Università
degli Studi
della Campania
*Luigi Vanvitelli*

# PhD Days 2023

## PhD Programme in Molecular Life Sciences

# Cancer biology, Immunology, Microbiology, Drug design

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Cationic lipopeptides for fighting antimicrobial resistance.
Potent and Selective CXCR4 Macrolactam peptide antagonists for anticancer intervention
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Tutor: Ivana D'Angelo

**Renata Esposito**
Similar programmed death ligand 1 (PD-L1) expression profile in mild chronic obstructive pulmonary disease (COPD) and non-small cell lung cancer (NSCLC)
Tutor: Bruno D'Agostino

**THE PHUC NGUYEN**
Monocytic fluorescent NFκB GFP U937 cell response to endotoxins
Tutor: Paola Italiani

**Tinghao Liu**
The innate immune memory of mast cells
Tutor: Diana Boraschi

**Vincenzo Mazzarella**
design and synthesis of anticancer peptides functionalizing gold surface of photonic biochips: towards a new potential detection strategy of cxcr4-overexpressing circulating tumor cells
Tutor: Salvatore Di Maro

**Saba Sadiq**
L.rhamnosus IMC501: Production of Biomass, EPS and Lactic Acid from simplified semi defined media and renewable waste resources
Tutor: Donatella Cimini

**Wenjie Yang**
The innate memory molecular mechanism of monocytes and macrophages
Tutor: Diana Boraschi

**Wenli Shi**
Anti-tumor mechanism of natural mushroom polypeptide Gymnopeptide A
Tutor: Paola Italiani

# Gene Regulation and Computational Biology

**Lucia Argenziano**
The role of the maternal-effect gene Padi6 in mouse female fertility, embryogenesis, and epigenetic reprogramming
Tutor: Andrea Riccio

**Sara Savaheli**
Elucidation of the link between RNA maturation and neurodegeneration by a cell-specific transcriptomic analysis in C. elegans
Tutor: Denis Dupuy, Andrea Riccio, Elia Di Schiavi

# Structure and Functions of Biomolecules

**Angela Clemente**
Isolation, Characterization and Biological Action of Type-1
Ribosome-Inactivating Proteins from Seeds of Atriplex hortensis L
Tutor: Antimo Di Maro

**Angela Oliver**
Structural and functional characterization of the recombinant FVII of coagulation
Tutor: Annamaria Sandomenico

**Anna Magri 10:05-10:20**
Development and application of new edible active formulations to preserve beneficial biomolecules and reduce fruit losses
Tutor: Antonio Fiorentino, Milena Petriccione

**Alessandra Del Bene**
Organic molecules with biological applications: ATP and GTP nucleopeptide binders and antimicrobial peptides
Tutor: Anna Messere

**Joyce Rodriguez**
Role of Allelopathy in the Success of Selected Invasive Plant Species in the Mediterranean Basin and Possible Applications.
Tutor: Monica Scognamiglio

**Chidoh Kootlole**
Plant specialized metabolites for treatment of Leukemia
Tutor: Monica Scognamiglio

**Hafiza Zumra Fatima**
Isolation, Characterization and Biological Action of an atyical Ribotoxin-like protein from fruiting bodies of Armillaria mellea
Tutor: Antimo Di Maro

**Clementina Acconcia**
The activation mechanism of the Melatonin MT1 receptor
Tutor: Luigi Russo

**Domenico Sgambati**
Study of eukaryotic and prokaryotic zinc-finger proteins
Tutor: Paolo Vincenzo Pedone

**Domenico Romano**
Specialized metabolites from natural sources as lead compounds to fight against emerging diseases
Tutor: Antonio Fiorentino

**Martina Slapakova**
MucR from S. meliloti: new insight into its DNA targets and its ability to oligomerize
Tutor:Paolo Vincenzo Pedone

**Rita Russo**
Insights into the identification of novel galectin inhibitors
Tutor: Emilia Pedone

**Eliza Kramarska**
Structural characterization of novel vaccine antigens
Tutor: Rita Berisio

**Giovanni Barra**
Biocompatible polimers for human health
Tutor: Rita Berisio , Alessia Ruggiero

**Nataliia Ventserova**
Understanding the conformational dynamics of the early stages of Human Prion Amyloid Fibril formation using Chemical Exchange Saturation Transfer
Tutor: Roberto Fattorusso

**Ivan Mercurio**
Development of a technological platform for the identification of modulators of the PED-PLD1 interaction
Tutor: Roberto Fattorusso

**Brunella Mongiardi**
Identification of sex-specific therapeutic strategies in aging and dementia
Tutor: Elvira De Leonibus

**Maryam Kamarehei**
Novel therapeutic tools preventing the neurotoxic Aβ/PrPC interaction
Tutor: Luigi Russo

**Francesca Guzzo**
Natural products in drugs discovery: extraction methods, NMR characterization and evaluation of their potential antimicrobial properties.
Tutor: Brigida D'Abrosca

**Raffaella di Vito**
Central glutamatergic dysfunction in spinal muscular atrophy
Tutor: Alessandro Usiello

**Isar Yahyavi**
DAspartate metabolism in autism spectrum disorder
Tutor:Alessandro Usiello

**Martina Dragone**
Characterization of protein molecular interactions with metal ions or cyclodextrins by NMR, CD and UV spectroscopies
Tutor: Carla Isernia

**Mercy Ebunoluwa, Ayinde**
Antibacterial and Antiviral Activities of Selected Nigerian Plants Against Clinically Important Human Pathogens
Tutor: Brigida D'Abrosca

**Vikram Pratap Singh**
Innovative mass spectrometry-based proximity labeling method for unraveling the Macrophage migration inhibition factor (MIF) interactome
Tutor: Angela Chambery

**Marco Barretta**
Microfluidic methods for the design of high-performance nanoparticles as drug delivery systems
Tutor: Assunta Borzacchiello

**Getasew Shitaye Ayalew**
Unmasking Viral RNA: targeting viral RNA capping machinery to tackle COVID-19 and future CoV emergencies
Tutor: Gaetano Malgieri

**Maria Marone**
Microbial and human lactonases for the control of virulence factors in pathogenic bacteria
Tutor: Giuseppe Manco

**Eunice Wairimu Maina**
Characterization of fniii domain of axl protein by NMR
Tutor:Gaetano Malgieri

**Antonello Prodomo**
Single-molecule biophysical study of the FANCJ DNA helicase
Tutor: Francesca Maria Pisani

**Mohammad Mahtab**
Elucidating the role of DDX11, the Warsaw breakage syndrome DNA helicase, at the DNA replication fork.
Tutor: Francesca M. Pisani

**Valentina Verdoliva**
Microwave-assisted solid-state procedure to covalently conjugate Hyaluronic acid to Curcumin: validation of a green synthetic protocol to prepare a biocompatible material
Tutor: Stefania De Luca

**Mehwish Kanwal**
Purification, structural and functional characterization of FV zymogen from plasma fraction concentrates
Tutor: Nunzianna Doti

**Manil Kanade**
Structural and biochemical studies of RTEL1, a FeS helicase
Tutor: Silvia Onesti

**Vincenzo Massimiliano Vivenzio**
Identification and characterization of novel γ-Carbonic Anhydrases in Pseudomonas aeruginosa
Tutor: Giuseppina De Simone, Simona Maria Monti

**Awet Ghebretinsae Tewelde**
Structural and functional studies of proteins involved in neurodegenerative diseases
Tutor: Roberto Fattorusso

# Molecular Cell Biology

**Antonietta Esposito**
Identify molecular pathway regulating cell proliferation through glycosphingolipids biosynthesis
Tutor: Seetharaman Parashuraman

**Seyedehnegar Parizadeh**
Investigation of cargo-specific autoregulatory and export systems in secretory pathway in mammalian cells
Tutor: Alberto Luini

**Anna Truda**
Liquid biopsy in precision oncology: an innovative workflow for the study of circulating nucleic acid
Tutor: Nicoletta Potenza, Giovanna Marchese

**Carmela Casale**
GADD45β influences the RIPK3-dependent regulation of NF-kB
Tutor: Alessandra Pescatore

**Debora Latino**
D-Aspartate attenuates cadmium-induced mitochondrial dysregulation in rat testis
Tutor: Maria Maddalena Di Fiore

**Ilenia De Leo**
RNA regulatory networks governed by miR-125a in hepatocarcinoma cells
Tutor: Nicoletta Potenza

**Maria Carannante**
Polystyrene microplastics's toxicity: in vitro study on human spermatozoa
Tutor: Lucia Rocco

**Giulia Grillo**
Microplastics impair the functionality of Sertoli cells by inducing mitochondrial damage
Tutor: Alessandra Santillo

**Nunzia Magnacca**
miR-18a-5p/SREBP1/PERK axis affects ER stress and autophagy in the early stages of Metabolic dysfunction-Associated Fatty Liver Disease (MAFLD)
Tutor: Antonia Lanni

**Nagendra Sai Kumar Achanta**
Paraoxonase2: Post-Translational Modifications and Protein-Protein Interactions
Tutor: Manco Giuseppe

**Mariagrazia Di Gennaro**
Unravelling the role of the TGN export machinery for basolateral proteins in Amyloid Precursor Protein (APP) transport and processing
Tutor: Alberto Luini

# Human genetics

**Sharon Russo**
ZNF687: a new player in skeletal growth
Tutor: Fernando Gianfrancesco

**Concetta Montanino**
Non-coding RNAs as versatile regulators in Neurodegenerative Diseases (NDDs). A focus on MCI (Mild Cognitive Impairment) and MS (Multiple Sclerosis).
Tutor: Bruna De Felice

**Emilia D'Angelo**
Multi-locus imprinting disturbances in patients affected by Beckwith-Wiedemann Syndrome
Tutor: Flavia Cerrato

**Angela Pagano**
Investigating the molecular mechanisms underlying the Silver-Russell syndrome
Tutor: Flavia Cerrato

**Abu Saadat**
Genome-wide studies for the molecular characterization of isolated Wilms tumor
Tutor: Andrea Riccio

**Cristina Somma**
Pharmacological stimulation of autophagy to rescue proteinopathy and cognitive decline in mucopolysaccharidosis-IIIA
Tutor: Elvira de Leonibus

**Ezia Spinosa**
The silent autoimmunity is a new aspect of phenotypic variability in Incontinentia Pigmenti
Tutor: : Francesca Fusco

**Giorgio Fortunato**
Dissecting the role of rare variants in Parkinson's disease pathogenesis trough
the generation of different cellular disease models
Tutor: Teresa Esposito

**Martina Di Guida**
AAV-mediated microRNAs modulation as gene-independent strategy in inherited retinal Dystrophies
Tutor: Sandro Banfi, Sabrina Carrella

**Pasquale Di Letto**
TUDP: diagnosis for undiagnosed
Tutor: Vincenzo Nigro

**Sarah Iffat Rahman**
Creation of novel allellic frequency database to optimise the diagnosis of rare genetic disorders
Tutor: Vincenzo Nigro

# Session 1:
## Cancer biology, Immunology, Microbiology, Drug design

# Novel molecular targets for Hepatocellular Carcinoma

PhD student: Beatrice Cavalluzzo (email: beatrice.cavalluzzo@unicampania.it )

Tutor: Luigi Buonaguro (email: l.buonaguro@istitutotumori.na.it )

PhD cycle: XXXVII cycle

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Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Session: Cancer biology, Immunology, Microbiology, Drug Design

Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancer and results, globally, as the third most common cause of cancer-related deaths. Few therapeutic options are currently available, mostly due to the absence of specific therapeutic targets. The aim of this project is to evaluate HCC specific overexpressed proteins to identify new targets for the development of active (vaccine) and/or passive (adoptive T-cell therapy) cancer immunotherapeutic strategies. To date, after the analysis of a large amount of data derived from a public dataset, we evaluated HCC-specific overexpressed proteins, identifying four HLA-A02:01 epitopes sharing a high level of sequence and structural homology with viral antigens. $CD8^+$ Tcells cross-reacting with homologous paired epitopes were identified by pMHC-tetramer staining analysis. Subsequently, a vector containing an MHC class I construct harbouring a signal peptide along with the epitope sequence and transmembrane domain was used to transfect the Hep-G2 cell line.

Experimental analysis of transfected cells is ongoing. Specifically:

- MHC-IAC experiments to validate the presence of the transfected construct carrying the selected peptide

- cytotoxicity experiments with peptide-stimulated PMBCs from HLA-A02:01 healthy donors to assess the ability of $CD8^+$ Tcells to cross-react, killing tumor cells expressing epitopes homologous to viral ones.

# Small Peptides for Pharmaceutical Purposes:

## Cationic lipopeptides for fighting antimicrobial resistance.

## Potent and Selective CXCR4 Macrolactam peptide antagonists for anticancer intervention

PhD student: Antonia D'Aniello (email: antonia.daniello@unicampania.it )

Tutor: Salvatore Di Maro (email: salvatore.dimaro@unicampania.it )

PhD cycle: XXXVI° cycle

Affiliation: Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania "Luigi Vanvitelli", Via Vivaldi 43, I-81100, Caserta

Session: Cancer biology, Immunology, Microbiology, Drug Design.

During my third year, I completed a project that focused on the development of lipopeptides as strategy for fighting Antimicrobial Resistance. Last year, I have synthesised and tested a library of short lipopeptides, which were designed as a simplification of the available antimicrobial peptides, including a common positively charged core conjugated with a spacer bearing a variable fatty acid. The first set of lipopeptidomimetics were preliminary tested *in vitro*, and the best candidates underwent structure-guided design, which allowed us to synthesise, this year, new derivatives endowed with different aromatic spacers and fatty acids. Among them, we observed promising results in terms of MIC values, which fallen in the low-μM range against gram (-) and gram (+) bacteria, and low-to-moderate toxicity versus host cells. On the other hand, I have been involved in the developed of potent, selective andplasma stableCXCR4 peptide antagonists as a potential anticancer agent. In particular, we investigated the structural and biological effects ensuing from the disulfide bond replacement of a CXCR4 parent peptide antagonist with a side-chain to tail macrolactamization, as the widely used disulphide bond showed some synthetic limitations, redox and metabolic instability. This strategy produced two promising candidates that displayed high affinity and selectivity against towards CXCR4, which were able to inhibit the CXCL12-dependent cell migration in the low-micromolar range. Inspired by the findings, molecular modeling studies will be conducted to obtain a theoretical model that could provide new analogs with higher affinity and stability.

# Insight into the probiotic potential of *Lactococcus lactis* strains isolated from natural whey starter cultures.

PhD student: Ida De Chiara (ida.dechiara@unicampania.it)

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PhD cycle: XVII° cycle

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

Lactic acid bacteria (LAB) play an important role in animal and human health by different mechanisms; among them, some exhibit antagonistic activity against pathogens. The probiotic potentials of eight *Lactococcus lactis* strains isolated from natural whey starter cultures (NWS), in terms of ability to survive to gastrointestinal conditions, cell surface properties and absence of antibiotic resistance, have been previously demonstrated. To gain more insight about the probiotic potential of these strains, we evaluated the adhesive capacity and the ability to inhibit pathogens. The adhesive capacity was evaluated via the auto and co-aggregation test, reporting that the auto-aggregation of LAB strains was higher compared to the pathogens' one, whereas poor or no co-aggregation was observed between pathogens and LAB strains. *Agar well diffusion* method revealed that all the LAB strains were able to inhibit the growth of the pathogen *S. sonnei*, but no antimicrobial activity was detected on the other tested pathogens. Finally, we studied the inhibition of pathogens adhesion in a model of co-cultured epithelial cells treated with 4 best performing *L. lactis* strains, revealing that all of them were able to significantly reduce the adhesion of enteric pathogens, *S. thypimurium* and *E. coli*, on gut epithelial cells. Moreover, studies on antiproliferative properties on human glioblastoma cell lines are in progress. The selection of new LAB probiotic strains from dairy products is functional to the production of novel functional foods with health-related properties.

# PD-L1 expression profile and mitochondrial activity in patients with COPD and lung cancer: what is the role of pollutants?

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Session: Cancer biology, Immunology, Microbiology, Drug Design

PD-1/PD-L1 are an immune checkpoint that plays a key role in lung immune homeostasis. The expression of PD-L1 on alveolar macrophages (AMs) has been implicated not only in the context of lung cancer but also in inflammatory diseases, such as chronic obstructive pulmonary disease (COPD), influencing mitochondrial activity. Notably, mitochondria are involved in the regulation of innate and adaptive immune responses, and mitochondrial integrity is critical for both the effector and memory phases of T-lymphocyte differentiation, which play a major role in COPD. COPD and lung cancer share pollutant exposure as a major risk factor and often occur as comorbidities; however, the molecular basis of this association is not well defined. We performed a prospective observational study on 150 age- and sex-matched subjects with a suspected diagnosis of lung cancer undergoing routine bronchoscopy and bronchoalveolar lavage (BAL). Of the 150 patients, only 75 met the inclusion criteria. We divided the patients into healthy never smokers (n = 15), smoker controls (n=16), lung cancer patients (n = 25), and COPD patients (n=19). The latter were then divided according to the GOLD guidelines into GOLD 1-2 (n=10) and GOLD 3-4 (n=9). So, we'll assess the presence of air pollutant in BAL collected from all patients investigating their impact on PD-L1 immune profile and mitochondrial activity in each group; we'll also assess the effect of air pollutants in vitro model of macrophages (THP-1) and in BAL-derived AMs.

# Insight into the glycopeptidolipids gene cluster in *Mycobacterium smegmatis*

PhD student: Milena Della Gala (milena.dellagala@unicampania.it)

Tutor: Lidia Muscariello (lidia.muscariello@unicampania.it)

PhD cycle: XXXVIII° cycle

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

In recent years the worldwide incidence of non-tuberculous mycobacteria (NTM) infections has dramatically increased. Among known NTM, *Mycobacterium abscessus* is the most difficult to manage. Glycopeptidolipids (GPLs) are the major surface exposed molecules found in several species of NTM and play an important role in both mycobacteria physiology and pathogenicity. Here we study the GPLs gene cluster using *Mycobacterium smegmatis* as model organism. All genes necessary for GPLs biosynthesis in NTM are clustered in a single highly conserved region of 65 kb. Using *in silico* analysis, we identified within this region, the *MSMEG_0394* gene, whose predicted protein product share 75% amino acid identity with *M. abscessus* MAB_4102c gene product. Both putative proteins are annotated as “hypothetical” with unknown function. By RT-PCR transcriptional studies we demonstrated that *MSMEG_0394* is the second gene of an operon that contains *MSMEG_0393*/*94*/*95* genes. In order to verify if *MSMEG_0394* gene product is involved in GPL biosynthesis, we have isolated a mutant strain of *M. smegmatis* carrying a null mutation in the *MSMEG_0394* gene using a two-step homologous recombination strategy. Preliminary analysis show that inactivation of the *MSMEG_0394* gene affects colony morphology and growth rate. Moreover, based on *in-silico* prediction, we suppose that both MSMEG-0394 and its orthologous MAB-4102c have a cytosolic localization and are probably involved in a protein-protein mechanism interaction. A deeper characterization of the mutant phenotype will shed light on the role of MSMEG-0394 in the GPL production.

# Metabolic rewiring in thyroid carcinomas induced by BRAF gene mutations

PhD student: Caterina Perfetto (caterina.perfetto@unicampania.it)

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PhD cycle: XXVI cycle

Affiliation: Institute of Genetics and Biophysics "Adriano Buzzati-Traverso", National Research Council, Naples, Italy

Session: Cancer biology, Immunology, Microbiology, Drug design

Distinct oncogenic alterations within the same tumor type can drive differential metabolic rewiring. For instance, BRAFV600E mutation - which drives the most aggressive thyroid cancer (TC) types - associates with increased glycolytic phenotype of tumor cells. Despite B-raf inhibitors (BRAFi) being effective as first treatment, acquired resistance limits their efficacy. We identified a peculiar signature in *BRAF*-mutated TCs consisting of increased expression of glycolysis-related genes, mirrored by a highly glycolytic phenotype, mostly mediated by Hif-1α. Accordingly, we found that Hif-1α stabilization counteracts the effects of vemurafenib on glycolysis-related genes and tumor cells' viability. Noteworthy, we tested diclofenac - an anti-inflammatory drug reported for its antiglycolytic activity in melanoma - also in the context of TCs (both the papillary and anaplastic forms) and we verified that in combination with vemurafenib it is able to potently restrain the glycolytic phenotype of *BRAF*-mutated TC cells. More interestingly, diclofenac maximizes the efficacy of BRAFi by synergistically impairing cell viability, even when compared to BRAFi+MEKi combination. Overall, these results highlight a new metabolic vulnerability of *BRAF*-mutated TCs and suggest new therapeutic approaches to maximize drug efficacy and reduce acquired resistance because of the synergistic ability of BRAFi and diclofenac to restrain the glycolytic metabolism.

# Identification of neuroprotective natural compounds for the development of nutraceuticals and drugs: screening of epi-drugs in *C. elegans* to identify novel pharmacological therapies for CHARGE syndrome.

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PhD cycle: 36° cycle

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

CHARGE syndrome (CS) is a rare genetic disease characterized by several neuronal and developmental malformations. CS is mainly due to mutations in the Chromodomain Helicase DNA binding protein 7 (*CHD7*) gene. Nowadays, no pharmacological treatments are available for CS, so identifying novel drugs is essential to improve patients' health and to better understand the molecular mechanisms underlying the syndrome. In this context, we used *C. elegans* as animal model to perform a drug screening to identify novel pharmacological hits for CHARGE syndrome. *chd-7* is the *C. elegans* homolog of the *CHD7* gene, and the *gk290* mutation in *chd-7* causes developmental defects, including a strong reduction in the number of eggs laid. We used this phenotype to set-up a medium-throughput drug screening *in liquido*, aimed at identifying new drugs from an epi-drug commercial library. We screened 234 molecules and identified 9 best candidate hits able to modify the aberrant number of eggs laid by *chd-7(gk290)* mutant. These hits will be further characterized for their role in *C. elegans* and also *in vitro* using an immortalized neuronal cell line in which, through CRISPR/Cas9, the knock-out of the *CHD7* gene was obtained.

# Targeting NSCLC cell-CAF interplay with newly selected aptamers

PhD student: Giuseppe Ciccone (email: Giuseppe.ciccone@unicampania.it; Giuseppe.ciccone@ieos.cnr.it; gciccone142@gmail.com)

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

Non-small cell lung cancer (NSCLC) is a prevalent and aggressive neoplasm characterized by important and intricate interactions between tumour cells and the surrounding tumour microenvironment (TME). The TME includes different cell populations. Among them, cancer-associated fibroblasts (CAFs) play a pivotal role in tumour progression, angiogenesis, and immune evasion. Therefore, targeting CAFs within the TME represents a very promising approach for the development of innovative therapeutic strategies with improved effectiveness. Aptamers are short single-stranded nucleic acids showing excellent specific binding properties that have emerged as very promising and advantageous targeting agents and therapeutics for precision medicine. Using a suitably cell-SELEX protocol optimized to isolate aptamers binding to cell surface antigens and undergoing target-specific internalization, we identified an RNA aptamer with improved stability in human serum and selective and effective binding and internalization in NSCLC-derived CAFs. The possibility to chemically modify and conjugate this aptamer to small chemical compounds or other RNA therapeutics may provide the model for the rationale design of innovative drugs with improved efficacy, introducing a new dimension in the fight against NSCLC.

# IL4 down-regulates the gluten-induced inflammation in the gut mucosa of celiac patients

PhD student: Ilaria Mottola (email: ilaria.mottola@unicampania.it)

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PhD cycle: 37° cycle

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

Celiac disease (CD) is a chronic intestinal inflammation caused by gluten ingestion in genetically predisposed individuals. Acute-CD and potential-CD are the two main forms of disease with different grades of intestinal mucosa lesion. We recently observed a marked infiltration of T cells releasing interleukin(IL)-4 in the intestinal mucosa of potential-CD compared to acute-CD patients, suggesting a protective effect of IL4 against the gluten-induced inflammation in gut mucosa. To further investigate the immune regulatory role of IL4, T-cell lines (TCLs) were generated from intestinal biopsies of both acute and potential CD-patients (N=12), by stimulation with gliadin and growth factors, in absence/presence of exogenous IL4 (control-TCLs and IL4-TCLs, respectively). Changes in the various cell subsets (CD4+, CD8+, TCRγδ+, and regulatory T cells), in cytokine production profile, and gliadin recognition pathways were evaluated in TCLs by multiparametric flow cytometry and/or ELISA. IFN-γ release was significantly reduced in culture supernatants of IL4-TCLs compared to control-TCLs. IL4 induced an expansion of CD4+ T-helper and a reduction of CD8+ and TCR γ/δ+ T-cells. A significant inhibition of IFN-γ production in response to gliadin and immunogenic peptides was measured in IL4-TCLs compared to control-TCLs. In conclusion, we demonstrated a hitherto unexplored immunoregulatory function of IL4 on gluten-induced inflammation in gut mucosa of CD patients. Further studies are required to dissect the role of IL4 in preventing villous atrophy in CD-predisposed individuals

# Vasculogenic Mimicry in Glioblastoma: mechanisms and novel compounds

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Session: Cancer biology, Immunology, Microbiology, Drug Design

Glioblastoma multiforme (GBM) is the most devastating primary CNS tumor, characterized by remarkable vascularization which occurs through different mechanisms, including Vasculogenic Mimicry (VM), the non-endothelial formation of new vessels causing resistance to anti-angiogenic therapies. Therefore, identification of new active compounds is challenging and urgent.

We study VM process in three GBM cell lines exploiting a quantitative tube formation assay on a 3D-matrix. The concomitant analysis of migration and invasion offers the possibility for a quantitative analysis of the underlying mechanisms.

Current analyses include a novel decapeptide denoted uPAcyclin, recently found to be anti-invasive in mouse models of tumor dissemination and *Ruta graveolens* water extract (RGWE), shown to kill human GBM cell lines while leaving neuronal cells unharmed.

Exposure to nanomolar concentrations of uPAcyclin or to sub-lethal doses of RGWE strongly inhibits migration as well as matrix invasion of both human and rat glioma cells. Furthermore, vascular-like structures are strongly reduced by uPAcyclin and RGWE. The combinations of these compounds with conventional drugs, like temozolomide, cisplatin or HDACi are also taken into consideration.

These studies shed light on the inhibitory mechanisms of VM in GBM and indicate that uPAcyclin and RGWE are promising candidates for anti-GBM therapies to overcome resistance to anti-angiogenic therapies.

# A machine learning approach using tool development for receptor-based virtual screening

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

Artificial intelligence algorithms are being employed in drug discovery domain to solve the high throughput compound screening program. PyRMD is a new fully automated tool designed to easily screen millions of compounds in hours through an automated workflow and intuitive input files, allowing fine-tuning of each parameter of the calculation by boosting the efficiency of its various steps. A high-performance AI method RMD algorithm specifically tailored for the identification of new ligands. This ligand-based virtual screening tool can be trained using target bioactivity data directly downloaded from the ChEMBL repository without manual intervention. PyRMD is a command-based automated tool that covers a limited community who knows the basics of python or at least command-line interfaces. I would like to work on its GU Interface so that It can connect with a major community of researchers belonging to different fields of study. In the GUI version of PyRMD, I will make the screening criteria more critical by applying a conserved binding pocket check (all active compounds will be subjected to bind at a specific binding site of receptor, only those compounds will be selected further which bind to that binding area). Like we can dock the PyRMD screened compounds with a particular binding pocket. A compound which gives better binding scores with assigned residues will be screened further and the rest of all compounds will be eliminated. This will save our time and financial resources to validate these compounds through wet lab.

# Overcoming the Infected Lung Barriers: Surface-Engineered PLGA Nanoparticles with Poly (vinyl alcohol) for Inhalation Delivery of Antimicrobial Peptide

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Session: Cancer biology, Immunology, Microbiology, Drug Design

Antimicrobial peptides (AMPs) administered via inhalation hold great promise in the treatment of lung infections; however, their effectiveness is restricted by infected lung barriers, including mucus and bacterial biofilm. To overcome these challenges, surface-engineered inhalable nanoparticles (NPs) emerge as the optimal choice, ensuring efficient delivery of AMPs to their intended site of action. Polyvinyl alcohol (PVA) is frequently employed as a stabilizing agent during the production of poly(lactide-co-glycolide) (PLGA) NPs, and it can also provide muco-inert properties to these NPs [1;2]. This study aims to comprehensively examine the impact of employing various types of PVA for PLGA NP coating on their interactions with model mucus and biofilm. To this aim, blank NPs were prepared using PVAs with different hydrolysis degree (HD) and molecular weight (Mw) and their interactions with mucin (the building blocks of mucus) and bacterial alginate (BA, the main component of biofilm) were assessed. Ultimately, optimized NPs were selected and loaded with a model AMP, colistin. Obtained results indicated that PVA with low HD and Mw forms an ideal shell on the NP surface, effectively protecting against interactions with mucin and BA. Additionally, these NPs exhibited rapid permeation through both artificial mucus and BA, as demonstrated by Transwell□-permeation assay across simulated lung barriers [3]. The findings from colistin-loaded NPs are in agreement with those obtained for the blank NPs. In conclusion, partially hydrolysed (HD 88%) low Mw (31KDa) PVA was revealed to be effective to achieve NPs capable to penetrate across mucus and biofilm. In vitro and in vivo studies are currently in progress to evaluate the antimicrobial capabilities of the optimized AMP-loaded NPs.

# Similar programmed death ligand 1 (PD-L1) expression profile in mild chronic obstructive pulmonary disease (COPD) and non-small cell lung cancer (NSCLC)

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Session: Cancer Biology, Immunology, Microbiology, Drug Design

COPD is considered a risk factor for NSCLC and both diseases share cigarette smoke (CS) as a major risk factor. Their pathological background presents an aberrant immune system with an immune checkpoint dysregulation. Notably, the expression of PD-L1 on alveolar macrophages (AMs) is critical in regulating the immunological tolerance in NSCLC. Moreover, AMs are crucial mediators of lung immune responses with PD-L1 overexpression following CS exposure, which may lead to the persistence of inflammation and emphysema, typical hallmarks of COPD. However, the PD-L1 role in CS-associated lung diseases associated with NSCLC, such as COPD is still unclear. The PD-L1 levels were evaluated in COPD and NSCLC patients, and ever and never-smoker controls by using:

-cutting-edge digital spatial proteomic and transcriptomic profiling (Geomx) of formalin-fixed paraffin-embedded lung tissue sections;

-immunofluorescence staining of AMs in peripheral lungs.

Lungs from patients with mild COPD and NSCLC are characterized by a similar strong PD-L1 expression signature in bronchioles and functionally active AMs compared to patients with severe COPD and controls. Within all the COPD patients, PD-L1 levels were associated with the up-regulation of genes involved in tumor progression and downregulation of oncosuppressive genes, and correlated with the FEV1% predicted, indicating higher PD-L1 expression in the milder vs. more severe COPD stages. Our data pave the way for future studies focused on the mechanisms by which CS promotes tumorigenesis and COPD.

# Monocytic fluorescent NFκB GFP U937 cell response to endotoxins

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Session: Cancer biology, Immunology, Microbiology, Drug Design

U937 represents a human monocytic cell line that emulates primary monocytes in the immune response. It can be activated by lipopolysaccharides (LPS), also called endotoxins. Previously, we designed and successfully obtained U937 transfected with fluorescent NFκB GFP. In this study, we investigated the capacity of these transfected cells to react to LPS in terms of both inflammatory cytokine induction by ELISA and counting of fluorescent cells imaged through microarray machine. NFκB GFP U937 were differentiated for 48h with phorbol myristate acetate (PMA) into tissue-like macrophage (NFκB GFP U937-PMA). Soon after, these cells were stimulated with different concentrations of LPS (from 0 to 10,000 ng/mL) from Escherichia coli and Klebsiella pneumoniae for 24h. The cells were able to detect both LPS types as low as 1 ng/mL as shown by the production of the cytokines TNF-α, IL-6 or IL-8. The measurement of relative fluorescence unit confirmed the sensitivity at 1 ng/mL. However, the fluorescent spots observed under the microarray imaging machine and counting the number of spots gave the better sensitivity as low as 0.02 ng/mL LPS. Based on the feasibility of monocytic cell line, NFκB GFP U937 could be applied in developing an alternative cell-based assay for LPS detection.

# The innate immune memory of mast cells

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Session: Cancer biology, Immunology, Microbiology, Drug Design

This project will focus on examining the immune memory of a very important population of innate immune cells, the mast cells. Mast cells are highly secretory innate immune cells that, when stimulated, release a large number of preformed and de novo synthesized mediators (such as histamine, heparin, tryptase, chymase, cytokines, prostaglandins, leukotrienes). Mast cells respond to many endogenous (cytokines, neurotransmitters) and exogenous agents (IgE-antigen complexes, toxins, allergens, parasitic infections) and can also be involved in pathological inflammation such as allergies. Mast cells are very sensitive to tissue signals and therefore have tissue-specific homeostasis and immunomodulatory functions. Whether mast cells can develop memory, that is, regulate their function in response to previously experienced stimuli, remains largely unexplored. Published data have shown that rat peritoneal mast cells can respond to repeated stimulation, but "memory" features have not been evaluated. Recent data on mouse bone marrow-derived mast cells suggest that pre-stimulation with LPS induces tolerance to subsequent exposure to LPS. This project will use human mast cells differentiated in culture from CD34+ precursors to study the capacity of these cells to generate innate immune memory upon priming, the memory mechanisms, its personalized characteristics, and specificity/lack of specificity. The focus will be on the development of models and biomarkers for predicting human innate immune memory, with a view to using relevant methods for disease prevention (vaccines) and treatment.

# Design and synthesis of anticancer peptides functionalizing gold surface of photonic biochips: towards a new potential detection strategy of cxcr4-overexpressing circulating tumor cells

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Session: Cancer biology, Immunology, Microbiology, Drug Design

Point-of-Care (PoC) tests based on biomarkers, useful to monitor tumor diseases are required for advices in medicine. In this perspective, during my first year of PhD course I was involved in a project aimed at implementing PoC Test devices for the development of diagnostic strategies that allow to early and effectively identify circulating tumor cells (CT). In particular, the CXCR4 chemokine receptor, overexpressed in more than 20 different types of solid and hematological tumors, was considered as "hallmark" to be detected by applying photonic biochips previously functionalized with CXCR4-ligands. Specifically, peptide L10 Ac-Arg-Ala-[Dap-Arg-2Nal-His-Glu]-COOH, a potent and selective CXCR4 antagonist, was modified to identify a suitable position within the sequence for introducing a residue of L-Cys, which could be employed to anchor the peptide on an Au-functionalized solid. The described peptides were endowed with the following general structure: Ac-Cys-X-Arg-Ala-[Dap-Arg-2Nal-His-Glu]-COOH, where X represents an amphiphilic linker that can be modulated in composition and length. The peptides were obtained through an ultrasound-assisted Fmoc/tBu solid-phase peptide synthesis protocol. Subsequently, they were tested in vitro on their ability to bind CXCR4 by the research group of Dr. Scala at the “Istituto Nazionale Tumori” "Fondazione Pascale”. All peptides displayed high affinity towards CXCR4. In the future, the most promising candidates will be used to functionalize photonic biochips in order to obtain Poc tests, which in turn will be preliminary assessed for the early detection of solid tumors that overexpress the CXCR4 receptor by using CXCR4+/CXCR4- CHO cells.

# *L.rhamnosus IMC501*: Production of Biomass, EPS and Lactic Acid from simplified semi defined media and renewable waste resources

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Session: Cancer biology, Immunology, Microbiology, Drug design

*Lactobacillus rhamnosus IMC501* is a commercially available probiotic strain that demonstrated an improvement in intestinal well-being of adult subjects, and it was recently shown to strengthen the intestinal barrier and inhibit the adhesion and invasion of *S. typhimurium* and EIEC in the intestinal mucosa. Therefore, the first part of the work regarded the evaluation of an industrially applicable batch process for the production and recovery of viable biomass and a preliminary purification strategy for the released exopolysaccharide for potential biological applications. This allowed us to obtain $5*10^{10}$ cfu/g of viable cells after spray drying. *L. rhamnosus IMC501* was also shown to be able to grow on lignocellulosic material converting glucose into lactic acid (LA) with yields that varied from 0.38 and 0.97g/g. LA is a key platform chemical, with applications in different industrial sectors (e.g. food, chemicals, pharmaceuticals, biopolymers). The second part of the study focused on the production of LA from *L. rhamnosus* IMC 501 on grape stalks, a non-agricultural biomass, massively present worldwide, in the perspective of a biorefinery concept. A combination of a steam explosion pre-treatment with optimized two-step hydrolysis conditions, allowed to obtain a cellulose conversion efficiency of about 37% and a yield of LA of about 0.98±0.05 g/g in small scale 2L batch fermentation processes.

# The innate memory molecular mechanism of monocytes and macrophages

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Session: Cancer biology, Immunology, Microbiology, Drug Design

The innate immune system is classically characterized as being nonspecific, generally devoid of memory functions, and serving to provide a mechanism to rapidly respond to diverse pathogens. For a long time, it was thought that immunological memory was the specific characteristic of adaptive immunity. However, more recent research shows that innate cells (mainly monocytes and macrophages) have the ability to retain memory of prior exposure to microbes and other agents and become primed to elicit a heightened, broad-spectrum response to subsequent infection/challenge. This phenomenon is termed “innate immune memory” and is particularly crucial to the survival of organisms that lack adaptive immune system (i.e., invertebrates and plants) but is also functional in the complex immune system of vertebrates. Monocytes and macrophages had proven to have the capacity of innate memory. The memory response can be induced by the infection or treatment and persist for weeks to years. Vaccination of children with Bacillus Calmette-Guérin (BCG) not only provides targeted protection from infection with M. tuberculosis but it can also protect from other respiratory pathogens, which is a strong suggestion of the broad-spectrum protection provided by innate memory. Epigenetic modifications (including histone acetylation and DNA methylation) associated with prior pathogen exposure have been described as the molecular basis for innate memory. In this study, we will focus on the capacity of BCG, and live bacteria in general, to cross-talk with human monocytes and macrophages, in particular by examining the role of non-coding RNAs and transposons in the establishment and persistence of memory. This will help us to understand and design innate memory-reprogramming immunotherapies.

# Anti-tumor mechanism of natural mushroom polypeptide Gymnopeptide A

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Session: Cancer biology, Immunology, Microbiology, Drug Design

Natural products are useful tools for the research of biological mechanisms and drug discovery. According to rough calculations, as of 2008, about 80% of the small molecule anti-cancer drugs newly marketed worldwide were originated from natural products or their analogs. Therefore, the development of anti-tumor drugs with natural products as lead compounds has great application potential. Gymnopeptide A (GA), a newly discovered cyclopeptide from the mushroom Gymnopus fusipes, it has been considered as a promising lead compound for cancer treatment due to its excellent tumor cell growth inhibitory properties and sub-nanomolar potency. However, the mechanism remains unknown. To further investigate the anti-tumor activity of GA in vivo, we synthesized GA with hydrophobic surface to mitigate uncertainty resulting from contaminants. First, we demonstrated that GA could significantly inhibit the growth and metastasis of tumor cells at very low concentrations in a mouse tumor-bearing model. Further studies showed that GA exerted an anti-tumor effect in a CD8-dependent manner. Previous studies revealed the importance of cellular metabolism in T cell differentiation, suggesting that CD8+ phenotype depends on glycolysis and OXPHOS for its metabolic needs, where mitochondria play distinctive roles in T cell development and differentiation. Thus, we determined the effect of GA on mitochondria. Our results showed that GA could not only target mitochondria, but also altered the morphology of mitochondria in CD8+ cells by limiting mitochondrial fusion. Therefore, revealing how GA achieves its anti-tumor immune function by regulating mitochondria will be the focus of our next work, which will promote the further application of GA in cancer therapy.

# Session 2:

## Gene Regulation and Computational Biology

# The role of the maternal-effect gene *Padi6* in mouse female fertility, embryogenesis, and epigenetic reprogramming

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Session: Gene regulation and computational biology

*Padi6* is a maternal-effect gene with a crucial role in female fertility. Studies in mice and humans showed that *Padi6* is highly expressed in oocytes and early embryos, essential for oocyte cytoplasmic lattice formation, mitotic spindle assembly, and chromosome alignment. PADI6 takes part with other maternal-effect proteins to the Subcortical Maternal Complex (SCMC), playing a crucial role in reproductive outcome. Loss-of-function mutations affecting SCMC components have been identified in healthy women with fertility issues and/or offspring with imprinting defects. Knockout female mice for SCMC genes show infertility, characterized by 2-cell stage embryonic arrest. We investigated gene expression and epigenetic modifications, using a transgenic murine line carrying a hypomorphic missense variant in *Padi6* gene. We found that *Padi6* transcription was maintained in both heterozygous and homozygous female lines' ovaries, but PADI6 protein was not detected in the homozygous line, suggesting the mutation affects protein stability. Notably, our mutant line shows normal ovarian development and oocyte maturation, despite PADI6 absence. Analysis of mutant oocytes and embryos obtained by *in vitro* fertilization revealed mislocalization of the epifactors DNMT1 and UHRF1, suggesting DNA methylation involvement in the 2-cell block. Our study may help to better understand the *Padi6* implications in female fertility and embryonic development.

# Elucidation of the link between RNA maturation and neurodegeneration by a cell-specific transcriptomic analysis in C. elegans

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Session: Gene regulation and computational biology

Spinal muscular atrophy (SMA) is a neuromuscular disease that causes the specific loss of lower motor neurons (MNs) in affected patients. Mutations in the Smn1 gene cause ~95% of all SMA cases. Smn1 controls the assembly of small nuclear ribonucleoproteins (snRNPs), essential for pre-mRNA splicing, and is ubiquitously expressed in the body, thus it is not clear why MNs are especially sensitive to SMN depletion. We are exploring the molecular origins of the distinct sensitivity of neurons to loss of SMN1 in C. elegans models using a neuron-specific RNA-interference approach to selectively knock-down smn-1 in 19 GABAergic motor neurons or in 6 glutamatergic touch receptor neurons. To identify genes differentially expressed in different classes of neurons in these SMN models, worm dissociation and cell sorting processes were implemented. Single cell cultures were performed in suspensions. Cell sorting by FACS, RNA extraction and sequencing of sorted cells is ongoing. We will also identify differential protein interactions involved in neuron survival using TurboID enzyme. We expressed SMN-1 fused to TurboID in MNs and TRNs and specifically Biotin-tagged proteins will be purified using streptavidin-beads, and identified by mass-spectrometry.

# Session 3:

## Structure and Functions of Biomolecules

# Isolation, Characterization and Biological Action of Type-1 Ribosome-Inactivating Proteins from Seeds of *Atriplex hortensis* L.

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Session: Structure and Function of Biomolecules

The ribosome-inactivating proteins (RIPs) from plants are RNA N-glycosylases (EC 3.2.2.22) that depurinate a specific adenine located in the sarcin-ricin loop of the large rRNA, blocking protein synthesis (1). Two main groups of RIPs are known: type-1 RIPs are single-chain proteins (~30-kDa with a basic pI) that possess rRNA N-glycosylase activity, type-2 RIPs consist of an A-chain (~30-kDa) endowed with enzymatic activity, linked to a B-chain (~34-kDa) with lectin properties. RIPs have antipathogenic effect against viruses and fungi, in stress and senescence responses (2).

Most of plant RIPs have been found in angiosperm families (3), recently my research group has found that type-1 RIPs are present in several species belonging to the Chenopodiaceae family (i.e.: Chenopodium quinoa L. (4) and Salsola soda L. (5)). In this scenario, we decided to investigate the presence of type-1 RIPs in Atriplex hortensis L., a leafy vegetable from the Chenopodiaceae family, which has historically been consumed as spinach.

During the last year of my PhD, we: i) purified type-1 RIPs (hereafter hortensis) from the seeds of A. hortensis; ii) evaluated the biochemical and enzymatic properties of hortensis; and iii) tested their potential cytotoxicity against primary cells NULU and ZAR from patient gliomas.

# Structural and functional characterization of the recombinant FVII of coagulation

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Session: Structure and Function of Biomolecules

Administration of Factor VII (FVII) is a first-line therapy for treating patients with haemophilia and high-titre inhibitors. Recombinant FVII (rFVII) is a post-translationally modified protein that must be produced in eukaryotic expression systems. During purification, rFVII self-converts into its active form rFVIIa, the product administered, not without safety concerns, to patients.

To explore alternative, cost-effective purification methods that prevent rFVII self-activation, we have designed and synthesized a non-inhibitory small synthetic ligand (F7-SPL) for use in affinity chromatography. We have produced some lots of rFVII in HEK293 cells and obtained F7-SPL by solid phase synthesis. F7-SPL binding to rFVII has been verified using fluorescence quenching and Biolayer Interferometry. F7-SPL immobilized on Sepharose 4B is able to capture rFVII from crude cell supernatants without protein self-activation showing its potential as a practical alternative to existing methodologies.

Using rFVII and a bacterial transglutaminase we are investigating the involvement of the protein in the final stages of the coagulation cascade. In preliminary experiments, we have observed that rFVII undergoes self-conjugation upon exposure to a transglutaminase, leading to the formation of covalent complexes of well defined molecular weight, corresponding to protein dimers and trimers. These observations could be relevant to understanding the final stages of the extrinsic coagulation pathway.

# Development and application of new edible active formulations to preserve beneficial biomolecules and reduce fruit losses.

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Session: Structure and Function of Biomolecules

Fruit and vegetables are an essential part of any balanced diet because of their nutritional value. In general, fruit and vegetables are rich in vitamins and minerals that provide a wide range of health benefits. These fresh foods have a relatively short post-harvest life due to physiological and biochemical deterioration, resulting in significant food losses. Demand for ready-to-eat foods has continued to grow in developed countries, and fresh-cut fruits and vegetables are following a similar trend. In recent decades, a significant increase in outbreaks of foodborne illness, mainly caused by Salmonella and Escherichia coli, has been linked to the use of fresh and minimally processed fruit. Therefore, the aim of my PhD project is to develop and test some novel eco-friendly edible formulations to preserve the important and health-promoting components of fruit while extending shelf-life and reducing food losses.

So far, the beneficial effects of a bi-layer coating containing carboxymethylcellulose, sodium alginate, citric acid and oxalic acid have been tested on minimally processed pears and apples, as well as on sweet cherries; in all cases, the coating has been shown to slow down the degradation and browning reactions of the fruit and to extend their shelf-life. In addition, a new coating consisting of chitosan and xanthan gum has been tested on ready-to-eat pears and the results show that the treatment slows down the senescence reactions of the fruit, controls lipid peroxidation and enzymatic browning caused by cutting. The final objective of this project will be to formulate a new nanomaterial capable of preserving fruit also from a microbiological point of view without varying the organoleptic characteristics of the fruit.

# Organic molecules with biological applications: ATP and GTP nucleopeptide binders and antimicrobial peptides

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Session: Structure and Function of Biomolecules

In the last year of my research, I focused on the synthesis of two distinct organic molecules with significant biological applications: nucleopeptides and antimicrobial peptides.

Nucleopeptides represent a novel class of biomolecules where a peptide backbone holds nucleobases in the side chains. The aim was to synthesize a set of nucleopeptide sequences as new ATP and GTP binders, as well as forming hydrogels. These nucleopeptides have diverse sequences but share common moieties: nucleobases, functionalized, cationic and hydrophobic amino acids. Preliminary CD studies revealed that T and C containing nucleopeptides exhibited binding affinity for ATP and GTP, respectively. Currently, NMR investigations are in progress to gain further insights into their secondary structure and conformational changes during their target recognition. Remarkably, the nucleopeptides, except for those bearing an acetyl group replacing the nucleobase in side chains, also exhibited gel-forming ability, highlighting the involvement of the nucleobase in sol-gel transition.

Considering the urgent need for alternative antibiotic molecules to address the issue of multidrug resistance of bacteria, my late study focused on the synthesis and biological evaluation of two series of new cyclic antimicrobial peptides, based on the β-hairpin structure. Starting from the dipeptide template pro-Pro, the first family was designed with the scaffold (YYY)-pro-Pro-(XXXX)-Asn, while the second one featured (XXX)-pro-Pro-(YYYY)-Asn (X= hydrophobic amino acids, Y= hydrophilic amino acids). During my research experience in Barcelona, I performed biological assays to test their antimicrobial activity, cytotoxicity, and their ability to interact with liposomes. The first family, particularly Lys-Lys-Lys-pro-Pro-Trp-Leu-Phe-Trp-Asn, displayed promising antibiotic activity, selectivity against Gram-negative bacteria and low toxicity levels.

# Role of Allelopathy in the Success of Selected Invasive Plant Species in the Mediterranean Basin and Possible Applications

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Session: Structure and Function of Biomolecules

Plants produce specialized metabolites for stress response, interactions, defense, and competition. Ailanthus altissima and Robinia pseudoacacia are highly invasive species in the Mediterranean basin that harm the biodiversity and economy. They produce allelochemicals that exert inhibitory effects on native species which benefits their invasiveness. Bioherbicides from the secondary metabolites of phytotoxic plants are alternatives to synthetic counterparts that damage environment and health. We used NMR-based metabolomics to study the phytotoxic effect of A. altissima and R. pseudoacacia on Aegilops geniculata and Lactuca sativa. This approach offers simultaneous qualitative and quantitative analysis of crude extracts which require minimal sample preparation and allow high sample throughput. In post-emergence bioassay, we determined that the extracts of A. altissima and R. pseudoacacia caused morphological changes (root necrosis, leaf chlorosis and wilting) to the receiving plants. NMR-based metabolomics revealed differences in metabolites present between treatments and control. The identity of the bioactive compounds was also hypothesized and ad-hoc chromatographic procedures were designed to obtain the pure putatively bioactive compounds in which their bioactivity was also determined. This study allowed to identify the potential allelochemicals, their effects on receiving plants and suitability of NMR-based metabolomics as a tool for phytotoxic studies and identifying the bioactive compounds.

# Plant specialized metabolites for treatment of Leukemia

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Session: Structure and Function of Biomolecules

Throughout the years, there have been great advancements in cancer medicine with an effort to treat leukemia. Regardless of these several immunotherapies, leukemia still remains the leading cause of cancer related deaths in the world. Some of these therapies are associated with adverse effects such as cardiac dysfunctions,[1] as well as surfacing of drug resistance [2]. Therefore, continuous search for new compounds remains crucial. Plant specialized metabolites and their derivatives provide key scaffolds for drug development. With the help of NMR based metabolomics, this project aims at identifying compounds from Botswana plants and further evaluating their anti-leukemic activity. Through ethnopharmacological knowledge, nine plant species from different families were identified and collected in Botswana. The plants extracts were subjected to NMR analysis for their chemical characterization. Partial purification of the plants' crude extracts using amberlite XAD-4 and XAD-7 was done. The effects of the obtained fractions on cell viability, cell cycle and cell death in human U937 leukemia cell line were explored using flow cytometry-based assays. Further experiments are currently ongoing to obtain pure compounds, including their structures, from the most active extracts.

# Isolation, Characterization and Biological Action of an atyical Ribotoxin-like protein from fruiting bodies of *Armillaria mellea*

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Session: Structure and Function of Biomolecules

*Armillaria mellea* [(Vahl) P. Kumm, 1871], known as honey fungus (chiodini in Italian), is an edible basidiomycete fungus from genus *Armillaria* (1). It causes Armillaria root rot in many plant species, resulting the production of mushrooms around the base of infected trees (2). The mushroom is edible, but some people may be intolerant to them (3). *A. mellea* is widely distributed in temperate regions of the Northern Hemisphere and typically grows on broadleaf trees but can also be found in open areas or around other living and dead wood.

Here, an atypical ribotoxin-like protein [RL-P; (4)] from the edible mushroom *A. mellea* has been purified and characterised. This RL-P, named melleatin, is a monomeric protein (~15-kDa) exhibiting specific ribonucleolytic activity. During the second year of my PhD, I: i) purified melleatin at homogeneity from fruiting bodies of *A. mellea*; ii) evaluated the biochemical and enzymatic properties of melleatin; and iii) tested its possible cytotoxic effects against some human tumour cell lines. Finally, to obtain structural information, when analysed by SDS-PAGE and stained for sugars, melleatin appeared to be glycosylated, while by Edman degradation the N-terminal amino acid sequence was determined up to 25 residues (1-AGPEFELXYR TYPQSSENIX YSXFD-25).

# The activation mechanism of the Melatonin MT1 receptor

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Session: Structure and Function of Biomolecules

Melatonin is a neurohormone secreted by the pineal gland in the brain. It is involved in different physiological functions, including sleep and mood regulation, through binding two G-protein coupled receptors named MT1 and MT2 (1-4). Preclinical studies using knockout animals for both MT1 or MT2 receptors showed that these receptors can mediate complementary and opposite physiological functions (4). Here, to understand how Melatonin interacts with the MT1 receptor underlying the molecular determinants involved in the activation mechanism, we investigated the MT1 recognition mechanism by Melatonin using Nuclear Magnetic Resonance (NMR), Molecular Modelling and Molecular Docking methodologies. Firstly, in order to identify the binding portions of Melatonin, we compared a series of NMR STD (Saturation Transfer Difference) and T1ρ-based experiments, acquired in the presence and absence of cell membrane containing the MT1 receptor. Then, we determined a three-dimensional model of the Melatonin/MT1 complex by combining computational methodologies with NMR data. Overall, the data demonstrate that the MT1 binding by Melatonin is driven by different regions of the ligand inducing significant conformational changes on the cytoplasmatic side of the receptor. Finally, the three-dimensional docking model of the Melatonin/MT1 complex indicates that upon MT1 activation the TM6 shows a structural displacement outward from the TM3.

# Study of eukaryotic and prokaryotic zinc-finger proteins

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Session: Structure and Function of Biomolecules

ZNF639 is a transcription factors identified in esophageal squamous cell carcinomas. It contains 9 C2H2 zinc-finger domains at the C-terminus, while the N-terminal region does not share similarities with the already known domains. ZNF639 has a role in cancer and interacts with the POZ-zinc finger protein ZBTB2, which is a partner of NuRD and is involved in cancer development. We obtained the interactome map of ZNF639 that confirms the interaction with ZBTB2 and shows many partners involved in chromatin remodelling, suggesting a role of ZNF639 in this function. We performed ChIP-seq of ZNF639 and ZBTB2 to uncover a DNA consensus sequence and genomic loci where the two proteins recruit chromatin remodelling multiprotein complexes. We are analysing ChIP-seq results.

Zinc-finger proteins are also present in prokaryotes and MucR protein is one of the best studied members of this family. MucR from S. meliloti contains a typical prokaryotic zinc-finger domain at the C-terminus. To perform structural studies of the zinc-finger domain contained in S. meliloti MucR, we tried to purify two N-terminal deletions of this protein (MucR51-143, MucR59-143). In both cases, MucR deletion mutants turned out to be degraded after gel filtration. New protocols to purify deletion mutants and to protect them from degradation are going to be tested.

# Specialized metabolites from natural sources as lead compounds to fight against emerging diseases

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Session: Structure and Function of Biomolecules

Nowadays the main emergencies concern the fight against tumours and antibiotic resistance and plants and fungus synthetize different type of specialized metabolites that also have both anticancer and antimicrobial activities and. In this context we investigated a plant pathogenic fungus, Drechslera gigantea, that causes eye spot disease on many host plants. This fungus produces specialized metabolites, ophiobolins, that have demonstrated antitumor and antibiotic activity. The first member of this group is Ophiobolin A, at first isolated by Drechslera orizae but the best source of it is through the fermentation of fungal strains D. gigantea. These metabolites are a group of sesterterpenes that were found to have a significant activity against apoptosis-resistant glioblastoma cells through the induction of a non-apoptotic cell death and it could be an interesting strategy to fight against this type of cancer. We fractionated the extract using different extracting and chromatographic technique, like SiO2 column chromatography, Sephadex-LH20 and HPLC RP-18. At the end, the different fraction obtained was analysed with 1H nuclear magnetic resonance spectroscopy, a technique used to identify the structures of compounds. During our work we obtained different ophiobolins, like ophiobolin A, 3-anidro-6-epiophiobolin A, ophiobolin I and biopolarolide A. Isolated compounds and any new compounds will be tested for antiproliferative and antimicrobial activity.

# MucR from *S. meliloti*: new insight into its DNA targets and its ability to oligomerize

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Session: Structure and Function of Biomolecules

The MucR/Ros protein family comprises zinc-finger transcriptional regulators with an N-terminal oligomerization domain and a C-terminal DNA-binding domain. Proteins of this family play important roles in regulating genes involved in infectious or symbiotic relationships with eukaryotic hosts.
We performed the first study by Mass Spectrometry, Light Scattering (LS) and EMSA of native MucR isolated from plant symbiont *S. meliloti*. Our results show that the native MucR has an oligomeric structure and can bind the DNA region upstream *ndvA* gene. These results show that the native protein is purified in its oligomeric active fold. Moreover, the interaction with DNA region upstream *ndvA* indicates this gene is a new MucR target.
Although the *S. meliloti* MucR DNA-binding activity was already investigated, we performed a detailed characterization of DNA targets. Analyses of sequences recognized by MucR in the promoter of *rem* target gene show that this protein recognizes AT-rich sequences and does not require a consensus sequence to bind DNA. Furthermore, we investigated the dependence of the MucR DNA-binding upon the length of DNA targets.
By mass spectrometry, we also identified in *S. meliloti* a putative new MucR/Ros family member showing a conserved primary structure compared to MucR. Secondary structure prediction indicates that this new MucR homolog contains fundamental structural elements to oligomerize and bind DNA. Low-specificity DNA-binding and oligomerization are characteristics of Histone-like Nucleoid Structuring proteins. These functional and structural similarities suggest MucR/Ros family potentially represents nucleoid-associated protein family in α-proteobacteria.

**Insights into the identification of novel galectin inhibitors**

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Session: Structure and Function of Biomolecules

Galectins are a family of emerging proteins involved in several cellular processes in both physiological and pathological conditions. These diffuse proteins are lectins that naturally interact with β-galactosides on glycoconjugates, inside and outside cells, through their Carbohydrate Recognition Domain (CRD). Based on their structure and oligomeric state, galectins are classified into three classes: Prototype, Tandem-Repeat and Chimera type. The human prototype Galectin-1 (hGal-1) and the human chimera type Galectin-3 (hGal-3) represent the most studied galectins due to their association with diseases such as inflammation, heart failure, neurodegeneration, metabolic disorders, tumors and many others. For this reason, in the last decade many researchers put efforts searching for new selective galectin inhibitors, both synthetic and natural-derived. Of course, a depth knowledge of the structure is mandatory to identify new inhibitors. To this end, we focused our attention on optimizing the expression and purification conditions of recombinant hGal-1 and hGal-3 and on studying the fold and the oligomeric state of both proteins using circular dichroism and light scattering techniques. Furthermore, a natural-derived extract, obtained from the seeds of Phaseolus coccineus through a chemical extraction process, was tasted for its biological effect on healthy and tumor cells with the future aim of understanding whether the observed effect is associated with the interaction with galectins.

# Structural characterization of novel vaccine antigens

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Session: Structure and Function of Biomolecules

The ESKAPEE group, comprising Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, Enterobacter sp. and Escherichia coli, poses a significant threat due to their rapid adaptation to antimicrobial agents. In the pursuit of innovative strategies to combat these bacteria, this research focuses on the structural, biophysical, and biochemical properties of three essential proteins within E. faecium—AdcA, SagA, and PpiC.

Utilizing a combination of computational tools, protein engineering techniques, and biophysical analyses, our investigation identified immunogenic regions within AdcA. This breakthrough led to the development of Sc(EH)3, a highly stable, and multi-antigen-presenting protein. Additionally, we explored an alternative delivery method by constructing glutamine-rich fibrils linked to this epitope. Sc(EH)3 was characterized as a monomeric protein with remarkable stability. Immunization trials using Sc(EH)3 as the antigen revealed its potential to induce selective and protective immunogenic responses against multiple bacterial species, as confirmed through opsonophagocytic killing and inhibition assays. Simultaneously, our research identified the interaction sites of SagA with monoclonal antibodies, leading to the development of a more robust antigen variant. Furthermore, we delved into the biophysical properties of PpiC, elucidating its native dimeric form and producing a monomeric variant to facilitate future structural characterizations. This multidisciplinary research underscores the importance of innovative approaches in vaccinology and presents promising avenues for combating ESKAPE bacteria through novel antigens and biophysical insights.

# Biocompatible polimers for human health

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Session: Structure and function of biomolecules

During the first year of my PhD project, we have set up the experimental conditions for the production and characterisation of bacterial cellulose [1-3], and in parallel we have also designed and produced enhanced antigenic protein fragments of the molecular HtpG chaperone [4,5] Based on these results, during this second year, I have focused the research activities on the structural characterization of this protein antigen to get a full knowledge of its structure and function. To this aim, we have set up different crystallization trials to achieve the best condition to obtain protein crystals, on both nucleotide-free and nucleotide-ligated states (AMPnP and ADP). Best crystals were obtained in nucleotide-ligated state and tested using in-house X-ray diffractometer [1, oral presentation]. Moreover, we are also are trying to investigate the conformational states of HtpG using Small-angle X-ray scattering (SAXS) at European Synchrotron Radiation Facility (ESRF, Grenoble). In collaboration with the company Knowledge for Business (K4B), I also used biophysical techniques to better characterize our produced biocellulose.

# Understanding the conformational dynamics of the early stages of Human Prion Amyloid Fibril formation using Chemical Exchange Saturation Transfer

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Session: Structure and Function of Biomolecules

The misfolding process of the prion protein is the cause of a unique group of rare and fatal neurodegenerative diseases. The conformational transition of the human prion protein from α–helical ($PrP^C$) to β–sheet-rich ($PrP^{Sc}$) structure is believed to be the crucial event in prion pathogenesis. Our prior research buttressed that the truncated HuPrP(90-231) misfolding pathway occurs through the formation of a β-enriched intermediate state (β-PrPI) involved in the initial stages of $PrP^C$ fibrillation of truncated HuPrP(90-231). However, there is no direct evidence linking the accumulation of β-PrPI to the fibril aggregation process and the underlying mechanism remains to be elucidated. Nuclear Magnetic Resonance (NMR) techniques have the ability to quantitatively probe exchange dynamics between interconverting states. We have shown here that Chemical Exchange Saturation Transfer (CEST) NMR experiments provide a sensitive approach for obtaining detailed kinetic and thermodynamic description of human prion conformational equilibria involving transient oligomeric species that drive amyloid fibril assembly mechanism. $^{15}N$ CEST data indicate that in the absence of oligomeric species, at low temperature (15°C), the native monomeric state rapidly interconverts with a minor conformation. On the contrary, in the presence of β-PrPI-oligomers we have indicated accurate excited-state chemical shift values precisely in the residues surrounding the native β-sheets and the first α-helix, which are involved in the formation of transient oligomeric species in turn govern the amyloid assembly mechanism. To get insight into the motion of the backbone of HuPrP(90-231) in solution at low temperature (15°C) $^{15}N$-$R_2$ relaxation rates were measured and the $^{15}N$ CEST NMR time window in which exchange parameters can be accurately quantified was validated.

# Development of a technological platform for the identification of modulators of the PED-PLD1 interaction

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Session: Structure and Function of Biomolecules

PED/PEA15 (Phosphoprotein Enriched in Diabetes/Phosphoprotein Enriched in Astrocytic) is 130 residues in length protein which is expressed ubiquitously in several human tissues. PED/PEA15 is known to modulate the function of several effectors involved in different cellular pathways. Recent studies demonstrated a correlation between insulin-resistance in individuals affected by type II diabetes and the PED/PEA15 overexpression in tissues. Upstream this resistance is the interactions between PED/PEA15 and phospholipase 1 (PLD1). Several studies aimed at understanding PED/PEA15 structure and the PED-PLD1 binding site and interaction were conducted. The authors of a recent study, Farina et al. (2021), screened a library of small molecular fragments with NMR analysis on cell lysate, and they identified a first promising lead compound binding in the same PED-PLD1 interaction site. Encouraged by these results, we conducted a deep in-silico analysis of the PED-lead compound interaction. We performed a cavity mapping analysis with Induced Fit docking, and the best scored pose from Induced Fit docking was subjected to perform equilibration Molecular Dynamic analysis of a 200 ns run in triplicate. These analyses showed that the lead compound binds the PED-PLD1 interaction residues with binding-induced conformational changes.

# Identification of sex-specific therapeutic strategies in aging and dementia

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Session: Structure and Function of Biomolecules

Aging leads to the accumulation of misfolded proteins such as β-amyloid and α-synuclein which are believed to underly the onset of neurodegenerative disorders. Most of the treatments for brain disorders come from pre-clinical evidence in males, although women are more vulnerable than men to Alzheimer' disease (AD). However, the mechanisms underlying these sex-differences are not known. To date, two types of treatments are highly considered to treat age-related cognitive decline: one pharmacological and one behavioral. The most widely used pharmacological treatments studied are autophagy enhancers, like Spermidine (SPD), a polyamine that can promote autophagy and lysosomal biogenesis through stimulation of the EB transcription factor (TFEB). Another therapeutic approach is voluntary exercise (ET). We studied a mouse model of aging (CD1) and AD (TG2576) and we evaluated how memory capacity (MC) is affected by aging underlining sex-differences. MC has been shown to have a prognostic value in the development of age-related memory decline and condition such as Mild Cognitive Impairment (MCI). We tested the two therapeutic strategies, and we found that they differently respond to the precognitive effects of SPD. This study suggests the need to understand how the role of biological sex regulates the efficacy of anti-dementia treatments.

# Novel therapeutic tools preventing the neurotoxic Aβ/PrPC interaction

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Session: Structure and Function of Biomolecules

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases known by the formation of amyloid plaques. Prion protein (PrPC) is a high-affinity membrane receptor for Aβ in oligomeric and fibrillar states. The misfolding and aggregation of PrPC to scrapie PrPSc is associated with a number of fatal neurodegenerative disorders defined as transmissible spongiform encephalopathies (TSEs). Intermediate conformations forming during the conversion of PrPC into PrPSc are key drivers of the misfolding process. Recently, NMR structural and dynamical data showed that the inter-domain coupling is the key mechanism in tuning long-range μs-ms conformational dynamics that in turn regulate the folding process avoiding PrPC misfolded states involved in the PrPC fibrillation. Growing experimental studies indicate PrPC as a toxic acceptor of Aβ(O). Yet, several pathological evidence indicates that PrPC deposits often accompany Aβ plaques in AD. In this scenario, the aim of this research is to develop a novel class of neuroprotective peptides able to prevent Aβ fibrillogenesis inhibiting the neurotoxic Aβ(O)/PrPC mechanism. To start, we undertook a NMR structural characterization of a series of trehalose-conjugated peptides (TCPs) able to inhibit the early stage of Aβ self-assembly. The collected results will allow us to understand the molecular mechanism by which TCPs are able to inhibit Aβ aggregation.

# Natural products in drugs discovery: extraction methods, NMR characterization and evaluation of their potential antimicrobial properties.

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Session: Structure and Function of Biomolecules

Plants are a proven source of pharmaceutical drugs (1). Nowadays, many efforts are looking into the extraction of plant material with solvents, toward isolating and identifying the specific compounds responsible for proven biological activities. Various aspects of plant extraction should be taken in consideration: this work proposes the study of two different family of plants, taking in account plant material selection and solvent system selection. Myrtaceae Juiss is known to be an everlasting source of phloroglucinols derivates, studied for their structural features and biological properties (2). From the bioactive dichloromethane extract of M. cisplatensis, three cinnamoylated alkylphloroglucinol glucosides, structurally related to Myrtus communis analogues (3), have been isolated for the first time (4), along with known coumarin derivatives, tricyclic sesquiterpene, pentacyclic triterpenes. The same way, tetronic acid derivative was isolated from P. friedrichsthalianum and characterized by 2D NMR (HSQC, COSY, H2BC). Agavaceae plants have been used since ancient times, showing a wide range of biological activities. Steroidal saponins have been described as the most abundant compounds in these species (5), showing also antimicrobial properties. The optimization of an extraction and isolation method of leaves of A. bracteosa is applyed to get pure saponins with lower purification steps and using green solvents. Box-Behnken experiment thourgh ultrasound-assisted extraction was necessary, using five variables (percentage of ethanol in the solvent, temperature, ratio "sample mass/solvent volume", potency and cycle). Two answers were obtained: the concentration of total saponins and the concentration of cantalasaponin-1, detected by UPLC-MSE analysis. Finally, this method was applyed to isolate other saponins present in the extract, whose characterization was reached throught HMAI method. This latter has developed for the identification of the most representative aglycones of Agave genus throught the experiments of 1H-NMR and HMBC.

# Central glutamatergic dysfunction in spinal muscular atrophy

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Session: Structure and Function of Biomolecules

Glutamatergic system abnormality is an emerging feature of different neuromuscular diseases. Particularly, by an HPLC approach, levels of excitatory amino acids involved in the glutamatergic signalling have been found altered in the cerebrospinal fluid (CSF) of spinal muscular atrophy (SMA) patients. Accordingly with this scenario, we determined D-/L- excitatory amino acid levels in the central nervous system and in peripheral tissues of a SMA preclinical model (i.e., SMNΔ7 mice) at both pre-symptomatic (post-natal day 3) and symptomatic stage (post-natal day 11). Remarkably, our results indicate that symptomatic mice exhibit a neurochemical signature similar to that found in the CSF of SMA patients, with the increase of D-serine in both brain and spinal cord. On the other hand, pre-symptomatic SMNΔ7 littermates shown a decrease of L-glutamate, glycine, and the D-serine precursor, L-serine only in the spinal cord, suggesting that the reduction of glutamatergic signalling may have a role in the degeneration of motor neurons prior to the disease manifestation.

# D-Aspartate metabolism in autism spectrum disorder

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Session: Structure and Function of Biomolecules

Autism spectrum disorder (ASD) is a neurodevelopmental condition described by altered social interaction, compromised communication, repetitive behaviors and comorbid features.

Recent studies suggest a potential involvement of D-aspartate (D-Asp) in ASD. D-Asp is an atypical amino acid acting as a co-agonist for NMDARs and mGluR5. It is abundant in the embryonic mammalian brain and decreases after birth, in concomitance with the expression of D-aspartate oxidase (DDO), the enzyme that catalyzes D-Asp degradation.

Evidence showed a striking increase of D-Asp content in the prefrontal cortex, hippocampus and serum of the ASD mouse model, BTBR. Accordingly, a reduction of Ddo mRNA levels was found in the same brain regions.

Another study reported brain abnormalities and social recognition memory impairment in a mouse model characterized by Ddo overexpression and D-Asp depletion.

Considering this evidence, we aim to study D-Asp metabolism in different genetic and pharmacological animal models of ASD, including Fmr1□ -exon 8, valproate- and lipopolysaccharide-treated rats.

At present, we are analyzing D-Asp levels in the serum and different brain regions, such as prefrontal cortex, nucleus accumbens, hippocampus, striatum at adolescence and adulthood (35 and 75 postnatal days) by an HPLC approach.

# Characterization of protein molecular interactions with metal ions or cyclodextrins by NMR, CD and UV spectroscopies

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Session: Structure and Function of Biomolecules

Molecular interactions are attractive or repulsive forces that occur between molecules or non-bonded atoms. They are essential in numerous processes, including protein folding and formation of molecular complexes. To better understand the involvement of molecular interactions in these processes, I have focused my investigation on two distinct systems: a) zinc finger domain / different metal ions; pentapeptides / cyclodextrins. In the first case, I analyzed how the change in metal coordinating residues could affect the stability of protein structure and folding mechanism. In particular, in this year, I studied Ros87_C27D, a mutant of the prokaryotic zinc finger, to understand the role of aspartate in the coordination sphere when it replaces one of the coordinating cysteine in the interaction with metal ion. The study of the second system is aimed at the development of methods to improve the stability and solubility of biomolecules by forming β-CD inclusion complexes. Here, we characterized the inclusion properties of five designed pentapeptides, bearing the aromatic amino acid Tyr in different position in the sequence. A combined NMR and Molecular Docking approach will unveil the molecular determinants of these interactions.

**Antibacterial and Antiviral Activities of Selected Nigerian Plants Against Clinically Important Human Pathogens**

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Session: Structure and Function of Biomolecules

Infections by microbes such as viruses and bacteria can cause debilitating illnesses in both humans and animals. These infections remain a source of concern to humans due to emergence and spread of resistance, resurgence of infections, evolution of new strains of disease-causing agents amongst others [1]. Hence, the need for novel therapeutic approaches towards the development of new pharmaceuticals or some potential source of novel drugs. Natural products, especially from plants, are known for their medicinal properties [2]. Commonly used medicinal plants of our community could profer a solution to this problem by being an excellent source of drugs.

In view of this, the aim of this project with the help of ethnopharmacological knowledge, is to evaluate the antibacterial and antiviral potential of medicinal plants from Nigeria. In particular *Nauclea latifolia, Andrographis paniculata, Azadirachta indica and Vernonia amygdalina* have been selected. These plants have a common use in the treatment of skin diseases, dysentery and diarrhea, malaria, cough, dental infections, herpes and diabetes [3,4]. The method employed involves the extraction of plant materials using solvents at increasing polarity: n-hexane, chloroform, and methanol. A bioguided approach through antibacterial/antiviral assays will allow the selection of the most promising extracts while 2D NMR analysis will be performed in order to identify the compounds potentially responsible for the recorded bioactivity.

# Innovative mass spectrometry-based proximity labeling method for unraveling the Macrophage migration inhibition factor (MIF) interactome

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Session: Structure and Function of Biomolecules

The proximity-dependent Biotin Identification (BioID2) approach, coupled with high-resolution tandem mass spectrometry, represents an elegant strategy for mapping local protein interactomes. This methodology provides a useful tool to explore the interaction network of a protein of interest, by expressing the bait as a fusion protein with a mutant form of the biotin ligase enzyme BirA (BirA). Following biotin incubation, proximal endogenous proteins are biotinylated by BirA, and streptavidin-purified biotinylated proteins are then identified by MS/MS. The aim of this project is to identify the interactome of MIF. U937 cells were transfected with plasmids expressing the BioID2-MIF fusion protein. Biotinylation of MIF proximal proteins was induced by biotin supplementation and potential interactors were isolated using streptavidin magnetic beads. By nano LC- high-resolution tandem MS, a selection of interactors was derived after applying stringent criteria for data filtering including the absence in BioID2 only (control), the number of matching peptides in replicate injections, and high confidence scores at both peptide and protein levels. Interestingly, among the interactors, a subset of proteins involved in ATP-dependent protein folding in the endoplasmic reticulum unfolded protein response (UPR) and intramolecular oxidoreductase the activity was identified using bioinformatics software tools (Cytoscape and Metascape). We further validated the direct interaction of MIF with GRP78, an ER resident chaperone, by Bio-layer interferometry (BLI) and by co-localization experiments. In vitro, assays of MIF chaperone activity were also investigated by measuring the anti-aggregation capability on different model protein substrates. These results provide novel insights into the local interactome of the MIF and its possible role as a molecular chaperone in stress-related stimuli in the cell.

# Microfluidic methods for the design of high-performance nanoparticles as drug delivery systems

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Session: Structure and Function of Biomolecules

Biopolymer-based nanoparticles (NPs) are widely employed as biomedical devices for controlled drug delivery and release. These systems can encapsulate and protect drugs, increasing their stability and delivery through biological systems until the pathological district is reached. An appropriate design of the physicochemical properties allows NPs to overcome biological barriers and recognize the damaged target tissue (active targeting). Thus, the improvement of these properties results in an enhanced therapeutic effect by reducing cytotoxicity and unwanted side effects. The traditional fabrication bulk methods are simple to perform, but do not allow control over the physicochemical properties of NPs. Contrarily, the modern microfluidic techniques ensure precise control of NPs properties such as size, size distribution and morphology, resulting into higher batch-to-batch reproducibility, drug encapsulation efficiency and yield. In this context, the aim of this work was the development of hyaluronic acid (HA)-based biodegradable NPs for active tumor targeting. Indeed, HA is well-known for its selective tropism towards CD44 receptor overexpressed by a plethora of tumor cells. In particular, design and optimization of fabrication strategy by means of microfluidic techniques was carried out.

# Unmasking Viral RNA: targeting viral RNA capping machinery to tackle COVID-19 and future CoV emergencies

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Session: Structure and Function of Biomolecules

SARS-CoV-2 is a member of the Betacoronavirus family along with SARS-CoV-1 and MERS-CoV. These viruses have similar genome and replication strategies but differ in their pathogenicity for humans. The Replication-transcription complex(RTCs) is one of the vital machinery that enables the virus to recruit diverse proteins to maintain its life cycle. The nonstructural protein 10 is the central player in the RTCs. Here, contributing to the development of new therapeutic tools, we aimed to design, synthesize, characterize and optimize peptide and peptidomimetic therapeutics (PPTs) capable of inhibiting the interaction of nsp10 with its partners' proteins nsp14 and/or nsp16. Utilizing computational alanine scanning method we performed the hotspot calculations or binding mutation energy on the interface and analyzed in detail NSP10/NSP16 and NSP10/NSP14 complexes. A cluster analysis of the available PDB structures of NSP10/NSP14 and NSP10/NSP16 complexes allowed us to select the structures based on the structural and conformational changes. Furthermore, to rationalise the design of the peptides based on the hotspot results we did also a detailed structural characterization including identification of conserved protein domain features/sites on NSP10 across corona viruses, disorder prediction, linear motive search and secondary structure prediction. The design of PPTs and the NSP10 heterologous expression is in progress.

# Microbial and human lactonases for the control of virulence factors in pathogenic bacteria

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Session: Structure and Function of Biomolecules

The emergence of multi-drug resistant (MDR) pathogenic bacteria has been a critical concern for public health in the last decades. Biofilm communities could represent a reservoir of MDR bacteria, one of the strategies to counteract MDR pathogen infections is to degrade the quorum sensing (QS) signal by using lactonases.

We are studying the Phosphotriesterase-Like Lactonases (PLLs), these enzymes were studied as phosphotriesterases at first, and subsequently it was discovered that their primary activity was toward lactones.

We focused on a AhlA, a PLL from a mesophilic bacterium *Rhodococcus erythropolis*, that was obtained from a synthetic his-tagged gene, expressed, purified, and further characterized. The enzyme shows: high thermophilicity and thermostability, a long shelf life at 4°C, and stability under oxidizing conditions. His-AhlA is a proficient quorum quenching enzyme, able to hydrolize acyl-homoserine lactones, 3oxo-C12-HSL and C4-HSL, and to inhibit the formation of *Pseudomonas aeruginosa* (PAO1) biofilm.

To improve the effect on PAO1 biofilm formation, we developed an enzymatic formulation consisting of three enzymes: human rPON2, microbial his-AhlA and archaeal SsoPox 4Mut. The formulation was tested in wound healing assays on immortalized HeLa cells in vitro. The positive results were encouraging, achieving a significant reduction in biofilm formation and wound closure.

# Characterization of FNIII domain of Axl protein by NMR

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Session: Structure and Function of Biomolecules

Axl receptor tyrosine kinase (RTK) and its ligand, growth arrest-specific protein 6 (Gas6), are associated with the development and progression of various malignancies and autoimmune disorders. Axl consists of two immunoglobulin-like(Ig-like) motifs and two fibronectin type III (FNIII) repeats linked, through the transmembrane domain, to a highly conserved cytoplasmic domain giving a crucial role in kinase domain activity,and discriminates it from its homologs. To date, there is a paucity of structural information regarding Axl as only a partial characterization has been done through high-resolution X-ray crystallography. Furthermore, although the NMR structure of the related Mer-FNIII domain is available in PDB, no information on FNIII motifs (AXL extracellular domains) is available. In this study, we used NMR spectroscopy and Alpha fold to characterize and predict the structure of the FNIII domain of the AXL protein. Axl and Gas6 have been demonstrated as attractive potential targets for diagnosing and developing novel biomolecules for therapeutic applications. However, the proposed systems are far from being fully realized from a drug discovery perspective. Within this context, there is a clear need to characterize AXL domains like the FNIII domain to unravel their role in various pathological conditions and to develop novel target molecules.

# Single-molecule biophysical study of the FANCJ DNA helicase

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Session: Structure and Function of Biomolecules

FANCJ is an Iron-Sulphur cluster DNA helicase involved in various genome maintenance pathways1 able to resolve different G-quadruplex (G4) DNA structures with high catalytic efficiency in vitro.

My objective so far has been to investigate the mechanism of FANCJ-mediated resolution of G4 DNA using an experimental approach based on correlative optical tweezers fluorescence microscopy (CTFM)3.

To be visualised by CTFM, the protein of interest needs to be fluorescently labelled. In our study, FANCJ was fused to a short peptide (ybbR-tag) that can be specifically labelled in vitro with a small fluorophore by a bacterial phosphopantetheinyl transferase (SFP)4. This strategy however has proven to be more challenging than anticipated, resulting in the loss of FANCJ helicase activity.

Design and production of a DNA molecule containing the G4 DNA of interest has been the subsequent crucial step. I generated a linear G4-containing DNA construction with biotin moieties at the ends. The biotinylated ends can be attached to streptavidin-coated optical beads that are trapped and micro-manipulated with elevated accuracy by the optical tweezers.

Since FANCJ was reported to directly interact with PCNA5, the replicative DNA polymerase sliding clamp, efforts have also been put on producing PCNA and the replication factor C (RFC) in purified recombinant form. RFC is a 5-subunit complex responsible for loading PCNA onto a DNA substrate containing a 3’-recessive terminus. I plan to reconstitute the PCNA-loading reaction mediated by RFC, to visualise it by CTFM and to test the effect of DNA-loaded PCNA on the helicase activity of FANCJ at a single molecule level.

# Elucidating the role of DDX11, the Warsaw breakage syndrome DNA helicase, at the DNA replication fork.

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Session: Structure and Function of Biomolecules

The dynamic processes of DNA replication, repair, and recombination are tightly orchestrated to maintain genome stability. The interplay between DNA helicases and accessory factors is pivotal in regulating these intricate processes. Central to these processes is the proliferating cell nuclear antigen (PCNA), a replicative DNA polymerase sliding clamp, that is responsible for recruiting many different protein factors to the DNA replication fork. DDX11/CHLR1 (Chl1 in yeast) belongs to the group of super-family 2 (SF2) DNA helicases characterized by the presence of an iron–sulphur cluster (Fe–S) domain and has 5′ to 3′ directionality in DNA unwinding. Bi-allelic mutations of the DDX11 gene cause Warsaw breakage syndrome (WABS), a rare autosomal recessive disease. DDX11 has been reported to have a key, although undefined, role in DNA repair/recombination pathways. PCNA interaction with binding partners is primarily mediated by a PCNA-interacting protein (PIP) motif, a short conserved amino acid sequence box. Biochemical studies have unveiled the presence of non-canonical PIP motifs, expanding our understanding of the PCNA “interactome” and its functional implications.

I have demonstrated that DDX11 associates with PCNA in cell extracts. This association is not enhanced upon treating cells with hydroxyurea (HU), a replication stressful agent. Co-pull down experiments with the purified recombinant proteins have revealed that DDX11 and PCNA directly interact. PCNA-binding by DDX11 requires the integrity of a newly-identified putative non-canonical PIP box at the C-terminal end of the DDX11 polypeptide chain. Substitution of invariant residues of this putative DDX11 PIP box abolishes PCNA-binding either in vitro or in cell extracts. Experiments aimed at exploring the physiological relevance of the DDX11/PCNA interaction in genome stability maintenance pathways are underway.

# Microwave-assisted solid-state procedure to covalently conjugate Hyaluronic acid to Curcumin: validation of a green synthetic protocol to prepare a biocompatible material

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Session: Structure and Function of Biomolecules

Curcumin has drawn a massive interest in research devoted to investigating its employment in several disciplines, due to its wide range of biological and pharmacological activities. CUR offers outstanding medicinal properties including anticancer, antioxidant, antimicrobial, anti-inflammatory etc. The main hurdle of using curcumin for treatment of diseases is its poor solubility and lack of stability in aqueous medium.

A microwave-assisted esterification reaction to prepare hyaluronan-curcumin derivatives by employing a fully solvent-free process was developed. While conventional organic protocols generally rely on the efficiency of organic liquid to dissolve the reactants, herein a solid-state strategy to react two molecules characterized by a totally different solubility was developed. Hyaluronic acid, strongly hydro-soluble, was reacted with the hydrophobic curcumin, instable in water, to improve its solubility and bio-availability, while providing its own additional biological benefits (antimicrobial, anti-inflammatory, anti-oxidant, wound-repairing effects). The new protocol can be considered efficient, fast, and also eco-friendly, since it avoids toxicity and hazard in handling, such as organic base besides solvents. A cytotoxicity test confirmed that the developed HA-CUR conjugate follows the requirements for the implementation of a new material.

# Purification, structural and functional characterization of FV zymogen from plasma fraction concentrates

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Session: Structure and Function of Biomolecules

Factor V (FV) is a key player of the coagulation process as, together with Factor Xa, is involved in the conversion of fibrinogen to fibrin. FV-deficient patients may undergo severe bleeding episodes which are currently only treated with fresh frozen plasma, given the unavailability of any clinically approved recombinant FV or FV concentrates. Plasma supplementation may however give rise to fatal allergic responses and alternate clinical option are still a strong medical need. This project strives to develop a stable FV zymogen concentrate potentially usable for clinical trials. In tight collaboration with a pharmaceutical company we work at developing a process for FV isolation or enrichment from plasma samples and at performing all analytical, structural and functional characterization of intermediates and final products. We have so far developed a new purification process that includes lipid extraction, immunoglobulin depletion and size exclusion chromatography steps that leads to a reasonably homogeneous product that has been characterized by SDS-PAGE, western blotting and mass spectrometry techniques. One additional strategy is based on the use of molecular probes compatible with affinity chromatography. For this purpose, a series of peptides mimicking some FV exposed regions have been designed and prepared in order to conduct screenings of phage display antibody libraries to isolate some with high affinity for FV zymogen and potentially usable as baits for the capture of the protein directly from plasma.

# Structural and biochemical studies of RTEL1, a FeS helicase

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Session: Structure and Function of Biomolecules

Helicases containing FeS clusters are ubiquitous, but their exact mechanism of action is not well understood. No high-resolution structural information of Human FeS helicase is available, comprising of the four medically-relevant paralogues involved in genetic diseases and cancer development. Among those, RTEL1 (Regulator of Telomere Length 1) has a role in DNA repair, homologous recombination, telomere metabolism, and DNA replication. Due to cluster lability and the presence of a number of low complexity regions, the expression and purification of the protein was challenging. However, I managed to obtain different fragments of RTEL1 suitable for structural studies. A full biochemical analysis is in progress. Preliminary experiments show that RTEL1 binds different substrates with a preference for G-quadraplexes and D-loops, and unwinds DNA, in a reaction that is dependent on the integrity of the cluster. Along with crystallization attempts we are performing Cryo-EM studies. Preliminary Cryo-EM analysis showed that the samples are promising and a low-resolution map 3D map was obtained with the initial dataset. We are also testing interactions with potential interacting partners to obtain molecular details of the interaction.

# Identification and characterization of novel γ-Carbonic Anhydrases in *Pseudomonas aeruginosa*

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Session: Structure and Function of Biomolecules

Focusing on P. aeruginosa genome, three potential γ-carbonic anhydrases (CAs) proteins were identified: PA0066, PA37533, and PA5540. Synthetic DNA of these genes was created and utilized for E. coli heterologous expression, leading to the successful purification of high-quality samples for biochemical and structural analyses, including oligomeric state, secondary structure composition, and thermal stability investigations.

Collaborating with the University of Florence, kinetic investigations confirmed that these three proteins exhibit CA biological activity. In addition, inhibition assays with various inhibitors were performed, yielding promising results.

Conclusively, PA0066, PA37533, and PA5540 were identified as novel γ-class CAs, showing potential as molecular targets for the development of antibacterial drugs. These findings offer significant insights for future research in devising innovative therapeutic approaches against P. aeruginosa, ultimately aiming to enhance human health and combat bacterial infections more effectively.

# Structural and functional studies of proteins involved in neurodegenerative diseases

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Session: Structure and function of Biomolecules

My Ph.D. project aims at developing a new experimental method, based on carbon dots (CD) fluorescence and diffusion coefficient (D) measurements, to investigate the aggregation features of amyloid-beta peptides (Aβ), the major culprit for the development of Alzheimer's disease (AD). Indeed, although it is widely reported that in AD the oligomeric Aβ species rather the fully formed fibrils must be the target of therapeutic intervention, the bioanalytical techniques commonly used to monitor protein aggregation, such as ThT fluorescence, circular dichroism, dynamic light scattering, among others, are not able to give qualitative and/or quantitative information about the early stages of protein aggregation, whose detection still remains a very challenging task. CD are a new class of fluorescent carbon nanomaterials with very attractive properties as they have high stability, strong and tunable fluorescence emission properties, good conductivity, low toxicity, environmental friendliness and simple synthetic routes. For these reasons, they have been extensively investigated and used for a variety of applications in many fields of research such as biomedicine, catalysis, and sensing. This project aims at contributing to the design of CD with specifically tuned fluorescent properties, which will enable to use such particles for monitoring protein aggregation.
NMR techniques, both in solution and in the solid phase, will be used to unveil the molecular features driving the changes in the fluorescent response of CD upon the different stages of protein aggregation. In addition, we plan to investigate the aggregation of Aβ at different experimental environmental conditions (pH, metal ions, buffer and protein concentrations) by using the newly synthesized CD and by measuring the D value in real time, applying NMR and SPR. Our results will open the way to new horizons into the field of conformational diseases as it will be applicable to any other aggregating protein.

# Session 4:

## Molecular Cell Biology

# Identify molecular pathway regulating cell proliferation through glycosphingolipids biosynthesis

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Session: Molecular Cell Biology

Glycosphingolipids (GSL) are a subtype of glycolipids localized on the plasma membrane. They have a role in the regulation of signal transduction and through this control several functions of cells including cell adhesion, cell motility, and growth. Contact Inhibition of Proliferation (CIP), a mechanism that ensures proper tissue homeostasis is known to regulate GSL biosynthesis, and the GSLs in turn exert feedback control on CIP. The molecular details of this feedback circuit are not known. We have (1) identified GRASP55, a Golgi matrix protein, to be a key molecular player in this feedback circuit. The absence of GRASP55 determines cell density-dependent alteration in GSL biosynthesis and also CIP. We are now dissecting the molecular details of how GRASP55 contributes to this feedback circuit.

**Investigation of cargo-specific autoregulatory and export systems in secretory pathway in mammalian cells**

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Session: Molecular Cell Biology

The secretory pathway (SP) contributes to polarity maintenance in cells by regulating export and localisation of distinct molecules to the corresponding membrane. Little is known about how the SP is internally regulated in polarized cells. In this project we aim to understand the organization and molecular determinants of the autoregulatory signalling pathways of the apical proteins. To this end, we have studied the autoregulation of GPI-Anchored Proteins (GPI-APs) and their export features within the SP. GPI-APs are one group of apical cargoes that are involved in many cellular processes. We identified and established a very good model to study autoregulatory mechanism of GPI-APs. Moreover, we demonstrated main altered signalling pathways for GPI-GFP export both in the level of TGN. Our next focus is mainly to recognize and validate other components of these signalling pathways and their contribution to the GPI-GFP transport. Last but not least, we investigated whether there is a segregation between apical and basolateral proteins in the Endoplasmic Reticulum (ER) in mammalian cells. Our results suggest that in mammalian cells, apical and basolateral proteins different classes of proteins do not segregate in the ER Exit Site (ERES) level.

# Liquid biopsy in precision oncology: an innovative workflow for the study of circulating nucleic acid

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Session: Molecular Cell Biology

In the era of precision oncology, molecular profiling of individual patients' tumors can direct treatment decisions, monitor subsequent responses, and alert to emergence of treatment resistance. In detail, liquid biopsies involve extraction and analysis of circulating nucleic acid to assess somatic mutations, copy number alterations, and gene fusions to select targeted therapies.

During the first year of activity, the study focused on the optimization of methods for the analysis of genomic alterations and gene fusions using liquid biopsy and next generation sequencing approach. The method was validated on a small cohort of 10 patients with pancreatic ductal adenocarcinoma (PDAC). In fact, systematic screening of KRAS WT tumors for oncogenic fusion genes will substantially improve the therapeutic prospects for a sizeable fraction of patients with PDAC1. Others studies show recurrent gene rearrangements such as NRG1 fusions as disease-driving events in KRAS WT tumors, thereby providing novel insights into oncogenic signaling and new therapeutic options.

Based on those findings, it was investigated the presence of mutations in a patterns of genes such as KRAS and/or the presence of novel gene fusions in patients with partial response to chemotherapy treatment. To this aim:

- Optimization of nucleic acid extraction methods from liquid biopsies have been performed;

- Optimization of sequencing libraries and short read sequencing have been performed;

- Oncology panel NGS experiments have been performed;

- For data analysis, an ad hoc bioinformatics pipeline will be developed.

# GADD45β influences the RIPK3-dependent regulation of NF-kB

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Session: Molecular Cell Biology

GADD45β is known as an NF-kB-induced pro-survival factor, meaning it promotes cell survival and counteracts cell death processes. It accomplishes this by inhibiting JNK (c-Jun N-terminal kinase) activity and cell death pathways triggered by TNFα (Tumor Necrosis Factor Alpha). A notable development is the identification of a D-tripeptide inhibitor called DTP3, which is capable of blocking the interaction between GADD45β and MKK7 (MAP kinase kinase 7). This interaction inhibition leads to the restoration of cell death and has shown promising results in inducing JNK-dependent apoptosis specifically in cancer cells, such as multiple myeloma cells, without causing apparent toxicity to normal cells. Although GADD45β is able to suppress caspase 8 activity, the specific molecular mechanisms underlying this inhibition remain unclear. We discover a specific interaction between GADD45β and RIPK3 (Receptor-Interacting Protein Kinase 3), a kinase protein involved in various cell death pathways, including necroptosis. We revealed that this interaction is independent of the RHIM (RIP homotypic interaction motif) region of RIPK3. Additionally, we found that the interaction between GADD45β and RIPK3 suppresses RIPK3-dependent NF-kB activation. Overall, our results highlight the complex interplay between GADD45β, RIPK3, and other proteins in regulating cell survival and death pathways in cancer cells.

## D-Aspartate attenuates cadmium-induced mitochondrial dysregulation in rat testis

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Session: Molecular Cell Biology

Recent studies have reported that rat testes are highly sensitive to cadmium (Cd) toxicity, since it causes testicular injury, associated with oxidative stress, cell death and alterations in both spermatogenesis and steroidogenesis processes. Furthermore, we have demonstrated the potential role of D-aspartate (D-Asp) in preventing or mitigating the toxic effects of Cd in rat testis by exerting its known beneficial action on spermatogenesis and steroidogenesis, also determining a reduction of apoptosis (1). However, at present, the mechanism underlying cadmium-induced toxicity in testes remains unclear. Reportedly, from spermatogenesis to fertilisation, reproductive function is strongly influenced by mitochondria and their interactions with the Endoplasmic Reticulum (ER) through the Mitochondria-Associated ER membranes (MAMs), which play important roles in mitochondrial morphology and in many cellular functions, such as lipid transfer and calcium signaling. Therefore, present research project aims to investigate the impact of Cd and D-Asp, administered alone or in combination, in rat testis mitochondrial compartment to understand which cellular responses may influence reproductive functions, potentially disrupting or maintaining mitochondrial homeostasis. Our results suggest that cadmium could induce testicular cell apoptosis through activation of mitochondrial fission and inhibition of mitophagy. Instead, the dynamic balance of fusion and fission events is preserved by D-Asp treatment. Furthermore, cadmium induces an imbalance in Ca2+ homeostasis and ER stress. In contrast, D-Asp could sustain mitochondrial homeostasis and functional interactions between the ER and mitochondria.

# RNA regulatory networks governed by miR-125a in hepatocarcinoma cells

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Session: Molecular Cell Biology

MiR-125a is emerging as an important player in the pathogenesis of hepatocellular carcinoma (HCC), acting as an oncosuppressor: it has antiproliferative activity; it mediates the activity of the antitumoral drug Sorafenib; it is downregulated in HCC biopsies in comparison to normal tissues; it can limit tumor growth; it is involved in hepatitis B virus infection. Different miR-125a oncotargets have been identified and some regulatory circuits based on coding and non-coding RNAs have been unveiled. This work aims to gain a genome-wide perspective of the whole miR-125a targetome with the ambitious goal to piece together the RNA regulatory networks governed by the miRNA impacting on hepatocarcinogenesis. HepG2 and HuH-7 cells have been transfected with miR-125a mimic to boost its intracellular level in comparison to cells transfected with a control molecule and RNA-Seq experiments have been performed. Multiple data comparisons identified coding and non-coding RNAs whose expression levels resulted changed in HepG2 and/or HuH-7 because of miR-125a increased level. Two candidate miR-125a targets were further validated, NR6A1 and NUP210, encoding for nuclear proteins, overexpressed in HCC tissues in comparison to normal livers with inverse correlation to miR-125a expression. Functional studies on the identified RNA regulatory networks and impact on HCC hallmarks are in progress.

# Polystyrene microplastics's toxicity: in vitro study on human spermatozoa

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Session: Molecular Cell Biology

Microplastics enter human body through food and water ingestion, inhalation and direct skin contact. It appears that male reproductive system is particularly susceptible to these pollutants. Recent studies highlighted microplastics presence in human fluids and organs; this is a cause for great concern as MPs could provoke alterations of several physiological functions, including reproduction. Data from previous PhD year showed that polystyrene MP caused damage to zebrafish genome; therefore, on the basis of these results and the data present in literature, the aim of the second PhD year was to test in vitro MP-PS reprotoxicity on human spermatozoa evaluating the effects on semen parameters and genome stability using RAPD-PCR technique.

Semen sample from 10 patients was treated with 105 and 210 μg/ml MP-PS for 30-60-90 minutes.

The results confirmed MP-PS genotoxic and cytotoxic effects. In particular, exposure to MP-PS caused a time and concentrations dependent sperm viability and motility decrease and a genomic instability at higher MP-PS concentrations and time tested. In addition, an increase of sperm agglutination was observed for both MP-PS concentrations tested. This could indicate an induced inflammatory state or a possible aggregation of polystyrene microparticles already adhered to the spermatic cell membrane impeding the ability to fertilize. These results suggest that MPs could affect semen quality and have a toxicological impact on male fertility. Fluorescent microplastics will be tested in the following PhD year in order to confirm these hypotheses. Further studies will focus on oxidative stress evaluation to investigate genome modifications and/or sperm DNA damage resulting in cell death after MP-PS exposure.

# Microplastics impair the functionality of Sertoli cells by inducing mitochondrial damage

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Session: Molecular Cell Biology

Microplastics (MPs) are known to impair testicular functions and fertility. However, the mechanism(s) underlying the testicular effects of MPs remains still largely unknown. The project aims to investigate the potential toxicity of MPs on Sertoli cells (SCs) functionality. SCs are somatic cells of testis that facilitate the progression of germ cells to spermatozoa via direct contact by controlling the environment milieu. By using a murine TM4 cell line, we found that the addition of MPs into the culture medium induced an inhibitory effect on SC viability in a dose-dependent manner. A decrease in androgen receptor expression as well as an increase in autophagy and apoptosis processes were observed in MP-treated TM4 cells compared to control cells. Furthermore, important factors related to the mitochondrial quality were markedly affected in MP-treated TM4 cells. Specifically, we found a decrease in the protein expression of SOD2, indicative of an impairment of mitochondrial antioxidant defense system. Increased protein levels of mtDNA repair-related factors suggested a mtDNA damage in MP-treated cells; decreased mitochondrial biogenesis and fusion/fission were also found; finally, increased mitophagy suggested the removal of damaged mitochondria. Therefore, the results suggest that mitochondrial damage is a factor responsible for impaired functionality of MP-treated SCs.

# miR-18a-5p/SREBP1/PERK axis affects ER stress and autophagy in the early stages of Metabolic dysfunction-Associated Fatty Liver Disease (MAFLD)

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Session: Molecular Cell Biology

Metabolic dysfunction-Associated Fatty Liver Disease (MAFLD) has emerged as a common public health problem in recent decades. Abnormal lipid accumulation is associated with perturbed endoplasmic reticulum (ER) proteostasis, known as “ER stress”. During the last years, studies on the relationship between ER stress and microRNAs (miRNAs) has burst on the scene. Based on these considerations, this study aimed to investigate the involvement of miR-18a-5p/SREBP1/PERK pathway in ER stress and how this pathway can affect autophagy and apoptosis in the early stages of MAFLD induced by high-fat diet (HFD). To this purpose, male Wistar rats fed on HFD for 5 weeks were used. In addition, to verify the central role of the miR-18a-5p/SREBP1/PERK axis, an in-vitro model of Hep-G2 cells transfected with mimic miR-18a-5p and subsequently exposed with an oleate/palmitate mixture for 24 hours was used. The results indicated that in both experimental models the miR-18a-5p expression was downregulated by excess fat. This downregulation was associated with activation of PERK signaling, highlighting a state of ER stress that in turn induced a decrease in the autophagic process and an increase in apoptosis. Overall, this study reveals the involvement of the miR-18a-5p/SREBP1/PERK axis in HFD-induced ER stress, suggesting promising pharmacological targets in fatty liver disease.

# Paraoxonase2: Post-Translational Modifications and Protein-Protein Interactions

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Session: Molecular Cell Biology

Paraoxonase2(PON2) is a membrane protein with a role in regulating mitochondrial oxidative stress by reducing ROS in addition to its lactonase activity. With its role in regulating the oxidative stress, Paraoxonase2 is reported to be upregulated in different Solid tumours. Recent studies highlighted the protective role of PON2 in neurodegenerative diseases. Its lactonase activity hydrolyses the Acyl Homoserine Lactones (AHLs), thereby inhibiting the quorum sensing of Bacteria resulting in prevention of Biofilm Formation.

Invitro studies identified the inactivation of the lactonase activity of PON2 in cells treated with Hydrogen Peroxide. We focussed on identifying the Post-Translational Modifications involved in Inactivation of the lactonase activity of Paraoxonase2. Mass spectrometry analysis identified the ADP-ribosylation of Paraoxonase2 and it was confirmed by Western Blot analysis. We earlier reported that Aspartate at D124 was ADP-ribosylated in PON2. With the advancement of Proteomic Data analysis pipelines, reanalysis of our existing mass spectrometry data and Bioinformatic analysis of Global Proteomic ADP-ribosylation data identified R101 as the ADP-ribosylation site. To validate this ADP-ribosylation and its role in Inactivation of PON2, site-specific mutations of Arginine at R101 and D124 was being carried out. In addition, Protein-Protein Interaction studies of Paraoxonase2 by Co-Immunoprecipitation followed by Mass Spectrometry analysis is performed to identify the PARP enzyme involved in ADP-ribosylation as well as the other Protein interactors, namely E3 ligases involved in Ubiquitination of PON2. We further aim to study the role of Ubiquitination of PON2, E3 ligases involved in PON2 Ubiquitination and if there is any crosstalk between ADP-ribosylation and Ubiquitination of PON2.

# Unravelling the role of the TGN export machinery for basolateral proteins in Amyloid Precursor Protein (APP) transport and processing

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Session: Molecular Cell Biology

The secretory pathway is involved in the synthesis, post-translational modification and transport into specific compartments of around 30% of human proteins. In the Trans-Golgi Network (TGN), basolateral proteins activate the orphan receptor GPRC5A to promote and regulate their own export and sorting, thanks to a series of signalling events that culminate with Protein Kinase D recruitment and activation. Indeed, the mis-sorting of basolateral proteins and/or the dysregulation in the TGN-sorting machinery have been associated with different human diseases, including neurodegeneration. Our aim is to test the role of the basolateral sorting system on the Amyloid precursor protein (APP) transport and processing, a basolateral protein normally processed by α-, β, and γ-secretases. We are characterising APP intracellular and extracellular levels and fragments in HeLa cells upon GPRC5A depletion and treatment with secretases inhibitors. Moreover, we are investigating the localisation of intracellular APP in GPRC5A-depleted cells. Here, our goal is to identify a possible mechanism that couples the TGN regulatory system with APP transport and processing to reduce the production of the amyloidogenic peptide, causative of Alzheimer's disease, and favour the production of the non-amyloidogenic peptide.

# Session 5:

## Human Genetics

# ZNF687: a new player in skeletal growth

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Session: Human Genetics

The germline p.P937R mutation in ZNF687 is causative for an aggressive form of Paget's disease of the bone (PDB). We recently reported the characterization of the Zfp687$^{P937R}$ knock-in mouse model, which mirrors the severe pagetic phenotype observed in P937R-mutated patients.

To pinpoint the mechanistic role of ZNF687 in bone metabolism, we generated the Zfp687 knock out (Zfp687$^{-/-}$) mouse model. As early as 1 month of age, mutant mice exhibited smaller size. At 3 months of age, mutant mice maintained their shorter body and bone length, along with reduced body weight. Given the post-natal role of growth plates (GPs) in bone elongation and the positive correlation between GP width and bone length, we histologically examined GPs. We found an 85% and 69% linear correlation for wild-type and Zfp687$^{-/-}$ GPs to bone length, respectively. Therefore, the linear correlation significantly decreased in Zfp687 deficiency.

Although bone mass remained unaffected in null mice, micro-computed tomography analysis revealed a decreased total cross-sectional area, resulting from a reduction in cortical bone area in Zfp687$^{-/-}$ femurs, indicative of narrower width. Since we have previously described ZNF687 as a positive regulator of osteoclastogenesis, we evaluated osteoclast activity to determine if the skeletal phenotype could be attributed to osteoclast failure. We analysed TRAP-stained femur sections, which showed a decreased osteoclast surface and a lower number of TRAP+-positive osteoclasts per bone surface in Zfp687$^{-/-}$ mice. This suggests that osteoclast differentiation is not completely abrogated but is still defective.

In conclusion, these results expand upon the established role of ZNF687 in bone remodelling by uncovering an unknown function in skeletal patterning.

# Non-coding RNAs as versatile regulators in Neurodegenerative Diseases (NDDs). A focus on MCI (Mild Cognitive Impairment) and MS (Multiple Sclerosis).

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Session: Human Genetics

Neurodegenerative Diseases (NDDs) are conditions characterized by degeneration of the neurons (impaired movement, memory loss, mood variations, impaired speaking). Initially, these dysfunctions do not impair patients; however, the progression of the diseases continue and the quality of life of the patients is reduced dramatically until they are in need of full-time care with a social and financial burden as a consequence. Emerging studies have suggested that various non-coding RNAs (such as microRNAs and long non-coding RNAs) play key roles in the pathogenesis of central nervous system (CNS) disorders and could become diagnostic/prognostic biomarkers and viable therapeutic targets. Thanks to a collaboration with Department of Advanced Medical and Surgical Sciences, University of Campania "L. Vanvitelli", Naples, I obtained Multiple Sclerosis (MS) samples and Mild Cognitive Impairment (MCI) samples. In MS samples, I evaluated the difference in expression levels of four microRNAs: miR-21, miR-338, miR-338-5p and miR-let7-a, in baseline conditions and at one year after the therapeutic treatment applied, while MCI samples were preliminary investigated, using a long non-coding array profiling, from which five long non-coding RNAs: HAR1A, HAR1B, MEG9, ST7-AS1, TUNAR, are emerged as differently expressed. Further studies aiming to identify networks in which the aforementioned non-coding RNAs are involved, are in progress.

# Multi-locus imprinting disturbances in patients affected by Beckwith-Wiedemann Syndrome

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Session: Human Genetics

Beckwith-Wiedemann syndrome (BWS) is a clinically heterogeneous imprinting disorder (ID) caused by genetic or epigenetic defects within the chromosome 11p15.5. The most common epigenetic mechanism causing BWS is loss of methylation at imprinting control region 2 (IC2-LoM). In one-third of IC2-LoM patients, methylation defects affect also other imprinted loci, a condition known as multi-locus imprinting disturbances (MLID),sometimes associated with atypical clinical features.

My project aims to characterize the MLID subgroup by performing methylation array and whole exome sequencing to determine the prevalence of MLID in our BWS cohort, the methylation profile and putative genetic variants predisposing to MLID.

Also, among the patients showing complex phenotypes we identified two cases: a young woman who developed early-onset colorectal cancer and a boy with co-occurrence of BWS and another ID, Pseudo-hypoparathyroidism type 1B. MLID and two pathogenic variants of the CFTR gene were detected in the first case, IC2-LoM, paternal heterodisomy of chromosome 20 (patUPhD20) and no MLID in the other one. Our results suggest that the co-occurrence of different pathologies arose through independent aetiologies in both patients, although the IC2-LoM might have accelerated tumorigenesis in the presence of predisposing genetic variants in one case, and a common undefined predisposing molecular lesion might have led to IC2-LoM and patUPhD20 in the other case.

# Investigating the molecular mechanisms underlying the Silver-Russell syndrome

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Session: Human Genetics

Silver-Russell syndrome (SRS) is a congenital imprinting disorder characterized by clinical and molecular heterogeneity. The clinical diagnosis is based on the presence of at least 4 out of the following 6 features: intrauterine and postnatal growth retardation, relative macrocephaly at birth, body asymmetry, protruding forehead, and feeding difficulties. SRS is caused by molecular defects affecting imprinted genes of chromosome 11p15.5 and 7. The most frequent molecular defects of SRS are loss of methylation (LOM) of the imprinting control region 1 (IC1) on chromosome 11p15.5, found in 50% of cases, and maternal uniparental disomy (UPD) of chromosome 7, found in 10% of cases. In about 40% of patients, the molecular defect is still unknown.

Aim of my project is the molecular characterization of an Italian cohort of about 130 SRS patients, so far tested for defects of 11p15.5 and chromosome 7. MS-MLPA testing methylation and copy number of 10 different imprinted loci will be performed on 32 IC1-LOM and 61 no defect cases to detect the involvement of further imprinted loci in SRS aetiology. SNP-array will be applied to reveal the heterodisomic or isodisomic nature of the mat(UPD)7. Whole exome sequencing will be performed to identify putative pathogenetic variants. The results will provide improvement in molecular diagnosis and genetic counseling.

# Genome-wide studies for the molecular characterization of isolated Wilms tumor

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Session: Human Genetic

Wilms tumor (WT), or nephroblastoma, is a relatively rare but the most common type of kidney cancer in children. It originates from embryonic kidney cells and is typically diagnosed at the age of 3 to 4 years. These tumors are characterized by intricate interplay between genetic and epigenetic factors that affect the normal development of kidney. In the present study we analyzed methylome of 27 normal-tumor paired tissues and expression of 18 paired, 4 unpaired tumors and 5 unpaired healthy kidneys (HK). We observed a general hypomethylation in these tumors. This trend was least significant in the epithelial histology tumors and may be attributed to being more differentiated and more similar to healthy kidney. Based on the methylome we identified a signature of ~9000 CpGs able to differentiate HK and WT. By transcriptomic analysis, we also found ~4600 genes differentially expressed between WT and HK. Upregulated genes were enriched for “Cell cycle”, “DNA replication” and “Pathways in cancer”, whereas downregulated showed general enrichment for “Metabolic pathways”. Part of this gene deregulation correlates with DNA methylation of promoters or gene body. With these results we try to extend deeper into the molecular landscape of Wilms tumor, highlighting the profound impact of both genetic and epigenetic factors in its development.

# Pharmacological stimulation of autophagy to rescue proteinopathy and cognitive decline in mucopolysaccharidosis-IIIA

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Session: Human Genetics

Mucopolysaccharidosis IIIA (MPS-IIIA) is a lysosomal storage disorder (LSD) characterized by the loss of function of the sulfamidase gene (SGSH), responsible for the degradation of the glycosaminoglycan (GAG) heparan sulfate (HS). Undegraded HS leads to the formation of primary and secondary storages responsible for neurodegeneration and dementia in children (1). Favouring the degradation of secondary storages is one of the most promising therapeutic strategies to prevent neurodegeneration. Genetic overexpression of the transcription factor EB (TFEB), through the control of genes involved in the autophagy/lysosomal degradation process, seems to promote the degradation of protein aggregates in animal models of neurodegeneration (2). However, few synthetic drugs are capable of stimulating TFEB and crossing the blood-brain barrier. We are testing a compound that in wild-type/control animal models has been shown to promote TFEB-mediated autophagy and lysosomal biogenesis. In this project, using validated animal models of MPS-IIIA, we have tested it in in vivo models of MPS-IIIA. The in vivo analysis shed light on the ability of the drug to improve some of the cognitive deficits associated to the accumulation of undegraded HS in the brain of MPS-IIIA animals, without any major side effect. These results suggest a new therapeutic approach for the treatment of MPS-IIIA.

# The silent autoimmunity is a new aspect of phenotypic variability in Incontinentia Pigmenti

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Session: Human Genetics

Incontinentia Pigmenti (IP, OMIM 308300) is a rare X-linked dominant neuroectodermal disorder caused by mutations in the IKBKG/NEMO gene. It leads to inflammatory skin (IP hallmarks) and, in 30% of cases, to central nervous system and ocular abnormalities (the severe form).

The aim of my research is to characterize the autoimmunity in IP as a novel clinical sign. Indeed, although IP patients do not suffer from overt autoimmunity, in their serum the presence of autoantibodies against two subtypes of IFN-α2 and/or IFN-ω has been revealed.

During my research (I and II year), we have collected and characterized 21 patients from the Incontinentia Pigmenti Genetic Biobank (IPGB). The patients' ages ranged from 1 to 56 years, with a mean age of 25 years. The IP phenotype analysis revealed ocular complications and neurological impairment in 7 patients (33%). Genetic investigation revealed that the deletion in IKBKGdel was present in 14 out of 21 patients (67%). Three variants were found: c.976_978del, c.628_651delinsCG and c.646del, the last two as new mutations. We did not identify any alteration in the IKBKG gene in 3 cases. Fourteen were sporadic (67%) and 4 familial (19%) cases. The immunological analysis, performed in collaboration with the "Imagine Institute" in Paris, revealed the presence of Auto-Abs against IFN-α2 and/or IFN-ω, in 11 patients (52%), while 10 (48%) resulted negative for both.

# Dissecting the role of rare variants in Parkinson's disease pathogenesis trough the generation of different cellular disease models

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Session: Human Genetics

We have recently reported the identification of new rare gene variants in Parkinson's Disease patients that support polygenic contribution to the disease, even in sporadic forms. We functionally investigated variants identified in TMEM175 gene to dissect the genetic heterogeneity of PD. Comprehensive genetic and molecular analysis of TMEM175-patients displayed: i) altered K+ conductance of the mutant channels, ii) altered binding affinity with AKT, iii) impaired autophagic flux, iv) activation of UPR markers and v) a more severe phenotype in the presence of polygenic mutations.

Overall, these data suggested that detrimental mutations in TMEM175 might be sufficient to cause the disease and supported the polygenic nature of the disease. To better investigate the pathogenic contribution of the novel identified variants, we generated a set of integration-free hiPSCs (6 of PD-patients and 3 of healthy subjects) carrying highly penetrant genetic combinations of variants in novel PD genes (AIMP2, HMOX2, IMMT, KIF21B, LRRK2, RHOT2, TMEM175, TOMM22, TVP23A, ZSCAN21).

A well-defined characterization protocol was performed to ensure high quality of collected iPSCs.

Finally, we demonstrated that these cells were able to generate of high-quality ventral midbrain dopaminergic progenitors offering the possibility to decipher the molecular basis underlying PD-neurodegeneration.

# AAV-mediated microRNAs modulation as gene-independent strategy in inherited retinal dystrophies

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Session: Human Genetics

Inherited retinal dystrophies (IRDs) are a group of disorders affecting the retina. They are characterized by photoreceptor (PR) cell death and progressive vision loss, and include different clinical subtypes, among which retinitis pigmentosa (RP). They are genetically heterogeneous with over 300 causative genes identified to date. For this reason, there is high need of gene-independent therapies aimed at delaying PR degeneration and that could be used in combination with genereplacement strategies.
In this respect, microRNAs (miRNAs) represent promising therapeutic tools due to their capability to simultaneously modulate multiple molecular pathways involved in human disease pathogenesis and progression. We previously demonstrated that the expression modulation of miRNAs miR-181a/b or miR-204 preserves retinal cells from death and ameliorates visual function in the RHO-P347S mouse, a model for an autosomal dominant form of RP.
We decided to apply our approach also to an autosomal recessive mouse model of RP, rd10, and we observed that both miR181a/b downregulation and miR-204 overexpression ameliorate retinal function and morphology, thus supporting the gene/mutation-independent protection exerted by this strategy, which seems to be effective in slowing down disease progression. Further ongoing experiments will indicate whether this approach could have additive effect in combination with gene-replacement strategies.

# TUDP: diagnosis for undiagnosed

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Session: Human Genetics

The Telethon Undiagnosed Diseases Program (TUDP) is a multicentre Italian national program, with the main purpose of identifying genes associated with undiagnosed paediatric rare monogenic diseases. To date, in the TUDP a total of 878 families were analysed with a diagnostic rate of almost 50%.
In the past year, we identified several candidate disease-causing genes unravelling a wholly new understanding of their pathological role.
Here we present three noteworthy cases. Firstly, in a 3-years-old male we found a mono-allelic pathogenic variant in PPFIA3, a novel disease-associated gene involved in a neurodevelopmental syndromic condition with only 14 individuals described so far.
The second case was a 13-years-old male with cognitive impairment and epilepsy in which we found a variant in LMAN2L, a known disease-gene for which we observed a dominant inheritance pattern rarer than the recessive one with a milder phenotypic manifestation.
Lastly, in a 10-years-old female we found the unique known pathogenic variant in CAPRIN1 (p.Pro512Leu), a recently discovered disease-gene, with few cases described worldwide, associated with early-onset ataxia and cognitive impairment.
Overall, our observations represent a step toward the global goal of enriching our current knowledge and offering the possibility to make known what is now unknown.

# Creation of novel allellic frequency database to optimise the diagnosis of rare genetic disorders

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Session: Human Genetics

Advancement of Next Generation Sequencing (NGS) technologies provides many options to diagnose rare diseases associated with several types of genetic variants. This massively parallel technique enabled a relatively cheaper sequencing of genomes and exomes of many patients, assisting in achieving an accurate diagnosis1.Variants reference population databases like gnomAD and 1000 Genomes are a powerful tool for the interpretation of genomic variants, their association with diseases or traits and to support the discovery of new disease–gene relationships2. Since we have observed that many variants exhibit a regional frequency distribution that differs from what is reported in international databases, an internal custom database that closely resembles the general population would offer a comprehensive and accurate genome-wide estimates of variant frequency, proving advantageous in evaluating the potential impact of selected variants on disease development among Italian subjects.

This PhD aims to create an updated regional database with allelic frequencies of variants found in more than 8000 individuals analyzed over the past years by both the Vanvitelli and TIGEM NGS facilities. So far, we have analysed 563 samples using an updated WES pipeline to ensure our investigations are up to date with the latest human genome version. We are also incorporating our findings into a clinician-friendly tabular output, reducing our dependence on external databases and providing more robust genetic insights.