UNIVERSITÀ DEGLI STUDI FIRENZE

Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino

Eccellenza 2023-2027

Pharma PhD-DAY

UNIVERSITY OF FLORENCE

Area of Drug and Innovative Treatments

Book of Abstracts

1st Edition of the Symposium

PhD in Drug Research and Innovative Treatments

January 23rd, 2026

Lecture Hall D4 – "Enrica Calabresi" Building

Campus Sesto Fiorentino, via Edoardo Detti 3

University of Florence

Pharma PhD-DAY
UNIVERSITY OF FLORENCE
Area of Drug and Innovative Treatments

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BOOK OF ABSTRACTS

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Pharma PhD-DAY
UNIVERSITY OF FLORENCE
Area of Drug and Innovative Treatments

Organizing Committee

The Symposium Organizing Committee was composed of PhD students enrolled in the Doctoral programme in the *Area of Drug Research and Innovative Treatments*, who volunteered to undertake this role. The event was autonomously conceived and organized by the Committee and was open to all students of the University of Florence. The initiative served as a dedicated forum for PhD students in the *Area of Drug Research and Innovative Treatments* to present, share, and critically discuss their research projects.

Francesca Catarzi, XXXIX cycle

Clara Ciampi, XXXIX cycle

Carmen Gallo, XL cycle

Elena Giulia Giugliano, XL cycle

Federica Panozzi, XL cycle

Pharma PhD-DAY
UNIVERSITY OF FLORENCE
Area of Drug and Innovative Treatments

Symposium Coordinators:
Lorenzo Di Cesare Mannelli, PhD coordinator
Serena Orlandini, PhD Review Group member

List of Participants

XXXIX cycle

- Emanuela Bocchi
- Irene Digiglio
- Silvia Fiani
- Ilaria Galli
- Maria Vincenza Mastrolia
- Alice Molli
- Gioele Renzi
- Lucrezia Sforzi
- Giulia Vagnoni

XL cycle

- Carmen Gallo
- Elena Giulia Giugliano
- Laura Gatti
- Lorenzo Mattolini
- Federica Panozzi
- Sara Pisini
- Camilla Vannucchi

XLI cycle

- Alessia Benucci
- Rebecca Castellacci
- Elisa Marinari
- Martino Montomoli
- Federica Polverini
- Sofia Quadrino

Pharma PhD-DAY UNIVERSITY OF FLORENCE Area of Drug and Innovative Treatments

Programme

09:00-09:15 Opening Remarks – *Lorenzo Di Cesare Mannelli*

09:15-09:45 Flash Talks – XLI cycle

Chairs: *Andrea Ammara* and *Riccardo Zecchi*

- **Alessia Benucci** - Study of the nutraceutical potential of phytocomplexes obtained from pre-germinated/germinated plant products and *microgreens*: chemical characterization and *in vitro* tests
- **Rebecca Castellacci** - Nanotechnology-based drug delivery systems for the modulation of cellular senescence and related disorders
- **Elisa Marinari** - Development of antioxidant casein kinase 1Δ inhibitors as novel therapeutic agents for amyotrophic lateral sclerosis
- **Martino Montomoli** - Drug response in movement disorders associated with developmental and epileptic encephalopathies: toward precision phenotyping and targeted treatments
- **Federica Polverini** - Gene × Environment interactions shaping neurodevelopment in SHANK3 deficiency
- **Sofia Quadrino** - Relevance of cellular senescence to the pathogenesis and treatment of multiple sclerosis

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09:45-10:45 Poster Session – XLI cycle

10:45-11:15 Coffee Break

11:15-12:00 Flash Talks – XL cycle

Chairs: *Dr. Andrea Di Santo* and *Dr.ssa Chiara Sasia*

- **Carmen Gallo** - Development of new melanogenesis-modulator peptides for dermocosmetic applications
- **Elena Giulia Giugliano** - Pharmacological and functional analysis of genes involved in colorectal cancer drug resistance
- **Laura Gatti** - Prognostic role of cytofluorimetry in pediatric connective tissue diseases: a prospective study
- **Lorenzo Mattolini** - Design and synthesis of new metallo-beta-lactamase inhibitors to fight antibiotic resistance

- **Federica Panozzi** - Role of adenosine A_{2B} receptors during de- and re- myelination process in different mouse brain regions: an in vivo study
- **Sara Pisini** - Modeling mTOR pathway dysregulation in patient-derived neurons
- **Camilla Vannucchi** - Natural active-based NLC hydrogels combining pomegranate oil and *Sedum telephium* polysaccharides for paediatric burn management

12:00-13:00 Poster Session – XL cycle

13:00-14:30 Lunch Break

14:30-15:15 Flash Talks – XXXIX cycle

Chairs: *Sara Calenda* and *Paraham Farahvachi*

- **Emanuela Bocchi** - A 3D air liquid interface lung model to assess IPF epithelial-mesenchymal crosstalk
- **Irene Digiglio** - Comparison of HS-SPME, Vac-HS-SPME and HiSorb pre-concentration techniques for profiling of volatile compounds in monovarietal extra virgin olive oil using GC×GC-MS
- **Silvia Fiani** - Thermosensitive multitarget hydrogels for the treatment of infected skin ulcers: the CUTESANA project
- **Ilaria Galli** - Use of precision therapy in neurodevelopmental disorders and epileptic encephalopathies related to mutation in the *GRIN* genes
- **Maria Vincenza Mastrolia** - Epigenetic profile of Pediatric Behçet Disease
- **Alice Molli** - SARM1-driven metabolic collapse as a novel anticancer strategy
- **Gioele Renzi** - Dual inhibition of carbonic anhydrase IX and glutathione peroxidase 4 as a novel strategy for ferroptosis-Induced tumor cell death
- **Lucrezia Sforzi** - Toward eco-friendly SPPS: a case study on Argireline™ and Pal-GHK-OH peptides
- **Giulia Vagnoni** - 5,7-Diaminothiazolo[5,4-d]pyrimidine derivatives as new CK1Δ Inhibitors for the treatment of neurodegenerative diseases and cancer

15:15-16:30 Poster Session – XXXIX cycle

16:30-16:45 Closing Remarks – *Lorenzo Di Cesare Mannelli*

Pharma PhD-DAY UNIVERSITY OF FLORENCE Area of Drug and Innovative Treatments

List of Abstracts

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Abstract 1

STUDY OF THE NUTRACEUTICAL POTENTIAL OF PHYTOCOMPLEXES OBTAINED FROM PRE-GERMINATED/GERMINATED PLANT PRODUCTS AND *MICROGREENS*: CHEMICAL CHARACTERIZATION AND *IN VITRO* TESTS

Alessia Benucci, Nadia Mulinacci, Maria Bellumori, Marzia Innocenti

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In recent years, the scientific community has shown increasing interest in developing of plant-based foods with high nutritional and nutraceutical value, produced through innovative and natural processes. This trend reflects the need for sustainable food solutions that can help prevent inflammatory, metabolic, and degenerative diseases, while also addressing the challenges posed by global population growth. In this context, seeds pre-germination and germination, as well as the still-emerging production of microgreens represent promising strategies for modulating the phytochemical profiles of plant species [1]. These processes can increase both the concentration and the bioavailability of bioactive compounds and micronutrients in the seeds and vegetative part of the plant.

Pre-germinated and germinated products, along with *microgreens*, represent intermediate stages between the seed and the mature plant, during which biochemical transformations change the phytochemical profile of the seed, influencing its nutritional profile and beneficial properties [1-2].

This PhD project aims to investigate the nutritional and nutraceutical profiles of these products obtained from selected plant species to assess their chemical reproducibility in different batches and their potential as functional foods.

To achieve this, a qualitative and quantitative characterization of phytocomplexes will be performed using advanced analytical techniques, including high-performance liquid chromatography coupled with diode-array detection (DAD) and mass spectrometry (MS), Nuclear Magnetic Resonance (NMR) and Dynamic Light Scattering.

In vitro bioassays will be conducted on characterized extracts to evaluate some of their potential biological activities.

The expected results should contribute to expanding current knowledge on these treatments and provide a detailed characterisation of the phytocomplexes extracted from pre-germinated and germinated seeds and from *microgreens* obtained from selected plant species, as well as their nutritional and nutraceutical potential.

[1] D. Günal-Köroğlu et al., J. *Food Meas. Charact.*, **2025**, *19*, 8144-8164.
[2] M. A. Johnson et S. Thankur, *Food Bioscience*, **2025**, *73*, 107579.

Abstract 2

NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS FOR THE MODULATION OF CELLULAR SENESCENCE AND RELATED DISORDERS

Rebecca Castellacci[a], Anna Rita Bilia[a], Elisa Landucci[b], Maria Camilla Bergonzi[a]

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Cellular senescence is a key biological process underlying aging and several age-related diseases, including Alzheimer's disease (AD). Senescent cells undergo irreversible cell cycle arrest and secrete pro-inflammatory and pro-oxidant factors collectively known as the Senescence-Associated Secretory Phenotype. In the brain, the accumulation of senescent neurons, astrocytes, and microglia promotes chronic neuroinflammation, oxidative stress, amyloid-β deposition, and tau hyperphosphorylation, all hallmarks of AD. Therefore, targeting senescent cells represents a promising strategy to modulate disease progression.
Ellagic acid (EA) is a natural polyphenolic compound mainly present in *Punica granatum* L. It is known for its antioxidant and anti-senescence effects demonstrated in cardiovascular, renal, and neurodegenerative models [1]. EA can scavenge reactive oxygen species, reduce lipid peroxidation, and modulate pro-inflammatory mediators. However, its clinical application is hindered by low aqueous solubility[2], poor bioavailability, and limited stability, which reduce its therapeutic potential.
This project aims to overcome these limitations by developing a liposomal formulation (LP-EA), further functionalized with a galactose derivative (Gal-LP-EA) for selective delivery to senescent cells. The targeting strategy exploits the overexpression of β-galactosidase in senescent cells, which cleaves the galactose-lipid bond and enables the controlled intracellular release of EA specifically in pathological cells [3].
LP-EA and Gal-LP-EA will be physico-chemically characterized in terms of particle size, polydispersity index, zeta potential, morphology, and encapsulation efficiency. Stability studies will be conducted under controlled storage temperature conditions, and *in vitro* release profiles will be evaluated in different release media using the dialysis bag method. Passive permeability across the blood-brain barrier will be assessed using the PAMPA test. Biological evaluation will include assessment of anti-senescence effects in models of senescent cells and neuroprotective activity in *in vitro* and *in vivo* models of neurodegenerative diseases.
By combining EA with targeted LP, this project proposes a therapeutic strategy to modulate senescence and potentially mitigate neurodegeneration associated with AD.

[1] N. Naghibi et al., *BMC Complement Med Ther.*, **2023**, *23*, 77.
[2] G. Zuccari et al., *Appl. Sci.*, **2020**, *10*, 3353.
[3] C. Battisegola et al., *Pharm.*, **2024**, *17*, 308.

Abstract 3

DEVELOPMENT OF ANTIOXIDANT CASEIN KINASE 1Δ INHIBITORS AS NOVEL THERAPEUTIC AGENTS FOR AMYOTROPHIC LATERAL SCLEROSIS

Elisa Marinari[a], Sara Calenda[a], Costanza Ceni[a], Daniela Catarzi[a], Flavia Varano[a], Letizia Trevisan[b], Stephanie Federico[b], Giampiero Spalluto[b], Veronica Salmaso[c], Gianluca Novello[c], Stefano Moro[c], Vittoria Colotta[a]

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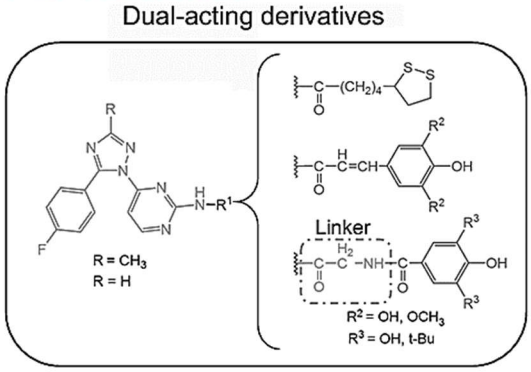
Amiotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons of the central nervous system. The pathogenic mechanisms underlying ALS are complex and multifactorial, and in this context casein kinase 1δ (CK1δ) emerges as a pharmacological target of great interest for combating ALS [1]. When overexpressed or deregulated, CK1δ hyper-phosphorylates the nuclear protein TDP-43, which forms neurotoxic cytoplasmic aggregates that damage motor neurons and accelerate the progression of the disease [2]. Preclinical studies performed in neuronal cells and motor neurons demonstrate that CK1δ inhibition reduces TDP-43 hyperphosphorylation, preventing its cytoplasmic accumulation and the aggregate formation. ALS, like many other neurodegenerative diseases, is associated with high levels of oxidative stress, in which the balance between free radical production and antioxidant defense is compromised. Therefore, the following PhD project will aim to identify new dual-acting compounds, designed by combining the structure of CK1δ inhibitors with different antioxidant moieties, such as Alpha-lipoic acid or polyphenolic carboxylic acids (***Figure 1***). Indeed, antioxidants, while often touted for their health benefits, have shown mixed results in clinical trials for ALS (National Institutes of Health). Moreover, the multifunctional compounds are considered innovative and believed to be more effective than single-target therapeutics, especially in multifactorial diseases such as ALS. Following this design approach, the dual-acting CK1δ inhibitors/antioxidants will be synthesized exploiting the coupling, direct or through a linker, of the pyrimidine amino group of our CK1δ inhibitors with the carboxylic function of powerful antioxidants. All the novel compounds will be characterized for their CK1δ inhibitory activities and, among them, the best inhibitors will be evaluated for their antioxidant activity using different methods depending on the antioxidant portion. The target compounds are expected to possess the ability to inhibit CK1δ and simultaneously counteract oxidative stress [3].



[1] D. Catarzi et al., *Curr. Med. Chem.*, **2022**, *29*, 4698-4737.
[2] V.I. Ko et al., *Neurobiol. Dis.,* **2024**, *15*, 106516.
[3] X.H. Makhoba et al., *Drug Des. Devel. Ther.*, **2020**, *14*, 3235-3249.

Abstract 4

DRUG RESPONSE IN MOVEMENT DISORDERS ASSOCIATED WITH DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHIES: TOWARD PRECISION PHENOTYPING AND TARGETED TREATMENTS

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Developmental and epileptic encephalopathies (DEEs) are severe pediatric-onset disorders characterized by drug-resistant seizures, profound developmental impairment, and heterogeneous genetic etiologies. Movement disorders (MDs)—including dystonia, chorea, stereotypies, ataxia, and myoclonus—are increasingly recognized as integral yet underdiagnosed components of the DEE phenotype. Despite advances in genetic diagnostics, therapeutic management of MDs in DEEs remains largely empirical, with inconsistent documentation of drug responsiveness and limited pediatric-specific evidence. Recent large-scale studies have emphasized the high prevalence of MDs in genetically defined DEEs and the critical need for structured phenotyping and genetically informed therapeutic strategies.

This project aims to characterize pharmacological responsiveness in MDs associated with genetically confirmed DEEs, integrating clinical, neurophysiological, and molecular data to identify predictors of treatment response. A multicenter, longitudinal observational design will include children and adolescents, with optional follow-up of adults with pediatric-onset DEEs. Clinical phenotyping will employ standardized scales adapted for pediatric and neurodevelopmentally impaired populations. Drug response will be assessed categorically (improved/no change/worsened), with secondary outcomes including tolerability, dose titration, and polytherapy requirements. Patients will be stratified by molecular mechanism—such as ion channel dysfunction, synaptic pathology, or transcriptional regulation defects—to assess pathway-specific therapeutic effects. Longitudinal follow-up will explore evolution of MDs and durability of treatment response.

By integrating multimodal data and exploratory machine learning models, the project seeks to define biomarkers predictive of drug responsiveness and to support precision medicine approaches in DEE-related MDs. The anticipated outcome is a clinically applicable framework to guide individualized therapeutic decisions and inform future stratified interventional trials.

[1] N. Specchio et al., *Brain*, **2021**, *144*, 32–43
[2] S. van der Veen et al., *Neurology*, **2023**, *101*, e1884–e1892
[3] B. Pérez-Dueñas et al., *Mov. Disord.*, **2022**, *37*, 2197–2209

Abstract 5

GENE × ENVIRONMENT INTERACTIONS SHAPING NEURODEVELOPMENT IN SHANK3 DEFICIENCY

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Autism Spectrum Disorder (ASD) emerges from complex interactions between genetic predisposition and environmental influences across early development. Among high-confidence ASD genes, SHANK3 plays a central role in organizing excitatory synapses and regulating neuronal circuit assembly [1]. Although SHANK3 haploinsufficiency alone can drive synaptic and behavioral abnormalities, environmental factors may substantially modulate neurodevelopmental trajectories, amplifying underlying genetic vulnerability.
Multiples evidences indicate that stress exposure during prenatal and early postnatal life has profound and lasting effects on brain maturation. Epidemiological studies show that maternal stress between 21–32 weeks of gestation, as well as traumatic or destabilizing events during the first months of life, are associated with increased ASD risk. Variations in early maternal care and postnatal stressors can additionally shape neurodevelopment through epigenetic regulation of stress-response pathways [2]. Stress during critical gestational windows alters fetal brain architecture and affects hippocampal and cerebellar development [3]. These findings support stress as a robust environmental risk factor that may interact with SHANK3-linked synaptic vulnerability.
This PhD project will investigate gene × environment (G×E) interactions between SHANK3 haploinsufficiency and stress using a translational, multi-system strategy. First, primary neuronal cultures from wild-type and Shank3+/– mice will be exposed to corticosterone to model stress responses, followed by analyses of synaptic function via MEA recordings, patch-clamp electrophysiology, Western blot, and immunocytochemistry. In parallel, patient-derived SHANK3-mutant iPSCs will be differentiated into neurons, characterized molecularly and functionally, and challenged with timed corticosterone paradigms to identify windows of heightened vulnerability. Finally, SHANK3+/– mice will be subjected to a social defeat stress protocol to examine whether stress exacerbates ASD-relevant behavioral phenotypes.
By bridging cellular, patient-derived, and in vivo models, this project aims to elucidate how stress influences SHANK3-dependent neurodevelopment, defining mechanisms and developmental windows that govern G×E interactions in ASD.

[1] P. Monteiro et al., *Nat Rev Neurosci*., **2017**, *18*, 147–157.
[2] A. Angelidou et al., *BMC Pediatr*., **2012**, *12*.

Abstract 6

RELEVANCE OF CELLULAR SENESCENCE TO THE PATHOGENESIS AND TREATMENT OF MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is a chronic autoimmune and inflammatory disease that affects the central nervous system (CNS), characterized by demyelination and neurodegeneration. Current treatments mainly modulate the immune response but don't prevent disease progression, particularly in its progressive forms[1]. Growing evidence indicates that post transcriptional regulation mediated by ELAV RNA-binding proteins (RBPs) plays a key role in neuroinflammation, neurodegeneration and remyelination. In particular, the pro inflammatory protein HuR is upregulated in activated microglia, whereas the neuronal ELAVs (HuB, HuC e HuD) promote neuronal differentiation, synaptic plasticity and neuroprotection. Their reciprocal regulation suggests that an imbalance in the HuR/nELAV axis may contribute to microglial senescence, impaired oligodendrocyte differentiation and MS progression[2].

This project aims to investigate the role of nELAV proteins as neuroprotective factors and to define how their interplay with HuR contributes to microglial senescence and neuroinflammatory damage.

Recent evidence demonstrate that chronically activated microglia develop a senescent phenotype characterized by SASP secretion, metabolic dysfunction and reduced capacity to support oligodendrocyte precursor cell differentiation. Importantly HuD downregulation increases susceptibility to stress induced senescence, linking ELAV dysregulation to senescence associated neurodegenerative cascades[3]. This project propose that an aberrant HuR/nELAV axis drives microglia-mediated senescence, amplifies neurotoxic inflammatory signalling, and contributes to both central and peripheral manifestations of MS, including cognitive decline, mood disturbances and gut dysbiosis.

To interrogate this hypothesis the project employs a precision gene regulatory strategy centred on antisense oligonucleotides (ASOs) targeting HuR and selectively modulating nELAV expression, with the aim of restoring a protective ELAV equilibrium. This approach, complemented by HuR-targeting small molecules, is benchmarked against established senotherapeutics to assess its senomorphic and neuroprotective potential. The theoretical framework proposes that ELAV modulation may simultaneously attenuate microglial senescence, enhance neuronal resilience and promote remyelination.

Overall, this work aims to clarify how ELAV-mediated post-transcriptional mechanisms regulate neuroinflammation and senescence-driven degeneration, providing a rationale for innovative antisense-based therapeutic strategies applicable to progressive MS.

[1] I.M. Dighriri et al., *Cureus*., **2023**, *15*, e33242.
[2] V. Borgonetti et al., *Neurotherapeutics*, **2021**, *18*, 412-429.
[3] S. Ryu et al., *Cell Death Dis*., **2022**, *13*, 329.

Abstract 7

DEVELOPMENT OF NEW MELANOGENESIS-MODULATOR PEPTIDES FOR DERMOCOSMETIC APPLICATIONS

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Melanogenesis is the physiological process by which melanin is synthesized in melanocytes and transferred to keratinocytes, playing a crucial role in skin photoprotection and color determination. Dysregulation of this process can lead to hyper- or hypopigmentation disorders, which, although typically benign, may cause significant psychological distress. Current pigmentation-modulating agents often suffer from cytotoxicity or poor bioavailability, highlighting the need for safer and more effective alternatives.
This PhD project focuses on the design and synthesis of novel melanogenesis-modulating peptides inspired by the multifunctional protein AIMP1 (Aminoacyl-tRNA synthetase complex-interacting multifunctional protein 1).[1] Previous studies demonstrated that a peptide derived from AIMP1 (residues 6–46) exhibits anti-pigmentation and anti-aging effects through the downregulation of melanin content and tyrosinase activity.[2]
Building on these findings, this work aims to identify minimal AIMP1-derived sequences that retain melanogenesis-regulating activity while avoiding fibroblast-proliferative effects, undesirable in cosmetic applications due to a possible mitogenic effect on dermal cells.
Eight overlapping peptides covering the AIMP1(6–46) region were synthesized by solid-phase peptide synthesis (SPPS), purified by RP-HPLC or flash chromatography, and characterized by analytical HPLC and mass spectrometry, achieving purities above 95%. Their activity was initially screened through a mushroom tyrosinase inhibition assay using L-DOPA as substrate.[3] None of the peptides displayed significant inhibitory activity, suggesting that melanogenesis modulation may occur through alternative, non-enzymatic pathways or through an indirect mechanism that could be evaluated only by *in vitro* cell model.
We have now begun *in vitro* experiments on B16F10 mouse melanoma cells to assess effects on melanin content, intracellular tyrosinase activity, and cytotoxicity. These investigations aim to determine which regions of AIMP1 (6–46) are responsible for regulating melanogenesis without stimulating fibroblast proliferation, thereby identifying the peptides with the greatest potential as safe and effective dermocosmetic ingredients.
This project is carried out in the framework of a collaboration with Ganassini Corporate.

[1] J. M. Han et al., *Biochem. Biophys. Res. Commun.,* **2006**, *342*, 113–118.
[2] J. Kim et al., *J. Cosmet. Dermatol.,* **2019**, *18*, 251-257.
[3] Y.Y. Liu et al., *Fish Physiol. Biochem.,* **2017**, *43*, 517-525.

Abstract 8

PHARMACOLOGICAL AND FUNCTIONAL ANALYSIS OF GENES INVOLVED IN COLORECTAL CANCER DRUG RESISTANCE

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Background: Colorectal cancer (CRC) ranks second in incidence and third in mortality worldwide. Over 20% of patients present with metastatic disease, and about 40% of early-stage cases relapse within 3 years after surgery. Adjuvant cytotoxic chemotherapy improves survival in high-risk stage II and in stage III patients although clinical outcomes vary widely due to marked CRC heterogeneity. No predictive biomarker of cytotoxic drug efficacy has been adequately validated, aside from a few with prognostic relevance [1]. Our previous studies [2,3] identified and validated *PNN* and *KCNQ1OT1* genes as predictive biomarkers of response to fluoropyrimidine-based chemotherapy in stage II-III CRC patients. Patients with low tumor mRNA expression levels of these genes showed significantly longer disease-free survival.
Aims: To assess drug sensitivity/resistance and *PNN* and *KCNQ1OT1* expression in established CRC cell lines;to define functional differences between the most sensitive and resistant 2D models.
Methods: Cytotoxicity was assessed by SRB assay (IC50) at 540nm. *PNN* and *KCNQ1OT1* gene expression was measured by real-time qPCR (7900HT Fast RT PCR System). Transcriptomic profiles were obtained by RNA-seq (NextSeq 500 Illumina) and analysed by R software.
Results: Among CRC cell lines, HT-29 was the most intrinsically sensitive to fluorouracil whereas H630 to oxaliplatin and SN-38; SW620 was the most resistant to fluorouracil and oxaliplatin, and LoVo to SN-38. Direct correlations were observed as higher IC50 values corresponded to *PNN* and *KCNQ1OT1* higher expression levels. A similar pattern in gene expression levels was found in panels including parental CRC cells and their drug-resistant sublines. Moreover, transcriptomic comparison of the most sensitive and resistant 2D models revealed distinct molecular profiles suggestive of different resistance mechanisms. **Conclusion**: This analysis identified the most suitable 2D models to further characterize CRC drug resistance mechanisms involving *PNN, KCNQ1OT1* and their related gene networks and to explore potential targets among key resistance-associated proteins.

[1] NCCN Guidelines – Colon Cancer – version 4.**2025**.
[2] Mini et al., **2019**, doi: 10.1002/ijc.32326.
[3] Lapucci et al., **2021**, doi: 10.3727/096504020X16056983169118.

Abstract 9

PROGNOSTIC ROLE OF CYTOFLUORIMETRY IN PEDIATRIC CONNECTIVE TISSUE DISEASES: A PROSPECTIVE STUDY

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Pediatric connective tissue diseases (CTDs) such as systemic lupus erythematosus (SLE), juvenile systemic sclerosis (jSSc), pediatric Sjögren's syndrome (pSS), and juvenile dermatomyositis (JDM) are rare, heterogeneous autoimmune disorders characterized by dysregulated immune responses and multi-organ involvement. Despite recent therapeutic progress, the underlying immunopathology of pediatric CTDs—particularly the contribution of B and T lymphocyte subsets—remains poorly defined.

This prospective monocentric study, conducted at the Rheumatology Unit of Meyer Children's University Hospital aims to (1) identify B- and T-cell markers, cytokine and chemokine profiles, and interferon signatures associated with disease activity and outcomes; (2) explore relationships between lymphocyte subsets, disease severity, and therapeutic response; and (3) evaluate whether cytofluorimetric profiles can guide precision medicine by differentiating patients likely to benefit from B-cell– or T-cell–targeted therapies.

During the reporting period, patient recruitment has begun in a secure electronic Case Report Form (eCRF). Laboratory procedures for blood sampling, flow cytometry, cytokine analysis, and interferon profiling were optimized in collaboration with the Immunology Laboratory. Pilot experiments confirmed the technical feasibility of lymphocyte subset characterization using markers such as CD27, CD45RA, CD25, and CXCR5. A research training period at the University of Liverpool provided advanced expertise in immunophenotyping and data integration with autoantibody profiles, strengthening the translational scope of the project. Next steps involve expanding recruitment, completing longitudinal sampling, and integrating immunological and clinical datasets to identify prognostic immune signatures that could inform tailored therapeutic strategies in pediatric CTDs.

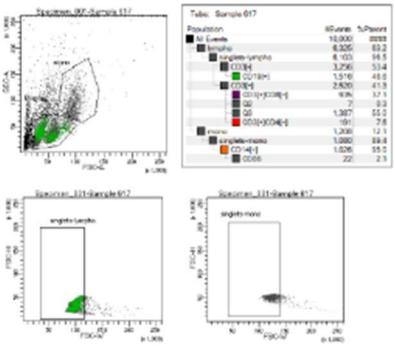

Fig.1 Example of cytofluorimetry analysis performed in Liverpool

[1]. M.D. Rosenblum et al., *J Clin Invest*., **2015**, *125*, 2228–2233.
[2]. A. Boni et al., *RMD Open*, **2024**, *10*, e003800.
[3]. L. Martin-Gutierrez et al., *Arthritis Rheumatol*., **2021**, *73*, 1626–1637.

Abstract 10

DESIGN AND SYNTHESIS OF NEW METALLO-BETA-LACTAMASE INHIBITORS TO FIGHT ANTIBIOTIC RESISTANCE

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The expression of β-lactamases, enzymes capable of inactivating β-lactam antibiotics through a hydrolysis reaction, is one of the most spread and concerning mechanisms of antibiotic resistance. Metallo-β-lactamases (MBL) belonging to the B1 subclass, such as NDM, VIM, and IMP, can hydrolyze β-lactam core thanks to the presence in their active site of two zinc ions, which are essential for the catalytic mechanism [1]. New Delhi Metallo-β-lactamase-1 (NDM-1) is the most concerning one, because of its rapid spread and broad spectrum of action.
A possible strategy to overcome this resistance mechanism is the co-administration of β-lactam antibiotics with MBL inhibitors (MBLI) in resistant bacterial strains. Several MBLI have been studied [1], but none are currently in therapy. Most of the developed inhibitors present a zinc binding group (ZBG), which can coordinate zinc ions [1]. To further investigate the structures of potent and selective MBLI, we designed and synthesized a new series of compounds characterized by the 1,2,4-triazole-3-thione nucleus, as ZBG, connected through an aromatic or aliphatic linker to different basic moieties. The triazolethione ring was selected because it demonstrated good activity on MBLs [2], while the basic groups confer good interaction with the enzyme. Moreover, a second ZBG, the hydroxyl group, was also introduced in some compounds, as derivatives displaying two ZBGs have been reported to be able to efficiently inhibit MBLs [3] (**Figure 1**).

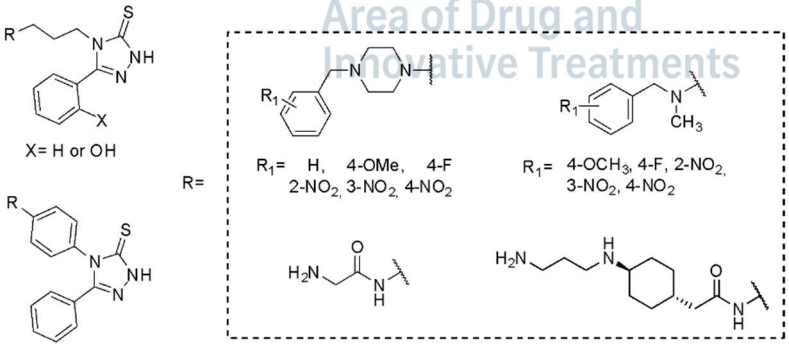

Figure 1: Structures of the designed MBL inhibitors

The inhibitory activity of these new compounds has been evaluated through enzymatic assays on NDM-1, IMP-1 and VIM-2 and through a cellular assay in co-administration with the β-lactam antibiotic Meropenem.

[1] Y.H. Yan et al., *Med. Res. Rev.,* **2020**, *40*,1558-1592.
[2] A. Legru et al., *J. Med. Chem.,* **2022**, *65*, 16392-16419.
[3] S. Dong et al., *J. Med. Chem.,* **2024,** *67*, 3400-3418.

Abstract 11

ROLE OF ADENOSINE A_{2B} RECEPTORS DURING DE- AND RE- MYELINATION PROCESS IN DIFFERENT MOUSE BRAIN REGIONS: AN IN VIVO STUDY

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Oligodendrocytes contribute to the formation and restoration of myelin sheaths in the central nervous system (CNS). They originate from oligodendrocyte precursor cells (OPCs), a pool of proliferating cells that, under appropriate stimuli, migrate throughout the CNS to sustain myelin turnover and repair, a process often impaired in demyelinating disorders. Adenosine signaling plays a pivotal role, through its four G-protein-coupled receptors (A_1, A_{2A}, A_{2B}, A_3), in modulating oligodendrogliogenesis and myelination. Adenosine A_{2B} receptor ($A_{2B}R$) is of interest due to its low affinity for the neuromodulator and its upregulation under pathological conditions related to elevated extracellular adenosine levels. It is known that $A_{2B}Rs$ are expressed in astrocytes, microglia, and oligodendrocytes, where they modulate the release of inflammatory mediators and influence glial reactivity. We studied the role of $A_{2B}R$ signaling on myelin turnover during de- and re- myelination processes induced by cuprizone intake and evaluated whether pharmacological modulation of this receptor could promote neuroprotection and/or glial regulation. Cuprizone is a toxin that triggers demyelination and subsequent remyelination following the cessation of its application. Demyelination in the mice was induced by administration of cuprizone for 5 weeks. PSB-603 (0.05mg/kg), a selective $A_{2B}R$ antagonist and BAY-606583 (0.1mg/kg), a selective $A_{2B}R$ agonist, were intraperitoneally administered starting from week 3 after beginning cuprizone treatment. Remyelination was studied by using the same cuprizone intake duration, but each $A_{2B}R$ ligand was applied for two weeks after the end of cuprizone intake. Immunohistochemical and behavioral analysis were performed. Our results demonstrated that PSB-603, administered during the demyelination phase, protected against behavior impairment, myelin loss and astrocytic and microglia activity caused by cuprizone in different brain regions, corpus callosum, cortex and striatum. On the contrary, no effects of PSB-603 on remyelination process was observed. These findings suggest that inhibiting $A_{2B}R$ may be an effective approach for treating demyelinating diseases.

Abstract 12

MODELING MTOR PATHWAY DYSREGULATION IN PATIENT-DERIVED NEURONS

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The MTOR (mammalian target of rapamycin) gene encodes a pivotal kinase that regulates key cellular processes, including growth, proliferation, motility, survival, transcription, and protein synthesis[1]. Pathogenic variants in MTOR, frequently occurring as somatic mosaicism and resulting in hyperactivation of the mTOR signaling pathway, have been associated with epileptogenic malformations of cortical development[2]. We have previously described a patient presenting with intellectual disability, megalencephaly, polymicrogyria, cutaneous pigmentary mosaicism, and focal epilepsy. The patient carried the MTOR p.Thr1977Ile variant, detected at varying levels of mosaicism across multiple tissues, including skin-derived fibroblasts. Previous studies on electroporated rat cortical neurons demonstrated that this variant induces mTOR pathway hyperactivation and increased cell size, phenotypes that were rescued by treatment with everolimus, an mTOR inhibitor[3]. To confirm functional consequences of the p.Thr1977Ile variant in a human cellular context, we analyzed patient's fibroblasts and confirmed mTOR signaling hyperactivation, which was reversed upon treatment with everolimus and metformin (another mTOR inhibitor). Here, we present preliminary data obtained studying neural progenitor cells (NPCs) differentiated from induced pluripotent stem cells (iPSCs) reprogrammed from patient's fibroblasts. In patient-derived NPCs, we observed mTOR pathway hyperactivation and increased cell size, corroborating the results obtained in rat cortical neurons and patient's fibroblasts. NPCs also exhibited enhanced proliferative capacity and reduced apoptosis compared to control cells, features that are in line with the megalencephalic phenotype observed in the patient. We are now differentiating NPCs in mature neurons to characterize their electrophysiological properties using multi-electrode array (MEA) technology. In the future we aim to evaluate the therapeutic effect of everolimus, metformin, and other targeted compounds that may mitigate mTOR hyperactivation. Our studies are expected to contribute to the development of innovative therapeutic strategies for epilepsy, particularly in drug-resistant cases associated with mTOR-related diseases.

[1] R. Guerrini et al., *Neurol Genet.,* **2020**, *7*, e540.
[2] A.J. Barkovich et al., *Brain,* **2012**,*135*, 1348- 69.
[3] G.M. Mirzaa, et al., *JAMA Neurol.,* **2016**, 73, 836-845.

Abstract 13

NATURAL ACTIVE-BASED NLC HYDROGELS COMBINING POMEGRANATE OIL AND *SEDUM TELEPHIUM* POLYSACCHARIDES FOR PEDIATRIC BURN MANAGEMENT

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Pediatric burns require early intervention to control infection, promote healing, and ensure adequate hydration. Currently, no formulations are specifically designed for children. In this study a natural hydrogel based on pomegranate oil—an active antimicrobial and antioxidant lipid [1] —and polysaccharides extracted from *Sedum telephium* [2] with wound-healing and co-gelifying properties, was developed. Nanostructured Lipid Carriers (NLCs) were employed to incorporate the lipophilic oil into the hydrophilic polysaccharide matrix. Several solid lipids were examined via visual inspection, DSC, and XRD to evaluate solid-state properties and miscibility with liquid lipid; Dynasan 118 was selected for its ability to maximize oil loading and stability. NLCs were prepared using high-shear homogenization and probe sonication [3], achieving mean particle size ~150 nm and PDI ≤ 0.3. *Sedum* polysaccharides were extracted via hot decoction (yield 17%) and characterized by ^{1}H NMR, UV-Vis, and DLS. The optimized NLC dispersion was incorporated into xanthan-based hydrogels (2.5–5% polysaccharides), displaying pH 5.2–5.5, suitable viscosity, spreadability, adhesion, and syringeability for painless pediatric application. Stability studies were performed on NLC and hydrogel over three months. The developed hydrogel has proven to be a promising, biocompatible, antibiotic-free system for early pediatric burn management, combining effective oil delivery with favorable rheological and application properties.

[1] J. Bekir et al., *Food Chem. Toxicol.*, **2013**, *55*, 470-5.
[2] V. Dalla Costa et al., *Molecules*, **2024**, *29*, 2472.
[3] M. Cirri et al., *Int. J. Pharm.*, **2017**, *521*, 73-83.

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Abstract 14

A 3D AIR LIQUID INTERFACE LUNG MODEL TO ASSESS IPF EPITHELIAL-MESENCHYMAL CROSSTALK

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive interstitial lung disease (ILD) with unclear causes and pathogenesis. Recent evidence indicates that IPF involves an abnormal airway epithelial response, including the presence of airway-like cells in the alveoli, epithelial-to-mesenchymal transition (EMT), and impaired epithelial–fibroblast communication. Current studies aim to clarify how this dysregulated crosstalk and EMT contribute to disease progression. New in vitro models that better reproduce patient conditions are urgently needed.
Objectives: Given the complexity of the respiratory tract, which limits high-throughput preclinical modeling, this work aimed to develop a 3D lung model using a human air–liquid interface (ALI) system able to reproduce epithelial–mesenchymal interactions. The model may help elucidate mechanisms relevant to IPF and identify optimal fibrotic phenotype markers through gene expression analysis.
Materials and Methods: Differentiated ALI models from a healthy donor were stimulated from the basal medium with a fibrotic cocktail containing TGF-β (5 ng/ml), PDGF-AB (10 ng/ml), TNF-α (10 ng/ml), and LPA (5 μM). Protein and gene expression analyses were performed on ALI inserts at 24h, 48h, 72h, and 96h after treatment.
Results: Gene expression analysis showed early upregulation of EMT-related genes (COL1A1, COL3A1, VIM, α-SMA) at 24h and 48h. Protein expression of Collagen I and Activin A increased over time, reaching maximal levels at 96h after treatment initiation.
Conclusions: This study examined temporal gene and protein expression changes in an ALI model exposed to fibrotic stimuli. The 3D model appears to be a suitable platform to study dysregulated epithelial–fibroblast crosstalk and EMT, key hallmarks of IPF. It may also support the identification of new therapeutic targets and the testing of potential antifibrotic drugs.

[1] C. Hill et al., *Journal of Lung Health and Diseases*, **2019**, *3*, 31–35.
[2] S.L. Barron et al., *Scientific Reports*. **2024**, *14*, 240.
[3] H.N. Alsafadi et al., *American Journal of Physiology-Lung Cellular and Molecular Physiology*, **2017**, *312*, L896–902.

Abstract 15

COMPARISON OF HS-SPME, VAC-HS-SPME AND HISORB PRE-CONCENTRATION TECHNIQUES FOR PROFILING OF VOLATILE COMPOUNDS IN MONOVARIETAL EXTRA VIRGIN OLIVE OIL USING GC×GC-MS

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Headspace solid-phase microextraction (HS-SPME) is a well-established approach for the extraction of volatile organic compounds (VOCs) in complex food matrices such as extra virgin olive oil (VOO), where active aroma molecules are crucial for quality assessment, sensory profiling and defect identification [1]. Since its introduction in the early 1990s [2], HS-SPME has undergone continuous refinement, with several sampling strategies emerging to improve extraction efficiency for analytes of different volatilities.
This study compares the extraction efficiency of three headspace-based microextraction techniques – standard HS-SPME (R-HS-SPME), vacuum-assisted HS-SPME (Vac-HS-SPME) and HiSorb – for the volatile profiling of monovarietal VOO samples from representative Italian cultivars. All analyses were performed using full two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS). R-HS-SPME and HiSorb were first optimized for extraction time, while Vac-HS-SPME was applied according to previously validated conditions [3]. The evaluation was conducted through a combined targeted/untargeted approach. The targeted analysis focused on 35 volatile organic compounds (VOCs) selected for their relevance in characterizing positive and defective sensory attributes in extra virgin olive oils (EVOO) [1]. Results indicated that Vac-HS-SPME was particularly effective in recovering semi-volatile compounds such as (E,E)-2,4-decadienal, 1-nonanol, 2-octanone, 1-heptanol, guaiacol, and 4-ethylguaiacol—compounds underrepresented by R-HS-SPME and HiSorb, which showed a preference in the extraction of more volatile species. Based on the 35 VOCs, statistical analyses, including one-way ANOVA, Tukey's HSD test, and hierarchical cluster analysis, revealed significant differences between the techniques and confirmed their influence on the resulting volatile fingerprints.
These findings underscore the importance of tailoring the extraction method to the chemical nature of the analytes and matrix complexity. The results contribute to refining analytical workflows for olive oil aroma profiling, and support the strategic application of VOC data for classification, quality control, and sensory guidance in the evaluation of virgin olive oils.

[1] L. Cecchi, M. Migliorini, *J. Agric. Food Chem.*, **2019**, *67*, 9112−9120.
[2] C.L. Arthur, J. Pawliszyn, *Anal. Chem.*, **1990**, *62*, 2145–2148

Abstract 16

THERMOSENSITIVE MULTITARGET HYDROGELS FOR THE TREATMENT OF INFECTED SKIN ULCERS: THE CUTESANA PROJECT

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Wound management represents a significant clinical challenge, with a considerable economic impact on healthcare systems. The onset of infection, which hinder and delay the healing process, accounts for a significant portion of the associated costs. Treatment of infected ulcers typically involves the administration of antibiotics. Unfortunately, the indiscriminate use of the latter has led to an increase in patients with skin lesions infected by antibiotic-resistant bacterial strains (1). Based on these premises, the CUTESANA project aimed to develop a thermosensitive antimicrobial hydrogel based on multi-target molecular hybrids obtained by combining β-lactam antibiotics with carbonic anhydrase inhibitors to counteract antibiotic resistance. The first phase of the study involved determining the Minimum Inhibitory Concentration of the synthesized molecular hybrids against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are among the primary bacterial species found in infected skin ulcers. Based on microbiological screening of 17 molecular hybrids, the compound MR-84 was identified as the most active against the tested strains. Pre-formulation studies were carried out to define the qualitative and quantitative composition of hydrogel. MR-84 at a final concentration of 0.25% (w/v) was loaded into previously optimized thermosensitive hydrogel composed of Poloxamer 407 (22% w/v) and chitosan lactate (0.5% w/v). The formulation was characterized in terms of content uniformity, gelation time, sol–gel transition temperature, and pH. The hydrogel also demonstrated good syringeability, hydration capacity, and adhesion to a simulated wound surface. In addition, both the erosion profile and MR-84 release profile were found to be optimal, ensuring therapeutic coverage for up to 48 hours. Antimicrobial activity was confirmed for both tested strains using zone of inhibition assays. Moreover, the same assay was also conducted against methicillin-resistant *Staphylococcus aureus*. Aligned with CUTESANA goals, the multitarget approach in topical therapy could be a useful alternative to traditional antibiotic therapy.

[1] Lipsky et al., *J. Antimicrob. Chemother.*, **2016**, *71*, 3026–3035.

Abstract 17

USE OF PRECISION THERAPY IN NEURODEVELOPMENTAL DISORDERS AND EPILEPTIC ENCEPHALOPATHIES RELATED TO MUTATION IN THE *GRIN* GENES

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GRIN-related neurodevelopmental disorders and epileptic encephalopathies are related to dysfunction of NMDA receptors (NMDARs) caused by pathogenic variants in *GRIN1*, *GRIN2A*, *GRIN2B*, and *GRIN2D*. Gain-of-function (GoF) mutations often result in early-onset epilepsy, severe developmental delay, intellectual disability, and movement disorders. Radiprodil, a selective negative allosteric modulator of the GluN2B subunit, represents a promising targeted treatment. This multicenter, open-label Phase Ib study investigates the safety, tolerability, pharmacokinetics, and preliminary efficacy of radiprodil as add-on therapy in children aged 6 months to 12 years with GoF *GRIN* mutations. Patients were enrolled in a Seizure Cohort (SC) or Behavioral Cohort (BC). The study included a 5-week screening, a titration phase with individualized dosing based on modeled exposure and safety, and a maintenance period. Three patients treated during the first year showed heterogeneous responses. One child with a *GRIN2A* mutation became seizure-free and exhibited improved interaction. He suddenly died during ongoing follow-up a few months ago; no Adverse Events of Special Interest (AESIs) ore Serious Adverse Events (SAEs) related to the study drug were observed. Two other patients did not show seizure improvement despite dose escalation, leading to treatment discontinuation. Interim analysis from 15 enrolled patients (8 SC, 7 BC) indicates that radiprodil is generally well tolerated. In the SC, 6 of 7 patients showed reductions in countable motor seizures, with a median 86% decrease during the 8-week maintenance phase. Clinically meaningful behavior improvement was also reported by clinicians and caregivers across both cohorts. These findings support further clinical development of radiprodil for *GRIN*-related disorders. Ongoing work includes continued follow-up in Phase III and a new study evaluating serine supplementation for patients with loss-of-function (LoF) *GRIN* variants.

[1] T.A. Benke et al., *Neuropharmacology*, **2021**, *199*, 108805.
[2] W. Han et al., *J. Pharmacol. Exp. Ther.*, **2022**, *381*, 54-66.
[3] H. Wolosker, *Adv. Pharmacol.*, **2018**, *82*, 325-348.

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Abstract 18

EPIGENETIC PROFILE OF PEDIATRIC BEHÇET DISEASE

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Background: Behçet disease (BD) is a chronic multisystem inflammatory disorder with a relapsing-remitting course. Pediatric BD (jBD) is rare, often presents with incomplete phenotypes, and lacks validated biomarkers. MicroRNAs (miRNAs), key post-transcriptional regulators of immune pathways, may play a role in BD pathogenesis.
Objectives: To characterize circulating microRNA (ci-miRNA) expression in pediatric BD and compare it with adult BD patients and healthy controls, exploring potential epigenetic signatures related to disease expression.
Methods: Fourteen pediatric BD patients were enrolled in a monocentric non-interventional prospective study at Meyer Pediatric Hospital IRCCS. Clinical data and peripheral blood samples were collected during the disease course. ci-miRNA levels were quantified by RT-qPCR and analyzed with the 2–ΔΔCq method. Comparisons were performed with 30 adult BD patients and 30 healthy adult controls using the Mann–Whitney U test ($p < 0.01$).
Results: The pediatric cohort had a median age of 85 months (IQR 46) and included 78.6% males. Clinical manifestations were: oral aphthosis 85.7%, genital ulcers 50%, cutaneous signs 57.1%, gastrointestinal involvement 71.4%, ocular manifestations 42.8%, neurological signs 28.6%, joint involvement 28.5%, cardiac signs 7.1%, and vascular thrombosis 7.1%. At the time of sampling, 64.2% of patients had active disease. ci-miRNAs were significantly downregulated in pediatric BD compared with adult BD patients and healthy controls ($p < 0.01$) (Figure 1). No correlation emerged between ci-miRNA patterns and specific clinical phenotypes. The mean interval between diagnosis and sampling was 36.7 ± 21.1 months.
Conclusions: Pediatric BD exhibits a miRNA expression profile markedly different from that of adult patients. Whether these differences reflect distinct age-related epigenetic mechanisms or a divergent underlying pathogenesis between pediatric and adult BD remains unknown. Nevertheless, the findings support ci-miRNAs as promising biomarkers for future precision-medicine approaches in juvenile BD.

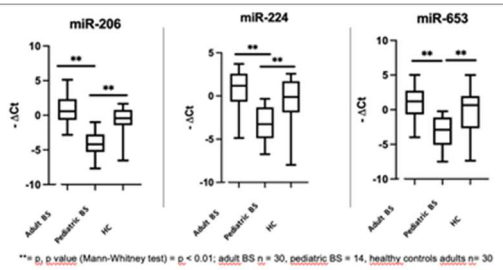

Figure 1 Comparison between mi-RNA expression in the three cohorts

[1] G. Emmi et al., *Ann. Rheum. Dis.*, **2022**, *81*, 386–397.

Abstract 19

SARM1-DRIVEN METABOLIC COLLAPSE AS A NOVEL ANTICANCER STRATEGY

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Cancer cells undergo metabolic reprogramming to sustain their uncontrolled proliferation, with increased NAD^+ synthesis representing a key event that sustains cellular bioenergetics and promotes tumor growth[1]. Attempts to inhibit NAD^+ biosynthesis within neoplasms have shown preclinical potential but limited clinical success due to systemic toxicity and redundancy of compensatory pathways leading to NAD^+ neoformation[2]. Here, we adopt an alternative strategy that triggers NAD^+ depletion from within tumor cells and demonstrate that gene therapy delivering a constitutively active form of the NAD hydrolase SARM1 collapses cancer cell NAD^+ contents and bioenergetics, inducing tumor regression in xenograft models.
In vitro, expression of constitutively active SARM1 in human cancer cells caused rapid reduction of intracellular NAD^+ levels, accompanied by mitochondrial depolarization, impaired ATP production and loss of cell viability. Real-time metabolic profiling using Seahorse assays revealed early impairment of oxidative phosphorylation, accompanied by decreased glycolytic flux and failure to activate compensatory glycolysis upon mitochondrial inhibition, indicating loss of metabolic flexibility and a consequent global energetic collapse. Flow cytometry analysis showed predominant Annexin V/propidium iodide double positive cells, consistent with energy-dependent necrotic cell death. In vivo, SARM1 activation prevented tumor formation and induced regression of established tumors in xenografts models. Bioluminescence imaging confirmed a rapid drop in ATP levels preceding tumor shrinkage, supporting metabolic failure as an early and causal event.
In conclusion, our findings show that forced activation of SARM1 induces catastrophic energetic failure in cancer cells, suppressing tumor growth both in vitro and in vivo. These results support SARM1-driven NAD^+ depletion as a novel promising metabolic gene-therapy approach for cancer treatment.

[1] J. Yong et al., *Front Immunol*., **2023**, *14*, 1269896.
[2] M.S. Ghanem et al., *Nutrients,* **2021**, *13*, 1665.

Abstract 20

DUAL INHIBITION OF CARBONIC ANHYDRASE IX AND GLUTATHIONE PEROXIDASE 4 AS A NOVEL STRATEGY FOR FERROPTOSIS-INDUCED TUMOR CELL DEATH

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In this study, we explored a dual-target strategy combining the inhibition of human carbonic anhydrase IX (hCA IX), a tumor-associated isoform[1], and glutathione peroxidase 4 (GPX4), a key regulator of ferroptosis[2]. We demonstrated that the simultaneous inhibition of hCA IX and GPX4 disrupts redox and iron homeostasis, thereby enhancing cell death via ferroptosis. Three series of compounds were rationally designed and synthesized based on the **ML162** scaffold using an integrated structural approach and their enzymatic inhibition was evaluated *in vitro*. Several dual-target compounds exhibited significant antitumor activity, with **18a–c**, **22abab** and **22abcb** inducing dose-dependent cell death. *In vivo*, intratumoral administration of the lead active compound, **22abcb**, significantly prevented the growth of CA IX-expressing human breast cancer xenografts, compared to inactive **22abbb**. The effect on tumour growth was significantly reversed by the ferroptosis inhibitor, **Fer-1**, confirming ferroptosis as the underlying mechanism[3]. These findings highlight the synergistic potential of dual-target inhibitors in disrupting tumor-specific metabolic pathways and position them as a promising therapeutic strategy for solid tumors.

[1] R. Ronca et al., *Biochim Biophys Acta Rev Cancer*, **2024**;*1879,* 189120.
[2] P.C. McDonald et al., *S. Front Mol Biosci*., **2023**, *10*, 1327310.
[3] M.T. Herrera-Abreu, *Nat Commun*., **2024**, *15,* 9550.

Abstract 21

TOWARD ECO-FRIENDLY SPPS: A CASE STUDY ON ARGIRELINE™ AND PAL-GHK-OH PEPTIDES

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In recent years sustainability has become a growing priority in the cosmetic sector, pushing the industry to reduce the environmentally friendly production of ingredients, including peptides, which are increasingly incorporated as cosmeceutical actives. To reduce the ecological impact of peptide manufacture, this study advances the development of greener solid-phase peptide synthesis (SPPS) workflows by optimizing solvents, reagents, protecting groups, and purification techniques. Two representative cosmetic peptides were chosen as models: i) Argireline, a neurotransmitter inhibiting peptide with botox-like activity due to the interaction with SNARE complex, Ac-EEMQRR-NH2, and ii) GHK, a carrier peptide, H2N-GHK-OH. In particular, we are addressing the following aspects:
i) use of greener solvents and solvent mixtures [1];
ii) specific synthetic problems, i.e. methionine oxidation, arginine protection [3];
iii) alternatives to trifluoroacetic acid to cleave the peptides from the resin [2];
iv) replacement of diethyl ether for the precipitation of the crude peptide;
v) alternatives to the classic HPLC purification such as catch and release technology.

The obtained results confirm that greener SPPS approaches are technically feasible and can maintain product quality. Future efforts will focus on eliminating hazardous piperidine during Fmoc removal and implementing resin and solvent recycling to further enhance sustainability and scalability in peptide production.

[1] L. Pacini et al., *J. Pept. Sci.,* **2024**, *30*, e3605.
[2] J. Pawlas et al., *Org. Lett,.***2024**, *26*, 6787–6791.
[3] M. Alhassan et al., *Int. J. Mol. Sci,.***2020**, *21*, 4464.

Pharma PhD-DAY UNIVERSITY OF FLORENCE Area of Drug and Innovative Treatments

Abstract 22

5,7-DIAMINOTHIAZOLO[5,4-D]PYRIMIDINE DERIVATIVES AS NEW CK1Δ INHIBITORS FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES AND CANCER

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Casein kinase 1δ (CK1δ) is a serine/threonine kinase belonging to the CK1 family. It is involved in different physiological processes, and Its dysregulation has been implicated in various pathological conditions, such as cancer and neurodegenerative disorders, including Parkinson's and Alzheimer's diseases, and amyotrophic lateral sclerosis. Consequently, CK1δ has emerged as a new promising pharmacological target for the development of novel therapeutic strategies [1]. Nevertheless, no CK1δ inhibitors are currently available on the market [1]. The aim of this project was to develop novel CK1δ ATP-competitive inhibitors. Exploiting the structural similarity between adenosine and ATP, and following a scaffold repurposing strategy, we focused attention on a series of our potent and selective adenosine A_{2A} receptor antagonists bearing a 5,7-diaminothiazolo[5,4-d]pyrimidine core [2].Taking inspiration from known CK1δ inhibitors [3], we synthetized a new series of 5,7-diaminothiazolo[5,4-d] pyrimidine derivatives, characterized by a free amino function at position 7 and an amino group at position 5 bearing an arylalkyl chain, or an ethylmorpholine or ethylpiperidine moiety. Furthermore, a phenyl ring, featuring methoxy or hydroxy group(s), was inserted at position 2 (***Figure 1***). The inhibition results confirmed the identification of a new class of potent CK1δ inhibitors (IC_{50} = 0.025–11.9 μM). Overall, the best-performing compounds were those featuring one or more hydroxy groups on the 2-phenyl ring. Among the various substituents introduced on the 5-amino group, the ethylpiperidine moiety emerged as the most favorable, yielding compounds with nanomolar potency. Ongoing molecular modelling studies will clarify the structural features that determine their biological activity and will guide the optimization of their pharmacodynamic and pharmacokinetic profiles. The toxicity of the most active compounds was assessed trough the MTT cell viability test, that highlighted their tolerability at a concentration up to 10 μM. The most promising compounds will be then selected to evaluate their antiproliferative and/or neuroprotective properties.

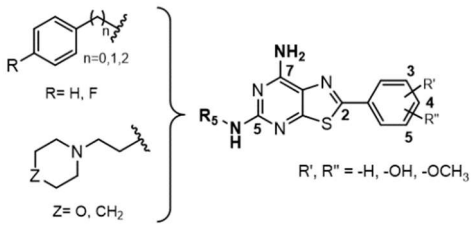

Figure 1

[1] D. Catarzi et al., *Curr. Med. Chem.*, **2022**, *29*, 4698-4737.
[2] F. Varano, et al., *J Med Chem.,* **2016,** *59,* 10564-10576.
[3] I. Grieco et al., *Eur J Med Chem.,* **2021**, *216*, 113331.