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ROME TECHNOPOLE

ROMA TRE UNIVERSITÀ DEGLI STUDI

FLAGSHIP PROJECT 7 MID-TERM PLENARY WORKSHOP

Advanced and automated innovation labs for diagnostic and therapeutic biopharma solutions

ABSTRACT BOOK

JUNE 13, 2024
9AM - 6PM

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FLAGSHIP PROJECT 7 MID-TERM PLENARY WORKSHOP
Advanced and automated innovation labs for diagnostic and therapeutic biopharma solutions
ROMA TRE UNIVERSITY (Aula Magna, via Ostiense 133, Roma) - JUNE 13, 2024

9.00-9.40 Welcome
Massimiliano Fiorucci, Magnifico Rettore, Università Roma Tre
Alberto Attanasio, Direttore Generale, Università Roma Tre
Sabrina Saccomandi, Direttrice Generale, Fondazione Rome Technopole
Franco Alberto Fossati, Direttore Scientifico, Fondazione Rome Technopole

9.40-10.00 Project overview and educational offer of Rome Technopole in Biopharma & Health
9.40-9.50 Augusto Giardini (Catalent), Giuseppe Roscilli (Takis Biotech), *FP 7 overview*
9.50-10.00 Silvia Conforto (Roma Tre University), *The educational offer of Rome Technopole for biopharma and health*

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10.10-10.20 Giovanni Bulfaro (Sapienza University of Rome) *Development and characterization of high-affinity monoclonal antibodies targeting ErbB3*
10.20-10.30 Valentina Peluzzi (Campus Bio-Medico University) *Evaluation of anti-HER3 antibody and bispecific T cell engager in a 3D model*
10.30-10.40 Daniela Giovannini (ENEA) *Targeted delivery of plant-produced biomolecules for cancer care*
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10.50-11.00 Valerio Chiarini (Takis Biotech) *An in-silico drive-through for mAbs discovery*

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Topic: Biosensors, Biomarkers, innovative medical device development, and bio-active molecules for biopharmaceutical/nutraceutical products (flash talks 11.30-12.30)
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11.40-11.50 Giovanni Antonini (Roma Tre University) *A new point-of-care test for the rapid antimicrobial susceptibility assessment of uropathogens*
11.50-12.00 Giulio Maria Bianco(Tor Vergata University) *Co-design of a four-chip NFC system and microfluidic channel for point-of-care electrochemical sensing*
12.00-12.10 Riccardo Mirabelli (Sapienza University of Rome) *Development of a new generation of radio-guided surgery probes for beta detection*
12.10-12.20 Selene Baschieri (ENEA) *Production and functional characterization of a detoxified gluten-protein for the development of innovative foods for special medical purposes*
12.20.12.30 Alberto Sinibaldi (Sapienza University of Rome) *Direct competitive assay for ERBB2 detection in cell lysates and human plasma using 1-D photonic crystals-based biochips*

Topic: Tech Transfer for bio-products and use of emerging technologies in the biopharma environment (flash talks 12.30-13.00)

12.30-12.40 Augusto Giardini (Chair, Calatent), *Overview of the research topic*
12.40-12.50 Claudia Cruciani (Catalent) *Tech transfer on a pre-filled syringe line: bioproducts and small molecules*
12.50 13.00 Matteo Monti (Catalent) *Electronic Manufacturing Batch Record and Manufacturing Execution System implementation*

Light lunch 13.00-14.00

Topic: New agents for infection control (flash talks 14.00-15.10)
14.00-14.10 Paolo Visca (Chair, Roma Tre University) *Overview of the research topic*
14.10-14.20 Silvia Cammarone (Sapienza University of Rome) *Identification of new therapeutic strategies against multi-drug resistance infections*
14.20-14.30 Massimiliano Lucidi (Roma Tre University) *Screening platforms for the identification of new antibacterial compounds*
14.30-14.40 Giordano Rampioni (Roma Tre University) *Drug repurposing to inhibit Pseudomonas aeruginosa adaptation to the cystic fibrosis lung environment*
14.40-14.50 Jacopo Forte (Sapienza University of Rome) *Versatile Essential Oil Nanoemulsions as new strategies to counteract "superbugs" infections*
14.50-15.00 Riccardo Polani (Sapienza University of Rome) *Bacterial genomics of clinically relevant Gram-Negative Bacteria exhibiting resistance to newest antibiotics*
15.00-15.10 Enzo Tramontano (Cagliari University), *Antiviral DiscoVery Initiatives: Educating Next-Gen Scientists (ADVISE)* (Spoke 3 Cascading Grant holder)

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15.40-15.50 Marco Fiocchetti (Roma Tre University) *Neuronal protective effects of soluble and vesicle-delivered extracellular neuroglobin*

Coffee break 15.50-16.20

Topic: Drug discovery, development, and delivery (flash talks 16.20-17.10)
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16.40-16.50 Eliana Capecchi (University of Tuscia) *Synthesis of pharmacologically active scaffolds using bioreactors based on sustainable lignin nanoparticles from sustainable sources*
16.50-17.00 Alessandro Rava (Roma Tre University) *Cannabidiol and positive effects on object recognition memory in an in vivo model of Fragile X Syndrome: obligatory role of hippocampal GPR55 receptors*
17.00-17.10 Beatrice Vallone (Sapienza University of Rome) *CryoEM complex of human ferritin with receptor CD71 drives protein engineering for protein-based nanoparticles*

General discussion (17.10-18.00) - All participants

Project overview and educational offer of Rome Technopole in Biopharma & Health

FP 7 Overview

Giardini A., Roscilli G.

Catalent, Takis Biotech

Rome Technopole, funded by the PNRR, is the innovation ecosystem of Rome and the Lazio region, comprising leading research and innovation entities organized in a network of state and private universities, public research entities, territorial public entities, and highly qualified and internationally recognized private enterprises. Flagship Project 7 (FP7) is one of eight flagship projects under the Rome Technopole plan. These projects are vertical innovation initiatives with industrial leadership, developed within strategic specialization areas such as Energy Transition, Digital Transition, and Health & Bio-Pharma, cutting across the Spokes. They integrate research capabilities, innovation needs, educational demands, and technological development into a common platform involving universities, research partners, and enterprises. FP7 is led by Takis Biotech and Catalent, working closely with CNR, Istituto Superiore di Sanità , Istituto Nazionale di Fisica Nucleare, and the Universities of Sapienza, Tor Vergata, Roma Tre, Tuscia, Cassino, and Campus Bio-Medico. FP7 aims to create a cohesive ecosystem by uniting nine existing research lines and identifying cross-cutting research and strategic partners. The project involves targeted meetings with students and researchers, to clearly outline objectives, therapeutic areas involved, and required technologies for each project. The objective is to contribute to the creation of an advanced open innovation ecosystem based on Joint and Open Labs. The focus on one side is on the accelerated development of biopharmaceutical solutions to enable innovative characterization of monoclonal antibodies for diagnostic and therapeutic applications, along with other solutions for relevant pathologies and, on other side, to support the industrial production of drugs, utilizing the most interesting technologies for drug delivery and applying the emerging technologies like artificial intelligence to industrial production. The project seeks to establish advanced and innovative laboratories open to all research partners, stakeholders of Rome Technopole, and entities interested in the development of innovative technologies and therapies. Additionally, it aims to engage all other partners and stakeholders of Technopole interested in technology transfer, innovation, and training activities. Through its collaborative approach, FP7 seeks to integrate research, education, and industrial innovation to develop cutting-edge biopharmaceutical solutions. By fostering a dynamic and inclusive research environment, it aims to drive advancements in diagnostics and therapeutics, ultimately improving health outcomes and technological progress in the bio-pharma sector. The Rome Technopole FP7 workshop is an interdisciplinary event designed to promote future collaboration and innovation in biopharmaceutical, biomedical, and pharmaceutical engineering research. This workshop will showcase a variety of cutting-edge research activities highlighting the latest advancements and breakthroughs in these fields. Highlights include developments in monoclonal antibodies targeting ErbB3 and plant-produced antibodies for glioblastoma, as well as antibody design for cutaneous T-cell lymphoma and strategies to combat antimicrobial resistance. Innovations in drug delivery, immunotherapy, and rapid diagnostic tools will also be featured. Additionally, the integration of digital technologies in pharmaceutical processes and studies on the gut-brain axis, neuroprotective proteins, and cognitive training for Parkinson's disease will be discussed. The Flaghship Project 7 workshop will encapsulate a wide range of pioneering research and technological advancements aimed at improving diagnostics, treatments, and understanding of various diseases. The collaborative efforts among universities, research institutes, and biotech companies will underscore the dynamic and innovative spirit driving the future of biopharmaceutical and biomedical research as well as the biopharmaceutical drug production.

The educational offer of Rome Technopole for biopharma and health

Conforto S.

Roma Tre University

The Ecosystem Rome Technopole is a project funded by the PNRR, Mission 4.2, and focuses on fostering research, technological development, and innovation in the Lazio Region. In Rome Technopole, Spoke3 deals with higher education and among its actions designed and developed advanced training initiatives for research lines in FP7 aiming to bridge the gap between academia and industry. Key components of the training initiatives include university courses and courses for PhD and researchers. These are tailored to address the evolving needs of the biopharma industry, covering topics such as drug discovery, molecular biology, bioinformatics, and regulatory affairs. Also, state-of-the-art technologies, AI and robotics have been considered of great importance. In the first half of the project several initiatives have been implemented and almost the 40% of the assigned PhD grants regard research lines in biopharma. Several initiatives have produced the activation of new degree programs and of minor courses the health and biopharma thematic area to update and complement the competences already provided in the Lazio Region. At the doctoral level new programs were activated and several courses were designed by Spoke 3 to enrich the educational offer for the PhD students. By investing in high-level training and research, Spoke3 and FP7 aim to cultivate a new generation of biopharma leaders who are well-equipped to advance the field and contribute to the development of novel therapies and medical solutions. This initiative represents a significant step forward in strengthening the biopharma industry in Lazio Region and driving scientific and technological advancements in Europe.

Topic: Monoclonal antibodies development and characterization

Overview of the research topic

Roscilli G.

Takis Biotech

The research activities in the Monoclonal Antibodies Development and Characterization line will be highlighted, emphasizing innovative approaches for therapeutic applications. This line focuses on developing and characterizing monoclonal antibodies to meet emerging therapeutic needs swiftly. Projects employ advanced techniques such as crystallographic structural studies, in-silico discovery platforms, antibody-drug conjugates, 3D modelling, direct competitive assays using photonic crystals, and targeted delivery of plant-produced biomolecules. These cutting-edge approaches target a range of diseases, including cancer, infectious diseases like COVID-19, and autoimmune conditions.

Development and characterization of high-affinity monoclonal antibodies targeting ErbB3

Bulfaro G.[a], Costanzo A.[a], Chiarini V.[b], Arriga R.[a], Montemiglio L.C.[a], Savino C.[a], Muzi A.[a], Vallone B.[a], Roscilli G.[a,b]

[a]Sapienza University of Rome, [b]Takis Biotech

Epidermal growth factor receptors (EGFRs), also called ErbB receptors, are considered the canonical receptor tyrosine kinases (RTKs). Notably, the dimerization of ErbB3 with ErbB1 or ErbB2 is known to induce the activation of the kinase signaling. ErbB1/2 are known to be involved in numerous forms of cancers and it has been shown that upon targeting ErbB1/2 by inhibitors-based therapies, ErbB3 is up-regulated and seems to participate in tumor progression and resistance. Furthermore, although ErbB3 is endowed with a tyrosine kinase domain, it lacks a proper kinase activity, making tyrosine kinase inhibitors (TKIs) ineffective. To provide an alternative anti-cancer therapy, a possible strategy is preventing ErbB3 heterodimerization with monoclonal antibodies. In this context, using the hybridoma technique, we developed two monoclonal antibodies targeting ErbB3, called hAb1 and hAb2. The aim of this work is to characterize the binding modalities of these monoclonal antibodies. We demonstrate the high affinity of hAb1 and hAb2 antibodies for the ErbB3 receptor through ELISA and BLI assays, and their effective suppression of ligand-dependent stimulation by in vitro experiments. Using X-ray crystallography we identified the epitope residues involved in the binding to the hAb1 antibody, while by means of single particle cryo-electron microscopy we determined the location of the binding site of hAb1 and hAb2 in the presence of the ligand NRG1β. In conclusion, we have identified the structural determinants that regulate the interaction of hAb1 and hAb2 with the ErbB3 receptor using a combined approach of structural biology techniques, functional and binding studies.

Evaluation of anti-HER3 antibody and bispecific T cell engager in a 3D model

Peluzzi V.[a], Muzi A.[b], Giannitelli S.M.[a], Bertani F.R.[c], De Ninno A.[c], Gerardino A.[c], Trombetta M.[a], Businaro L.[c], Rainer A.[a], Roscilli G.[b]

[a]Campus Bio-Medico University, [b]Takis Biotech, [c]CNR

Over the years, many research efforts have focused on immunotherapy to gain better understanding on immunological anticancer mechanisms. Research approaches include the characterization of tumor-specific antigens and the analysis of the patient's immune profile, to develop targeted immunotherapies with improved efficacy. Yet, several treatments show limited response rate, and cannot be "long-term delivery". In this regard, studies on the

HER3 receptor demonstrated its significant contribution to tumorigenesis and cancer progression. Therefore, targeting HER3 downmodulation could be a viable strategy to treat drug resistant tumors. Furthermore, recent studies suggest that resistance to treatments may be influenced by the composition of the tumor microenvironment (TME). Although traditional 2D in vitro models have long represented relevant tools in modern biology, they are still not fully predictive of human responses, limiting the understanding of the interplay between different cell populations. Thus, understanding the dynamic relationships between TME components is essential for the development of new precision therapeutic strategies, including monoclonal antibody-based cancer immunotherapies. This project aims to evaluate the efficacy of anti-HER3 antibody therapy and to gain insights into the enhancement of T-cell activity with bispecific T cell engager antibodies (BiTEs), alone and in combination with an anti-PD-L1 immune checkpoint inhibitor in co-culture systems. Indeed, to overcome the limitations of 2D in vitro models, a tumor-on-a-chip approach is proposed for the evaluation of innovative cancer therapies. The findings could inform future research and clinical strategies, highlighting the value of combination therapies in overcoming tumor resistance and enhancing the immune response.

Targeted delivery of plant-produced biomolecules for cancer care

Giovannini D., Donini M., Marusic C., Baschieri S., Lico C., Camera F., Simonelli F., Triggiani D., Merla C., Mancuso M.

ENEA

The aim of the project is to develop non-invasive and targeted treatments (integration between device and plant-derived biomolecules) to be used in the care of difficult-to-reach solid tumors, such as glioblastoma. In this work, we describe an efficient system for producing the human monoclonal antibody mAbH10, in plants. This antibody targets the C domain of Tenascin-C, a tumor marker expressed by several types of cancer, including glioblastoma. The production of complex heterologous proteins in plants, known as Molecular Farming, offers several advantages over traditional expression systems based on mammalian cells, such as lower costs, ease of production, scalability and limited risk of contamination by human pathogens and the possibility to engineer the glycosylation profile of the recombinant protein. The antibody was purified and characterized biochemically. Furthermore, its binding capacity to the recombinant C domain of Tenascin C, as well as full Tenascin-C released by glioblastoma U87 cells, was tested. The next step will be to evaluate the functionality of the anti-tumor antibody produced in plants in a more-complex in vitro cell systems, such a spheroids. To this aim, spheroids were successfully generated, using glioblastoma U87 cells, and several electroporation protocols were tested to determine that most suitable one for inducing cell permeabilization. A further step will be to test the biological activity of the plant-produced anti-tumor antibody linked to a toxic drug and delivered into spheroids by an electroporation device.

Design of class-G monoclonal antibodies for the treatment of cutaneous T-cell lymphoma (DOGMA)

Gallo V., de Lauro A., Croce N., Salvatore G., Arienzo A., Antonini G., Pitaro M., Narducci M.G., Polticelli F.

Roma Tre University, INBB, IDI IRCCS

The research focuses on the development of an innovative computational protocol for the design of engineered monoclonal antibodies targeting biomarkers and for the development of personalized therapies. The research is conducted within the DOGMA project (Design Of Class-G Monoclonal Antibodies for the Treatment of Aesthetic T-cell Lymphoma). We designed monoclonal antibodies to develop personalized therapies for the Sézary syndrome, a rare and orphan oncological disease. In Sézary syndrome and other T-cell lymphoma, the TCR uniquely distinguishes neoplastic cells from healthy cells. Based on this, we targeted T cell receptors (TCRs) on CD4-positive T-lymphocytes from Sézary patients. T-lymphocytes were isolated from peripheral blood and cDNA libraries were produced and sequenced to reconstruct the variable regions of TCRs. These data were used for the computer-aided design of the

corresponding anti-idiotype antibodies. For each of the neoplastic TCRs, 5000 different antibodies were initially generated which differed in the sequence and structure of the hypervariable regions (CDRs). These were classified based on the value of free energy of formation of the complex (separated dG). The antibody displaying the highest predicted binding affinity has been produced and analysed by SPR, demonstrating nanomolar binding affinity for the target. Flow cytometry analyses are ongoing to confirm the specificity of the antibodies for the neoplastic clones. The project is on TRL4, with upcoming steps including antibody design and validation on TCRs from additional patients, GMP antibody production and purity/safety assessment, obtaining authorization for patients' administration, and progressing to phase 1 clinical trials.

An in-silico drive-through for mAbs discovery

Chiarini V., Arcangeli C., Marchio S., Giusepponi S., Mancuso M., Roscilli G.

Takis Biotech, ENEA

The work carried out for joint lab MATCH was focused on the realization of bioinformatic pipelines for the acceleration of therapeutic biomolecules discovery. In particular, the joint lab consists of scientists from both Takis srl and ENEA, each one with specific backgrounds and expertise, encompassing structural biology, bioinformatic, physics and IT science. The proposed pipelines are conceived to aim and guide experimental discovery and reducing its costs. MATCH's goal is to create a computing architecture performing in silico analysis for both high-throughput pre-screening purposes and to optimize promising candidates. The two afore-mentioned cases apply for a) de-novo design of antibodies and b) optimization of Takis-proprietary molecules for further development. Up to now, antibodies discovery was led by experimental protocols exploiting the immunization of mice in vivo, which results in laborious, time-consuming and expensive procedures. As an alternative, to tackle this problem the pipelines under construction in MATCH, simultaneously integrate several steps of molecular modelling and artificial intelligence, allowing to broad by far the sampling of potential candidate for in vitro studies.

Beetles-derived cantharidin as potential therapeutic agents for solid tumor treatment: design and development of antibody-drug conjugates

Proietti R., Presaghi A., Sperati A., Carpinelli L., D'Ezio V., Bologna M.A., Roscilli G., Persichini T., Colasanti M.

Roma Tre University, Takis Biotech

Cantharidin (CTD) is a beetle's derived terpene with several healing properties whose exploitation is limited due to its toxicity. Here, we have investigated the anti-tumoral effect of CTD on glioblastoma, the most common type of primary malignant brain tumor characterized by a very severe prognosis. The aim of this study is, on the one hand, to analyze the cellular mechanisms underlying the cytotoxic effects of CTD in glioblastoma cells and, on the other one, to find a way to reduce its important side effects. The preliminary results show that CTD is highly cytotoxic for tumor cells. In particular, CTD was observed to reduce cell viability of glioma cell lines in a dose-dependent manner. However, CTD resulted to be cytotoxic also for normal human glial cells (HA), thus indicating its side effects. To reduce the toxicity of CTD and increase its specificity, we proposed the use of advanced biotechnological drug-delivery systems, with a focus on Antibody-Drug Conjugate (ADC), and sought to identify possible tumor markers (e.g., System XC-, CD44, and EGFR) as potential targets to selectively direct the effects of CTD. In particular, we are focusing on CD44, which is significantly more expressed in glioma cells, and we are evaluating the expression of CD44 splicing isoforms to understand their possible role in tumor progression. As a whole, CD44 might be a good candidate as a selective tumor marker and an effective target for the development of antibody-cantharidin conjugates.

Development and characterization of neutralizing antibodies against SARS-CoV2

Costanzo A.[a], Bulfaro G.[a], D'acunto E.[a], Chiarini V.[b], Muzi A.[b], Montemiglio L.C.[a], Savino C.[c], Vallone B.[a], Roscilli G.[b]

[a]Sapienza University of Rome, [b]Takis Biotech, [c]CNR

The research aim of this project is the structural characterisation of neutralising antibodies against SARS-CoV2. We used hybridoma technology to obtain a library of murine antibodies (more than 2000). We examined these antibodies for their ability to bind RBD and its variants and their affinity. The most promising antibodies were humanised using a bioinformatics algorithm. Further screening of the humanised antibodies allowed us to identify four antibodies with high affinity (in the nanomolar range) and low IC50 for the wild-type antigen and/or the alpha, beta, gamma and delta variants. Two of them bind the Omicron variant and neutralise it, as demonstrated in our pseudo-virus neutralisation assay. The CryoEM structure of the Fab of these antibodies in complex with the Spike protein will make it possible to identify the epitope and thus the structural basis that enables them to neutralise even multiple Spike variants.

Analysis of tumor-infiltrating B lymphocytes for the design of new therapeutic strategies based on synthetic antibodies

Principato E.[a], Muzi A.[a], Ferrara F.[a], D'Acunto E.[a], Serrao S.[a], Cappelletti M.[a], Arriga R.[a], Pantano F.[b], Vincenzi B.[b], Iuliani M.[b], Simonetti S.[b], Valeri S.[b], Pagnoni C.[b], Roscilli G.[a]

[a]Takis Biotech, [b]Campus Bio-Medico University

At the dawn of the millennium, it became evident that immune tumor microenvironment, including density, localization, and functional orientation of lymphocytes, plays a crucial role in controlling cancer growth and metastasis. This discovery led to the introduction of immunotherapies aimed mainly at enhancing T cell activity against cancer cells. While these treatments have been effective against various tumours, they are insufficient for some other types, necessitating the development of new therapeutic approaches. Recently, the importance of B cells has been recognized. Studies show that the presence of B cells infiltrates, correlates with a favourable prognosis, and predicts therapeutic response, even in tumours with low mutational burdens. For instance, sarcomas, traditionally considered immune-quiescent tumours with low mutational rates, have shown high immunogenicity driven by B cells. However, the heterogeneity and molecular roles of B cells in these tumours are not well understood. Our study aims to evaluate presence and phenotypes of B infiltrating lymphocytes (BIL) in soft tissue sarcomas. Identifying distinct cellular populations can reveal mechanisms underlying tumor development or regression, potentially correlating with predictive outcomes for patients. Multiple transcriptomic analyses will identify possible biomarkers and the immunoglobulin repertoire. Antibodies identified in patients will be pooled to generate synthetic libraries for in vitro testing to assess their binding capacity to cancer antigens. These antibodies will also be studied to understand their interaction with tumor cells and other microenvironment components. Promising candidates will be synthesized on a large scale and processed via preclinical testing to evaluate their potential as new immunotherapeutic targets.

Topic: Biosensors, Biomarkers, innovative medical device development, and bio-active molecules for biopharmaceutical/nutraceutical products

Overview of the research topic

Battistoni A.

Tor Vergata University

The abstracts presented in this workshop provide elegant examples of the many research activities included in this line and which focus on three main areas: developing advanced biosensors and medical devices, identifying new biomarkers and bio-active molecules, and creating nutraceutical products. In biosensor and medical device development, a major aim is to enhance health monitoring through innovative sensors, wearable devices, and point-of-care testing systems. The identification of biomarkers and bio-active molecules seeks to improve disease diagnosis and treatment by discovering new biological markers and compounds, including those for Alzheimer's and cancer. The nutraceutical products area aims to develop dietary interventions, such as detoxified gluten, to treat conditions like celiac disease. Overall, the research integrates cutting-edge technology and biology to advance medical diagnostics, treatment, and preventive healthcare.

A new point-of-care test for the rapid antimicrobial susceptibility assessment of uropathogens

Arienzo A.[a], Murgia L.[b], Cellitti V.[b], Ferrante V.[b], Stalio O.[b], Losito F.[c] , Gallo V.[b] , Tomassetti F.[b], Marino R.[d], Cristofano F.[d] , Orrù M.[a] , Visca P.[b], Di Somma S.[d], Silvestri L.[e], Ziparo V.[e], Antonini G.[a,b]

[a]Interuniversity Consortium Biostructures and Biosystems National Institute (INBB), [b]Roma Tre University, [c]MBS srl, [d]Sapienza University of Rome, [e]Istituto Dermopatico dell'Immacolata.

We have developed a Point-of-Care Test (POCT) to facilitate rapid and easy diagnosis of urinary tract infections, along with an automatic in vitro diagnostic device (IVD) for conducting antibiotic susceptibility tests. Bacterial resistance to antimicrobials is a significant global concern, often resulting in treatment failure for urinary tract infections, which are prevalent in both community and healthcare settings. Empirical therapy against uropathogens is common but may lead to ineffective treatment, recurrence, and the development of antibiotic resistance. Two open-label, monocentric, non-interventional clinical trials enrolled 349 patients. The results of urine sample analysis using the Point-of-Care Test (POCT) were compared with those of routine Antibiotic Susceptibility Testing (AST) conducted on culture-positive samples. The POCT demonstrated high accuracy (>90%) for all tested antimicrobial drugs, providing reliable results in under 12 hours from urine collection. The project has reached Technology Readiness Level (TRL) 7. Further stages will commence following agreements with a biomedical company for production and marketing, as well as licensing for non-European countries. These stages include: i) patent filing and setting up serial production; ii) streamlined CE certification (as an In Vitro Diagnostic device); and iii) development of new applications for both laboratory and home use.

Co-design of a four-chip NFC system and microfluidic channel for point-of-care electrochemical sensing

Bianco G. M., Mazzaracchio V., Fiore L., Arduini F., Marrocco G., Occhiuzzi C.

Tor Vergata University

Fast, on-site chemical sensing is essential for various applications, including point-of-care analyses that can exploit NFC communications for ease of use with commercial smartphones. Here, we report the layout design of a novel four-chip NFC system integrated with microfluidics for electrochemical sensing, enabling simultaneous analysis of up to

four chemical species. Last-generation commercial-off-the-shelf NFC boards capable of performing open circuit potentiometry are utilized. However, based on the microfluidic circuit, more liquid samples may be required, hindering measurement accuracy. On the other hand, the arrangement of the commercial-off-the-shelf NFC boards themselves must adapt to the channel's shape to minimize liquid losses and avoid eventual interferences between the boards. Hence, three layouts of the NFC-microfluidic system are initially considered, and the best one was experimentally selected to optimize i) the amplitude of the read areas, ii) the communication quality, quantified by passive load modulation, and iii) the number of liquid samples required by the microfluidic. The selected system layout is finally validated by quantifying sodium in a standard solution, showing promising results. The resulting system can be used multiple times by simply changing the sensing electrodes and the low-cost, paper-based microfluidic. Ongoing work includes optimizing the microfluidic circuit for blood sensing and testing the system in clinical settings by integrating all the components into an ad-hoc 3D-printed case.

Development of a new generation of radio-guided surgery probes for beta detection

Mirabelli R., Cavoto G., Collamati F., Mancini-Terracciano C., Morganti S., Pandolfi F., Faccini R.

Sapienza University of Rome

Radio-Guided Surgery (RGS) is a surgical technique that enables the surgeon to evaluate during the surgical procedure the completeness of the tumor lesion resection, thus minimizing the amount of healthy tissue removed. Traditional RGS approaches use a combination of γ (photons) emitting tracers with a γ detection probe. Since γ radiation can traverse large amounts of tissue, any uptake of the tracer in the nearby healthy tissues represents a non-negligible background, often preventing the practical usage of this technique. In lasts years we developed a new concept of RGS beta probe based on the detection of β+ (positrons) emitters. Indeed, the limited penetration power of the β radiation (electrons) allows for smaller background radioactivity from nearby healthy tissues. Furthermore, a β-probe, as it operates with lower background, provides a clearer delineation of margins of the radioactive tissue, requires administration of a lower activity of radiopharmaceutical and it is smaller and easier to handle in the surgical environment. In the first part of this contribution the results of the first clinical trial of the RGS probe based on 15 patients affected by prostate cancer will be presented. In particular we are able to obtain a Sensitivity of 86% and a Specificity of 93%. Moreover, the first results on the development of a new generation of probes based on the use of Solid State Detector (SSD) technologies and on a multichannel architecture will be also described.

Production and functional characterization of a detoxified gluten-protein for the development of innovative foods for special medical purposes

Baschieri S., Donini M., Giovannini D., Lico C., Marusic C., Vitali R.

ENEA

Celiac disease (CD) is an immune-mediated life-long disorder in which chronic inflammation of the small intestine is triggered in genetically predisposed subjects by the ingestion of gluten (gliadins and glutenins) and gluten-like proteins found in wheat, barley and rye. The complete, rigorous and permanent elimination of gluten from the diet is the only currently available treatment allowing to achieve remission of symptoms and prevent complications. ENEA, through a multidisciplinary approach, has previously developed and patented the sequence of a "detoxified" gliadin (GliaMUT). The "detoxification" should have resulted in the definition of a "new" protein safe for coeliac patients but with technological properties (ability to form a network mimicking gluten structure) unaltered as far as possible and contributing, when added to naturally gluten-free flours, to the creation of doughs and products comparable to those obtained with gluten-containing flours. The aim is to set-up and scale-up the production of GliaMUT to then evaluate its inflammatory (on human colonic epithelial cells co-cultured with lymphocytes from celiac patients) and technological (on naturally gluten-free flours) properties.

Direct competitive assay for ERBB2 detection in cell lysates and human plasma using 1-D photonic crystals-based biochips

Sinibaldi A.[a,b], Occhicone A.[a,b], Chiappetta D.[a,b], Di Matteo P.[a,b], Gaganina A. [a], Danz N.[c], Sonntag F.[d], Munzert P.[c], Allegretti M.[e], Giordani E. [e], Giacomini P.[e], Michelotti F.[a,b]

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This research introduces the development and characterization of a disposable biochip designed for detecting monoclonal antibodies against the ERBB2, a crucial target in diagnosis and therapeutic research in breast cancer. The biochip relies on a one-dimensional photonic crystal (1DPC) placed on a plastic substrate, engineered to support Bloch surface waves (BSW) within the visible spectrum. The experimental phase involved employing the biochip alongside a custom optical read-out platform capable of real-time refractometric detection and fluorescence-based end-point measurements. To bio-conjugate our biochips, we immobilized the recombinant ERBB2 protein onto the surface through a functionalization strategy using a silane and glutaraldehyde-based activation. After an initial study using breast cancer cell lysates, we proceeded to test human plasma samples from breast cancer patients, including both ERBB2-positive and negative samples. The experimental findings reveal that the biochip effectively distinguishes between ERBB2-positive and negative samples using fluorescence mode, at 1:10 dilution of the plasma and spiked plasma samples. One of the main advantages of the assay developed in this work lies in the single-step detection procedure that reduces assay turnaround time to less than 20 minutes. These results underscore the potential of the disposable biochip for sensitive and specific detection of ERBB2 through monoclonal antibodies. The approach outlined has exhibited potential not only in ERBB2 diagnostics but also in a range of other life sciences applications. Through adapting the assay format, the authors have recently showcased that this approach can be applied to the assessment of ischemic stroke-related miRNA as well as SARS-CoV-2 antibodies.

Detection of cell oxidative stress changes and scavenging strategies by Raman Spectroscopy in cytological thyroid samples

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Thyroid gland plays a key role in the metabolic regulations through hormones synthesis, thus inducing a physiological production of reactive oxygen species (ROS) in thyrocytes. Therefore, the detoxification system plays a crucial role in protecting thyrocytes from an ROS excess. The imbalance between ROS production and the defence system results in the cell oxidative stress (OS). The increased OS levels are a risk factor for carcinogenesis. In recent years, Raman spectroscopy (RS) has been successfully used for thyroid cancer diagnosis. Here, RS is proposed as a tool to investigate the thyroid disease development through a cell OS study. We enrolled 28 patients, submitted to a first and second thyroid fine needle aspiration (FNA) during follow up. The cytological samples were investigated by RS and morphological examination. On the basis of the evolution of the Raman features over the two FNAs, the 28 patients have been classified into 4 categories, evidencing a different cell OS. In particular, RS shows the presence of carotenoids bands whose uptake may be activated to mitigate the ROS increase, as suggested by the intensity ratio of the peaks assigned to oxidized/reduced cytochrome c. Moreover, the presence of fatty acids droplets in papillary thyroid carcinoma samples is evidenced, as an additional pathway against oxidative stress. Overall, our results suggest a correlation among changes in oxidative stress, carotenoids uptake, fatty acid droplet and thyroid diseases, which could inspire new fundamental research on biomarkers and signalling pathways involved in thyroid OS.

Multiomics approach to identify novel fecal biomarkers of Alzheimer's disease

Vitali R., Merla C., Mancuso M.

ENEA

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disease. It is the most common form of dementia observed in elderly patients (60-70% cases of Dementia). Fifty milion of people worldwide have AD, the incidence and prevalence are steadily increasing, the number of AD is expected to increase to more 150 million by 2050. Currently, a specific diagnostic test for AD is not available. AD diagnosis is a long process that requires several visits for patient evaluation and the execution of various clinical and instrumental tests (Cognitive tests; Brain neuroimaging: computed tomography, magnetic resonance imaging, positron emission tomography; Cerebrospinal fluid (CSF) analysis). To date, the possibility of prevention, early diagnosis with prompt management of patients is almost impossible. Although CSF is considered the ideal sample for the evaluation of biomarkers of AD its collection is very invasive, poorly tolerated by patients and very expensive. Consequently, research is currently committed to identifying new biomarkers in easier-to-obtain biological samples such as blood and saliva. Recent knowledge highlights the role of the intestinal microbiota in neurodegenerative diseases (microbiota-intestine-brain axis). Our hypothesis based on interplay between gut and brain (gut-brain axis) is that modification in microbiota could influence stool composition, so our interest is focused on the fecal sample to identify biomarkers of AD.
Aim: The aim of the project is to identify diagnostic and prognostic biomarkers for Alzheimer's disease (AD) using a multiomics approach (proteome and miRnome) on fecal samples.
Methods: The study was conducted on a transgenic mouse model of AD (3×Tg-AD) with three mutations associated with familial AD (APP Swedish, MAPT P301L, and PSEN1 M146V). miRNome and Proteomic analysis were performed by Next Generation Sequencing (NGS) and high definition mass spectrometry techniques, respectively.
Results and conclusion: By Omics analyses we identified 31 microRNAs and 81 proteins differentially modulated in fecal samples from subjects with AD compared to controls, these molecules are potential biomarkers for AD. Their validation by Real-Time PCR, ELISA or immunoblotting is in progress. By this study, we demonstrated that the fecal sample is suitable to identify new biomarkes, also in neurodegenerative diseases as AD. The use of the fecal sample implies multiple advantages. In fact, feces are simply to collect, non-invasive and repeatable over time. Furthermore, once biomarkers have been validate, the resulting diagnostic analysis is performed with accessible and standardized methods in diagnostic laboratories at low or relatively low costs.

Optimization, prototyping and validation of a new device for the monitoring of water

Tomassetti F., Gallo V., Arienzo A., Antonini G.

Roma Tre University

Water safety is crucial for the survival and well-being of the global population. Water systems provide essential safe drinking water for human health, agriculture, and industry, but an increasing number of poor countries are affected by water shortages, and water pollution caused by man-made chemicals is a pressing issue in wealthy countries. Investing in sustainable water resource management and pollution prevention is important to ensure access to water for the entire global population. In this context, the Micro Biological Survey Method (MBS) is a robust and accurate test for microbiological analysis, originally developed and patented by "Roma Tre" University. The MBS method is a colorimetric system for quantitative and qualitative microbiological analyses that provides rapid and reliable results, facilitating procedures and interpretation of data, and allowing analyses directly on-site without sample pre-treatments. This project aims to extend the application of the MBS method to biomonitoring water microbiological and chemical safety. The production of the reaction media for microbiological analysis of water has been optimized, and a new reaction media was developed to evaluate the presence of heavy metals such as cadmium, lead and

mercury in water samples through the colorimetric detection of bacterial metabolism. The method is based on heavy-metal inhibition of *B. subtilis* α-amylase and could detect the presence of cadmium, lead and mercury at concentrations much below the safety limits, representing an excellent starting point to improve the monitoring of water sources.

Topic: Tech Transfer for bio-products and use of emerging technologies in the biopharma environment

Overview of the research topic

Giardini A.

Catalent

The research line on tech transfer for bio-products is related to the industrial production of drugs. The experimental development performed aimed to identify the working condition to produce and inspect pre-filled syringes (PFS). The PFS are the most recent system to deliver sterile product to patients. A PFS line is a complex equipment with hundreds of control instrument inside. The presentation will cover the challenge that an industry has to face with to reach the ultimate target that is the industrial production of sterile drug in PFS. The research line on use of emerging technologies in the biopharma environment is about how the new technologies may help the industrial production of drugs. The topics covered are related to digitalization of Batch Record, the temperature mapping for cold chain drugs, and the Artificial Intelligence applied to human writing, and the application of digital twin to industry planning.

Tech transfer on a pre-filled syringe line: bioproducts and small molecules

Cruciani C.

Catalent

Case Studies of tech transfer projects to a new PFS line and relevant to Biological molecules and Small Molecules Drug products are presented with the aim to highlight the main aspects of the implementation of a combination product (DP product in Syringes) on a filling machine with all the troubleshouting required to gain knowledge about the new technology and to design a robust manufacturing process.

Electronic Manufacturing Batch Record and Manufacturing Execution System implementation

Monti M.

Catalent

This project aims to digitalize the most important document produced during production in the pharmaceutical industry: the Batch Record. The document will be digitalized through to implementation of a Manufacturing Execution System. Starting from the production of oral solid dose and syringes products, the system will be progressively deployed to all production processes. By re-designing the legacy paper batch record we will include all MES capabilities, simplifying data acquisition, reducing data transcription, eliminating all manual calculation and implementing a modular procedure approach designing decision trees. For all data captured will be introduced live system controls and ranges. All these activities aim to reduce manual errors and reduce process deviations while removing paper documents from the process. Within the scope falls the integration of the MES with internal ERP, environmental monitoring system and line equipment as well as the internal training program.

Digital Twin and Artificial intelligence

Giardini A., Santolamazza A.

Catalent

Within Rome Technopole Project a Joint Lab with Tor Vergata University has been initiated on Digital Twin and Artificial Intelligence. The activities are focused on simulation of different process of pharmaceutical industry: Packaging Operations, Technology Transfer, Setup and Maintenance. The first two model have been developed and we can start to see the firsts results. In the digital world, we are not able yet to transform everything handwritten in a digital information with the accuracy level requested by the pharmaceutical industry. A startup coming from Salerno University has created a software able to read the human writing giving a level of reliability of the interpretation of the results. The software is capable of interpreting human writing and transforming it into letters and numbers regardless of who wrote it. The result is a transcription with a level of reliability which in the highly regulated world of the pharmaceutical industry is extremely important because it would allow the automatic acquisition of data, reducing verification only to those data whose reliability is low.

TIR TOR Track system

Belli S.

Catalent

Test the possibility to use in the industrial contest a brand new software for material tracking and evaluation of the time outside of the cold room. In the pharmaceutical environment the control of temperature condition of a thermos-sensible material is connected to a series of calculation that must be done for each single vials. These calculations are complex due to the number of activities performed in the pharmaceutical production. The project aims to delivery automatic results thanks to a real time monitoring.

Topic: New agents for infection control

Overview of the research topic

Visca P.

Roma Tre University

Antimicrobial resistance (AMR) is a growing public health concern, with more than 5 million deaths being attributable to AMR worldwide and an increasing trend in the next years. While AMR invariably grows in major pathogens, the antimicrobial discovery pipeline runs almost dry. Consequently, WHO is constantly updating the list of priority pathogens for which antibiotics are urgently needed. Most of these pathogens are bacteria responsible for healthcare-associated infections, including those belonging to the ESKAPEE group, namely *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*. Early stages of antimicrobial drug discovery are typically driven by academic and SME research. Advancing cooperation between academia, health foundations, and industry, while promoting national antimicrobial research networks are essential paths to identifying and developing new antimicrobials. Within the FP7 framework, a collaborative network aimed at searching for and developing new agents for infection control has been established. The network includes nearly all partners of the FP7 consortium and encompasses expertise in microbiology, microbial genetics and molecular biotechnology, chemistry, biochemistry, and drug delivery. The network strives to identify novel therapeutic strategies and to stimulate dialogue with the scientific community to address the AMR global challenge. The dissemination of results in this field aims to improve the awareness of a broader and more diverse audience and to promote the training of PhD students and researchers in combating AMR. Contributions provided by individual partners will be overviewed with a focus on the synergisms and perspectives of further integration and collaboration among partners.

Identification of new therapeutic strategies against multi-drug resistance infections

Cammarone S.
Sapienza University of Rome

The research activity of FP7_Line5_Project Sapienza aims at identifying new antimicrobial compounds or auxiliary agents. The project involves the creation of a shared library for screening bioactive compounds by expanding an existing internal library of natural products with small molecules and macromolecules, including peptides from member laboratories and the market. In this context, the intervention strategies employed so far have involved various approaches such computer-aided screening procedures which allowed to filter chemical compound libraries based on their theoretical binding affinities with biological targets responsible for resistance to the most common antibiotics used in clinical practice, whose three-dimensional structures are known. Specifically, we identified two biological targets; one is the KPC3 enzyme, that is a beta-lactamase capable of conferring resistance to carbapenems and cephalosporins in gram-negative bacteria; for this project, we are still in a preliminary phase of structural and in vitro characterization of those compounds which showed activity in silico. The second target that we identified is the ArnT enzyme, which is a glycosyl-transferase responsible for colistin resistance in *Pseudomonas aeruginosa* bacterial strains. This project is in an advanced stage of candidate hit optimization. Indeed, a natural ent-beyerene diterpene was identified as a promising inhibitor of the ArnT enzyme and based on this finding, semisynthetic analogues of hit were designed, synthetized, and tested against colistin-resistant *Pseudomonas aeruginosa* strains. Microbiological assays indicated that, for an efficient colistin adjuvant activity, an ent-beyerane scaffold is required and that the oxalyl group has a key role in the interaction with the enzyme. In order to optimize the hit compound, two different strategies have been elaborated: the development of suitable drug delivery systems to improve the therapeutic potential of the ent-beyerene based ArnT inhibitors and a simplification and a

chemical modification of the ent-beyerene scaffold. This study explores the use of abietane scaffold, characterized by the presence of conjugated systems that allow the analysis of the compounds by HPLC. Among the abietane-type diterpenoids abietic, dehydroabietic and podocarpic acid have been selected as a starting point for the development of a second generation of ArnT inhibitors. A small focused library of abietane-type derivatives has been designed and synthesized, combining the abietane scaffold with the functional groups essential for efficient inhibition of the ArnT activity.

Screening platforms for the identification of new antibacterial compounds

Lucidi M., Visaggio D., Visca P.
Roma Tre University

The rise of antibiotic-resistant bacteria is a new pandemic that jeopardizes the success of modern medicine, prompting the World Health Organization to rank antimicrobial resistance among the top ten global health threats. The absence of an effective arsenal of antibiotics to counteract bacterial resistance underscores the urgent need to discover novel antimicrobials to fight multidrug-resistant pathogens. Here, we report the development of two single-blind screening approaches to identify new antimicrobial agents. The first approach was aimed at identifying bacterial growth inhibitors. By determining the minimum inhibitory concentration (MIC) according to the broth microdilution method, 119 compounds have been tested against different Gram-positive (*Staphylococcus aureus*, *Bacillus spizizenii*, *Enterococcus* spp.) and Gram-negative (*Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa*) bacterial species. Nine compounds exhibited a strong activity (MIC≤16 µg/mL) on Gram-positive species together with negligible haemolytic effects on human erythrocytes (<3% haemolysis at 64 µg/mL), representing promising candidates for future development. The second approach was aimed at developing a user-friendly screening platform for the identification of β-lactamase inhibitors to impair resistance to β-lactams which are among the most frequently prescribed antibiotics. For this purpose, two plasmids for the controlled expression of the serine β-lactamase TEM-1 or the metallo-β-lactamase NDM-1, with a narrow and a broad spectrum of activity against β-lactams, respectively, have been separately cloned in *E. coli*. Out of 125 candidates tested, none displayed inhibitory effects against the two *E. coli* strains expressing TEM-1 or NDM-1, posing the need to expand the screening campaign to a broader range of compounds.

Drug repurposing to inhibit *Pseudomonas aeruginosa* adaptation to the cystic fibrosis lung environment

Mellini M., Ridolfi C., Renzi L., Visca P., Imperi F., Leoni L., Rampioni G.
Roma Tre University

The success of a bacterial pathogen in establishing hard-to-eradicate infections strictly relies on its ability to use the host environment as a growth medium, to produce virulence factors, and to survive antibiotic treatment. The opportunistic human pathogen *Pseudomonas aeruginosa* uses the airway sputum as a nutritional source during cystic fibrosis (CF) lung infection, and finely modulates the formation of antibiotic-resistant biofilms and virulence factors production in response to stimuli associated to the host environment through c-di-GMP and quorum sensing (QS) signaling systems. The CF sputum has been characterized and reconstituted as a synthetic CF sputum medium (SCFM). Genes required for *P. aeruginosa* growth in SCFM are dispensable in standard laboratory media. Moreover, *P. aeruginosa* displays similar biofilm formation and QS activation during growth in the CF sputum and in SCFM. Hence, unexplored molecular pathways are required for growth, virulence, and biofilm formation in the CF sputum, and molecules inhibiting these pathways in SCFM have the potential to reduce P. aeruginosa load and pathogenicity in the CF lung. Here, we will present the construction and validation of a *P. aeruginosa*-based biosensor strain in which molecules hampering c-di-GMP or QS signaling systems decrease light or fluorescence emission, respectively. Data collected during the screening of a library of more than 3,000 FDA-approved drugs using the biosensor strain grown in SCFM will be also presented.

Versatile Essential Oil Nanoemulsions as new strategies to counteract "superbugs" infections

Forte J.

Sapienza University of Rome

Bacterial infections caused by "superbugs" are increasing globally and conventional antibiotics are becoming less effective against these bacteria, such that we risk entering in a post-antibiotic era. In recent years, antimicrobial peptides, multifunctional therapeutic agents, which are effective for a broad spectrum of microorganisms, have gained significant attention for their clinical potential as new class of antimicrobial substances. On the other hand, their clinical use is still limited by some shortcomings, such as low bioavailability, instability and cytotoxicity. Oil in water nanoemulsions could be excellent and versatile drug delivery system to overcome these limitations, increasing the bioavailability of loaded molecules and targeting them to the disease area by exploiting specific characteristics (e.g., thermosensitivity or pH sensitivity), minimizing their degradation or inactivation after administration and preventing unwanted side effects. In the first part of the research, the right amounts of Thyme essential oil and Rosemary essential oil as the oil phase, Tween®85 and Hepes buffer as the aqueous phase were determined as well as the choice of suitable concentrations of essential oils, selected by evaluating not only their antibacterial activity on *Klebsiella pneumoniae* and *Escherichia coli* ATCC 25922, but also the absence of their cytotoxicity on epithelial human cell A549-CCL-185. Subsequently, the selected nanoemulsions were properly characterized evaluating size, PDI, ζ-potential, fluidity, microviscosity, polarity, stability over time at different storage temperatures or in presence of biological fluids and the release capacity using NileRed, a fluorescent probe capable of emulating the behavior of a possible peptide to be delivered.

Bacterial genomics of clinically relevant Gram-Negative Bacteria exhibiting resistance to newest antibiotics

Polani R., De Francesco A., Carattoli A.

Sapienza University of Rome

The project leverages genomics to identify resistance mechanisms to last-generation antibiotics in clinically relevant Gram-negative bacteria. This approach relies on Whole Genome Sequencing (WGS) techniques, using rapid methods like Oxford Nanopore Technologies, complemented with Illumina sequencing. WGS is applied to identify clones circulating in hospitals, studying their global evolution. The main research focus is *Klebsiella pneumoniae*, a public health threat microorganism often displaying resistance to various antibiotics. In Italy, the dissemination of multidrug-resistant *K. pneumoniae* is driven by few high-risk clones, expressing the KPC carbapenemase enzyme, such as those belonging to the Clonal Groups (CGs) 101, 147, 258 and 307. To face KPC-producing *K. pneumoniae* infections, in 2018 ceftazidime/avibactam (CZA) has been introduced in clinical practice. 33 K. pneumoniae strains, belonging to different major Sequence Types and encoding different inhibitor resistant KPC-3 variants, were studied at genome level. A total of 12 KPC variants conferring CZA resistance were detected from clinical K. pneumoniae strains. Recently, the project is moving toward the identification of mechanisms conferring resistance or reduced susceptibility to Cefiderocol (FDC). Analyzing the genomes of high-risk *K. pneumoniae* sequence type 512 during CZA, Meropenem/vaborbactam, and FDC treatment, we described in vivo evolution of the *K. pneumoniae* sequence type 512 resistome occurred through plasmid loss, outer membrane porin alteration, and a nonsense mutation in the cirA siderophore receptor gene, resulting in high levels of cefiderocol resistance. This strains collection and a library of the respective blaKPC allele cloned in isogenic *Escherichia coli* could be used to test new antimicrobial molecules.

Antiviral DiscoVery Initiatives: Educating Next-Gen Scientists (ADVISE) (Spoke 3 Cascading Grant holder)

Corona A., Esposito F., Distinto S., Maccioni E., Mandalari G., Sciortino M.T., Tramontano E.

Cagliari University

ADVISE is an innovative educational network with a multidisciplinary program in which trainees are embedded in a case-study research effort and exposed to activities comprising a summer school, an international congress, and specialized workshops. ADVISE main goal is to implement an innovative educational network for four young undergraduate researchers (trainees) to be formed in the field of drug development. The proposal will reach its goal by two means. On the one side, ADVISE will introduce the trainees in a highly qualified research environment involving scientists from UNICA and UNIME, through a “on the field” case-study dedicated to the development of novel broad spectrum antiviral agents targeting a cellular protein. On the other side, ADVISE will directly organize two main events, an International Summer School and a Workshop, involving very highly qualified scientists in the antiviral drug discovery and big data fields from universities and companies. In addition, ADVISE will allow the trainees to participate to the Congress of the International Union of the Microbiological Societies (IUMS 2024) that will take place in Italy. In ADVISE the theoretical and practical activities of the trainees will be highly interconnected: the theoretical ones will enhance trainees’ competencies with inter- multi- and trans-disciplinarity, while their applications will strengthen their skills promoting their personal development and future employability. Overall, ADVISE activities will allow the trainees to be immersed in the work of scientific teams of excellence within a highly stimulating environment that will provide an intense training.

Identification of the diadenosine tetraphosphate hydrolase ApaH as a promising target for antivirulence drugs against *Pseudomonas aeruginosa*

Cervoni M., Sposato D., Ferri G., Bähre H., Leoni L., Rampioni G., Visca P., Recchiuti A., Imperi F.

Roma Tre University

Novel therapeutic interventions are urgently needed to address the threat of antimicrobial resistance. Inhibition of the virulence potential of bacterial pathogens, the so called antivirulence approach, is an alternative or complementary strategy to standard antibiotic-based therapies. The identification of suitable targets is essential to promote and advance antivirulence drug discovery programs. By combining in vitro analyses and in vivo infection assays, in this study we demonstrate that the enzyme ApaH, which is responsible for the degradation of the intracellular signaling molecule diadenosine tetraphosphate, is crucial for the virulence of the important human pathogen *Pseudomonas aeruginosa*. Indeed, loss of ApaH drastically reduces the production of several virulence factors in both laboratory and clinical isolates, and impairs *P. aeruginosa* ability to cause infection in plant, insect and rodent models. These results, together with the absence of ApaH homologues in mammals, make ApaH a promising target for the development of anti-P. aeruginosa antivirulence drugs. Studies are in progress to characterize *P. aeruginosa* ApaH at the biochemical and structural levels and to generate cell-based biosensors to identify potential ApaH inhibitors.

The synergistic activity of colistin and clofoctol against Gram-negative pathogens

Fortuna A., Guiducci A., Imperi F., Mori M., Cutarella L., Visca P., Rampioni G., Leoni L.

Roma Tre University

Colistin is an antibiotic active against Gram-negative pathogens (GNP). Although the toxicity of this antibiotic is a worry due to its limited therapeutic range, it has been used in recent years as a last-line defence to treat infections caused by GNP, when no other treatment options are available. However, resistance to colistin has inevitably occurred

among clinical isolates, making the research for colistin adjuvants extremely important. Clofoctol is an FDA-approved synthetic antibiotic active against Gram-positive bacteria, with well-established pharmacological characteristics such as low toxicity and high airway tropism. We have shown that clofoctol restores colistin susceptibility not only in colistin-resistant clinical isolates of the ESKAPE pathogens *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumanii*, but also in other pathogens such as *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia* (isolated from the lungs of people with cystic fibrosis). In parallel, the mechanism of action of the colistin-clofoctol combination has been investigated by in silico and wet lab approaches. Molecular dynamics studies suggested that colistin could help clofoctol cross the outer membrane of Gram-negative bacteria. To validate this model and identify possible molecular targets of clofoctol, *P. aeruginosa* mutants resistant to the colistin-clofoctol combination were evolved in vitro and their genomes were sequenced. Experiments are in progress to confirm the involvement of identified mutations in the resistance to the colistin-clofoctol combination.

Mechanisms of resistance to gallium, a recently repurposed antibacterial agent

Visaggio D., Lucidi M., Visca P.

Roma Tre University

Iron uptake and metabolism are vital processes for bacteria, hence potential therapeutic targets. Gallium [Ga(III)], the active component of the FDA-approved citrated gallium nitrate formulation, is a ferric iron-mimetic that inhibits bacterial growth by disrupting iron uptake and metabolism. Ga(III) is active against several pathogens, including *Acinetobacter baumannii*, which has been posed by the World Health Organization at the top of the list of priority pathogens requiring urgent new antibiotic treatment options. Investigating Ga(III) resistance and its underlying mechanisms is paramount for the prospective utilization of Ga(III) in treating A. baumannii infections. To this aim, the *A. baumannii* type strain ATCC 19606 was exposed to high concentrations of Ga(III), and 5 spontaneous Ga(III)-resistant mutants were selected, namely Ab-GaNR1, Ab-GaNR2, Ab-GaNR3, Ab-GaNR4, and Ab-GaNR5. Whole genome analysis of Ga(III)-resistant *A. baumannii* mutants revealed that Ab-GaNR1 possessed a mutation in lpxC, an essential gene encoding an enzyme involved in lipid A biosynthesis. Ab-GaNR2, Ab-GaNR3, and Ab-GaNR4 displayed mutations mapping in the essential gene tamB, involved in transporting glycerophospholipids across the outer membrane. Lastly, mutant Ab-GaNR5 had a mutation in oprD, encoding an outer membrane porin. The contribution of individual mutations to Ga(III) resistance was confirmed by genetic complementation assays. Phenotypic assays revealed that Ga(III)-resistant mutants exhibited a growth defect in human biological fluids, remarkable membrane damage, and increased antibiotic susceptibility. The deleterious effects of the mutations associated with Ga(III) resistance encourage the clinical development of Ga(III) as an antimicrobial agent for treating *A. baumannii* infections.

Topic: Toolboxes for testing metabolic, inflammatory, neurologic, and tumoral disorders

Overview of the research topic

Limatola C.

Sapienza University of Rome

An overview of the research activities running in the Line 9 will be given, with special emphasis on the projects focused on new technological approaches for the study of metabolic, inflammatory, tumoral and neurological diseases. The Projects in this line aim at to find solutions to rapidly respond to the need for new drug testing with in vitro and in vivo approaches using multi-photons microscopy, advanced electrophysiology, omics technologies, optogenetica, multigenes sequencing, brain organoids and preclinical models.

Two-week computerized cognitive training affects resting-state electroencephalographic rhythms in Parkinson's disease patients with cognitive deficits

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The present exploratory study tested the hypothesis that computerized cognitive training (CCT) in home telemonitoring may beneficially affect eyes-closed resting-state electroencephalographic (rsEEG) rhythms in Parkinson's disease patients with cognitive deficits (PDCD). A Eurasian database provided clinical-demographic-rsEEG datasets in 40 PDCD patients, 29 PD patients without cognitive deficits (PDNCD), 40 Alzheimer's disease patients with cognitive deficits (ADCD), and 40 cognitively normal older adults (Healthy). Sixteen of the 40 PDCD patients performed a cross-over unsupervised CCT program with simple, serious video games (over a sham program), consisting of 14 daily sessions lasting about 20 minutes each at patients' homes. Compared to the Healthy, PDNCD, and ADCD groups, the PDCD group was characterized by greater rsEEG delta (about 2-4 Hz) and theta (about 4-7 Hz) source activities diffusely. The PDCD patients undergoing the CCT program showed improvement in video game performances and a reduction in those delta-theta source activities after that program over the control condition. In conclusion, these results suggest that the 2-week CCT program in home telemonitoring may mitigate abnormal "slowing" of rsEEG rhythms in PDCD patients, possibly enhancing the regulation of brain arousal and quiet vigilance.

Acute gut dysbiosis induces alterations in neuronal network excitability and triggers neuroinflammation in the brain

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Sapienza University of Rome

Inflammatory bowel diseases affect the gastrointestinal tract and can lead to extra-gut effects, including depression and anxiety related to the brain. During the peak of inflammation, certain brain regions, such as the hippocampus, respond to bowel inflammation by modulating neuronal and glial activity. Additionally, bowel inflammation compromises the integrity of the blood-brain barrier, allowing immune cells and metabolites to infiltrate. However, a comprehensive understanding of the signaling pathways involved in this axis remains incomplete. Thus, we developed a mouse model of peripheral inflammation to investigate the impact of the gut-brain axis's impact on brain functioning and identify the mediators of cerebral dysfunctions. First, we validated our model by characterizing gut inflammation. Our analyses revealed the presence of reactive enteric glia and disruption in gut integrity. Metabolomic analysis confirmed gut dysbiosis, showing alterations in the levels of SCFA. We then explored whether peripheral inflammation induces neuroinflammation in the brain. Studying glial cell responses, we observed changes in glial morphology and density. These alterations are associated with changes in hippocampal excitatory and inhibitory synaptic transmission. In conclusion, we have demonstrated that peripheral gut inflammation disrupts synaptic functionality and induces a neuroinflammatory in the hippocampus.

Neuronal protective effects of soluble and vesicle-delivered extracellular neuroglobin

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High levels of neuroglobin (NGB) exhibit cell-autonomous anti-apoptotic effects within neurons. Based on recent findings indicating the presence of the protein extracellularly, here we investigated the release of NGB and its potential function outside the cells. Results obtained in neuron-derived cells (SH-SY-5Y) indicate that different globin inducers (e.g., H_2O_2, 17β-Estradiol) trigger the secretion of NGB as a soluble protein and as part of small extracellular vesicles (sEVs) cargo, supporting a paracrine function of the protein. To reinforce this idea, we examined the impact of extracellular NGB by utilizing conditioned media (CM) or sEVs from wild-type (WT) and NGB overexpressing cells. Obtained data demonstrated that CM enriched with NGB prevents early mitochondrial fragmentation and diminishes apoptosis in SH-SY-5Y cells when subjected to oxidative stress or the mitochondrial toxin 3-nitropionic acid (3NP). Similarly, favorable anti-apoptotic effects were induced by NGB-enriched sEVs or the EVs-deprived CM fractions. This implies that the release of NGB, whether as soluble or vesicle-encapsulated protein, holds the potential for neuroprotection, independently of the mechanism of the extracellular release. Altogether, the obtained results strengthen the idea that NGB could operate in the extracellular compartment as a transmission factor of neuroprotection evidencing exogenous NGB as a new targetable neurotrophic protein in neurodegenerative disease.

Abnormal electroencephalographic rhythms from quiet wakefulness to light sleep in alzheimer's disease patients with mild cognitive impairment

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Alzheimer's disease patients with mild cognitive impairment (ADMCI) show abnormal resting-state eyes-closed electroencephalographic (rsEEG) alpha rhythms (8-12 Hz) and may suffer from daytime sleepiness. The present exploratory study tested the hypothesis that they may have characteristic EEG rhythms from quiet wakefulness to light sleep during diurnal EEG recordings. Datasets of 34 ADMCI and 24 matched healthy elderly (Nold) subjects were obtained from an international archive. The AD diagnosis was based on cerebrospinal fluid Abeta/p-tau biomarkers. Diurnal EEG recordings lasted approximately30 minutes. Transitions of EEG activity from quiet wakefulness (alpha dominant) to light sleep (voltage flattening to theta-dominant ripples) were scored according to Hori's stages. EEG delta (< ≈ 4 Hz), theta (≈ 4-7 Hz), and alpha (≈ 8-12 Hz) source activities were estimated. The following results were found: (1) the ADMCI over Nold participants were characterized by greater frontal EEG delta source activities from quiet wakefulness to ripples and lesser reduction (reactivity) in the posterior EEG alpha source activities from quiet wakefulness to ripples. Notably, EEG delta source activities during quiet wakefulness were also greater in those ADMCI patients than in the ADMCI patients who remained in quiet wakefulness. These results suggest that ADMCI patients with a greater susceptibility to daytime sleepiness may show characteristic ongoing EEG delta and alpha rhythms in the transition from quiet vigilance to naps during daytime.

Topic: Drug discovery, development, and delivery

Overview of the research topic

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The path of novel drugs to reach marketing authorization comprises several steps: it begins with the drug discovery phase in which basic research aims at understanding disease mechanisms, identifying possible targets and searches strategies that interfere the investigated disease. Once targets and strategies have been defined and before reaching clinical evaluation, the drug development stage takes place where the mode of action of the drug candidates is clearly defined, the potential toxicity and efficacy on various in vitro and in vivo models are explored, and where drug manufacturing, formulation and delivery are defined. All these steps are essential for the technology transfer to pharmaceutical companies in order to begin the clinical evaluation. In the FP7, several lines of research are pursued to define novel disease targets, develop novel manufacturing processes and drug formulation, and exploit novel strategies for drug delivery. Taken together, the expertise of the partners involved spans over almost all the sectors of life science: from structural biology to bioinformatics, from pathophysiology to biotechnology. Similarly, the therapeutic strategies explored within the topic comprise small molecules, biologics and advanced therapies against a variety of diseases. In spite of such heterogeneity, the multidisciplinary background of the groups involved and the interconnection with pharmaceutical companies represent a unique opportunity to foster the development of novel drugs for unmet clinical needs.

In search of a new therapeutic target for Niemann-Pick disease

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Niemann-Pick type C1 disease (NP-C1) is a fatal and rare autosomal recessive lysosomal disorder resulting from mutation in npc1 gene. It is caused by an abnormal lipid accumulation of unesterified cholesterol in endosome-lysosome system. To date there are no curative therapy. Most of the NPC1 protein mutations produce proteins which are immediately degraded but presenting residual activity. Therefore, increasing the availability of mutant NPC1 protein could be an emerging therapeutic approach. We found out, that an epigenetic pathway regulated by BET (Bromodomain and Extra-Terminal motif) proteins controls NPC1 protein expression. The aim of our work is to investigate whether the modulation of BET proteins can increase NPC1 protein level and reduce cholesterol accumulation in both human-derived cells and transgenic mice carrying the most common NPC1 mutation. Therefore, fibroblasts derived from NP-C1 patients and mice carrying I1061T mutation on npc1 gene (Npc1(tm(I1061T)dso) have been treated with a specific BET protein inhibitor (JQ1). Our results show that BET inhibition, modulates NPC1 protein expression, lysosomal size, and NPC1 localization in patient-derived fibroblasts. Moreover, JQ1 treatment seems to rescue some locomotor parameters in mutated mice. Our data suggest that BET proteins are regulators of cholesterol metabolism and could be used as a target for NP-C1.

Synthesis of pharmacologically active scaffolds using bioreactors based on sustainable lignin nanoparticles from sustainable sources

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Biocompatible and eco-certified lignin nanoparticles, from pulp and paper and biorefinery industries, exhibit unique redox properties resulting from electron interactions arising from the ordered organization of lignin's aromatic groups. These nanoparticles have been employed as a multifunctional platform in drug discovery. These Biotechnological Platforms have been functionalized with single or cascade enzymatic systems acting as nanobioreactors for the one-pot synthesis of drugs containing hydroxytyrosol, benzoxazine, and flavanones identified as privileged scaffolds with pharmaceutical activity.

Cannabidiol and positive effects on object recognition memory in an in vivo model of Fragile X Syndrome: obligatory role of hippocampal GPR55 receptors

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Cannabidiol (CBD), a non-psychotomimetic constituent of Cannabis sativa, has been recently approved for epileptic syndromes often associated with Autism spectrum disorder (ASD). However, the putative efficacy and mechanism of action of CBD in patients suffering from ASD and related comorbidities remain debated, especially because of the complex pharmacology of CBD. We used pharmacological, immunohistochemical and biochemical approaches to investigate the effects and mechanisms of action of CBD in the recently validated Fmr1-Δexon 8 rat model of ASD, that is also a model of Fragile X Syndrome (FXS), the leading monogenic cause of autism. CBD rescued the cognitive deficits displayed by juvenile Fmr1-Δexon 8 animals, without inducing tolerance after repeated administration. Blockade of CA1 hippocampal GPR55 receptors prevented the beneficial effect of both CBD and the fatty acid amide hydrolase (FAAH) inhibitor URB597 in the short-term recognition memory deficits displayed by Fmr1-Δexon 8 rats. Thus, CBD may exert its beneficial effects through CA1 hippocampal GPR55 receptors. Docking analysis further confirmed that the mechanism of action of CBD might involve competition for brain fatty acid binding proteins (FABPs) that deliver anandamide and related bioactive lipids to their catabolic enzyme FAAH. These findings demonstrate that CBD reduced cognitive deficits in a rat model of FXS and provide initial mechanistic insights into its therapeutic potential in neurodevelopmental disorders.

CryoEM complex of human ferritin with receptor CD71 drives protein engineering for protein-based nanoparticles

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Human transferrin receptor-1 (CD71) guarantees iron supply by endocytosis upon binding of iron-loaded transferrin and ferritin to CD71. Viruses and the malaria parasite exploit CD71 for cell invasion and epitopes on CD71 for interaction with transferrin and pathogenic hosts were recently identified. We provide the molecular basis of the human ferritin-CD71 interaction by the 3.9 Å resolution single-particle cryo-electron microscopy structure of their complex and by validating our structural findings in cellular context. The structure of the H-Ft/CD71 complex revealed the specific sites on CD71 to be hooked by ferritin for physiological access to cell through the CD71 “iron door”. Moreover, it accounts for a Tf-independent binding of ferritin to the receptor, allowing differential regulation of iron uptake, and indicates a physiological role for the CD71 apical domain, unassigned to date. The contacts between the heavy-chain ferritin and CD71 largely overlap with arenaviruses and Plasmodium vivax binding regions in the apical part of the receptor ectodomain. Our data account for transferrin-independent binding of ferritin to CD71 and suggest that select pathogens may have adapted to enter cells by mimicking the ferritin access gate and it provides a

sound structural basis to elaborate on the possibility of developing alternative ferritin-like anti-viral or anti-parasite therapeutic ligand, be it an antibody or a peptidomimetic capable of blocking the “common contacts” epitope on CD71 residue, and to further engineering ferritins as nanocarriers and theranostic agents.

Mitochondrial carriers, master regulators of cell metabolism. Insight from in silico studies

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The Mitochondrial Carrier Family, also known as Solute Carrier Family 25 (SLC25), is involved in the translocation of a wide range of molecules across the inner mitochondrial membrane (IMM). In doing so, these carriers act as “gates” that regulate cell metabolism through the trafficking of metabolic intermediates in and out of the mitochondria, driving anabolic and catabolic reactions such as glycolysis, Krebs cycle, beta oxidation of fatty acids, etc.. Thus, members of this family are key players in physiological and pathological states and their activity is often deregulated in various cancer types. Structural information on these carriers is scarce and therefore computational studies represent a valuable approach for a better understanding of their function and for the development of drugs to target them to treat various human diseases. The application of computational methods to members of this protein family, such as protein structure prediction, molecular dynamics simulations and molecular docking, allowed us to get deeper insight into their structure and function and to devise strategies aimed at developing lead compounds for drug development. Examples of these studies will be illustrated with particular emphasis on the basic amino acids transporter (SLC25A29) and the citrate/isocitrate transporter (SLC25A1).

Development of Natural Killer cells-based immunotherapy for cancer

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Advanced therapies represent a cutting-edge approach in medical treatment. In oncology, redirecting immune cells against tumor cells have revolutionized the treatment landscape, offering new avenues for cancer patients with limited options. Natural Killer cells (NK) are cells of the innate immune response that play a crucial role in the defense against tumors and virally infected cells. Besides being exploited for their natural antitumor activity, NK cells can be employed in immunotherapies in which autologous or allogeneic cells acquire enhanced cytotoxicity, persistence, and tumor targeting properties, by means of chimeric antigen receptor (CAR) engineering. In our project, we plan to establish a cellular and molecular platform for the development of CAR-NK immunotherapies based on non-viral delivery system. As a proof of principle, the NK- cell line NK-92 was selected for the development of allogeneic CAR-NK targeting the Human Epidermal Growth Factor Receptor 2 (HER2), a key oncogene in various cancers. Several constructs, with different combinations of CAR elements, were designed. Quality controls were set up to assess the expression of anti-HER2 CAR and functional assays were developed to test cell activation against HER2+ tumor cells. Different approaches are now being tested to optimize the efficiency of transfection and the expansion of CAR expressing cells. Our findings highlight the potential of non-viral delivery systems for the development of CAR-NK cells and pave the way for the design of novel advanced cell therapies against cancer.

Macrophages treated with interferons induce different responses in lymphocytes via extracellular vesicles

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Background and aims: Cellular response to pathogens influences the production of cytokines, chemokines, and Extracellular Vesicles (EVs). Interferons (IFNs) are fundamental effectors of antimicrobial innate immunity and important regulators of the adaptive immune response. Limited information exists regarding the impact of IFNs on the information carried by EVs. This study aimed to investigate whether IFNα2b, IFNβ, IFN-γ, and IFN-λ1/2 alter the content and function of EVs released by primary monocyte-derived macrophages (MDM).
Methods: Small-EVs (sEVs) were purified by size exclusion chromatography from supernatants of MDM treated with IFNs. To characterize the concentration and dimensions of vesicles, Nanoparticle Tracking Analysis was used. The expression of surface markers was evaluated on MDM and sEVs by flow cytometry. Next, sEVs produced by IFNs-stimulated MDM were used to treat autologous PBMC and secreted cytokines were quantified by flow cytometry.
Results: IFNs treatments induced a significant down-regulation of the exosomal markers CD9, CD63, and CD81 on sEVs, and a significant modulation of some adhesion molecules, major histocompatibility complexes and pro-coagulant proteins. The sEVs-treated PBMCs showed significant modulation of lymphocyte activation and IL-17 release.
Conclusions: Altogether, our results suggest that IFNs influence biogenesis of EV. In addition they affect composition and activity of sEVs produced by MDM showing significant modulation of lymphocyte activation and a potential role in the immune response to microbes.

Production of plant-made molecules for disease diagnosis, therapy and prevention

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Plants have emerged as a convenient, safe and economical way to produce high amounts of recombinant proteins (‘Molecular Farming’). Proofs of principle and efficacy exist for many proteins. Recombinant proteins can be expressed in plants either following stable plant transformation or using transient expression systems, and can be delivered as plant material or following purification. The aim of this project is to evaluate the efficiency of the ‘Molecular Farming’ in the production of recombinant Humanin, a small animal mitochondrial polypeptide. Humanin exhibits protective effects against stress conditions and apoptosis in numerous cell types through regulation of signalling processes and is currently investigated as a potential therapeutic target in the treatment of different diseases, including neurodegenerative ones. In this study, two constructs were prepared for transient and stable expression of recombinant Humanin in plants. For transient expression, Humanin gene was introduced in a plant viral vector in a way to express the recombinant protein in fusion with two tags (an N-terminal and a C-terminal) which may facilitate purification and confer protection from proteolysis. This vector will be used to agroinfiltrate tobacco plants, and the recombinant protein, following purification, will be analyzed for its neuroprotective effect on cell lines modelling Parkinson’s disease. For stable expression, Humanin gene in fusion with a sequence encoding a signal peptide for targeting to the apoplast was introduced in a binary vector for Agrobacterium tumefaciens-mediated transformation of tomato plants. Tomato fruits expressing recombinant humanin will be used in strategies for disease prevention and therapy through oral delivery.