R. (2017) A Novel RNA Polymerase-binding Protein that interacts with a Sigma-Factor Docking Site. *Molecular Microbiology*, 105(4):652-662.

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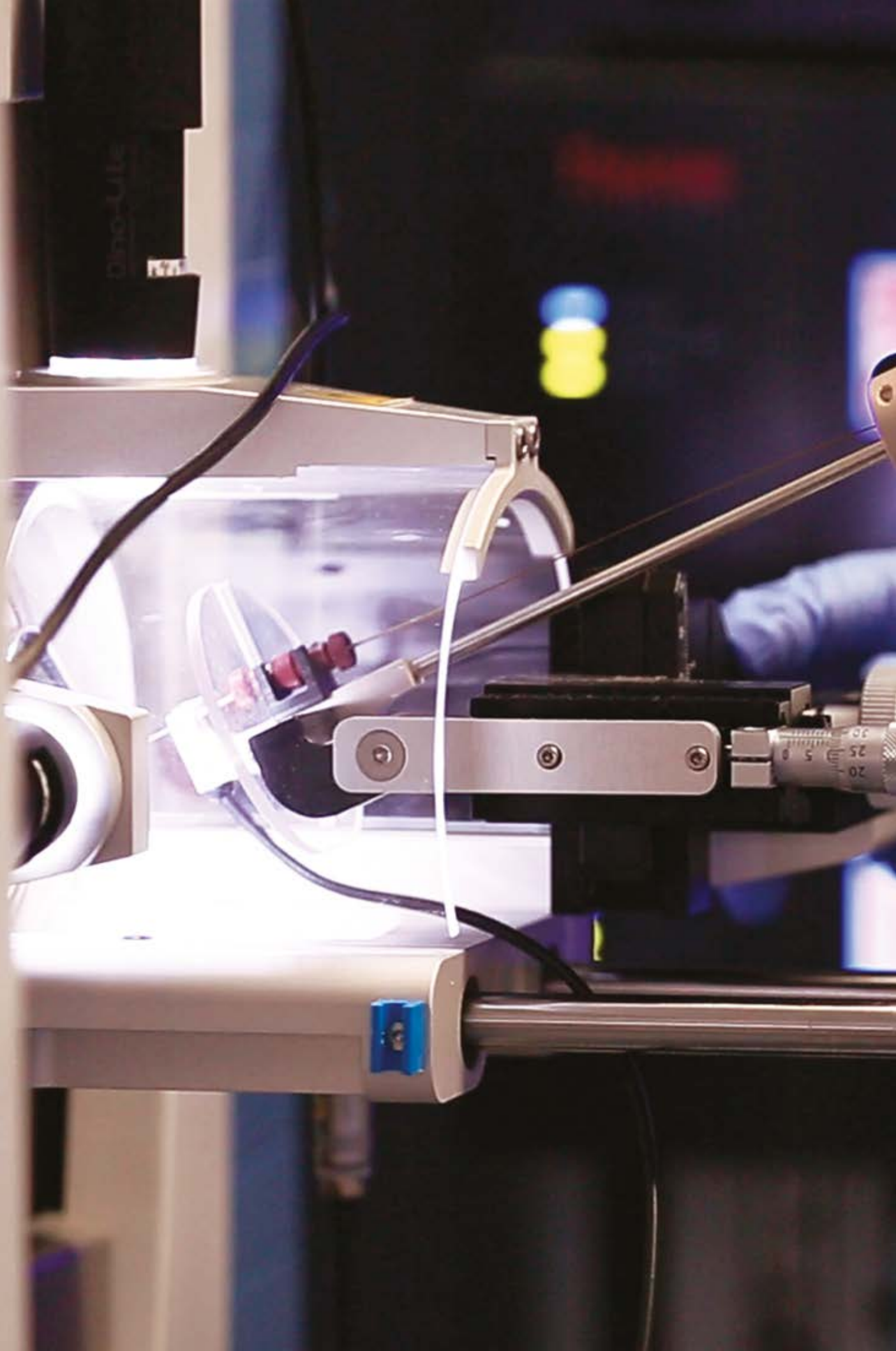
Goals for 2018

Engineering of three different recombinant proteins from *Perna viridis* mussels with potential adhesive properties.



TECHNOLOGY PLATFORMS

The engine of translational research of the Fondazione Ri.MED envisages the development of competences and technological platforms to support discovery projects and preclinical development for traditional approaches of drug discovery, regenerative medicine and immunotherapy approaches and medical devices.
During 2017 the foundations of computational chemistry, bioinformatics, biophysics & structural biology and bioengineering platforms were laid.
Today the computational chemistry platform allows to perform molecular dynamic simulations to study bimolecular protein-protein and protein-ligand interactions, as well as to select potential hit and lead ligands through in silico screening of millions of molecules on therapeutic targets of interest. The chemo-informatic infrastructure for the structured analysis of molecules has been implemented for the chemico-physical properties, 3D conformations, commercial availability, etc.
The bioinformatics platform exploits open source software as well as proprietary applications to drive the identification and validation of new therapeutic targets, as well as for the analysis of biological matrix data.
The biophysical and structural biology platform is dedicated to the production and purification of proteins of interest, as well as to the 3D elucidation of proteins structure and to study the interactions protein-small molecule, protein-peptide or protein-protein. It is emphasized that the biophysical and structural biology platform plays a fundamental role both in screening and in the 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 bio-mechanic simulations integrated with clinical and preclinical data. The models developed by the bioengineering team are already used today in the stratification of patients at clinical risk, in supporting the decision-making system in biotech companies and in the preclinical development of new biomedical devices.
The platforms will be further potentiated during 2018 both in terms of human capital, specific skills and infrastructure.

BIOINFORMATIC PLATFORM

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Brief Description

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. In order to better satisfy the collaborators needs we are going to acquire the most diffused high throughput data analysis software, i.e. GeneSpring and Ingenuity Pathway Analysis.

Hardware

- 3 workstations
- Server: 80 CPUs e 2 x NVIDIA Tesla K80

Active research Projects

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

MicroRNA-mRNA interaction network

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

RISC proteins RIP-Chip prediction

We developed a machine learning based algorithm useful to predict the differentially expressed genes in the RIP-Chip data. The algorithm is currently optimized to predict the outcome of 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

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BIOENGINEERING PLATFORM

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Brief Description

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.

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 based at the Advanced Technologies Network Center (ATeN-Center, http://www.atencenter.com/en/), and is currently undertaking a major expansion of its technology platform, integrating the following facilities:

Computational facilities

- Computer-aided design (CAD) software;
- Segmentation toolbox of medical image data for patient-specific modelling;
- Codes for linear and non-linear finite element analysis, based on both implicit and explicit formulations;
- Codes for thermo-fluid-dynamic, multiphysics and fluid-structure interaction analyses;
- 4-node server and 72 cores processors.

Experimental facilities

- Hydro-mechanical pulse duplicator for the fluid dynamic characterisation of therapeutic solutions, designed to meet ISO 5840, ISO 5840-3, ISO-17845, and FDA requirements.
- Particle Image Velocimetry (PIV) system;
- Accelerated wear tester system for the estimation of cardiovascular implants' functional durability;
- Uniaxial testing machine for the mechanical characterisation of soft tissues and biomaterials, equipped with media container with temperature control unit, and pneumatic grips;
- Biaxial test system for mechanical characterisation of soft tissues and biomaterials, equipped with media container with temperature control unit.

Prototyping facilities

- 3D printing technologies based on fused deposition modelling and stereolithography;
- Ovens suitable for the thermal treatment of biomaterials and the thermos-setting of Ni-Ti alloys.

Active research Projects

- Risk prediction of right ventricular failure in patients with pulmonary hypertension and ventricular assist devices (VAD).
- Non-invasive evaluation of portal hypertension in patients with liver cirrhosis;
- Development of a clinical decision support system for the risk-stratification of ascending thoracic aortic aneurysm
- Development of a novel transcatheter aortic valve.
- Development of a novel alfa-gal free xenograft heart valve.
- Development of a novel transcatheter mitral valve.
- Analysis of the left atrial appendage to predict thrombosis risk.
- Prediction of the ischaemic lesions potential after heart valve therapy.

Publications

Maneas, E., Xia, W., Nikitichev, D., Daher, B., Manimaran, M., Wong, R.Y., Chang, C.W., Rahmani, B., Capelli, C., Schievano, S., **Burriesci, G**., Ourselin, S., David, A., Finlay, M., West, S., Vercauteren, T., Desjardins, A. (2018) Anatomically realistic ultrasound phantoms using gel wax with 3D printed moulds. *Physics in Medicine and Biology* 63:015033(10pp).

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Pasta S, Zingales M. Special Issue on "Frontier Biomechanical Challenges in Cardiovascular Physiopathology". Medical engineering & physics. 2017;47:1.

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Malvindi PG, **Pasta S**, Raffa GM, Livesey S. Computational fluid dynamics of the ascending aorta before the onset of type A aortic dissection. European journal of cardio-thoracic surgery: official journal of the European Association for Cardio-thoracic Surgery. 2017;51:597-9.

STRUCTURAL BIOLOGY AND BIOPHYSICS PLATFORM

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Brief description

The Structural Biology and Biophysics Group aims to provide an added value to a great variety of biological projects by investigating the structural/functional relationships in biomolecular systems with particular focus on molecular mechanisms linked to human diseases which could lead to the identification of potential therapeutic targets. We use an interdisciplinary approach that combines state-of-the-art biophysical techniques such as nuclear magnetic resonance, calorimetry, surface plasmon resonance, high resolution atomic force microscopy, X-Ray as complemented by well-established technical and methodological expertise in molecular biology and protein science to provide biophysical and structural insights into biological phenomena that are driven by protein folding, aggregation and interactions with a particular emphasis on drug discovery. In particular, we employ a variety of biochemical and biophysical techniques that allow the characterization of the intrinsic properties of macromolecules and their assemblies and of the interactions in which they are involved.

Expertise

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

Technology Platform

The Structural Biology and Biophysics Group is based at the Advanced Technologies Network Center (ATeN-Center, http://www.atencenter.com/en/).
We are in process of equipping the Platform with:

- 800 MHz NMR spectrometer with cryogenically-cooled probe for 1H/13C/15N multiple-resonance experiments;
- Surface Plasmon Resonance spectrometer
- Isothermal Calorimetry
- Bio-Layer Interferometry technology;
- High throughput crystallization platform.

Active research Projects

- Research Project 1: Molecular mechanisms of polyglutamine diseases.
- Research Project 2: Elucidation of the binding mode of molecules able to interfere with the oligomerization process of NPM1.

- Research Project 3: Generation of mussel-inspired bio-adhesives molecules able to work in wet environment
- Research Project 4: Impact of molecular crowding on protein folding

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*, accepted.

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COMPUTER AIDED DRUG DESIGN PLATFORM

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Brief description

The CADD group at Ri.MED Foundation main is 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.

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

- 3 Workstations
- Server: 80 CPUs e 2 x NVIDIA Tesla K80

Calculation capability:

- Library optimisation ~ 3,000 molecules/min
- Virtual Screening HTVS ~ 2,500 molecules/min
- Virtual screening SP ~ 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 actually involved in several projects focused on the retrieval of active modulators on the following areas:

Ophthalmology

The project aims to find selective inhibitors of the CD14 target involved in Age-related Macular Degeneration (AMD) to be tested through biological assays. Delivables - targeted libraries on CD14.

Anti-inflammatory Diseases

NLRP3 inflammasome is one of the most important complex within the inflammation process, found to be responsible for several pathologies. This project is based on the search and the rational design of selective inhibitors of the NLRP3 protein activation. Deliverables – targeted libraries of small molecules; homology model validated of NLRP3 protein.

Oncology

Epigenetic modulation of cancer is one of the main machinery studied for chemotherapeutics. At Ri.MED foundation, we are currently working on an anticancer strategy focused on the search of SIRT1 and KDM4 modulators. Deliverables – targeted libraries of small molecules.

CDK1 has been demonstrated to be one of the key players in the breast cancer progress. This project aims to target selectively CDK1 starting from rationally designed molecules analogues to natural product nortopsentin.

Deliverable: Deliverables – targeted libraries of small molecules.

PTEN-Null prostate cancer cells are one of the most studied cell type for this type of cancer. In this project we are focusing on the research of selective molecules targeting the most important proteins involved in the cancer progression.

EPH-B4 is a well-known protein involved in promoting angiogenesis in tumours with the consequent tumour progression and metastasis formation. The target structural analysis together with the molecular similarity with known ligands is carried out to design new molecules active on this target. Deliverables – targeted libraries of small molecules.

MUC1/CIN85 interaction is one of the most important players within the modulation of metastasis formation in cancer. The use of computational techniques is used in this case to assess the protein-protein interaction (PPI) and to guide the rational design of PPI modulators.

Neurodegenerative diseases

TOM20/alpha-synuclein has been recently discovered to be a fundamental protein-protein interaction (PPI) crucial for the modulation of Parkinson's disease. Unfortunately, no 3D structures of this proteins are actually available. The use of computational molecular modeling allow the homology modeling building of the proteins and the consequent use in a dynamic way. Therefore, the interaction between the two partners is assessed opening new insights into the molecular design of new modulators.

CB1 has been demonstrated to be one of the responsible protein in the modulation of epileptic disorders, especially within mitochondria. The CADD approach is used in this case to virtually screen small molecules in order to find out modulators that can be selective. One of the most important challenge when targeting endocannabinoid system is in fact the selectivity toward only one of the two main receptors. Deliverables – targeted libraries of small molecules.

Publications

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PROTEOMICS PLATFORM

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Brief description

At Ri.MED, we are setting up a Proteomics facility due to the awareness that the data obtained by genomic approaches in recent years has provided the ability to correlate the different levels of gene expression to multiple diseases, but such information is unfortunately "static" and unable to define the different biochemical processes that take place inside a cell and which lead to the post-translational modifications (PTM) of the expressed proteins, with consequent physio-pathological involvement and / or the ability to influence the cellular microenvironment.

To address this issues, our team has developed techniques and skills to perform either systematic proteomics studies, aimed at identifying all the proteins present within a cell population, and differential proteomics studies, aimed at the analysis of qualitative and quantitative expression differences between two or more cell populations.

Nowadays, the State of the Art for proteome study is Mass Spectrometry coupled to high performance Liquid Chromatography (LC-MS), an analytical technique used to indentify unknown products, quantitative measurements of known compounds and to elucidate structural and chemical properties of the molecules. This technique is based on the separation of a complex protein mixtures and on consecutive ionization of a molecule and its fragmentation into ions with different mass/charge ratio, which data becomes accessible through a diagram, called a mass spectrum. Ions and their relative charge intensity are subjected to a bioinformatic analysis using dedicated software and surveyed in the most up-to-date databases, thus allowing us to establish the molecular weight and structure of the target compounds.

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Expertise

- Protein concentration from conditioned means
- Spectrophotometric Measurement (Bradford, BCA, micro BCA)
- Precipitaion and sample chemical processing
- In solution and in gel proteolysis
- 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

The unit is equipped with:

Hardware devices:

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

Software devices:

- Chromeleon
- Xcalibur
- Proteome Discoverer
- MAX QUANT

Active research Projects

- Dermal fetal cells for the treatment of chronic skin lesions: analysis of conditioned media for identification of proteins involved in regenerative pathways such as angiogenesis and wound healing by mass spectrometry and bioinformatics.

- Molecular and cellular pathways underlying the inhibition of HCV-induced NK cell function in order to restore the correct immune response in the HCV+ chronic patient: analysis of conditioned media from NK cells with various stimulations for the identification of the involved molecular mechanisms in the recovery of the immune response.

- Isolation and in vitro characterization of human epithelial and mesenchymal cells with characteristics of staminality. Analysis of conditioned media and pellets resulting from primary cells isolated from placenta, label free and labeled with TMT, to identify possible staminality protagonists produced, and confirm if modulations at different co-culture conditions between mesenchymal and epithelial stem cells occur.

Publications

Gaetani M., **Chinnici C.**, **Carreca A.P.**, **Amico G.**, Conaldi P.G. Unbiased and quantitative proteomics reveals highly increased angiogenesis induction by the secretome of mesenchymal stromal cells isolated from fetal rather than adult skin. J. Tissue Eng. Regen. Med. 2017 Jan 19. Doi. 10.1002/term. 2417 (IF: 3.989)


BILANCIO DI PREVISIONE
TRIENNALE 2018 - 2020

GRANTS

Ri.MED is aiming at a sustainable R&D model thus is constantly seeking funding opportunities, to support its research activities, offered by public and private bodies at regional, national and international institutions. The work aimed at obtaining research funding is considered a strategic activity for the Foundation. The Grants office first identifies funding opportunities at European, ministerial and regional calls also in collaboration with other bodies and then liaises with scientists in order to identify and submit suitable projects.

The Grant Area promotes and encourages all initiatives aimed at raising funds for the development of project proposals in the research areas of the Foundation, taking care of identifying the needs and potential of Ri.MED in terms of planning for the procurement of funds, selecting financial programs to support biomedical research, specialized training, international cooperation. Furthermore, the Grant office manages relations with public and private administrations holding financing programs, coordinating and supervising all projects eligible for funding.

In 2017, five projects were accepted for funding. Thanks to these grants, the Foundation will be able to build its technology platforms, train highly specialized professionals and carry out research, thus fulfilling its mission for human health, having a socio-economic impact and broadening and strengthening the international network of collaborations.

In addition, the 2017 was a year characterized by strong planning: eight projects were submitted for PO FESR Sicilia funding, four projects based on PON Ricerca e Innovazione and eleven proposals for PhD with industrial characterization.

RESEARCH PROJECTS FUNDED DURING 2017

Funding institution:
Sicilian Department of Productive Activities
Patto per il Sud

CHEMIST - Computational Molecular Design e Screening

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.

Funding institution:
Italian Ministry of Health
Ricerca finalizzata 2013

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

A prospective, double-blinded, randomized phase 2 study of 2 parallel groups designed to include 106 consecutive subjects who will undergo LT for the first time with administration of simvastatin during liver procurement, to evaluate the efficacy of simvastatin 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.

Funding institution:
Sicilian Department of Productive Activities
PO FESR Sicilia 2014-2020

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

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.

Funding institution:
European Commission
Research and Innovation Action SC1-PM-17

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

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 behaviour modelling and social/environmental risk factors.

Funding institution:
Italian Ministry of Health
Ricerca finalizzata 2016

Genetic variations that predict cognitive impairment related to copper failure

The aim is to identify and validate genes and biological variables, associated to Alzheimer's Disease, Mild Cognitive Disorder and Diabetes Type 2, that can predict cognitive decline.

RESEARCH PROJECTS SUBMITTED DURING 2017

Funding institution:
Sicilian Department of Productive Activities
PO FESR Sicilia 2014-2020
Action 1.1.5 – OT1-. Scienze della Vita

SEnsore Nanstrutturato per Stress Ossidativo - SE.N.SO
Lead partner: Di Pietro GROUP

Immunoterapia contro Klebsiella - KLEBS
Lead partner: Charybdis s.r.l.

Oncological therapies through Biological Interaction Network Discovery - OBIND
Lead partner: Exprivia s.r.l.

Prodotti Medicinali Derivati da Placenta per Terapie Avanzate e per Patologie Epatiche e Endometriali - PROMETEO
Lead partner: Casa di Cura Candela S.p.A.

Rete Integrata Clinico-Biologica- Elettronica di Biobanche per Medicina Rigenerativa- e-RIMedRi
Lead partner: Pyxis s.r.l.

Piattaforma Computazionale per la Valutazione non Invasiva e il Supporto Clinico Decisionale in Pazienti con Aneurisma Toracico dell'Aorta Ascendente - AORTIVIEW
Lead partner: Orobix s.r.l.

Test Exosome-based per medicina di precisione in malattie di origine autoimmune e cardiovascolari - EXO-PREMIA
Lead partner: Locorotondo s.r.l.

Valorizzazione biotecnologica dei sottoprodotti del settore Ittico - BIOITTIVO
Lead partner: Profineco s.r.l.

Funding institution:
Italian Ministry of Education, University and Research
PON Research and Innovation 2014-2020

Advanced manufacturing technologies for regenerative and reconstructive medicine - Regen-MED
Specialization line: Salute
Lead partner: Università degli Studi di Catania

Processi green per l'estrazione di principi attivi e la depurazione di matrici di scarto e non
Specialization line: Chimica Verde
Lead partner: Università degli Studi di Palermo

4FRAILTY - Sensoristica intelligente, infrastrutture e modelli gestionali per la sicurezza di soggetti fragili
Specialization line: Tecnologie per gli ambienti di vita
Lead partner: Distretto Tecnologico Micro-Nano

TaRgeting cAnCer Epigenome - TRACE
Specialization line: Salute
Lead partner: Università degli Studi della Campania "L. Vanvitelli"

Funding institution:
European Commission
Horizon 2020

BIOMITRAL - Engineering the mitral valve: bioinspired control of structure and function for enhanced in-vivo performance.
Principal Investigator: Antonio D'Amore
Host Institution: Fondazione Ri.MED
Partner: IRCCS ISMETT

MAMMA - Marine Alkaloids for anti-Malignant Mesothelioma Activity
Applicant: Università degli Studi di Palermo
Principal Investigator: Girolamo Cirrincione
Partners: Fondazione Ri.MED; Fondazione IRCCS Istituto Nazionale Dei Tumori; Istituto Nazionale Biostrutture e Biosistemi (INBB).

INNOVATIVE PhDs WITH INDUSTRIAL CHARACTERIZATION

STARTED IN 2017

Understanding the relationship between normal function and aberrant aggregation: the case of ataxin-3

Promoter University: Università degli Studi di Palermo, ITA
Foreign University: University of London, UK
Industrial partner: IRCCS ISMETT
Tutor: Alfano Caterina, Fondazione Ri.MED

SUBMITTED IN 2017

Interaction network from Biological Big Data: customized miRNoma analysis

Promoter University: Università degli Studi di Palermo; PHD in Economics and Statistics; PhD coordinator: Andrea Consiglio
Foreign University: University of Pittsburgh, USA
Industrial partner: UPMC Italy
Tutor: Claudia Coronnello, Fondazione Ri.MED

Characterization and engineering of myocardium

Promoter University: Università degli Studi di Palermo;PhD in Technology and Sciences for Human Health; PhD coordinator: Maurizio Leone
Foreign University: University of Pittsburgh, USA
Industrial partner: UPMC Italy
Tutor: Antonio D'Amore, Fondazione Ri.MED

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; PhD in Neuroscience; PhD coordinator: Salvatore Salomone
Foreign University: University of Pittsburgh, USA
Industrial partner: UPMC Italy
Tutor: Pier Giulio Conaldi, Fondazione Ri.MED

Development of a new immunotherapy against *Klebsiella pneumoniae* based on *Saccharomyces cerevisiae* probiotic yeasts genetically engineered

Promoter University: Università degli Studi di Messina; PhD in PhD in Medical and Surgical Biotechnologies; PhD coordinator: Giovanni Raimondo
Foreign University: Centre of Molecular Inflammation Research, NOR
Industrial partner: UPMC Italy
Tutor: Pier Giulio Conaldi, Fondazione Ri.MED

Development of microfisiological systems for the modeling of joint diseases

Promoter University: Università degli Studi di Palermo; PdD in Information and Communication Technologies; PhD coordinator: Ilenia Tinnirello
Foreign University: University of Pittsburgh, USA
Industrial partner: UPMC Italy
Tutor: Pier Giulio Conaldi, Fondazione Ri.MED

Development of active drugs in molecular target therapy

Promoter University: Università degli Studi di Messina; PhD in Research in Applied Biology and Experimental Medicine; PhD coordinator: Salvatore Cuzzocrea
Foreign University: University of Vienna, AUT
Industrial partner: Sterling Farmaceutici Srl
Tutor: Alessandro Padova, Fondazione Ri.MED

Design and synthesis of molecules active in the epigenetic control of tumor pathology

Promoter University: Università degli Studi di Palermo;PhD in Research in Molecular and Biomolecular Sciences; PhD coordinator: Patrizia Diana
Foreign University: University of Vienna, AUT
Industrial partner: UPMC Italy
Tutor: Alessandro Padova, Fondazione Ri.MED

Knowledge management techniques for federating and querying large distributed databases to support pharmaceutical design

Promoter University: Università degli Studi di Palermo; PhD in Technological Innovation Engineering; PhD coordinator: Salvatore Gaglio
Foreign University: University of Vienna, AUT
Industrial partner: QWince
Tutor: Alessandro Padova, Fondazione Ri.MED

Micromechanical modeling of aortic diseases

Promoter University: Università degli Studi di Palermo; PhD in Technology and Sciences for Human Health; PhD coordinator: Maurizio Leone
Foreign University: Ecole Mines Saint Etienne, FRA
Industrial partner: UPMC Italy
Tutor: Salvatore Pasta, Fondazione Ri.MED

Tolerogenic dendritic cells derived from bone marrow and residents for the promotion of operative tolerance in liver transplantation

Promoter University: Università degli Studi di Messina; PhD in Research in Medical and Surgical Biotechnologies; PhD coordinator: Giovanni Raimondo
Foreign University: Centre of Molecular Inflammation Research, NOR
Industrial partner: UPMC Italy
Tutor: Pier Giulio Conaldi, Fondazione Ri.MED

RI.MED'S PATENTS PORTFOLIO UP TO 31 12.2017

DRUG DISCOVERY

Nitro-oleic acid (NO2-OA) controlled release platform to induce regional angiogenesis in abdominal wall repair.
Ownership: Fondazione Ri.MED and University of Pittsburgh

Novel nitro-nitrate-lipid intermediates that mediate nitrosating and alkylating reactions
Ownership: under discussion

REGENERATIVE MEDICINE AND IMMUNOTHERAPY

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

Probiotic yeasts as novel vaccination vectors.
Ownership: Fondazione Ri.MED and University of Pittsburgh

TISSUE ENGINEERING AND BIOMEDICAL DEVICES

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

Method and system for the evaluation of the risk of an ascending thoracic aortic aneurysm.
Ownership: Fondazione Ri.MED

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

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

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

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

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

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

Prevention of soft tissue ossification by controlled release.
Ownership: Fondazione Ri.MED and University of Pittsburgh

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

Recruitment of mesencymal stem cells using controlled release systems.
Ownership: Fondazione Ri.MED and University of Pittsburgh

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

An organ chip to model mammalian joint.
Ownership: Fondazione Ri.MED and University of Pittsburgh

High throughput mechanical activation device.
Ownership: Fondazione Ri.MED and University of Pittsburgh

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

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

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

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

Fondazione
Ri.MED

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