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Phd Day Academic Year 2025-2026 of National Institute of Health

Istituto Superiore di Sanità
Rome, October 21, 2026

Edited by
A. Ambrosone, C. Lugli, L. Minghetti and L. Scotti

ISTITUTO SUPERIORE DI SANITÀ

Phd Day Academic Year 2025-2026 of National Institute of Health

Istituto Superiore di Sanità
Rome, October 21, 2026

ABSTRACT BOOK

Edited by
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This volume collects the abstracts of the contributions presented at the "PhD Day academic year 2025-2026 of National Institute of Health. The presence of doctoral students is an important stimulus for the National Institute of Health's research, fostering collaboration and interaction with the university research community. Since 2022, the National Institute of Health has annually funded approximately 40 doctoral scholarships through agreements with various universities across the country, hosting approximately 130 doctoral students. Through this initiative, National Institute of Health aims to enhance its public value by contributing to the increase in the number of doctoral degrees in our country. A PhD Working Group, composed of senior researchers and representatives elected by the PhD students works together to strengthen and support the PhD student community at National Institute of Health.

Key words: PhD Day, Open day

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Questo volume raccoglie i riassunti dei contributi presentati in occasione della "Giornata dei dottorandi anno accademico 2025-2026 dell'Istituto Superiore di Sanità". La presenza dei dottorandi rappresenta un importante stimolo per la ricerca dell'Istituto Superiore di Sanità, favorendo collaborazioni e interazioni con la comunità di ricerca universitaria. Dal 2022, l'Istituto Superiore di Sanità finanzia ogni anno circa 40 borse di dottorato attraverso accordi con varie università del Paese e ospita circa 130 dottorandi e dottorande. Attraverso questa iniziativa, l'Istituto Superiore di Sanità mira ad accrescere il proprio valore pubblico contribuendo all'aumento del numero di dottorati di ricerca nel nostro Paese. Un Gruppo di lavoro costituito da ricercatori senior e rappresentanti eletti dai dottorandi, lavora insieme per rafforzare e sostenere la comunità dei dottorandi e dottorande dell'Istituto Superiore di Sanità.

Parole chiave: PhD Day, Open day

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TABLE OF CONTENTS

Programme iii

Notes for the reader vii

Abstracts 1

Author's index 165

PROGRAMME

Wednesday, 21 Oct 2026

9.15 Opening address
Luisa Minghetti
ISS PhD Committee

Stefano D'Amelio
Sapienza University of Rome

Session I
RARE DISEASES

Chairs: **Maria Luisa Scattoni and Ilenia Laterza**

9.30 *Functional characterization of novel Rho GTPases related mutations causing a heterogeneous spectrum of rare conditions*
Maria Chiara Barone

9.40 *Development of brain organoids from human induced pluripotent stem cells for studying the rare Leukodystrophy Megalencephalic Leukoencephalopathy with subcortical cysts*
Elena Sofia Caprini

9.50 *Muscular and neurological phenotyping of CHKB deficiency: insights from the flipper mouse model*
Raffaella Flammia

10.00 General discussion

Session II
NON-COMMUNICABLE AND METABOLIC DISEASES

Charis: **Francesca Cirulli and Tommaso Bertossi**

10.15 *Dietary supplementation with polyphenols prevents cognitive decline in aged subjects with metabolic syndrome: evidence from animal and human findings*
Letizia Giona

10.25 *Assessment of the risk/benefit of the intake of nutrients and food contaminants with endocrine action on hypothalamic-adipose tissue signalling*
Irene Masciola

10.35 *Effects of the neonicotinoid acetamiprid on the adipose tissue/microglia cross-talk in obesity*
Roberta Alessi

10.45 General discussion

Session III
DIAGNOSTIC AND HEALTH TECHNOLOGY

Chairs: **Alessandro Palombo and Matilda Hushi**

11.00 *Gold nanorods loaded with radiopharmaceuticals for the development of innovative theragnostic techniques in nuclear medicine*
Marco Ranaldi

11.10 *Amantadine-Functionalized copper complexes against glioblastoma: synthesis, characterization, biological evaluation and liposomal encapsulation*
Sofia Migani

11.20 *Wearable technologies for monitoring the cardiovascular system in extreme environments*
Federica Ricci

11.30 *A Torpor-Like Hypometabolic State as a Protective Countermeasure Against Ionizing Radiation and Microgravity in Drosophila melanogaster*
Claudia Di Dio

11.40 General discussion

Session IV
MOLECULAR AND CELLULAR BIOLOGY

Chairs: **Mauro Biffoni and Allen Amburose Stephen**

11.55 *Investigating macroH2A1.1 and macroH2A1.2 in neurodevelopmental trajectories*
Irene Coppola

12.05 *RAD52 overexpression is linked to elevated PRIMPOL recruitment and repriming*
Giorgia Marozzi

12.15 *The E. coli Toxin CNF1 Promotes Colorectal Carcinogenesis via Genomic Instability and Inflammation: Modulation by Olive Oil-Derived Polyphenols*
Michela Tozzi

12.25 *Immunological analysis of the tumor microenvironment in glioblastoma*
Sara Gualdi

12.35 *Role of Neuregulin 1 in the postnatal immune activation mouse model of autism: focus on gut-brain axis*
Maria Ilaria Marino

12.45 General discussion

13.00 Lunch break and Poster session

Session V
PUBLIC HEALTH AND CLINICAL AND EPIDEMIOLOGICAL RESEARCH

Chairs: **Giovanni Capelli and Yanelys Cantillo Villa**

15.00 *Professional and patient care outcomes of e-learning in healthcare continuing education: A systematic review*
Sara Dentice

15.10 *Statistical perspectives in medical diagnostics: challenges and opportunities*
Gloria Remoli

15.20 *Assessing Municipal Fragility through Partially Ordered Sets: A Non-Compensatory Approach for evaluating Territorial Health Inequalities*
Sofia Urbani

15.30 *Cause-specific mortality attributable to summer heat in Italy, 2004-2023: a nationwide Bayesian spatiotemporal study across ecoregions*
Emiliano Ceccarelli

15.40 *Polygenic liability to eating dysregulation and body mass index (BMI) trajectories during the first three years of life: a longitudinal study of the MUBICOS cohort*
Gianmarco Giacomini

15.50 *Assessing dietary intake according to level of food processing: validation study of a novel food-frequency questionnaire in the Italian adult population*
Erica Cardamone

16.00 General discussion

Session VI
INFECTION AND IMMUNITY

Chairs: **Loredana Ingrosso and Corinna Perini**

16.15 *Host immune preparedness shapes disease severity and vaccine responsiveness in respiratory infections*.
Anita Muglia

16.25 *Association Between Immunosuppressive Cell Populations and Immune Response to Influenza Vaccination in Younger and Older Adults*
Daniela Di Placido

16.35 *Interaction of Uropathogenic and Shiga Toxin-Producing* Escherichia coli *Outer Membrane Vesicles with Host Cell Models and Evaluation of Early Inflammatory Signaling*
Giorgia Barbieri

16.45 *Development and implementation of an innovative and safe Pseudovirus-based neutralization assay platform for the analysis of anti-orthoflavivirus neutralizing antibodies in human and animal samples*
Chiara Falce

16.55 General discussion

17.10 Conclusions
ISS PhD Committee and participants

NOTES FOR THE READER

This volume contains the abstracts of all the contributions presented at the 2025-2026 PhD Day of National Institute of Health, held in Rome on 21st October 2026. Contributions have been submitted on a voluntary basis by PhD students conducting their research projects at National Institute of Health and therefore are not exhaustive of all projects and PhD students currently active at the Institute.

For ease of reference, the abstracts are organized as follows. Abstracts of oral presentations are marked with an “O” and ordered as presented in the six sessions of the PhD Day; abstracts of the posters are marked with a “P” and are ordered alphabetically by the authors’ surnames.

A final section contains the abstracts, marked with an "A", of the projects, which are still in their early stages, of the PhD students of National Institute of Health in the 41st doctoral cycle. These are ordered alphabetically by the authors' surnames.

An index of all the authors of each contribution is included at the end of the volume.

ABSTRACTS

O FUNCTIONAL CHARACTERIZATION OF NOVEL RHO GTPASES RELATED MUTATIONS CAUSING A HETEROGENEOUS SPECTRUM OF RARE CONDITIONS

Barone Maria Chiara (1,2), Ronco Alessia Bruna Petronilla (1,2), Ferruti Emma (1,2), Zara Erika (1), Coppola Simona (1)

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Rho GTPases are key regulators of fundamental cellular processes belonging to the RAS super family of small GTPases. Among them, CDC42 and RAC1 control cytoskeletal remodeling, intracellular trafficking, MAPKs signaling, and apoptosis, variably regulating neurodevelopment, skeletal and immune-hematopoietic morphogenesis. In addition, RAC1 plays a key role in innate immunity by promoting the formation of the NADPH oxidase complex (NOX) by interacting with its subunit p67phox. Pathogenic mutations in CDC42, RAC1 and the p67phox encoding gene NCF2 are extremely rare, with CDC42 mutants giving rise to a broad phenotypical range of developmental disorders, RAC1 variants causing neurodevelopmental conditions of different degrees of severity and p67phox mutants being associated with a form of primary immune deficiency, namely chronic granulomatous disease. The aim of this work was to clinically and molecularly characterize novel missense variants in CDC42, RAC1, and NCF2 genes associated with individuals affected by a heterogeneous range of rare conditions. To assess the molecular mechanisms underlying these disease-causing variants, ectopic expression of the mutant proteins was induced in suitable cellular systems. The functional consequences of each variant were then analyzed by assessing protein and cellular function dysregulation (i.e. variants expression levels and activity, interactors binding capability, subcellular localization, MAPK activation and cell spreading). The results were also validated in patient-derived primary cells or in the in vivo model C. elegans (for RAC1 and CDC42 variants, respectively), strengthening the pathophysiological relevance of the findings. Notably, distinct pathogenic mutations within the same Rho GTPase gene were found to differentially perturb protein function and multiple molecular pathways, resulting in diverse functional outcomes, ranging from context-dependent gain-of-function effects to dominant-negative behavior. This work contributes to defining the pathogenic mechanisms underlying the observed phenotypes, thus validating the pathogenicity of newly identified NCF2, CDC42 and RAC1 variants, and ultimately providing affected patients with a molecular diagnosis. Overall, these results broaden the spectrum of pathogenic NCF2, CDC42 and RAC1 variants and provide new insights into the complex molecular behaviors underlying their associated disorders, improving clinical management and genetic counseling in rare diseases.

◉ DEVELOPMENT OF BRAIN ORGANOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR STUDYING THE RARE LEUKODYSTROPHY MEGALENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS

Caprini Elena Sofia (1,2), Lanciotti Angela (1), Brignone Maria Stefania (1), Rosicarelli Barbara (1), Sposito Sara (1), Alfieri Lavinia (1), Veroni Caterina (1), Meloni Chiara (1), Santia Lorenzo (1), Scipioni Angela (3), Nicita Francesco (4), Bertini Enrico (4), Serafini Barbara (1), Ambrosini Elena (1)

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Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC) is a rare leukodystrophy characterized by cerebral edema, subcortical cysts, myelin vacuolation, and astrocyte swelling. Patients show macrocephaly and progressive cognitive and motor impairment featuring ataxia, spasticity, and epilepsy. This disease belongs to astrocytopathies, a group of leukodystrophies distinguished by progressive cystic degeneration of myelin and caused by a primary astrocyte dysfunction. The pathology is mainly associated with mutations in the gene encoding MLC1, an astrocyte-specific protein highly expressed at the astrocyte-astrocyte junctions and the perivascular astrocytic end-feet. Although the precise function of MLC1 remains unclear, several studies have demonstrated its critical role in regulating astrocyte volume, proliferation, and activation in response to injurious stimuli. Using astrocytes differentiated from patient-derived induced pluripotent stem cells (iPSCs), we recently showed that MLC1 mutations lead to astrocyte maturation defects. Given the important roles of astrocytes during neurodevelopment, including the regulation of neurogenesis, synaptogenesis, oligodendrocyte differentiation, myelination, and blood-brain barrier formation, we hypothesized that the resulting dysfunctional astrocytes may be incapable of properly supporting brain development. In this study, we generated iPSC-derived brain organoids to investigate whether impaired astrocyte maturation also affects oligodendrocyte differentiation/maturation, potentially contributing to the myelin abnormalities observed in MLC. Brain organoids were generated from two healthy controls and four MLC patients and analyzed at days 50 and 70 of differentiation using Western blotting, quantitative PCR, and immunofluorescence to assess the expression of astrocytic, oligodendrocytic, and neuronal markers. Both control and MLC-derived organoids successfully developed the major brain cellular populations. However, MLC organoids exhibited reduced expression of mature astrocyte markers at both time points compared to controls. In addition, immunofluorescence at day 50 revealed a marked downregulation of oligodendrocyte markers in MLC organoids. These findings confirm impaired terminal differentiation of MLC astrocytes and suggest that this dysfunction may negatively affect

oligodendrocyte development, a process essential for proper myelination. iPSC-derived brain organoids therefore represent a valuable model to study MLC pathophysiology and a robust platform for future therapeutic screening.

O MUSCULAR AND NEUROLOGICAL PHENOTYPING OF CHKB DEFICIENCY: INSIGHTS FROM THE FLIPPER MOUSE MODEL

Flammia Raffaella (1,2), Macchioni Giorgia (3), Casagrande Andrea (1), Bertuccini Lucia (4), De Luca Gabriele (1), Iorio Egidio (4), Chirico Mattea (4); Pisanu Maria Elena (4), Canese Rossella (4), Palombelli Gianmauro (4), Serafini Barbara (5), De Stefano Maria Egle (6), De Santis Eleonora (7), Calamandrei Gemma (3), Tartaglione Anna Maria (3), Crescenzi Marco (4), Pajalunga Deborah (1)

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Megaconial Congenital Muscular Dystrophy (MCMD) is a rare genetic disorder caused by biallelic loss-of-function mutations in the Choline Kinase beta (CHKβ) gene. In addition to progressive muscular dystrophy, MCMD patients exhibit intellectual disability and behavioral abnormalities, highlighting a significant but poorly understood involvement of the Central Nervous System (CNS). Although transgenic expression of Chkβ has been shown to rescue the muscular phenotype in mouse models, the molecular and cellular mechanisms underlying CNS dysfunctions remain largely unexplored. To investigate the neurological aspects of the disease, we characterized a murine MCMD model, the flp/flp ("flipper") mouse, which carries a loss of function mutation in the Chkβ gene. Initially, we focused on a deeper chatacterization of the muscle defects and then addressed the main goals of our studies: i) define whether the flipper mouse recapitulates the neurological deficits observed in human MCMD, thereby providing a model to initiate the study of CNS defects; ii) identify neurological biomarkers useful in future gene therapy approaches. We showed that Flipper mice exhibited early-onset and persistent motor deficits. Their skeletal muscles present typical dystrophic features like wasting, atrophy, and inflammation. Transmission electron microscopy and cytochrome c oxidase histochemistry revealed giant and abnormal localized mitochondria in skeletal muscle fibers. To identify neurological and brain structural alterations associated to Chkβ deficiency, we performed behavioral tests, histological analyses, and in vivo Magnetic Resonance Imaging (MRI) with our MCMD model. These analyses revealed that flipper mice exhibited increased arousal, as evidenced by intensified ultrasonic vocalizations, during the neonatal period, and increased rearing/jumping behaviors, in adulthood. Importantly, histological and MRI analyses unveiled a significant reduction in cerebral ventricles volume in mutant mice. In addition, ultrastructural analyses

identified enlarged mitochondria in brain tissues as described in muscle fibers. Collectively, we established that the flipper mouse recapitulates several neurological features of human MCMD, providing a valuable model for studying CNS defects. Furthermore, we identified candidate neurological biomarkers that could support the developmen and evaluation future gene therapy approaches.

O DIETARY SUPPLEMENTATION WITH POLYPHENOLS PREVENTS COGNITIVE DECLINE IN AGED SUBJECTS WITH METABOLIC SYNDROME: EVIDENCE FROM ANIMAL AND HUMAN FINDINGS

Giona Letizia (1,2), Zanchi Giulia (3), Monteleone Valentina (3), Musillo Chiara (1), Tait Sabrina (3), Martellacci Noemi (4), Gistro Maria (4), Splendore Amelia (5), Sonnino Rebecca (5), Marra Camillo (4), Giaccari Andrea (5), Varì Rosaria (3), Cirulli Francesca (6), Berry Alessandra (1)

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The lifespan increase observed in the last century has not been paralleled by a similar healthspan trajectory. This is mainly due to the high incidence of age-related pathologies, including dementia and the often observed metabolic comorbidities, characterised by chronic inflammation and Oxidative Stress (OS). In this context, the use of polyphenols has becoming even more attracting due to their multi-target, pleiotropic anti-oxidant and anti-inflammatory effects. However, the precise mechanisms of action of these natural compounds are still unclear. The aim of this research was to evaluate whether dietary supplementations with polyphenols (Rosmarinic Acid - RA - and Curcumin - CUR) may prevent cognitive decline by targeting inflammation and oxidative stress pathways in at-risk individuals with metabolic frailty. In a first set of studies, we assessed the ability of RA to prevent cognitive decline and metabolic alterations in a mouse model of aged mice with Metabolic Syndrome (MetS) induced by Western Diet (WD). We characterised spatial memory, emotionality and glucose homeostasis. In addition, we investigated the transcriptome as well as targeted molecular changes in brain areas involved in cognitive processes and metabolic control (i.e. the Hippocampus - HPC - and the Prefrontal Cortex - PFC). RA prevented WD-mediated memory impairments in all mice, buffering emotionality in females and insulin resistance in WD males. These changes were associated with sex-specific modulations of OS, immune, sex hormone and metabolic signalling in the HPC, and with a boost of NRF2-mediated antioxidant capacity specifically in the PFC of males. A second and most important part of the research project (run in collaboration with a company specialized in phytochemical formulations) was aimed at assessing the efficacy of curcumin with improved bioavailability (MERIVA®) to preserve cognitive functions and to buffer metabolic alterations, inflammation and OS in aged men and women with MetS. Participants were enrolled in a double-blind controlled trial and supplemented with MERIVA® (1g/day p.o.) or placebo for

6 months. Changes in cognitive performance, plasmatic inflammatory and OS markers, glucose and lipid homeostasis and gut microbiota composition was assessed. MERIVA® improved verbal memory and attention span and stabilized glycaemia in women, while improving leptin and insulin sensitivity in men; microbiota composition shifted towards a healthier phenotype upon MERIVA® supplementation. Overall, our data strengthen the body of evidence on the efficacy of polyphenols to prevent age-related cognitive decline and metabolic issues. Intriguingly, the observed effects were long-lasting and gender-specific, suggesting to counteract aging through the modulation of sexually dimorphic pathways. Our data also underline the importance of pro-active healthcare rather than reactive sick care to prevent the burden of chronic age-related diseases.

O ASSESSMENT OF THE RISK/BENEFIT OF THE INTAKE OF NUTRIENTS AND FOOD CONTAMINANTS WITH ENDOCRINE ACTION ON HYPOTHALAMIC-ADIPOSE TISSUE SIGNALLING

Masciola Irene (1,2), Tammaro Alessia (1), Varì Rosaria (1), Silecchia Gianfranco (3), Manella Sara (3), Scazzocchio Beatrice (1), D'Archivio Massimo (1), Tait Sabrina (1)
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Acetamiprid (ACE) is a pesticide representing a risk factor for obesity being a possible metabolic disruptor. In obese subjects, altered secretion of adipokines in adipose tissue (AT) may trigger inaccurate responses from hypothalamus, which regulate the Hypothalamic-Pituitary-Gonadal (HPG) axis through GnRH neurons. Therefore, any alteration of AT-hypothalamus crosstalk may affect reproduction. Nutrients in the diets, like Protocatechuic Acid (PCA), may counteract this effect by its antioxidant activity. Within the PNRR project "HEAL-Italia spoke 7", this PhD study investigated the effects of ACE and PCA on hypothalamus-AT signalling using murine GnRH neurons (GT1-7 cells) and two experimental stages: 1) direct assessment; 2) addition of treated conditioned media of AT from obese patient. 1) GT1-7 neurons were exposed (72 h) to ACE (1-150 nM), PCA (10-1000 μM), or their mixture (ACE 150 nM + PCA 100 μM) then assessing secretion of GnRH and IL-6 by ELISA, gene expression of neuroendocrine (GnRH, Erα, Erβ, Ar, Nkx2.1, Kiss1R) and metabolic (LepR, Igf1, Igf1R, TlR4, AdipoR1) markers by qPCR, and immunofluorescence analysis of Kiss1R, AdipoR1, Igf1R. 2) Human adipocytes from male (n=11) and female (n=12) patients were treated (24 h) with ACE (150 nM), PCA (100 μM), their mix, or culture medium, then assessing leptin, adiponectin, and FFAs secretion. Untreated and treated Adipocyte Conditioned Media (ACM) were then added to GT1-7 cells (72 h) evaluating same endpoints. Drect treatment of GT1-7 cells with ACE stimulated GnRH release but decreased its gene expression; ACE also induced the expression of Kiss1r, Tlr4 Nkx2.1, Igf1, Lepr and AdipoR1. PCA direct treatment reduced IL-6 secretion and Gnrh expression, increasing Lepr and Nkx2.1. Direct treatment with ACE+PCA decreased almost all the genes (ERs, Ar, Nkx2.1, Kiss1r, Igf1, Adipor1, and Igf1r), increasing only LepR. Treatment of ACM samples showed that only in males ACE reduced leptin and increased FFAs, while PCA enhanced leptin levels. The addition of these ACM samples to GT1-7 cells revealed a sexual dimorphism. With untreated samples, only male ACM decreased Ar, LepR and Igf1 gene expression, while both sexes decreased GnRH. For ACM treated with ACE, only male samples induced GnRH secretion and decreased ERs expression; both sexes inhibited GnRH, Ar, Kiss1R, Igf1, AdipoR1 and induced Tlr4. Also for PCA-treated ACM, only male samples decreased GnRH and LepR expression, inducing Kiss1R protein expression; both sexes increased ERa expression while decreasing almost all the genes (Erb,

Ar, Nkx2.1, Kiss1R, Igf1, Tlr4, AdipoR1 and Igf1R). ACM samples treated with the MIX did not exert sex-specific effects, inhibiting the gene expression of all the markers, except GnRH, LepR and Tlr4. Overall, these results evidenced differences in direct and adipocyte-mediated effects, with sex-specific features, providing evidence of metabolic and endocrine disruption exerted by ACE, PCA and their mixture.

O EFFECTS OF THE NEONICOTINOID ACETAMIPRID ON THE ADIPOSE TISSUE/MICROGLIA CROSS-TALK IN OBESITY

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The study investigated the cross-talk between microglia, the brain's immune cells, and the Adipose Tissue (AT) in the context of obesity and exposure to the neonicotinoid pesticide Acetamiprid (ACE), which has recently emerged as harmful to human health. By employing the Human Microglia Clone 3 (HMC3) cell line and Conditioned Media (CM) collected from AT biopsies obtained from obese male and female patients, we evaluated: i) the effects of obesity-related AT signals on microglial immune profile; ii) the impact of molecular signals from ACE-exposed AT on microglia; iii) the potential protective role of the phenolic compound Protocatechuic Acid (PCA); and iv) the direct effects of those substances on microglia. To assess the AT-microglia interaction, we analyzed the expression of inflammatory cytokines (IL-6 and IL-1β), which play key positive roles in the central regulation of glucose homeostasis and energy metabolism. Recent research has demonstrated that the increase of these cytokines in microglia, at least during early phases of obesity, may serve as a protective mechanism that opposes further metabolic alterations. Consistently, we found that CM from AT of obese patients of both sexes induced the expression of IL-6 and IL-1β mRNA in microglia. In addition, our results suggest that ACE may disrupt this homeostatic mechanism both by influencing the AT secretome and by acting directly on microglia, activating pathways that reduce the expression of both cytokines. PCA did not reverse these effects. Furthermore, CM ACE significantly reduced TGF-β gene expression, upregulated the gene expression of the insulin receptor (InsR), and reduced the levels of Glucose Transporter 1 and 4 (GLUT1 and GLUT4). We observed that glucose uptake was impaired in microglia exposed to all CMs from both sexes, suggesting that the obesity-related AT secretome may alter the microglial metabolic profile and glucose sensing. Regarding the direct effects of ACE on microglia, we found that this pesticide altered microglial viability and functional homeostasis, modulated pro-inflammatory and anti-inflammatory cytokines by reducing IL-6 and elevating TGF-β, and increased phagocytosis by altering ITGAM. By contrast, ACE exerted the opposite effects in the presence of lipopolysaccharide (LPS)-induced inflammatory activation, pointing to a context-dependent immunotoxic effect of the

pesticide. To elucidate the underlying mechanism, we investigated the involvement of the nuclear receptor PPARγ, a central hub in the control of microglial activation. We demonstrated that these responses were not mediated by PPARγ, as evidenced by the failure of the selective PPARγ antagonist GW9662 to prevent these effects. A toxic effect of chronic exposure to ACE in microglia was also evident in our data, raising further concerns about the human safety of this pesticide. PCA weakly mitigated the ACE-induced alterations.

O GOLD NANORODS LOADED WITH RADIOPHARMACEUTICALS FOR THE DEVELOPMENT OF INNOVATIVE THERAGNOSTIC TECHNIQUES IN NUCLEAR MEDICINE

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Glioblastoma is a highly aggressive brain tumor characterized by marked radioresistance, which severely limits the efficacy of conventional radiotherapy. To address this clinical challenge, gold nanorods (AuNRs) have emerged as highly promising theragnostic platforms. Due to their unique physicochemical properties and their high atomic number (Z=79), can generate complex radiation-matter interactions, such as the photoelectric effect and Auger electron production, that significantly amplify the biological effects of ionizing radiation. Within the framework of the INFN SEGNAR project, we developed biocompatible cysteine-coated gold nanorods (AuNRs-Cys) to overcome the inherent cytotoxicity of the Cetyltrimethylammonium Bromide (CTAB) surfactant used during seed-mediated synthesis. These nanocarriers are specifically engineered for the loading of radiopharmaceuticals, such as 99mTc-sestamibi, enabling targeted delivery and advanced radiocatalytic applications in nuclear medicine. The final structural configuration of the AuNRs, including successful ligand exchange and radiopharmaceutical/dye conjugation, was rigorously characterized using X-ray Photoelectron Spectroscopy (XPS), alongside UV-Vis and fluorescence spectroscopy. Efficient cellular internalization and intracellular distribution within the T98G human glioblastoma cellular model were verified via transmission electron microscopy (TEM) and confocal imaging. Once internalized, the AuNRs can act as radiocatalytic agents. Upon exposure to gamma irradiation (Cs-137) or the decay of loaded radiopharmaceuticals, the nanorods stimulate the emission of secondary electrons, drastically enhancing localized energy deposition. To fully understand the extent of the cellular damage, the impact on oxidative and metabolic stress following the combined treatment (AuNRs + radiation) was evaluated and compared to control cells subjected to radiation only. Subsequently, the investigation employed high-resolution 1H Nuclear Magnetic Resonance (NMR) for

comprehensive metabolic profiling and the semi-quantification of reduced glutathione (GSH), highlighting a significant decrease in GSH signal intensity in irradiated cells previously exposed to AuNRs-Cys compared to irradiated-only controls. This depletion, coupled with free radical generation, is consistent with enhanced antioxidant consumption and increased oxidative stress, strongly validating the potential of AuNRs-Cys as a highly effective, integrated radiopharmaceutical delivery and radiocatalytic platform to overcome glioblastoma radioresistance.

O AMANTADINE-FUNCTIONALIZED COPPER COMPLEXES AGAINST GLIOBLASTOMA: SYNTHESIS, CHARACTERIZATION, BIOLOGICAL EVALUATION AND LIPOSOMAL ENCAPSULATION

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Glioblastoma is an aggressive malignant primary brain tumor. Despite the combination of surgery, radiotherapy and systemic therapy it is characterized by poor prognosis. In order to overcome the development of resistance and tumour recurrence there is an urgent need to identify less toxic and more effective therapeutic alternatives. In this contest, metal complexes have emerged as promising pharmaceutical agents for cancer therapy. Among them, copper complexes are particularly interesting given that copper is an essential element for cellular growth and development. In this study, novel copper(I) and copper(II) coordination complexes derived from the conjugation between the bis(pyrazol-1-yl) acetate ligand and the biomolecule amantadine, were synthesized. The copper(I) species were stabilized utilizing either lipophilic triphenylphosphine or hydrophilic 1,3,5-triaza-7-phosphaadamantane co-ligands, whereas copper(II) chloride and copper(II) bromide served as acceptors for the copper(II) complexes. The antitumoral efficacy of these newly synthesized Cu(I) and Cu(II) complexes has been evaluated on U87 and LN18 glioblastoma cell lines. Copper complexes bearing PPh3 co-ligands proved to be the most promising in inhibiting cell viability and were therefore selected for further investigation. Cell cycle profile, apoptosis induction, reactive oxygen species production and glutathione levels were investigated through flow cytometry. Morphological and ultrastructural alterations associated with cytotoxic response induced by selected copper complexes were investigated via scanning electron microscopy and Confocal Laser Scanning Microscopy (CLSM). Obtained data showed the induction of cell death through mitochondrial dysfunction and redox-mediated pathways, more evident in LN18 than U87MG cell line. Unfortunately, an obstacle to the clinical use of copper ionophores is represent by their poor solubility and limited accumulation in the tumor site. Indeed, the design of selective and low-toxicity nanocarriers of copper complexes has become an innovative key research focus. With this aim, the most promising copper complex was encapsulated into diverse zwitterionic and anionic liposomal formulations synthetized via thin-film hydration method. Particle size distribution and zeta potential in different dispersions were assessed using multi-angle

dynamic light scattering. Encapsulation efficiencies were quantified via 1H NMR spectroscopy. The biocompatibility of the formulations was evaluated on HaCaT human keratinocyte cell line. MTT assay was selected to prove the efficacy of the copper-loaded liposome formulations on glioblastoma cell lines. Finally, mechanistic validation using the selective copper chelator tetrathiomolybdate confirmed copper-dependent cell death, while CLSM analyses validated intracellular internalization and targeted lysosomal trafficking of the liposomal formulations, as previously demonstrated by flow cytometry.

O WEARABLE TECHNOLOGIES FOR MONITORING THE CARDIOVASCULAR SYSTEM IN EXTREME ENVIRONMENTS

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Extreme environments are significantly challenging to both human physiology and biomedical monitoring technologies. The hyperbaric environment experienced by SCUBA divers represents an extreme scenario characterized by elevated ambient pressure (either water or air), thermal stress, and limited accessibility to conventional medical instrumentation. These conditions require the development of dedicated wearable systems capable of reliably monitoring physiological functions underwater. The main objective of this PhD project is the design, development, and validation of innovative technologies for monitoring the cardiovascular and autonomic system of SCUBA divers. The research focuses on the integration of multiple sensing systems into wearable devices capable of acquiring physiological parameters during underwater activities. During the PhD, various solutions have been developed and tested. A wearable single-lead Electrocardiographic (ECG) system has been implemented to continuously monitor cardiac activity during diving and investigate cardiovascular adaptations to pressure exposure. A non-invasive blood pressure monitoring device has been designed and validated for operation under elevated ambient pressure. Furthermore, an innovative pulse oximetry solution integrated into the SCUBA mouthpiece is being developed for underwater monitoring of peripheral oxygen saturation (SpO2). The current design is based on a preliminary body mapping study in which reflective-mode Photoplethysmographic (PPG) signals were acquired from multiple anatomical sites, including the finger, wrist, ear, temple, nose, and upper and lower lips. Signal quality analyses demonstrated that the lip region provided some of the highest values in terms of Signal Quality Indices (SQIs), supporting its integration into the mouthpiece and enabling physiological monitoring without interfering with diving activities. Beyond cardiovascular and autonomic monitoring, the project explores the assessment of cognitive performance underwater. A novel interactive device is under development to evaluate divers' reaction times at a specific depth. The system consists of a visual panel that generates randomized light stimuli. Divers are required to identify and touch the illuminated target as quickly as possible. This approach aims to provide objective information on responsiveness under hyperbaric exposure. The integration of these technologies represents a step toward the realization of advanced wearable devices for underwater applications. Such systems could support real-time safety assessment, improve risk prevention strategies, and provide new insights into human performance in extreme environments.

A TORPOR-LIKE HYPOMETABOLIC STATE AS A PROTECTIVE COUNTERMEASURE AGAINST IONIZING RADIATION AND MICROGRAVITY IN DROSOPHILA MELANOGASTER

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Long-duration space missions require effective countermeasures against major space-related stressors, particularly ionizing radiation and microgravity. Hypometabolic states have emerged as a promising strategy, as they are associated with increased resistance to oxidative stress, DNA damage and radiation-induced tissue injury. Drosophila melanogaster is a valuable model for space biology due to its genetic conservation with humans and experimental tractability. Here, we investigated whether reversible hypometabolism induced in Drosophila by water immersion produces torpor-like protective features, including increased radioresistance, and whether simulated microgravity modulates these effects. Young adult males and third-instar larvae were induced into a reversible hypometabolic state by immersion in water. Flies and larvae were exposed to 50 Gy and 10 Gy of gamma radiation, respectively, under hypometabolic or normometabolic conditions, and/or subjected to simulated microgravity using a Perfusion Bioreactor System. Survival, fertility, locomotor performance and chromosomal damage were assessed and compared between experimental conditions. Water immersion induced a reversible state of profound metabolic suppression characterized by immobility and complete post-treatment recovery. Larvae tolerated immersion for up to 2 h and adults for up to 6 h while maintaining full viability. Hypometabolism markedly increased radioresistance. In irradiated larval brains cells, IR-induced chromosome breaks were reduced 2.5-fold, accompanied by lower γH2Av levels compared with normometabolic controls. Following 50 Gy irradiation, survival/emergence from larvae was substantially higher under hypometabolic conditions than under normometabolic conditions (80% *vs* 5%). Similarly, young adult males irradiated with 50 Gy retained greater residual fertility when hypometabolic than when normometabolic (49% *vs* 27%). To investigate the molecular basis of this response, RNA-seq analyses are ongoing, with a focus on conserved pathways involved in DNA damage signalling, oxidative stress and tissue-specific protection. We also found that simulated microgravity partially attenuated the radioprotective effect of hypometabolism on residual fertility (49% *vs* 35%), although microgravity alone during transient immersion did not significantly affect viability, lifespan

or locomotor performance. These findings indicate that microgravity can modulate, but does not abolish, the protective effects of hypometabolism. Future studies will dissect the molecular mechanisms underlying this attenuation and employ genetic screening to identify conserved suppressors or enhancers of the response. Overall, our findings establish Drosophila as a promising model to investigate the mechanisms underlying hypometabolism-associated radioresistance and its modulation by microgravity, potentially informing the development of innovative countermeasures for long-duration human space exploration.

O INVESTIGATING MACROH2A1.1 AND MACROH2A1.2 IN NEURODEVELOPMENTAL TRAJECTORIES

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MacroH2A1 is a histone variant expressed as two alternatively spliced isoforms, macroH2A1.1 and macroH2A1.2, both increasingly implicated in brain function and neurodevelopment. Here, we explored the involvement of these two macroH2A1 isoforms in neurobehavioral development by investigating macroH2A1.1 deletion in the context of prenatal Valproic Acid (VPA) exposure, an environmental risk factor for Autism Spectrum Disorder (ASD), and characterizing macroH2A1.2 Knockout (KO) mice under baseline conditions. In both models, male and female Wild-Type (WT) and KO offspring were longitudinally assessed from the neonatal stage to adulthood for ASD-like behavioral phenotype and neurotransmitter levels. For macroH2A1.1, offspring were derived from heterozygous matings, and pregnant females received a single injection of VPA (500 mg/kg) or vehicle on gestational day 12.5. For macroH2A1.2, offspring were obtained from genotype-matched matings. Behavioral alterations associated with macroH2A1.1 loss emerged across development. At the neonatal stage, macroH2A1.1 deficiency increased locomotor activity, an effect modulated by prenatal VPA exposure, which also elevated the number of ultrasonic vocalizations in KO females. During adolescence, VPA induced a significant reduction in both social interaction and anxiety-like behavior across sexes, whereas the impact of macroH2A1.1 deletion was characterized by sex-specific behavioral profiles, with KO males showing a selective increase in repetitive behaviors and KO females exhibiting heightened anxiety-like responses. Beyond the behavioral phenotype, VPA-exposed WT mice showed impaired hippocampal long-term depression at the neonatal stage and reduced serotonin levels in the hippocampus and striatum, with the striatal reduction persisting into adulthood. Notably, similar neurochemical alterations were observed in vehicle-treated KO mice. In the parallel macroH2A1.2 model, neonatal assessment across different postnatal days revealed that deletion of this isoform reduced body weight gain, increased ultrasonic vocalization emission, and selectively altered spontaneous motor behavior, without evidence of a generalized increase in locomotor activity. At the

neurochemical level, KO mice showed a significant reduction in striatal serotonin levels compared with WT mice. Overall, these findings support the involvement of macroH2A1 isoforms in shaping brain and behavioral development, suggesting that macroH2A1.1 loss may modulate susceptibility to neuropsychiatric disorders, while preliminary findings in macroH2A1.2 KO mice point to alterations in early neurobehavioral responses.

O RAD52 OVEREXPRESSION IS LINKED TO ELEVATED PRIMPOL RECRUITMENT AND REPRIMING

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RAD52 is frequently overexpressed and mutated in tumors, although its precise functional role in these contexts remains unclear. Since RAD52 is an emerging regulator of SMARCAL1 recruitment at perturbed forks, we investigated how RAD52 overexpression affected the response to replication stress. Using inducible RPE1 or MRC5-derived cell models overexpressing RAD52 at tumor-relevant levels, we show that elevated levels of RAD52 lead to increased accumulation of DNA gaps compared to control cells. This effect is reversed by PRIMPOL knockout or its transient depletion, indicating that the observed gaps originate from PRIMPOL-mediated repriming events rather than from fork degradation. Consistently, RAD52 overexpression stimulates PRIMPOL recruitment at perturbed forks and its interaction with RPA. Both DNA gaps and PRIMPOL accumulation are prevented in cells overexpressing RAD52 mutants that are unable to form the fork-associated double-ring structure, consistent with functional requirement of the RAD52 gatekeeper activity at replication forks. Consistent with these results, RAD52-overexpressing cells exhibit a significantly increased RAD52 fork localization and RPA interaction compared to control cells. Notably, RAD52 overexpression can prevent fork-reversal-related replication slowing although it stimulates SMARCAL1 fork recruitment, suggesting interference with a productive engagement of SMARCAL1 at stalled forks. Most importantly, RAD52-overexpressing cells exhibit increased sensitivity to Olaparib, suggesting a functional dependence on PARP activity, likely due to the accumulation of unrepaired ssDNA gaps. Collectively, our results suggest that elevated RAD52 expression suppresses productive fork reversal, promoting replication fork restart through PRIMPOL-mediated repriming and conferring sensitivity to Olaparib, making RAD52 levels one potential predictive biomarker for PARPi therapeutic efficacy.

O THE *E. COLI* TOXIN CNF1 PROMOTES COLORECTAL CARCINOGENESIS VIA GENOMIC INSTABILITY AND INFLAMMATION: MODULATION BY OLIVE OIL-DERIVED POLYPHENOLS

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Colorectal Cancer (CRC) is a multifactorial disease driven by the interplay of genetic alterations, chronic inflammation, and environmental factors. Among the bacterial virulence factors increasingly implicated in colorectal carcinogenesis, the *Escherichia coli* toxin CNF1 has been associated with colorectal neoplastic lesions, although its pathogenic role remains only partially understood. This work investigated the contribution of CNF1 to colorectal carcinogenesis and tumor progression in vivo, and assessed the preventive effect of a mixture of olive oil-derived polyphenols (Poly-NaDES) obtained through an eco-friendly, totally biocompatible extraction method. CNF1 induces the release of reactive oxidizing species and chromosomal instability in non-neoplastic intestinal epithelial cells, and alters intestinal permeability by disrupting tight junction distribution in 2D Caco-2 monolayers and impairing the differentiation of 3D Caco-2 spheroids. In vivo, repeated intrarectal administration of

CNF1 induces dysplastic aberrant crypt foci and promotes the formation of colorectal adenomas in an inflammatory-bowel disease mouse model. These effects are accompanied by increased neutrophilic infiltration in colonic tissue, a mixed pro- and anti-inflammatory cytokine profile, and a pro-tumoral shift in fecal microbiota composition. In a colitis-associated CRC mouse model (AOM/DSS), repeated intrarectal administration of CNF1 accelerated tumor progression, as adenocarcinomas were already detected at 6 weeks, whereas only adenomas were observed in AOM/DSS controls, increased the inflammatory infiltrate and supports the development of an adaptive immune response in the spleen. Interestingly, concomitant Poly-NaDES oral administration counteracts tumor development, reduces the inflammatory infiltrate and reduces the activation state of both the innate and adaptive immune response in the spleen suggesting the attenuation of inflammation-driven immune dysregulation. Single-cell transcriptomic analyses are currently being performed on paraffin-embedded colon tissues, to gain deeper insight into the underlying mechanisms, and fecal microbiota analysis are ongoing. In vitro, Poly-NaDES reduces CNF1-induced oxidative stress, attenuates DNA damage response activation, and exerts anti-inflammatory effects by limiting NF-κB nuclear translocation and cytokine release in both intestinal epithelial and immune cells. Overall, these findings identify CNF1 as a bacterial driver of colorectal carcinogenesis acting through genomic instability and inflammation and provide preliminary evidence that olive oil polyphenols can counteract CNF1-induced carcinogenesis, supporting their potential as dietary agents against colorectal cancer.

O IMMUNOLOGICAL ANALYSIS OF THE TUMOR MICROENVIRONMENT IN GLIOBLASTOMA

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Despite advances in oncology, the standard treatment for Isocitrate Dehydrogenase (IDH)-wildtype Glioblastoma (GB) has not changed in over fifteen years, with median survival still below fifteen months, underscoring the urgent need for novel strategies to extend survival and Quality of Life (QoL). GB shows extensive heterogeneity within and across patients, grows in a diffusely invasive manner resisting complete surgical removal, and recurs in nearly all cases. This aggressiveness relates to a microenvironment that reduces anti-tumour immunity, favouring tumour expansion over immune clearance. The lack of reliable biomarkers further limits clinical benefits and hinders immunotherapy strategies, which benefit only a limited number of patients. My doctoral work, within the European IPerGlio project, characterizes the immune landscape of the Tumor Microenvironment (TME) in GBM patients to identify patient-specific biomarkers predictive of immunotherapy response. Business Intelligence (BI) approaches integrate multidimensional datasets, including Lifestyle (LE) data, and Artificial Intelligence algorithms identify clinically relevant biomarker signatures. Blood and tumor samples are collected from GB patients at Fondazione Policlinico Universitario Agostino Gemelli IRCCS following signed informed consent. Blood is processed to isolate Peripheral Blood Mononuclear Cells (PBMCs) and serum; tumor samples yield viable cells and paraffin-embedded tissue blocks. For a subset of patients, bulk RNA-seq was performed on tumor samples and a 78-plex immune Bioplex assay on serum. RNA-seq data were deconvoluted using three independent methods (EPIC, MCP-counter and the GBM-specific GBMdeconvoluteR), consistently showing myeloid/tumour-associated macrophage predominance linked to inflammatory signaling. Patients were classified according to the molecular subtypes (classical, mesenchymal or proneural), revealing distinct cell distributions linked to stratification The Bioplex analysis identified 30 of 78 serum analytes significantly elevated in patients versus healthy donors (FDR<0.05). As a complementary functional approach, co-cultures of patient-derived tumor cells and amplified autologous Tumor-Infiltrating Lymphocytes (TILs) were established. Patient-derived 3D cultures investigated tumour–immune interactions directly, showing TIL homing to the tumor only in selected patients. Overall, these data represent a crucial advancement towards personalized immunotherapy, identifying patient-specific biomarkers to guide treatment selection. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement *ERAPERMED2022-245*.

O ROLE OF NEUREGULIN 1 IN THE POSTNATAL IMMUNE ACTIVATION MOUSE MODEL OF AUTISM: FOCUS ON GUT-BRAIN AXIS

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Neuregulin 1 (Nrg1) is a key developmental neurotrophin with neuroprotective and anti-inflammatory properties. Interaction between Nrg1 mutations and environmental factors, including early life immune activation, may lead to pathophysiological processes typical of Autism Spectrum Disorders (ASD), a heterogeneous group of neurodevelopmental disorders for which genetic susceptibility, neuroinflammation and altered peripheral and gut immune responses have been described. To investigate the role of the interaction between Nrg1 and early immune activation in the pathophysiology of ASD, we used trans-membrane domain Nrg1 mutant mice (Nrg1 TM HET) exposed to Postnatal Immune Activation (PIA), consisting in a postnatal administration of lipopolysaccharide (LPS) to mimic a bacterial infection at the neonatal stage. Additionally, to assess the contribution of gut inflammation in this model, we tested gut-brain axis modulation by the gut-local anti-inflammatory drug Mesalamine (MES). Four weeks after LPS subcutaneous administration, PIA or control mice received either MES or vehicle by oral gavage for 2 weeks. At the end of the treatment, we evaluated the expression of inflammatory and homeostatic genes in brain and gut, as well as their behavioural profile. In the brain, both sexes revealed a strong genotype-related pattern in the expression of the analyzed markers, with Nrg1 TM HET mice showing an overall altered neuroinflammatory and synaptic/neurotransmission-related profile. PIA induced sex-, genotype-, and brain-region-specific alterations, partially restored by MES. Remarkably, Nrg1 TM HET mice, particularly males, failed to mount a typical neuroimmune molecular response following the immune challenge. In the colon of Nrg1 TM HET male mice, baseline mRNA levels of IL-1β and IFN-γ were significantly higher than in WT mice. LPS treatment further increased IFN-γ mRNA expression and MES treatment selectively reduced its expression in these mice. In females, cytokine expression was comparable between genotypes, and PIA increased IL-1β levels in both genotypes. Focusing on behaviour, male Nrg1 TM HET mice exhibited increased locomotor activity and time spent in the central area of the open field, indicative of a disinhibitory/anxiolytic-like profile. Notably, independently of PIA, MES reduced center time in both WT and Nrg1 TM HET mice. Male Nrg1 TM HET mice also showed increased grooming and reduced time spent sniffing the social stimulus, suggesting alterations in repetitive and social behaviours. In contrast, no genotype-related behavioural differences were observed in female mice. Overall, these findings highlight that dysregulated Nrg1 signaling has a profound, sex-dependent impact on brain and gut homeostasis, as well as on reactivity to early-life immune challenges, with important consequences for behavior. Furthermore, MES shows a protective role, especially in males, suggesting a potential beneficial effect that deserves further investigation.

O PROFESSIONAL AND PATIENT CARE OUTCOMES OF E-LEARNING IN HEALTHCARE CONTINUING EDUCATION: A SYSTEMATIC REVIEW

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Background. E-learning is increasingly used for continuing healthcare education, yet its perceived effectiveness on professional and patient outcomes has not been summarised. The aim of the review was to synthesize evidence on the perceived and objectively measured effectiveness of e-learning for continuing educational purposes among healthcare professionals, with particular attention to professional and patient care related outcomes.

Methods. A systematic review was conducted. The review protocol was registered in PROSPERO (*CRD42024545255*) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed. MEDLINE-PubMed, CINAHL, Scopus and the Cochrane Library were searched for studies published from January 2016 to June 2024. Eligible studies included quantitative, qualitative and mixed-methods designs, using comparative and non-comparative approaches, assessing e-learning interventions in postgraduate or continuing education for healthcare professionals and reporting professional and/or patient care outcomes. Study quality was appraised using Joanna Briggs Institute tools. Findings were synthesized narratively and organized using the adapted Kirkpatrick/Barr framework.

Results. Seventeen studies from 16 countries were included, nine quasi-experimental, six cross-sectional and two randomized trials. E-learning was consistently perceived as effective in improving knowledge, confidence, attitudes and selected skills, while evidence of practice change was less consistent and only partly documented in routine care. However, only four studies examined patient safety, quality of care, satisfaction, or infection outcomes, using heterogeneous indicators and follow-up periods.

Conclusions. E-learning appears useful for continuing professional development, especially for improving knowledge, confidence, attitudes, perceived competence and selected skills. However, evidence remains largely self-reported and data on sustained practice change and patient-related outcomes are still limited, heterogeneous and inconsistently measured.

O STATISTICAL PERSPECTIVES IN MEDICAL DIAGNOSTICS: CHALLENGES AND OPPORTUNITIES

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Statistical analyses in medical research often have to deal with departures from the ideal assumptions underlying standard methods. Such departures may arise both from the structure of the problem itself or from limitations in the data available for the analysis. This contribution presents two projects addressing these challenges. The first project examines how covariates can alter diagnostic-test behaviour and the interpretation of accuracy measures. Within the three-class ROC analysis framework, we address the problem of making inferences about the sensitivity at an early stage, fixing the true class fractions, namely the probabilities of correct classification, for the first and third classes. Rather than reporting marginal performance only, the aim is to estimate the covariate-specific true class fraction at early stage conditionally on the remaining two. This approach allows us to investigate how classification accuracy varies with patient characteristics and whether these covariates are associated with the fixed thresholds. It can also help identify situations in which the thresholds may need to be adjusted for clinical use. Theoretical properties of the proposed inferential procedures are established, and their finite-sample performance is assessed through a simulation study. Furthermore, we apply the proposed methodology to data from two independent studies, namely the Alzheimer's Disease Neuroimaging Initiative (ADNI) and the A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF). The second project addresses a different practical problem: the external validation of a prognostic model when no fully-comparable validation cohort is available. The INTERCEPTOR project developed a prognostic model for progression from mild cognitive impairment to Alzheimer's disease. We used data from ADNI for external validation. Although ADNI provides an independent cohort with longitudinal follow-up and several relevant measurements, it does not reproduce the derivation setting exactly: some predictors are unavailable, biomarker measurements rely on different platforms and individual missing data are present. We examine the consequences of these limitations for external validation, combining an applied validation study with a systematic assessment of the statistical strategies used to handle missing and non-comparable information. Overall, the two projects highlight different challenges that arise when statistical methods are applied to real medical data. The first requires methodological development to answer a non-standard inferential question, whereas the second examines how standard validation procedures are affected by limitations in the available data.

O ASSESSING MUNICIPAL FRAGILITY THROUGH PARTIALLY ORDERED SETS: A NON-COMPENSATORY APPROACH FOR EVALUATING TERRITORIAL HEALTH INEQUALITIES

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Background Multidimensional indicators of area-level fragility can support the assessment of health burden and inequalities associated with multiple health determinants. Conventional composite indicators generally allow compensation across dimensions and may conceal differences between territorial profiles. Partially ordered sets (Posets) provide a non-compensatory alternative based on dominance relations. Objectives This study aims to develop a Poset-based indicator of municipal fragility and examine its relationship with mortality outcomes. It also investigates how alternative specifications of the dominance relation affect municipal rankings and classifications. Methods The methodology was applied to the 404 municipalities of Calabria, Southern Italy, using six indicators covering socioeconomic, demographic, housing, and accessibility dimensions. Indicators were discretized into three ordinal fragility levels, generating a full evaluation space of 729 theoretical profiles. In the first stage, profiles were compared using a Component-Wise Poset specification (CW), in which dominance is determined by comparing corresponding indicator levels without introducing a priority structure. Mutual Ranking Probabilities based on lexicographic linear extensions were estimated, and separation scores were normalized to [0,1]. Municipalities were classified into four fragility levels, from the lowest Green to the highest Black, through a two-stage procedure combining an upper-tail threshold for extreme fragility with Jenks natural breaks for the remaining classes. A second analytical stage introduces an alternative dominance relation assigning greater importance to selected indicators. Its comparison with CW will assess the sensitivity of rankings and classifications to the dominance structure. Associations between the fragility indicator and four broad groups of mortality outcomes are also being investigated. Results Under the CW specification, 24.8% of municipalities were classified as Green, 37.1% as Yellow, 27.5% as Red, and 10.6% as Black. Municipalities were distributed across all four levels, with the largest proportion in the Yellow class and more than one-third in the two highest-fragility classes. Conclusions Preliminary results indicate that the CW specification distinguishes different degrees of municipal fragility without reducing the six indicators to a fully compensatory aggregate measure. Comparison with the alternative specification will clarify how indicator priorities affect rankings and classifications. Linking the fragility measures to mortality outcomes will provide an empirical assessment of their relevance for identifying vulnerable territories and health inequalities, potentially supporting evidence-based public health planning and resource allocation.

O CAUSE-SPECIFIC MORTALITY ATTRIBUTABLE TO SUMMER HEAT IN ITALY, 2004-23: A NATIONWIDE BAYESIAN SPATIOTEMPORAL STUDY ACROSS ECOREGIONS

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Background. Italy is among the European countries most affected by heat-related mortality, yet the assessment and quantification of the burden across specific causes of death and different climatic settings remain limited.

Methods. We analysed daily mortality during June-August, 2004-2023, across all inland Italian municipalities, considering eight major ICD-10 chapters (endocrine and metabolic, mental and behavioural, nervous system, circulatory, respiratory, digestive, genitourinary diseases, and external causes) and two age groups (18-64 and ≥65 years). Heat-mortality associations and attributable burden were estimated using Bayesian hierarchical Poisson models, separately across five Italian ecoregions to account for the wide climatic heterogeneity across Italy.

Results. Among adults aged ≥65 years, high temperatures were associated with increased mortality across all causes examined, with the strongest increases observed for endocrine and metabolic diseases, mental and behavioural disorders, and diseases of the nervous system. At the national level, the highest heat-attributable fractions were estimated for nervous system diseases (15.27%, 95% credible interval 14.48-16.07), mental and behavioural disorders (14.63%, 13.61-15.64), and respiratory diseases (12.50%, 11.85-13.16). However, circulatory diseases accounted for the largest absolute burden, with an estimated 83,624 heat-attributable deaths (80,819-86,224) across the 20 summer periods. Heat-related burden varied geographically and was generally greater in the Tyrrhenian and Islands and Apulia and Adriatic Coast ecoregions. Positive associations were also observed among adults aged 18–64 years for several causes, particularly nervous system diseases, although estimates were less precise, with wider credible intervals.

Conclusions. Overall, heat-related mortality extended beyond cardiovascular and respiratory diseases and varied across ecoregions. Our findings reinforce the evidence linking high temperatures to mortality and highlight the need for future research to focus on cause-specific outcomes and high-resolution geographical analyses to better identify vulnerable populations and underlying mechanisms. Heat-related mortality is not confined to older

adults, with evidence of increased mortality also observed among the working-age population for selected causes of death. Accounting for heterogeneity across ecoregions provides a more accurate assessment of populations at risk and offers a framework that can be applied to other countries and regions characterised by similar climatic variability. These findings can support public health authorities in prioritising prevention measures and allocating resources where they are most needed under a warming climate.

O POLYGENIC LIABILITY TO EATING DYSREGULATION AND BODY MASS INDEX (BMI) TRAJECTORIES DURING THE FIRST THREE YEARS OF LIFE: A LONGITUDINAL STUDY OF THE MUBICOS COHORT

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Background. Polygenic liability to eating disorders and BMI has been associated with growth trajectories across childhood and adolescence. However, it remains unclear whether such associations are already detectable during infancy and early childhood.. Therefore, we investigated between an Eating-related Polygenic Score (ED-related PGS) and longitudinal BMI trajectories from birth to 36 months in an Italian twin cohort.

Methods. The analytic sample included 129 children and 677 BMI observations from the MUBICOS cohort. An eating-disorder-related PGS, reported for "recent poor appetite or overeating", was used and a BMI-PGS was included to account for genetic liability to higher body mass. Sex-stratified linear mixed-effects models with natural cubic splines were used to model log-transformed BMI trajectories, including eating-disorder-related PGS, BMI-PGS, genome build, and eating-disorder-related PGS × age spline interactions.

Results. BMI followed a strong non-linear trajectory in both sexes, increasing through the first year of life and declining thereafter. No statistically significant association was observed between ED-related PGS and BMI trajectories in either males or females. The omnibus ED-PGS by age spline interaction was close to significance in males ($F = 2.32$, $p = 0.057$) and not statistically significant in females ($F = 2.07$, $p = 0.085$). Individual spline interaction terms showed nominal associations in both sexes, not supported by the corresponding omnibus tests.

Conclusions. We found non clear evidence that broad eating-related genetic liability is associated with BMI level or BMI trajectory shape during the first 36 months of life, suggesting that associations between genetic liability to eating-related traits and body-size development may not yet be clearly detectable during this early developmental period, although the limited sample size reduces the ability to exclude small-to-moderate effects. Larger longitudinal studies integrating genetic, growth, and behavioral measures are needed to clarify the developmental timing of these associations

O ASSESSING DIETARY INTAKE ACCORDING TO LEVEL OF FOOD PROCESSING: VALIDATION STUDY OF A NOVEL FOOD-FREQUENCY QUESTIONNAIRE IN THE ITALIAN ADULT POPULATION

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Despite the growing body of evidence on the adverse health impact of food processing, particularly Ultra-Processed Food (UPF) consumption, accurately characterising food processing and incorporating this dimension into dietary assessment instruments remains a major methodological challenge. This study aimed to validate a Food-Frequency Questionnaire (FFQ) integrating food-processing information into habitual dietary intake assessment among Italian adults. Based on a comprehensive literature review and previously validated tools, an initial FFQ version was developed and pilot-tested, supporting face and content validity and yielding an improved version. The online, semi-quantitative, self-administered FFQ includes 8 sections and 54 main items, each corresponding to branching questions collecting data on consumption frequency, portion size, and product type, resulting in a total of 116 food items. Food processing was assessed by asking participants to specify the product consumed, including brand and whether it was artisanal, homemade, or industrially produced, and classifying each item a priori using a modified Nova classification accounting for nutritional profile. This version was administered to a sample of Italian adults aged ≥18 years recruited via snowball sampling through institutional mailing lists and social media nationwide. To assess reliability, the FFQ was administered twice, at least 2-3 months apart. Internal consistency was planned to be evaluated using Cronbach's alpha and test-retest reliability by comparing estimates obtained at the two administrations. After each FFQ, participants completed a 4-day Weighed Dietary Record (WDR). Criterion validity was planned to be assessed by comparing FFQ-T0 estimates with WDR data using correlation and agreement analyses. Of the 336 participants who completed the FFQ-T0, 132 completed the first 4-day WDR, corresponding to an attrition rate of 60.7%. Of these, 111 subsequently completed the FFQ-T1, with an attrition rate of 15.9% between the first WDR and the second FFQ administration. To date, 96 participants have completed all procedures (both FFQs and WDRs); however, data collection has not yet been completed. Retention strategies, particularly to reduce attrition between T0 and T1, included direct email contact, individual feedback reports based on FFQ responses, and a healthy lifestyle booklet upon completion. These findings reflect the challenges commonly encountered in similar epidemiological studies, including the substantial participant commitment required and the difficulty of

maintaining engagement throughout all study phases, as well as the conceptual challenges surrounding food processing. They underscore the need to achieve an appropriate balance in dietary assessment tools between simplicity, participant burden, and scientific rigour, while establishing objective and standardised criteria for food-processing classifications.

O HOST IMMUNE PREPAREDNESS SHAPES DISEASE SEVERITY AND VACCINE RESPONSIVENESS IN RESPIRATORY INFECTIONS

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Background and Objectives. Respiratory infections caused by viral and bacterial pathogens such as RSV, IAV, SARS-CoV-2, and Bordetella pertussis are among the leading causes of morbidity and mortality globally. Immune responses to infection and clinical outcomes are shaped by the interaction between pathogen-specific factors and host immune preparedness. This study investigates how host immune profiles and pre-existing immune history influence disease severity and vaccine response, while exploring the mechanisms involved in dysregulated inflammatory and antiviral responses to respiratory infections.

Materials and Methods. We integrated: (i) retrospective analyses of hospitalized clinical cohorts, including infants with pertussis and adult COVID-19 patients across different pandemic periods; (ii) prospective longitudinal studies in healthcare workers assessing early cytokine responses and humoral immunity up to 12 months after XBB.1.5 mRNA vaccination; (iii) *ex vivo* whole-blood stimulation assays in older adults (>65 years) using UV-inactivated respiratory viruses; and (iv) in vitro infection models using A549 and A549-hACE2 respiratory epithelial cells infected with RSV, IAV, or SARS-CoV-2, including pharmacological inhibition of CD38 NADase activity with compound 78c. Cytokines and proteins were assessed by ELLA or western blotting, gene expression by RT-qPCR, antibodies by CLIA, and neutralizing activity by microneutralization.

Results. CXCL10 emerged as an independent predictor of mortality, Intensive Care Unit (ICU) admission, and pneumonia in COVID-19 patients and was also associated with disease severity in infants with pertussis. In vaccinated healthcare workers, early levels of IL-6 and IL-8 were inversely associated with anti-Spike IgG levels at three and six months. Longitudinal analysis at 12-months showed that pre-existing immune history influenced responsiveness, with highly primed individuals displaying attenuated antibody expansion. Over time, antibody trajectories converged, while neutralizing capacity remained stable. Ex vivo stimulation of whole blood from older adults revealed distinct virus-specific cytokine and chemokine profiles. In airway epithelial cells, RSV and IAV, but not SARS-CoV-2, induced CD38 upregulation, while CD38 inhibition modulated virus-induced inflammatory and antiviral responses.

Conclusions. Our findings highlight how different immune response patterns and pre-existing immune history contribute to variability in disease severity and vaccine responsiveness. Overall, these observations support a conceptual framework aimed at

deciphering the determinants of host immune preparedness and their contribution to individual responses to respiratory infections and vaccination, with potential implications for risk stratification and vaccination strategies.

O ASSOCIATION BETWEEN IMMUNOSUPPRESSIVE CELL POPULATIONS AND IMMUNE RESPONSE TO INFLUENZA VACCINATION IN YOUNGER AND OLDER ADULTS

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Seasonal influenza vaccination shows highly variable effectiveness across individuals, particularly in older adults, in whom age-related immune decline compromises protective responses. This study evaluated immune responses to the 2019/2020 quadrivalent influenza vaccine in 75 individuals aged 25-89 years. Influenza-specific antibody titers and virus-neutralizing activity were measured before and four weeks after vaccination, and participants were stratified as high or low responders. We observed that older individuals exhibited increased frequencies of Myeloid-Derived Suppressor Cells (MDSCs) and activated regulatory T cells (Treg) expressing HLA-DR and CD38, consistent with enhanced immunosuppressive activity. Importantly, higher levels of these immunoregulatory populations correlated with poor vaccine responsiveness in both adult and older participants. Additionally, circulating T follicular regulatory (Tfr) cells were elevated in older participants and in low responders, whereas T follicular helper (Tfh) cells remained unchanged, resulting in an increased Tfr/Tfh ratio associated with impaired antibody production. However, when adjusting for possible confounders, most immunoregulatory subsets did not remain independently associated with responder status, except CD38+ Treg cells. These findings suggest that immunoregulatory networks, while essential for controlling chronic inflammation during aging, may limit vaccine-induced immunity. Identifying biomarkers such as MDSC frequency, activated Treg phenotype, and Tfr/Tfh balance may contribute to the identification of individuals with different levels of vaccine responsiveness and could help inform future personalized vaccination strategies.

O INTERACTION OF UROPATHOGENIC AND SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* OUTER MEMBRANE VESICLES WITH HOST CELL MODELS AND EVALUATION OF EARLY INFLAMMATORY SIGNALING

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Uropathogenic *E. coli* (UPEC) and Shiga Toxin-producing *E. coli* (STEC) exploit Outer Membrane Vesicles (OMVs) to deliver virulence factors and engage host tissues. Understanding how these vesicles physically interact with different host cells and activate immune pathways is essential to unravel host-pathogen dynamics. We investigated the structural engagement of OMVs with target cells and characterized the resulting inflammatory response across epithelial and macrophage models. OMVs were isolated from overnight cultures of UPEC (LC2, EC73) and STEC (ED1374) strains via size-exclusion chromatography and characterized by electron microscopy, nanoparticle-tracking analysis, and protein content. Cell viability was assessed to establish safe, sublethal OMV doses. Vesicle-host internalization was verified in human bladder epithelial cells (T-24) and differentiated THP-1 macrophages using microscopy. Pattern recognition receptors and pro-inflammatory mediators screening was carried out via ELISA (4PL regression), with differences analyzed by One-Way and Two-Way ANOVA, followed by Dunnett's/Tukey's post-hoc tests. Cell viability assays revealed that OMV concentrations above 1.25 µg/mL induced cytotoxicity in T-24 cells, while THP-1 macrophages showed no cytotoxicity up to MOI 5000 equivalents across all pathotypes. Fluorescence microscopy confirmed vesicle internalization in THP-1 cells, showing a marked perinuclear localization. Preliminary ELISA screening highlighted distinct kinetics for the first quantified targets, TLR5 and TNF-α. In T-24 cells, both UPEC OMVs significantly shifted TLR5 expression dose-dependently ($p<0.0001$), showing a transient decrease at 0.3 µg/mL followed by a robust upregulation up to the 1.25 µg/mL non-toxic threshold; epithelial TNF-α remained below the limit of detection. Conversely, macrophages exhibited clear pathotype-specific behavior ($p<0.0001$ for OMV-dose interaction). Differentiated THP-1 stimulation with LC2 OMVs triggered a significant increase in TLR5, peaking at 740 pg/mL at MOI 1000, accompanied by a massive TNF-α release up to 264 pg/mL. In contrast, EC73 OMVs did not significantly modulate TLR5 (p=ns) and induced a lower TNF-α response that stabilized around 100 pg/mL. Finally, STEC OMVs left TLR5 undetectable but demonstrated a strong, dose-dependent induction

of TNF-α, peaking at 95 pg/mL at MOI 5000 ($p<0.05$). These findings indicate that *E. coli* OMVs physically engage with host cells, traffic to the perinuclear region of macrophages, and trigger specific inflammatory signals under sublethal doses. Activation profiles vary significantly based on both vesicle concentration and pathotype, outlining distinct host-pathogen interaction dynamics. Further characterization of the complete panel of immune mediators is ongoing to fully map the signaling networks driven by these vesicles.

O DEVELOPMENT AND IMPLEMENTATION OF AN INNOVATIVE AND SAFE PSEUDOVIRUS-BASED NEUTRALIZATION ASSAY PLATFORM FOR THE ANALYSIS OF ANTI-ORTHOFLAVIVIRUS NEUTRALIZING ANTIBODIES IN HUMAN AND ANIMAL SAMPLES

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Background. Emerging and re-emerging arthropod-borne viruses (arboviruses) are an increasing global public health threat, driven by climate change, urbanization and international travel, which facilitate the spread of competent vectors into previously non-endemic areas. In Italy, arboviruses including DENV, WNV, ZIKV and TBEV cause both imported and autochthonous infections, highlighting the need for rapid, accurate and safe diagnostic tools. Molecular assays are essential for arbovirus detection but are limited by the short duration of viremia. Serological detection of anti-viral Antibodies (Abs) is therefore pivotal, although cross-reactivity may complicate diagnosis. Plaque Reduction Neutralization Tests (PRNT) are used as confirmatory assays to detect specific neutralizing Abs (nAbs), but are laborious, time consuming, have low throughput and require live infectious viruses in BSL-3 laboratories.

Aim. This project aims to develop a safe, high-throughput, pseudovirus-based neutralization assay for detecting nAbs against clinically relevant orthoflaviviruses under BSL-2 conditions. We produced Yellow Fever Virus (YFV)-based non-replicating single-round infectious particles expressing luciferase, pseudotyped with envelope prME glycoproteins from representative orthoflaviviruses (SRIP-Luc/prME).

Methods. SRIP-Luc/prME were produced by co-transfecting 293T cells with a luciferase-expressing YFV replicon plasmid, a plasmid expressing the YFV capsid protein, and plasmids encoding the prME proteins of representative orthoflaviviruses. Particles were harvested, titrated on VERO cells and used in a pseudovirus-based neutralization test (SRIP-NT). Serial dilutions of human and animal sera were incubated with SRIP-Luc/prME and added to VERO cells. After 72 hours, nAb titres were expressed as the serum dilution reducing Relative Light Units (RLU) by 50%, 75% or 90% (ID50, ID75 or ID90) compared with SRIP-only treated cells.

Results and Conclusions. SRIP-Luc/prME pseudotyped with orthoflavivirus prME glycoproteins were successfully generated and showed high infectious titres in VERO cells. The SRIP-NT was evaluated using sera from individuals involved in the 2024 DENV-2 Marche outbreak, sera from experimentally TBEV-infected goats, and field animal samples collected

through surveillance programs. The assay efficiently detected and quantified virus-specific nAbs, showing excellent agreement with reference methods. Importantly, the assay was entirely performed under BSL-2 conditions, eliminating the need to handle replication-competent viruses in BSL-3 laboratories. Overall, SRIP-NT represents a safe, robust and versatile platform for rapid quantification of anti-orthoflavivirus nAbs, supporting differential serodiagnosis, surveillance and monitoring of arboviral infections.

P PROMPTING RARE CARE: MAPPING AND ASSESSING GENERATIVE AI PROMPTING PRACTICES AMONG PAEDIATRIC HEALTHCARE PROFESSIONALS IN RARE DISEASE CONTEXTS

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Background and aims. The increasing use of Large Language Models (LLMs) in healthcare raises questions about the competencies required for their responsible use. This is especially relevant in rare diseases, where fragmented expertise and complex clinical trajectories require careful integration and communication of heterogeneous information. This PhD project aims to characterise how healthcare professionals understand, use and formulate prompts for LLMs in rare disease care, and to compare real-world practices with strategies reported in the literature.

Methods and results to date. A preliminary cross-sectional study included 95 paediatric professionals using a shortened 8-item GAAIS and a personal AI interest measure. Positive AI attitudes correlated with greater interest in its use (Spearman ρ=.59; Holm-adjusted p<.001). Rehabilitation (43.2%) and diagnosis (33.7%) were the most anticipated application domains. A systematic review, following PRISMA 2020 guidelines, spans PubMed, Scopus, Web of Science and Cochrane Library (2020-present) to identify peer-reviewed, English-language studies applying LLMs to a rare disease-related clinical task. After deduplication and eligibility screening, 81 unique studies were identified; 20 analysed to date. Diagnosis was the most represented domain (35%), followed by patient/family communication (20%), and long-term monitoring and screening (15% each). Commercial models were used exclusively in 65% of studies; 25% combined them with a medically dedicated model. The 80% of analysed studies reported three or more prompting strategies; zero-shot (85%) and structured/templated prompting (80%) were most common. Full reporting transparency was observed in 40%. Planned activities and expected outcomes. Review findings will inform a bilingual cross-sectional survey of 100-120 paediatric professionals in Catania and Dublin, recruited via the Policlinico G. Rodolico network and Dublin-based networks. The survey will assess real-world LLM use, AI literacy, attitudes (GAAIS), usability needs (BUS-11) and prompt-formulation skills through simulated clinical scenarios. Prompt formulations will be coded against the review's strategy families and compared across participants. Beyond characterising current practice, these findings will inform a prototype clinical LLM interface, developed at IADT drawing on its recognised expertise in human-centred interaction and UX design, to meet clinicians' needs and improve LLM usability in clinical practice. Expected outcomes are recurrent prompting profiles, evidence-practice gaps, and training needs for responsible LLM use.

Conclusions. Preliminary findings indicate broad openness toward AI but substantial heterogeneity in how LLMs are used and reported. Integrating literature-based evidence with clinicians' observed prompting practices can inform targeted educational and methodological recommendations for safer and more effective use of generative AI in paediatric rare disease care.

P LEGAL RESPONSES TO NEW PSYCHOACTIVE SUBSTANCES: IMPACT OF THE REITOX NETWORK AND DIFFERENT REGULATORY APPROACHES IN EUROPE

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The unpredictability of New Psychoactive Substances (NPS), designed to evade existing legislation, challenges drug control and requires rapid regulatory responses. Within the European Union (EU), the REITOX network and the EU Early Warning System (EWS) support monitoring, risk assessment, and scheduling harmonization, while countries outside this system display heterogeneous regulatory approaches. To comparatively analyse the national legal frameworks governing the control of psychoactive substances across European countries both within and outside the REITOX network. By mapping geopolitical trajectories, the study explores how various regulatory systems influence the ability to detect and control emerging substances. The analysis covers REITOX countries (EU Member States, Norway and Türkiye) and strategically relevant non-REITOX countries grouped into four geopolitical trajectories: the Western Balkans, the United Kingdom (UK), Moldova and Ukraine, and Russia and Belarus. Data were collected from primary legal sources, including national drug laws, ministerial regulations, scheduling lists, and reports from the European Union Drugs Agency and the UNODC. Within REITOX countries, EWS-supported systems ensure systematic detection, but responsiveness varies: legislative procedures may delay control by 6-12 months, whereas administrative and emergency measures enable intervention within weeks. Countries such as the Netherlands, Hungary, and the Baltic States use temporary control mechanisms as regulatory bridges until formal scheduling. While most rely on individual substance listing, others adopt generic or analogue-based approaches (e.g., Germany, Ireland), enabling broader and faster coverage. Outside REITOX, fragmentation is more evident. Some Western Balkans countries have irregular updates, such as Bosnia and Herzegovina, which had no revisions from 2011 to 2025 before adding over 100 NPS, whereas Serbia shows more dynamic updating. Russia and Belarus use centralized rapid-scheduling models. Overall, the findings reveal a heterogeneous but structured European regulatory landscape, shaped by the active role of REITOX network and by varying degrees of alignment among non-REITOX countries. Regulatory paths range from gradual alignment in Western Balkans, Moldova, and Ukraine, to post-integration decoupling in the UK, to centralized systems in Russia and Belarus. Although international or REITOX standards offer important reference points, their adoption does not guarantee consistent operational effectiveness, especially where updates are irregular or institutions fragmented. The results suggest that regulatory responsiveness depends more on interactions among legal frameworks, update mechanisms, and institutional coordination than on formal standardization.

P ECOTOXICOLOGICAL ASSESSMENT IN URBAN AQUATIC ECOSYSTEMS EXPOSED TO EXTREME WEATHER EVENTS

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Rome, with a population approaching 3 million, is experiencing an increase in the chemical substances released and released in ways that remain poorly understood. In addition, ongoing climate change is leading to extreme weather events, ranging from heatwaves to extreme rainfall. The environmental fate and dispersion patterns of these pollutants are still largely unexplored and pose potential risks to human health and aquatic ecosystems. Although the Water Framework Directive (WFD) establishes a list of priority substances to be monitored, rapid urbanisation and changing climatic regimes mean that many emerging compounds are not yet adequately monitored. Moreover, the synergistic effects between emerging contaminants and extreme weather conditions are only partially predictable, and many substances remain unidentified. The city of Rome is an emblematic case study: in recent years, intense rainfall events have been recorded, associated with fish mortality events and the detection of contaminants, even at low concentrations. For this reason, this PhD project aims to develop an integrated multidisciplinary strategy to identify these dynamics. The methodology involves a preliminary meteorological characterization and an analysis of urban anthropogenic pressures in the city of Rome, followed by systematic water and biota sampling in areas with high and low anthropogenic impact under baseline conditions (no rainfall for >10 days) and during extreme storm events. The water samples undergo a comprehensive battery of ecotoxicological bioassays: algal growth inhibition tests using Raphidocelis subcapitata (OECD 201), immobilisation tests on Daphnia magna (OECD 202) and neurotoxicity assays, and the Coiling Activity Tail (CAT) test on zebrafish (Danio rerio). In addition, fish specimens (Squalius squalus and Abramis brama) sampled in the same stretches of the River Tiber and timeframes undergo chemical analysis to detect targeted priority compounds (PFAS and priority pesticides) and non-target compounds (emerging contaminants, industrial intermediates and transformation products). Preliminary results confirm the effectiveness of the event-driven sampling strategy and show evident sublethal effects in the organisms tested. Further analyses, combining high-resolution chemical profiling with ecotoxicological endpoints, will provide critical insights into the impacts of climate extremes on urban aquatic environments. The integration of meteorological, chemical and ecotoxicological data will represent the basis for the overall assessment and for the subsequent development of predictive models for the management of urban aquatic ecosystems, also in compliance with the EU WFD objectives.

P EXPOSURE OF WORKERS TO HIGH LEVELS OF ELECTROMAGNETIC FIELDS

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This thesis aims to address the issue of high Electromagnetic Field (EMF) workers' exposures, which can approach and potentially exceed occupational exposure limits. Such exposures can occur within specific occupational categories of workers in industrial (e.g., arc welding), healthcare (e.g., electrosurgery), and telecommunications (e.g., maintenance of base station antennas) sectors. These conditions pose risks of the only currently established health effects of EMF: acute responses related to the electrical stimulation of nerve and muscle tissues at lower frequencies, and body heating at higher frequencies. Compliance with internationally established regulations prevents these health risks through a two-tier system of restrictions based on reference levels and basic restrictions, set respectively on radiometric quantities (measured in the absence of the human body) and dosimetric quantities (internal to the exposed body and linked to biological effects). Nevertheless, exceedances of exposure limits cannot be ruled out, either due to accidental events or because regulations permit derogations from compliance with the Exposure Limit Values (ELVs) for operational requirements (Article 212 of Legislative Decree 81/2008). Investigating exposure in high-exposure occupational scenarios is therefore essential. In some circumstances-such as when reference levels are exceeded, or for highly localized exposures-accurate risk assessment requires evaluating internal physical quantities. Since those quantities cannot be measured directly, numerical dosimetry-utilizing advanced computational techniques and anthropomorphic 3D anatomical models-enables the calculation of dosimetric distributions within the exposed body. The research focuses on radio frequency fields, with emphasis on millimeter-wave frequencies associated with 5G technology. Attention was devoted to Radio Base Stations (RBS) and, looking ahead to Industry 4.0/5.0 scenarios, standalone visors operating at 26 GHz. Simulation studies of maintenance workers exposed to 26 GHz fields and workers wearing these visors constitute the core of this investigation. In parallel, an experimental measurement campaign is being conducted jointly by the ISS, ARPA Lazio, and ISPRA at shared RBS rooftop sites in Rome. The activities cover technologies from 2G to 5G, including co-located 4G and 5G antennas. Broadband and narrowband measurements were performed in restricted maintenance areas to evaluate electric field contributions, focusing on active 5G bands around 3.5 GHz. This thesis integrates numerical dosimetry with field measurements to offer comprehensive insights into exposure levels and health risks, supporting the development of guidelines, practices, and protocols that ensure workers' health in workplace environments.

P EBV INFECTION DRIVES NEUROINFLAMMATION IN THE BRAIN OF MULTIPLE SCLEROSIS DONORS THROUGH IMMUNE EVASION MECHANISMS

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Multiple Sclerosis (MS) is a chronic central nervous system disorder driven by genetics and environment. Epstein-Barr Virus (EBV) infection is a prerequisite for MS and likely sustains chronic neuroinflammation and disease progression, though the exact pathogenic mechanisms remain unclear. This study explored the EBV-MS pathogenetic relationship in progressive MS brain tissue. Objectives: (1) to investigate EBV presence, status, and the related immune response; (2) to analyze intrameningeal Tertiary Lymphoid Organs (TLOs) structure, as EBV-infected B-cell reservoirs, and their role in chronic brain inflammation. The presence of EBV-infected B-cells and EBV-specific cytotoxic T-cells in MS brain indicates failed viral clearance, potentially driven by viral-induced immune evasion. Because PD-1/PD-L1 mediates EBV immune evasion in tumors, we used immunohistochemical technique to check for a similar strategy in MS brains. We found that EBV, both in latent and reactivation phases, upregulates PD-L1 on infected B cells. PD-L1+B cells interact with PD-1+ T cells in inflammatory infiltrates, mainly within meningeal TLOs, suggesting local inhibitory interactions favouring viral persistence. Within TLOs, we found $CD35^{+}$follicular dendritic cell expressing PD-L1, frequently in close contact to $PD\text{-}1^{+}CD4^{+}$ T cells. As in peripheral lymphoid organs, this interaction, may contribute to follicle organization, germinal center formation and B cell (also EBV-infected) survival in MS brain. To further characterize the MS-related immune response, we analysed the presence and distribution in MS brain of the atypical B cells, an antigen-experienced B-cell subset, involved in pathogen-directed immune responses, active infection control and antigen presentation, characterized by the expression of T-bet/TBX21 and CXCR3+. Preliminary results show that CXCR3+ and T-bet+ cells are present in the inflamed meninges, mainly within TLOs. To quantify viral load in pMS brain, we established a ddPCR-based method for EBV DNA detection in post-mortem brain tissue. EBV DNA was detected in a large proportion of analysed samples, with viral load varying according to the degree of tissue B cell inflammation. High-resolution spatial transcriptomic analysis, using the 10x Genomics Visium HD platform, enabled the molecular characterization of EBV-rich inflammatory niches. Preliminary data identified spatially defined gene-expression clusters corresponding to distinct tissue areas. In meningeal TLOs signatures associated with mature B cells and plasma cells, T-cell recruitment and activation, cytotoxic responses, and lymphoid-organizing chemokines have been identified. These complementary approaches aim to provide an integrated view of EBV persistence in the MS brain, linking viral immune evasion, immune-cell

composition, viral burden, and organization of meningeal TLOs, to provide new insights into therapeutic strategies targeting the mechanisms sustaining chronic intracerebral inflammation.

P ERICOV CIRCULATION IN ITALIAN URBAN ECOSYSTEMS: WILDLIFE SURVEILLANCE AND POTENTIAL RISK OF CROSS-SPECIES TRANSMISSION

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Introduction. Since 2002, three zoonotic Coronaviruses (CoVs) - SARS-CoV, MERS-CoV, and SARS-CoV-2 - have emerged, each first described in bat reservoirs, causing severe outbreaks and, in the case of SARS-CoV-2, a pandemic with an estimated 19 million excess deaths. In Europe, the *Erinaceus coronavirus* (EriCoV), a MERS-CoV-like *Betacoronavirus*, has recently been described in the European hedgehog (Erinaceus europaeus) and is considered a potential candidate for future spillover events. In 2022, an insertion coding for the CD200 ortholog - an immunomodulatory cytokine that may help the virus evade the immune system - was identified within the EriCoV genome. This study investigated the presence of CoVs in hedgehog feces from urban wildlife rescue centers, mapped the prevalence of the CD200 insertion, and used molecular modelling and docking to assess the zoonotic potential of EriCoV, evaluating RBD binding affinity to DPP4, ACE2, and APN.

Materials and Methods. Fecal samples from 211 European hedgehogs were collected (2025-2026) from LIPU wildlife rescue centers across six Italian locations (Rome, Magenta, Livorno, Asti, Ficuzza, Ferrara). RNA was extracted (QIAamp Viral RNA Mini Kit) and screened using a pan-coronavirus RT-PCR/nested PCR (RdRp gene), followed by an EriCoV-specific RT-PCR targeting the CD200 insertion site (amplicon length varies with insertion presence/absence). The RBD region was Sanger-sequenced from five positive samples (Lazio) to study its interaction with host receptors. 3D models of DPP4, ACE2, and APN from multiple species (hedgehog, dog, cat, fox, badger, human), and of the hedgehog RBD, were obtained via PDB or homology modelling (SWISS-MODEL); protein-protein docking was performed with HADDOCK 2.4. Given evidence that EriCoV entry via DPP4 may be limited, ACE2 and APN were also included to explore alternative tropism among predators (foxes, badgers) and species sharing the hedgehog's urban habitat (dogs, cats, humans).

Results and Discussion. Pan-CoV screening detected EriCoV exclusively in Rome (24/72, 33.3%). The EriCoV-specific RT-PCR showed 56/211 positive hedgehogs (26.5%), with prevalence ranging from 0% (Ficuzza) to 51.4% (Rome, 37/72), followed by Ferrara (45.5%, 5/11), Livorno (20.0%, 8/40), Asti (12.5%, 3/24), and Magenta (6.4%, 3/47). The CD200 insertion was detected in three areas: Ferrara (n=4), Livorno (n=2), Magenta (n=1).

Docking of five EriCoV RBD variants against host receptors revealed distinct profiles: two variants showed favourable scores against fox and human DPP4; the EriCoV RBD showed strong ACE2 affinity for badger and fox; and fox/badger APN showed strong structural convergence with the hedgehog RBD. These findings suggest wild carnivores could represent potential susceptible hosts, warranting further targeted surveillance. The aim for the future is to test RBD from different Italian regions and analyse their interaction with the described host receptors.

P G6PD DEFICIENCY IS ASSOCIATED TO *PLASMODIUM FALCIPARUM* TRANSMISSION FROM THE HUMAN HOST TO THE ANOPHELES VECTOR

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Malaria remains the most lethal parasitic disease worldwide. According to the World Health Organization, an estimated 263 million cases and nearly 600,000 deaths occurred in 2023. Sub-Saharan Africa accounts for over 90% of global mortality, with young children under five enduring the heaviest burden, representing more than 70% of total deaths, which translates to approximately 2,000 young lives lost each day. Malaria transmission relies on complex interactions among human hosts, Anopheles vectors, and Plasmodium parasites. Indeed, the dynamics of malarial infection do not depend exclusively on the pathogen, but represent the result of an intricate interplay where human host determinants play a decisive role. Peripheral gametocyte density is a key determinant of transmission, as it correlates with the load of the subsequent diploid stage, the ookinete, developing in the mosquito midgut within 24 hours post-blood meal. Although extensive research has demonstrated that malaria has shaped human genetic adaptation, the impact of host genetic diversity on parasite transmission from humans to mosquitoes remains poorly understood. Studies have shown that haemoglobin variants such as HbC and HbS, which are associated with reduced susceptibility to severe malaria compared with wild-type haemoglobin, are also linked to increased gametocyte prevalence or sexual-stage parasite loads, potentially affecting transmission to mosquitoes. In contrast, for glucose-6-phosphate dehydrogenase (G6PD) deficiency, another genetic trait associated with protection against severe malaria, its potential impact on transmission remains unexplored. In this study, we investigated malaria transmission using a novel molecular approach based on the analysis of semi-gravid mosquitoes collected within 24 hours of blood feeding in a malaria-endemic area of Burkina Faso. By extracting nucleic acids from individual mosquitoes, we simultaneously assessed parasite infection and burden, identified the human origin of the blood meal, and characterized selected host genetic traits. Specifically, this study aimed to: (i) detect and

quantify Plasmodium falciparum ookinetes in field-caught, human-fed Anopheles mosquitoes using ookinete-specific molecular markers; (ii) characterise host traits from the blood meal, including sex and the most prevalent G6PD deficiency variant in sub-Saharan Africa, using targeted real-time PCR assays; and (iii) assess the association between these host genetic traits and ookinete burden in mosquitoes. Overall, our study provides a sensitive molecular approach to investigate how human genetic factors influence parasite development within the mosquito vector, providing new insights into host determinants of malaria transmission.

P CYANOBACTERIA AS POTENTIAL RESERVOIRS OF ANTIMICROBIAL RESISTANCE: FROM PHENOTYPIC TO GENOMIC ANALYSIS

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In 2015, the World Health Organization (WHO) classified antibiotic resistance as one of the world's leading health threats. This led to the creation of a global plan based on the "One Health" concept. This approach emphasizes the link between human, animal, and environmental health. Cyanobacteria (CB), ubiquitous photosynthetic prokaryotes mainly found in freshwater habitats, may play a role in the dynamics of antibiotic resistance. They can produce large blooms, that due to eutrophication and climate changes, are increasing in spatial and temporal extension. As prokaryotes, their abundance makes them a possible relevant factor in the spread of antimicrobial resistance. We evaluated the susceptibility and resistance profiles of six freshwater CB strains to the clinically relevant erythromycin (macrolide), ceftazidime and amoxicillin (beta-lactams). Test concentrations ranged from 0.0008 to 0.8 mg/L to simulate antibiotic environmental contamination. Minimum inhibitory concentration assays tests were conducted using an adapted microdilution method in 96-well plates, with 14 days exposure. The organisms tested included three Microcystis aeruginosa strains (PCC 7806, CCAP 1450/6 and CCAP 1450/16) and three filamentous one, Planktothrix rubescens (F1 and F9) and Planktothrix serta (PCC 8927). Whole-genome sequencing and bioinformatic analyses, using a specialized bioinformatics pipeline, were then performed to identify the structural and genetic determinants corresponding to the observed responses. Phenotypic tests revealed inter- and intraspecific variability in antibiotic susceptibility, which spanned over the whole range of concentrations. Filamentous strains exhibited high resistance (>0.8 mg/L) to beta-lactams whereas the CCAP 1450 strains proved significantly more susceptible. Genomic analysis identified a highly conserved intrinsic resistome, dominated by tripartite efflux pumps and the presence of redundant Type II toxin-antitoxin systems, which suggests an enhanced capacity for metabolic dormancy and phenotypic tolerance. The presence of inactivation enzymes spanning all four Ambler classes of beta-lactamases only in the filamentous CB, was consistent with their higher resistance to beta-lactams. The mobilome was found to be potentially highly dynamic, containing abundant active transposases and stable plasmid maintenance markers, underscoring its potentiality to maintain and reshuffle functional traits. Based on these results, this study highlights the potential role of CB as reservoirs of resistance traits and vectors for the horizontal spread of antibiotic resistance genes in aquatic ecosystems, showing the need of more studies to possibly include these photosynthetic prokaryotes in environmental monitoring initiatives as part of the "One Health" approach. Aknlwledgements: this work is the result of the project ISS20-6e08998e0c0e Bando Ricerca Indipendente ISS, in collaboration with DMI

P GOLD NANORODS FOR NUCLEAR MEDICINE THERANOSTICS: ENHANCED RADIATION-INDUCED DNA DOUBLE-STRAND BREAKS IN GLIOBLASTOMA CELLS

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The development of targeted radiotherapy strategies requires a deep understanding of radiation-induced DNA damage mechanisms at the cellular level. Gold Nanorods (AuNRs) functionalized with gamma-emitting radiopharmaceuticals, such as technetium-99m (Tc-99m), represent a promising theranostic platform capable of enhancing localized cytotoxic effects through Auger electron emission. The aim of this work is to investigate the enhancement of DNA damage in T98G glioblastoma cells treated with AuNRs and exposed to gamma radiation. To simulate Tc-99m decay (gamma rays and Auger electrons), cells were irradiated using an external cesium-137 source. DNA damage was assessed through γH2AX immunofluorescence analysis, quantifying foci formation at different time points post-irradiation. Preliminary results demonstrate a significant increase in γH2AX foci in cells treated with the combined AuNRs + gamma radiation compared to radiation alone, suggesting that AuNRs effectively amplify DSB induction. The kinetics of foci resolution also indicate a potential impairment of DNA repair mechanisms in the presence of AuNRs. These findings support the hypothesis that AuNR-mediated Auger electron emission enhances localized DNA damage, providing a rationale for the development of nuclear-targeted nanosystems for improved radiotherapeutic efficacy.

P NEGATIVE INTERACTION BETWEEN CEFTRIAXONE AND ACCELERATED RTMS: IMPLICATIONS FOR GLUTAMATERGIC MODULATION IN TREATMENT-RESISTANT DEPRESSION

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Treatment-Resistant Depression (TRD) remains a major clinical challenge, prompting the investigation of combined pharmacological and neuromodulation strategies. Accelerated repetitive Transcranial Magnetic Stimulation (A-rTMS) is an established intervention for TRD, while ceftriaxone, a β-lactam antibiotic that modulates glutamatergic transmission, has shown antidepressant-like effects in preclinical models of depression. Although both approaches exert beneficial effects independently, their combined impact has not been investigated. We evaluated the effects of ceftriaxone alone and in combination with A-rTMS on depressive- and anxiety-like behaviour in a preclinical model of TRD. Socially-isolated Wistar-Kyoto rats underwent a 4-week Chronic Mild Stress (CMS) paradigm, followed by a 2-day A-rTMS protocol. Animals were assigned to 4 groups: ceftriaxone+active-A-rTMS, ceftriaxone+sham-A-rTMS, vehicle+active-A-rTMS, vehicle+sham-A-rTMS. Ceftriaxone (200mg/kg) or vehicle (saline) were administered intraperitoneally (i.p.) for 8 consecutive days: during the last 6 days of CMS and on the 2 A-rTMS days (prior to stimulation). The A-rTMS protocol consisted of 4 sessions/day with a 55-min inter-session interval (10Hz, 40 pulses/train, 1600 pulses/session, 8 sessions, 16s inter-train interval). Sham groups were exposed to the same manipulation/acoustic effect. Behavioural tests investigating motivational- and apathy-like states, helplessness, social behaviour, anhedonia and anxiety-like behaviour were conducted over the 3 days following the last A-rTMS session. In sham-stimulated animals, ceftriaxone (vs vehicle) improved apathy-like state (splash test,ST), reduced helplessness (forced swim test,FST), and increased motivation (novelty-seeking test, NST) and social engagement (social interaction test,SIT). In contrast, under active A-rTMS, ceftriaxone (vs vehicle) increased anxiety-like behaviour (light-dark test,LDT), reduced

coping-related behaviour (FST), and decreased motivation (NST). Similarly, within ceftriaxone-treated animals, active A-rTMS (vs sham) increased apathy-like state (ST) and anxiety-like behaviour (LDT). Neither ceftriaxone alone nor its combination with A-rTMS affected anhedonia (female urine sniffing test, FUST). This is the first evidence that ceftriaxone alone exerts a broadly beneficial behavioural profile in a TRD model. In contrast, its combination with active A-rTMS, compared to either treatment alone, resulted in an overall worsening of behavioural outcomes, highlighting a negative interaction between the two interventions. Ceftriaxone enhances glutamate uptake via astrocytic GLT-1 upregulation, whereas rTMS modulates cortical excitability and synaptic plasticity, influencing glutamatergic neurotransmission and astrocyte-mediated glutamate regulation. Together, these mechanisms may interact in a non-additive manner, potentially disrupting glutamate homeostasis and leading to impaired behavioural outcomes.

P INTEGRATED ANALYTICAL STRATEGIES FOR PFAS OCCURRENCE AND EXPOSURE ASSESSMENT IN MARINE ECOSYSTEMS

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Emerging chemical contaminants pose significant challenges to environmental protection and human health, particularly in marine ecosystems, where dilution coexists with long-term persistence and accumulation. Among these, per- and Polyfluoroalkyl Substances (PFASs) are of particular concern due to their extreme persistence, high water solubility, and resistance to degradation, which promote widespread occurrence and long-range transport. This study aimed to develop and validate a sensitive multi-residue analytical strategy for PFAS determination in seawater, integrating chemical occurrence data to support exposure assessment in marine ecosystems. A Solid-Phase Extraction–Liquid Chromatography–Tandem Mass Spectrometry (SPE–UPLC–MS/MS) method was optimised for the simultaneous quantification of 30 PFASs. Water samples (500 mL) were subjected to freezing and thawing prior to extraction using Oasis WAX cartridges, followed by LC–MS/MS analysis. Chromatographic separation was achieved using a dual-column setup comprising a Luna Omega PS C18 analytical column coupled with its delay column, with a methanol–water gradient containing ammonium acetate and acetic acid. This configuration enabled the retention of both short- and long-chain PFASs while minimising background contamination. The method demonstrated high analytical reproducibility, consistent chromatographic performance, and high sensitivity, with detection limits ranging from 0.002–0.036 ng/L. A matrix-adapted limit of quantification was applied to account for background contamination in blank seawater, ensuring reliable quantification in environmental samples. The analytical performance achieved supports the generation of high-quality occurrence data suitable for integration into exposure assessment models. The validated method was applied to seawater samples collected during the SeaCare project, developed jointly by the Italian National Institute of Health and the Italian Navy, across the Mediterranean Sea, revealing a widespread but spatially heterogeneous distribution of PFASs. Concentrations were generally below 10 ng/L, with localised maxima reaching 156.71 ng/L in industrialised and port-influenced areas of the Adriatic and Tyrrhenian Sea. The same analytical protocol was subsequently extended to a markedly different environmental context in the Arctic Ocean, providing an opportunity to investigate PFAS occurrence in one of the world's most remote marine environments. Despite the low environmental concentrations observed, the method enabled detection and quantification of a broad range of PFASs across the investigated Arctic samples. The simultaneous determination of regulated PFASs and emerging substitutes demonstrates the capability of the proposed analytical strategy to characterise PFAS occurrence across contrasting marine environments, providing robust occurrence data to support exposure assessment, environmental surveillance and risk-based approaches for emerging contaminants.

P GENOMIC ANALYSIS AND IDENTIFICATION OF LEGIONELLA USING OXFORD NANOPORE TECHNOLOGY IN CLINICAL AND WATER SAMPLES

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Introduction. The global incidence of legionellosis is increasing worldwide, and the need for rapid detection of Legionella is critical to prevent infections and limit contamination of water systems. The aim of this study is to evaluate the direct detection of Legionella in clinical and environmental samples using third-generation sequencing based on Oxford Nanopore Technology (ONT), in comparison with Illumina, possibly distinguishing *Legionella pneumophila* (*Lp*) serogroups and *L. non-pneumophila* (*L. spp*). Here, we present the results from the first year of the study, focusing on environmental samples.

Materials and Methods. To verify the reliability of ONT in identifying Legionella, 20 water samples were collected from five different water systems and analysed by culture and real-time PCR. The protocol with barcoding-24-V14 kit for rapid PCR sequencing, useful for very low concentrations of input DNA, was applied for ONT sequencing. The same DNA samples were sequenced on an Illumina MiSeqDx platform using the Nextera XT library preparation kit with a shotgun approach. To evaluate ONT performance in distinguishing Lp serogroups and *L. spp,* reference ATCC Legionella strains were grown on specific media, and the extracted DNA used for ONT sequencing with the SQK-RBK114.24 protocol on an R10.4.1 flow cell without prior amplification. Sequences were then compared with those present in database. For *L. spp.* discrimination, the discriminatory power of eight gene markers was evaluated.

Results. Sequencing of DNA extracted from water samples yielded comparable results with ONT and Illumina. ONT sequencing of the reference ATCC Lp serogroups demonstrated good genomic coverage compared with the corresponding sequences available in the NCBI database, although a higher number of SNPs was observed. Among the gene markers selected to discriminate *L. spp*, rpoB gene provided good species-level discrimination although a similarity of ≥90% was observed among *L. gormanii, L. bozemanae* and *L. parisiensis*. Two primer panels were evaluated for Legionella pneumophila serogroup discrimination but yielded conflicting results. Indeed, strains belonging to the same serogroup showed variability in the aligned genomic regions, suggesting the need for further investigation for accurate molecular characterization.

Conclusions. ONT represents a promising approach for the identification of Legionella in water samples, although low DNA concentration remains a limiting factor, even though the DNA concentration is of concern for reliable results. Some *L. spp* need further investigation to be correctly discriminated, while for *Lp* serogroups new strategies are required.

P DESIGN AND OPTIMIZATION OF A FULLY WEARABLE PROTOTYPE SYSTEM FOR DOSE MONITORING IN MEDICAL TREATMENTS WITH RADIONUCLIDES

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The use of ionizing radiation in medicine requires accurate monitoring of radiation exposure to assess treatment effectiveness while minimizing the dose delivered to healthy tissues. This is particularly relevant in molecular radiotherapy, where the distribution and kinetics of the administered radiopharmaceutical can substantially differ among patients, resulting in significant variability in the absorbed dose delivered to both target and healthy organs. Patient-specific dosimetry is therefore essential to estimate organ-level absorbed dose and support treatment personalization. Within this framework, the WIDMApp (Wearable Individual Dose Monitoring Apparatus) project aims to develop an innovative system for continuous monitoring of activity distribution inside the patient's body during treatment. By integrating measurements from radiation detectors with patient-specific computational models and dedicated data-analysis algorithms, WIDMApp aims to reconstruct organ-level Time-Activity Curves, providing information for personalized dosimetric assessments. The current research activity focuses on the development of a detection system tailored to the requirements of the WIDMApp framework, combining stable and sufficiently sensitive measurements over prolonged acquisition periods with the compactness and wearability required for clinical applications. The first system was designed as a modular technology in which detectors positioned on the patient's body were connected through cables to a medical-grade trolley housing the multi-channel readout electronics, control unit and power supply. This configuration provided the flexibility required to characterize and validate the detection chain and identify the hardware functionalities needed for the final device. The system is currently operational and has been used for experimental campaigns on an anthropomorphic phantom with controlled dynamic activity distributions, demonstrating reliable measurements under realistic conditions and providing feedback for defining the specifications of the next-generation device. The new prototype, currently under development, features a fully wearable architecture in which the entire acquisition system can be worn by the patient. The compact four-channel readout board integrates analog front-end, digital processing and battery supply into a lightweight device. The main objective is to minimize dimensions and enable prolonged and comfortable use while maintaining the required measurement performance. The electronic architecture, component selection and board specifications have been defined based on the experience gained during the characterization and experimental validation of the first system. The ongoing development represents a significant step towards the clinical translation of WIDMApp, enabling continuous patient monitoring through a practical wearable device and providing the technological foundation for an innovative tool for personalized dosimetry.

P PHAGES AND PHAGE-DERIVED DEPOLYMERASES AS STRATEGIES AGAINST HIGH-RISK CLONES OF CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIA*

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Phage-based strategies are emerging as promising alternatives or adjuvants to antibiotics against multidrug-resistant bacterial infections. This is particularly relevant for Carbapenem-Resistant-*Klebsiella pneumoniae* (CR-Kp), a World Health Organization top-priority pathogen. Among its high-risk lineages, the New Delhi Metallo-beta-lactamase (NDM)-producing ST147 clone caused a major outbreak in Tuscany (2018-2019), highlighting the need for novel therapeutics. This study aimed to isolate and characterize novel phages active against CR-Kp and to investigate the therapeutic potential of their depolymerases. Phages were isolated from river samples collected in Tuscany using clinical CR-Kp hosts and characterized by efficiency-of-plating and lysis kinetics. Candidate depolymerases were identified bioinformatically, recombinantly expressed and evaluated for capsule degradation, complement sensitization and antibiofilm activity. Two phages, Nargle and Mononoke, were isolated using an NDM-producing ST147 strain (cKp33) and a Klebsiella Pneumoniae Carbapenemase (KPC)-producing ST307 strain (cKp1196), respectively. Genome analysis predicted that both phages were strictly lytic and lacked antimicrobial resistance and virulence-associated genes, supporting therapeutic suitability. Nargle infected 29/32 NDM-producing ST147 isolates and targeted KL64 strains, while Mononoke infected KL107 isolates. Both phages suppressed growth in all susceptible isolates, with inhibition lasting from a few hours up to 24 h depending on strain. Halos around phage plaques and capsular specificity suggested the presence of phage-encoded depolymerases. Putative depolymerases selected based on PhageDPO and DePP predictions were recombinantly expressed. Their activity was confirmed by halo formation on bacterial lawns and reduced capsule thickness. Given the capsule's role in serum resistance, the depolymerase effect on complement-mediated killing was investigated. Treatment enhanced complement-mediated killing of the serum-resistant cKp33 isolate, while no additional effect was observed on the intrinsically serum-sensitive cKp1196. Because of depolymerases' potential to degrade polysaccharides within the biofilm matrix, their antibiofilm activity was evaluated on preformed biofilms. However, no reduction in biofilm biomass was observed; conversely, cKp1196 biomass increased after 24 h. In conclusion, this study identifies two novel lytic phages and their

capsule-degrading depolymerases as promising candidates against CR-Kp. While the phages showed direct antibacterial activity, the depolymerase enhanced complement-mediated killing of the serum-resistant cKp33, supporting depolymerase potential in promoting immune-mediated clearance. Although no antibiofilm activity was observed, the different responses of the two strains emphasize the need for strain-specific evaluation. Synergy studies with antibiotics will help clarify the role of depolymerases as antimicrobial adjuvants.

P EX VIVO FUNCTIONAL ASSESSMENT OF RARE CFTR GENOTYPES PREDICTS RESPONSE TO MODULATOR THERAPY

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Cystic Fibrosis (CF) is a life-limiting autosomal recessive disorder caused by pathogenic variants in the CFTR gene, resulting in defective chloride and bicarbonate transport across epithelial cells. The consequent alteration of ion and fluid homeostasis leads to mucus accumulation, chronic inflammation, and progressive multi-organ damage, particularly affecting the respiratory system. CFTR modulators, including Ivacaftor (IVA) and the highly effective triple combinations Elexacaftor/Tezacaftor/Ivacaftor (ETI) or Vanzacaftor/ Tezacaftor/Deutivacaftor (VTD) (commercial names Kalydeco, Kaftrio/Trikafta or Alyftrek, respectively) have transformed CF treatment by targeting the underlying molecular defect. However, therapeutic response may vary among patients, and it is crucial to identify the most effective therapeutic option for each patient, particularly for rare uncharacterized CFTR variants. Patient-derived nasal organoids represent a valuable tool for CFTR theratyping, allowing functional characterization of individual genotypes and their response to available modulators. We previously established and optimized the Forskolin-Induced Swelling (FIS) assay in nasal organoids and evaluated the response of 38 rare CFTR genotypes to CFTR modulators. We found concordance between CFTR activity, measured by nasal organoid FIS assays, and genotype eligibility for the clinical modulators, supporting the use of nasal organoids as a valuable predictive platform for theratyping. Interestingly, S549R/G542X and G551D/621+1G>T genotypes showed a greater response to ETI than to IVA. Here, we further investigated these two genotypes also by optimizing the Ussing chamber assay, the gold-standard approach for measuring CFTR-dependent ion transport. Our results proved concordance between FIS and Ussing chamber assays measurements. Moreover, additional experiments showed marked efficacy of VTD (comparable to ETI). Based on these ex vivo findings, both patients were selected for Kaftrio therapy, considering the limited clinical benefit obtained with the previous prolonged Kalydeco treatment. Preliminary observations (one month time point) showed improved clinical status for both patients. Follow-up is ongoing and will allow to estimate the extent of Katrio efficacy and patient clinical improvement. These findings support the use of patient-derived nasal organoids as a robust and clinically relevant platform for predicting individual responses to the different CFTR modulators. By providing results comparable to those obtained with the Ussing chamber assay and accurately anticipating clinical outcomes, nasal organoids represent a valuable tool for theratyping and precision medicine, particularly for patients with rare or uncharacterized CFTR variants.

P COMPUTATIONAL AND CELLULAR SCREENING OF SARS-COV-2 NSP13 INHIBITORS TO COUNTERACT VIRAL EVASION OF INNATE IMMUNE RESPONSES

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The innate immune response represents the first line of defense against viral infections and is mainly mediated by the production of type I Interferons (IFN-I) following the recognition of Pathogen-Associated Molecular Patterns (PAMPs) by cellular receptors. This response involves the activation of Interferon Regulatory Factor 3 (IRF3), a key transcription factor for IFN-I synthesis. Secreted IFN-I subsequently activate the Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway, inducing the expression of Interferon-Stimulated Genes (ISGs) and establishing an antiviral state. SARS-CoV-2 possesses several proteins with immune evasion functions that promote efficient viral replication.Among them, NSP13 is an superfamily-1B helicase with ATPase activity essential for viral RNA replication within the Replication and Transcription Complex (RTC) and acts as an antagonist of the host interferon response. NSP13 inhibits IFN-I production by impairing IRF3 phosphorylation and activation and interferes with downstream signaling through inhibition of JAK1-mediated STAT1 phosphorylation.The aim of this project is to develop an integrated computational and cellular strategy for the identification and characterization of antiviral molecules able to counteract NSP13-mediated innate immune evasion. Human HEK293T and A549 cells were transiently transfected with an NSP13 expression vector and stimulated with RIG-I agonists, such as M8 and 3p-hpRNA.The effect of selected compounds was evaluated by Western blotting analysis of IRF3 phosphorylation and by luciferase assays based on the IFN-β promoter using the constitutively active phospho-mimetic form of IRF-3 (IRF3-5D).Preliminary results show that NSP13 reduces IRF3 phosphorylation following RIG-I stimulation and inhibits IFN-β promoter activity even in the presence of IRF3-5D. Treatment with selected compounds, previously identified for their capacity to inhibit NSP13 ATPase activity, was found to partially restore IRF3 phosphorylation and IFN-β promoter activity, supporting their potential to counteract NSP13-mediated immunosuppression.In parallel, docking and molecular dynamics studies were performed to characterize the interaction between NSP13 and the selected compounds. Protein-protein docking of NSP13 and IRF3, in the presence and absence of compounds, was also performed to identify interaction surfaces potentially involved in NSP13-mediated inhibition.These analyses provide a structural basis for future validation experiments, including the generation of site-specific mutants of NSP13 and delete forms of the protein, aimed at understanding the molecular mechanism by which NSP13 interferes with the function of IRF3.Finally, a retroviral vector is being generated to obtain stably transduced cell lines constitutively expressing NSP13. This model will provide a more robust and reproducible platform for studying NSP13-mediated mechanisms and screening novel antiviral molecules.

P EVALUATION OF THE CORRELATION BETWEEN CFU AND RLU FOR *ESCHERICHIA COLI* ATCC 25922 USING AN ATP BIOLUMINESCENCE MONITORING SYSTEM

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Verifying the efficacy of sanitization protocols is crucial to preventing infectious risks in high biological risk environments, such as healthcare facilities and catering services. ATP bioluminescence analysis using a bioluminometer is currently widely employed as a rapid surface hygiene monitoring method, with results expressed in Relative Light Units (RLU). However, scientific literature highlights conflicting results, and quantitative conversion between RLU values and actual microbiological contamination (Colony Forming Units, CFU) remains controversial and poorly characterized for individual bacterial species. This study aims to evaluate this method by defining the calibration curve (RLU *vs* CFU correlation) and measurement repeatability for *Escherichia coli* ATCC 25922, thereby defining the analytical reliability limits of bioluminescence technology in surface sanitization monitoring. Instruments used included Hygiena System SURE Plus and Hygiena UltraSnap™ surface ATP Test swabs. For the calibration curve, *E. coli* ATCC 25922 was resuspended in PBS to exclude culture matrix (LB) interference. Bacterial suspensions of known concentrations (1.6×105 - 1.5×107 CFU) were analyzed via plate count and bioluminometer readings (RLU). Concurrently, surface recovery was simulated: 50 µL aliquots of suspension were deposited onto glass slides, dried for 30 minutes, and sampled with ATP swabs. Statistical analysis was subsequently performed. Results: Direct inoculum analysis demonstrated a linear response between RLU signal and bacterial load in the range 1.6×105 - 1.5×107 CFU (R2=0.9108). Repeatability analysis revealed a marked concentration dependence: below 3.8×106 CFU, measurements exhibited high random variability (CV% 34.9 - 67.0%). Above 3.8×106 CFU, signal dispersion decreased significantly, recording a CV% <17% (down to 9.1% at maximum concentrations). Surface contamination RLU values differed significantly from direct inoculum measurements ($p<0.05$ for high loads, $p<0.001$ for low loads). Discussion: The results indicate that the bioluminometer represents a rapid tool for hygiene monitoring, but for *E. coli*, quantitative precision (CV% <17%) is guaranteed only at high bacterial loads (>3.8×106 CFU). At lower loads, high signal dispersion restricts the instrument to qualitative screening. The discrepancy observed upon surface recovery can be attributed to several factors requiring further investigation, including variations in bacterial susceptibility to the lysis reagent under exponential growth or stress conditions, reagent lysis efficiency, surface-induced changes in cellular ATP levels, and other potential mechanisms. Overall, these data provide experimental insights into the efficacy of bioluminescence monitoring for *E. coli*, clarifying its potential and limits. Further experiments will be conducted using other bacterial species to confirm whether the trend observed in these preliminary experiments holds true.

P NGS AND SANGER SEQUENCING FOR THE VIROLOGICAL CHARACTERIZATION OF HBV- AND HCV-POSITIVE BLOOD DONATIONS

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Epidemiological surveillance of transfusion-transmissible infections is an indispensable tool to assess the safety of donated blood. All blood donations must be screened by law for serological and molecular markers of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Viruses (HIV-1 and HIV-2), as well as serological markers for syphilis (*Treponema pallidum*). Prior to donation, donors are selected according to a standard set of eligibility criteria, which are verified through a questionnaire. Due to screening procedures, blood donors represent a healthy subset of the population; still, they serve as a valuable sentinel population for tracking infection dynamics in low-risk demographics. Both HBV and HCV exhibit significant genetic variability: ten HBV genotypes (A to J) have been recognized, with at least 8% nucleotide divergence across the genome. For HCV, eight genotypes (1-8) have been defined, displaying 30-35% nucleotide divergence. The high genomic variability of viruses demands a greater focus on advanced molecular epidemiology, as there is a potential risk for the emergence of strains capable of evading detection by current diagnostic tests. In this context, next-generation sequencing (NGS) enables high-throughput characterization of viral whole genome sequences, going beyond routine screening, which is limited to detecting the presence of the infectious agent. Molecular data can provide updated information on circulating genotypes and subgenotypes in blood donors population, supporting the early detection of any emerging viral strains. Such data can also be integrated with an in-depth analysis of risk factors and mutational analysis in relevant genomic regions for serological/vaccine escape and drug resistance, providing insights for updating diagnostic tests and clinical protocols. Samples from 2022 to 2024 from blood donations testing positive to the mandatory screening for HBV or HCV markers through the transfusion network were included in this study. Epidemiological and demographic data were collected through the SISTRA (Transfusion Services Information System). HBV and HCV molecular characterization by sequencing is ongoing using NGS technologies (Ion Torrent, Oxford Nanopore), for full-length viral genome characterization, and Sanger sequencing, for targeted analysis of a specific genomic region and cross-validation of NGS results. Together, these

approaches allow both high-fidelity targeted sequencing and comprehensive whole-genome analysis, enhancing the accuracy and completeness of viral genetic characterization. The project is part of the Central Action programme of the "Centro nazionale per la prevenzione e il controllo delle malattie" (CCM) of the Ministry of Health.

P NEXT GENERATION PESTICIDE RISK ASSESSMENT IN EUROPE: A DECADE-LONG ANALYSIS OF THE USE OF NEW APPROACH METHODOLOGIES

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The integration of New Approach Methodologies (NAMs) into risk assessment represents a key component of the transition toward Next Generation Risk Assessment (NGRA). NAMs include a broad range of non-animal approaches, e.g. in vitro assays, in silico models, read-across and omics technologies, which aim to reduce reliance on animal testing while providing mechanistic and human-relevant information to support regulatory decision-making1. Within the European Pesticide Risk Assessment (PRA) framework, under Regulation (EC) No 1107/2009, several initiatives are currently underway to promote the integration of NAMs into PRA. This study presents the results of the PARC case study "PESTNAM". The Partnership for the Assessment of Risks from Chemicals (PARC) aims to strengthen the scientific basis of chemical risk assessment by bridging research and regulatory needs and facilitating the uptake of innovative approaches, including NAMs, into regulatory practice. PESTNAM is part of WP6 "Innovation in regulatory risk assessment", which aims to identify research needs and facilitate the uptake of innovative approaches into risk assessment. A retrospective analysis of pesticide dossiers and assessment reports from the last decade was conducted to investigate the use of NAMs across the main toxicological endpoints, including acute and repeated toxicity, genotoxicity, and carcinogenicity, reproductive and developmental toxicity, neurotoxicity, toxicity of metabolites, and endocrine-disrupting properties. In addition, specific analyses were performed on In Vitro Comparative Metabolism (IVCM) studies 2. The analysis indicates that NAMs are increasingly included in regulatory submissions, although their use varies considerably across toxicological endpoints. NAM-derived evidence was most frequently used to support weight-of-evidence evaluations, provide mechanistic information, investigate species relevance, and support metabolite and endocrine disruption assessments. In contrast, examples where NAMs directly informed regulatory conclusions or were used as stand-alone evidence were less frequent. The analysis also identified several factors influencing regulatory uptake, including the availability of endpoint-specific guidance, methodological maturity, standardization, and the ability of NAM outputs to address specific regulatory

questions. Stakeholder involvement, ongoing EFSA initiatives and collaborative efforts at the European level are discussed, highlighting the need for harmonized guidance, dedicated OECD Test Guidelines, and targeted training to support a consistent regulatory implementation of NAMs in PRA.

P STATISTICAL LEARNING FOR RT-QUIC KINETICS PHENOTYPING IN PRION DISEASE

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Prion diseases, such as Creutzfeldt-Jakob disease, are invariably fatal neurodegenerative disorders whose clinical heterogeneity makes early diagnosis challenging. The Real-Time Quaking-Induced Conversion (RT-QuIC) assay has revolutionized antemortem detection by amplifying the pathogenic prion protein (PrPSc) with near-perfect specificity. However, routine diagnostics typically reduce the continuous RT-QuIC fluorescence curve to just two metrics: the lag phase and the maximum fluorescence intensity. This two-parameter reduction discards the striking heterogeneity of the full temporal profile, erasing latent biological information regarding pathological burden and strain properties. Exploiting this hidden data requires new analytical methods that keep reaction kinetics biologically legible. To address this, we propose an end-to-end statistical framework that captures the complete fluorescence distribution over time. We modeled the PrPSc seeding activity of cerebrospinal fluid samples from 301 patients using a five-parameter growth-decay kinetic model. We then applied a three-way (Tucker3) tensor clustering approach to the unreduced time courses across all technical replicates. This methodology successfully identifies distinct latent kinetic phenotypes and provides a compact, interpretable description of curve morphology. By capturing the complete reaction regime, this data-driven grouping can distinguish prion disease variants. Ultimately, this framework can help move neurodegenerative diagnostics away from simple binary thresholds and toward precision medicine.

P DESIGN AND DEVELOPMENT OF AN ANALYTICAL WORKFLOW FOR THE CHARACTERIZATION OF POLYSTYRENE NANOPLASTICS

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Nanoplastics (NPs) represent a major analytical challenge due to their nanoscale dimensions, structural heterogeneity, and complex physicochemical behavior in suspension. Since no single technique can fully characterize their morphological, optical, and colloidal properties, reliable assessment requires an integrated workflow combining complementary analytical approaches. To this end, this study presents a multi technique framework designed and developed for the characterization of Polystyrene (PS) NPs. Monodisperse PS standards with nominal diameters of 20, 60, 100, and 200 nm were first characterized individually to establish a baseline of their physicochemical properties. Field Emission Scanning Electron Microscopy (FESEM) was used to assess particle morphology and dry state dimensions, Dynamic Light Scattering (DLS) to determine ensemble averaged hydrodynamic size and colloidal stability, and Nanoparticle Tracking Analysis (NTA) to obtain number weighted size distributions and particle concentrations. The standards were subsequently combined into a polydisperse suspension to simulate a heterogeneous NP sample and separated using Asymmetric Flow field flow fractionation (AF4). Online coupling of AF4 with Multi-Angle Light Scattering (MALS), Quasi-Elastic Light Scattering (QELS), and UV Diode-Array Detection (UV-DAD) provided complementary information throughout the elution profile, enabling the characterization of particle populations in terms of hydrodynamic size, radius of gyration, and concentration-dependent optical responses. AF4 fractions were then collected and analyzed offline by FESEM and NTA to further evaluate separation performance and investigate potential changes induced by the fractionation process. These analyses provided additional information on particle morphology, size distributions, and concentrations after separation, allowing comparison with the initial characterization and assessment of consistency among the different analytical techniques. Overall, the integration of complementary analytical methods enhances confidence in NP characterization, facilitates cross-validation among measurements, and contributes to the development of more harmonized strategies for the assessment of NPs in complex environmental and biological matrices.

P QUALITY AND SAFETY OF LIVE BIOTHERAPEUTIC PRODUCTS: MOLECULAR AND GENOMIC TOOLS UNDER PH. EUR. MONOGRAPH 3053

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Introduction Ph Eur 3053 establishes strict quality and safety criteria For Live Biotherapeutic Products (LBPs), i.e. medicinal products containing one or multiple live microbial strains (bacteria or yeasts) intended for human use. Quality compliance necessitates identity, quantity and viability determination of the microorganisms comprising the product. Concerning safety, transferable Antibiotic Resistance Genes (ARGs) and genes encoding virulence factors should be excluded. Aims 1) Development of a novel multiplex-vPCR method for the concomitant identification and quantification of viable bacteria in LBPs. 2) In silico investigation of selected probiotic bacteria forARGs distribution, their association with Mobile Genetic Elements (MGEs), and the occurrence of CRISPR-Cas and R-M defense systems. Multiplex-vPCR A triplex viability PCR (vPCR) protocol was developed for the simultaneous identification and quantification of viable Bifidobacterium bifidum, Lactobacillus acidophilus, and Streptococcus thermophilus. The assay revealed three distinguishable signals and the generated standard curves for quantification purposes proved good linearity ($R2>0.98$) and reaction efficiency ($90\%< E <110\%$). Sample pretreatment with 25 μM propidium monoazide (PMA) selectively prevented DNA amplification from dead cells. Applied to commercial formulations, the multiplex vPCR demonstrated high efficiency and reproducibility, yielding higher live cell counts than traditional plate counting. Resistome Characterization A phylogenetic tree was built from 70 non-redundant probiotic genomes (ANI<99.5%) belonging to 15 different species. Bioinformatic screening identified 14 different ARGs in 27/70 (39%) probiotic genomes of 8 different species. Acquired ARGs were confirmed in 16/70 genomes. Notably, 11/16 (69%) lacked CRISPR-Cas systems and 2/16 (12.5 %) lacked R/M systems. Furthermore, 10 ARGs were associated with MGEs in 7/70 genomes, of which 6/7 (86%) lacked CRISPR-Cas systems and 1/7 (14,3%) lacked R/M systems. A statistically significant association was found between the absence of CRISPR-Cas defense systems and the acquisition of mobile resistance determinants (one-tailed Fisher's exact test, $p<0.05$). Conclusions 1) The multiplex vPCR assay allowed the simultaneous identification, quantification and viability assessment for the three target microorganisms. The method overcomes the drawbacks of culture-dependents techniques, representing a reliable alternative for quality control and regulatory assesement. 2) The in-silico analysis revealed that some probiotic strains used in commercial products harbor potentially transferable ARGs associated with MGEs, which may represent a risk to public health. CRISPR-Cas systems may act as a natural barrier against the acquisition of transferable ARGs.

P EFFECTS OF TWO EMERGING MYCOTOXINS, CITRININ AND BEAUVERICIN, IN HEPATIC (HEPG2) AND RENAL (HK-2) CELL LINES

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The primary goal of this PhD project is to evaluate the chemical safety profile of 70 Italian bovine milk samples, from 10 distinct farms in the Marche region, through a comprehensive environmental biomonitoring screening. While persistent organic pollutants (i.e., PCBs, dioxins, and bisphenols) were not detected, quantifiable levels of some heavy metals and mycotoxins were assessed. Two of the most prevalent emerging mycotoxins among the top four overall detected at the highest concentrations, Citrinin (CIT) and Beauvericin (BEA), were selected to characterize their toxicological profiles and potential role as metabolic disruptors. Two human cell lines, HepG2 (human hepatocarcinoma cells) and HK-2 (human immortalized proximal tubule epithelial cells), were used to perform in vitro experiments. Three integrated cell-based assays were carried out: the MTS assay to evaluate cell viability, the Nile Red assay to quantify intracellular lipid accumulation, and Fibroblast Growth Factor 21 (FGF21) secretion by ELISA assay as a marker of cellular stress linked to lipid dysregulation. In addition, 200 μM Palmitic Acid (P.A.) was used as a key metabolic stimulus - either as a positive control for lipid accumulation or as a pre-treatment to establish a model of mild steatosis - owing to its distinctive intracellular uptake mechanism in HK-2 cells. The results demonstrated that both single and binary interaction profiles are non-linear and strictly concentration-dependent. Using a broad concentration range (from low nM to middle μM) of single-exposure profiles on non-pretreated cells, individual mycotoxins exhibited non-monotonic, biphasic concentration curves, with marked toxicity or intracellular lipid accumulation peaks at intermediate concentrations. These effects were significantly exacerbated by the binary mixture, which triggered marked interaction peaks at intermediate levels (maximal lipid accumulation at 96 μM CIT + 38 μM BEA for HepG2, $p \leq 0.01$; 48 μM CIT + 19 μM BEA for HK-2, $p < 0.001$), while shifting to an antagonistic behavior at both lowest and highest concentrations (as assessed by the Bliss independence model). Regarding baseline cytotoxicity, HK-2 cells displayed greater vulnerability (CIT 96 μM, $p < 0.001$; BEA ≥1.9 μM, $p < 0.001$) than HepG2 cells (CIT 192 μM, $p < 0.001$; BEA ≥7.6 μM, $p < 0.001$). Notably, in the hepatic model, P.A. pre-treatment shifted the toxicological window: the binary mixture exacerbated intracellular lipid accumulation at significantly lower doses, triggering a spiked lipotoxic response already at nanomolar concentrations (CIT ≥4.8 nM + BEA 1.9 nM, $p < 0.05$) - directly matching the mean environmental levels detected in the

sampled bovine milk. In the renal model, a key finding was that the HK-2 cell line actively secreted FGF21 in response to mycotoxin-induced lipotoxicity, suggesting this protein as a potential biomarker of metabolic injury in the kidney proximal tubules.

P MODULATION OF INNATE IMMUNE CELL FUNCTION BY REDUCED BACKGROUND RADIATION: INSIGHTS FROM UNDERGROUND RADIOBIOLOGY STUDIES AT LNGS

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Whether and how low-dose ionizing radiation affects biological systems remains an open question, and there is growing interest in the extent to which natural environmental background radiation shapes molecular and cellular responses. Within this framework, the completed INFN-DISCOVER22 project and its ongoing continuation, ECLIPSE, address the effect of reduced background radiation environments on innate immune responses. The work was performed at the INFN Gran Sasso National Laboratories (LNGS), an underground facility whose rock overburden and dedicated ventilation system strongly attenuate cosmic-ray and neutron flux, providing a rare setting in which environmental radiation exposure can be experimentally controlled at very low levels. Human HL60 cells were maintained in parallel cultures, one at a surface reference laboratory and one within the underground LNGS environment, with cells passaged twice weekly over a 30-day period. At three defined adaptation time points, 7, 15 and 30 days, differentiation toward innate immune-like phenotypes (monocytes and neutrophils) was evaluated through CD marker expression. The functional properties of the differentiated cells were then examined under both chemical stimulation and bacterial challenge, so as to more closely mimic in vivo conditions.

Challenge with Escherichia coli was used to assess activation of immune-related pathways and the ability of differentiated cells to limit bacterial growth and suppress infection-like processes, evaluated by immunofluorescence microscopy, DCFH-DA-based Reactive Oxygen Species (ROS) production assays, and Colony-Forming Unit (CFU) counts after in vitro exposure to E. coli strain ATCC 25922. Immune cells derived from HL60 cultured underground showed a more pronounced functional response after 15 days, an effect that diminished by day 30, indicating a time-dependent adaptation to the low-background radiation environment. These findings support the hypothesis that environmental background radiation plays a role in modulating key molecular and cellular immune functions. Future work, pursued within the ECLIPSE project, will further characterize the kinetics and mechanisms underlying this adaptive process, with particular attention to its temporal dynamics and to the regulation of the pathways involved.

P CK2 INHIBITION SENSITIZES BRCA-PROFICIENT CANCER CELLS TO PARP INHIBITORS BY STIMULATION OF GAP ACCUMULATION

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CK2 is a constitutively active Ser/Thr kinase that is ubiquitously expressed across eukaryotes and implicated in multiple cellular processes. Recent studies have highlighted its crucial role in ensuring optimal activation of the ATR–CHK1 checkpoint and in preserving genome integrity by promoting WRN-RPA interaction. Despite these findings, the contribution of CK2 to the control of replication-associated DNA lesions remains incompletely characterized. Here, we identify a previously unrecognized role of CK2 in restraining replication gap formation during perturbed DNA replication. Using a selective CK2 functional inhibitor, we show that loss of CK2 activity under mild replicative stress results in marked accumulation of replication gaps. These extended ssDNA regions represent a critical cellular vulnerability, enhancing sensitivity to PARP inhibition and closely resembling the phenotype of BRCA-deficient cancer cells. Notably, this phenotype was observed even in the absence of HU-induced fork stalling, upon PARPi treatment alone. However, CK2 inhibition did not affect RAD51 or BRCA2 localization at perturbed replication forks, or fork degradation, suggesting a BRCA-independent mechanism. Based on these findings, we explored the therapeutic potential of combining CK2 inhibition with PARP inhibitors. Co-treatment of Olaparib-resistant/BRCA2 revertant PEO4 cells with a CK2 inhibitor and Olaparib significantly reduced cell viability and proliferation, demonstrating that CK2 inhibition is sufficient to sensitize otherwise PARPi–resistant cells. Overall, our work uncovers CK2 as a key regulator of replication gap suppression and identifies CK2 inhibition as a promising strategy to sensitize BRCA-proficient tumors to PARP inhibitors, potentially extending the clinical applicability of PARP inhibitor–based therapies.

P 3D MULTICELLULAR SPHEROIDS AS PREDICTIVE MODELS FOR MELANOMA THERAPY: COMPARING TWO GENERATION METHODS

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Melanoma is a highly aggressive form of skin cancer derived from the malignant transformation of melanocytes, accounting for approximately 60,000 deaths worldwide each year. A key challenge in developing effective therapies is the limited ability of current preclinical models to reproduce the complex interactions of the tumor microenvironment (TME), which plays a crucial role in melanoma progression. This highlights the need for more advanced and physiologically relevant in vitro models to better study melanoma biology and predict therapeutic response. In this study, three-dimensional (3D) multicellular melanoma spheroids (MCTs) were generated using two melanoma cell lines: A375, derived from a primary tumor carrying the common BRAF V600E mutation, and WM266-4, a metastatic cell line harboring the BRAF V600D mutation. Four MCT types were established for each melanoma cell line by culturing melanoma cells alone or in combination with keratinocytes (HaCaT) and fibroblasts (HDF). To investigate different approaches for spheroid generation, two methods were employed: flat-bottom Ultra-Low Attachment (ULA) plates and the MicroTissue® Mold system. In the first approach, melanoma monoculture spheroids were treated with Vemurafenib (VEM), a selective BRAF V600 inhibitor, and cell viability was assessed by measuring metabolic activity using the MTT assay. However, ULA-generated spheroids showed variability in size and morphology. The MicroTissue® Mold system was therefore used to generate more consistent and standardized MCTs, which were the focus of all further analyses. MCTs were morphologically and functionally characterized by assessing their diameter, solidity, homogeneity, and metabolic activity over a 10-day culture period. The analyses revealed distinct morphological features between the two melanoma cell lines and showed that co-culture influenced their growth and compactness. H&E staining showed pyknotic nuclei, preserved spheroid architecture, and possible Extracellular Matrix (ECM) deposition. Live/Dead staining identified day 4 as the optimal time point for drug testing, with higher viability than at day 7. Fluorescent labeling revealed distinct spatial organization in triple MCTs, with keratinocytes mainly embedded within the structure and melanoma cells localized at the periphery. Finally, VEM response was evaluated in MicroTissue® Mold-generated MCTs using the CellTiter-Glo 3D assay. MCTs generated from both melanoma cell lines responded to VEM treatment in a dose- and time-dependent manner, with WM266-4 MCTs showing greater sensitivity, particularly at 100 μM, as indicated by a stronger reduction in metabolic activity and increased cell

detachment. These findings support the use of MCTs as relevant preclinical 3D melanoma models for studying TME interactions and drug responses, while contributing to the reduction of animal use in research.

P DEVELOPMENT AND VALIDATION OF A QUESTIONNAIRE ON DIETARY HABITS FOR CARDIOVASCULAR RISK ASSESSMENT

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Cardiovascular Diseases (CVDs) remain the leading cause of morbidity and mortality worldwide. Extensive evidence has shown that greater adherence to the Mediterranean Diet (MD) is associated with a lower risk of all-cause mortality, as well as cardiovascular morbidity and mortality. Nevertheless, MD adherence has progressively declined over recent decades, highlighting the importance of large-scale epidemiological studies to monitor adherence and guide evidence-based public health strategies for cardiovascular disease prevention. In Italy, since 1998, the CUORE Project has periodically conducted Health Examination Surveys (HESs) to monitor the distribution of major NCD conditions (e.g., hypertension, diabetes, and obesity) and related risk factors (diet, physical activity, smoking, and alcohol use), providing nationally representative data on cardiovascular risk in the Italian general adult population. Using data from HESs conducted in 2008-2012, 2018-2019, and 2023-2025, the project aims to: - evaluate distribution and temporal trends in MD adherence from 2008 to 2025; - assess the association between MD adherence and cardiovascular risk and its determinants in the Italian general adult population, identifying population groups at higher diet-related cardiovascular risk; - develop and validate a novel MD adherence index aimed at addressing the limitations of available tools. To achieve these objectives, the project comprises three phases: (1) conducting a systematic review of the literature to map and critically appraise available MD adherence indices; (2) selecting and implementing MD adherence indices in the HESs; and (3) comparing their performance to support the development and validation of a novel MD adherence index. The systematic review identified 66 indices, including 28 MD adherence scores and 38 MD questionnaires, 25 of which were cross-cultural adaptations of pre-existing indices. Over half of the indices were developed in Mediterranean countries (52%), particularly Spain (n=15), Italy (n=9), and Greece (n=6), while outside the Mediterranean basin, the United States accounted for the largest number (n=7). Indices differed substantially in the type, number, and grouping of components, as well as in scoring systems. None of the indices underwent complete validation, with construct validity (n=45) and criterion validity (n=42) being the most frequently assessed domains. Based on these findings, three MD adherence indices with different methodological characteristics were selected for implementation to evaluate whether these differences affect their ability to discriminate groups at higher diet-related cardiovascular risk. Descriptive and comparative analyses are currently underway to evaluate the performance of selected indices. The findings will support the development of a novel MD adherence index, enabling a more reliable assessment of MD adherence in epidemiological studies and public health surveillance.

P AN ADVANCED *IN VITRO* MODEL COMPOSED OF PBMC AND MUSCLE CELLS TO EVALUATE THE RESPONSE TO ADJUVANTS AND VACCINES FOR HUMAN USE

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In spite of few systems vaccinology studies showing a correlation between innate immune response and the magnitude of vaccine-stimulated adaptive immunity, the impact of adjuvant type and administration route remains poorly explored. In the present project, by taking advantage of a human-cell based in vitro model consisting of Human Skeletal Muscle Cells (HSkMC) and Peripheral Blood Mononuclear Cells (PBMC), we aim to recapitulate the early immune signature observed in vivo after vaccine intramuscular administration and clarify adjuvant contribution. A differentiation protocol for HSkMC was optimized to identify the conditions promoting efficient myotube formation, including cell density, fibronectin coating requirements, differentiation kinetic and fusion index. The analysis of muscle cell marker expression (Titin, Myosin Heavy Chain, Desmin, Laminin, Actinin, Paired Box 7 and Myogenin) was performed on Day 6, 10 and 14 using quantitative real-time PCR (qPCR) and confocal microscopy, with all results compared to baseline (Day 0). Day 6 was selected as the best time point for HSkMC differentiation given the statistically significant increase in Myosin Heavy Chain and Titin marker expression as compared to Day 0. Multinucleated fibers were clearly visible by performing confocal microscopy and the multinucleated myotube phenotype was further confirmed by the fusion index that was 40% for almost all muscle cell differentiation markers and raised up to 60% with Laminin. In addition, myotubes showed a higher mitochondrial membrane potential compared to myoblasts suggesting a differentiation-specific metabolic plasticity. Once defined the optimal differentiation conditions, HSkMC were stimulated with different doses of vaccine adjuvants, i.e. Aluminum Hydroxide (AlOH3), MF59 and AS01, as well as with two intramuscular administered vaccines, namely Encepur, the anti-Tick-Borne Encephalitis virus, and Comirnaty, the vaccine against SARS-CoV-2. A cytotoxic effect was observed with both AS01 and MF59 while AlOH3 as all as AlOH3 containing vaccine, Encepur, did not affect cellular viability as the non-adjuvanted Comirnaty vaccine that, however, elicited an impairment of mitochondrial membrane potential. To avoid the introduction of bias related to the cytotoxicity, further analyses were conducted by using Comirnaty, Encepur and AlOH3 only. Interestingly, Comirnaty significantly upregulated interferon stimulated genes and RNA sensor expression as well as the secretion of several cytokines, chemokines and

growth factors, while Encepur/AlOH3 elicited an opposite effect, showing a specific vaccine-driven signature. These findings support the advancement of the project toward the analysis of vaccine/adjuvant-driven PBMC responses and the development of an integrated HSkMC–PBMC co-culture system to model the early immune events occurring after intramuscular vaccination.

P VULNERABLE POPULATIONS AND THE ACCESS TO HEALTH SERVICES FOR CHRONIC RESPIRATORY DISEASES: A SYSTEMATIC REVIEW

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Introduction. As part of the Joint Action on REspiratory Diseases (JARED), funded by the European Union, a systematic literature review was conducted to analyze sociodemographic characteristics, barriers, and solutions related to access to care for chronic respiratory diseases. The review focuses on vulnerable populations such as migrants, refugees, ethnic minorities, and socially disadvantaged populations.

Methods. The systematic review was conducted according to the PRISMA 2009 framework. Medline, Embase, and SciSearch databases were searched for studies conducted between 2015 and 2025 in countries of the WHO European Region. Qualitative, quantitative, and mixed-method articles in English, Italian, Portuguese, and Spanish were included. The Joanna Briggs Institute checklists were used to assess study quality.

Results. Of the 3,696 articles evaluated, 37 were included in the review, covering Tuberculosis (TB) (31 articles), asthma (5 articles), and Chronic Obstructive Pulmonary Disease (1 article). Out of the included studies, 25 were assessed as high quality, 9 as medium quality, and 3 as low quality. Many articles addressed multiple levels of care, particularly access to screening and treatment. The preliminary results of the review focus on TB.

Discussion. The main barriers identified for access to TB screening were lack of knowledge about the disease or the possibility of screening, linguistic and cultural barriers, fear of being identified or deported, and the stigma of a TB diagnosis. The main facilitators for access to TB screening included staff training, the use of cultural mediation services, community involvement, and already existing care for other conditions. Barriers to access to TB treatment include linguistic and cultural differences, discrimination by professionals, the cost of treatment, and insufficient knowledge of the disease. Language has also been identified as a

barrier to treatment completion, as well as arriving in the new country during a period of high influx of migrants. Solutions to improve antibiotic treatment completion include professional support, shorter treatment durations, and Directly Observed Therapy (DOT).

Conclusions. Chronic respiratory diseases are associated with significant morbidity, mortality, and disability, particularly among disadvantaged populations. The preliminary findings of this review confirm that, especially for a disease as common in vulnerable populations as tuberculosis, addressing inequalities in access to services requires a multidisciplinary route, including the use of linguistic and cultural mediation, professional training, and a systems approach to healthcare.

P CHARACTERIZING ELEMENT RELEASE IN PREMISE PLUMBING: ICP-MS CONCENTRATION-VOLUME PROFILING AND STAGNATION EFFECTS

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Internal Drinking Water Distribution Systems (DWDSs) are intrinsically complex and heterogeneous, comprising components made of different materials and operating under variable hydraulic conditions. During water residence, physicochemical interactions at water–material interfaces may promote the mobilization of inorganic elements, including species of toxicological relevance. Characterizing element release is therefore important for protecting drinking-water consumers. Release kinetics are governed by multiple factors, including material composition, water chemistry, hydraulic conditions and, critically, stagnation time. However, such spatially and temporally variable processes are not fully captured by conventional sampling strategies. Random Daytime, spot and composite sampling characterize water quality under defined conditions but do not resolve release dynamics within building distribution systems, potentially masking concentration gradients, localized release events and stagnation effects. To address these limitations, an analytical and sampling approach was developed to investigate elemental release with greater temporal and volumetric resolution. An exploratory semiquantitative Inductively Coupled Plasma Mass Spectrometry (ICP-MS) screening of 80 elements was initially performed. Based on screening results and relevance to materials-related release processes, a multielement ICP-MS quantitative method was developed for the determination of 14 selected trace and major elements. Following controlled stagnation, consecutive small-volume fractions were collected at the tap and analysed. Element concentrations were assessed as a function of cumulative withdrawn volume, yielding analyte-specific concentration-volume profiles. The approach was applied to several residential case studies differing in DWDSs and water supplies. Stagnation effects were investigated by sequential sampling after different controlled stagnation periods, conducted overnight to improve reproducibility by limiting variability in temperature and hydraulic conditions and reducing water use within the building. Preliminary results showed that, for nearly all elements of interest, such as Pb, Ni, Zn and Cu, the highest concentrations occurred in the initial fractions, followed by a progressive decrease during flushing. For selected analytes, secondary concentration maxima occurred at higher cumulative volumes, although their occurrence and position varied across the investigated systems, reflecting the site-specific nature of element release. The main concentration–volume features remained identifiable across repeated experiments, while increasing stagnation time primarily influenced release magnitude. This approach provides insights into localized release phenomena and stagnation-dependent variations not resolved by conventional single-sample strategies, supporting a more representative assessment of materials-related contamination and consumer exposure.

P PEROVSKITE MATERIALS FOR GAMMA RADIATION DETECTORS IN NEXT GENERATION MEDICAL IMAGING TECHNOLOGY

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Gamma radiation detectors are relevant and challenging components in the development of new medical imaging technologies. Current systems applied in clinical routine predominantly rely on conventional indirect γ-ray detection systems, where a scintillator (e.g., NaI:Tl, CsI:Tl, or BGO) converts incident radiation into detectable light that is subsequently captured by a photomultiplier or Si-based photodiode array. On the other hand, direct detection technologies based on semiconductor materials such as Cadmium Zinc Telluride (CdZnTe) have already applied in medical imaging systems; however, these technologies require costly materials, facilities and processes such as high-temperature crystal growth, vacuum deposition, and complex material purification, which not only limit scalability but also result in persistently high manufacturing costs, motivating current research to investigate new emerging materials and production techniques. Among the halide scintillators, copper-based Perovskite Inspired Scintillators (PISs), notably Cs3Cu2I5 have attracted growing interest as sensors for γ-ray spectroscopy, primarily due to their efficient self-trapped exciton emission, yielding a high light yield, a large Stokes shift and fast decay times. During the research study at ISS, copper-based PISs materials and processes were investigated and realized at centimeter scale with high transparency, comparable rise time to traditional thallium doped Cesium Iodide (CsI (Tl)) and faster decay around 68 ns, making them promising scintillators. Gamma-imaging evaluation and characterizations were conducted on produced samples and prototypes in laboratory setup, and the experimental and computational outcomes demonstrated the feasibility for the next generation medical imaging systems. Conversely, halide perovskites and particularly FAPbBr3, have recently emerged as alternative materials due to their stability, tunable optoelectronic properties, low-temperature solution-processing under ambient conditions and low cost. Thus, FAPbBr3 based γ-direct detectors properties were studied and improved, and crystals were grown by approaching different methods and techniques at Shanghai University. The optimal configuration achieved exhibited a longer carrier lifetime (~25 ns), lower dark counts (~10^{-8} A) and a higher mobility-lifetime product (~10-4 cm2·V^{-1}). The research carried out further investigated the main challenges including halide vacancies (VBr) and ion migration, as well as compositional engineering strategies aimed at extending the operational lifespan of the manufactured sensors. Results showed the potential for stable, efficient and low-cost medical imaging radiation detectors.

P IDENTIFICATION AND ANALYSIS OF THE PROTEIN-PROTEIN INTERACTION NETWORK AT THE BRADYZOITE STAGE OF *TOXOPLASMA GONDII*

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Inference of Protein-Protein Interaction (PPI) networks is a major challenge in bioinformatics, bridging protein functions and complex cellular behaviors. In parasites, stage-specific PPI networks could reveal how the interactome is remodeled during adaptation to different host environments. *Toxoplasma gondii*, the causative agent of toxoplasmosis, establishes chronic infections through transition to a latent, tissue cyst-contained bradyzoite stage, which permits long-term persistence within hosts. Despite its biological importance, PPI networks for this stage remain unavailable. To address this gap, we generated the first bradyzoite-stage interactome of *T. gondii* from in vitro cultured cysts, combining co-fractionation/mass spectrometry approach, followed by data integration with phylogenetic profiling and RNA sequencing data. Size-Exclusion Chromatography coupled with Mass Spectrometry (SEC-MS) in a label-free, data-independent acquisition mode was performed, generating a protein elution profile for each protein in our sample, based on the principle that proteins in the same complex exhibit similar elution profiles. In parallel, phylogenetic profiles were constructed comparing parasite proteins with 699 genomes from the EggNOG database, under the premise that proteins sharing similar phylogenetic profiles are probably interacting. A bradyzoite transcriptomic data time-course dataset was generated by selecting RNA sequencing data from ToxoDB. Multiple metrics were applied to all datasets to generate a series of similarity matrices which were used as input for PrInCE, an R/Bioconductor package for supervised ML-based PPI inference. Gold-standard datasets were constructed using physical interactions from StringDB as true positives and shortest-path–based network imputation to derive true negatives. Several classifiers (RF, SVM, NB, LR) were evaluated and their corresponding predictive performance was compared to identify the best performing model. A final predictive performance assessment derived a high confidence interactome using a false discovery rate (FDR) threshold of <5%. To assess network organization, an evaluation framework based on the gold standard was developed to determine the optimal clustering approach. Markov Clustering (MCL) was evaluated at different granularity values calculating the ratio of observed-to-expected true positive interactions within each cluster. Integration with *T. gondii* spatial proteomics data (HyperLOPIT), revealed that proteins sharing subcellular localizations tend to interact and form distinct subnetworks. Functional analyses based on Gene Ontology Biological Process, showed that proteins with similar biological functions tend to cluster together,

including clusters enriched for dense granules, rhoptries and apicoplast proteins, as well as invasion-associated pathways. Finally, some potential candidates were selected for experimental validation.

P PROJECTING COMPLETE CANCER PREVALENCE AND HEALTHCARE COSTS BY PHASE OF CARE IN VENETO TO 2031

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Cancer prevalence, which is defined as the number of people alive on a certain date following diagnosis, is a key indicator for healthcare planning. However, an overall prevalence estimate does not distinguish between the different care needs that arise throughout the cancer care pathway. For this reason, cancer prevalence can be divided into three clinically relevant phases of care. The initial phase covers the first 12 months after diagnosis, the final phase corresponds to the 12 months before death, and the continuing phase covers the rest of the time. The epidemiological analysis projects complete cancer prevalence by phase of care in Veneto to 2031 for colorectal, lung and thyroid cancers in both sexes and for female breast cancer. The economic component, developed exclusively for female breast cancer, proposes a methodology for estimating the associated healthcare costs. The Veneto Cancer Registry provided Limited-Duration Prevalence (LDP) estimates as of 1 January for each year from 2019 to 2023. The maximum follow-up period common to all five observations was 29 years. For each cancer site and sex, the LDP proportion P was projected according to a previously published method and applied to ISTAT population projections for Veneto in 2031 and 2032. The totals were redistributed by age and time since diagnosis based on the LDP observed in 2022 and 2023, respectively. The two resulting matrices were processed using COMPREV to estimate complete prevalence by phase of care in 2031. The continuing phase was predominant for all cancer sites. In lung cancer, however, the final-phase share was higher than in the other cancer sites considered. Complete prevalence of female breast cancer in 2031 was estimated at 100,966 women: 6,059 in the initial phase, 91,896 in the continuing phase and 3,011 in the final phase, representing a 24% increase compared with 2022. Healthcare unit costs were adjusted to 2031 prices by combining historical trends in healthcare expenditure with observed and projected inflation. Initial-phase costs were weighted by stage at diagnosis using published stage-specific costs and the 2022 stage distribution, resulting in €56.3 million. For the continuing phase, a sensitivity analysis based on the already cured indicator produced costs ranging from €50.8 million to €203.3 million. Final-phase valuation was restricted to cancer deaths. Two EPICOST cost profiles, expressed in 2011 and 2018 prices and based on the same healthcare cost components, were adjusted to 2031 prices, resulting in estimates of €12.9 million and €17.3 million, respectively. Overall, the projected economic burden of female breast cancer is driven mainly by the continuing phase, while final-phase estimates remain sensitive to the cost profile adopted. The proposed methodology quantifies the economic implications of projected cancer prevalence and supports healthcare planning.

P THE *ESCHERICHIA COLI* TOXIN CNF1 PROMOTES COLORECTAL CANCER PROGRESSION THROUGH DNA DAMAGE, CELLULAR SENESCENCE, AND METASTATIC DISSEMINATION

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Background. Colorectal Cancer (CRC) progression is increasingly recognized as being shaped by complex interactions between tumor cells and the gut microbiota. Beyond their established role in tumor initiation, bacterial toxins are emerging as important modulators of tumor evolution and metastatic dissemination. We previously demonstrated that Cytotoxic Necrotizing Factor 1 (CNF1), a toxin produced by pathogenic *Escherichia coli*, promotes inflammation-driven colon carcinogenesis in vivo. Here, we investigated whether CNF1 may also contribute to CRC progression by promoting cellular senescence and metastatic traits.

Methods. Human CRC cell lines were exposed to CNF1 and analysed for DNA damage, activation of the DNA Damage Response (DDR), senescence, and secretion of Senescence-Associated Secretory Phenotype (SASP) factors. The effects of CNF1 on stem cell-associated features was also evaluated. Extracellular matrix remodelling, and metastatic dissemination were further evaluated using metastatic CRC cells and an experimental mouse model.

Results. In Caco-2 and HT29 cells, CNF1 induced DNA damage, evidenced by increased γH2AX and 53BP1 foci, accompanied by activation of the DDR. Persistent genotoxic stress triggered cellular senescence and the acquisition of a pro-inflammatory SASP, although the amount of the response differed between the two cell lines. CNF1 also enhanced stem cell-associated features and clonogenic potential in responsive CRC cells, supporting increased cellular plasticity. In metastatic SW620 cells, toxin exposure increased MMP-9 expression and enzymatic activity, consistent with enhanced extracellular matrix remodelling and invasive potential. Importantly, these findings were validated in vivo, where CNF1-pretreated metastatic cells generated a significantly higher metastatic burden than untreated controls following intrasplenic injection.

Conclusions. Our findings identify CNF1 as a microbiota-derived virulence factor that promotes CRC progression beyond tumor initiation. By inducing DNA damage and cellular senescence in primary CRC models while enhancing the invasive behaviour of metastatic

cells, CNF1 contributes to the establishment of a tumor-promoting microenvironment and facilitates metastatic dissemination. These results provide mechanistic evidence linking bacterial toxins to CRC malignancy and progression, highlighting the microbiota as a promising therapeutic target for limiting disease progression and metastatic spread.

P DECIPHERING THE CONTRIBUTIONS OF FAECAL MICROBIOTA TO DISEASE PROGRESSION IN A HUMAN MICROBIOTA-ASSOCIATED ALS MOUSE MODEL

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Background. Amyotrophic Lateral Sclerosis (ALS), like other neurodegenerative diseases, is caused by a combination of genetic and environmental factors. The intestinal microbiota is increasingly recognized as a key modulator of the gut-brain axis. Alterations in the gut microbiome composition have been linked to the progression of ALS, and it is debated whether this is a cause or consequence of the disease.

Methods. To investigate the role the gut microbiome plays in ALS pathogenesis, we generated a human microbiota-associated mouse model by transplanting faecal microbiota from ALS patients and controls into microbiota-depleted transgenic SOD1G93A mice. Clinical phenotype was monitored until the onset of motor symptoms. Gut microbiota composition, intestinal permeability, inflammatory markers, and the serum metabolome were also evaluated.

Results. Transplantation of microbiota from ALS patients exacerbated disease progression in SOD1G93A mice in a sex-dependent manner. Female mice receiving ALS patient microbiota showed earlier motor onset compared to mice receiving autologous or human control faecal microbiota transplantation. Clinical phenotypes did not differ among male mice by donor type. Mice receiving ALS patient faecal material exhibited significantly higher levels of pro-inflammatory gene expression in the motor cortex, spinal cord, and skeletal muscles, in combination with a reduction in the Foxp3 transcription factor. Dimension reduction analyses revealed distinct immunological profiles among mice who received different faecal donors, and distinct metabolic profiles in male mice.

Conclusion. The gut microbiome may contribute to ALS onset in female transgenic mice. Microbial transplantation differentially affected inflammatory markers and metabolic pathways, highlighting the broader regulatory influence of the gut microbiota on other biological pathways in ALS.

P WHOLE GENOME SEQUENCING IMPROVES DIAGNOSTIC YIELD AND REVEALS PHENOTYPIC EXPANSION IN INHERITED RETINAL DISEASES

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Abstract Purpose Inherited Retinal Diseases (IRDs) remain genetically unresolved in a substantial proportion of cases despite widespread use of gene panels and exome sequencing, limiting accurate diagnosis and patient stratification. We evaluated the diagnostic utility of Whole-Genome Sequencing (WGS) combined with integrated genomic and functional approaches in previously unsolved IRD cases. Methods We performed WGS in 43 affected individuals from 30 unrelated families with IRDs who remained unsolved after prior genetic testing. Variant interpretation was supported by multidisciplinary clinical re-evaluation (deep phenotyping) and complementary in silico and functional analyses, including patient-derived cellular models. Results WGS established a molecular diagnosis in 20 of 30 families, achieving a diagnostic yield of approximately 67%. Pathogenic or likely pathogenic variants were identified in established IRD genes (including ABCA4, PROM1, CNGB1, CDHR1, and HMCN1) as well as in genes previously associated with syndromic conditions (e.g., TUB, BBS9, POMGNT1, IFT52, INTS11, PLAA, ARL3, and ALPK1). Notably, nearly half of the solved cases exhibited phenotypic expansion, with genes traditionally associated with multisystem disorders underlying isolated or predominant retinal disease. In addition, we identified candidate genes, including PRKCZ and BAIAP3, supported by genetic and functional evidence, suggesting additional mechanisms relevant to retinal degeneration. Conclusion Our findings demonstrate that WGS improves diagnostic resolution in IRDs and

provides clinically relevant insights into genotype-phenotype relationships. The frequent identification of atypical presentations associated with genes classically linked to multisystem disorders underscores the need to reconsider existing disease classifications. Integrating genomic data with deep phenotyping and functional validation is critical for accurate diagnosis and characterization of rare retinal disorders.

P STUDYING THE HUMAN REPLISOME UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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The replisome is a multiprotein complex built around the CMG helicase (CDC45-MCM2-7-GINS), which unwinds parental DNA and serves as the structural hub for the fork protection complex - Timeless-Tipin, AND-1, and Claspin, which couples helicase movement to fork stability. This physical coupling is thought to prevent premature CMG disassembly and to coordinate helicase and polymerase activities under replication stress. However, whether stress causes Timeless to actually dissociate from MCM2-7, rather than remain bound while repositioning within a rearranged fork, remains unresolved. Understanding this mechanism is fundamental to gain more insights into replisome dynamics connected to DNA repair. Here we asked whether Timeless remains physically associated with MCM2-7 during replication stress, and which fork-remodelling factors account for changes in this association. We used Proximity Ligation Assay (PLA), co-immunofluorescence, and chromatin-based approaches to measure Timeless-MCM7 proximity and abundance in Wild-Type (WT), and in the absence of RAD52 or SMARCAL1, two key factors involved in fork remodeling upon replication stress. Experiments were performed in unperturbed conditions and Hydroxyurea (HU)-induced replication stress, with or without CDC7 kinase inhibition (CDC7i) to block firing of new/dormant origins. Timeless-MCM7 PLA signal was never abolished in any condition tested, though HU alone reduced it substantially in WT and RAD52 KO cells, while shSMARCAL1 cells showed stable or increased signal. CDC7i alone reduced both proximity and chromatin-associated signal similarly across genotypes, consistent with fewer active origins rather than loss of proximity. Combined with HU, the signal recovered above the CDC7i-alone level. Co-immunofluorescence confirmed this pattern, with Timeless-MCM7 colocalization decreasing, but never reaching zero, independent of changes in total protein levels. Moreover, RAD52 loss further reduced colocalization beyond active replication forks. And also produced a distinct, genotype-specific pattern under CDC7i + HU treatment that was not apparent from proximity data alone, pointing to a radical change of replisome dynamics if fork reversal is deregulated and fork stability is impaired. Together, these data suggest that Timeless remains physically coupled to MCM2-7 throughout replication stress, repositioning rather than dissociating. Future work will use proximity-dependent biotinylation (TurboID/UltraID tagging) to map how core replisome and fork-protection components reposition relative to one another under replication stress and transiently engage new partners at the stalled fork, providing a dynamic, unbiased view of fork-protein interactions beyond the Timeless-MCM7 pair studied here.

P TEACHING CLIMATE AND ENVIRONMENTAL HEALTH: A PROBLEM- AND COMPETENCY-BASED EDUCATIONAL APPROACH

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Environmental changes such as climate change threaten human health in multidimensional ways, directly or indirectly, conditioning health determinants. A health workforce capable of acting on Environmental Health (EH) is fundamental to build climate-resilient health systems and services. Lifelong learning is a key instrument to develop competencies. In Italy, this is made more equitable and accessible through distance-learning (DL). Italy's Complementary National Plan funded a training program of 14 DL courses to create an EH-literate workforce nationwide. Topics were determined through training-need assessment. A panel of experts was asked to identify course objectives and competencies, as suggested in competency-based education. The courses were built through a problem-based learning approach. The National Institute of Health supplied the first edition of the courses via EDUISS, its DL platform, from April to December 2025. This work investigates Kirkpatrick's first 3 levels of training evaluation: 1) "Did learners enjoy training?"; 2) "Did learning transfer occur?"; 3) "Did the training change behavior?". Of the 31˙696 users enrolled, 67% were women, 58% had ≥46 years of age and 35% were nurses. Of those who started the course, 53% completed it. On a Likert scale from 1 to 5, mean cumulative satisfaction was 4.47. The difference between pre- and post-test evaluation was 10%. In addition, a statistically significant difference in Knowledge Attitude Practice Behaviour (KAPB) questionnaires before and after the course was found with Wilcoxon signed-rank test (K: $z=79$, $p<0.001$; A: $z=27$, $p<0.001$). To our knowledge, this is the first lifelong DL program on EH in Italy. Preliminary results show high satisfaction, acceptable learning transfer and good behavioral change. Nevertheless, to answer Kirkpatrick's fourth question on performance, a 6-month post-course follow-up is necessary.

P HYPOXIC HUMAN NEURONS AND ASTROCYTES RELEASE EXTRACELLULAR MIR-25/MIR-106A THAT ACTIVATE NEUROGENESIS-RELATED PATHWAYS IN NPCS

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Stroke is the second leading cause of death worldwide, and ischemic stroke is the most common type, accounting for 84% of cases. Ischemic injury may promote endogenous repair mechanisms, including neurogenesis and neuroplasticity, which are regulated by microRNAs (miRNAs) and guidance molecules. Ischemic like hypoxia reshapes intercellular communication within the human neural niche, but the contribution of extracellular miRNA remains incompletely defined. We generated neurons and astrocytes from human induced pluripotent stem cell-derived Neural Progenitor Cells (NPCs) and exposed them to hypoxia followed by reoxygenation. We quantified miR 25 3p and miR 106a in culture media and assessed whether conditioned media from hypoxic and reoxygenated cells modulate neurogenesis related pathways in naïve NPCs. Under hypoxia/reoxygenation, both neurons and astrocytes showed a significant increase in extracellular miR 25 3p and miR 106a, indicating active release. NPCs exposed to miRNA enriched conditioned media displayed selective regulation of specific targets and pathways: HMGB1 expression was significantly reduced, most notably after reoxygenation, whereas p57 (CDKN1C) levels were significantly increased, consistent with miRNA mediated post transcriptional control of cell cycle and differentiation programs. In parallel, BDNF levels were markedly increased in the culture media of NPCs exposed to reoxygenated conditioned media, indicating enhanced paracrine trophic signaling. These findings identify a hypoxia induced extracellular miRNA-based communication mechanism whereby neurons and astrocytes influence NPC signaling through miR 25 and miR 106a dependent regulation of neurogenesis related pathways.

P ECOSYSTEM SERVICES AND THE NATURE RESTORATION REGULATION IN ENVIRONMENTAL AND HEALTH IMPACT ASSESSMENTS

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Ecosystem services are defined as the multiple benefits provided by ecosystems to humans (Millennium Ecosystem Assessment). This definition highlights how the functions performed by ecosystems are closely linked to the fulfilment of human needs and, more generally, to the well-being of society, which depends on them. It is therefore essential to develop a greater understanding of the links between the environment and human health, and to recognise the "true value" of ecosystems in decision-making processes. From this perspective, the legal interest of the environment cannot be regarded as merely the sum of the natural resources of which it is composed, but as a system of ecological relationships with its functions and benefits. Article 191 of the Treaty on the Functioning of the European Union (TFEU) sets out the objectives of the Union's environmental policy, emphasising, amongst other things, the importance of the prudent and rational use of natural resources. Directive 2014/52/EU has reinforced the need to assess the effects and impacts of authorised projects on the environment and human health. In Italy, this principle was transposed by Legislative Decree No. 104/2017, which introduced the Health Impact Assessment (HIA), driven by the guidelines adopted by the Ministry of Health and the National Institute of Health. The HIA integrates knowledge relating to health and environmental conditions with an assessment of the potential effects that specific projects may have on human health. Even during the scoping phase and the subsequent exposure assessment and monitoring phases, the Italian HIA takes into account several factors relating to ecosystem services, such as ecotoxicological aspects and the assessment of the quality of surface water, groundwater and soil. Similarly, natural features, habitats and biodiversity are essential components of the Environmental Impact Assessment (EIA). The PhD project highlights, amongst other things, how the new EU Nature Restoration Regulation 2024/1991 is making the link between nature conservation and human health ever more compelling. The protection and restoration of aquatic and terrestrial ecosystems, to be carried out within specific timeframes, is in fact an obligation for all Member States, which will therefore have to adopt significant measures to restore, for example, green spaces even in heavily urbanised industrial areas where the HIA is currently in force, thereby helping to improve environmental quality, resilience to climate change and, consequently, to support the protection of public health. Under the new nature law, these aspects – relating to the benefits to human health derived from ecosystem services – could therefore be incorporated in a more structured manner into sector-specific legislation relating to the protection of human health in the future.

P EXPLORING THE POTENTIAL OF SAND FLY MICROBIOTA FOR SUSTAINABLE PATHOGEN CONTROL: EVIDENCE FROM ASAIA SPP. DYNAMICS

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Phlebotomine sand flies (*Diptera: Psychodidae*) are expanding their geographic range towards higher latitudes and altitudes, driven by climate and environmental changes, with consequent spread of pathogens such as *Phleboviruses* and *Leishmania spp*. into previously non-endemic areas. In Italy, the distribution of sand flies was extensively documented in the mid-1990s, providing an important historical baseline, while subsequent studies have reported their occurrence and expansion towards new areas. Although Italy remains endemic for sand fly-borne diseases, the role of sand fly gut microbiota in pathogen development is still poorly investigated. The endosymbiont *Asaia spp*., already studied in Aedes and Anopheles mosquitoes, represents a promising candidate for investigating vector competence in sand flies. Two experimental co-infections were conducted using *Phlebotomus perniciosus*, *Toscana Virus* (TosV) and the genetically modified Asaia strain (ApH-W) expressing the Wolbachia Surface Protein (WSP), to evaluate a possible pathogen interference. In the test cage, 10-day-old specimens were exposed to a 30% glucose solution containing ApH-W [5 ml - 107], three days before the infection with a mixture of rabbit blood and TosV [5 µL - 10^8]. Control specimens were exposed only to TosV. The bacterial load was assessed by plating 5 µL of insects homogenates on rich solid media, while the viral detection was performed by an automatic total nucleic acids extraction, from day 1 to day 8 post infection. During the first trial, feeding rates were 37% in the test cage and 60% in the control cage. In the test, 21/54 samples were analyzed: half tested positive for ApH-W and negative for TosV, while the remaining half tested positive for Asaia without WSP and negative for TosV, indicating possible plasmid curing. In the control, 11/32 individual samples were analyzed, with 9.1% positive for TosV. Further analyses are ongoing. In the second trial, feeding rates of 36.7% in the test and 29.2% in the control were recorded. In the test, 18/38 individually analyzed samples tested positive for ApH-W. Additionally, 17 females exposed to both agents, but not fed on the virus, were analyzed and 12 were positive for ApH-W. In the control, 19 engorged females were selected. Viral analyses are ongoing. The complete absence of TosV and the presence of ApH-W in the test, together with the opposite pattern observed in the control during the first trial, may suggest an exclusion or competitive interaction between the virus and the bacterium, although the sample size is

small. Further investigation is required. This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. *PE00000007, INF-ACT*).

P WASTEWATER SURVEILLANCE OF FOODBORNE PATHOGENS IN ITALY: TRENDS IN *SALMONELLA SPP.* AND *YERSINIA ENTEROCOLITICA*

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Wastewater monitoring is increasingly recognized as a valuable tool for assessing the circulation of human pathogens at the community level. The FoodZoo project evaluated the potential of wastewater-based surveillance to characterize the epidemiology of foodborne zoonotic pathogens, including Salmonella spp. and Yersinia enterocolitica, in an urban population in Italy. A total of 66 wastewater samples were collected from three Wastewater Treatment Plants (WWTPs) between January and December 2025. Samples were analyzed using a five-tube, three-dilution most probable number (MPN)-PCR method for the detection of *Yersinia enterocolitica* and *Salmonella spp.,* followed by the characterization of *S. Enteritidis* and *S. Typhimurium*. Data were normalized by daily flow rate and population size. *Salmonella spp*. was detected in 100% of the samples, whereas *Y. enterocolitica* was detected in 63.3%. The mean concentrations were 7.14 $\log_{10}$ CFU/day/inhabitant for Salmonella spp. and 6.65 $\log_{10}$ CFU/day/inhabitant for *Y. enterocolitica.* Occasional but substantial increases in pathogen concentrations were observed in specific samples and WTPs, potentially reflecting localized increases in pathogen circulation within the population served by the three WTPs. The results of the study confirm the consistent occurrence of *Salmonella spp* in urban wastewater and highlight the non-negligible presence of *Y. enterocolitica.* Further investigations will assess the potential of wastewater-based data to estimate the incidence of salmonellosis and yersiniosis in the human population. Acknowledgements: Funded by National Institute of Health, project "Foodborne transmission of zoonotic agents: a proof-of-concept study on the use of urban wastewater monitoring for epidemiological estimates and comparative genomic" (*ISS Independent Research 2023 - ISS20-f238f7c58314*).

P EPSTEIN-BARR VIRUS AND MULTIPLE SCLEROSIS: SEARCHING FOR NEW VIRUS-RELATED DISEASE BIOMARKERS AND INTERACTIONS WITH GENETIC PREDISPOSITION

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Multiple Sclerosis (MS) is the most prevalent chronic inflammatory demyelinating disease of the central nervous system, resulting from the interplay between genetic and environmental factors. Infection with the B-lymphotropic Epstein-Barr virus (EBV) is a prerequisite for MS, although the underlying mechanisms remain unclear. Most MS risk alleles are associated with immune-related genes. Among these, the genetic variant BAFF-var of the TNFSF13B gene, encoding for the B-cell Activating Factor (BAFF), is associ\ated with increased soluble BAFF levels and expansion of circulating memory B-cells, the main EBV reservoir. This suggests that BAFF-var may promote the survival of EBV-infected memory B cells, favouring viral reactivation and leading to immune homeostasis disruption, contributing to MS pathogenesis. This study aims to investigate the associations between EBV dysregulation and immune activation in People with MS (PwMS), focusing on the impact of BAFF-var on these mechanisms, by assessing EBV status and immune gene marker expression in the peripheral blood of PwMS and Healthy Donors (HD). Peripheral Blood Mononuclear Cells (PBMCs) and sera were collected from 215 therapy-naïve PwMS and 135 HD. BAFF-var prevalence was assessed by SNP genotyping. EBV DNA was quantified by droplet digital PCR. Expression of EBV latency and lytic transcripts, as well as transcripts linked to BAFF signalling and other immune-related genes, was analyzed by enhanced real-time RT-PCR. Serum levels of soluble BAFF, EBNA1 IgG, and VCA IgG were measured by ELISA. Statistical analyses were performed using non-parametric tests. In this cohort, we extended and validated our previous findings showing increased EBV DNA load and a higher

prevalence of transcripts associated with viral dysregulation, as well as elevated levels of both EBNA1 and VCA IgG in PwMS, compared to HD. Consistent with previous studies, BAFF-var carriers had higher serum BAFF levels ($p<0.001$). BAFF-var was more frequent in PwMS (33%; 30% heterozygotes, 3% homozygotes) compared to HD (23%; 20% heterozygotes, 2% homozygotes), while 67% of PwMS and 77% of HD were Wild-Type (WT). Within the PwMS group, BAFF-var carriers showed significantly higher expression of BAFF-related genes and genes associated with antiviral type I interferon signaling compared to WT individuals. Notably, VCA IgG titers were higher in BAFF-var carriers ($p=0.02$), suggesting a shift toward EBV reactivation. Although EBV viral load did not differ between groups, BAFF-var carriers exhibited increased expression of the EBV BZLF1 transcript, indicative of lytic infection. These results support EBV dysregulation in MS and suggest that elevated BAFF levels associated with BAFF-var may promote EBV latency disruption and reactivation in PwMS, accompanied by alterations in immune gene expression. This study reinforces the role of impaired EBV control in MS and highlights the contribution of genetic risk factors to viral dysregulation.

P ARCHIVES AS A SYSTEM: TOWARDS A GUIDE TO THE HISTORY OF THE ITALIAN NATIONAL INSTITUTE OF HEALTH (ISS)

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The documentary collection of the National Institute of Health (Istituto Superiore di Sanita, ISS) is the primary source for reconstructing its history and for understanding the role played by the organisation in the development of scientific research and health policies in Italy, since its foundation in 1934. However, studying the ISS's records means dealing with a fragmented context, both in terms of where the records are held and the records themselves, due to institutional and political changes which, over time, have prevented the records from being preserved and enhanced in the best possible way. It is within this context that the current doctoral research is carried out, aimed at producing a guide to sources for the history of the ISS, intended as a tool to provide an overview of the Institute's documentary heritage and related sources. The research focused primarily on the ISS's paper-based records held at the Archivio Centrale dello Stato, with particular attention to conditions of access and consultability. As regards the Institute's archival holdings, information was gathered on the status of reorganisation, the availability of finding aid and the level of detail they provide, to define the practical possibilities for using the documents; where it was possible to consult the material, useful information was collected to survey the archival units held. At the same time, the study led to the identification of related sources. These include a number of institutional archives, such as ministerial archives and parliamentary sources, as well as the personal archives of figures who, in various capacities, have left their mark on the history of the ISS, such as the archives of the directors Domenico Marotta and Giovanni Battista Marini Bettolo, whose papers are held at the Accademia Nazionale delle Scienze detta dei XL, or the archives of scientists who collaborated with the Institute, such as Mario Ageno, who was Head of the ISS Physics Laboratory, and Edoardo Amaldi, held at the Physics Library of Sapienza University of Rome. This outlines a system of interrelationships between the documentary collections, structured around two central cores: the ISS's paper-based records held at the ACS and the photographic material brought together in ArchISS, the ISS's multimedia archive. This system aims to present the related sources not as a series of isolated collections, but as a network of interconnected documentary fonds, whose records are interwined with the ISS's documentation, helping to define its evolution and history.

P GLOBAL ASSESSMENT OF ANTHROPOGENIC ORGANIC POLLUTANTS IN MARINE WATERS

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Large amounts of organic contaminants are released into the environment every year by industrial activities, urban settlements, maritime transport, agriculture and consumer products, with a significant proportion ultimately reaching the sea. Within the Ocean and Human Health framework, marine pollution is both an environmental and public health concern. Contaminants can impair ecosystem functioning, accumulate in marine organisms, enter food webs and potentially affect humans through seafood consumption and recreational or occupational exposure. However, their occurrence, distribution and fate in offshore waters and remote marine regions remain poorly understood. The Sea Care project is an Italian initiative coordinated by the Italian National Institute of Health in collaboration with the Italian Navy and several national research institutions. It aims to assess anthropogenic contamination in seas and oceans worldwide and improve understanding of its potential effects on marine ecosystems and human health. This study investigated selected organic contaminants in seawater samples collected from 133 sites worldwide, including the Mediterranean Sea, Atlantic Ocean, Arctic region, Red Sea, Persian Gulf, Pacific Ocean and Indian Ocean. At each site, samples were collected near the surface and at approximately 50 m depth. They were immediately frozen on board and subsequently processed in the laboratory using solid-phase extraction for analyte preconcentration and matrix clean-up, enabling trace- and ultra-trace-level detection. Target analytes included Polycyclic Aromatic Hydrocarbons (PAHs), di(2-ethylhexyl) Phthalate (DEHP), Polybrominated Diphenyl Ethers (PBDEs) and Alkylphenols (APs). These substances are of concern because of their persistence, widespread use, bioaccumulation potential and possible toxic or endocrine-disrupting effects. APs, PAHs and DEHP were quantified by Gas Chromatography-Mass Spectrometry (GC-MS), whereas PBDEs were analyzed by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS)[1]. APs were the most frequently detected contaminants, occurring at 71 sites: octylphenol was found at 66 sites and nonylphenol at 19, at concentrations of 3-90 ng/L. PAHs were detected at 60 sites, generally below 0.1 µg/L, with a maximum of 73 ng/L. DEHP occurred at 29 sites and reached 6.5 µg/L in the Red Sea. PBDEs were detected at 20 sites at concentrations of 0.04-0.4 ng/L. Their occurrence in both coastal and remote waters suggests long-range dispersal through ocean circulation, atmospheric transport, maritime activities and land-based inputs. Overall, this study highlights the importance of harmonized, multi-depth marine sampling combined with

advanced analytical methods for assessing anthropogenic contamination globally. Within the Sea Care framework, these data can help identify contamination patterns and priority areas, guide future monitoring strategies and support the protection of marine ecosystems and human health.

P MULTIDISCIPLINARY CHARACTERIZATION OF CLINICALLY RELEVANT ANTIBIOTIC-RESISTANT BACTERIA IN WASTEWATER TREATMENT PLANTS

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Antimicrobial Resistance (AMR) is a growing global health concern requiring a One Health approach due to the close interconnection between human, animal, and environmental health. As important interfaces between human activities and the environment, Wastewater Treatment Plants (WWTPs) represent relevant sites for AMR surveillance, as wastewater can serve as a reservoir of Antibiotic-Resistant Bacteria (ARBs) and their resistance determinants. This study aimed to combine conventional microbiological methods with spectroscopic techniques to characterise clinically relevant ARBs in wastewater and to evaluate the potential of vibrational spectroscopy as a complementary tool for the rapid differentiation of resistance phenotypes. Five WWTPs distributed across Italy were included in the study, one of which consisted of two hydraulically independent treatment lines. Seasonal samples of influent wastewater and treated effluent collected between 2024 and 2026 were analysed for the presence of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-Ec) and Vancomycin-Resistant Enterococci (VRE). Target bacteria were isolated by filtration and culture on selective media, and selected isolates were identified by MALDI-TOF MS. Resistance phenotypes were assessed according to EUCAST guidelines. The relative abundance of ESBL-Ec and VRE was assessed against total *E. coli* and total enterococci, respectively. Median ESBL-Ec/total *E. coli* ratios were 1.75% in influent and 1.31% in effluent samples, while median VRE/total enterococci ratios were 3.45% and 2.95%, respectively. The recovered ARB isolates were subsequently subjected to spectroscopic characterization. Spectra acquired using the MicroNIR OnSite-W system were analysed by Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) to explore discrimination between resistant and susceptible isolates. Preliminary PLS-DA models showed the highest performance for plate-grown isolates, with validation Non-Error Rate (NER%) values ranging from 98.8% to 100% and Validation Root Mean Square Error (RMSE) values ranging from 0.14 to 0.17. Lower performance was observed for spectra obtained from broth-grown isolates. In addition to NIR analysis, exploratory Fourier-Transform Infrared (FT-IR) spectroscopy of environmental vancomycin-resistant E. faecium isolates suggested spectral heterogeneity, warranting genomic characterization to assess whether it reflects distinct genetic lineages. Overall, these preliminary findings suggest that integrating conventional microbiological methods with vibrational spectroscopy could provide a complementary approach for the rapid characterisation and screening of resistance phenotypes in wastewater. Further studies involving a larger number of isolates are required to assess the robustness, reproducibility, and potential applicability of this approach to environmental AMR surveillance.

P WRN INHIBITION DRIVES GENOMIC INSTABILITY BUT SUPPRESSES INNATE IMMUNEACTIVATION IN CANCER CELLS

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Replication Stress (RS) is a hallmark of cancer and fuels tumor progression through theaccumulation of genetic alterations. RS can also contribute to the innate immune responses viathe cGAS-STING pathway. The WRN exo/helicase is one of the replication caretakers in human cells limiting RS andgenome instability, and it was recently discovered to be synthetic-lethal with microsatelliteinstability in cancer. A role of WRN in the RS-generated immune response has not beenpreviously explored. Using human cancer cell lines, we examined the eff ects of pharmacologicalWRN inhibition on innate immune signaling by a combined approach of immunofl uorescence,RNA sequencing, migration assays, and ELISA. Our results indicate that WRN inhibition caused accumulation of cytoplasmic DNA, consistentwith increased genomic instability, but unexpectedly, this didn't activate the cGAS-STINGpathway or induce a pro-infl ammatory transcriptional program. Instead, transcriptomic analyserevealed broad repression of innate immune genes, including interferons, cytokines, andchemokines such as CXCL10. Notably, WRN inhibition attenuated this response even undergenotoxic stress induced by etoposide, which is otherwise suff icient to strongly activate cGAS-STING signaling. Consistently, cGAS accumulated in the chromatin in WRN-inhibited cells, alikely inactive state, suggesting defective immune sensing under RS. Moreover, conditionedmedia from cells inhibited of WRN strongly dampens the migration of immune cells, consistentwith reduced expression of infl ammatory mediators. Our fi ndings identify an unexpected implication of the loss of WRN function as a driver of animmunosuppressive state, providing new insights into the interplay between RS and immuneescape, with potential therapeutic implications.

P INFLAMMATION IS ASSOCIATED WITH REDUCED NETWORK-BASED PLASTICITY AND CONSTRAINED CAPACITY FOR CHANGE IN MAJOR DEPRESSIVE DISORDER

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Plasticity, the capacity to modify mental state, plays a crucial role in the transition from psychopathology to well-being. The Network theory of plasticity1 proposes that individual plasticity can be operationalized as the inverse of global network connectivity strength (e.g., symptom co-occurrence): weak connectivity reflects high plasticity, and vice versa1,2. Given the reported interplay between immune functioning and plasticity in Major Depressive Disorder (MDD)3, the present study aims to validate the inflammatory trap hypothesis, investigating whether an increase in inflammatory markers reduces plasticity and constrains the capacity for change in individuals with MDD, using a symptom network-based operationalization of plasticity. Data were derived from the Netherlands Study of Depression and Anxiety-Ecological Momentary Assessment substudy (NESDA-EMA). Of 384 participants, 353 were included to validate the network theory of plasticity, with no more than 4 missing assessments and variance different from zero. A subsample of 337 with available inflammatory markers was used for the inflammatory analysis. Participants completed a 31-item questionnaire 5 times/day for 14 days, including 16 momentary affect states (7-point scale) plus contextual items. Main outcomes were mood change in week 2 (days 9-14): absolute/directional net change and maximum change. We calculated the Connectivity Strength as the sum of all edge weights, and it was estimated from baseline affect states (days 2-8) using Dynamic Time Warping (DTW), converted into similarity matrices via a Radial Basis Function kernel (σ=0.5). Inflammatory markers included C-Reactive Protein (CRP), Interleukin (IL)-6, IL-1β, TNF (Tumor Necrosis Factor)-α, and a composite of the first two (inflammatory index). A one-month current depression diagnosis, assessed via the Composite International Diagnostic Interview (CIDI), was used to stratify the subsample into MDD and no-MDD. Higher Connectivity Strength (lower plasticity) significantly predicted smaller mood changes (|Maximum-change|: R=-0.47; |Net-change|: R=-0.31, both $p<0.001$), independently of the emotional trajectory. The inflammatory index was positively associated with Connectivity Strength in MDD (R=0.53, $p<0.001$), but not in no-MDD (R=0.02, p=0.72). CRP and IL-6 individually were also significantly associated with reduced plasticity (R=0.47 and R=0.50, both $p<0.01$), whereas no association was observed for IL-1β (R=0.27, p=0.10) and TNF-α (R=0.03, p=0.85). In MDD, higher inflammation was linked to reduced mood-change capacity (|Maximum-change|: R=−0.34,

p<0.05; |Net-change|: R=-0.27, p=0.09). These findings support network-based plasticity as a predictor of individual capacity for change1,2, offering a promising tool for anticipating disease trajectories. Inflammation appears to reduce plasticity in MDD, consistent with the inflammatory trap hypothesis3. Replication in larger longitudinal samples is warranted.

P BIALLELIC MUTATIONS IN TBCD, ENCODING THE TUBULIN FOLDING COFACTOR D, PERTURB MICROTUBULE DYNAMICS AND CAUSE A WIDE NEURODEVELOPMENTAL/NEURODEGENERATIVE DISEASE SPECTRUM

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Mutations in genes encoding tubulins and microtubule-associated proteins are known to cause neurodevelopmental and neurodegenerative disorders. Our group demonstrated that biallelic mutations in TBCD, encoding one of the five co-chaperones required for assembly and disassembly of the αβ-tubulin heterodimer, the structural unit of microtubules, cause a disease with neurodevelopmental and neurodegenerative features. Here, we present the functional and clinical profiling of disease-associated TBCD variants identified in a large series of newly enrolled patients and affecting different domains of the chaperone. Biochemical analyses performed on patients' fibroblasts showed variably reduced levels of TBCD, indicating relative instability of a subset of mutant proteins, and defective β-tubulin binding. Cytogenetic analyses were carried out to test chromosome stability and chromosomal damages, showing severe genome instability, which was associated with a significant delay in the progression into mitosis, indicating a transient blockade occurring during spindle formation. Polymerization and depolymerization assays

demonstrated the occurrence of variable aberrant Microtubule (MT) dynamics characterized by delayed depolymerization and accelerated repolymerization, which correlated with the severity of disease. Defective MT dynamics affects necessarily the organelles distribution and primary cilium biogenesis. Accordingly, we observed an evident Golgi apparatus fragmentation and aberrant cilium morphology in primary fibroblasts from patients with mild and severe phenotype. Our findings confirm that defective TBCD function underlies a recognizable encephalopathy, characterized by altered microtubules dynamics affecting chromosomes stability, organelles distribution and cilium biogenesis, with is expected to impact neuronal function and survival. The analysis of these variants allow to more accurately defining the consequences of the disease-causing mutations on TBCD function and microtubule arrangement, and appreciating how altered microtubule dynamics contributes to pathogenesis.

P POSTBIOTIC SUPPLEMENTATION IN AUTISM: THE B(AUT)YRATE MULTICENTER RANDOMIZED CONTROLLED TRIAL

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Background. Autistic individuals frequently present with Functional Gastrointestinal Disorders (FGIDs), gut–brain interaction conditions with altered motility, visceral hypersensitivity and other functional abnormalities without organic cause. Often co-occurring with sleep and behavioral disturbances, they impair functioning and complicate autism management. FGIDs and autism share partially overlapping mechanisms: gut microbiota imbalance, immune dysregulation and reduced butyrate, a neuroactive Short-Chain Fatty Acid (SCFA) with anti-inflammatory, epigenetic and mitochondrial functions. Postbiotic butyrate is a promising, safe way to restore gut-brain communication and improve gastrointestinal and behavioral outcomes. Objectives: To evaluate the efficacy, safety and tolerability of oral sodium butyrate in reducing gastrointestinal and behavioral symptoms in preschool autistic individuals with FGIDs, and to identify clinical and biological signatures of treatment response by integrating microbiome, immune and behavioral data.

Methods.The multicenter, randomized, double-blind, placebo-controlled b(AUT)yrate trial is recruiting 128 autistic individuals aged 3.0-6.11 years with FGIDs at two Italian academic hospitals (Rome and Naples), randomized 1:1 to oral sodium butyrate or placebo for 16 weeks; clinical and biological assessments at baseline, 4 and 8 months. Primary endpoint: clinically meaningful FGIDs improvement (≥4-point reduction on the 6-item Gastrointestinal Severity Index at 4 months *vs* baseline). Secondary endpoints: core autism symptoms, adaptive functioning, sensory processing, sleep quality and parental quality of life (standardized validated tools). Biological outcomes: gut microbiota composition, fecal SCFAs, immune and

inflammatory biomarkers, peripheral blood mononuclear cell phenotypes. Analyses use mixed-model repeated measures, multivariate approaches and machine learning to identify predictors and mediators of response.

Results. The ongoing trial has enrolled 98% (N=126) of the planned sample, with 6 withdrawals and no adverse effects to date; study completion is anticipated in April 2027. Butyrate supplementation is expected to reduce FGIDs severity and improve behavioral, adaptive and sleep outcomes, reflecting normalized gut–brain axis function, and to partly restore the microbiome, recover butyrate-producing taxa and shift cytokines toward an anti-inflammatory profile. Integrated clinical–molecular analysis will identify the phenotypes most responsive to butyrate, enabling targeted interventions and enhancing children's and parents' quality of life, ultimately reducing the healthcare burden of autism.

Conclusions. This first large-scale, rigorous RCT of a postbiotic for autism-associated gastrointestinal dysfunctions targets a key pathway linking gut health to neurobehavioral regulation, offering an innovative, non-pharmacological approach to improving gastrointestinal and behavioral outcomes in autistic individuals.

P ALTERED FACIAL DYNAMICS AS AN EARLY BIOMARKER OF RETT SYNDROME

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Rett Syndrome (RTT) is a rare, female-prevalent neurodevelopmental disorder affecting motor, cognitive and social skills with wide clinical variability. It is caused by de-novo mutations in the X-linked Methyl-CpG-binding Protein 2 (MECP2) gene. Although no cure is currently available, an increasing number of promising therapeutic options are emerging, with several clinical trials currently underway and trofinetide approved in 2023 as the first symptomatic treatment. RTT clinical progression is characterized by a perinatal period of apparently normal development, followed by a regression of previously acquired skills around 2 years of age. Due to the late onset of overt symptoms and the need for genetic confirmation, diagnosis occurs late in infancy, precluding any type of early intervention. Evidence from retrospective home video analysis suggests that subtle abnormalities in facial and body dynamics may already be present in RTT infants well before overt symptom onset, though these are often too subtle to be reliably detected by non-expert clinicians. Automated computer vision approaches have been increasingly applied to detect behavioral and motor alterations in the context of neurodevelopmental disorders. However, no studies have attempted to leverage this promising strategy to enable fine-grained discrimination between RTT and Typically Developing (TD) newborns. To investigate whether computer vision tools can detect early alterations in facial dynamics, we analyzed retrospective home videos recorded during the first six months of life of newborns later diagnosed with RTT (n=19) alongside those of TD peers (n=33) as controls. Videos were analyzed using Baby FaceReader 10 (Noldus), a software that automatically encodes individual facial muscle movements (Action Units, AUs) based on the Facial Action Coding System for Infants and Young Children (Baby FACS). Frequency and duration of each AU, normalized to the total recording duration, were compared between RTT and TD using mixed models. We found that RTT newborns exhibit detectable alterations in the duration and frequency of lower face AUs involving movements of the mouth and chin, compared to TD peers, confirming early abnormalities in facial motor control. Overall, these results highlight the potential of automated tools for anticipating the identification of RTT. Indeed, by making screening tools more widely accessible to the general population, the implementation of digital approaches may increase awareness of early signs of RTT, prompting timely clinical referral and access to care. Funded by the European Union - Next Generation EU - NRRP M6C2 - Investment 2.1 "Enhancement and strengthening of biomedical research in the NHS" (*PNRR-MR1-2022-12376808*).

P PATIENT-DERIVED HUMAN IPSC ASTROCYTES RECAPITULATE MEGALENCEPHALIC LEUKOENCEPHALOPATHY WITH SUBCORTICAL CYSTS AND REVEAL DEFECTS IN ASTROCYTE MATURATION AND KIR4.1 CHANNEL FUNCTION

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Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC) is a rare leukodystrophy caused by astrocyte dysfunction and is predominantly associated with pathogenic variants in the MLC1 gene, which encodes the astrocyte-specific membrane protein MLC1. The disease is characterised by diffuse brain oedema, myelin vacuolation and subcortical cyst formation. Clinically, patients commonly present with motor impairment, including ataxia and spasticity, together with epilepsy. Although the causative gene has been identified, the physiological role of MLC1 and the molecular mechanisms underlying disease development remain poorly understood, thereby limiting the identification of effective therapeutic strategies. This is particularly relevant given that partial clinical recovery has been reported in a subset of patients. To overcome the limitations of existing animal models, which only partially reproduce the human phenotype, we established a patient-specific in vitro model by generating astrocytes from induced Pluripotent Stem Cells (iPSCs) reprogrammed from fibroblasts obtained from three healthy donors and three MLC patients. Using complementary molecular, biochemical, electrophysiological and imaging approaches, we confirmed that MLC astrocytes exhibit impaired cell volume regulation, cytoplasmic vacuolation and altered Epidermal Growth Factor Receptor (EGFR) expression, consistent with observations from previous disease models. Importantly, our findings also reveal unrecognised pathological features, including endosomal abnormalities, increased cellular proliferation, and dysregulated expression of key astrocyte maturation markers, such as EAAT1, GFAP, Cx43, AQP4, and Kir4.1. Notably, alterated Kir4.1 expression was associated with impaired potassium currents, indicating defective potassium homeostasis in patient-derived astrocytes. These findings provide the first evidence that MLC1 mutations alterate astrocyte maturation and potassium buffering, two mechanisms that are likely to contribute to disease pathogenesis. Overall, the patient-derived iPSC astrocyte model generated in this study provides new insights into the molecular and cellular consequences

of MLC1 deficiency and identifies, for the first time, a role for MLC1 in astrocyte development. Furthermore, it represents a valuable platform for preclinical drug screening and supports the development of personalised therapeutic strategies for this rare leukodystrophy.

P STUDY OF THE EFFECTS OF PHYTOCOMPOUNDS PRESENT IN ESSENTIAL OILS ON BIOFILM FORMATION AND THE PATHOGENICITY OF THE ENTEROHEMORRHAGIC STRAIN *ESCHERICHIA COLI* O157:H7

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Escherichia coli O157:H7 is a major enterohemorrhagic pathogen responsible for foodborne enterocolitis and hemolytic uremic syndrome (HUS) worldwide. Cattle represent the primary reservoir of this pathogen, with human infections occurring mainly through the consumption of contaminated bovine-derived foods or fresh produce exposed to bovine fecal material, particularly in the Western Hemisphere. The pathogenicity of *E. coli* O157:H7 is largely associated with three major virulence determinants: *Shiga* toxins, the Locus of Enterocyte Effacement (LEE) pathogenicity island, and the pO157 virulence plasmid. Together, these factors contribute to the severe intestinal and systemic manifestations associated with infection, including hemorrhagic colitis and HUS. The absence of effective specific therapeutic options, combined with the capacity of this pathogen to form biofilms and tolerate conventional antimicrobial treatments, emphasizes the need for alternative strategies to control *E. coli* O157:H7 infections. Phytocompounds, naturally occurring bioactive molecules derived from plants, have emerged as promising antimicrobial candidates against a wide range of pathogenic microorganisms. In the present study, we investigate three major phytocompounds obtained from essential oils of plants belonging to the genera Cinnamomum and Cymbopogon. Previous studies have demonstrated the antimicrobial activity of these compounds against both planktonic bacteria and biofilms of several pathogens, including *Shigella, Klebsiella* and *staphylococcus spp*. In this PhD project, we evaluate their antimicrobial efficacy against planktonic *E. coli* O157:H7 strain EDL933, as well as their ability to inhibit biofilm formation and eradicate established mature biofilms. Furthermore, the effects of these phytocompounds on the expression of key virulence-associated genes and genes involved in the early stages of biofilm development are investigated. Finally, their therapeutic potential is assessed *in vivo* using a *Caenorhabditis elegans* infection model, providing further insight into the efficacy and biological relevance of these phytocompounds against *E. coli* O157:H7.

P DESCRIPTIVE STATISTICAL DISTRIBUTIONS OF ALPHA AND BETA-EMITTING RADIONUCLIDES, RADON AND TRITIUM ACTIVITY CONCENTRATIONS IN DRINKING WATER: RESULTS FROM DATA ANALYSIS

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Italian Legislative Decree No. 28/2016, transposing Directive 2013/51/EURATOM, sets the requirements for the protection of public health regarding radioactive substances in water intended for human consumption. According to this decree, the Regions and Autonomous Provinces design and implement Control Programmes (PdC) for monitoring radioactivity in drinking water. The results are reported annually to the Ministry of Health and to the National Instituto of Health (Istituto Superiore di Sanità, ISS), the national public health institute, which maintains the national archive of both planned schedules and measurements actually performed. The statistical distributions of the activity concentrations of gross alpha, gross beta, radon and tritium in drinking water were analysed from measurements carried out by the regional environmental protection agencies (ARPA) under the first and second PdC during 2018-2022. The main challenge is the treatment of measurements below the Minimum Detectable Activity (MDA), about 55% of the total, with wide variability across parameters: from 28% for radon to 88% for tritium. These left-censored data are handled by two families of methods: substitution with a conventional value, under four assumptions (MDA, MDA/2, 0.7 MDA and MDA_Random), and adaptive methods, which treat censoring explicitly without assigning values to non-detects (maximum likelihood, MLE, and Kaplan-Meier). The Shapiro-Wilk test and the Departure Index identify the log-normal as the most appropriate distribution for all four parameters. The result is confirmed independently: the normal, gamma and Weibull families were fitted to the same measurements by censored likelihood and compared using the Akaike Information Criterion (AIC), requiring no substitution of non-detects. Observed quantiles align with the expected ones fully for gross alpha and radon, only partially for gross beta and tritium. Comparing the standard deviation estimated by MLE with that obtained under the four assumptions shows that substitution methods systematically compress dispersion, increasingly so as censoring grows: the MLE estimate exceeds the substituted one by 20-30% for radon and by almost a factor of three for tritium. The log-normality assumption is therefore verified where the data allow it and extended to Supply Zones (SZ) with few measurements, where a distributional check is not feasible; the intensively monitored sample does not differ appreciably from the overall set of SZs. The distribution thus identified will provide the basis for estimating the probability that future measurements exceed the parametric values. The results are intended for integration into the system used by the ISS to assess the adequacy of current drinking water monitoring, particularly the coverage of supply zones to be monitored.

P MAMMALIAN CELL-BASED PRODUCTION OF RECOMBINANT VIRAL ANTIGENS FOR SEROLOGICAL ASSAY DEVELOPMENT: WEST NILE VIRUS ENVELOPE DOMAIN III (EDIII) AND NON-STRUCTURAL PROTEIN 1 (NS1) AS A MODEL

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The choice of expression system can significally influence the folding pathway and final conformation of recombinant proteins. Although prokaryotic systems based on E. coli as well as eukaryotic platforms utilizing insect cells and plants are widely employed owing to their high production efficiency, scalability, and cost-effectiveness, only mammalian cell systems reliably support the biosynthesis of recombinant proteins endowed with functional, native-like post-translational modifications, thereby preserving their antigenic properties. In this context, the HEK-293F mammalian expression platform uniquely combines the native post-translational processing capacity of mammalian cells with high-yield, scalable protein production. West Nile Virus (WNV) Non-Structural Protein 1 (NS1) and the Envelope protein Domain III (EDIII) were selected as model antigens to validate the platform. Both NS1 and EDIII represent suitable antigenic targets owing to their high immunogenicity and the presence of epitopes that may confer greater specificity for WNV serological detection. The coding sequences of NS1 and EDIII were cloned into an expression plasmid containing an Igκ signal peptide and a C-terminal 6×His tag. HEK-293F cells were transiently transfected, and culture supernatants were collected 96 h post-transfection. Recombinant proteins were recovered by Ni-NTA affinity chromatography and characterized by SDS-PAGE, Western blotting, and ELISA. Both proteins were efficiently secreted into the culture supernatant and purified to high purity. Analyses under reducing and non-reducing conditions revealed differences in electrophoretic mobility that were consistent with the presence of disulfide bonds. Moreover, deglycosylation of rNS1 resulted in an electrophoretic shift consistent with the removal of its two conserved N-glycosylation sites, whereas rEDIII, which lacks N-glycosylation sites, showed no appreciable change in electrophoretic mobility. Both recombinant proteins retained reactivity with specific monoclonal antibodies, confirming the preservation of relevant antigenic properties. These results indicate that the developed expression system provides a versatile platform for the production of high-quality viral antigens, with the potential to improve the sensitivity and specificity of existing serological assays.

P SUPPORTING PERIADOLESCENT BRAIN DEVELOPMENT THROUGH OMEGA-3 AND MEMBRANE LIPID SUPPLEMENTATION: EFFECTS ON COGNITION AND PREFRONTAL CORTEX INTEGRITY IN MICE

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Periadolescence represents a critical period of brain maturation characterised by synaptic pruning, myelination and the refinement of higher cognitive functions. These developmental processes make individuals vulnerable to environmental factors, including diet. The latter can can exert both beneficial and detrimental effects on brain structure and function. Several lipids such as docosahexaenoic acid, an omega-3 polyunsaturated fatty acid, and sphingomyelin, a a sphingolipid involved in membrane and myelin formation, play essential roles in neuronal membrane integrity and synaptic signalling, while Palmitoleic Acid (POA), an omega-7 monounsaturated fatty acid, has recently emerged as a potential neuroprotective lipid. This study aimed to investigate the effects of a nutrient-enriched diet containing these specific lipids during periadolescence on cognitive function in mice and to evaluate whether further supplementation with POA, provides additional cognitive benefits. At weaning (Postnatal Day (PND) 21), male and female CD1 mice were randomly assigned to one of three experimental groups: (i) Control Diet (CD), standard laboratory chow diet with balanced macronutrient content; (ii) Enriched Diet (ED), supplemented with DHA and sphingomyelin; (iii) Enriched Diet + Palmitoleic Acid (ED+POA), same formulation as the ED, further supplemented with POA. From PND35 to PND60, mice underwent behavioural assessment including the attentional set-shifting task (cognitive flexibility), Barnes maze (spatial memory and reversal learning), and elevated zero-maze (anxiety-like behaviour). Structural and metabolic brain alterations associated with behavioural performance were examined using in vivo neuroimaging techniques, such as magnetic resonance spectroscopy and diffusion tensor imaging. Data were analysed using repeated analysis of variance for split-plot designs followed by Tukey's post hoc test. Mice receiving either of the enriched diets showed improved cognitive flexibility compared with controls, as demonstrated by fewer errors committed during the extra-dimensional shift phase of the attentional set-shifting task and reduced latency to find the escape box during the reversal phase of the Barnes maze. These behavioural improvements were accompanied by increased N-acetylaspartate concentrations and higher fractional anisotropy in the prefrontal cortex of ED mice, indicating enhanced neuronal metabolism and improved microstructural organisation. Dietary supplementation with selected lipids during periadolescence enhances cognitive flexibility and learning performance in mice while

promoting favourable neurobiological changes in the prefrontal cortex. These findings support the potential role of targeted nutritional interventions in optimising periadolescent brain development and cognitive maturation.

P CLEARING THE PATH TOWARDS A MORE QUITABLE HEALTH CARE: EVIDENCE FROM THE ITALIAN PITER AND MASLD COHORTS

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Background. Social Determinants of Health (SDoH) are non-clinical factors shaping inequities in risk exposure and outcomes. This interim analysis evaluates their role in liver disease severity in two cohorts of Italian patients in care: the ITA-MASLD (Italian Metabolic Dysfunction-Associated Steatotic Liver Disease) cohort (n=169) and the PITER (Italian Platform for the Study of Viral Hepatitis Therapies and Outcomes) cohort of chronic viral hepatitis patients (n=81), focusing on fibrosis progression as a proxy of liver cancer risk.

Methods. SDoH were assessed using standardized questionnaires based on domains including education, social context, environment and financial stability. A composite indicator of economic insecurity was derived from individual items. These metrics were integrated with anthropometric data to explore associations with clinical risk factors. Severe fibrosis was defined as F3-F4.

Results. Across cohorts, better socioeconomic conditions were associated with milder disease, whereas vulnerable groups showed higher F4 prevalence (56.3% *vs* 39.5%). In MASLD, severe fibrosis was associated with economic insecurity (p=0.0258) and low education (p=0.0106), while no association was observed with occupational status (p=0.4616). In PITER, vulnerability was higher, with unemployment at 20% *vs* 6.6% and difficulty coping with unexpected expenses at 50% *vs* 21.9%. A multivariable logistic regression model was implemented for the MASLD cohort. No significant associations were found between SDoH indicators and clinical risk factors, except for a significant relationship between obesity (binary, normal *vs* overweight/obese) and economic insecurity (p=0.034). Obesity was consequently included in the multivariable model for adjustment. Multivariable analysis confirmed the association of economic insecurity (OR=2.48, 95% CI 1.12 to 5.70) and age (OR=1.07/year, 95% CI 1.02 to 1.12) with severe fibrosis in the MASLD cohort. Conversely, occupational status (OR=2.11, 95% CI 0.79 to 5.95), low education (OR=2.13 95% CI 0.99 to 4.75), obesity (OR=1.01, 95% CI 0.22 to 4.04) and sex (OR=0.50, 95% CI 0.21 to 1.15) were not significantly associated with severe fibrosis. The model successfully established that specific socioeconomic stressors maintain statistical significance even after controlling for traditional metabolic risk factors, age and sex.

Conclusions. Social disadvantage is a key determinant of liver disease severity across etiologies. These findings support risk-based approaches that integrate SDoH evaluation with clinical factors. Embedding social vulnerability in care pathways is essential to ensure no one is left behind and to reduce inequities in liver disease and cancer risk.

P STUDY OF THE ROLE OF INFLUENZA A INFECTIONS IN NEURODEGENERATION

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Influenza A Virus (IAV) represents a major global health issue, known for causing both seasonal epidemics and worldwide pandemics. Although the virus primarily affects the respiratory system, its ability to infect various cell types and tissues allows it to spread to other sites, including the Central Nervous System (CNS). Within the CNS, it induces acute neurological effects mainly mediated by neuroinflammation, which is reported as a potential risk factor for long-term sequelae linked to neurodegenerative diseases, including Alzheimer's Disease (AD) and Parkinson's Disease (PD). Interestingly, proteins associated with neurodegeneration, such as Amyloid-beta peptide (Aβ) and phosphorylated tau (ptau), key molecular markers of AD, or α-synuclein (α-Syn), linked to PD, have recently been suggested to play a protective role in response to pathogen brain invasion, whereas their chronic dysregulation leads to pathological events. In this context, the short- and long-term consequences of IAV brain infection remain poorly understood. To address this issue, in vitro and ex vivo models of infection consisting of rodent primary neuron-glia co-cultures and mouse organotypic brain slice cultures were used, and the expression and distribution of neurodegeneration-related proteins was assessed following IAV infection. Specifically, exploiting Western blot, confocal immunofluorescence, real-time PCR, and virological assays, we evaluated the expression levels and patterns of Amyloid Precursor Protein (APP), Aβ, tau and ptau, as well as α-Syn, were evaluated. Additionally, synaptic integrity was assessed using the post-synaptic marker PSD-95, alongside the effects on inflammation and the involvement of autophagy. Following IAV infection, we observed an increase in the expression of interleukin-1β, a key mediator of the inflammatory response, alongside the upregulation of peptides with a well-established neurodegenerative potential and a recently suggested antimicrobial role. Specifically, acute IAV infection induced altered processing of APP and the subsequent accumulation of Aβ. Furthermore, an increase in ptau p was detected alongside its nuclear translocation, suggesting that such compartmentalization may alter cellular physiology during infectious stress. Concurrently, an elevation in tau aggregated forms was observed. A similar upregulation was recorded for α-Syn. In parallel, IAV infection modulated autophagic flow, suggesting that altered cell clearance may contribute to the accumulation of these peptides. While the production of these peptides may reflect a host defence attempt to counteract IAV acute infection, their accumulation may lead to neurodegeneration. Consistently, a significant decrease in PSD-95 expression levels was observed, indicative of potential synaptic impairment. Overall, our results indicate that IAV infection triggers neurodegenerative pathways during the acute phase of infection.

P UNCONVENTIONAL T CELL IMMUNITY AGAINST ANTIBIOTIC-RESISTANT BACTERIA

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Introduction. Unconventional T cells are a subset of T lymphocytes recognizing non-peptide antigens, lipids and metabolites presented by non-classical MHC-like molecules through a conserved TCR repertoire. They include Mucosal-Associated Invariant T cells (MAIT), invariant Natural Killer T cells (iNKT) and γδ T which are important in infections because they recognize highly conserved microbial antigens. Acting as a bridge between innate and adaptive immunity, especially at mucosal and barrier sites, they rapidly produce cytokines and cytotoxic molecules. These properties make them promising effectors against antibiotic-resistant bacteria. Antimicrobial resistance is a global health threat. *Mycobacterium abscessus* and *Mycobacterium avium* are highly antibiotic-resistant mycobacteria causing chronic pulmonary disease. *Escherichia coli* and *Acinetobacter baumannii* are Gram-negative bacteria responsible for resistant urinary tract infections and bloodstream infections. However, the unconventional T cell response to these pathogens remains poorly understood.

Aims. 1. Analyse MAIT, γδ T and iNKT responses to *M. abscessus, M. avium, E. coli and A. baumannii*. 2. Determine the impact of sex on unconventional T cell responses to antibiotic-resistant bacteria. 3. Evaluate unconventional T cell responses to clinical strains of M. abscessus isolated from cystic fibrosis patients.

Methods: CD14+ monocytes were purified from PBMCs of healthy donors by magnetic bead isolation and stimulated with heat-killed bacteria at different ratios. The CD14- fraction containing T lymphocytes was labelled with CFSE to evaluate cell proliferation. Cocultures were analysed on day 11 by CFSE dilution and multiparametric flow cytometry using anti-CD3, CD4, CD8, CD161, CD56, TCR Vα24/Jα18, TCR Vα7.2 and TCR γδ antibodies. Intracellular staining measured IFN-γ, TNF-α, IL-17 and granzyme B production. Bacteria were cultured in appropriate media. Clinical strains were obtained from the National Institute of Health strain library.

Results. MAIT and γδ T, but not iNKT, proliferated after stimulation with autologous monocytes pulsed with *M. abscessus, M. avium, E. coli* and *A. baumannii*, suggesting differential expression of microbial antigens recognized by unconventional T cells. MAIT and γδ T also produced IFN-γ, TNF-α, IL-17 and granzyme B, supporting their role in antibacterial defence through inflammatory and cytotoxic functions. No sex differences were observed in unconventional T cell responses. The evaluation of unconventional T cell responses to clinical strains of *M. abscessus* is in progress.

Conclusions. MAIT and γδ T recognize antibiotic-resistant bacteria. Although sex can influence the incidence and outcome of bacterial infections, no differences were found in unconventional T cell responses between men and women. Studying clinical strains is essential because they better represent the microorganisms responsible for human infections compared with standard laboratory strains.

P UNCOVERING ERYTHROCYTE DETERMINANTS OF SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* INVASION

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Malaria is one of the deadliest infectious diseases worldwide, causing 282 million cases and 610,000 deaths in 2024, mainly due to Plasmodium falciparum. This parasite invades Red Blood Cells (RBCs), where it develops and replicates before releasing new extracellular invasive forms, called merozoites. These must rapidly invade new RBCs through coordinated parasite–host interactions and disruption of this process can severely impact parasite survival, making invasion a point of weakness in the parasite life cycle. Malaria has been a strong selective pressure on the human genome, and several erythrocyte traits are known to confer resistance to malaria. For instance, erythrocytes from individuals with the Dantu blood group or hereditary ovalocytosis display increased membrane tension or rigidity and reduced susceptibility to invasion. Here, we aim to identify novel erythrocyte determinants of P. falciparum invasion by combining *P. falciparum* invasion assays on RBCs from individual donors with a comprehensive physicochemical characterization of the same samples. We established and validated an integrated experimental pipeline in which invasion is quantified by flow cytometry and normalized to a pooled RBC reference sample. Donor RBCs are further characterized by osmotic fragility testing, complete blood count, rheometry, and membrane lipid and protein analyses, allowing invasion phenotypes to be correlated with membrane biophysical properties, deformability, and molecular composition. Erythrocytes from thirteen donors were tested in three independent biological invasion assays. Assay reproducibility was assessed by calculating the Coefficient of Variation (CV) across the three biological replicates for each donor, resulting in a mean within-donor CV of 7.4% (range, 2.0-16.0%). Pearson's correlation analysis revealed significant associations between P. falciparum invasion rates and some RBC characteristics. The strongest correlations were observed with the membrane proteins spectrin and ankyrin, whose higher levels were associated with increased *P. falciparum* invasion efficiency and lower levels with reduced invasion. Overall, these results demonstrate that our approach provides a robust and quantitative framework for assessing individual susceptibility to *P. falciparum* invasion and reveals measurable inter-individual variability. These preliminary findings support the potential of this platform to identify novel host determinants of malaria susceptibility and enable large-scale screening in malaria-endemic populations.

A A MULTIPARAMETRIC APPROACH COMBINING NIR SPECTROSCOPY, THERMOGRAVIMETRY AND RHEOLOGY FOR THE QUALITY CONTROL OF FACTOR VIII AND VON WILLEBRAND FACTOR CONCENTRATES

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Factor VIII (FVIII) and von Willebrand Factor (vWF) are two plasma proteins that play a fundamental role in hemostasis, being involved in coagulation and platelet adhesion, respectively. FVIII and vWF concentrates can be obtained from human plasma through industrial manufacturing processes and are used as life-saving therapies for the treatment of several bleeding disorders. Quality control of these plasma-derived medicinal products is essential to ensure their quality, safety and efficacy. In the present study, rapid analytical techniques, including Thermogravimetric Analysis (TGA), near-infrared spectroscopy (NIRs), and rheological analysis, were investigated as part of a multiparametric approach for quality control of FVIII and vWF concentrates. Thermogravimetric Analysis (TGA), performed using a TGA 8000 thermobalance, was used to characterize the thermal behavior of the medicinal products by monitoring mass changes as a function of temperature under controlled experimental conditions. NIR spectroscopy, based on the absorption of near-infrared radiation by the samples, provided characteristic spectral profiles that were rapidly acquired using the portable MicroNIR spectrophotometer in the 950-1650 nm wavelength range. Chemometric tools were applied to interpret the obtained thermogravimetric and spectroscopic data, including Principal Component Analysis (PCA) for exploratory analysis and Partial Least Squares - Discriminant Analysis (PLS-DA) for sample classification and discrimination. PCA highlighted distinctive patterns in the thermal and spectral profiles, revealing clusters among the samples, while discriminant analysis enabled the development of accurate predictive models. Rheological analyses were performed using the Anton Paar Physica MCR 301 Rheometer, Parallel Plate System. Rotational viscosity measurements were used to evaluate the flow behavior of the formulations under shear rates representative of microcirculatory and macrocirculatory conditions, while oscillatory tests were performed to characterize their viscoelastic properties by measuring the storage modulus (G') and loss modulus (G"). Overall, the integration of thermal and spectral profiles with rheological parameters, combined with multivariate statistical analysis, enabled the development of a rapid and complementary multiparametric analytical approach, with potential application in the quality control of FVIII and vWF concentrates.

A FROM ANIMAL PRE-CLINICAL MODELS TO NAMS: REGULATORY CHALLENGES AND OPPORTUNITIES IN ATMP NON-CLINICAL DEVELOPMENT

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The non-clinical development of medicinal products traditionally relies on in vitro and in vivo models to characterize pharmacological activity and identify safety concerns before first-in-human administration. However, the translational relevance of conventional animal models may be limited by interspecies differences, particularly for complex products such as Advanced Therapy Medicinal Products (ATMPs), whose species-specific mechanisms of action, cellular interactions, biodistribution, persistence, and immunological properties may hinder identification of pharmacologically relevant animal models and reduce their predictive value for human responses. Within a regulatory landscape shaped by the 3Rs principles and the aim of reducing animal use, New Approach Methodologies (NAMs) offer opportunities to generate more human-relevant non-clinical evidence. Among these, advanced in vitro systems such as Organ-on-Chip (OoC) technologies may reproduce key aspects of human tissue architecture, functionality, and intercellular interactions, potentially improving the predictivity of non-clinical studies. This regulatory research aims to assess CTA submissions to identify emerging trends and opportunities in integrating NAMs into ATMP non-clinical development, drawing on observations from training activities involving assessment of early-phase Clinical Trial Applications (CTAs) at the National Institute of Health (Istituto Superiore di Sanità - ISS). This experience indicates growing interest in incorporating NAMs into non-clinical development programmes, reflecting the increasing need for more predictive, human-relevant testing strategies. Nevertheless, scientific innovation alone is not sufficient for regulatory integration: implementing new testing systems requires adequate evidence that the method is reliable and fit for its intended purpose. Validation is therefore critical to establishing an appropriate context of use and demonstrating robustness, reproducibility and biological relevance. Regulatory acceptance subsequently determines whether data generated through a validated methodology can be considered adequate to address specific regulatory questions and support decision-making. Preliminary observations from CTA assessments suggest that, despite growing interest in NAMs, their application remains largely confined to pharmacodynamic investigations, used mainly as complementary tools alongside conventional approaches to provide additional evidence of pharmacological activity. Their implementation in non-clinical safety assessment, by contrast, remains limited. A comprehensive, granular evaluation of CTA submissions may offer insight into emerging NAM applications and the scientific and regulatory barriers still constraining broader adoption. Expanding NAM use in safety assessment represents a critical milestone for implementing the Replacement principle of the 3Rs and for transitioning towards more human-relevant, animal-free testing strategies in ATMP development.

A IMPROVING RETINOBLASTOMA BRACHYTHERAPY TREATMENT WITH A DEDICATED MONTE CARLO TOOL

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Retinoblastoma is the most prevalent intraocular malignancy in children and poses a severe risk of vision loss or enucleation. While the standard of care often involves a combined regimen of chemotherapy, cryotherapy, and focal treatments, brachytherapy is highly effective for tumors localized in specific retinal regions. The application of episcleral radioactive plaques containing Ru-106 isotopes delivers a high, localized radiation dose directly to the tumor, significantly improving ocular preservation rates. However, its clinical implementation is limited by the lack of patient-specific 3D treatment planning. Clinicians currently rely on a 1D dosimetric calibration provided by the plaque manufacturer, which lacks comprehensive spatial dose characterization near the plaque surface. This leads to uncertainties regarding the actual dose administered to the tumor apex and adjacent tissues at risk, often restricting brachytherapy to a secondary treatment option. The development of a simulation-based planning algorithm is crucial to improve dose personalization and accessibility. The “Innovative RadIation Dose Estimation for pediatric retinoblastoma treatment enhancement” (IRIDE) project aims to overcome these dosimetric limitations by developing an advanced Monte Carlo (MC) treatment planning tool. This tool will determine the optimal plaque position and required exposure time, combining high-resolution 3D dose profile calculations with diagnostic eye images that highlight the patient's specific tumor bulk. The foundational step is the development of a robust simulation algorithm utilizing the Geant4 toolkit. Unlike conventional mathematical dosimetry approaches that rely on simplified approximations, this innovative method simulates individual particle tracks. This allows for an accurate characterization of radiation interactions within heterogeneous tissues and plaque backing materials, yielding a true 3D dose distribution map. For testing and validation, the simulation incorporated highly detailed anatomical structures derived from the 4D XCAT digital phantom library (Duke University), which is based on real patient imaging. Additionally, an accurate model of a CCX Ru-106 plaque was digitally reconstructed using manufacturer specifications. Integrating the virtual phantom with the plaque model enabled the successful reconstruction of a comprehensive 3D dose profile, proving the software's capability on realistic anatomical data. In parallel, the hardware phase

of IRIDE is moving toward the study of dedicated detector solutions to sample the 3D activity distribution in proximity of the plaque. Inputting this empirical data back into the MC code will ultimately provide medical physicists with a fully personalized dosimetric tool, maximizing tumor control while preserving pediatric vision.

AN AIRWAY EPITHELIUM-MONOCYTE CO-CULTURE MODEL TO INVESTIGATE THE IMPACT OF CCR2 AND CCR5 INHIBITION ON SARS-COV-2 REPLICATION AND INFLAMMATORY RESPONSES

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Several clinical studies have highlighted that excessive release of chemokines, including CCL2 and CCR5 ligands, plays a key role in driving the hyperinflammatory response and Acute Respiratory Distress Syndrome (ARDS) that characterize severe COVID-19. This study assessed the impact of CCR2 and CCR5 blockade during SARS-CoV-2 infection using a Three-Dimensional (3D) co-culture model consisting of fully differentiated primary Human Airway Epithelial Cells (HAECs) and primary human monocytes. HAECs cultures were generated from primary bronchial Conditionally Reprogrammed Cells (CRCs) derived from three healthy donors. CRCs were seeded onto 0.4-µm pore transwell inserts and cultured at Air-Liquid Interface (ALI) for 5 weeks to generate a well differentiated, polarized epithelium (HAECs-ALI). Cultures were apically infected with SARS-CoV-2 (BetaCov/Italy/CDG1/2020). Twenty-four hours post-infection, cells were treated with the monoclonal antibodies plozalizumab (anti-CCR2) or PRO-140 (anti-CCR5). In selected experiments, freshly isolated primary monocytes (3×10^5/100 µL/well) from healthy donors were added at the same time point to the apical surface of infected HAEC-ALI cultures in the presence or absence of the antibodies. After 4 hours at 37°C, the apical medium was removed and cultures were maintained under ALI culture conditions. Apical washes, cell lysates, basolateral media and cells were collected every 24 h up to 96 h post-infection for RT-qPCR quantification of the SARS-CoV-2 N gene as well as cellular genes, ELISA of cytokines/chemokines, and analysis of activation markers by flow cytometry. Infected HAECs-ALI cultures obtained from different donors displayed distinct expression levels of CCR2 and CCR5 and diverse kinetics of viral replication. SARS-CoV-2 infection increased IL-6 and CCL2 secretion and type I IFNs mRNA levels at 96 h post-infection. Treatment with plozalizumab or PRO-140 induced type I IFNs at earlier time points and reduced viral replication. Co-culture of infected HAECs-ALI with primary monocytes reduced viral replication and IL-6 secretion, suggesting an active role of monocytes in controlling infection within this model. In conclusion, CCR2 and CCR5 blockade influenced SARS-CoV-2 replication while

modulating epithelial inflammatory responses in a 3D airway epithelium cell model. These findings highlight the dual role of CCR2/CCR5 signaling in antiviral immunity and inflammation and support further investigation of chemokine receptor blockade as an immunomodulatory strategy for severe COVID-19. This work was supported by the Italian Ministry of Health (*R.I.Pr.E.I. 2023_88a81b92ca9f to LF*).

A EVALUATION OF THE ANTIVIRAL EFFICACY OF A POTASSIUM PEROXIMONOSULFATE-BASED DISINFECTANT AGAINST HEPATITIS E VIRUS (HEV)

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Potassium peroxymonosulfate-based disinfectants are widely used in livestock farming. They are characterized by a broad biocidal spectrum, rapidity of action, tolerability and ecological safety characteristics. The aim of this study was to evaluate their efficacy against HEV under controlled laboratory conditions. In this in vitro study, HEV viral suspensions were exposed to a potassium peroxymonosulfate-based disinfectant at four different contact times: 5 seconds, 10, 20 and 30 minutes. The tests were conducted both in the presence and absence of Bovine Serum Albumin (BSA), used as an organic interferer. The results showed that a contact time of 5 seconds is not sufficient to inactivate the virus, while after 30 minutes complete viral inactivation is observed. Intermediate time of 10 minutes produced variable results, with virus survival in some experimental replicates. Furthermore, the presence of BSA appears to exert a protective effect against the virus, reducing the effectiveness of disinfectant treatment.

A THE DEVELOPMENT AND ASSESSMENT OF HARMONISED REGIONAL AND LOCAL DIABETES REGISTRY MODELS WITHIN THE ITALIAN PILOTS OF JACARDI: TOWARDS A NATIONAL FRAMEWORK FOR DIABETES SURVEILLANCE

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Background. Diabetes (DM) represents a major public health challenge due to its increasing prevalence, chronic complications, and impact on healthcare systems. High-quality, harmonised health data is essential to support epidemiological surveillance, healthcare planning, and evidence-informed policymaking. In Italy, regional heterogeneity in data availability, quality, and interoperability limits comparable epidemiological evidence. In this context, 11 Italian partners (regional and local authorities) are participating in the EU-funded Joint Action on Cardiovascular Diseases and Diabetes (JACARDI), coordinated by the Italian National Institute of Health (ISS), with the aim of developing harmonised and interoperable Diabetes Registry models to strengthen national DM surveillance.

Aim. The project aims to document and assess the development of harmonised Regional and Local Diabetes Registry models within the Italian JACARDI pilots, including the co-design process leading to a common methodological framework and the validation of a shared algorithm for estimating prevalence and incidence of Type 1 Diabetes (T1DM), type 2 diabetes (T2DM), and Gestational Diabetes (GDM). The project will further assess registries' capacity to generate policy-relevant evidence on health inequalities, their economic value and long-term sustainability and scalability beyond JACARDI.

Methods. The study adopts an implementation science approach within the JACARDI methodological framework. The harmonisation process, encompassing the co-design pathway, governance mechanisms, and technical standardisation activities across the Italian pilots, will be documented, and the resulting algorithm validated using routinely collected administrative data. The registries' ability to identify socioeconomic inequalities in DM burden will also be evaluated, alongside a multidimensional assessment of various outcome domains.

Expected Results. The project will produce: a comprehensive description of the harmonisation process and validation of the identification algorithm for T1DM, T2DM, and GDM; evidence on DM registries' economic value; an assessment of their capacity to monitor health inequalities; and insights into the factors influencing long-term sustainability and scalability.

Conclusions. By generating harmonised epidemiological, economic, and implementation evidence, this research will contribute to strengthening DM surveillance and health information systems in Italy, supporting evidence-informed decision-making, future national scale-up of interoperable diabetes registries, and transferable methodological and policy lessons for the implementation of chronic disease registries across Europe. The JACARDI project received funding from the EU4Health Programme 2021–2027 under Grant Agreement No. 101126953. The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Health and Digital Executive Agency (HaDEA).

A SELICICLIB AS A DRUG-REPOSITIONING CANDIDATE FOR CDKL5 DEFICIENCY DISORDER: PRECLINICAL EVIDENCE OF THERAPEUTIC EFFICACY

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CDKL5 Deficiency Disorder (CDD) is an ultra-rare neurodevelopmental condition predominantly affecting females, for which no cure currently exists. Symptomatology includes early-onset epileptic seizures, intellectual disability and motor impairments. CDD is caused by de novo mutations in the X-linked Cyclin-Dependent Kinase-Like 5 (CDKL5) gene, which encodes a homonymous serine/threonine kinase highly expressed in the brain. Studies on Cdkl5 Knockout (Cdkl5-KO) mouse models, carrying a loss-of-function mutation of the Cdkl5 gene, and patient-derived induced pluripotent stem cell neurons have revealed profound neuronal alterations, including altered neuronal morphology, defective dendritic spine maturation and synapse formation, suggesting a key role of the Cdkl5 protein in the development and maintenance of neuronal integrity. Although the underlying mechanisms remain unclear, accumulating evidence points to brain cytoskeleton defects as crucial pathogenic factors that contribute to CDD neuronal alterations. Cytoskeletal dynamics, which play a key role in dendritic development and synaptic plasticity, are regulated by Cyclin-dependent kinase 5 (Cdk5), a serine/threonine kinase, whose dysregulation has been implicated in several neurological diseases that share alterations in neuronal architecture and cognition with CDD. Nevertheless, the potential role of this kinase in CDD pathogenesis has not been explored so far. In the present study, we investigated whether behavioral, neuroanatomical and peripheral oxidative alterations in a CDD mouse model at a fully symptomatic stage of the disease can be rescued by systemic administration of seliciclib, a brain-penetrant small molecule with high inhibitory potency toward Cdk5. This compound is currently undergoing phase I/II clinical evaluation for five different indications, thus bearing a high repositioning potential. Female heterozygous Cdkl5-KO (Cdkl5-Het) mice and wild-type littermates were daily treated with intraperitoneal injections of seliciclib (50 mg/kg) or control solution for 14 consecutive days at 1 year of age. Treatment efficacy was assessed through a battery of behavioral tests followed by molecular and morphological analyses of the hippocampus, a brain region highly compromised in CDD. Peripheral

oxidative stress in whole blood was also assessed and considered as a systemic readout of treatment efficacy. Systemic seliciclib administration in Cdkl5-Het mice significantly improved spatial reference and working memory deficits, rescued dendritic spine maturation and cytoskeletal-related molecular alteration in the hippocampus. Furthermore, seliciclib treatment normalized peripheral oxidative stress in whole blood of Cdkl5-Het mice. Present results highlight seliciclib as a novel therapeutic strategy for CDD, warranting further studies to perform a more-in-depth characterization of its effects at both molecular and behavioral levels. Funded by CDKL5 Insieme verso la Cura Association.

A DEVELOPMENT OF A BIOLUMINESCENT IMMUNOASSAY FOR THE EVALUATION OF *PLASMODIUM FALCIPARUM* TRANSMISSION POTENTIAL IN ASYMPTOMATIC CARRIERS

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Asymptomatic carriers of *Plasmodium falciparum* can harbour transmissible gametocytes and sustain malaria transmission despite the absence of clinical disease. Assessing whether these asymptomatic carriers can infect mosquito vectors currently relies largely on mosquito-feeding assays, which require specialized facilities, mosquito infections and dissections, and provide a relatively late readout based on oocyst development. These requirements limit throughput and complicate the evaluation of transmission-blocking drugs and vaccines. This doctoral project aims to develop and validate a quantitative, scalable bioluminescent immunoassay that estimates *P. falciparum* transmission potential through an early mosquito-stage readout, approximately 24 hours after infection. The project will first use public transcriptomic and proteomic datasets to identify parasite proteins preferentially expressed during fertilization and early mosquito-stage development, particularly in zygotes and ookinetes. Candidate biomarkers will be prioritized according to stage specificity, predicted protein features and conservation in *Plasmodium berghei*. Their temporal and spatial expressions will then be assessed experimentally using CRISPR/Cas9-generated reporter parasite lines. Validated antigens will be used to produce monoclonal antibodies and identify suitable antibody pairs for immunodetection. These antibodies will be labelled with complementary NanoBiT subunits to establish a Lumit-based add-and-read assay, followed by optimization of assay conditions, calibration curves and determination of analytical sensitivity, including limits of detection and quantification. The expected outputs include a prioritized panel of early mosquito-stage biomarkers, one or two validated reporter parasite lines, antigen-specific monoclonal antibodies and a prototype bioluminescent immunoassay. By providing an earlier and potentially higher-throughput measure of transmissibility, this approach could support the evaluation of transmission-blocking interventions and contribute to improved malaria surveillance and control.

A IDENTIFICATION OF POTENTIAL MOLECULAR TARGETS FOR THE DEVELOPMENT OF NEW STRATEGIES TO CONTROL THE CHRONIC PHASE OF *TOXOPLASMA GONDII* INFECTION

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Toxoplasma gondii, an Apicomplexa parasite that causes severe congenital disease and deaths in immunocompromised individuals, is one of the most widespread human pathogens, infecting up to one-third of the world population. Toxoplasma has three walled forms: oocysts, shed in cat faeces, sporocysts, produced when oocysts sporulate in the environment, and latent tissue cysts containing bradyzoites, located in the brain and muscles of warm-blooded animals. Drug treatment of chronic toxoplasmosis, caused by the bradyzoites, remains challenging due to a limited drug arsenal against this stage. The overall objective of my PhD project is to improve intervention strategies and new drug development against the growth, latency and/or functions of the bradyzoites through the application of OMICS tools, molecular and cellular biology and advanced imaging. A genomic shotgun proteomic experiment was recently performed in the lab comparing in vivo brain cysts collected at 30-days infected mice (late bradyzoites) to in vitro formed cysts (early bradyzoites), collected at 9 days post induced switching. Bioinformatic analysis suggested the selection of four over-expressed in vivo bradyzoite-specific proteins to be explored as potential candidates with a functional role in the establishment/development of the chronic stage. During my first year as a PhD student, I generated mutant KO lines of selected genes using CRISPR/Cas9 tools to assess the function and possible role in tissue cyst viability, persistence, wall components and/or with a role in bradyzoite reactivation. The guide RNA sequences targeting each of the four selected genes were generated from annealed ssDNA oligonucleotides and subsequently introduced into a prefabricated plasmid using either the Golden Gate or Gibson assembly technique. Donor DNA templates, containing a resistance cassette for mutant selection, were generated simultaneously as PCR products. For each target gene, the corresponding plasmid and donor DNA were co-transfected into Toxoplasma gondii tachyzoites to generate independent transgenic lines. Transfection was performed using the Amaxa nucleofection system in the *T. gondii* Prugnaud-ΔKu80 strain (type II), which reduces random genomic integration and promotes precise genome editing. Currently, two knocked out transgenic lines have been successfully cloned, ready for further characterization of the phenotypical pattern in in vitro/vivo bradyzoites, comparing to wild-type. The identification of potential molecular targets involved in the chronic phase of *T. gondii* infection will pave the way for the development of novel biochemical approaches, including the design and evaluation of molecules capable of interfering with the molecular processes identified in this project.

A TOWARDS NEXT-GENERATION HUMAN HEALTH RISK ASSESSMENT: CRITERIA AND STRATEGIES FOR THE REGULATORY INTEGRATION OF NAMS

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The paradigm shift reshaping predictive toxicology has led to sophisticated testing strategies as alternatives to in vivo studies, including in vitro assays, in silico methods and omics technologies. Collectively defined as New Approach Methodologies (NAMs), these approaches have the potential to provide more human-relevant and mechanistically informative evidence while reducing reliance on animal testing. However, their regulatory uptake requires confidence that NAM-derived evidence is robust, reliable and fit-for-purpose within a clearly defined context of use. When NAMs are intended to support quantitative risk assessment, in vitro biokinetics need to be integrated with bioactivity data to enable their translation into exposure metrics informing dose-response assessment and Point of Departure (PoD) derivation. In this context, this PhD research contributes to the Regulatory_NAM Project within the European Partnership for the Assessment of Risks from Chemicals (PARC), which supports the transition towards Next Generation Risk Assessment (NGRA). A structured workflow was developed to guide NAM-based case studies from their early design stages. Nine case studies covering different toxicological endpoints, regulatory purposes, contexts and implementation timeframes were selected according to feasibility and relevance criteria. Key methodological elements were defined through continuous interaction with regulatory stakeholders, allowing the approach to be tailored to regulatory needs and expectations. Starting from the regulatory question and intended framework, it addresses data collection and quality assessment, NAM readiness and robustness, chemical selection, evidence integration, uncertainties and data gaps, with particular attention to transparent reporting. The quantitative component is currently being explored through quantitative in vitro-to-in vivo extrapolation (qIVIVE) combined with physiologically based kinetic (PBK) modelling to translate in vitro concentration-response data into human-equivalent external doses. Within one case study focused on the aneugenic potential of bisphenols P and E, ongoing work focuses on the identification and quality assessment of physiological, physicochemical and toxicokinetic model parameters and the definition of an appropriate internal dose metric to support reverse dosimetry and derivation of a human-equivalent PoD relevant for risk assessment. Overall, this work contributes to defining a structured and transparent path from the design and evaluation of NAM-based strategies to the quantitative interpretation of NAM-derived evidence, supporting their fit-for-purpose use in NGRA. This project is co-funded by PARC.

A MASKED IMPUTATION OF ANTIMICROBIAL SUSCEPTIBILITY WITH A BIPARTITE GRAPH DEEP MARKOV MODEL

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Antimicrobial resistance surveillance produces sparse matrices of Susceptible\Intermediate\ Resistant (S/I/R) outcomes for pairs of pathogen and antibiotic, stratified by country and year. Completing these matrices is often treated as a classical matrix completion problem, overlooking the fact that a substantial share of the empty cells is not missing at random but structurally inestimable. We propose a two part pipeline: (i) a system of structural masks that, by combining intrinsic resistance (European Committee on Antimicrobial Susceptibility Testing: EUCAST/AMR), specific of the site reporting rules (Clinical and Laboratory Standards Institute: CLSI) and empirical coverage, separates biologically impossible cells and excluded ones by the protocol (Missing Not at Random: MNAR) from genuinely imputable cells (Missing at Random MAR); (ii) a Deep Markov Model over a bipartite pathogen-antibiotic graph, with binomial emission, a bilinear decoder on node embeddings and a learned weight α for the Intermediate category, trained by maximising the ELBO with parameters shared across countries. On the ATLAS dataset (roughly 1.0 million isolates, 16 species, 17 antibiotic families, 55 countries, 2014-2024), restricting the universe to effectively sampled blocks, 36.2% of the 88,978 candidate cells are observed, 41.9% imputable and 21.8% to be excluded: nearly a quarter of the gaps are not a legitimate imputation target. Across the 30 largest countries the model reaches a MAE of 0.059 on held-out cells versus 0.096 for the pooled binomial baseline (-39%); the learned weight α=0.68 places Intermediate (I) closer to Susceptible (S). An ablation compares the bilinear decoder with a variant whose embeddings come from a message-passing encoder over the graph. On a random 15% held-out split the two are equivalent (0.060 *vs* 0.062), whereas under the cold-pair protocol, with entire (species, family) pairs withheld from both training and graph adjacency, the graph is more accurate and markedly more stable (0.132±0.029 *vs* 0.194± 0.093): the relational structure helps precisely where generalization to rare or unobserved combinations is required, even without external features. Estimates are qualitatively aligned with European Centre for Disease Prevention and Control (ECDC) reports. Validation also surfaced a coding artefact for colistin, which has no Susceptible outcomes following the EUCAST removal of category S, and led to a curated override correcting the failure to exclude penicillins for Klebsiella. We discuss limitations (an 11-year window, MNAR assumptions, the two-category simplification) and next steps: temporal splits, geographic correlation, multinomial emission and external node features.

A DEVELOPMENT OF AN EIS-BASED MOLECULAR SYSTEM FOR MIR-25 LOADING INTO EXOSOMES: TOWARD A NOVEL STRATEGY FOR POST-STROKE NEUROGENESIS

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Ischemic stroke is one of the leading causes of mortality and long-term disability worldwide. Although cerebral ischemia stimulates endogenous neurogenesis through the activation of Neural Stem Cells (NSCs) within the subventricular and subgranular zones, this regenerative response is insufficient to replace lost neurons and achieve complete functional recovery. Therefore, developing therapeutic strategies capable of enhancing endogenous brain repair represents a major challenge for improving outcomes after ischemic stroke. MicroRNAs have emerged as key regulators of neural plasticity and regenerative processes. Among them, miR-25 has attracted increasing interest because of its involvement in neurogenesis and its potential role in recovery after cerebral ischemia, making it a promising candidate for RNA-based therapeutic approaches. Based on this rationale, the aim of this study was to develop a molecular system designed to promote the selective loading of miR-25 into exosomes, with the long-term goal of exploiting these extracellular vesicles as delivery vehicles to enhance neurogenesis in in vitro models of ischemia. To this end, two expression constructs were generated by cloning the mature miR-25 sequence either upstream or downstream of an RNA segment derived from the HIV-1 Gag p17 region, previously shown to mediate selective RNA sorting into exosomes and referred to here as the Exosome Incorporating Sequence (EIS). Following molecular cloning, recombinant plasmids were transformed into competent bacterial cells, and positive clones were identified by molecular screening and confirmed by Sanger sequencing. Sequence-validated plasmids were subsequently amplified, purified, and transiently transfected into HEK293T cells. RT-qPCR analysis of total RNA isolated from transfected cells confirmed the expression of both engineered miR-25-EIS transcripts, demonstrating the successful expression of the constructs in a mammalian cell system. Overall, these findings establish the molecular basis for the development of an EIS-mediated miR-25 exosomal delivery system. Future studies will determine whether the engineered transcripts are efficiently incorporated into exosomes and whether miR-25-enriched exosomes can enhance neurogenesis and contribute to functional recovery in in vitro models of ischemic stroke.

A COMPARATIVE PATHOGENESIS OF *GIARDIA DUODENALIS* ASSEMBLAGES A/E AND GLV+ ISOLATES IN PORCINE IPEC-J2 INTESTINAL CELL LINE

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Introduction. *Giardia duodenalis* is a widespread intestinal parasite causing giardiasis in humans and animals, yet the host-pathogen interactions remain incompletely understood. Particularly, the virulence characteristics of various *G. duodenalis* assemblages and the potential role of Giardia-infecting viruses have not been systematically compared in non-cancerous intestinal cell models. This research aimed to employ the porcine IPEC-J2 cell line as a non-transformed in vitro system to investigate the effect of *G. duodenalis* infection on intestinal barrier and tight junction integrity.

Materials and Methods. We compare assemblages A and E, and assemblage E strain infected with the specific *Giardiavirus* (GLV). Confluent IPEC-J2 monolayer was subjected to apical infection with an MOI of 3:1 and incubated for various time periods. To assess barrier disruption and permeability, cell culture barrier assays such as transepithelial electrical resistance and luciferase yellow assay were applied, parasite burden was quantified by direct cell counting. Tight junction protein localization and expression were examined by confocal immunofluorescence microscopy.

Results. Infection with both assemblages led to a significant reduction of TEER values and an increase in permeability compared to controls, indicating barrier disruption. Confocal imaging confirmed the alteration of tight junction proteins. Parasite colonization was observed between three specimens, revealing the differential outcomes of assemblages A and E.

Conclusions. These findings demonstrate that the porcine IPEC-J2 cell line is a suitable in vitro model of animal giardiasis, providing a basis to advance our knowledge on disease pathogenesis linked to host-specific and zoonotic assemblages.

A EXPANDING THE PHENOTYPIC SPECTRUM OF GNAO1-RELATED DISORDERS: FUNCTIONAL CHARACTERIZATION OF PARKINSONISM-ASSOCIATED VARIANTS IN *CAENORHABDITIS ELEGANS*

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GNAO1 encodes the Gα subunit of the abundant neuronal inhibitory Go protein (Gαo), which negatively regulates Neurotransmitters (NTs) release downstream of several G protein-coupled receptors and plays a crucial role in neuronal circuits. De novo mutations in GNAO1 were initially associated with early-onset neurological disorders characterized by hyperkinetic movement disorders (MDs), with or without epilepsy. More recently, the phenotypic spectrum has been expanded to include milder adolescent-onset dystonia. Here, we characterize two rare GNAO1 pathogenic variants, p.Leu13Pro and p.Leu23Pro, affecting the N-terminal α-helix, identified in patients presenting with a parkinsonian syndrome characterized by bradykinesia and rigidity, in contrast to the hyperkinetic MDs more commonly associated with the disease. We previously established *C. elegans* as a powerful model for studying GNAO1-related disorders and exploring potential therapeutic approaches, thanks to the high conservation between human GNAO1 and its worm ortholog goa-1. CRISPR/Cas9 engineered worms carrying the N-terminal variants were generated and phenotypically characterized. Mutant animals showed hyperactive locomotion, with increased reversal rate and speed, enhanced egg-laying, and hypersensitivity to aldicarb-induced paralysis (aldicarb is an acetylcholinesterase inhibitor), indicating excessive NT release across multiple neuronal classes. These phenotypes resemble those observed in goa-1 knock-out worms and in animals carrying GNAO1 pathogenic variants associated with the canonical form of the disease. In contrast, transgenic animals overexpressing the gain-of-function Gαo variant p.Arg179Cys exhibited opposite phenotypes, including egg retention in the uterus, impaired locomotion, and resistance to aldicarb. Overall, these findings demonstrate that p.Leu13Pro and p.Leu23Pro exert a loss-of-function effect, similarly to the more common GNAO1 pathogenic variants associated with hyperkinetic phenotypes. These data support the hypothesis that GNAO1-related disorders represent a phenotypic continuum ranging from hyperkinetic MDs to hypokinetic/parkinsonian manifestations, rather than distinct disorders driven by opposite molecular mechanisms.

A PRECLINICAL DEVELOPMENT OF AN INNOVATIVE EXTRACELLULAR VESICLE (EV)-BASED VACCINE STRATEGY AGAINST RESPIRATORY INFECTIONS

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Respiratory infections remain a major global health challenge, highlighting the need for innovative vaccines able to induce strong and durable lung immunity. While the BCG vaccine provides incomplete protection against pulmonary TB, particularly in adults, protection induced by current COVID-19 vaccines can wane over time and against emerging variants. This PhD project investigates an Extracellular Vesicle (EV)-based vaccine strategy against respiratory infections, using Mycobacterium tuberculosis (Mtb) and SARS-CoV-2 as models. The proposed strategy is based on Nefmut, a functionally inactive mutant of the HIV-1 Nef protein that efficiently incorporates into EVs, even when fused to heterologous proteins. This property enables EV-mediated antigen delivery through two approaches: (i) DNA vaccination, which drives the endogenous production of engineered EVs, or (ii) the direct administration of in vitro-produced engineered EVs. These EVs are efficiently taken up by antigen-presenting cells, promoting specific T-cell responses. Previous studies by our group showed that this platform induces strong, protective and long-lasting CD8+ T-cell responses against different antigens. During the first phase of the project, three Mtb antigens (Ag85B, ESAT-6 and TB10.4) were selected based on their immunogenicity. Following transfection, all Nefmut-antigen fusion proteins were expressed and efficiently incorporated into EVs, as confirmed by western blot. The resulting DNA vaccines, either alone or in combination, will be administered intramuscularly followed by electroporation and evaluated for immunogenicity with a focus on specific T-cell responses included lung-resident memory T cells, and protective efficacy. Mtb growth inhibition will be measured ex vivo using a Mycobacterial Growth Inhibition Assay (MGIA), while protective efficacy will be assessed in vivo following an Mtb challenge. For SARS-CoV-2, building on previous evidence of protective N-specific CD8+ T-cell immunity obtained with the DNA-based platform, a novel vaccination approach based on the direct intranasal administration of Nefmut-N-engineered EVs will be evaluated for its ability to induce lung T-cell immunity and control virus replication following challenge with SARS-CoV-2. As part of the first phase of the project, these EVs were produced at the University of Turin and purified by tangential flow filtration and nanofiltration. To simplify and standardize EV production, a stable Nefmut-N-expressing cell clone was used, resulting in lower but still detectable immunogen levels. Overall, the project will extend the preclinical evaluation of Nefmut-based DNA vaccination to Mtb and investigate a novel intranasal EV-based vaccination strategy against SARS-CoV-2. Together, these studies will further support the versatility of the platform across different pathogens, particularly those affecting the respiratory tract.

A GENETICS, ENVIRONMENT, AND WORK: A TWIN STUDY ON EMOTION REGULATION AND MENTAL HEALTH

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Studying the environmental and genetic foundations of burnout and emotion regulation is essential to debunk the myth of the "one-size-fits-all" model of occupational stress. Emotion regulation acts as the primary mechanism linking genetic vulnerability to the environment in the development of burnout. Investigating the overlap between individual biological vulnerability and psychological mechanisms of managing work-related stress is essential to shift intervention strategies from generic, reactive self-care toward targeted, biologically informed preventive training programs. Since twin studies in this field are still scarce and none has ever been conducted in Italy, this work represents an element of originality in the literature of the sector. The project aims to investigate the genetic and (familial and individual-specific) environmental factors underlying the relationship between job characteristics, emotional states, and mental health. We hypothesize a strong association between the burnout score - assessed using three different scales (MBI-9, CBI, BAT4) - and occupational depression, supporting the substantial conceptual redundancy between the two constructs. Regarding the genetic component of burnout and target phenotypes, we hypothesize a significant heritability, with a higher intra-pair correlation in monozygotic compared to dizygotic twin pairs. Secondary research hypotheses involve evaluating: the role of personality (BFI-10), motivation (MWMS), self-esteem (RSES, OBSE), self-efficacy (RESE), and emotional reflexivity as moderators/predictors of burnout and general or occupational depression (PHQ-9, ODI); the association between organizational characteristics (COPSOQ) and outcomes (UWES); the convergent validity of the three burnout measurement tools. Further exploratory hypotheses include job boredom (DUBS), work addiction (DUWAS), and turnover intention. The study sample will be comprised of at least 250 twin pairs of working age (18-67 years), previously enrolled in the Italian Twin Registry. Among these, at least 30 discordant twin pairs (i.e., pairs in which co-twins differ in the outcome of interest) are expected to be identified. Data collection will take place via an online questionnaire, implemented on the LimeSurvey platform, encompassing the above-mentioned validated scales. Statistical analysis will involve a preliminary individual-level analysis of association based on multivariate regression models, an intra-pair analysis based on quantitative genetic models, and a discordant-twin analysis based on tests and models for matched data. Estimating genetic and environmental components will contribute to clarifying the etiology of burnout and identifying key protective factors, thereby informing public health policies and laying the groundwork for targeted intervention strategies in populations at risk.

A STUDIES ON THE PRESENCE OF HAZARDOUS SUBSTANCES IN TATTOO AND PMU INKS IN THE FRAMEWORK OF REACH REGULATION

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Background. Since 2022, Regulation (EC) No 1907/2006 (REACH) has restricted the use of a vast array of chemical substances in tattoo and Permanent Make-Up (PMU) inks, considering their hazardous properties with the aim of guaranteeing a high level of consumer health protection in the EU. Since then, ink formulation has undergone several changes, as substances used for decades can no longer be used. The aim of this PhD project is to investigate the presence of hazardous substances in inks, evaluate the impact of REACH on tattoo and PMU ink composition over recent years, and study the levels of substances consumers are potentially exposed to. Among the substances studied, Formaldehyde (FA) and Acetaldehyde (AA) are of particular interest. Due to their harmonised classification as carcinogenic and mutagenic, FA and AA are only permitted at concentrations below 0.5 mg/kg, which is a considerably low limit for such technically unavoidable contaminants. Even if high-purity ethanol or glycerol are used for ink formulation, contamination with FA and AA cannot be completely avoided. From an analytical perspective, verifying product compliance with the 0.5 mg/kg limit requires highly sensitive techniques and careful accounting for contamination during sample preparation. In addition, gamma-irradiation, applied to inks to reduce microbial contamination, has been demonstrated to increase FA levels.

Materials and methods. LC-MS/MS and LC-DAD analytical methods were set up to quantify substances of interest in inks purchased on the Italian market. Commercially available inks and laboratory-formulated inks (prepared by mixing different percentages of purified water, glycerol, ethanol, and selected pigments) were subjected to low-dose gamma irradiation (1 kGy) to study its effect on aldehyde levels.

Results and discussion. Preliminary results showed that around 70% of analyzed commercial samples (14/21) contained levels of FA above 0.5 mg/kg (conc. interval 0.12-1.92 mg/kg) and all the samples exceeded the same limit for AA (conc. interval 0.45-47.9 mg/kg), considering measurement uncertainties. Inks formulated in the lab contained levels of FA and AA below 0.5 mg/kg, attributable to their presence in glycerol and ethanol: after gamma irradiation, concentrations increased up to 1.87 mg/kg for FA and 3.71 mg/kg for AA. Gamma-irradiation of commercial samples resulted in a 5-fold and 8-fold increase in FA and AA levels, respectively.

Conclusion. Limits established by REACH for FA and AA represent a critical aspect of restriction enforcement, as demonstrated by the number of non-compliant samples on the market and the issues related to contamination and degradation following gamma irradiation.

A STATISTICAL METHODS FOR THE DEVELOPMENT OF AN EARLY WARNING SYSTEM FOR RESPIRATORY SYNDROMES IN ITALY BASED ON EMERGENCY DEPARTMENT ADMISSION FLOWS

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Within the EMUR-PANFLU system, we develop statistical methods for early warning based on individual emergency department ED admissions for respiratory syndrome, reported by over 600 healthcare facilities across Italy since 2019 and aggregated into weekly counts by geographic area and age group. The goal is a robust, warning system able to distinguish ordinary fluctuations in healthcare demand from genuine epidemiological anomalies. Among the various methods currently existing in the literature, we have chosen, as a starting point, the Farrington/Noufaily method, which seemed, at least in theory, the most suitable for handling this type of data. Analyses conducted to date show markedly inflated dispersion parameters and strong sensitivity of baseline estimates to pandemic-era data contamination (2020-2021), motivating a broader methodological development effort along three interconnected directions. The first concerns missing data: facilities began reporting at different times, and reporting gaps are not randomly distributed in time or space. Beyond structural censoring (facility activation/deactivation, straightforward to handle), a substantial share of gaps reflects genuine temporary non-compliance - facilities that stop and resume reporting without any real service interruption. Cross-regional comparison reveals highly heterogeneous patterns in frequency and duration of these gaps, making standard missing-completely-at-random assumptions inadequate. Planned work includes imputation or joint modeling strategies that explicitly account for the missingness mechanism and its territorial variability. The second concerns notification delays: recent weeks are progressively consolidated, so real-time counts systematically underestimate final ones, destabilizing alerts on current weeks. Planned work includes explicit modeling of the consolidation process to correct real-time counts and stabilize alert generation. The third concerns spatial dependence: current methods, including recent site-level random-effects extensions, treat geographic units as exchangeable rather than spatially correlated, discarding relevant information on the spatial diffusion of epidemic phenomena. Planned work includes spatio-temporal dependence structures and hierarchical formulations for correlated counts across neighboring regions. Together, these developments aim to more effectively distinguish ordinary fluctuations from early warning signals, reducing false positives and detection delays, and yielding more stable baseline estimates under variable facility reporting coverage. The resulting methods are intended for integration into the ISS national ED surveillance network, supporting the operational early warning system envisioned under the National Pandemic Plan.

A NEW CHALLENGES FOR HEALTH AND HYGIENE SAFETY IN SWIMMING POOLS: EMERGING CONTAMINANTS, INTEGRATED CONTROL AND OPERATOR TRAINING

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Introduction. Swimming pools are shared environments where public health protection requires adequate hygienic and sanitary conditions of water, indoor air, and surfaces. Recent scientific literature has shown increasing interest in Emerging Contaminants (CECs), Disinfection By-Products (DBPs), and Pharmaceutical and Personal Care Products (PPCPs), highlighting new challenges for pool management. In Italy, the regulatory framework is currently evolving, also following the approval, in July 2025, of the framework Bill on health and safety in swimming pools, aimed at promoting harmonized requirements for safety, self-monitoring, and operator training nationwide. This study aims to systematically analyze the available evidence on CECs and non-conventional indicators, assessing their potential implications for monitoring, self-monitoring, and operator training systems.

Materials and Methods. A systematic literature review was conducted according to the PRISMA 2020 guidelines. Bibliographic searches were performed in PubMed, Scopus, ScienceDirect, and Web of Science, while Google Scholar was used to identify grey literature. Articles published between 2002 and 2025 in English and Italian were considered. The search strategy included terms related to swimming pools, CECs, PPCPs, DBPs, environmental monitoring, and health risks.

Results. Approximately 3,000 records were identified. After duplicate removal, PRISMA screening, and eligibility assessment, 31 studies were included in the final review. The evidence indicates that conventional monitoring parameters do not always provide a comprehensive characterization of emerging contaminants. Substances of potential health and toxicological concern were detected at concentrations ranging from ng/L to µg/L, despite substantial methodological heterogeneity among studies. The findings highlight the need for integrated monitoring approaches combining chemical, microbiological, and management-related parameters, together with specific training programs for pool operators.

Conclusions. Emerging challenges to swimming pool safety require a shift towards integrated and multidisciplinary control systems consistent with the One Health approach. Current evidence supports updating prevention and control tools through the inclusion of new indicators capable of detecting emerging risks. Operator training, continuous technical and regulatory updates, and the implementation of innovative monitoring strategies are key elements for strengthening public health protection in swimming pool environments.

A DISSECTING REACTOGENIC AND IMMUNOGENIC PROPERTIES OF INNOVATIVE RNA-BASED PLATFORMS FOR FUTURE PANDEMIC EMERGENCY IN IN VITRO HUMAN PRIMARY CELL MODELS

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RNA-based therapies represent an advancing frontier in medicine, offering accelerated development, high translational efficiency, scalable manufacturing, and platform flexibility, as demonstrated by the rapid deployment of COVID-19 mRNA vaccines. Building on this success, platforms have emerged including self-amplifying RNA, trans-amplifying RNA, and circular RNA, each offering distinct features regarding stability, expression durability, and dosage control. To advance these platforms safely, analytical frameworks are essential to evaluate their reactogenicity and immunogenicity. Because in vivo vaccine responses are influenced by the administration route and complex crosstalk between immune and non-immune cells, in vitro models must accurately reflect these tissue dynamics. Thus, by using a human cell-based in vitro model that mimics intramuscular administration route and is composed of Human Skeletal Muscle Cells (HSkMCs), fibroblasts and Peripheral Blood Mononuclear Cells (PBMCs), we aim at evaluating both reactogenicity and immunogenicity of RNA-based therapeutics formulated for pathogens with epidemic and pandemic risk. The research project will be conducted in two phases. First, the most promising RNA platforms and dosage will be selected by treating HSkMCs, PBMCs, and fibroblasts and evaluating cytotoxicity, alongside GFP/Antigen Of Interest (AOI) expression by immunofluorescence and flow cytometry. Subsequently, the selected platforms encoding the AOI will be tested in single- and co-culture systems, using the BNT162b2 COVID-19 vaccine as a control. Key readouts include inflammatory mediator profiles, interferon-stimulated gene expression, immune cell phenotyping, and cell migration dynamics. Over the past few months, we focused on immune cell isolation, primary cell culture maintaining and optimization of differentiation protocol for HSkMCs. Currently, we are evaluating the effects of different LNP-based formulations loaded with mRNA or saRNA on fibroblasts and PBMC in terms of AOI expression and modulation of secretome and transcriptional programs. Additionally, our ongoing work involves the optimization of a co-culture 3D system combining HSkMCs/fibroblasts/PBMCs, through which we will then investigate cell types locally expressing or targeted by the RNA-based therapeutics; characterize profiles of inflammatory and acute-phase factor associated with reactogenicity; assess local tissue toxicity and damage; identify early protective immune signatures that can be correlated with the immunogenicity of the novel RNA-based

formulations. This approach enables comparative profiling of reactogenicity and immunostimulatory features across RNA platforms, providing mechanistic insights to translate in in vivo responses. Aligning with the 3Rs principles and EU Directive 2010/63, this work contributes to establish an integrated, immune-relevant in vitro model for the preclinical safety and immunogenicity assessment of intramuscular RNA therapeutics.

A OXIDATIVE STRESS AS A MODULATOR OF DNA DAMAGE-INDUCED INNATE IMMUNITY: INSIGHTS INTO THE CGAS-STING PATHWAY

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Ionizing Radiation (IR)-induced DNA damage promotes micronuclei (MNi) formation and activates cGAS, a cytosolic double-stranded DNA sensor that synthesizes cGAMP, leading to STING activation and the production of type I interferons and pro-inflammatory cytokines. To investigate how IR modulates cGAS recruitment and activation, immortalized human keratinocytes (HaCaT) were cultured under Natural Background Radiation (NBR) or Below Background Radiation (BBR) at the Gran Sasso National Laboratories and then exposed to X rays. Cells adapted to BBR for 60 days showed a delayed peak of cGAS-positive micronuclei ($cGAS^+$ MNi) at 120 h, compared with 48 h in NBR, without changes in total cGAS protein levels. Moreover, IFN α, IFN β, and ISG15 was evaluated to verify if this delayed localization agreed with a different expression of immune system. Consistently, BBR-adapted cells exhibited delayed immune activation. We previously showed that cGAS is recruited to MNi from the nucleus later under BBR conditions, and that cGASP + P MNi display defective lamina, highlighting a structural role of MNi in cGAS binding. Given that the autophagy receptor p62 inhibits micronuclear membrane repair and regulates cGAS activity we investigated the interplay between p62 and cGAS. Preliminary data in NBR conditions indicate that p62 localizes to MNi earlier than cGAS, suggesting that micronuclear rupture is an initial step enabling cGAS recruitment. Since p62 is sensitive to oxidative stress, antioxidant treatment (NAC) reduced both p62 and cGAS localization to MNi, supporting a ROS-dependent mechanism. We therefore propose that, under BBR conditions, delayed cGAS recruitment may result from altered p62 dynamics at MNi, likely reflecting differences in oxidative stress metabolism and suggesting a potential adaptive response to radiation.

A SYSTEMATIC REVIEW OF FOOD-SYSTEM PATHWAYS LINKING MEDITERRANEAN CLIMATE HAZARDS WITH DIET-RELATED NON-COMMUNICABLE DISEASES IN OLDER ADULTS

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Introduction. Climate change and Non-Communicable Diseases (NCDs) form an interconnected public-health crisis. In Mediterranean countries, population ageing intensifies this interaction because older adults are more likely to live with chronic disease and have less capacity to adapt to climate shocks. Climate hazards may disrupt food production, affordability and access, altering diet and metabolic health and increasing the risk of cardiovascular disease, type 2 diabetes, overweight and obesity. Understanding these pathways can uncover complex pathways between CC and NCDs and inform intervention design and implementation.

Objectives. To identify and synthesise the causal relationships through which Mediterranean CC hazards affect diet-related NCD risks and outcomes among older adults through food-system pathways, and to develop a causal pathway map. Methods: We conducted a PRISMA-guided systematic review of empirical and modeling studies from Mediterranean countries. Eligible studies reported at least one directional relationship linking a CC hazard with food-system change, diet or metabolic risk, or a diet-related NCD outcome. Upstream studies examined food production, supply, prices or availability without age-specific data; downstream studies of food access, diet, and health outcomes examined findings for adults aged 60 years or older. Evidence was organised into a relationship database and pathway map.

Results. Preliminary findings suggest that evidence is concentrated in separate parts of the pathway. Mediterranean studies link drought, extreme heat and marine heatwaves with lower cereal, olive and shellfish production and, in some settings, higher food prices. Separate older-adult studies associate food insecurity, limited socioeconomic resources and the higher cost of healthy diets with poorer food access or diet quality. Stronger Mediterranean-diet adherence is associated with lower obesity, metabolic syndrome, blood pressure, diabetes prevalence and cardiovascular mortality. Evidence documenting the complete pathway from climate hazard through food-system and dietary change to an NCD outcome is limited.

Conclusions. The review clarifies how climate hazards may influence diet-related NCDs among older adults through food systems in Mediterranean countries. The pathway map illustrates pathways between CC-NCDs and will inform future participatory systems dynamics modelling and implementation-research.

A NEURONAL RESPONSE TO SPACE STRESS: STUDY OF PROTEIN QUALITY CONTROL AND AGGREGATION IN A HUMAN NEUROBLASTOMA CELL MODEL

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Space travel is one of the most advanced aspects of humanity. In recent years, longer missions in space have been taken into consideration. During space missions, astronauts face extreme environmental conditions, such as altered gravity and ionizing radiation, which could put astronauts' health at risk, causing disorders of the central nervous system. Therefore, how the health of astronauts is affected by this extreme environment is key to programming longer expeditions and reaching further targets in space. This project focuses on exposing Retinoic Acid (RA) and Brain-Derived Neurotrophic Factor (BDNF) differentiated human neuroblastoma SH-SY5Y cells to stress factors, including ionizing radiation and altered gravity. Once differentiated, neurons will be chronically exposed (up to 72 hours) to high and low Linear Energy Transfer (LET) radiation, alpha particles and gamma rays, respectively. Altered gravity will be assessed by utilizing a Perfusion Bioreactor System (RCCMax, Synthecon) to create a microgravity environment, in which SH-SY5Y cells will form and differentiate into 3D spheroids. Therefore, the first goal is to establish a model of chronic stress condition exposure. Next, the project aims to assess alterations to the protein quality control system and to evaluate the aggregation of proteins in amyloid structures following chronic exposure to space-mimicking conditions.

A IMPACT OF ENVIRONMENTAL CONTAMINANT EXPOSURE AND DIET ON METABOLIC ALTERATIONS AND IDENTIFICATION OF SEX-SPECIFIC EFFECTS IN AN IN VIVO EXPERIMENTAL MODEL

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Introduction. Metabolic Syndrome (MetS) is a multifactorial condition affecting 20-25% of the adult population, characterized by obesity, insulin resistance, hypertension and dyslipidemia, increasing the risk of type 2 diabetes and cardiovascular diseases. Sex significantly influences MetS pathophysiology, with women more prone to post-menopausal visceral obesity and men showing earlier cardiovascular complications. Industrial activities in the Basilicata region raise concerns about exposure to toxic metals, some of which act as metabolic and/or endocrine disruptors interfering with hormonal regulation and energy metabolism in a sex-dependent manner. Lifestyles, such as the diet, may play a pivotal modulating role in MetS: The Western Diet (WD), rich in saturated fats, simple sugars and ultra-processed foods, exacerbates metabolic damage, amplifying insulin resistance and systemic inflammation. Conversely, the Mediterranean diet, characterized by a high intake of fiber, polyphenols, and monounsaturated fatty acids, appears to exert protective effects in humans through antioxidant and anti-inflammatory mechanisms.

Experimental design. Within the framework of the project LucAS, the in vivo study aims to evaluate the sex-specific effects of heavy metals and trace elements in a mixture at realistic environmental exposure combined with two different dietary regimens, a Standard Diet (SD) or a WD. Adult male and female Sprague-Dawley rats are exposed for 90 days via drinking water to two mixtures: 1) cadmium, arsenic, lead, mercury, nickel, and 2) chromium, zinc, and copper, formulated to mimic the real-world exposure scenarios monitored in high-impact areas of Basilicata. Dose levels are selected based on the highest exposure data from the general population. The following endpoints are evaluated: serum biochemical parameters, histopathology of target tissues, and gene and protein expression related to lipid metabolism, inflammation, and oxidative stress. All the analyzed endpoints aim at elucidating the sex-specific endocrine and metabolic effects associated with exposure to the mixtures and dietary regimens, alone or in combination.

Aim and expected outcomes. The objective of the in vivo study is to provide novel insights into the sex-related synergistic mechanisms through which environmental pollutant exposure and dietary patterns can interact to drive metabolic dysfunctions. Specifically, the research aims to identify and validate sex-specific tissue biomarkers that reflect early

metabolic and inflammatory distress. Ultimately, these findings will support the development of predictive, preventive, and personalized nutritional and therapeutic strategies tailored to individual metabolic profiles and exposure risks. The study is supported by the LucAS project *EA89/3 CUP: I85E23001430005.*

A USE OF ADVANCED IN VITRO MODELS TO STUDY THE IMPACT OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFASS) ON LIPID METABOLISM

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Per- and Polyfluoroalkyl Substances (PFASs) are persistent synthetic chemicals widely used in industrial and consumer products. Human exposure occurs mainly through food and drinking water, and increasing evidence links PFAS exposure to altered lipid metabolism, hepatic steatosis, oxidative stress and renal injury. PFASs can interact with nuclear receptors, including PPARα/γ, LXR and FXR, modulating pathways involved in lipid synthesis, β-oxidation and lipid trafficking. Co-exposure to other potential obesogens may further contribute to these metabolic alterations. This PhD project aims to develop a dynamic multi-organ in vitro model integrating liver- and kidney-on-chip systems to investigate the effects of PFASs and other potential obesogens on hepatic and renal lipid metabolism. The experimental workflow will initially involve conventional Two-Dimensional (2D) static cell cultures to characterize PFAS-induced effects and establish a baseline for comparison. The study will then progress towards dynamic systems: the commercial millifluidic MIVO® platform (Multi-In Vitro Organ, React4Life, Genoa, Italy) will first be used to investigate and optimize flow and perfusion conditions for the selected cellular models. The information obtained from these experiments will guide the subsequent development and optimization of the microfluidic Organ-on-Chip (OoC) system, with the aim of establishing physiologically relevant dynamic conditions and shear stress. Human primary hepatocytes (PHH) and/or hepatic cell lines will be used to represent the liver compartment, while human primary proximal tubule epithelial cells (RPTEC) and/or renal cell lines will be used to represent the kidney compartment. Cell viability, lipid accumulation, gene and protein expression, and protein secretion will be assessed under static and dynamic conditions, focusing on PPARα/γ-, LXR- and FXR-dependent pathways. Targeted analyses will be complemented by lipidomic and metabolomic approaches to identify metabolic alterations and potential biomarkers of PFAS toxicity. The project will also investigate the protective potential of Mediterranean diet-derived compounds, particularly olive-derived polyphenols. The integrated platform is expected to provide a more physiologically relevant representation of PFAS-induced hepatic and renal dysfunction than conventional static cultures. Comparison of 2D, MIVO® and OoC models will clarify the influence of increasingly complex and dynamic conditions on cellular and toxicological responses and define the advantages and limitations of each approach. Overall, the project aims to identify molecular mechanisms and biomarkers of PFAS toxicity and provide preliminary evidence for the potential use of natural compounds as preventive or nutraceutical strategies against pollutant-induced metabolic alterations.

A EARLY PREDICTION OF VACCINATION OUTCOME IN MULTIPLE SCLEROSIS: LINKING PRE- AND POST-VACCINATION INNATE IMMUNE STATES TO PROTECTIVE ADAPTIVE RESPONSES ACROSS VACCINE PLATFORMS

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Introduction. The COVID-19 pandemic accelerated the development of mRNA vaccines, such as Pfizer-BioNTech BNT162b2, and highlighted the potential of systems vaccinology to identify molecular and cellular predictors of vaccine responses. People with Multiple Sclerosis (pwMS) receiving Disease-Modifying Therapies (DMTs) with immunosuppressive effects may experience reduced vaccine efficacy and an increased risk of infection. Precision medicine aims to develop prevention and treatment strategies tailored to defined groups of individuals and may improve vaccination outcomes. Given that vaccine-induced antibody responses in pwMS vary according to DMTs, personalized vaccination strategies are becoming increasingly important. Objective: This study will investigate whether different DMTs are associated with distinct pre-vaccination immune profiles, whether different vaccine platforms [i.e. mRNA based-COVID-19 or subunit based- Herpes zoster (HZV) vaccines] induce specific or shared early immune signatures in pwMS, and whether pre- and early post-vaccination profiles can predict the magnitude and quality of protective responses to different vaccines.

Methods. Peripheral blood will be longitudinally collected from pwMS and matched healthy subjects (HS) before and after vaccination at early and later time points (enrolment at Sant'Andrea Hospital, Policlinico Tor Vergata). Serum will be cryopreserved to quantify cytokines and chemokines using next-generation multiplex ELISA (ELLA system, Bio-Techne) and vaccine-specific antibody responses. Peripheral blood mononuclear cells (PBMCs) will be analyzed by RNA sequencing, real-time PCR, and multiparametric flow cytometry to characterize vaccine-induced transcriptional signatures and innate and adaptive immune cell populations. In parallel, HZV vaccine-specific T-cell responses will be assessed by IFN-γ ELISpot.

Results. Both retrospective and prospective studies will be conducted. The retrospective study on COVID-19 vaccinated MS subjects is in progress, with RNA sequencing already performed on PBMCs from HS and pwMS collected at baseline and 24 or 48 h after COVID-

19 mRNA vaccination. Enrolment of patients for the prospective study on HZV vaccination is starting. This will include vaccine-naïve pwMS receiving different vaccine types, with blood collected at baseline for RNA sequencing and assessment of pre-vaccination immunity, and 30 days after vaccination to evaluate adaptive immune responses.

Conclusion. This study aims to provide a comprehensive understanding of the immune mechanisms underlying vaccine responsiveness in pwMS. Identifying pre- and early post-vaccination immune profiles predictive of vaccine-specific protective immunity may contribute to the development of personalized vaccination strategies for pwMS and other immunosuppressed populations, with potential benefits for public health and healthcare systems.

A SHARED FUNCTIONAL DEFECTS OF CDC42 VARIANTS UNDERLYING NOCARH-LIKE PHENOTYPES

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CDC42, a GTPase of the RAS family, regulates the development and homeostasis of tissues, including the immune and hematopoietic systems, by controlling multiple key cellular processes, such as cytoskeletal dynamics and inflammatory cytokines trafficking. We recently characterized several pathogenic CDC42 missense variants associated with a broad spectrum of rare conditions, including a severe phenotype characterized by pancytopenia, autoinflammation and hemophagocytic lymphohistiocytosis (NOCARH syndrome). To define the molecular mechanisms underlying this disorder, we functionally profiled the NOCARH-like phenotype-associated variants p.C81Y, p.R186C and p.C188Y. We performed a panel of biochemical and functional assays in cells overexpressing mutants. p.C188Y and p.C81Y markedly reduced protein levels, in part due to proteasomal degradation. GTPase activity was enhanced in p.R186C and p.C188Y mutants expressing cells, while p.C81Y increased binding to the positive regulator of activity IQGAP1. All the variants showed an impaired binding to the cytoplasmic sequester RhoGDI, presenting an aberrant intracellular localization, with p.C81Y accumulated in the perinuclear area. Moreover, all variants increased ERK1/2 activation, indicating enhanced crosstalk with the RAS-MAPK signalling pathway. In contrast, the pathogenic variants behaved similarly to the dominant negative variant p.T17N with respect to N-WASP function (variably altered N-WASP localization, its binding to CDC42 and cell spreading), ultimately leading to N-WASP signalling impairment. Significantly, both pERK upregulation and N-WASP dysfunction have been shown to mediate pro-inflammatory signals in immune cells. Overall, this study delineates shared pathogenic mechanisms underlying CDC42-related NOCARH-like phenotypes and indicates that these variants differentially perturb multiple molecular pathways, resulting in distinct functional outcomes, ranging from context-dependent gain-of-function to dominant negative effects. These findings widen the spectrum of CDC42 pathogenic variants exerting complex molecular behavior.

A SEX-SPECIFIC DIFFERENCES IN VACCINE-INDUCED IMMUNE RESPONSE: IN SEARCH OF RESPONSE AND PREDICTIVE BIOMARKERS

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Background. Biological sex significantly influences immune responses, with females generally mounting stronger innate and adaptive immune responses to infections and vaccinations than males. In particular, women can present a higher production of cytokines (i.e. IFN-α) and antibodies as well as an increased number of T-Cells and a higher CD4+/CD8+ ratio. These differences may be attributed to the effect of several genetic, epigenetic and hormonal factors. However, the mechanisms driving these sex-specific disparities in immune responses to antiviral and antibacterial vaccines remain incompletely understood.

Aim. This PhD project aims to: 1) evaluate sex differences in the immune response to seasonal influenza and pertussis vaccines in healthcare workers (HCWs). Seasonal Influenza vaccination represents a model of repeated annual revaccination, as it requires annual reformulation due to continuous viral antigenic drift. Pertussis vaccination instead represents a model in which the rapid waning of immunity following acellular vaccines necessitates booster doses in HCWs to prevent nosocomial transmission. 2) Investigate the role of sex hormones and immune-related circulating microRNAs as potential sex-specific biomarkers of vaccine-induced immune response Methods: A cohort of male and female HCWs will be enrolled at Ospedale Pediatrico Bambino Gesù and Policlinico di Tor Vergata. The experimental approach will be structured on several levels: First, plasma samples will be collected before and after vaccination to determine vaccine-specific antibody titers, circulating sex hormone levels, and selected immunoregulatory microRNAs, known to be encoded on X-chromosome or modulated by 17-estradiol or testosterone. Statistic regression analyses will be performed to evaluate the associations between vaccine-specific antibody titers and sex, plasma levels of sex hormones and circulating microRNAs.

Expected Results. Based on our group's previous works, we expect to observe sex-specific differences in vaccine-induced antibody responses and sex-specific associations between humoral immunity and circulating levels of sex hormones and/or selected microRNAs following both influenza and pertussis vaccination. Our results may enable the identification of molecular markers associated with the magnitude and quality of vaccine responses.

Conclusions. This multi-level analysis aims to provide an integrated characterization of the molecular mechanisms underlying sex differences in vaccine response. The identification of sex-specific biomarkers of vaccine response may contribute to the development of more personalized, sex-informed vaccination strategies and support the implementation of precision medicine approaches in vaccinology.

A TARGETING MITOCHONDRIAL DYSFUNCTION AS A NOVEL THERAPEUTIC STRATEGY FOR NIEMANN-PICK TYPE C DISEASE

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Niemann-Pick type C1 (NPC1) is a rare, progressive neurodegenerative disorder caused by pathogenic variants in the NPC1 or NPC2 genes, resulting in impaired intracellular lipid trafficking and lysosomal cholesterol accumulation. This is associated with a cascade of cellular dysfunctions, including mitochondrial impairments, which contribute to the clinical manifestations of NPC1 (neurodegeneration, cerebellar ataxia and cognitive decline). The main objective of this project is to verify if targeting mitochondrial dysfunction could represent a new therapeutic approach for NPC1. To this aim, we will use an inhibitor of ENT1 (i.e an adenosine transporter) called J4, a brain-penetrant compound whose mechanism of action relies on its ability to increase adenosine levels and, subsequently, rescue mitochondrial bioenergetics. J4 has already demonstrated efficacy in models of Alzheimer's disease by targeting dysregulated adenosine homeostasis and, subsequently, ameliorating mitochondrial energy failure. Importantly, J4 has now entered a first-in-human Phase I clinical trial in healthy adults (NCT06938763). Npc1-deficient mice (BALB/cNctr-Npc1m1N/J) received J4 (3 mg/kg/day) in drinking water from Postnatal Day (PND) 28 to PND63. WT vehicle-treated (N=30), NPC1 vehicle-treated (N=8) and NPC1 J4-treated mice (N=10) were longitudinally assessed using coat hanger, vertical screen, and balance beam tests on 1.6- and 0.6-cm beams to monitor cerebellar-associated motor function. Hippocampal-dependent recognition memory was assessed by novel object recognition test (NORT) at PND52–54. At PND63, cerebellum and hippocampus were collected to assess Purkinje cell preservation, neuroinflammatory and autophagy markers, and mitochondrial bioenergetics by Seahorse analysis of isolated mitochondria. Preliminary data confirmed progressive motor impairment in NPC1 mice. In the 1.6-cm balance beam, a significant time × group interaction was detected ($p<0.0001$). J4 reduced traversal latency versus NPC1 vehicle-treated mice after three weeks of treatment (81.5 *vs* 135.6 s; adjusted $p=0.0048$), corresponding to an approximately 40% improvement, with a trend already evident at the preceding weeks (76.3 *vs* 114.9 s; adjusted $p=0.0619$). The effect was not maintained at later time points. On the 0.6-cm beam, NPC1 mice showed marked impairment without significant improvement with J4. No consistent treatment effect was observed in coat hanger or vertical screen performance. NORT data analysis is ongoing. In conclusion, J4 at 3 mg/kg induces a transient and task-dependent improvement in motor coordination in NPC1 mice. The limited magnitude and persistence of the effect suggest that this dose may be suboptimal, supporting

evaluation of a higher dose in subsequent experiments. Integration of motor, cognitive, neuropathological and mitochondrial endpoints will determine whether ENT1 modulation can provide neuroprotection and ameliorate mitochondrial dysfunction in NPC1 disease.

A CHEMICAL AND TOXICOLOGICAL RISK ASSESSMENT OF ENDOCRINE-DISRUPTING CHEMICALS: FROM CELLULAR EFFECTS TO BIOMARKER DISCOVERY

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Endocrine-Disrupting Chemicals (EDCs) are environmental contaminants capable of interfering with hormonal signalling and potentially contributing to the development of hormone-dependent diseases. Among them, phthalates and bisphenols are widely used plasticizers associated with endocrine-disrupting activity. Molecular mechanism(s) underlying their endocrine disrupting activity has been correlated mainly with their binding to either Peroxisome Proliferator-Activated Receptors (PPARs) and/or Estrogen Receptors (ERs), although the molecular pathways underlying their biological effects continue to be investigated. This research investigates the toxicological and molecular effects of selected phthalates and bisphenols, including di(2-ethylhexyl) Phthalate (DEHP) and Bisphenol A (BPA), in comparison to phthalate and bisphenol alternatives in androgen-responsive prostate cancer in vitro experimental models. Following chemical exposure, cell viability, androgen receptor-mediated responses, such as Prostate-Specific Antigen (PSA) secretion and intracellular lipid accumulation are assessed to characterize endocrine-disrupting activity and identify relevant biological endpoints. In parallel, bioinformatic analyses are being performed to identify and prioritize candidate microRNAs as potential biomarkers of exposure and biological effects. Candidate miRNAs are selected through literature mining and database analysis. Preliminary findings indicate differential cellular responses among the tested compounds, supporting further mechanistic investigations. The integration of experimental and in silico approaches will facilitate the identification and validation of molecular biomarkers, contributing to a better understanding of endocrine disruptor-mediated toxicity and supporting innovative approaches to chemical and toxicological risk assessment.

AUTHOR'S INDEX

Acchioni Chiara 64
Acchioni Marta 64
Adimari Gianfranco 29
Adriano Cristina 127
Ahmad Ibrar 86
Aiello Chiara 116
Ajmone-Cat Maria Antonietta 12; 27
Albanese Naomi Ciano 27
Albertini Chiara 127
Alessandri Guido 146
Alessi Roberta 12
Alfieri Lavinia 4
Alfò Marco 148
Allen Amburose Stephen 118
Ambroni Elena 116
Ambrosini Elena 4
Ambrosone Alessandra 128
Amendola Antonello 41
Amici Roberta 131
Ammendolia Maria Grazia 14; 55
Amoresano Angela 73
Anastasio Gennaro 43
Andersson John 92
Andreotti Alessandra 89
Andreotti Mauro 131
Anello Pasquale 75
Angelici Laura 134
Anticoli Simona 161
Antoccia Antonio 78
Antonini Massimiliano 129
Antonucci Caterina 131
Aquilina Valeria 45
Arancia Silvia 120
Arasi Maria Beatrice 93; 111
Arcà Bruno 52
Arcopinto Ludovica 66
Arecchi Serenella 96
Armida Monica 92
Armocida Benedetta 134
Attili Andrea 14; 55
Avellino Pamela 52
Azim Gada Mohamed Abdel 102
Baiocco Giorgio 75; 152
Baldassari Arianna 83
Baldassarri Lucilla 65; 147; 164
Balestrieri Emanuela 113
Barbano Lucilla 93
Barbarulo Sara 133
Barbieri Giorgia 39
Barbieri Giulia 89
Barone Maria Chiara 3; 160
Barra Melissa 46
Battistini Beatrice 71
Battocchio Chiara 14; 55
Bava Cecilia Irene 102
Belfiore Marcello 116
Bella Antonino 148
Bellosono Luca 47
Benincasa Lucia 48; 102
Benoni Roberto 83
Berardinelli Francesco 75; 152
Bernard Geneviève 111
Bernardini Ilaria 99
Berni Canani Roberto 113
Berry Alessandra 8
Berselli Irene 50
Bertini Enrico 4; 111; 116
Bertossi Tommaso 134
Bertuccini Lucia 6
Bessereau Jean Louis 144
Bevivino Giulia 52
Bianchi Gloria 54
Bianchi Riccardo 99
Biffoni Mauro 63; 90
Binelli Ludovica 14; 55
Blaconà Giovanna 63
Blangiardo Marta 31
Bocca Beatrice 71
Boe Alessandra 24; 160
Boffa Marta 56
Boirivant Monica 92
Bollella Alessandro 58

Bombelli Cecilia 16
Bona Roberta 131; 160
Bonanno Giulia 83
Bonazza Enza Bruna 59
Bongiorno Gioia 99
Borghi Lorenzo 50
Borghi Martina 41
Bortone Giuseppe 105
Boussadia Zaira 162
Bozzuto Giuseppina 16
Bracci Laura 24; 90
Branchi Igor 109
Bravaccio Carmela 113
Brescianini Sonia 33
Brignone Maria Stefania 4; 116
Brkić Diandra 121
Bruni Roberto 66
Bruselles Alessandro 93
Buiarelli Francesca 147
Bullitta Silvia 92
Busani Luca 141
Busardò Francesco Paolo 45
Butera Alessia 27; 92
Caccia Barbara 129
Cacioppo Andrea 141
Caimi Stefano 71
Caissutti Daniela 154
Calamandrei Gemma 6
Calcabrini Annarica 16
Calcagni Maria Lucia 14; 55
Calcagnini Giovanni 18
Camerini Serena 111
Camillocci Elena Solfaroli 129
Camillocci Solfaroli Elena 60
Campioni Ilaria 86
Campoli Giulia 59
Candelise Niccolò 154
Canese Rossella 6; 121
Canham Natalie 111
Canini Irene 26
Canitano Andrea 41
Cannatà Vittorio 129
Canonico Annalisa 115; 136
Cantillo Yanelys 138
Capocefalo Antonio 120
Cappella Marisa 97
Caprini Elena Sofia 4; 116
Caprio Mario 119
Cara Andrea 41; 90
Carbone Elena 92; 97
Carbonetti Francesco 139
Cardamone Erica 34
Cardillo Valentina 33
Carere Mario 46; 98
Carlston Colleen Mara 111
Carollo Maria 24; 81; 150
Carpentieri Carmen 119
Carpentieri Giovanna 111
Carrozzo Rosalba 116
Carruezzo Marina 60; 129
Caruso Angela 113
Carvetta Mattia 93
Casadei Viola Turroni 83
Casagrande Andrea 6
Casata Alice 61
Castelli Germana 63; 131
Castiello Luciano 24
Castrucci Maria Rita 120
Catinari Cristina 140
Catone Stefania 72
Ceccarelli Emiliano 31
Ceccherelli Renato 50
Cecchetti Serena 12; 75; 93; 111; 131; 143
Ceci Aurora 63
Cellitti Francesco 141
Cenci Giovanni 19
Cencini Benedetta 64
Centola Carmela 83
Cerio Anna Maria 63; 131
Cerra Sara 71
Cerri Carola 56
Cerri Matteo 19
Cese Martina 142
Chern Yijuang 162
Chervyakova Natalya 143
Chiappinotto Stefania 28
Chiodi Valentina 21
Chiogna Monica 29
Chiozzini Chiara 145
Chiriatti Luigi 93
Chirico Mattea 6; 92

Chirullo Barbara..... 39
Ciabattini Annalisa 81; 150; 158
Ciani Elisabetta.....136
Ciaramella Antonio..... 39
Ciardiello Andrea 141
Ciccaglione Anna Rita.....39; 66
Ciccozzi Alessandra..... 65
Cimino Giuseppe..... 63
Cipressa Francesca..... 19
Cipriani Chiara 113
Cirulli Francesca..... 8
Cisbani Evaristo 86
Ciucci Davide.....129
Coccia Anna Maria.....107
Coccia Eliana Marina..... 81; 150; 158
Cocco Eleonora 102
Colone Marisa 16
Columba Cabezas Sandra.....142
Cometa Maria Francesca.....128
Compagnucci Claudia 111
Condello Maria 78
Conte Antonella.....102
Conte Valeria 75; 152
Contessa Gian Marco..... 47
Coppola Irene 21
Coppola Lucia 155
Coppola Simona 3; 93; 160
Cordeddu Viviana..... 93
Corsa Manuel..... 41
Cosentino Livia 115; 136
Costanzi Giulia..... 66
Costanzo Matteo..... 70
Cozzetto Domenico 24
Craxi Lucia.....123
Crescenzi Marco..... 6
Crestini Alessio 97
Creti Roberta..... 61
Cruz Andreia V.S. 50
Currá Chiara.....139
D'Alessandro Manuela.....144
D'Amelio Stefano.....46; 98
D'Amore Teresa 68
D'Andrea Mattia..... 93
D'Angelantonio Benedetta..... 12
D'archivio Massimo 155
D'Archivio Massimo..... 12
D'Ippolito Cristina 146
D'Onofrio Matteo 146
D'Andrea Gabriele 70
Danese Giorgia 71
D'Archivio Massimo 10
De Angelis Marta..... 124
De Bartolo Ilenia..... 102
De Berardis Barbara..... 14; 55
De Chiara Giovanna..... 124
De Filippis Bianca.....115; 136
De Gara Laura..... 65
De Luca Gabriele 6; 90; 92
De Michele Manuela..... 97
De Nuccio Chiara.....81; 116; 162
De Risio Luisa..... 56
De Sabato Luca..... 50
De Santis Eleonora..... 6
De Simone Roberta 12; 27
De Simoni Micol..... 129
De Stefano Maria Egle 6
Declich Silvia..... 83
Del Pio Luca..... 72
Delibato Elisabetta 101
Dell'Aquila Carmen..... 93
Della Libera Simonetta..... 107
Delli Colli Claudia 109
Dentice Sara 28
Desogus Eleonora 96
Dettin Monica..... 55
Dezi Giulia..... 41
Di Bartolo Ilaria..... 50; 133
Di Benedetto Laura 73; 157
Di Carlo Christian 119
Di Carlo Nazzareno..... 92
Di Chio Daniele 75; 152
Di Consiglio Emma..... 68; 140
Di Dio Claudia..... 19
Di Feo Flavia 77
Di Lorenzo Giorgio..... 56
Di Luca Mariagrazia..... 61
Di Muccio Trentina..... 99
Di Netta Alice 78
Di Nucci Annalisa..... 80
Di Placido Daniela..... 39
Di Plinio Giada 158
Di Pucchio Aalessandra..... 96

Di Risola Daniel 24
Di Rocco Martina.....144
Di Sapio Alessia 102
Di Trana Annagiulia 45
Di Veroli Jacopo Niccolò..... 73
Dini Valentina 14; 55; 75; 152
Dioguardi Marika..... 89
Dipaola Maria Greta 52
Domenici Maria Rosaria 21
Donati Simona.....24; 26
Donfrancesco Chiara..... 80
Donnini Micaela 145
Dorrucci Maria 39; 161
Draisci Rosa 65
Duc-Khanh To..... 29
Dupuis Maria Luisa 16
Dussi Gaia..... 28
Epis Sara 99
Equestre Michele 66
Eramo Adriana.....63; 131
Esposito Giuseppe 19; 154
Etna Marilena Paola..... 81; 150; 158
Fabbri Alessia.....24; 90
Fabbri Andrea.....14; 55
Fabiani Monica.....81; 150
Fagnani Corrado 146
Falce Chiara 41
Famele Marco.....147
Fang Fang..... 92
Fantuzzi Laura.....131
Faragalli Andrea 83
Farchi Francesca 39
Farina Iole..... 41
Fausti Elisa..... 61
Fausti Giulia..... 50
Favazzi Ughetta..... 96
Fecchi Katia 125
Fedele Giorgio..... 36
Federici Cristina..... 39
Federico Maurizio.....145
Fei Giulia..... 83
Ferrante Antonella 162
Ferrantelli Flavia 145
Ferrara Fulvio.....105
Ferrari Caterina..... 83
Ferrarini Alessandro 55
Ferraro Bianca 148
Ferrazzano Gina..... 102
Ferri Maurizio..... 146
Ferrigno Luigina 123
Ferrucci Daniela.....96
Ferruti Emma..... 3; 160
Fillo Silvia 59
Fiore Alessia..... 24; 108
Fiorentino Eleonora.....99
Fiori Federica.....34
Fiori Micol Eleonora 90
Flammia Raffaella 6
Flavia Porreca.....70
Flex Elisabetta 93; 111
Formenti Beatrice..... 134
Fornari Antonella..... 101
Fortini Daniela.....54
Fortuna Claudia 99
Fracella Matteo 52
Franchitto Annapaola.....23; 77; 108
Franciosa Giovanna.....72
Francisci Silvia.....89
Frasca Claudia 149
Fratini Federica.....71
Fratoddi Ilaria 71
Frau Jessica..... 102
Fulceri Francesca 113
Gabbianelli Roberta 118
Gabbianelli Rosita.....73
Gabriele Lucia 26
Gaetani Silvana..... 164
Gagliardi Maria Cristina..... 125
Galano Diletta..... 102
Galanti Sergio.....19
Galati Giulia 113
Gallinaro Alessandra.....41
Galluzzo Clementina Maria..... 131
Galosi Serena..... 144
Gambardella Lucrezia 24; 90
Garbini Alice 85
Gauzzi Maria Cristina 131
Gebara Elias..... 121
Genovese Domenico 72
Gentile Amalia Egle.....43
Germinario Elena Angela Pia 24; 90
Gesuita Rosaria.....83

Gherardi Giovanni 61
Giaccari Andrea..... 8
Giacomini Gianmarco..... 33
Gianfelici Ester..... 50
Giannitelli Sara Maria 157
Giantin Mery.....143
Giardina Giovanni 50
Gigante Guido 70; 141
Gila Letizia.....113
Giona Letizia..... 8
Giordani Francesco..... 59
Giorgi Lucia 102
Giovagnoli Tommaso.....161
Giovinazzo Alessandro.....155
Girolamo Antonietta 59
Gistro Maria..... 8
Giuseppetti Roberto 39
Goletti Benedetta..... 83
Grande Sveva 14; 55
Grasso Felicia..... 75
Graziano Silvia..... 45
Gualdi Sara..... 26
Guelbeogo Moussa..... 52
Gullifa Giuseppina.....127
Guzzinati Stefano..... 89
Hauser Jonas.....121
Hushi Matilda.....81; 150
Iacobino Angelo 125
Iaconelli Marcello.....101
Iacoponi Francesca 34
Ianiro Giovanni.....50; 133
Iannetta Marco.....158
Ingre Caroline..... 92
Inserra Antonio 56
Iorio Egidio 6; 81; 92
Iris Federica 152
Isanti Lorenzo..... 52
Iucci Giovanna 14; 55
Iurilli Martin..... 28
Joyce Emily E..... 92
Juel Rachel 153
Kayserili Hülya.....111
Kondili Loreta A.....123
Konstantinoudis Garyfallos..... 31
Koukouli Despoina 87; 139
Kyriaki Machera..... 68
Kyriakopoulou Katerina..... 68
La Maida Nunzia.....21
La Penta Caterina.....26
La Rocca Cinzia..... 155
La Rosa Giuseppina 101; 107
La Valle Roberto..... 75; 99
Lacchetti Ines.....46
Lalle Marco..... 143
Lanciotti Angela 4; 116
Lanciotti Lucrezia 65
Landi Doriana..... 158
Lanni Alessio..... 125
Lasinio Giovanna Jona 31
Lasorsa Lorenzo..... 89
Laterza Ilenia 24; 90
Lauri Antonella 111
Lauricella Alessio 92
Lelli Davide.....41
Leodori Giorgio 102
Leone Alessandro.....93
Leone Pasqualina 36
Leone Patrizia 145
Leoni Omar.....90
Leuzzi Vincenzo 144
Libri Valentina.....24
Lista Florigio Romano 59
Lo Cicero Stefania 63; 131
Loi Manuela..... 136
Lombardo Fabrizio.....52
Lombardo Flavia.....29
Longhi Catia.....39
Lorenzetti Stefano.....73; 157; 164
Lorenzini Patrizia.....29
Lori Gabriele 155
Luca Vincenzo.....65
Lucarelli Marco 63
Lucchesi Simone.....150; 158
Lucentini Luca.....85; 105; 107
Luchetti Luisella 127
Lugli Camilla.....96
Luijten Mirjam..... 140
Macchia Daniele 24
Macchioni Giorgia.....6
Macrì Simone 121
Madonna Elisabetta 66
Maggino Filomena 30

Magro Gaia 28
Maiolo Francesca..... 97
Mancini Aurora 98
Mancini Cecilia 93
Mancini Fabiola..... 59
Mancini Flavia 64
Mancini Laura 46
Mancosu Cristina.....102
Mancuso Elisa 99
Mandarino Luciano.....127
Mandolini Enrico.....154
Manella Sara.....10; 12
Manfredi Francesco 145
Manganelli Maura..... 54
Mangiapelo Claudia..... 99
Mannan Samra.....101
Manteleone Valentina.....155
Maramao Luna..... 39
Maranghi Francesca.....155
Marcantonio Cinzia..... 66
Marchetti Giulia..... 83
Marchi Serena 39
Marcocci Maria Elena.....124
Marcon Francesca.....24; 111
Marcucci Isabella..... 54
Marfia Girolama A. 158
Marinaccio Jessica 152
Marino Maria Ilaria..... 27
Marinovich Marina 68
Mariotti Sabrina.....125
Maroccia Zaira 90
Marozzi Giorgia..... 23
Marra Camillo 8
Marra Manuela 24
Marroccia Zaira 24
Marsili Giulia 99
Martellacci Noemi 8
Martinelli Simone 144
Martinotti Giovanni..... 56
Martire Serena.....102
Martorano Benedetta.....155
Masciola Irene..... 10
Massimi Sara.....126; 127
Mastrogiuseppe Chiara 141
Mattei Eugenio 18
Mattei Fabrizio 108
Mattei Giorgia..... 85
Mattioli Benedetta..... 123
Mattioli Roberto..... 24
Maurizi Linda..... 39
Mazzaccara Alfonso 28; 96
Mazzone Luigi..... 113
Medaglini Donata.....81; 150; 158
Medda Emanuela 146
Meloni Chiara.....4; 48; 102; 116
Mesquita João..... 50
Micai Martina 113
Micale Vincenzo..... 21
Michelini Zuleika.....41; 90; 131
Migani Sofia..... 16
Mignini Luca 93
Mikhail Milena 73; 157
Minelli Giada..... 31
Minerva Valentina..... 104
Misasi Roberta..... 154
Miteva Mariya T 124
Modiano David 52; 126
Molinaro Francesca..... 96
Monini Marina..... 50; 133
Monte Annella 59
Monteleone Valentina 8
Montomoli Emanuele 39
Morabito Stefano..... 39
Moracci Gabriele 70
Morciano Patrizia..... 75; 152
Moreal Chiara..... 28
Moretto Angelo..... 68
Mosca Luciana..... 24
Moschella Federica..... 24
Motta Marialetizia..... 93
Mouzaki-Paxinou Akrivi-Chara..... 68
Muglia Anita..... 36
Muratore Anna..... 105
Musillo Chiara 8
Negri Donatella..... 41
Nencioni Lucia..... 124
Niceta Marcello..... 93
Nicita Francesco 4; 111; 116
Nigro Di Gregorio Federica..... 58; 149
Notargiacomo Lorenza..... 105
Nunziata Giovanna..... 143
Olivetta Eleonora 36

Olivieri Anna.....52; 126
Onder Graziano 134
Ostanello Fabio.....133
Pacchione Guglielmo..... 58
Pacini Mariairene..... 50
Padula Lorenzo Pio 56
Pagano Maria Teresa 125
Pajalunga Deborah..... 6
Palamara Anna Teresa . 36; 81; 124; 150; 158
Palese Alvisa..... 28
Palma Alessandra 14; 55
Palombelli Gianmauro 6; 121
Panebianco Concetta..... 24
Panico Federica 147
Pantaleoni Sofia..... 68
Pantanetti Alisia.....126
Pantano Valentina..... 46
Paone Silvio 126
Papa Elena.....127
Parisi Vincenzo..... 93
Parpinel Maria 34; 80
Pasetto Roberto..... 30
Pasquini Luca 24; 160
Pasquino Maria Teresa.....127
Patera Vincenzo.....60; 86
Pati Ilaria..... 66
Pazienza Valerio..... 24
Pellegrini Manuela..... 21
Pellei Maura 16
Peluso Alessia 107
Penninx Brenda WJK.....109
Penny Philip 43
Perdichizzi Benedetta.....108
Perini Corinna 158
Perrone Federica..... 97
Perugini Eleonora 52
Petrilli Ilaria.....164
Petrucci Paola 39
Petrucciani Niccolò..... 12
Petruccioli Giulia.....109
Pettorruso Mauro..... 56
Pezzoni Giulia 41
Pezzotti Patrizio.....39; 148
Piccinini Vanessa..... 66
Piccinno Ludovica 111
Pichierri Pietro 23; 77; 108
Pichini Simona..... 45
Picozza Mario 24
Pietraforte Donatella..... 24; 136
Pietrantoni Agostina.....39; 81; 111
Pirillo Maria Franca..... 41
Pisa Edoardo..... 121
Pisani Giulio 66; 72
Pisanu Maria Elena 6; 92
Piscopo Paola..... 92; 97
Pitzalis Maristella..... 102
Pizzarelli Scilla 83
Pizzi Elisabetta..... 87; 139
Poggini Silvia 109
Poleggi Anna 70
Polichetti Alessandro 47
Polito Claudia 129
Polizzi Agata 43
Poma Noemi..... 61
Pombi Marco 52
Potenza Rosa Luisa 92
Pozzi Silvia..... 129
Protto Virginia 124
Pugliese Katherina 145
Puliani Natascia 83
Pulici Stefania..... 50
Puopolo Maria 27; 92; 113
Putzolu Giordano 127
Quaranta Maria Giovanna 123
Quattrini Maria Cristina 24; 136
Rainaldi Gabriella 24; 26
Ranaldi Marco 14; 55
Rappuoli Rino..... 150
Remoli Gloria 29
Repouskou Anastasia 68
Retrosi Diletta..... 111
Rezza Guglielmo..... 45
Ricceri Laura 27
Ricci Daniela 81; 150; 158
Ricci Elena 139
Ricci Federica 18
Ricci Maria Luisa..... 59
Riccio Maria Pia 113
Riccomi Antonella..... 39
Righi Elena..... 96
Risoluti Roberta 85; 105; 107; 127

Rivabene Roberto 97
Rizza Teresa 116
Rizzello Margherita 143
Rizzo Gaia 127
Romagnoli Giulia 26
Ronco Alessia Bruna Petronilla 3; 160
Rosato Stefano 123
Rosicarelli Barbara 4; 48
Rossella Briancesco 107
Rossi Angelo 50
Rossi Laura 34
Rossi Roberta 96
Rossi Stefania 24; 90
Rouault Charles-Edoard 121
Rovetto Claudia 66
Ruggieri Anna 161
Ruggiero Andrea 161
Ruocco Marialuisa 127
Sabatini Alessia 52
Sabbioni Mara 113
Sacco Chiara 148
Salvati Anna 16
Salvetti Marco 158
Samà Marianna 162
Sanarico Nunzia 39
Santia Lorenzo 4
Santini Carlo 16
Santoro Filippo 113
Sarmento Bruno 78
Sartorelli Jacopo 116
Sbriccoli Marco 70
Scagnolari Carolina 52
Scansalegna Lisa 115; 136
Scattoni Maria Luisa 113
Scaturro Maria 59
Scazzocchio Beatrice 10; 12; 155
Schiavoni Giovanna 108
Schiavoni Ilaria 36
Scipioni Angela 4
Scotognella Teresa 14; 55
Seitz Christina 92
Serafini Barbara 4; 6; 48
Serini Paola 52
Sette Giovanni 63
Severa Martina 81; 150; 158
Sgarbanti Marco 64
Sgura Antonella 75; 152
Siciliano Giulia 138
Silano Marco 34; 80; 134
Silecchia Gianfranco 10; 12
Silva Laura 50
Simeoni Matteo 66
Simonis Beatrice 16
Simporé Jacques 52
Siniscalchi Ester 24; 111
Siracusano Martina 113
Sonnino Rebecca 8
Spada Massimo 24; 90
Spadaro Francesca. 24; 81; 131; 150; 160
Spica Vincenzo Romano 149
Spinelli Michele 73
Splendore Amelia 8
Sposito Sara 4; 116
Stazzoni Samuele 150
Stefanelli Paola 36
Stellacci Emilia 93
Stoppa Giorgia 31
Stringaro Annarita 16
Suffredini Elisabetta 101
Suzuki Ryosuke 41
Tabolacci Claudio 113
Tait Sabrina 8; 10
Tammaro Alessia 10; 12; 155
Tarantino Michela 39
Tartaglia Marco 93; 111; 160
Tartaglione Anna Maria 6; 21
Tassinari Roberta 155
Teatini Maria Federica 119
Telesca Matteo 164
Teloni Raffaella 125
Testai Emanuela 68
Tirelli Valentina 75; 90
Tocilj Flora 50
Tomao Paola 161
Tomei Roberta 120
Tondo Mariateresa 144
Tonelli Cristiana 121
Toni Danilo 97
Tortora Luca 55
Toschi Elena 29
Tosti Maria Elena 83
Tozzi Michela 24; 90

Travaglione Sara.....24; 90
Trazzi Stefania.....136
Trombetta Claudia Maria..... 39
Trombetta Marcella.....157
Trupia Mariagrazia 12
Turatto Federica..... 83
Unim Brigid..... 83
Urbani Sofia 30
Urbinati Chiara.....115; 136
Urdiales Alberto Mateo.....148
Vaccari Gabriele..... 50
Vaiano Andrea.....123
Valdarchi Catia..... 39
Valentino Paola 102
Valeriani Federica.....149
Vanacore Nicola 29; 31
Varì Maria Rosaria 45
Varì Rosaria 8; 10; 12; 155
Varotto Boccazzi Ilaria 99
Vendetti Silvia..... 39
Venditti Iole 14; 55
Venturi Giulietta 41; 99
Verachi Paola..... 63
Verin Ranieri..... 24
Veroni Caterina 4; 48; 102
Veroni Caterini 116
Veschetti Enrico..... 85
Vicenza Teresa..... 101
Vichi Maurizio..... 70
Viegas Juliana 78
Viglione Aurelia 109
Villani Annacandida..... 24
Vincentini Olimpia..... 24
Vinciguerra Manlio..... 21
Viollet Louis..... 111
Visentin Sergio 116
Vitanza Luca..... 107
Vivarelli Cecilia..... 18
Voso Domenico..... 56
Wassmer Evangeline..... 111
Zanchi Giulia 8; 12; 155
Zanzi Francesco 124
Zappitelli Alice..... 125
Zara Erika.....3; 93; 160
Ziccardi Lucia..... 93
Zini Maurizio..... 21; 71
Zongo Davy..... 126
Zoratto Francesca 56
Zychlinsky Arturo 75

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