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PhD day 2026 of Istituto Superiore di Sanità

Istituto Superiore di Sanità
Rome, January 19, 2026

ABSTRACT BOOK

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

ISTITUTO SUPERIORE DI SANITÀ

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This volume collects the summaries of the contributions presented at the "2026 PhD Day of Istituto Superiore di Sanità". The presence of doctoral students is an important stimulus for the Istituto Superiore di Sanità's research, fostering collaborations and interactions with the university research community. It is also a key aspect of the institution's mission and specifically of the ongoing Three-Year Activity Plan, through which the institution intends to enhance its public value by contributing to the increase in the number of doctoral degrees in our country. Since 2022, the Istituto Superiore di Sanità has annually funded approximately 40 doctoral scholarships through agreements with various universities across the country and since 2024, it is now hosting approximately 130 doctoral students. To promote and undertake this activity, a PhD Working Group has been established, in which senior researchers and representatives elected by the PhD students work together.
Key words: PhD Day, Open day

Istituto Superiore di Sanità
2026 PhD Day of Istituto Superiore di Sanità. Istituto Superiore di Sanità, Roma, 19 gennaio 2026. Riassunti.
A cura di Alessandra Ambrosone, Alice Di Netta, Camilla Lugli, Luisa Minghetti e Lorenza Scotti.
2026, vii, 121 p. ISTISAN Congressi 26/C1 (in inglese)

Questo volume raccoglie i riassunti dei contributi presentati in occasione del "PhD Day 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. È inoltre un aspetto chiave della missione dell'Istituto e, in particolare, del Piano Triennale di Attività in corso, attraverso il quale l'Istituto intende accrescere il proprio ruolo pubblico contribuendo all'incremento del numero di dottorati di ricerca nel nostro Paese. Dal 2022, l'Istituto Superiore di Sanità ha finanziato circa 40 borse di dottorato attraverso convenzioni con diverse università su tutto il territorio nazionale e, dal 2024, ospita circa 130 dottorandi. Per promuovere e svolgere questa attività, è stato istituito un Gruppo di Lavoro per il Dottorato, in cui collaborano ricercatori senior e rappresentanti eletti dai dottorandi.
Parole chiave: PhD Day, Open day

Scientific Responsible: Luisa Minghetti

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Director General of Istituto Superiore di Sanità

Stefano D'Amelio
Sapienza University of Rome

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NOTE FOR THE READER

This volume contains the abstracts of all the contributions presented at the 2026 PhD Day of Istituto Superiore di Sanità, held in Rome on 19th January 2026. Contributions have been submitted on a voluntary basis by PhD students conducting their research projects at Istituto Superiore di Sanità 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 are 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.

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Abstracts

O BAYESIAN HIERARCHICAL SPATIO-TEMPORAL MODELS FOR THE ANALYSIS OF INTERREGIONAL MOBILITY OF PATIENTS UNDERGOING ANKLE ARTHROPLASTY IN ITALY

Adriano Cuccu (1,2)
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(2) Scientific Secretariat, Presidency, Istituto Superiore di Sanità, Rome, Italy

Patient mobility across regions represents a critical dimension of healthcare systems, particularly for highly specialized surgical procedures, where differences in availability and organization may strongly influence access to care. This work examines inter- and intra-regional patient flows in Italy, with a specific focus on total ankle replacement, using nationwide administrative hospital discharge data provided by the Italian Ministry of Health for the period 2001-2023. The primary objective is to develop a robust statistical framework for modeling the spatio-temporal dynamics of patient mobility over time. The aim is not only to describe mobility patterns, but also to identify and quantify their main underlying components in a coherent and probabilistic way. Traditional descriptive indices, such as attraction and escape indices, are highly sensitive to volume fluctuations and may be unstable for regions with small case numbers. The proposed Bayesian approach is designed to address these limitations by explicitly accounting for spatial dependence and temporal evolution through random effects, while incorporating regional healthcare-related covariates as fixed effects. Several alternative representations of spatial structure are systematically compared, including conditional autoregressive models, Matérn covariance models, and B-spline formulations. Temporal dynamics are captured through autoregressive processes, while covariates such as resident population, number of hospitals and surgical volume are included to reflect healthcare demand and organizational capacity. An additional methodological contribution is the introduction of soft constraints on annual national totals, reflecting known aggregate volumes. Results indicate that parsimonious spatio-temporal models provide the most reliable and interpretable description of patient mobility. Simple CAR and Matérn specifications achieve a favorable balance between fit and predictive performance, while more complex formulations tend to overfit and offer limited gains. The estimated latent components yield interpretable measures of regional attractiveness and retention that are inherently adjusted for volume effects, thereby addressing key limitations of traditional mobility indices. Soft penalization of annual totals plays a secondary but non-detrimental role. These findings suggest that, in this context, robust, interpretable spatio-temporal summaries are preferable to highly parameterized models for monitoring patient mobility and supporting healthcare planning.

ESTIMATION OF MORBIDITY INDICATORS IN POPULATION-BASED CANCER STUDIES: METHODOLOGICAL DEVELOPMENTS

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(2) Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

Cancer survival analysis in population-based studies relies on the relative survival framework, which enables the estimation of survival specific to the disease of interest in the absence of information on the individual cause of death. Within this framework, the overall hazard is decomposed into the excess hazard due to cancer and hazard due to the other competing causes of death, the latter derived from routine sources such as national or regional life tables. The survival function associated only with the excess hazard corresponds to the quantity of interest, known as net survival. Over the years, researchers in cancer epidemiology have emphasized the value of model-based approaches, as they allow flexible modelling, improved interpretability, and the ability to compare different demographic groups. In this sequence of contributions, we first focus on estimating the proportion of cured patients in cancer populations and propose two novel mixture cure models. The first is a cure model that identifies distinct groups among fatal cancer patients. The second models the cure fraction as a flexible function of population characteristics using smoothing splines and neural networks. We apply these methodologies to a cohort of colon cancer patients diagnosed in the municipality of Varese, Italy, between 1993 and 2013, providing relevant insights into colon cancer survival outcomes. We then address the problem of cancer prevalence estimation and projection using an illness–death model, in which incidence and net survival are combined within a forward calculation framework to estimate this quantity of interest. We improve the state-of-the-art procedure by applying regularization techniques for incidence model selection and by estimating net survival within the framework of flexible parametric relative survival cure models. The resulting novel and classical pipelines for estimating prevalence are compared using data from colon cancer patients diagnosed in Sweden between 1958 and 2019. All proposed methodologies are also evaluated through simulation studies to assess their statistical properties and to compare their small-sample efficiency with respect to classical methods.

O IMPACT OF A HIGH-FLAVONOID, HIGH-FIBER DIET IN COMBINATION WITH GLP-1 ANALOGUES THERAPY ON THE IMMUNOMETABOLIC PROFILE IN SUBJECT AFFECTED BY METABOLIC SYNDROME AND TYPE 2 DIABETES

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(2) Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy

Metabolic Syndrome (MetS) is a chronic metabolic disorder characterized by the co-occurrence of several risk factors (obesity, insulin resistance, chronic inflammation) that, together, increase the risk of Type 2 Diabetes (T2D) and Cardiovascular Diseases (CVD). Environmental factors, such as dietary habits and sedentary behaviour, play a crucial role in the progression of MetS. Consequently, primary treatment strategies involve lifestyle modifications. This study evaluated the effects of a diet enriched with fibers and flavonoids, combined with GLP-1 analogue therapy, on metabolic, inflammatory, and immunological parameters in subjects affected by MetS and T2D, with a specific focus on gender-related differences. Thirty subjects (men/women ratio 1:1) with MetS were randomized into two groups: DIET-A (standard diet for T2D: 30-40 g fiber, ≤100 mg flavonoids/day) and DIET-B (45-50 g fiber, >200 mg flavonoids/day. At baseline (T0) and after 8 months (T1), anthropometric parameters were monitored, and metabolic and inflammatory markers were analyzed from blood samples. Plasma biomarkers (IL-6, TNF-α, adiponectin, leptin, ROS, FFA, and fasting glycerol) were analyzed via ELISA and biochemical assays. Furthermore, PBMCs were isolated to assess monocyte subset frequencies via FACS and subjected to ex vivo stimulation (LPS, β-glucan, R848, and Poly(I:C)) to evaluate cytokine secretion (IL-10, IL-6, IL-1β, CCL2). At T0, women exhibited significantly higher BMI, waist circumference, PhA (phase angle), adiponectin, TNF-α, and glycerol levels ($p<0.05$), highlighting an impaired adipose tissue function. Elevated levels of leptin, INF-γ, and IL-8 were also higher in women. At T1, DIET-B subjects showed promising metabolic improvements, including reduced leptin, IL-8, IFN-γ, and glycerol levels and increased adiponectin secretion. Immunological parameters remained unchanged. In parallel, the effects of Protocatechuic Acid (PCA), an anthocyanin-derived metabolite, were evaluated on Adipose Tissue (AT) biopsies from bariatric subjects (9 men and 9 women). PCA demonstrated anti-inflammatory and antioxidant activities specifically in female subjects, reducing FFA production (colorimetric assay) and upregulating PPARγ expression (Western Blot), and leptin and adiponectin secretion (ELISA assay). pNF-kB expression decreased in both sexes (Western Blot). In conclusion, our findings demonstrate that women with MetS and T2D present a more severe immune-metabolic dysfunction at baseline. DIET-B treatment improved metabolic parameters in both sexes, whereas PCA treatment on AT results more effective on female subjects. These results underscore the critical necessity for gender-specific research to develop effective, personalized nutritional and pharmacological therapeutic strategies.

O THE ROLE OF THE CLINICIAN'S SUBJECTIVE EXPERIENCE IN THE INTERACTION WITH THE PATIENT: A PILOT STUDY ON NEUROBIOLOGICAL CORRELATES

Giovanni Videtta (1,2,3)
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(2) Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
(3) Centre for Behavioural Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy

The Assessment of Clinician's Subjective Experience (ACSE) is a questionnaire designed to assess the clinician's subjective experience during patient interactions. Although previous studies have supported its reliability and clinical validity, its neurobiological underpinnings remain unexplored. Two clinicians and ten patients were recruited in a three-phase study: (1) a first clinical interview, (2) ACSE administration, and (3) listening to the first clinical interview recording. Electroencephalography (EEG) data were recorded from clinicians during both the first clinical interview and the ACSE administration. EEG data were analyzed using relative power spectral density, node strength, and global efficiency derived from imaginary coherence. Clinicians were psychiatrists, while patients were diagnosed with depressive and anxiety disorders. ACSE scores were highest for Difficulty in Attunement and Engagement dimensions, aligning with prior findings. EEG analyses revealed distinct frequency- and region-specific patterns associated with each ACSE dimension: the dimension of Disconfirmation elicited the most heterogeneous neural responses across all EEG metrics and both clinicians. Connectivity analyses highlighted divergent network profiles: the female clinician showed higher variability in global efficiency, while the male clinician exhibited more stable patterns. Finally, correlational analyses identified specific associations between ACSE dimensions and EEG features. These preliminary findings provide the first neurobiological evidence linking the clinician's subjective experience with measurable brain activity. They suggest that subjective dimensions in clinical encounters may be partially encoded in distinct EEG patterns, laying the groundwork for future research on the neurobiology of intersubjectivity processes.

O VIRUS-HOST INTERPLAY IN SARS-COV-2 INFECTION: GAINING INSIGHTS INTO INNATE IMMUNE RESPONSES AND MECHANISMS OF IMMUNOPATHOGENESIS INDUCED BY VARIANTS OF CONCERN USING A HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC)-BASED *IN VITRO* MODEL

Giada Cairo (1), Martina Severa (1), Elena Criscuolo (2), Matteo Castelli (2), Marilena Paola Etna (1), Daniela Ricci (1), Anna Teresa Palamara (1), Nicola Clementi (2,3), Eliana M. Coccia (1)

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(3) IRCCS San Raffaele Scientific Institute, Milan, Italy

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and caused the COVID-19 pandemic. Due to its high mutational plasticity, SARS-CoV-2 has generated multiple Variants Of Concern (VOC) with distinct replication capacities and immune-modulatory properties. Disease severity is closely linked to host immune responses; therefore, understanding how viral evolution reshapes innate immune activation is critical for identifying aberrant immune responses. My PhD project aimed to characterize innate immune responses to SARS-CoV-2 VOC using a human PBMC-based *in vitro* model and to identify variant-specific immune signatures by single-cell multi-omic profiling. Cytokine and chemokine release was quantified 24 hours post-PBMC exposure to Alpha, Beta, Gamma, Delta, Omicron BA.1 and the recombinant variant XBB.1. D614G and Alpha variants induced strong type I Interferon (IFN-I) responses, whereas Beta, Gamma, and Delta failed to elicit IFN-I production, suggesting the induction of VOC-specific immune evasion strategies. Omicron BA.1 triggered robust IFN-α, IL-6, and TNF-α responses, along with increased IL-8 and RANTES secretion, while Beta was the weakest inducer of the majority of the secreted factors. Interestingly, this comparative analysis revealed that XBB.1 induced stronger IFN-α and uniquely triggered IL-12p70 production. In line with this evidence, flow cytometric analysis showed that XBB.1 promoted Dendritic Cell maturation and differentiation towards the $BDCA3^+$ subtype promoting $CD8^+$ T cell activation; while BA.1 reduced expansion of $CD14^{low}CD16^{high}$ inflammatory monocytes. Indeed, single-cell multi-omic profiling of enriched HLA-DR^+/NK immune cells stimulated with BA.1 revealed extensive monocyte reprogramming, affecting differentiation state, adhesion, and antigen presentation. Viral RNA preferentially accumulated in $CD14^+$ monocytes, where stress, inflammatory, and metabolic pathways were up-regulated, while IFN α/β signalling and antigen presentation process were specifically suppressed by the virus. However, SARS-CoV-2 stimulation was shown to undergo only transient and abortive replication in monocytes without productive infection. Overall, these findings demonstrate that SARS-

CoV-2 VOC differentially modulate innate immune pathways, with monocytes emerging as key targets of immune reprogramming. This work provides insights on how viral evolution balances immune evasion and innate immune pressure, with potential implications for host-directed therapeutic strategies.

Supported by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT) and by the Italian Ministry of Health (RIPrEI project, L106/2021).

O VALIDATION OF INTRABODIES AGAINST THE E6 AND E7 ONCOPROTEINS: A TARGETED THERAPEUTIC APPROACH FOR THE TREATMENT OF HPV16 ASSOCIATED LESIONS

Susanna Falcucci (1), Sabrina Tait (2), Maria Vincenza Chiantore (1), Anna Rita Ciccaglione (1), Carla Amici (3), Paola Di Bonito (1), Luisa Accardi (1)
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High-risk Human Papillomavirus type 16 (HPV16) is the most prevalent oncogenic HPV type. Collectively, high-risk HPVs cause nearly all cervical cancers and a substantial proportion of other anogenital and oropharyngeal cancers. Persistent HPV infections remain a major clinical challenge, as vaccines cannot treat established lesions or cancers. The viral E6 and E7 oncoproteins drive transformation by inactivating p53 and pRb tumor suppressors and reprogramming host pathways. Intrabodies-recombinant antibodies in single-chain format expressed intracellularly- can provide a strategy to neutralize viral oncoproteins and restore tumor suppressor function. Two intrabodies were developed at Istituto Superiore di Sanità: nuclear I7NUC targeting E6 partially restores p53, while ER-localized 43M2SD targeting E7 sequesters E7 and interferes with pRb degradation. We investigated their transcriptional and functional effects after expression in HPV16-positive SiHa cells. Genome-wide RNA sequencing revealed marked differences between the intrabodies. I7NUC induced extensive reprogramming, with over twelve thousand genes differentially expressed. Downregulated pathways included proteostasis, nucleocytoplasmic transport, ER protein processing, chromatin remodeling, ubiquitin-mediated proteolysis, ribosome biogenesis, senescence, and metabolism. Network analysis showed that many downregulated genes are E6 interactors, indicating disruption of the E6 oncogenic network. Instead, 43M2SD had a limited transcriptional footprint, affecting approximately one thousand genes and enriching only cytokine–receptor interaction pathways. No direct E7 interactors were detected. In three-dimensional (3D) SiHa spheroids, which closely mimic tumor heterogeneity and hypoxia, I7NUC accumulated rapidly and moderately inhibited the spheroid growth. Time-course RT-qPCR confirmed sustained p53 activation and a dynamic interferon-γ response. Prolonged expression also downregulated ER stress and ferroptosis defense genes, weakening adaptive survival pathways. In comparison, 43M2SD accumulated slowly and had more modest effects on the spheroid growth. Overall, nuclear inhibition of E6 by I7NUC triggers a broad antitumor program, restoring p53 activity, suppressing oncogenic pathways, perturbing metabolism and proteostasis, and modulating immune signaling. Although further confirmation is needed, I7NUC seems to be quite efficacious even in 3D spheroids, and supports intrabody-based strategies as a promising therapeutic approach for HPV16-associated malignancies.

O ANTITUMORAL EFFECT OF IL-33 AND ITS ROLE AS COMBINATORY AGENT WITH DECITABINE (5-AZA-2'-DEOXYCYTIDINE) AGAINST METASTATIC MELANOMA. A MULTIDISCIPLINARY STUDY INVOLVING *IN VITRO*, *IN VIVO* AND "ON-CHIP" APPROACHES

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The treatment of metastatic melanoma is still a challenge in clinical oncology. Immunotherapy and "targeted" therapies against tumor antigens have proven notable efficacy but on a limited number of patients. Immune Checkpoint Inhibitors (ICI) therapy limits tumor immune escape yet only for approximately a third of patients and in most cases for a limited time. Several approaches to overcome resistance to ICI are being investigated among which the addition of epigenetic drugs that are expected to act on both immune and tumor cells. In melanoma, aberrant DNA hypermethylation is frequently observed, resulting in the silencing of several genes involved in cell cycle regulation, apoptosis, tumor growth and drug resistance. The use of DNMTi holds promise for melanoma treatment due to their ability to promote immune recognition by re-activating silenced genes. Combination of Decitabine (DAC) with immunostimulatory cytokines, is highly effective in contrasting the growth of mouse and human melanoma *in vitro* and *in vivo* through direct effects and by remodeling the tumor-immune microenvironment. IL-33 is an atypical alarmin belonging to the IL-1 cytokine family that plays multiple roles in allergies, autoimmunity and inflammation. Due to its oncologic role recently redefined, IL-33 is an excellent candidate as an immune modulator for the development of new combined therapies, which can lead to encouraging results in the treatment of metastatic melanoma. Expression of IL-33 by tumor cells stimulates CD8 T cell-mediated anti-tumor activity in various cancer models, including colorectal cancer pancreatic ductal adenocarcinoma, melanoma and breast cancer and is required for ICI efficacy. The aim of this study is to explore the combination of IL-33 with DAC, to restore tumor suppressor genes and to increase immune recognition, highlighting their potential to improve the efficacy of immune checkpoint inhibitors in pre-clinical models of melanoma. We evaluated the efficiency of this combined therapeutic approach *in vitro*, *in vivo* and by Organ-On-Chip (OOC) system. Specifically, in spheroids of mouse and human melanoma cells, we investigated the direct anti-tumor activities of DAC alone or combined with IL-33. *In vivo*, we explored the benefits of DAC/IL-33 combination in contrasting tumor growth in mice transplanted with B16.F10 melanoma cells. We dissected how the combined DAC/IL-33 treatment affected the immune recruitment (i.e., T cells and eosinophils) at the tumor site, PD-1 expression, and its potential to ameliorate the therapeutic response to PD-1 blockade *in vivo*. We further evaluated the requirement of endogenous DAC/IL-33 axis for

DAC *in vivo* therapeutic response and cancer-immune crosstalk by employing ST2-deficient ($ST2^{-/-}$) mice. In a microfluidic-based dual-TME competitive migration assay, we assessed the chemotactic response generated by DAC/IL-33 to spleen cells from naïve WT and $ST2^{-/-}$ mice, as well as in human PBMCs. We also conducted methylation studies to explore epigenetic control of IL-33 expression by DAC in mouse and human melanoma cells. Finally, to improve the translational potential of this project, we used Organix™, an advanced Organ-on-Chip platform study vascularized melanoma spheroids within a 3D TME. These experimental settings allowed us to analyze the effect of this combinatory therapy in a more complex but still reproducible human model. Through confocal imaging, flow cytometry and RT-qPCR analyses, we confirmed more accurately the effect of DAC/IL-33 combination in the recruitment of immune cells within the TME. Overall, by a multidisciplinary approach we could demonstrate the synergistic anti-tumor action between IL-33 and DAC in promoting immune cell recruitment and modulating the tumor immune microenvironment to overcome resistance to ICBs in melanoma, and the crucial role of IL-33/ST2 signaling for the efficiency of immune migratory response to DAC. The results of our studies could provide new knowledge useful for the development of therapeutic strategies based on the combination of epigenetic and immunotherapeutic drugs with higher efficiency for melanoma patients.

O CONSEQUENCES OF MICROGRAVITY ON AMYLOID AGGREGATE FORMATION USING TRANSGENIC LINES OF CAENORHABDITIS ELEGANS

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With the increasing challenges posed by pollution, overpopulation, and climate change, long-term space travel may soon become a necessity. In this scenario, environmental stressors such as microgravity (μg) and cosmic radiation are expected to exert profound effects on human physiology. While space biomedicine has advanced, the molecular and genetic bases of disease processes, particularly neurodegenerative disorders, remain poorly understood under these conditions. μg has the potential to disrupt protein folding and aggregation, processes that are tightly regulated and genetically determined. *C. elegans*, a genetically tractable model organism, provides a powerful system for dissecting these effects at the molecular level. To investigate how space-like conditions influence the aggregation of beta-amyloid (Aβ), a hallmark of Alzheimer's disease, we employed transgenic *C. elegans* lines constitutively expressing human $A\beta_{1-42}$ in either neuronal or muscle tissues. This allowed us to examine tissue-specific genetic responses and phenotypic consequences of Aβ expression under both normal and simulated μg conditions. Our data show that both neuronal and muscular expression of Aβ lead to locomotion impairments compared to the relative isogenic controls. However, only the muscle-expressing line exhibits reduced pharyngeal pumping, which is associated with an unpredicted extension of lifespan. The latter effect is likely due to caloric restriction caused by impaired pharyngeal function. Conversely, neuronal Aβ expression has no effect on pharyngeal pumping, whereas it significantly shortens lifespan. Immunofluorescence analysis performed in the transgenic line expressing $A\beta_{1-42}$ in muscles highlighted β-amyloid deposition in the target tissue, validating this model at the histological level. Notably, we implemented the NASA-developed Rotary Cell Culture System (RCCS) to expose animals to simulated μg from early adulthood to mid-life. This setup offers a unique opportunity to explore how altered gravity modulates gene expression, protein aggregation, and neurodegenerative phenotypes *in vivo*. Preliminary data regarding neuronal isogenic control line in simulated μg conditions show a slight reduction in locomotion at T8 (T0 corresponds to the young adult stage; T1 to the first day of adulthood; T2 to the second day of adulthood and so on) compared with the same line not exposed to simulated μg. Our findings contribute to the understanding of gene-environment interactions relevant to

neurodegeneration. By integrating a genetically defined model with a controlled space analog system, this study lays the groundwork for screening antibody-based therapies targeting protein aggregation, both in space and on Earth.

O MGST1 REGULATES PD-L1 EXPRESSION TO PROMOTE IMMUNE EVASION IN NON-SMALL CELL LUNG CANCER (NSCLC)

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Over the past decades, immunotherapy has revolutionized cancer treatment, establishing itself as one of the most promising therapeutic strategies for previously incurable malignancies, including Non-Small Cell Lung Cancer (NSCLC). Among the most innovative approaches is the use of Immune Checkpoint Inhibitors (ICIs), monoclonal antibodies that neutralize the immunosuppressive function of receptors such as PD-1 (Programmed Death-1), its ligand PD-L1 (Programmed Death-Ligand 1), and CTLA-4 (Cytotoxic T-Lymphocyte Antigen-4), thereby restoring antitumor immune responses. However, to date only a fraction of patients derives durable clinical benefit from these therapies. A deeper understanding of the underlying mechanisms is therefore essential to extend the efficacy of immunotherapy through the identification of resistance mechanisms, predictive biomarkers, novel combinatorial strategies, and new therapeutic targets. Moreover, accumulating evidence supports a tumor-intrinsic, pro-tumorigenic role of PD-L1 in promoting stemness-associated features, including unlimited clonogenic potential and resistance to therapy, underscoring the central relevance of this molecule in immuno-oncology. In this study, using a Two-step co-Immunoprecipitation (TIP) approach to identify novel PD-L1–interacting proteins, Microsomal Glutathione S-Transferase 1 (MGST1) was identified as a new regulator of PD-L1 expression in NSCLC tumor cells. Silencing of MGST1 by short hairpin RNAs (shRNAs) led to a marked reduction of PD-L1 levels in multiple NSCLC cell lines, mediated by enhanced ubiquitin–proteasome–dependent degradation. Mechanistically, MGST1 was shown to compete with the E3 ubiquitin ligase HRD1 for binding to PD-L1, thereby inhibiting its polyubiquitination at the cytoplasmic domain. This process promotes PD-L1 maturation and facilitates tumor immune evasion. Immunohistochemical analyses of human NSCLC tumor specimens further revealed a positive correlation between MGST1 levels and PD-L1 expression, while in silico analyses of clinical datasets showed that high MGST1 expression is associated with poor patient survival. Collectively, these findings identify MGST1 as a novel modulator of PD-L1 and suggest it as a potential therapeutic target for future cancer immunotherapy strategies.

O DEVELOPMENT OF HORIZONTAL ANALYTICAL PLATFORMS TO BE USED FOR MONOCLONAL ANTIBODIES QUALITY CONTROL

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Monoclonal Antibodies (mAbs) currently represent one of the most important classes of biotherapeutic drugs and are widely used in the treatment of oncological, autoimmune, and inflammatory diseases. However, unlike chemically synthesized drugs, mAbs are intrinsically heterogeneous molecules. Throughout the entire product life cycle, from production to storage, they may undergo numerous post-translational modifications, such as deamidation, oxidation, terminal clipping, or variations in glycosylation. These transformations contribute to the formation of charge and mass variants that increase product complexity and may have a direct impact on quality, stability, safety, and clinical efficacy. The PhD project, carried out in collaboration with the National Center for the Control and Evaluation of Medicines (CNCF) of the Istituto Superiore di Sanità, focused particularly on charge variant analysis, which is recognized as a Critical Quality Attribute (CQA) and therefore must be carefully monitored during manufacturing and post-marketing surveillance. The primary objective was the development of transversal analytical platforms for mAbs characterization and Quality Control (QC), in accordance with ICH guidelines and following the Analytical Quality by Design (AQbD) approach. This approach enables the design of robust, scientifically sound methods capable of ensuring consistent performance and a better understanding of the method itself. The evaluation of charge variants was conducted using the Imaged Capillary Isoelectric Focusing (icIEF) technique, available at the CNCF and considered one of the most promising techniques for separating charge heterogeneities based on their isoelectric point (pI). Starting from the conditions described in the European Pharmacopoeia (Ph. Eur.), two novel methods were developed and optimized: the first following a classical One Factor at a Time approach, and the second introducing the innovative AQbD methodology. Both methods were able to provide a more accurate measurement of pI values and, above all, applied to different mAbs. This aspect is particularly relevant, as it meets the growing need for "horizontal" methods, generic approaches applicable to different classes of biomolecules, rather than product-specific procedures. The results show that the development of horizontal icIEF platforms, in line with Ph. Eur. recommendations, can represent a fundamental support to QC and post-marketing surveillance activities. They enable batch monitoring and assessment of the impact of process variations, harmonize analytical strategies, facilitate data comparison, and strengthen the overall QC of mAbs throughout their entire life cycle.

Progetto di Ricerca di Interesse Nazionale (PRIN) – Bando 2022 Prot. 2022af8kz3: "Quality by Design approach for the development of validated analytical platforms to be used for recombinant proteins characterization and Quality Control (QubyD4Prot)".

O THE DIAGNOSTIC ROLE OF MULTIMODAL MRI (1H-MRS, AND STRUCTURAL MRI) IN ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT

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Alzheimer's Disease (AD) is the leading cause of dementia in older adults and is characterized by a long preclinical phase during which biological, metabolic, and structural brain alterations precede cognitive symptoms by several years. In this context, the identification of non-invasive biomarkers capable of distinguishing normal aging from prodromal stages such as Mild Cognitive Impairment (MCI), and from overt AD, is essential for early diagnosis, patient stratification, and monitoring disease progression and treatment response. This contribute evaluates the diagnostic and prognostic value of a multimodal magnetic resonance approach combining structural MRI, Diffusion Tensor Imaging (DTI), and Magnetic Resonance Spectroscopy (MRS), which provide complementary information on anatomical, microstructural, and metabolic brain changes associated with AD. In Cognitively Normal (CN) individuals, these techniques typically show preserved brain volumes, intact white matter microstructure, and stable neurometabolic profiles. In contrast, subjects with MCI exhibit early but detectable alterations: MRS reveals reduced N-Acetylaspartate (NAA), reflecting neuronal dysfunction, and increased myo-Inositol (mI), indicative of glial activation; DTI demonstrates increased Mean Diffusivity (MD) and reduced Fractional Anisotropy (FA), suggesting early microstructural disruption; structural MRI identifies selective atrophy of AD-vulnerable regions, including the hippocampus, entorhinal cortex, and posterior cingulate cortex. In clinically manifest AD, these abnormalities become more pronounced and widespread. MRS consistently shows marked neuronal loss (decreased NAA) and enhanced neuroinflammatory and membrane turnover markers (increased mI and choline). DTI reveals extensive white matter degeneration, particularly affecting limbic and posterior associative tracts, while structural MRI demonstrates progressive cortical and subcortical atrophy extending beyond the medial temporal lobe. Longitudinal studies indicate that MCI patients who convert to AD show faster rates of metabolic decline, microstructural damage, and volume loss compared with stable MCI subjects. Importantly, the integration of metabolic, microstructural, and volumetric biomarkers improves the ability to differentiate CN, MCI, and AD, and enhances the prediction of conversion from MCI to AD. Multimodal MRI metrics have also been shown to correlate with cognitive performance, clinical severity, and specific neuropsychiatric symptoms, supporting their relevance as surrogate markers of disease burden. In conclusion, a combined MRI–DTI–MRS approach represents a powerful, non-invasive strategy for the early detection and characterization of Alzheimer's disease. Despite remaining

methodological challenges, particularly for MRS standardization, multimodal MRI holds significant promise for improving diagnostic accuracy, understanding disease heterogeneity, and supporting personalized therapeutic monitoring in both clinical and research settings.

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O GOLD NANORODS: SYNTHESIS, CHARACTERIZATION, FUNCTIONALIZATION WITH RADIOPHARMACEUTICAL AND *IN VITRO* TESTING FOR APPLICATION IN NUCLEAR MEDICINE

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This research focuses on the development and validation of a nano-radiosensitization strategy using gold nanorods functionalized with the TAT peptide (AuNR-TAT) to enhance the efficacy of radiotherapy. The core objective was to exploit the high atomic number of gold to increase radiation absorption and induce localized biological damage through the emission of Auger electrons. The study successfully demonstrated that the AuNR-TAT system possesses high colloidal stability and low intrinsic toxicity, making it a safe candidate for clinical applications. A central part of the investigation involved the use of physical-chemical characterization to optimize the conjugated system and Transmission Electron Microscopy (TEM) to track the intracellular fate of the nanoconstructs. The analysis revealed that while the TAT peptide facilitates high cellular uptake, the nanorods are predominantly sequestered within endo-lysosomal vesicles in the perinuclear region rather than translocating into the nucleus. Radiobiological assays showed a significant increase in γ-H2AX foci following irradiation, indicating enhanced DNA damage. This finding suggests that the radiosensitization is mediated by an indirect mechanism: Auger electrons generated by irradiation of AuNRs in the cytoplasm produce reactive species that migrate into the nucleus to induce complex, clustered DNA breaks. In conclusion, this work indicates that nuclear proximity could be sufficient to promote radiobiological enhancement; however, these findings should be considered as preliminary and may represent a possible proof of concept for future targeted nano-radiotherapies.

O INTEGRATED PROTEOMICS AND METABOLOMICS APPROACHES FOR THE IDENTIFICATION OF NOVEL MOLECULAR TARGETS OF DRUGS WITH ANTITUMOR ACTIVITY

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Triple-Negative Breast Cancer (TNBC), lacking estrogen, progesterone, and human epidermal growth factor receptors expression, fails hormonal therapies and frequently develops chemotherapy resistance. Given the limited therapeutic options available for TNBC, this study investigated a novel drug repurposing strategy based on the combination of Metformin (Met) and D609. Met is an antidiabetic drug known to exert antiproliferative effects in several BC cell lines. D609 is an inhibitor of phosphatidylcholine-specific Phospholipase C (PC-PLC) activity, which is increased in TNBC and associated with aberrant PC metabolism in malignant BC phenotypes. To evaluate the effects of Met and D609, individually or in Combination (Combo), on TNBC, biological assays, label-free Mass Spectrometry (MS)-based proteomic, and 1H-Nuclear Magnetic Resonance (NMR) metabolomic analyses were performed on a model of TNBC, the MDA-MB-231 cells, treated for 24 or 48 hours. Met and D609, alone or in Combo, reduced cell viability and exerted a cytostatic effect. Notably, the combined treatment also induced a cytotoxic effect. A MS-based proteomic strategy was optimized to provide efficient and robust evaluation of proteomic changes induced by the drugs. Statistical analysis showed a time-dependent shift in drug dominance: D609 drove the proteomic signature of Combo at 24 hours, whereas Met emerged as the principal modulator at 48 hours. The cell cycle emerged as one of the most significantly modulated pathways at both time points, with the abundance of several cell cycle related proteins decreased, especially in Combo treated cells for 24 hours. Moreover, apoptotic signaling proteins were modulated by D609 and Combo at 24 hours and by Met and Combo at 48 hours, inducing a pro-apoptotic response that was mostly enhanced with Combo at 24 hours. Furthermore, metabolic pathways were affected, with Combo increasing a greater number of glycolytic enzymes at both 24 and 48 hours of treatment compared with either drug alone. In addition, aspartate aminotransferase, involved in amino-acid degradation, was increased in 24 hours Combo and 48 hours Met and Combo treated cells. This result is consistent with the decrease of aspartate levels, measured by NMR, in cells treated with Met and Combo for 24 hours and with Met for 48 hours. This study provides a wide-ranging picture of the effects exerted by Met and D609, administered individually or in combination, on MDA-MB-231 cells. It highlights the antiproliferative, pro-apoptotic, and metabolic modulations induced by Combo, supporting its potential as a drug repurposing strategy with promising therapeutic applications in TNBC.

O CRUCIAL ROLE OF CXCL4 AND TYPE I INTERFERON IN EARLY SYSTEMIC SCLEROSIS: WAYS TO BLOCK THEM VIA NEW EXPERIMENTAL SMALL MOLECULES

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Introduction. Systemic Sclerosis (SSc) is a rare, chronic, orphan autoimmune disease, characterized by specific autoantibodies, vascular abnormalities or damage. SSc has no effective treatment that block its progression. Chemokine (C-X-C motif) Ligand 4 (CXCL4), also known as Platelet Factor 4 (PF4), is an antimicrobial chemokine with multiple effector functions mainly released after platelets activation and is currently considered as a biomarker in systemic sclerosis (SSc). In SSc, the presence of CXCL4, at an early stage, correlates with poor prognosis and severe disease progression. CXCL4 contributes to the interferon-I (IFN-I) signature in the disease by forming pro-inflammatory liquid crystalline complexes with self-DNA, which activate TLR9 and induce IFN-α in plasmacytoid Dendritic Cells (pDCs). Here, we evaluated, *in vitro*, the role of CXCL4 tetramerization on its interferogenic function in complex with DNA.

Results and methods. We used small-angle X-ray scattering (SAXS) to characterize the corresponding peptide-DNA structures in various mutated/truncated CXCL4 derived peptides. PDC were isolated from buffy-coats and the read-out or their activation was measurement of IFN-a secretion measured by ELISA. We tested CXCL4 and its mutated/truncated peptide versions on pDcs and found that the physiological pre-assembly of CXCL4 into tetramers, before DNA interaction, is crucial for stimulation of TLR9 and amplified IFN-I secretion by pDCs, the main IFN-I producing cells in the body. At the same time, our results of homemade ELISA assay, which detected CXCL4-DNA complexes in SSc plasma, showed that small molecules that destabilize CXCL4 tetramerization block the pDC-IFN-α response to natural CXCL4-DNA complexes present in SSc blood, suggesting new pharmacological interventions in SSc, and possibly in other autoimmune conditions characterized by the presence of high circulating CXCL4 and CXCL4-DNA complexes expression.

Conclusions. This study suggests a novel approach to block the CXCL4-driven inflammation: the disruption of the CXCL4 tetrameric assembly, which in turn abrogates IFN-I amplification in SSc, and possibly in other chronic diseases characterized by increased CXCL4 expression and platelets activation.

O DEVELOPMENT OF ORGAN-ON-CHIP SYSTEM TO STUDY THE INTERACTIONS BETWEEN IMMUNE CELLS AND TUMOR CELLS IN RESPONSE TO DRUG TREATMENTS

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The initiation of an effective anti-cancer immune response critically depends on the dynamic interactions between cancer cells, Dendritic Cells (DCs), and T lymphocytes. These interactions represent a pivotal step in tumor immune surveillance and remain essential even in the context of therapeutic interventions. Microfluidic-based platforms, such as immune system–cancer-on-a-chip models, have emerged as powerful tools to investigate these processes, as they can faithfully recapitulate key structural and functional features of the Tumor Microenvironment (TME). In this context, we have developed an advanced immune system–cancer-on-a-chip platform. The device comprises two tumor chambers flanked by parallel perfusion channels designed to supply complete culture medium, and connected to a central immune chamber through a series of short, narrow capillary-like migration channels. This architecture enables controlled immune cell trafficking and intercompartmental communication. The immune system–cancer-on-a-chip allows real-time monitoring of dendritic cell migration and Peripheral Blood Lymphocyte (PBLs) recruitment toward cancer cells, as well as the characterization of their mutual interactions, both under basal conditions and in response to pharmacological treatments. Overall, this microfluidic platform enables the investigation of immune-cancer cell interactions within three-dimensional tumor-like environments, the evaluation of anticancer treatment efficacy, and the assessment of cancer cell invasiveness and immune cell recruitment. As such, it represents a versatile and robust system for performing functional assays within a single, integrated microfluidic device.

O TOWARDS NEW APPROACH METHODOLOGIES FOR MICRO- AND NANOPLASTICS HAZARD IDENTIFICATION: COMPARATIVE INSIGHTS ON POLYSTYRENE AND BIODEGRADABLE PARTICLES

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Micro- and Nano-Plastics (MNPs), which result from the fragmentation processes of plastic waste, are solid polymers with an average size of <5 mm and <100 nm, respectively. MNPs are persistent environmental contaminants that have colonized every ecological niche. They are detected worldwide in marine and terrestrial ecosystems, including oceans, rivers, air, drinking water, sediments, and food. In addition, MNPs have been found in soil and earthworms living on the surface and in deep soil layers. The presence of these pollutants in the marine, terrestrial and atmospheric environment is an emerging risk that requires urgent risk assessment and management strategies to mitigate their consequences on human health and ecosystems. New Approach Methodologies (NAMs) are one of the first choices to generate the information needed to improve mechanistic understanding of nanoscale processes, as they can support chemical risk assessment by informing on the hazard of a chemical without the use of animal testing. Since ingestion is considered one of the main routes of exposure to MNPs, this study investigated the biological effects of Polystyrene (PS-NPs) and Polycaprolactone (PCL-NPs) Nanoplastics (NPs) using complementary *in vitro* intestinal barrier models and the *in vivo* 3R-compliant Caenorhabditis elegans (C. elegans) model. Cytotoxicity, DNA damage, nanoparticle internalization, and paracellular permeability were evaluated in the *in vitro* intestinal barrier models. In C. elegans, the analyzed endpoints included oxidative stress response and locomotor behaviour, along with the assessment of potential multi- and trans-generational effects. Results indicated that both PS-NPs induced DNA damage and barrier impairment *in vitro*, whereas PCL exhibit comparatively lower biological impacts. Consistent findings emerge in the *in vivo* model, where PCL causes milder effects than PS-NPs, suggesting that chemical characteristics of biodegradable polymers and surface chemistry play a key role in determining the biological impact of NPs, underscoring the potential advantages of biodegradable nanomaterials for safer environmental and biomedical applications. The present study, through *in vitro* and *in vivo* approaches, intends to contribute to the development and standardization of reliable and new approach methodologies suitable for the assessment of human health risk resulting from exposure to MNPs in a "One Health" perspective, in the framework of BioPlast4SAFE Project.

O NEW APPROACHES METHODOLOGIES APPLIED FOR THE ASSESSMENT OF GENOTOXIC POTENTIAL: CASE-STUDIES WITH EMERGING ENVIRONMENTAL CONTAMINANT

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Genotoxicity refers to the ability of chemical, physical or biological agents to damage genetic material, potentially leading to gene mutations, chromosomal aberrations and severe health outcomes such as cancer and reproductive disorders. Assessing genotoxicity is a crucial part of chemical safety evaluation and relies on a battery of *in vitro* and *in vivo* tests to detect gene mutations, structural chromosome aberrations and numerical chromosome changes. In recent years, New Approach Methodologies (NAMs) have emerged as innovative tools to support genotoxicity and risk assessment while adhering to the principles of 3Rs in animal testing. NAMs, including advanced cell culture systems, high-throughput screening and computational models, provide mechanistic insights into the molecular and cellular events underlying genotoxicity, enabling more predictive, human-relevant and ethical evaluations. Attention has increasingly focused on Emerging Environmental Contaminants (EECs), chemicals present in water, soil, air and biota that may pose risks to human health or ecosystems. Some of them are not yet fully regulated and their toxicological properties, including genotoxicity, are still under investigation. This research project investigated the genotoxic potential of specific EECs, selecting as case studies phthalates (DEHP), Neoacids (NDA), Bisphenol A alternatives (BPE, BPP), and two non-volatile nitrosamines (NTCA and NMTCA). DEHP and NDA induced an increase in micronuclei without a corresponding rise in structural chromosomal aberrations. CREST analysis revealed multiple signals within the micronuclei, consistent with clusters of mis-segregated chromosomes, while cytological observations showed multipolar anaphases and anaphase bridges, suggesting interference with the mitotic spindle. BPA alternatives also induced an increase in micronuclei, multiple CREST signals and anaphases abnormalities. These findings indicate that these substances may induce numerical chromosomal aberrations (i.e., aneuploidy), therefore showing a genotoxic activity and posing a concern for human health; since aneuploidy is a genotoxic mode of action where a threshold can be identified, it is possible to define a safe level of exposure. Conversely, NTCA and NMTCA did not induce chromosomal damage or gene mutations; thus, they do not raise concern for genotoxicity. In conclusion, the project results highlight how integrating NAMs with traditional genotoxicity assays enhances the initial phase of risk assessment, namely hazard identification, by offering detailed insights into the molecular mechanisms underlying the effects of EECs and providing crucial data to support regulatory frameworks. These findings are crucial in guiding the definition of exposure limits and supporting informed decisions regarding safety measures for products intended for human use or food contact, ultimately facilitating the definition of reliable safety thresholds.

O EARLY IL-17A ANTIBODY TREATMENT MITIGATES AUTISM-LIKE BRAIN AND BEHAVIORAL ABNORMALITIES INDUCED BY MATERNAL IMMUNE ACTIVATION IN MICE

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Autism Spectrum Disorder (ASD) is an early-onset neurodevelopmental condition characterized by impairments in social communication and interaction, accompanied by restricted, repetitive behaviors or interests. Prenatal environmental insults, such as maternal exposure to pollutants, drugs (e.g., the antiepileptic valproic acid), or infections, affect fetal brain development and increase the risk of ASD. Maternal infection, especially early in pregnancy, and the consequent Maternal Immune Activation (MIA), increase proinflammatory cytokines that cross the placenta, thus inducing persistent brain changes as well as long-lasting ASD-like behavioral alterations. Recent preclinical studies implicate T helper 17 cells and their cytokine, Interleukin-17A (IL-17A), as key mediators of MIA-induced behavioral abnormalities in offspring. Elevated IL-17A levels have also been detected in maternal blood and offspring brain of MIA models, and higher circulating IL-17A levels correlate with the severity of symptoms in ASD individuals. These findings support testing IL-17A antibody in pregnant mice to protect against or mitigate MIA effects in their offspring. We assessed the therapeutic potential of early IL-17A antibody administration after MIA induction to mitigate ASD-like phenotypes in both male and female mouse offspring. MIA was induced by administering polyinosinic:polycytidylic acid [Poly(I:C)], a double-stranded RNA analog mimicking viral infection, to pregnant mice on gestational day 12.5, followed twenty-four hours later by treatment with an IL17A antibody. Offspring from both sexes underwent behavioral testing across three developmental cohorts, from early neonatal stages to late adolescence, using batteries targeting ASD core symptoms (social deficits, repetitive behaviors) and comorbidities (anxiety-like behavior, cognitive deficits). Hippocampal markers of synaptic plasticity (i.e., neurotrophin BDNF and synaptic proteins) and neuroinflammation (proinflammatory cytokines IL-6 and TNF-α) were also analyzed. Prenatal anti-IL-17A treatment significantly attenuated early motor abnormalities, namely hyperactivity and reduced motor control, in MIA-exposed males. Additionally, it reduced repetitive behaviors selectively observed in MIA male offspring and mitigated social deficits emerging in late adolescence in both sexes. These improvements coincided with

normalized early MIA-induced changes in hippocampal synaptic plasticity and neuroinflammation markers in both male and female offspring. These findings indicate that precisely timed IL-17A blockade, one day after MIA induction, effectively prevents behavioral and molecular alterations in offspring. IL-17A neutralization thus emerges as a promising therapeutic strategy for ASD core symptoms, warranting further preclinical and translational studies.

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

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The study aimed to investigate the interaction between Adipose Tissue (AT) and microglia, the brain's immune cells, in the context of obesity and exposure to environmental toxicants, focusing on the neonicotinoid insecticide 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 flavonoid Protocatechuic Acid (PCA); and iv) the direct effects of those substances on microglia. Specifically, we analyzed the expression of the cytokines IL-6 and IL-1β for their key positive role in central regulation of glucose homeostasis and energy metabolism. Recent research has demonstrated that the elevation 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 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 expression of the Insulin Receptor (InsR) gene, and reduced the levels of Glucose Transporter 4 (GLUT4) gene. We observed that glucose uptake was impaired in microglia exposed to all CMs, suggesting that the obesity-related AT secretome may alter the microglial metabolic profile and glucose sensing. Regarding the direct effects of ACE on microglia, our data demonstrated that ACE reduced the mRNA levels of IL-6 and IL-1β. In addition, reduced phagocytic activity was observed in HMC3 treated with ACE under inflammatory conditions with Lipopolysaccharide (LPS). 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.

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P FOSTERING EXPERTISE IN PUBLIC HEALTH THROUGH ANDRAGOGICAL DISTANCE LEARNING: EVALUATION OF COMPETENCY-ORIENTED CONTINUING MEDICAL EDUCATION

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The continuous transformation of healthcare systems has reinforced the strategic role of lifelong learning for public health professionals. In this context, distance-based Continuing Medical Education (CME) has become a key instrument to ensure accessibility, scalability, and responsiveness to emerging health challenges. However, the expansion of digital and distance learning formats has also raised critical questions regarding training quality, learning effectiveness, and the adequacy of evaluation practices, particularly in large-scale public health programs. This contribution (Extract from my Doctoral Thesis, successfully defended on 17th December 2025) addresses these challenges by proposing an integrated, andragogy-informed perspective on the design and evaluation of competency- oriented distance learning for healthcare professionals. Building on adult learning principles, the approach emphasizes learner autonomy, prior professional experience, problem orientation, and immediate applicability to practice. A particular focus has been placed on the combined use of Problem-Based Learning and Competency-Based Learning as complementary strategies capable of aligning educational processes with professional performance requirements in public health systems. From an evaluation standpoint, the contribution critically examines the widespread reliance on learner satisfaction as a primary indicator of training effectiveness. While satisfaction represents a relevant dimension of learners' reactions, evidence from large-scale educational settings suggests that it cannot be interpreted as a universal or linear proxy for learning outcomes. Rather, its relationship with learning is shaped by contextual factors such as instructional design, facilitation quality, learner motivation, and organizational support. This perspective calls for more nuanced and evidence- informed evaluation models that capture how and under what conditions training generates meaningful learning. Empirical insights derived from the analysis of extensive distance CME programs for healthcare professionals highlight the importance of integrating objective learning measures with reaction data, as well as the need to account for heterogeneity in learner profiles and training contexts. These findings support a shift away from satisfaction-driven evaluation toward multi-level assessment strategies that better reflect the complexity of adult professional learning. Finally, the contribution translates theoretical and empirical insights into an operational framework for competency- oriented CME in public health. By aligning distance learning design with internationally recognized public health competency frameworks and adapting them to national institutional contexts, it provides actionable guidance for training providers and policymakers. Overall, this work contributes to strengthening the effectiveness, accountability, and strategic value of distance-based CME, supporting the development of a competent and resilient public health workforce.

P NEUROCOMPUCARE: AN INTEGRATE CLINICAL, COMPUTATIONAL AND HEALTH HUMANITIES FRAMEWORK FOR PERSONALIZED CARE IN PEDIATRIC RARE DISEASES

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Rare paediatric neurological diseases generate complex and long-term impairments across cognitive, motor, emotional, and family domains, yet they remain poorly characterized and insufficiently supported by current clinical and technological models of care. In particular, underrecognized neurocognitive syndromes, limited access to specialized follow-up, and the lack of integrated digital and family-centred approaches represent major unmet needs. NeuroCompuCare was designed to address these gaps by developing an integrated framework that combines clinical neuropsychology, computational analysis, digital health technologies, and health humanities to improve assessment, monitoring, rehabilitation, and family-centred care in children with rare neurodevelopmental disorders.

The project pursues four interconnected objectives:

1. to design and evaluate home-based and remote follow-up and rehabilitation strategies through a digital narrative medicine platform;
2. to explore underrecognized neurocognitive profiles, including the cerebellar cognitive-affective syndrome, and to develop innovative technological tools for cognitive assessment;
3. to implement advanced motor assessment methods based on motion analysis and mathematical modeling;
4. to generate interdisciplinary knowledge integrating clinical data, family experience, ethical reflection, and health-humanities perspectives on rare pediatric neurological diseases.

Within the project, the doctoral work has so far produced the following empirical, methodological, and interdisciplinary results:

- ***Remote and home-based care****:* A digital narrative medicine platform was adapted to support remote clinical and neuropsychological assessment in children with rare neurological diseases. The study defined a **structured model of remotely deliverable clinical and neuropsychological examinations**, enabling follow-up and family-centred monitoring through a dedicated digital environment. A PRISMA-based systematic review of pediatric telemedicine in neurological and neuropsychological settings found **partial to full effectiveness**, improved accessibility and reduced family burden, despite methodological heterogeneity. In parallel, an exploratory study evaluated the feasibility of a low-frequency electrical stimulation neuro-suit (Mollii) as a home-based intervention for children with Ataxia-Telangiectasia.
- ***Cognitive assessment:*** Cognitive assessment first targeted the characterization of **underrecognized cerebellar cognitive-affective profiles in pediatric populations**

through the contribution to the development and refinement of **CCAS-informed testing tools adapted for children**. In parallel, the Mini-Mental State Examination was experimentally adapted for **robot-assisted administration** via a NAO social robot using a Wizard-of-Oz paradigm, demonstrating **high feasibility and engagement of pediatric users.**

- ***Motor assessment:*** Motor function in children and adolescents with rare ataxic disorders was assessed using **markerless motion capture** combined with the **φ-Bonacci gait index**, which successfully discriminated pathological from typical gait patterns, supporting **for the first time its use as a digital biomarker of gait harmonicity**.
- ***Human, educational and ethical dimensions:*** The human and ethical dimensions of care were investigated through mixed-methods studies. An observational NICU survey linked **parental educational level** to awareness of **art-related benefits in neurodevelopment**. A qualitative bioethical study identified **communication, trust and shared decision-making** as core themes among parents of children with rare diseases. A caregiver study in **GNAO1-related disorders** revealed **depression as a central psychological burden**, alongside preserved family functioning. Finally, a narrative review on **MED-related disorders** combined literature synthesis with **generative artificial intelligence** to enhance the representation of complex phenotypes.

NeuroCompuCare establishes an interdisciplinary framework in which digital health, computational analysis and health humanities support personalized and family-centered care for children with rare neurodevelopmental disorders. In this context, the project strengthens the link between technologies, health humanities, and clinical practice to support health in rare diseases.

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P BIOPHYSICAL FEATURES OF OUTER MEMBRANE VESICLES FROM PATHOGENIC *ESCHERICHIA COLI*

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Outer Membrane Vesicles (OMVs) blebbing from bacterial outer membrane represent a key weapon in interspecies and interkingdom communication, virulence and spread of antimicrobial resistance. OMV isolation is a fundamental step to the study of their functions; however, the yield, purity, and structural integrity of OMVs can be influenced by the purification procedure. In this work, we compared the efficacy of two commonly used isolation techniques, Size-Exclusion Chromatography (SEC) and differential Ultracentrifugation (dUC), in separating and concentrating vesicles produced by Shiga Toxin-producing (STEC) and Uropathogenic (UPEC) *Escherichia coli* strains. The obtained OMVs were characterized using a comprehensive multi-analytical approach including Scanning and Transmission Electron Microscopy (SEM, TEM), Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA), Polydispersity Index (PDI) and ζ-potential measurements, and protein quantification for purity assessment. dUC-derived samples were characterized by broader particle size distributions, higher protein concentration, and more noticeable contamination by non-vesicular material. In contrast, SEC-derived samples yielded structurally well-preserved and morphologically homogeneous vesicles, higher particle-to-protein ratios, and lower total protein content, showing reduced co-isolation of protein-aggregates. NTA and DLS analyses unveiled polydisperse populations in samples obtained with both isolation methods, with DLS measurements emphasizing the presence of larger or transient aggregates. ζ-potential values were close to neutrality for all samples, coherent with limited electrostatic repulsion and with the aggregation tendencies observed in some preparations. Of interest, a dense proteinaceous matrix was observed in all samples, more pronounced in UPEC samples, suggesting strain-specific biophysical interference. Overall, the two methods provided complementary information, emphasizing the trade-offs between yield, purity and vesicle integrity, exerting also strain-dependent effects. These findings may support informed selection of OMV isolation strategies and reproducible characterization of bacterial vesicles for downstream application in host-pathogen interaction and vesicle-based studies.

P FUNCTIONAL CHARACTERIZATION OF A NOVEL RAC1 VARIANT CAUSING A NEURODEVELOPMENTAL CONDITION WITH NOONAN SYNDROME FEATURES

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RAC1 is a member of the Rho GTPase subfamily within the RAS superfamily of small GTP-binding proteins, regulating the actin cytoskeleton architecture, cell spreading, migration and proliferation. De novo missense variants in RAC1 are associated with rare neurodevelopmental disorders characterized by developmental delay/intellectual disability and brain abnormalities and a wide range of additional features. Previous studies documented a possible role of RAC1 in contributing to the ectodermal features associated with Noonan syndrome. Here, we describe a familial case (affected mother and daughter) with a previously unreported missense RAC1 variant (p.A13D). We functionally demonstrate its pathogenicity proving a Gain-of-Function (GoF) effect. In particular, the mutation increases RAC1 activation and spreading in HEH293T and COS1 overexpressing cells. A significant enhancement of cell spreading was also documented in primary fibroblasts isolated from the patient carrying the heterozygous RAC1A13D, compared to control fibroblasts. The individuals carrying the mutation present with developmental delay and facial features resembling Noonan syndrome. Consistently, we demonstrate a strong hyperactiving effect of the variant with respect to MAPK signaling, providing a possible explanation for the RASopathy-like features of the mutated subjects.

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) exposures which significantly exceed the ambient levels experienced by the general population. Such exposures can occur among specific 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 potentially pose a risk of the only currently established effects of EMF: acute effects related to the stimulation of electrically excitable nerve and muscle tissues at lower frequencies, and body heating at higher frequencies. Compliance with internationally established and nationally transposed regulations prevents the known effects of electromagnetic fields through a two-tier system of restrictions based on reference levels and basic restrictions, respectively set on radiometric quantities (measured in the absence of the exposed human body) and dosimetric quantities (more directly related to biological and health effects, but more difficult to evaluate) internal to the exposed human body. Nevertheless, the possibility that exposure limits may be exceeded in certain circumstances cannot be ruled out, either due to accidental events or because the regulations themselves permit derogations from the Exposure Limit Values (ELVs) for specific operational requirements in occupational settings (Article 212 of Legislative Decree 81/2008). Therefore, it is essential to investigate occupational EMF exposure in scenarios where exceedance of regulatory limits may occur. In order to perform an accurate risk assessment, it is necessary to consider not only external exposure levels, but also internal physical quantities such as the Specific Absorption Rate (SAR) or the induced electric field. Since these physical quantities are not easily measurable, there is a need to introduce computational approaches such as the numerical dosimetry, which-through advanced numerical calculation techniques and anthropomorphic modelling (e.g., 3D anatomical models from the Virtual Population)-enable the estimation and calculation of dosimetric quantity distributions within the exposed human body. The objective of this thesis is to provide comprehensive insights into exposure levels and possible health effects by integrating numerical dosimetry with field measurements in exposure scenarios where exceedances of occupational exposure limits are possible. The results of this research may contribute to the development of guidelines, practices and protocols that ensure the safety and well-being of workers in occupational environments.

P OPTIMIZING ACCELERATED RTMS: BEHAVIORAL EFFECTS OF VARYING THE DOSE TEMPORAL DISTRIBUTION IN A RAT MODEL OF TREATMENT-RESISTANT DEPRESSION

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The advent of accelerated repetitive Transcranial Magnetic Stimulation (a-rTMS; ≥2 sessions/day) has greatly advanced neuromodulation for treating Treatment-Resistant Depression (TRD), allowing to address practical limitations (financial/time burdens) of standard protocols (1 session/day). However, heterogeneity of clinical a-rTMS protocols and response variability highlight the urgency of identifying the parameters that most influence efficacy. In a validated TRD rat model, we investigated short-term effects on depressive-/anxiety-like behavior of three differently concentrated rTMS protocols administering the same dose: St-rTMS, matching human standard protocols (1 session/day, 8 days); (ii) a-rTMS, replicating human accelerated protocols (4 sessions/day, 2 days, 55-min intersession interval); (iii) super Accelerated (sA-rTMS) to assess potential limits in dose temporal distribution (8 sessions in 1 day, 15-min intersession interval). Corresponding sham groups were included in each protocol. Both active (*vs*. sham) St-rTMS and a-rTMS normalized heightened anxiety/reduced motivation in the novelty-suppressed feeding test whilst sA-rTMS had no effect. Only a-rTMS was able to promote the recovery of the helplessness behavior in the forced-swim test. All three protocols reduced the motivational/apathy-like states in the splash test. Neither protocol affected the locomotor activity. As for anxiety-related parameters in the open-field test, no clear effects were observed following either St-rTMS or a-rTMS while increased anxiety and immobility were noticed after sA-rTMS. While these results support the notion that "concentrating" the dose over fewer days can improve the efficacy, they highlight the importance of maintaining adequate intersession intervals to allow the activation of

neuroplastic processes by a-rTMS protocols. These findings could contribute to the optimization of a-rTMS protocols, ultimately increasing their efficacy in the treatment of clinical depression.

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P UNVEILING PLASMODIUM FALCIPARUM GAMETOCYTE DYNAMICS IN ASYMPTOMATIC MALARIA: INSIGHTS FROM THE HUMAN BONE MARROW MICROENVIRONMENT

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Plasmodium falciparum gametocytes, the parasite transmission stages to mosquitoes, preferentially accumulate and mature within the human bone marrow, where interactions with stromal and endothelial cells are thought to regulate their development and release into circulation. Asymptomatic malaria infections are highly prevalent in Ghana, yet the contribution of bone marrow-resident gametocytes to transmission and their relationship with host and parasite factors remain poorly defined. This PhD project aims to: (i) identify the presence, maturation stage and spatial localization of P. falciparum gametocytes in bone marrow biopsies from asymptomatic individuals; (ii) dissect how bone marrow stromal and endothelial cells, and their soluble factors, influence gametocyte induction and maturation *in vitro*; and (iii) link bone marrow gametocyte carriage to epidemiological and immunological parameters in a malaria transmission season in Ghana. To address these aims, histo-morphological and immunohistochemical analyses with gametocyte- and host cell-specific markers are being optimized on decalcified human bone biopsies, supported by a troubleshooting phase using parasite-containing clot preparations to refine fixation, embedding and staining protocols. In parallel, 3D transwell co-culture systems combining transgenic *P. falciparum* lines with human bone marrow stromal and endothelial cells are being established to quantify sexual conversion and early gametocyte development. Field activities in Ghana will collect paired bone marrow, aspirate and peripheral blood samples from asymptomatic orthopaedic patients during anticipated transmission season, with molecular and histological characterization of parasite burden, gametocyte stage composition, host demographics, and parasite population structure. Despite initial delays due to ethics approval and suboptimal quality of early biopsy material, refined histology workflows, consolidated parasite culture skills and developing RT-qPCR assays have laid the technical foundation for the forthcoming surveys and co-culture experiments. The expected outcome is an integrated view of how the human bone marrow niche shapes *P. falciparum* gametocytogenesis and transmission potential in asymptomatic infections. Given that asymptomatic carriers sustain malaria transmission even in low-endemicity areas, characterizing the bone marrow as a privileged niche for sexual stage development could reveal novel intervention targets that complement current blood-stage therapies.

These biological insights may inform future transmission-blocking strategies that address hidden parasite reservoirs in deep tissues, thereby contributing to malaria elimination efforts.

P TARGETING CHOLINE AND ENERGY METABOLISM IN TRIPLE-NEGATIVE BREAST CANCER: EFFECTS OF PC-PLC INHIBITION AND METFORMIN

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Triple-Negative Breast Cancer (TNBC) is an aggressive subtype of breast cancer characterized by poor clinical outcomes, limited therapeutic options, pronounced chemoresistance, and extensive metabolic reprogramming. Among the metabolic alterations associated with TNBC, altered Phosphatidylcholine (PC) metabolism has emerged as a distinctive hallmark, reflecting the profound remodeling of membrane lipid pathway that supports tumor growth and survival. This metabolic phenotype is partly driven by aberrant activation of phosphatidylcholine-specific phospholipase C (PC-PLC), a key enzyme responsible for PC hydrolysis and the generation of Phosphocholine (PCho), a well-established marker of malignancy. Using high-resolution ^{1}H NMR spectroscopy, we investigated choline and energy metabolism in the TNBC cell line MDA-MB-231. Markedly elevated intracellular PCho levels were detected both *in vitro* and *in vivo*, confirming altered choline metabolism as a defining metabolic feature of TNBC. We then evaluated the metabolic consequences of pharmacological PC-PLC inhibition using D609, administered either alone or in combination with metformin, an antidiabetic drug currently under extensive investigation for repurposing in oncology. Metformin exerts anticancer effects, at least in part, through inhibition of mitochondrial respiratory complex I, thereby inducing energetic and redox stress in tumor cells. Comprehensive NMR-based metabolomic analyses revealed that both D609 and metformin significantly perturbed cellular energy metabolism. Metformin treatment was associated with enhanced glycolytic flux, consistent with compensatory metabolic responses to mitochondrial dysfunction. Notably, inhibition of PC-PLC resulted in additional metabolic alterations affecting high-energy phosphate compounds. Combined treatment with D609 and metformin produced a more pronounced antiproliferative effect than either agent alone and induced deeper disruptions in energy-related metabolites, including ATP and ADP, indicative of impaired cellular energy homeostasis. Overall, our findings demonstrate that aberrant phosphatidylcholine metabolism plays a functional role in sustaining the metabolic phenotype of TNBC and that pharmacological targeting of PC-PLC significantly impacts tumor cell energy metabolism. The combination of PC-PLC inhibition with mitochondrial complex I blockade further exacerbates metabolic stress, revealing a potential metabolic vulnerability that may be therapeutically exploitable. This study underscores the power of ^{1}H NMR spectroscopy as an integrative tool for characterizing drug-induced metabolic reprogramming and supports phosphatidylcholine-specific phospholipase C as a metabolically relevant and promising target in triple-negative breast cancer.

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

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Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC) is a rare neurological disorder that belongs to astrocytopathies, a group of leukodystrophies distinguished by progressive cystic degeneration of myelin and mainly due to astrocyte dysfunctions. Disease features include cerebral oedema, the formation of subcortical cysts, myelin vacuolation, and astrocyte swelling, which together result in clinical manifestations such as macrocephaly, progressive cognitive and motor impairment featuring ataxia, spasticity, and epilepsy. The condition is primarily linked to mutations in the *MLC1* gene, which encodes the MLC1 protein. In the human brain, this protein is almost exclusively localized at the astrocyte-astrocyte junctions and the perivascular astrocyte end-feet, where it is believed to play a crucial role in regulating cellular volume in response to physiological or pathological stimuli and consequently in controlling astrocyte activation. While the exact role of MLC1 and the mechanisms underlying MLC pathogenesis remain poorly understood, it is hypothesized that the resulting dysfunctional astrocytes may be incapable of properly supporting brain development. Indeed, astrocytes fulfil critical roles during neurodevelopment, including the regulation of neurogenesis, synaptogenesis, oligodendrocyte differentiation, myelination, and blood-brain barrier formation. In this study, we sought to investigate how MLC1 mutations impact early neurodevelopment by utilizing human brain organoids generated from human induced pluripotent stem cells (hiPSCs) derived from three healthy individuals and four MLC patients carrying mutations in the MLC1 gene. Western blotting, quantitative PCR, and immunostaining analyses confirmed that the organoids successfully developed the major cell populations of the central nervous system. This was evidenced by the expression of astrocyte markers (MLC1, SOX9, Vimentin, GFAP, Glutamine synthetase, EAAT1, EAAT2, Cx43, Kir4.1, and AQP4), neuronal markers (NEFH and MAP2), and oligodendrocyte markers (PDGFRA, CNPASE, OLIG2, and MBP). After 50 and 70 days of culture, organoids derived from MLC patients showed a reduced expression of some astrocyte-specific markers (MLC1, GFAP, Cx43, AQP4, Kir4.1, and SOX9) compared to controls, suggesting that the absence of functional MLC1 leads to defects in terminal differentiation and maturation of astrocytes. Furthermore, immunofluorescence staining of 50-day-old organoids revealed a downregulation

of oligodendrocyte markers (PDGFRA and MBP). These findings point to a "cascade effect" where the primary dysfunction in astrocytes could negatively influence the differentiation of oligodendrocytes, which are essential for proper myelination. In conclusion, these hiPSC-derived brain organoids represent a sophisticated and powerful system for dissecting the pathophysiological mechanisms of MLC, marking a critical step toward the discovery of effective pharmacological treatments.

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P PLASMA BIOMARKERS ASSOCIATED WITH NEUROCOVID AND COGNITIVE DECLINE

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SARS-CoV-2 infection has been associated with persistent neurological manifestations in a substantial proportion of patients. Growing evidence suggests that NeuroCOVID may increase the risk of developing neurodegenerative disorders or accelerate their progression through mechanisms involving neuroinflammation, mitochondrial dysfunction, and altered post-transcriptional regulation. This study aimed to investigate a potential link between NeuroCOVID and cognitive decline by analysing circulating plasma biomarkers, with particular emphasis on molecular alterations shared with Mild Cognitive Impairment (MCI), to identify common pathophysiological features and potential therapeutic targets. The study cohort consisted of 15 healthy controls, 8 patients diagnosed with NeuroCOVID, and 7 individuals with MCI. Total RNA was extracted from plasma samples and reverse-transcribed for the quantitative analysis of selected microRNAs (miRNAs) by RT-qPCR. Relative expression levels of miRNAs previously implicated in neurodegenerative processes were assessed. Circulating cell-free mitochondrial DNA (ccf-mtDNA) was isolated from plasma using the Norgen extraction kit. Absolute quantification of ccf-mtDNA copy number per microliter was performed by digital droplet PCR using TaqMan probes (Life Technologies). Previous work identified three circulating miRNAs (miR-92a-3p, miR-320a, and miR-320b) that regulate the MAPT gene and are differentially expressed in patients with frontotemporal dementia compared with healthy controls and/or patients with Alzheimer's disease. In the present cohort, expression analysis revealed a significant upregulation of miR-320a and miR-320b in NeuroCOVID patients compared with healthy controls. In addition, miR-320b expression was significantly increased in individuals with MCI. No significant differences in miR-92a-3p expression were observed among the groups. Analysis of circulating mitochondrial DNA showed a significant reduction in ccf-mtDNA copy number in MCI patients compared with controls, with a similar decreasing trend also observed in the NeuroCOVID group. Collectively, these findings support the hypothesis that NeuroCOVID and MCI share common molecular alterations associated with cognitive impairment. The concurrent dysregulation of specific circulating miRNAs and mitochondrial DNA in both conditions suggests the involvement of converging biological mechanisms potentially linked to neurodegenerative risk. Further investigation of these circulating biomarkers in larger cohorts may contribute to a better understanding of NeuroCOVID-related cognitive decline and support the development of novel diagnostic and therapeutic strategies.

P DEVELOPMENT AND VALIDATION OF A FOOD-FREQUENCY QUESTIONNAIRE FOR THE ASSESSMENT OF ULTRA-PROCESSED FOOD CONSUMPTION IN THE ITALIAN ADULT POPULATION

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Over the last decade, growing evidence of the detrimental effects of Ultra-Processed Food (UPF) consumption has highlighted significant methodological limitations in the quality of available research, particularly regarding the conceptualization and measurement of dietary processing. Starting from a critical reassessment of the NOVA classification, this project aims to develop and validate a Food Frequency Questionnaire (FFQ) based on food processing and specifically designed to assess UPF consumption and dietary intake of macro- and micronutrients in the Italian adult population. The online, semi-quantitative, self-administered FFQ will capture the past year's intake, distinguishing industrial, artisanal, and home-made products. The project comprises two phases. The initial pilot phase involved 52 Italian volunteers recruited from two institutions, between March and May 2025, to assess face and content validity of the FFQ. Participants self-completed the FFQ followed by semi-structured interviews, during which a trained interviewer assessed their understanding of the questionnaire and conducted 24-hour dietary recalls (24HR) to characterize eating habits of the target population. Although all foods reported in the 24HRs were represented in the FFQ, confirming comprehensive dietary coverage, quantitative analyses enabled refinement of the food list by removing four food items consumed by less than 1% of Italian adults. Qualitative analyses revealed that respondents generally perceived the questionnaire as clear and easy to understand; however, thematic analysis of participant feedback identified several areas for improvement in questionnaire structure and wording. The refined FFQ from the pilot study will be tested in a subsequent phase, involving at least 436 healthy males and females aged ≥18 years. To assess reliability, the FFQ will be administered twice (3–10 months apart) using the test–retest method, with a 7-day weighed dietary record (WDR) completed after each administration. Criterion validity will be evaluated by comparing WDR data against the first FFQ administration. This project is expected to provide a novel, valid, and reliable tool, useful for future studies in the Italian adult population, addressing the current uncertainty surrounding UPF conceptualization and measurement.

P COMPARING SPATIOTEMPORAL TEMPERATURE MODELS FOR HEAT-RELATED MORTALITY RISK ASSESSMENT IN LAZIO, ITALY

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The aim is to investigate how different spatiotemporal temperature models affect the estimation of heat-related mortality in Lazio, Italy (2008-2022). Three methods are compared to reconstruct daily maximum temperature at the municipality level: Two Bayesian station-based approaches, a quantile autoregressive model with spatial interpolation, a Gaussian model via INLA-SPDE, and a satellite-based method using ERA5. Station based models show higher and more spatially variable temperatures than satellite-based ones, especially in warmer provinces. Using individual mortality data for cardiovascular and respiratory causes, temperature-mortality associations are estimated through Bayesian conditional Poisson models in a case-crossover design. Exposure is defined as the mean maximum temperature over the previous three days. Additional models include heatwave definitions combining different thresholds and durations. All models show a marked increase in relative risk at high temperatures, but the temperature of minimum risk varies notably across methods. Station-based models estimate higher minimum-risk temperatures compared to ERA5. Stratified analyses reveal higher RR increases in females and the elderly (80+). Heatwave effects depend on definitions, but all methods capture the prolonged heat exposure effect. Overall, results confirm the importance of temperature model choice in epidemiology and provide insights for early warning systems and climate-health adaptation strategies.

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P MACROH2A1.1 CONTRIBUTES TO NEUROBEHAVIORAL DEVELOPMENT AND PRENATAL VALPROATE-INDUCED PHENOTYPES IN MICE

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MacroH2A1 (mH2A1) is a H2A histone variant, expressed as two isoforms, mH2A1.1 and mH2A1.2. Although mH2A1 has been implicated in cellular differentiation and development, its involvement in atypical neurodevelopmental conditions, such as autism spectrum disorder (ASD), remains largely unexplored. This study aimed to evaluate the contribution of mH2A1.1 to neurobehavioral development in mice prenatally exposed to the antiepileptic drug Valproate (VPA), a well-established environmental risk factor for ASD.Pregnant Heterozygous (HT) mH2A1.1 female mice, mated with HT mH2A1.1 males, received a single intraperitoneal injection of VPA (500 mg/kg) or Vehicle (VEH) on gestational day 12.5. Offspring of both sexes were assessed during the neonatal and juvenile stages for: (i) ASD-like behavioral phenotype, (ii) hippocampal synaptic plasticity, and (iii) neurotransmitter levels. Behavioral phenotyping did not reveal any effects of mH2A1.1 deletion or prenatal VPA exposure on the behavioral responses assessed during the neonatal stage. In contrast, during the juvenile stage, Knock-Out (KO) males exhibited increased repetitive behaviors, whereas KO females showed enhanced anxiety-like behavior compared to their sex-matched Wild-Type (WT) counterparts.While mH2A1.1 deletion did not impact social interaction, prenatal VPA exposure induced social deficits exclusively in males (both WT and KO), who spent less time interacting with a conspecific and emitted fewer ultrasonic vocalizations. VPA-exposed WT mice displayed impaired hippocampal long-term depression in the neonatal stage and a significant reduction in serotonin levels in the hippocampus and striatum across developmental phases. Interestingly, similar neurochemical alterations were observed in VEH-exposed KO mice. Overall, these findings suggest a role for mH2A1.1 in shaping brain and behavioral development, potentially modulating susceptibility to neuropsychiatric disorders. Its deletion appears to partially mimic the effects of prenatal VPA exposure, pointing to a potential epigenetic mechanism underlying ASD-related phenotypes.

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P SUPRAMOLECULAR SOLVENTS: A GREEN ALTERNATIVE FOR TARGET, SUSPECT, AND NON-TARGET SCREENING OF URINARY BIOMARKERS

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Human exposure to complex and evolving chemical mixtures represents a critical challenge for public health biomonitoring. Traditional methods, focused on a limited set of target analytes, fail to capture this complexity. While high-resolution mass spectrometry (HRMS)-based Suspect and Non-Target Screening (SNTS) offers a solution, its effectiveness is often constrained by conventional sample preparation, which can be solvent-intensive, selective, and ill-suited for broad chemical discovery. This study introduces Supramolecular Solvents (SUPRAS) as a sustainable, efficient, and comprehensive extraction procedure designed for exposomics. A SUPRAS-based method, employing a tetrahydrofuran/1-hexanol mixture, was optimized and validated for the multi-class analysis of 11 representative urinary biomarkers, including bisphenols, phthalates, and per- and Polyfluoroalkyl Substances (PFAS). In a direct comparison with a standard Solid-Phase Extraction (SPE) clean-up protocol, the SUPRAS approach demonstrated superior performance in target analysis, achieving higher average extraction recoveries (75% *vs*. 65% for SPE), excellent precision (RSD < 25%), and high sensitivity (limits of quantification 0.04-1 ng mL^{-1}). The key strength of SUPRAS lies in its ability to deliver broad-spectrum extraction for SNTS, enabling comprehensive chemical discovery beyond conventional methods. Thanks to its non-selective nature, SUPRAS enabled the identification of 27 additional unique compounds via HRMS-more than twice the 11 detected using SPE. Chemical space evaluation revealed that SUPRAS covered a wider range of hydrophobicity (LogP 1.6-9.6) compared to SPE (LogP 1.6-7.4), proving particularly efficient at capturing more hydrophobic substances often missed by conventional approaches. This positions SUPRAS as a powerful tool for uncovering emerging and unknown chemical risks. This work establishes SUPRAS extraction as a transformative, green alternative that successfully unifies quantitative target analysis and comprehensive chemical discovery into a single, streamlined workflow. By drastically improving mixture detection while reducing organic solvent consumption, this method provides a critical innovation for advancing large-scale, high-throughput exposure assessment and public health biomonitoring.

P INDUCED TORPOR AS A COUNTERMEASURE FOR IONIZING RADIATION EXPOSURE AND MICROGRAVITY IN DROSOPHILA MODEL

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Recent advances in aerospace technologies have emphasized the need to understand the physiological challenges associated with long-duration space missions, particularly exposure to microgravity and ionizing radiation. Prolonged exposure to these conditions can severely compromise human health, increasing the risk of cardiovascular, neurodegenerative and oncological diseases. Among the proposed countermeasures, the artificial induction of a hypometabolic state resembling natural hibernation, referred to as synthetic torpor, has attracted increasing interest, even in non-hibernating species. Hibernation is a well-characterized adaptation that allows organisms to survive adverse environmental conditions through a profound reduction in metabolic activity and energy expenditure. Recent pharmacological and neuromodulatory studies have demonstrated the feasibility of inducing torpor-like states in non-hibernating animals, suggesting potential applications in space and clinical medicine. Experimental evidence indicates that hypometabolism is associated with increased tolerance to ionizing radiation. In this context, Drosophila melanogaster represents a valuable model organism for investigating hypometabolism-mediated protection under space-relevant stressors. Drosophila has been widely used in space biology research due to its short life cycle, high genetic homology with humans, and strong experimental tractability. Moreover, large cohorts of genetically homogeneous individuals can be analyzed, providing robust statistical power. Under specific conditions, such as water immersion, Drosophila can enter a reversible torpor-like state, characterized by reduced energy consumption, suppression of oxidative metabolism, reliance on anaerobic pathways, and neuronal inactivity, resembling metabolic hibernation. The present study investigates the combined effects of simulated microgravity and ionizing radiation in young adult flies and third instar larvae of Drosophila maintained in a hypometabolic state. Both developmental stages were subjected to induced torpor and subsequently exposed to ionizing radiation. Preliminary data indicate that torpor confers radioprotective effects, as shown by a marked reduction in radiation-induced chromosomal aberrations in larvae exposed to 10 Gy and increased tolerance to high-dose irradiation in adult males, reflected by higher residual fertility compared to irradiated normometabolic controls. In parallel, the potential of hypometabolism to mitigate microgravity-induced effects is being evaluated. Adult males exposed to short-term simulated microgravity while in torpor did not display locomotor impairments and exhibited survival and lifespan comparable to control groups. Overall, these findings support the hypothesis that

hypometabolic states can modulate biological responses to radiation and microgravity and may contribute to the development of torpor-based countermeasures for long-duration human spaceflight.

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, including cell cycle regulation, DNA damage response, and the maintenance of genome stability. Recent studies have highlighted its crucial role in ensuring optimal activation of the ATR–CHK1 checkpoint pathway during replication stress and in preserving genome integrity by promoting the interaction between WRN helicase and Replication Protein A (RPA). 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 in response to perturbed DNA replication. By using a selective functional inhibitor of CK2, we show that loss of CK2 activity during conditions of mild replicative stress, characterized by slowed replication fork progression, results in a marked accumulation of replication gaps. Notably, we demonstrate that the accumulation and processing of replication gaps lead to increased exposure of single-stranded DNA (ssDNA) but does not stimulate DNA damage. The presence of extended ssDNA regions represents a critical cellular vulnerability, as it enhances cellular sensitivity to PARP inhibition, closely resembling the phenotype observed in BRCA-deficient cancer cells, which are characterized by impaired handling of replication-associated DNA damage. However, inhibition of CK2 did not affect localisation of RAD51 or BRCA2 to perturbed replication forks. Based on these observations, we explored the therapeutic potential of CK2 inhibition in combination with PARP inhibitors. Our results show that co-treatment of Olaparib-resistant/ BRCA-proficient cancer cells with a CK2 inhibitor and Olaparib significantly reduces cell viability and proliferation. These findings indicate that CK2 inhibition is sufficient to sensitize otherwise PARP-inhibitor–resistant cells. Overall, our work uncovers CK2 as a key regulator of replication gap suppression during replicative stress and identify CK2 inhibition as a promising strategy to sensitize BRCA-proficient tumours to PARP inhibitors, thereby potentially extending the clinical applicability of PARP inhibitor–based therapies.

P PUBLIC HEALTH INITIATIVES FOR THE CARE OF PEOPLE LIVING WITH DEMENTIA: THE ALZHEIMER'S AND DEMENTIA FUND AND NEW NATIONAL INTERVENTION STRATEGIES

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Dementia represents one of the major global public health challenges, currently affecting around 50 million people worldwide and projected to exceed 150 million by 2050. In Italy, more than 1.1 million People Living With Dementia (PLWD) and approximately 900,000 with Mild Cognitive Impairment (MCI), with a substantial impact on health, social, and economic systems. A large share of care is provided by family caregivers, exposing them to significant emotional, physical, and work-related consequences. To address this complex scenario, Italy established the first Alzheimer's and Dementia Fund, in 2020. The Fund promoted several strategic actions, including the development of national guidelines for the diagnosis and treatment of dementia and MCI; the update and implementation of the National Dementia Plan; surveys on dementia services; the promotion of prevention strategies; training initiatives for health and social care professionals and caregivers; the creation of a national dementia information system; and the monitoring of regional activities. The doctoral project falls within this framework, contributing to research on the care of PLWD and their caregivers. The specific objectives were to analyse bio-psycho-social needs of PLWD, service availability and use, and training needs of caregivers and health and social care professionals; and to describe caregivers' characteristics, types of services used, levels of satisfaction, diagnostic pathways, and the use of legal support measures. The research adopted a mixed approach, including focus groups with caregivers and health and social care professionals across Italian regions and a national survey targeting caregiver. Findings highlighted systemic shortcomings and marked territorial inequalities. The needs of PLWD are only partially met, with significant barriers for migrants, including linguistic obstacles, lack of culturally adapted diagnostic tools, and limited access to cultural mediation services, resulting in delayed diagnoses and stigma. The national survey confirmed the heavy caregiving burden, with negative effects on caregivers' health, social lives, and employment. Gaps in service provision, long waiting times for diagnosis, and limited use of legal support mechanisms were also identified, increasing feelings of isolation, stress, and risk of burnout. In conclusion, dementia should not be addressed solely as a clinical condition, but as a social, cultural, and political phenomenon. The findings point to the need for a paradigm shift towards an integrated, person-centred, and culturally sensitive care model, capable of strengthening local care networks, professional training, and structured support for caregivers, in order to ensure equity, dignity, and quality of life for PLWD.

P INFLUENZA VACCINATION IMPROVES THE IMMUNE RESPONSE TO THE SARS-COV-2 VACCINE

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Vaccines are historically developed to target specific pathogens; however, growing evidence indicates that certain formulations, such as BCG or measles vaccines, may confer broad, non-specific protection against unrelated infectious diseases. In line with this concept, recent observational studies have suggested an inverse association between influenza vaccination and the incidence and severity of COVID-19, implying that the flu vaccine may confer partial non-specific protection. However, the immunological mechanisms driving this cross-protection remain unclear. This study investigates the effects of the 2021/2022 seasonal quadrivalent inactivated influenza vaccine on the humoral immune response to the booster dose of the SARS-CoV-2 vaccine in a cohort of healthy adults. The study recruited 113 participants, of whom 74 met the eligibility criteria (no prior COVID-19 diagnosis, no significant comorbidities, and no immunosuppressive therapy). Participants were assigned to two groups: those receiving the influenza vaccination followed by the SARS-CoV-2 booster, and those receiving only the SARS-CoV-2 vaccine. Blood samples were collected at baseline, four weeks post each vaccination, and 12 weeks after the SARS-CoV-2 booster. The immune response was assessed analysing anti-flu and anti-spike-specific antibody titers and *in vitro* influenza and SARS-CoV-2 neutralization capacity. Results indicated that individuals who received both vaccines exhibited increased reactivity and sustained anti-Spike antibody titers up to 12 weeks post-vaccination compared to the group that received only the SARS-CoV-2 vaccine. Anti-nucleoprotein antibody titer was also evaluated to assess whether an asymptomatic COVID-19 infection occurred. Double-vaccinated participants were stratified into "high responders" (HR) and "low responders" (LR) based on their HI titers against the four influenza vaccine strains. The HR group demonstrated significantly higher anti-SARS-CoV-2 antibody titers and neutralization capacity at both 4- and 12-weeks post-vaccination compared to LRs and the control group. Conversely, LRs showed responses comparable to those who received the SARS-CoV-2 vaccine alone. A linear regression analysis confirmed a positive association between the magnitude of the influenza-specific response and anti-Spike antibody levels. In conclusion, these findings suggest that administration of the influenza vaccination potentiates the humoral response to the SARS-CoV-2. One possible mechanism underlying this observation is the emerging concept of "trained immunity," a functional reprogramming of innate immune cells that enhances responsiveness to heterologous

stimuli. These data indicate that both external stimuli, such as the administration of the influenza vaccine, and the host's intrinsic ability to respond to stimuli play a critical role in determining vaccine efficacy.

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P A PROSPECTIVE AUDIT AND FEEDBACK APPROACH USING ROUTINE DATA TO IMPROVE OBSTETRIC PRACTICE AND REDUCE CAESAREAN SECTION RATES IN THE CALABRIA REGION

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The progressive increase in Caesarean Section (CS) rates represents a major public health challenge, as high and potentially inappropriate use is associated with adverse maternal and neonatal outcomes and increased healthcare costs. Italy continues to report among the highest CS rates in Europe, with marked regional variability. The Calabria Region, in Southern Italy, has persistently recorded CS rates well above the national average. In this context, internationally recommended non-clinical interventions, including the Robson Ten-Group Classification System (TGCS) and structured Audit and Feedback (A&F) strategies based on routine data, are promoted to improve the appropriateness of obstetric care. This PhD research project, conducted within the Easy-Net program, adopts a prospective audit and feedback approach to improve obstetric practice and reduce CS rates in the Calabria Region. The program is structured in three phases, with a strong emphasis on pre-intervention assessment. The project aimed to generate robust, context-specific evidence to inform the design and implementation of a multi-strategic A&F intervention. The pre-intervention phase consisted of a comprehensive baseline evaluation integrating multiple quantitative and qualitative data sources. A population-based cross-sectional study analysed CS rates and temporal trends using data from National and Regional Birth Registers, categorizing women with Robson's TGCS. In parallel, a multicentric cross-sectional survey and a qualitative study were conducted. These studies explored healthcare professionals' perceptions and attitudes toward CS, A&F, Robson TGCS, and routine data use, as well as determinants of CS decision-making. Baseline routine data analyses confirmed persistently high CS rates, with women with a previous CS representing one of the largest contributing groups. Findings (n=427; n=92) showed that, although attitudes toward physiological maternal care practices, A&F, and data-informed quality improvement were generally positive, exposure to structured A&F activities using routine data and use of the Robson TGCS in clinical practice were limited. Professional role, perception of CS appropriateness, and exposure to audit and data strategies were consistently associated with attitudes toward CS practices and quality improvement. Key healthcare professional determinants influencing high CS rates included medicalisation of birth, women's reported fear of childbirth, family pressure, cultural beliefs, organisational constraints, and medico-legal concerns. This integrated baseline assessment directly informed the implementation of the subsequent multi-strategic intervention, combining structured A&F meetings, training activities, and communication initiatives. Post-intervention data will enable evaluation of changes in obstetric practices and CS rates.

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P IMPLEMENTATION OF AN INNOVATIVE ASSAY PLATFORM FOR THE SAFE EVALUATION OF ANTI-ORTOFLAVIVIRUS NEUTRALIZING ANTIBODIES IN HUMAN AND ANIMAL SAMPLES

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Introduction. The spread of orthoflaviviruses like Dengue (DENV), Zika (ZIKV), West Nile (WNV), and Tick-borne Encephalitis (TBEV) into new temperate regions has increased significantly in recent years, driven by factors such as climate change, urbanization and global travel. These viruses are transmitted by arthropod vectors like mosquitoes and ticks, posing a serious public health threat to both humans and animals worldwide. Plaque Reduction Neutralization Test (PRNT) is the gold standard for orthoflavivirus serologic diagnosis but is performed using live infectious viruses in high Biosafety Level Laboratories (BSL-3), raising safety and technical issues. To address these limitations, our objective is to implement a high-throughput, safe and versatile platform based on non-replicating Single-Round Infectious Particles (SRIPs) expressing luciferase and pseudotyped with orthoflavivirus prME glycoproteins to quantitatively detect neutralizing Antibodies (nAbs). SRIPs mimic the viral entry and early replication stages but are incapable of producing infectious progeny, allowing assays to be performed in BSL-2 settings.

Materials and Methods. To generate SRIPs, 293T Lenti-X cells were co-transfected with a Yellow Fever Virus (YFV)-derived replicon plasmid encoding the luciferase reporter protein, a YFV capsid plasmid and plasmids encoding the prME glycoproteins from selected orthoflaviviruses, including DENV, ZIKV, WNV and TBEV. The produced SRIPs were titrated on VERO cells and employed in a neutralization assay using sera from TBEV-infected goats and PRNT-positive sera from individuals infected with WNV, ZIKV, and DENV. Human and animal sera were serially diluted, incubated with SRIPs, and then applied to VERO cells. After 72 hours, nAb titers were expressed as the serum dilution that caused a 50% reduction in RLU (ID50) relative to cells treated with SRIPs alone.

Results. SRIPs were successfully generated and exhibited high infectivity in VERO cells. The SRIP-based Neutralization Test (SRIP-NT) was successfully exploited to evaluate the presence of nAbs in human PRNT- positive serum samples, in sera from TBEV experimentally infected goats and in field sera samples collected for surveillance purpose, confirmed by competitive ELISA.

Discussion and Conclusions. After optimization and standardization, this platform can be rapidly adapted, by substituting the prME glycoproteins with those of different orthoflaviviruses. This innovative and versatile tool will enable the rapid and safe quantification of antiviral nAbs and the simultaneous screening of multiple sera against different viruses, which could support the tracing and surveillance of emerging and re-emerging infections.

P DISPOSITION OF HEXAHYDROCANNABINOL EPIMERS AND THEIR METABOLITES IN BIOLOGICAL MATRICES FOLLOWING A SINGLE ADMINISTRATION OF SMOKED HEXAHYDROCANNABINOL: A PRELIMINARY STUDY

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In 2023, the semi-synthetic cannabinoid Hexahydrocannabinol (HHC) attracted the attention of the European Drug Agency owing to its rapid diffusion on the illicit drug market and its involvement in an increasing number of intoxication cases. Although first described in the 1940s, limited data are still available on the pharmacology of its two naturally occurring epimers, 9(R)-HHC and 9(S)-HHC. Recent clinical observations and reports from poison centres describe recurrent cardiovascular manifestations following HHC exposure, notably sinus tachycardia and palpitations, consistent with CB1R-mediated dysregulation of autonomic control and cardiac electrophysiology. Given the increasing evidence supporting the pharmacological activity of HHC, characterization of its disposition in human matrices is essential to inform the interpretation of emerging clinical and forensic cases. This preliminary study investigated the pharmacokinetic disposition of HHC epimers and their metabolites in whole blood, urine, and oral fluid following a single smoked administration. The study was approved by the Institutional Ethics Committee (IRCCS-INRCA Ancona). Six healthy non-user volunteers smoked 25 mg of a 50:50 mixture of 9(R)-HHC and 9(S)-HHC admixed with 500 mg of tobacco. Blood and oral fluid were collected up to 3 h post-intake, while urine was collected over 0–2 h and 2–6 h intervals. Samples were analyzed using a validated HPLC–MS/MS method targeting parent compounds and eight putative metabolites. A marked stereoselective disposition was observed across all biological matrices. 9(R)-HHC consistently showed higher systemic exposure than 9(S)-HHC, with C max and $AUC_{0–3}$h values approximately threefold greater. No metabolites were detected in oral fluid, whereas phase I and phase II metabolites were identified in blood and urine with distinct epimer-specific profiles. 11-nor-9(R)-HHC predominated in blood, while 8(R)-OH-9(R)-HHC was the main urinary metabolite. Conversely, 11-nor-9(S)-COOH-HHC was detected exclusively in blood, whereas 8(S)-OH-9(S)-HHC was observed only in urine, underscoring stereoselective elimination pathways. This controlled human pharmacokinetic study should be regarded as a foundational step toward comprehensive pharmacological investigations necessary to elucidate the mechanistic basis of HHC activity, clarify epimer-specific effects, and assess potential therapeutic opportunities. Such efforts must be balanced against growing safety concerns surrounding emerging cannabinoids, particularly foreseeable cardiovascular risks. Within this framework, the predominance of 9(R)-HHC in blood during the early post-intake phase may be especially relevant to acute psychoactive effects and cardiovascular vulnerability. In

conclusion, from a forensic toxicology standpoint, the present findings provide robust reference data for interpreting HHC concentrations across biological matrices, supporting the assessment of recent consumption, acute impairment, and time since intake, while strengthening the scientific basis for forensic interpretation and medico-legal evaluation of adverse events associated with these compounds.

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P A NEW MOUSE MODEL TO STUDY NEUROBEHAVIORAL DEFECTS ASSOCIATED WITH MEGACONIAL CONGENITAL MUSCULAR DYSTROPHY

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Megaconial Congenital Muscular Dystrophy (MCMD) is a rare autosomal recessive disorder caused by loss-of-function mutations in the *CHKβ* gene. MCMD is characterized by muscle weakness, intellectual disability, behavioral abnormalities, and accumulation of enlarged mitochondria in muscles fibers. Although muscular pathology has been rescued in mouse model, the neurological component of the disease remain largely unexplored. We characterized a new MCMD mouse model, the *flipper* mouse, which carries a point mutation in the *CHKβ* gene resulting in lack of protein expression. The study aims to assess whether *flipper* mice recapitulate the muscular and neurological deficits of human MCMD, and to identify biomarkers useful for the development of targeted gene therapy strategies. Skeletal muscles characterization revealed atrophy, inflammation and dystrophic features in *flipper* skeletal muscles. Transmission electron microscopy demonstrated the presence of giant mitochondria at the periphery of muscle fibers in *flipper* mice. Furthermore, cytochrome c oxidase (COX) histochemistry revealed an abnormal distribution of mitochondrial enzymatic activity. Metabolic profiling indicated alterations in muscles metabolism, including reduction in phosphocholine levels. Motor, cognitive, and behavioral abilities were assessed over the first six months of life. *Flipper* mice exhibited early-onset and persistent motor deficits, as well as pronounced alterations in emotional and affective state, including increased neonatal rearing and ultrasonic vocalization. These phenotypes were accompanied by altered anxiety-like behavior and jumping in adulthood. Both histological and *in vivo* magnetic resonance imaging revealed a significant reduction in cerebral ventricles volume in *CHKβ* mutant mice. Additionally, ultrastructural analyses identified enlarged

mitochondria. Collectively, the *flipper* mouse faithfully recapitulates key muscular and neurological defects of human MCMD, providing neurological biomarkers to use in preclinical gene therapy approaches.

P ANTIBIOTIC RESISTANCE GENES IN WATER MATRICES: QUANTITATIVE ANALYSIS AND NEXT-GENERATION SEQUENCING

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Antibiotic resistance is a growing threat across human, animal and environmental sectors. Wastewater Treatment Plants (WWTPs) act as important reservoirs of antibiotic resistance genes (ARGs), which may persist throughout treatment and be released into receiving water bodies. As part of an ongoing doctoral project, this study investigates the distribution, persistence and seasonal dynamics of 12 clinically relevant ARGs, along with bacterial community composition, in influent and effluent wastewater collected across Italy. To date, 46 wastewater samples (23 raw influents and 23 treated effluents) have been collected and analyzed from six WWTPs located in Northern, Central and Southern Italy. Twelve ARGs conferring resistance to clinically relevant antibiotic classes (blaOXA-48, blaCTX-M-1group, blaTEM, blaKPC, and blaNDM; sul1; tetA; vanA, vanB; mecA, and mecC), together with the integrase gene Int1, were quantified by digital PCR, as both absolute concentrations and values normalised to 16S rRNA gene copies. Full-length 16S rRNA sequencing was employed to characterize the bacterial community composition and explore potential associations between ARGs occurrence and microbiome composition. Most ARGs were consistently detected across samples, with the exception of mecC, which was never detected. Influent wastewater was dominated by Int1, sul1 and tetA, followed by clinically relevant β-lactamase and glycopeptide resistance genes. Treated effluents generally showed reductions of one to two log units, although ~ 40% of normalized measurements (102/276) exhibited an increase of ARGs in effluents, particularly for blaOXA-48 and blaTEM. Distinct geographic patterns were observed, with Int1, blaCTX-M-1 group and tetA more abundant in Southern Italy and blaNDM more prevalent in the North. Seasonal variability was generally limited, although selected ARGs displayed WWTP-specific increases during spring or summer. Full-length 16S rRNA gene sequencing revealed distinct bacterial community compositions across WWTPs, including the presence of several species belonging to the World Health Organization Priority Pathogens List, a classification that identifies pathogens of greatest concern due to their clinical relevance and limited treatment options. Overall, these results show that clinically relevant ARGs and priority bacterial pathogens can persist through wastewater treatment, with clear geographic and plant-specific patterns. This highlights the value of wastewater surveillance as a complementary tool to clinical data for tracking antimicrobial resistance at the community level and identifying potential hotspots of environmental dissemination.

P SUPPLEMENTATION WITH NUTRACEUTICALS SEX-DEPENDENTLY PROMOTES HEALTHSPAN IN AGED MICE FED A WESTERN DIET

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Aging is a multifactorial process characterized by increased low-grade systemic inflammation and oxidative stress, leading to a progressive decline in homeostasis. In this context, there is an increased risk of developing chronic age-related non-communicable diseases, including cognitive decline and Metabolic Syndrome (MetS). There is evidence that the consumption of diets rich in fats and sugars (Western-style diet, WD), by precipitating chronic inflammation and oxidative stress, is a risk factor for unhealthy aging, generating a vicious circle between metabolic alterations and brain functions. As changes in lifestyles (e.g.: physical exercise and balanced diets) often result in poor compliance, particularly at old age, current research is testing the efficacy of nutraceuticals with antioxidant and anti-inflammatory properties, such as polyphenols, as a means to slow down the aging process. In this study we assessed the effectiveness of Rosmarinic Acid (RA) to prevent WD-induced pathological aging in 21-month-old male and female C57BL/6N mice. Animals were supplemented with RA (500 mg/kg *per os*) or vehicle for 6 weeks; after the first 2 weeks on RA, they were started on a WD (5.28 kcal/g) or a control diet (CD, 4.25 kcal/g) until the end of the experiment. Cognitive performance was assessed by means of the Morris Water Maze (MWM) spatial task. At sacrifice, trunk blood was collected to evaluate metabolic homeostasis while the hippocampus, a brain area involved in cognitive processes, was dissected out to investigate RA-mediated central mechanisms of action. Overall, RA improved learning abilities in all subjects and prevented WD-induced memory impairment in the MWM. Moreover, RA prevented the increase in insulin resistance and in the leptin/adiponectin ratio characterizing WD-fed males, counteracting the development of a MetS-like phenotype. Transcriptomic analysis of the hippocampus revealed strong sex differences, with RA modulating oxidative and immune-related pathways respectively in males and females, while affecting sex hormone signaling cascades in both sexes. The validation analysis with RT-qPCR showed that RA reduced the expression of androgen and estrogen receptors gene in males, while increasing it in females. Similarly, RA decreased *Glp1r* gene expression in males while increasing its expression in females, with no differences due to WD consumption. Interestingly, this latter gene was recently identified as pharmacological target to prevent metabolic derangements and cognitive decline during aging. Overall, these data point to RA as a promising compound in preventing WD-induced metabolic stress and in promoting healthy aging with sex-specific effects that deserve further investigation.

P SANITIZATION STRATEGIES FOR INDOOR ENVIRONMENTS: INNOVATIVE TECHNOLOGIES TO ENHANCE HEALTHCARE SETTINGS

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Background. Indoor environmental sanitation, including microclimate improvement (Italian Decree 7 July 1997, No. 274), is essential to ensure healthy spaces, directly impacting human health, particularly in vulnerable contexts such as healthcare facilities. The COVID-19 emergency highlighted critical limitations of traditional methods, including discontinuous sanitation, incompatibility of certain products and technologies with human presence, lack of operational parameters, high costs, and poor sustainability, promoting the development of innovative and green solutions.

Rationale. Among innovative technologies, systems emitting visible light (400-420 nm, Soret band) have demonstrated microbicidal efficacy against Gram-positive and Gram-negative bacteria, including multidrug-resistant strains, spores, molds, fungi, and viruses, without inducing harmful photobiological effects and contributing to total microbial load control. The mechanism relies on photoexcitation of intracellular chromophores (porphyrins), generating Reactive Oxygen Species (ROS) capable of damaging cellular membranes, representing a non-invasive and sustainable approach. Visible light offers several advantages for healthcare environments, including continuous sanitation without displacing occupants, reduced chemical pollution and antimicrobial resistance, lower risks of intoxication due to chemicals. Research has shown that visible light, through LED-based systems, can be applied for environmental sanitation and infection control in healthcare settings; however, current evidence mainly derives from *in vitro* tests on specific indicator pathogens.

Objectives. One of the main objectives of this PhD project is to demonstrate the sanitizing efficacy of LED devices, operating at specific frequencies within the visible spectrum, in real-world environments, particularly in selected healthcare facilities. Furthermore, to ensure safe implementation of these systems, project activities include drafting guidance documents defining operational requirements, target environments, and compliance parameters for safety and efficacy, as outlined in UNI 173:2025 and relevant UNI EN ISO standards.

Expected impacts. The results will contribute to redefining the crucial role of sanitation processes in improving indoor healthcare environments and provide a scientifically relevant example of validating innovative sanitation systems for public health protection.

P UPFAS RESTRICTION PROPOSAL AND THE TULAC SECTOR: SOCIO ECONOMIC COMPARISON BETWEEN COMPANIES

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Background. On 13 January 2023, the competent authorities of Denmark, Germany, the Netherlands, Norway, and Sweden sub-mitted a proposal to ECHA for a universal restriction on PFAS (uPFAS restriction) aiming to significantly reduce environmental emissions and human exposure across the EU. The proposal received over 5,600 scientific and tech-nical comments. On 27 August 2025, ECHA published an updated version submitted by the aforesaid five Member States expanding the scope of the original proposal to include eight additional sectors (e.g. military and medical applications, tech-nical textiles, etc.) and introducing a third restriction option (RO3), allowing PFAS use under strict conditions to minimize emissions through their life cycle. Including these additional sectors would require significant evaluation beyond 2026, while RAC and SEAC are expected to adopt opinions by March 2026 and the end of 2026, respec-tively, with a public consultation on the SEAC draft opinion in the first half of 2026.

Materials and Methods. Within this context, the PhD project "socioeconomic analysis as a tool to support the finalization of the uPFAS restriction proposal", developed with CNSC-ISS and UCBM, applies socioeconomic analysis to assess the impact of the uPFAS restriction in the TULAC sector, balancing environmental and health protection with industrial needs. The project began with a literature review and two surveys targeting textile and leather companies nationwide.

Results. The first survey evaluated companies' awareness of the essential use concept, as defined in the European Commis-sion's Guiding Criteria, whether PFAS use was considered essential in production, and whether alternatives for non-essential uses had been explored. The second focused on challenges and benefits in identifying PFAS alternatives, the progress of feasibility and sustainability studies, and anticipated impacts of the proposed uPFAS restriction on competitiveness, production costs, quality of life, and public health. The second survey recorded higher participation (34 *vs*. 21), with predominant involvement from the leather sector (26 *vs*. 8) and a reduced representation of textile companies (8 *vs*. 13). Both surveys indicate continued intentional use of PFAS due to the limited alternatives offering equivalent water- and oil-repellent performance or client re-quirements. Nonetheless, adoption of substitutes based on silicones, waxes, resins, and hybrid blends is increasing; although sometimes less performant, they are considered acceptable for EU regulatory compliance and luxury brand requests. The

second survey also revealed contrasting opinions on the restriction's potential impact on costs and competitiveness. Only a few companies conducted total fluorine analyses often with uncertain results highly dependent on sampling methods.

P 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), which consists of a postnatal administration of lipopolysaccharide (LPS) to mimic a bacterial infection at the neonatal stage. Additionally, to assess the contribution of gut inflammation to the altered neuroinflammatory and behavioral profile in this model, we tested gut-brain axis modulation by anti-inflammatory drug mesalamine (MES), used to treat human inflammatory bowel disease. Four weeks after LPS subcutaneous administration, PIA or control mice received either MES or vehicle by oral gavage for 11 days. At the end of the treatment, we evaluated the expression of inflammatory and homeostatic genes in the brain and gut, as well as their behavioral profile. We observed genotype-related differences in gene expression, with Nrg1 TM HET mice showing an overall altered neuroimmune profile. Remarkably, LPS induced specific effects mainly in wild type (wt) mice and MES treatment partially restored the mRNA levels of the altered genes whereas Nrg1 TM HET mice seemed to be less responsive to both PIA and MES. At the colon level, Nrg1 TM HET mice exhibited higher mRNA IL-1β levels than wt mice. LPS increased IFN-γ expression in Nrg1 TM HET mice but not in wt mice. Regardless of LPS, MES administration reduced IFN-γ levels in Nrg1 TM HET mice, with no effect on wt mice. Focusing on behavior, in the open field test male Nrg1 TM HET mice exhibited increased locomotor activity levels, as well as greater distance traveled and more time spent in the central area of the arena - indicative of an anxiety-related response. LPS exposure increased the distance traveled in the central area in both wild type and Nrg1 TM HET mice, an effect that was not reversed by MES. Remarkably, MES treatment reduced the time spent in the central area of both genotypes. These findings indicate that Nrg1 dysfunctional signaling: i) affects the expression of genes relevant for brain and gut homeostasis, as well as the behavioral phenotype, ii) induces a distinct pattern of long-term effects in Nrg1 TM HET mice in response to postnatal immune activation compared to wild type mice. They also suggest a potential beneficial effect of MES in modulating the gut-brain axis in the context of neurodevelopmental disorders, which deserves further investigation.

Nrg1 TM HET mice were kindly provided by Professor Tim Karl, Western Sydney University.

P RAD52 PREVENTS ACCUMULATION OF RAD51 AND POLA-DEPENDENT DNA GAPS AT PERTURBED REPLICATION FORKS

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RAD52 is a recognized DNA repair factor contributing to a specialized DNA double-strand break repair pathway called single-strand annealing. RAD52 depletion is synthetic lethal with that of BRCA1 and 2, two genes commonly mutated in early-onset breast and ovarian cancers, highlighting the relevance of RAD52 in cancer biology and therapy. Accordingly, inhibitors of RAD52 have been identified and are being improved for clinical use. Recently, we discovered a novel function for RAD52 at stalled replication forks as a regulator of replication fork reversal and fork degradation. Since the absence of RAD52 still allows cells to replicate and survive following replicative stress, we wondered how they could recover from the excessive degradation of the replication fork. To this aim, I exploited molecular cell biology and advanced imaging in cells in which RAD52 was inhibited, depleted or knocked out to assess the response to perturbed replication under this pathological condition. Our experiments indicated that loss or inhibition of RAD52 results in a striking accumulation of parental ssDNA representing daughter-strand DNA gaps, consistent with the engagement of repriming at the perturbed fork as an alternative pathway of replicative stress response. In humans, repriming is mainly mediated by the specialized primase/polymerase PRIMPOL; however, we discovered that loss of RAD52 engages repriming by Polα rather than PRIMPOL. Moreover, inhibition of RAD51 prevents Polα recruitment and Polα-dependent DNA gap formation in the absence of RAD52. RAD51 and Polα interact through the N-Terminal Domain (NTD) of the POLA1 subunit of Polα. We therefore generated cells expressing a POLA1 mutant missing the NTD region (ΔNTD) and demonstrated that the recruitment of Polα at DNA is lost in RAD52-deficient cells and double breaks accumulate when POLA1 and RAD51 could not properly associate, leading to poor fork progression and cellular viability under replication stress. Since RAD51 is a pleiotropic protein whose function requires formation of nucleoprotein filaments, we investigated if nucleofilament formation was essential in our condition by analyzing daughter-strand gap formation in RAD52-inhibited cells expressing a functional mutant of RAD51 that abrogates nucleofilament and D-loop formation. We found that the ability of RAD51 to form stable nucleofilaments is relevant for the association with POLA1 and Polα-dependent gap formation in RAD52-inhibited cells. Collectively, our findings indicate that RAD52 inhibition uncovers a novel repriming pathway functioning during extensive degradation of reversed forks without fork cleavage, mediated by Polα and RAD51 and engaged downstream the assembly of nucleofilaments, likely after strand invasion. Using inhibition or genetic depletion of RAD52 as a model, we propose to investigate the mechanistic framework of RAD52 as a gap-modulating protein and determine if this type of DNA gaps contribute to chemosensitivity in cancer cells.

P COMPREHNSIVE CHARACTERIZATION OF ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC) AGAINST THE HIV-1 TAT AND ENV ANTIGENS

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Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) and Antibody-Dependent Cellular Phagocytosis (ADCP) are key immune mechanisms contributing to the control of HIV-1 infection beyond antibody neutralization. Traditional vaccine strategies focused on eliciting neutralizing antibodies against the highly variable Env protein have shown limited success, prompting the exploration of alternative antigens and combined immune mechanisms. In this context, the HIV-1 Tat protein plays a crucial role in viral replication and immune regulation. Although naturally occurring anti-Tat antibodies are uncommon in infected individuals, their presence correlates with slower disease progression, suggesting a protective role. These findings have driven the development of Tat-based vaccination strategies aimed at inducing robust, multifunctional antibody responses to complement conventional approaches. To assess antibody effector functions, a Rapid Fluorometric ADCC (RFADCC) assay was adapted and employed using sera from two cohorts: People Living Without HIV (PLWoH) and people living with HIV (PLWH). Peripheral Blood Mononuclear Cells (PBMCs) were evaluated for their ability to mediate ADCC against CEM NKr CCR5+ target cells pulsed with HIV antigens. Dual labeling and opsonization with specific antibodies or cohort sera enabled quantitative analysis of cytotoxicity by flow cytometry, while fluorescence, confocal, and live-cell time-lapse microscopy provided complementary visualization of effector–target interactions and cellular dynamics. The RFADCC assay proved to be reliable, rapid, quantitative, sensitive, reproducible, and suitable for high-throughput screening. Both monoclonal and polyclonal antibodies, as well as human sera, mediated ADCC responses against Env- or Tat-pulsed target cells, with specificity determining the pattern of activity. Monoclonal antibodies preferentially induced cytotoxicity toward defined Env conformations, while polyclonal antibodies triggered broader, cross-clade responses against multiple antigenic forms, independent of clade or tier classification. Similarly, sera from PLWH demonstrated cross-clade ADCC activity against

diverse Env variants. Robust killing was also detected in Tat-pulsed target cells incubated with hyperimmune rabbit serum raised against Tat, highlighting Tat as a promising ADCC target. Combined analysis revealed that $CD14^+$ monocytes play a pivotal role in Tat-specific ADCC, forming stable conjugates with target cells and cooperating with other effector subsets to amplify cytotoxicity. These observations underscore the importance of monocyte-mediated mechanisms and cooperative immune networks in antibody-driven HIV immunity. Overall, this study validates the RFADCC assay as a versatile tool for evaluating Fc-mediated functions and supports the inclusion of Tat as a potential immunogen to enhance antibody-dependent antiviral responses in future HIV vaccine design.

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

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The bidirectional signalling between the hypothalamus and Adipose Tissue (AT) is fundamental to energy homeostasis and appetite regulation. The hypothalamus integrates metabolic and hormonal signals from AT, which acts as a complex endocrine organ; in addition, it governs the Hypothalamic-Pituitary-Gonadal (HPG) axis. Obesity-related AT dysfunction often leads to impaired secretion of GnRH, FSH, and LH, thus unbalancing the HPG axis. Exogenous factors, such as the neonicotinoid Acetamiprid (ACE) which is a suspected metabolic disruptor, may alter AT functionality. In contrast, dietary antioxidants like Protocatechuic acid (PCA) may counteract this disruption by reducing inflammation. As part of the PNRR "HEAL-Italia spoke 7" project, my PhD study utilizes the GT1-7 mouse GnRH neuronal cell line and conditioned media (CM) from bariatric surgery patients (10 males, 11 females) to elucidate how ACE and PCA alter hypothalamus–AT signalling and reproductive regulation. Initial assessments of direct effects on GT1-7 neurons involved 72-hour treatments with ACE (1 nM-100 μM) or PCA (10-1000 μM). CyQuant and MTS assays confirmed no significant cytotoxicity. At 150 nM, ACE significantly stimulated GnRH secretion, despite stable gene expression levels; furthermore, ACE treatment significantly up-regulated *Igf1* (at 1, 10 nM) and *Tlr4* (at 150 nM) gene expression, while decreasing *Igf1R* (at 1 nM). PCA alone showed no direct effect on the gene panel. Effects on the hypothalamus-AT signalling were evaluated by exposing GT1-7 cells to CM from human adipocytes previously treated with ACE (150 nM) or PCA (100 μM). Results revealed that CM exerts a predominantly inhibitory influence, often distinct from direct treatments. *GnRH* and *Igf1* gene expression were significantly reduced across all CM groups, with the decrease in *GnRH* being particularly pronounced in male-derived samples. ACE-treated CM from both sexes significantly inhibited *Ar* and *AdipoR1* gene expression. PCA-treated CM from both male and female samples exhibited complex modulations, significantly inducing *Erα,* and reducing *Erβ*, *Nkx2.1*, *Kiss1R*, *Igf1R*, and *Tlr4*. Overall, the present findings indicate that ACE direct treatment stimulates GnRH neurons, whereas by adipose-mediated signals it predominantly suppresses hypothalamic and HPG axis markers.

P LINKING EPSTEIN-BARR INFECTION STATUS TO THE GENETIC RISK VARIANT BAFF-VAR IN MULTIPLE SCLEROSIS

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MS results from the interplay between genetic and environmental factors. Among these, Epstein-Barr Virus (EBV) infection is a prerequisite for MS development. The genetic variant BAFF-var of the *TNFSF13B* gene, encoding for the B-cell activating factor (BAFF), is linked to an increased MS risk, elevated serum BAFF levels and expansion of memory B cells, the main EBV reservoir. The aim is to explore the interplay between EBV and BAFF-var in MS by assessing EBV status in the peripheral blood of People with MS (PwMS) and Healthy Donors (HD) and the impact of BAFF-var on EBV biology. Peripheral Blood Mononuclear Cells (PBMC) and sera were collected from 149 therapy-naïve PwMS and 105 HD. BAFF-var prevalence was assessed by SNP-genotyping. EBV DNA was quantified by droplet-digital PCR, while the expression of EBV latency/lytic transcripts and BAFF/B cell-related genes were analyzed using enhanced real time RT-PCR. Soluble BAFF, EBNA1 IgG and VCA IgG levels in serum were measured by ELISA. Statistical analyses used non-parametric tests. BAFF-var was present in 30% of PwMS (26% heterozygotes, 4% homozygotes) and 22% of HD (20% heterozygotes, 2% homozygotes), while 70% of PwMS and 78% of HD were Wild-Type (WT). As expected, BAFF-var carriers had higher serum BAFF levels ($p<0.001$). In addition, BAFF-var PwMS showed significantly increased expression of the B cell marker CD20 and key BAFF pathway genes (BAFF, BAFF-R, APRIL, TACI) in PBMC, indicating a broader effect on B cell regulation. PwMS had higher

levels of EBNA1 IgG ($p<0.001$) and VCA IgG ($p=0.03$) compared to HD. In PwMS, VCA IgG titers were higher in BAFF-var carriers than in WT ($p=0.02$), suggesting potential shift towards EBV reactivation. Both EBV DNA and RNA were more frequently detected in PwMS than in HD, with PwMS showing higher viral DNA load ($p=0.004$) and prevalence of transcripts associated with EBV latency disruption and lytic reactivation ($p<0.001$). While viral load did not differ between BAFF-var and WT PwMS, BAFF-var carriers had higher expression of EBV genes involved in latent (EBNA3A) and lytic (BZLF1) infection. These results support EBV dysregulation in MS and suggest that elevated BAFF levels associated with BAFF-var might promote EBV latency disruption/reactivation in PwMS. This study reinforces the idea that impaired control of EBV infection is related to MS and influenced by genetic risk factors, helping identify new disease biomarkers and more targeted therapies.

P SYNTHESIS AND ANTITUMORAL ACTIVITY OF PROMISING COPPER COMPLEXES BEARING MEMANTINE AND AMANTADINE

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Metal complexes, due to their chemical and biological diversities, have attracted increasing attention in recent years as pharmaceutical agents for cancer diagnosis and therapy. Copper is an essential element for cellular growth and development, and it is involved in three typical tumoral pathways: cell proliferation, stimulation of angiogenesis and metastasis. Copper-based complexes have demonstrated noteworthy antitumor and antimetastatic effects against various solid tumors, through several action mechanisms. In this context, memantine and amantadine have been conjugated to the bifunctional species bis(pyrazol-1-yl)acetic acid (LH), affording the novel ligands LMem and LAd, which have been subsequently used as coordinating agents for the synthesis of novel Cu(II) and Cu(I) complexes. In particular, for the synthesis of Cu(I) complexes to stabilize copper in the +1 oxidation state, triphenylphosphine (PPh3) and 1,3,5-triaza-7-phosphaadamantane (PTA) were used as lipophilic and hydrophilic co-ligands, respectively, which confer different solubility to the corresponding complexes. The antitumoral efficacy of the new Cu(I) and Cu(II) complexes has been evaluated on U87, LN18, U251 and T98 glioblastoma cell lines. To study the mechanism of cancer cell death induced by the synthesized copper complexes, high-resolution imaging techniques, such as Scanning Electron Microscopy (SEM) and Laser Scanning Confocal Microscopy (LSCM) and flow cytometric analysis have been employed to study morphological alterations, apoptosis induction, cell cycle progression, ROS production and GSH levels evaluation.

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P FROM MALARIA TO COVID: SOURCES FOR THE HISTORY OF THE ISTITUTO SUPERIORE DI SANITÀ

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The archive of the Istituto Superiore di Sanità (ISS), currently held at the Archivio Centrale dello Stato (ACS), contain the ISS's historical documentation and recount the research and monitoring activities in the field of public health that the ISS has carried out since its foundation in 1934, as well as a significant part of the history of social and health policies implemented in Italy. Since their transfer to the ACS, the documents have been studied and discussed at length, particularly with regard to the challenges and problems that the preservation of paper documents from a research institution such as the ISS can present in relation to scientific research and the history of public health. The idea of creating a guide to sources on the history of the ISS aims to provide the community of scholars and researchers with a new research tool that serves as a guide and a first step towards overcoming the critical issues caused by the fragmentation of storage locations and the documentation itself due to institutional and political changes which, as always, affect the production, preservation, and valorization of the documentary memory of institutions. Added to this fragmentation is the almost total absence of archival research tools to support research on the social and health policies implemented by the ISS. The guide, through a more or less detailed description of the archival collections, aims to provide an overview of the ISS's complex documentation and related archives. These include the archives of numerous researchers and personalities who have written the history of the ISS, such as Domenico Marotta and Giovanni Battista Marini Bettolo, whose papers are kept at the Accademia Nazionale delle Scienze detta dei XL, the archives of Mario Ageno, Edoardo Amaldi, and Giovanni Battista Grassi, preserved respectively at the Department of Physics and the Department of Biology and Biotechnology Charles Darwin at Sapienza University of Rome. A significant part of the research will also focus on some of the historical documentation preserved at the ISS, in particular the documentary collection of Maria Ester Alessandrini, chemist, researcher at the ISS Chemistry Laboratory, and lecturer in analytical chemistry at the University of Rome. The collection, which is currently being reorganized and described, will shed light on Alessandrini as a woman and a scientist, in relation to her work at the ISS and, more generally, to the world of scientific research in the 20th century.

P ROLE OF CD38 IN SHAPING INNATE IMMUNE RESPONSES TO RSV INFLUENZA VIRUS AND SARS-COV2 IN AIRWAY EPITHELIAL CELLS

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Introduction. CD38 is an ectoenzyme and receptor involved in multiple immune processes, acting as a major consumer of cellular NAD^+ and regulating intracellular calcium mobilization. Infections caused by Respiratory Syncytial Virus (RSV), Influenza Virus (IV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), are characterized by an exaggerated release of pro-inflammatory mediators which may contribute to disease complications. The aim of this study was to compare CD38 expression and activity in human airway epithelial cells infected with RSV, IV, and SARS-CoV-2.

Material and Methods. Human A549 airway epithelial cells were infected with RSV-A or IV (H1N1), while engineered A549 cells expressing the ACE2 receptor were infected with SARS-CoV-2 (EPI_ISL_412974). In selected experiments RSV-infected cells were treated with 5 μM of the specific CD38 inhibitor 78c. Whole-cell lysates and culture supernatants were collected 24 hours post-infection (p.i.).

Results. RSV and IV induced a significant upregulation of CD38, at both protein and mRNA levels, whereas no modulation was observed in SARS-CoV-2 infected cells at 24 and 48 hours p.i. Comparative analysis of antiviral and inflammatory responses revealed a delayed and overall reduced activation of selected mediators in SARS-CoV-2 infected cells compared with RSV and IV, including CCL5, IL-6, TNF-α, IFN-β1, and ISG15. The functional impact of CD38 inhibition was evaluated in RSV-infected cells. Treatment with the 78c inhibitor reduced CD38 protein and mRNA levels and markedly decreased the expression of antiviral genes (IFN-β, MX-1, and ISG15) and pro-inflammatory mediators (IL-6, CCL5), while IL-8 expression was unaffected. Consistent with gene expression data, the multiparametric ELISA analysis showed reduced secretion of INF-β and IL-6.

Discussion and Conclusions. Overall, these results indicate that CD38 expression parallels the induction of antiviral and pro-inflammatory responses in respiratory epithelial cells infected *in vitro* with RSV or IV. In contrast, SARS-CoV-2 infection was characterized by a lack of CD38 upregulation and reduced activation of antiviral and inflammatory pathways. Together with functional inhibition data, these findings support a role for CD38 in amplifying antiviral and inflammatory responses in airway epithelia. Moreover, reduced CD38 activity may contribute to delayed antiviral and inflammatory activation in during SARS-CoV-2 infection.

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P ELUCIDATING THE MOLECULAR BASIS OF INHERITED RETINAL DYSTROPHIES VIA WHOLE GENOME SEQUENCING

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Inherited Retinal Dystrophies (IRDs) represent a broad group of genetically and clinically heterogeneous disorders characterized by progressive retinal degeneration, ultimately leading to visual function loss. Due to their extensive genetic heterogeneity, elucidating the complex molecular mechanisms underlying these conditions remains challenging; therefore, genetic diagnosis is crucial for effective clinical management. The introduction of Next Generation Sequencing (NGS) has significantly improved the diagnostic yield for IRDs, but a high percentage of cases remain genetically unsolved.

This project aims to:

I. provide a molecular diagnosis using Clinical Exome Sequencing (CES) and Whole Genome Sequencing (WGS) for superior detection of structural variants, copy number variations and deep intronic mutations;

II. identify novel disease-associated genes.

To date, 76 patients have been enrolled following comprehensive clinical evaluation. CES analysis revealed six patients with a clear mismatch between their clinical phenotype and the genetic findings, including negative results, heterozygous variants in recessive genes, and Variants of Uncertain Significance (VUS). To further investigate these unresolved cases, the six selected patients will undergo WGS in trio with their parents through an external service, aiming to detect variants not identifiable with previous approaches and to provide a more accurate molecular interpretation of the observed clinical presentations. The WGS approach is expected to enhance the understanding of the genetic basis of IRDs and enhance the precision of genetic counselling and clinical management.

P SAMPLING AND ANALYSIS METHODS FOR EMERGING CONTAMINANTS IN AQUEOUS MATRICES

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Bisphenol A (BPA) is a synthetic chemical compound that is widely used in numerous industrial applications, particularly in the production of epoxy resins and polycarbonates. Its pervasive presence in the environment give raise to significant concerns for public health and marine ecosystems, as BPA acts as an endocrine disruptor with documented effects on development, reproduction, and the nervous system. Further toxicological studies have indicated the potential for teratogenic and carcinogenic actions. While a substantial body of data exist on the presence of BPA in freshwater surface waters, information on its occurrence in offshore marine waters remains limited and fragmentary. The study proposes innovative methods for analyzing BPA using spectroscopic sensors operating in the near-infrared transmittance range. The sample analysis with this approach is characterized by its simplicity, speed, and non-destructive nature. Furthermore, the small size and robustness of the instrument enable in situ use, even under harsh conditions. A MicroNIR spectroscopic sensor with a PAT-L probe was employed. The probe operates within a wavelength of 900 to 1700 nanometers in transmittance mode. It is equipped with a thermostatically controlled cell maintained at 40°C, with a flow rate of 2 milliliters per second and an optical path length of 3 millimeters. Experiments followed a progressive design: starting with standard solutions in distilled water acquired under both static and dynamic conditions, then advancing to samples prepared in artificial seawater (prepared by adding 35 g/L of salts to distilled water and sonicating for over 30 minutes), also in static and dynamic modes to mimic real operational scenarios. The acquired spectral data were subsequently analyzed using chemometric models. Exploratory Principal Component Analysis (PCA) assessed sample distribution in multivariate space and identified outliers. A Partial Least Squares-Discriminant Analysis (PLS-DA) classification model discriminated BPA presence/absence in the matrix. Concurrently, a Partial Least Squares Regression (PLS) quantification model accurately estimated analyte concentrations. The models were subjected to rigorous evaluation using various figures of merit including slope, R^2, RMSE, accuracy, precision, LOD, and LOQ across the calibration, validation, and prediction phases. This process yielded satisfactory results. In a subsequent phase, a method was developed for the analysis of BPA in seawater using a MicroNIR analyzer in transflectance mode, the PAT-U. An analogous experimental plan was applied, with acquisitions initially in distilled water and subsequently in artificial seawater. The chemometric processing reflected the prior strategy (PCA, PLS-DA, PLS), enabling a direct comparison of the model performances between the two analyzers to evaluate their suitability for in situ deployment. Finally, to complete and validate the methodological framework, a GC-MS-based method was optimized for the determination of BPA in aqueous matrices.

P IMPLEMENTATION OF AN ADVANCED *IN VITRO* GIARDIA DUODENALIS/PIG ODM CO-CULTURE SYSTEM TO EVALUATE ANTIGIARDIAL DRUG(S) EFFICACY AND SAFETY

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Giardiasis is a One Health enteric disease caused by the protozoan Giardia duodenalis, affecting a wide range of mammalian hosts, including humans. Transmission occurs via the faecal-oral route through ingestion of cysts, the environmentally resistant stage of the parasite. The infection may be asymptomatic or present with acute symptoms such as watery diarrhoea, abdominal pain, weight loss and in some cases, it can become chronic. In livestock, infection is linked to reduced feed efficiency, impaired weight gain, and intestinal dysfunction, resulting in significant economic losses. Currently, chemotherapeutic treatments for giardiasis in veterinary medicine rely almost entirely on the same drug classes used for human infections, including benzimidazoles derivates (e.g., albendazole) and nitroimidazoles (e.g. metronidazole). This increases the risk of emergence and spread of drug-resistant isolates. Moreover, significant limitations exist in the available experimental models. In this study, we evaluated the suitability of porcine intestinal Organoid-Derived Monolayers (ODMs) as a physiologically relevant *in vitro* model for giardiasis. Using this system, we assessed the efficacy of selected antigiardial compounds and the impact of different G. duodenalis isolates on the epithelial barrier. A boron-based compound showed a strong inhibitory effect on trophozoite proliferation without compromising epithelial viability. Furthermore, infection with different isolates resulted in variable levels of barrier disruption, indicating strain-specific pathogenic effects. Overall, our findings support the pig ODM model as a robust, animal-free platform for studying host-parasite interactions and for the screening of novel, selective antigiardial strategies.

P DIAGNOSTICS AND EPIDEMIOLOGICAL INVESTIGATIONS OF EMERGING ANIMAL PRION DISEASES IN TUNISIA

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Prion diseases, or Transmissible Spongiform Encephalopathies (TSEs), are fatal neurodegenerative diseases that affect both humans such as Creutzfeldt Jacob disease (CJD), Fatal Familial Insomnia (FFI), and Gerstmann-Sträussler-Scheinker syndrome (GSS) and animals, including scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and Chronic Wasting Disease (CWD) in cervids. The etiological agent is PrP^{Sc}, an infectious misfolded isoform of the normal cellular Prion Protein (PrP^{C}). The recent identification of Classical Scrapie (CS) in sheep in Tunisia and in Libya, as well as a new prion disease affecting dromedary camels (CPrD) in Algeria and Tunisia, that has never been described before 2018, has raised significant concerns about the distribution and potential spread of these diseases in North Africa. Within the framework of the "Italy-Africa Doctorates" program, a research project has been launched to investigate the transmission dynamics and ecological impact of animal prion diseases in Tunisia. The study is a pilot focusing on the epidemiological characterization of CS and CPrD, to support the development of future surveillance programme. This includes general demographic analysis of Small Ruminants (SR) and dromedary camels in Tunisia, case detection, biochemical and histopathological characterization, determination of PrP genotype, and investigation of prion distribution in dromedaries affected by CPrD. Field investigations were conducted to collect data, with a particular focus on putative risk factors linked to farming practices and environmental exposure. Ongoing work includes active sampling of high-risk groups. Collected specimens are being investigated through genetic, neuropathological, and biochemical analyses, including molecular PrP^{Sc} typing. This study aims to generate new scientific data on the prevalence, pathological features, and risk factors associated with CS and CPrD in Tunisia. The findings will support the establishment of targeted surveillance strategies and inform public and animal health policies at both national and regional levels.

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P PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA IN WASTEWATER TREATMENT PLANTS

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Antibiotic Resistance (AR) is a growing global concern involving the three pillars of the One Health approach: human, animal, and environmental health. Investigating AR across these domains is crucial to understanding its dissemination and developing effective containment strategies. In the environmental component, wastewater act as both a reservoir and a hotspot for Antibiotic-Resistant Bacteria (ARB), contributing to their persistence and the horizontal transfer of resistance genes. Consequently, the effective removal of ARB in Wastewater Treatment Plants (WWTPs) is critical to minimizing their release into the environment. This aligns with the objectives of the Italian National Action Plan on Antimicrobial Resistance (PNCAR 2022-2025), which promotes integrated One Health surveillance and environmental monitoring of antimicrobial resistance. This study assessed the presence and abundance of clinically relevant antibiotic-resistant bacteria in raw influent and treated effluent from wastewater treatment plants, to investigate the potential environmental release of resistant strains and the role of wastewater treatment in their persistence. The study specifically focused on extended-spectrum β-lactamase–producing *Escherichia coli* (ESBL-Ec), Carbapenem-Resistant *Escherichia coli* (CREC), and Vancomycin-Resistant Enterococci (VRE). Influent and effluent samples were collected from six WWTPs across Italy, with seasonal sampling conducted in 2024 and 2025 (n = 40–76, depending on the parameter). Bacteria were isolated by filtration and culture on selective media: Tryptone Bile X-GLUC (TBX) agar supplemented with cefotaxime (4 µg/mL) or meropenem (4 µg/mL) for ESBL-Ec and CREC, respectively, and Slanetz & Bartley agar supplemented with vancomycin (4 µg/mL) for VRE, with confirmation on Esculin Iron Agar. Typical colonies were enumerated and a subset was identified by MALDI-TOF MS (Bruker MALDI Biotyper® Sirius One); phenotypic resistance was confirmed according to EUCAST guidelines. All investigated ARB were detected in both influent and effluent samples across all WWTPs. Mean influent concentrations were ~ 3-5x10^4 CFU/100 mL for ESBL-Ec, CREC, and VRE. Treatment resulted in 2-3 $\log_{10}$ reductions for ESBL-Ec, 1-4 $\log_{10}$ for CREC, and 1-3 $\log_{10}$ for VRE, despite the persistence of resistant bacteria in effluents. Resistant populations were also quantified relative to the total *Escherichia coli* and enterococci populations, including both resistant and susceptible cells. Although wastewater treatment resulted in an overall reduction of antibiotic-resistant bacteria, removal was incomplete, leading to the release of resistant microorganisms into the environment. These findings highlight the importance of ongoing monitoring and the need for further investigation into wastewater treatment processes to limit the environmental dissemination of antimicrobial resistance.

P INTRACELLULAR PATHWAYS INVOLVED IN EXOSOME BIOGENESIS: A STUDY OF THEIR ROLES AND MECHANISMS

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Exosomes are small Extracellular Vesicles (sEV) formed within late endocytic compartments/Multivesicular Bodies (MVB) by invagination of the limiting membrane into the lumen. Various molecular machineries have been implicated in the regulation of exosome biogenesis, including the ESCRT machinery, the syntenin–alix pathway, tetraspanins and lipids, such as ceramide. However, several aspects of these regulatory processes remain incompletely understood. In our laboratory we developed a methodology to obtain fluorescent exosomes (Bodipy exo) of endosomal origin by using Bodipy FL C16, a fluorescent palmitic acid. Upon internalization by cells, C16 is converted into phospholipids that are incorporated into the bilayer of secreted vesicles. Bodipy exo can be precisely quantified by Flow Cytometry (FC) and further characterized. To gain insight into the different mechanisms participating in exosome biogenesis, we combined this labeling technique with the use of a panel of known inhibitors of intracellular pathways, investigating the release of fluorescent exosomes and analyzing their protein profile. Significant differences in Bodipy exo secretion were observed by flow cytometry following treatment of melanoma cells with selected intracellular pathway inhibitors. Compounds targeting cholesterol and Lysobisphosphatidic Acid (LBPA) metabolism, ceramide synthesis, endolysosomal acidification, and intracellular trafficking were evaluated. The strongest increase in fluorescent exosome release was seen with U18666A in contrast with Thioperamide, suggesting that cholesterol, rather than LBPA, is the main driver of this effect. Ceramide metabolism inhibitors GW4869 and Desipramine, which target neutral and acid sphingomyelinases (SMase), respectively, showed opposite effects, highlighting distinct roles for SMase isoforms in exosome regulation. Notably, GW4869 selectively reduced Bodipy exo secretion without affecting total sEV release. Inhibitors of endolysosomal acidification had variable outcomes: Bafilomycin A1, a V-ATPase inhibitor, increased Bodipy exo secretion, whereas Monensin, acting via a different mechanism, had no effect, supporting a specific role for V-ATPase in exosome biogenesis. Across most conditions, the percentage of Bodipy exo positive for tetraspanins (CD63, CD81, CD9) remained stable, suggesting minimal effect on tetraspanin loading. However, Western blot analysis revealed marked differences in sEV protein composition, even when secretion levels were unchanged, indicating that inhibitors can selectively affect cargo sorting. Finally, TEM analysis showed notable variations in the number of Multivesicular Bodies (MVBs) and Intraluminal Vesicles (ILVs), reflecting structural changes linked to the modulation of intracellular pathways involved in exosome biogenesis. In summary, the use of selected inhibitors targeting intracellular pathways affects not only the secretion of sEV, but also their protein

composition. Our results, in addition to the known effects of cholesterol and ceramide, pinpoint the involvement of V-ATPase that is not associated with an increase in intracellular pH; rather, we hypothesize that it functions as a regulator of the intracellular sorting of extracellular vesicles. This suggests that our experimental approach provides valuable insights into the molecular mechanisms underlying exosome formation and offers a foundation for further exploration of the regulatory pathways involved.

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P GOLD NANORODS LOADED WITH RADIOPHARMACEUTICALS FOR THE DEVELOPMENT OF INNOVATIVE THERANOSTIC TECHINQUES IN NUCLEAR MEDICINE

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This project focuses on the development of innovative theranostic systems based on Gold Nanorods (AuNRs) for targeted applications in nuclear medicine. AuNRs are investigated as advanced drug delivery systems due to their unique physicochemical properties, such as a high surface-area-to-volume ratio and the ease of surface functionalization with several capping agents. These features enable the simultaneous conjugation of fluorescent dyes and specific radiopharmaceuticals, creating a multifunctional platform for molecular imaging and therapy. The theoretical rationale relies on exploiting the high atomic number of gold and the energy emitted during the radioactive decay of the conjugated isotopes. The primary objective is to direct the emission of Auger electrons low-energy electrons with a nanometric biological range, directly into the cell nucleus. By releasing this energy in close proximity to the genome, the system aims to induce direct DNA damage in malignant cells, maximizing localized therapeutic efficacy while minimizing collateral damage to healthy tissues. A cornerstone of this research involves the application of Nuclear Magnetic Resonance (NMR) spectroscopy to monitor the biological response to the treatment. NMR is employed to evaluate the effects of both nanoparticles and radiation on cells by analyzing variations in the intensity of metabolite signals, allowing for the precise mapping of induced biochemical alterations. The distinct advantages of NMR in this field lie in its intrinsically quantitative, non-destructive, and highly reproducible nature, which provides a comprehensive spectral "fingerprint" of the cellular state with minimal sample preparation. Unlike other analytical techniques, NMR facilitates an untargeted analysis capable of delivering detailed structural information and tracking dynamic changes in metabolic pathways in an unbiased manner. This integrated approach not only validates the effectiveness of the theranostic system but also offers profound insights into the biocompatibility and metabolic impact of the therapy at the molecular level.

P EVALUATION OF SUPRAS AS AN ALTERNATIVE TO SPE FOR URINARY EXPOSOME PROFILING

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Comprehensive characterization of the human exposome by High-Resolution Mass Spectrometry (HRMS) remains strongly constrained by sample preparation, which can limit chemical space coverage. Supramolecular Solvents (SUPRAS) have emerged as a green-chemistry option with potential for broad-spectrum extraction, yet their suitability for multi-class exposure profiling in human urine has not been systematically assessed within integrated non-target and suspect screening workflows. Here, we present the development and optimization of a SUPRAS-based urinary extraction approach designed to maximize chemical coverage while maintaining low solvent consumption. A tetrahydrofuran/1-hexanol mixture applied to diluted urine provided robust performance compatible with HILIC/PR liquid chromatography coupled to HRMS. Method performance was benchmarked against a conventional Solid-Phase Extraction (SPE) workflow using a panel of isotopically labeled reference standards and a large set of annotated chemicals at confidence levels 1 and 2. Although SPE generally showed higher extraction recoveries for labeled standards, SUPRAS achieved comparable overall chemical coverage and outperformed SPE for more lipophilic compounds retained on the RP column. In contrast, SUPRAS showed reduced suitability for highly polar compounds. To support structure-based extraction selection, an ordinal machine-learning model was developed using molecular descriptors to predict the preferred extraction method. In repeated stratified cross-validation, the model showed moderate overall performance, with its strongest predictive power observed for the predominant SPE-favored class. Overall, this study demonstrates that SUPRAS provides an efficient, low-solvent, chemically broad sample-preparation alternative and highlights the potential of machine-learning tools to guide extraction-method selection.

P STATISTICAL PERSPECTIVES IN MEDICAL DIAGNOSTICS: CHALLENGES AND OPPORTUNITIES

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Medical research increasingly relies on empirical evidence to inform clinical practice, thereby posing persistent methodological challenges. Clinical datasets are often complex and heterogeneous, and they are also commonly incomplete; furthermore, patient responses may vary substantially, and covariates may modify both treatment effects and diagnostic performance; finally, models intended for clinical deployment require validation across diverse and non-standard settings, which demands methods tailored to the underlying data and study structures. This work addresses these issues through three interconnected projects, each focusing on a distinct aspect of clinical research. The first study is part of the Horizon project: "BIOmarker based diagnostic TOOLkit to personalize pharmacological approaches in congestive heart failure" (BIOTOOL-CHF). A cluster analysis is performed to identify subgroups of patients with distinct congestion profiles and expected responses. A central obstacle is substantial missingness in biomarker measurements, which motivates the use and assessment of clustering approaches that jointly handle incomplete data. The second 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 fraction, i.e., 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, given the remaining two. This provides a way to quantify how classification accuracy changes across patient characteristics, allows for the evaluation of the significance of such covariates on the fixed thresholds and provides a framework to clinically assess which thresholds may need to be adjusted based on the existing covariates. Theoretical properties of the proposed inferential procedures are investigated. The third project introduces a new separation index for settings involving multiple disease classes with non-standard ordering structures. The index is proposed as a complement to more classic tools based on the ROC analysis within the framework of tree or umbrella ordering, and it is associated with a classification rule. Related statistical inference is discussed, and simple estimation and interval estimation procedures are derived, both in a parametric and a non-parametric setting. Statistical properties of the estimators are studied theoretically and through simulations. Collectively, these contributions provide methodological insights for the analysis of medical data under common complexities encountered in clinical research, with implications for the evaluation of diagnostic and prognostic tools.

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P WEARABLE TECHNOLOGIES FOR MONITORING THE CARDIOVASCULAR SYSTEM IN EXTREME ENVIRONMENTS

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Extreme environments present significant challenges to the monitoring of the cardiovascular system, due to the combined effects of altered physical conditions and physiological adaptations. Hyperbaric exposure, such as that experienced during professional and recreational diving, induces marked cardiovascular responses, including bradycardia and autonomic nervous system modulation, while simultaneously imposing technical constraints on wearable monitoring devices. The aim of the PhD research project is to develop wearable technologies for cardiovascular monitoring in extreme environments, with a primary focus on hyperbaric conditions. One part of this work addresses the physiological aspects of cardiovascular regulation in hyperbaric environments, with particular emphasis on autonomic nervous system responses assessed through Heart Rate (HR) and Heart Rate Variability (HRV). We designed, validated and implemented a series of experimental protocols to investigate the effects of pressure, depth, body position, breathing pattern, and environmental conditions on cardiovascular variables. Experiments were conducted both in a dry hyperbaric chamber and during real dives in warm water, allowing the isolation of pressure-related effects from thermal stress and immersion-related factors. The results consistently showed a pressure-dependent bradycardia and a modulation of autonomic balance, with a reduction in the LH/HF ratio during compression and a distinct temporal behavior of HR and HRV during decompression. Additional experiments investigating postural changes in water suggested that body position does not induce the same autonomic shifts observed in normobaric conditions. Furthermore, the marked reduction in respiratory rate during diving emerged as a critical limitation for frequency-domain HRV analysis. Another topic of the PhD research activities focuses on technological aspects related to wearable monitoring in hyperbaric conditions. The performance and reliability of wearable ECG systems were evaluated, identifying critical issues related to sensor sealing, signal quality, and usability underwater. In addition, a wearable system for Non-Invasive Blood Pressure (NIBP) monitoring was designed and tested in a hyperbaric chamber to assess its functionality and accuracy across different pressure levels. The device demonstrated stable operation and consistent pressure measurements throughout compression and decompression phases, conforming its suitability for integration into wearable monitoring system intended for hyperbaric applications. Overall, this work contributed to 1) develop devices for cardiovascular monitoring in extreme environment; 2) provide experimental data for a better understanding of cardiovascular regulation in hyperbaric environments and

methodological and technological insights for the development of wearable systems capable of reliable operation under extreme conditions. These findings support wearable cardiovascular monitoring for safety assessment, risk mitigation, and physiological research in hyperbaric environments.

P MOLECULAR INVESTIGATION OF MALARIA TRANSMISSION DYNAMICS IN FIELD-COLLECTED ANOPHELES COLUZZII MOSQUITOES

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Malaria remains the world deadliest parasitic disease, causing about 263 million cases and nearly 600,000 deaths in 2023, mainly among African children. Transmission relies on complex interactions between the human host, *Anopheles* vectors, and *Plasmodium* parasites. A critical determinant is the density of gametocytes in peripheral blood, which correlates with the load of the subsequent diploid stage, the ookinete, developing in the mosquito midgut within 24 hours after an infectious blood meal. Extensive evidence demonstrates the role of malaria in shaping human genetic adaptations; however, the impact of the human genetic background on parasite transmissibility from humans to mosquitoes remains poorly understood. Several studies have shown that carriers of hemoglobin variants, such as Hemoglobin C (HbC) and Hemoglobin S (HbS), are less susceptible to severe malaria compared to individuals with the wild-type genotype. These variants have also been associated with increased gametocyte carriage, which potentially enhances malaria transmission. Similarly, Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency has been linked to altered susceptibility to severe malaria and to higher levels of parasite sexual stages. In this study, we focus on malaria transmission by conducting molecular analyses of semi-gravid mosquitoes, collected in the first 24 hours post blood feeding in an endemic area of Burkina Faso. Nucleic acids are extracted from single mosquitoes to simultaneously investigate parasite infection, human blood meal composition, and mosquito-related factors that influence transmission dynamics. Specifically, our objectives are: (i) the identification and quantification of *Plasmodium falciparum* ookinete stages in field-collected *Anopheles* mosquitoes fed on human hosts throughout molecular amplification of ookinete markers; (ii) the detection of human features within the mosquito blood meal, including host sex and the presence of genetic variants prevalent in sub-Saharan Africa, such as HbC, HbS, and G6PD deficiency (e.g., G6PDA-) throughout specific Real Time PCR assays; and (iii) the correlation of these human features with parasite ookinete load in mosquitoes, to assess their contribution to malaria transmission. Overall, this work aims to develop and apply sensitive molecular tools based on the analysis of single infected mosquitoes to elucidate the relationships between human host conditions and *P. falciparum* transmission. By improving our understanding of how human genetic factors influence parasite development within the mosquito, this study may contribute to a more comprehensive view of malaria transmission biology and support the development of targeted control strategies.

P DEVELOPMENT OF COMPUTER VISION APPROACHES TO CHARACTERIZE ECOLOGY AND BEHAVIOR OF MOSQUITO VECTORS IN ITALY

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The traditional methods of mosquito surveillance require a large labor input and logistical burden constraining the capacity to perform appropriate and uniform entomological surveillance. Advances in computer vision and deep learning provide potential new solutions to this challenge; these methods are a powerful approach for distinguishing classes of images and there is a growing interest in applying it to characterise species and delimit their distribution, particularly in the identification of mosquito vectors. My PhD research project aims to develop an AI approach, based on computer vision technology and deep learning network, to perform in real time the classification of adult mosquitoes from field captures at the genus and species levels and to perform proof-of-principle applications to address questions on mosquito ecology. The goal of the project, in the frame of the «MOSquito Artificial Intelligence COntrol» (MOSAICO) project of the Istituto Superiore di Sanità (ISS), is to produce and validate a portable automatic mosquito-detection device for local health authorities/research institutes able to capture, analyse and communicate to an interactive database multiobject images of trapped mosquitoes (82 specimens per image), to improve, speed up and harmonize mosquito surveillance activities nationwide. The present prototype consists of two distinct components: 1) a “local part” or “image system” (MOSAICO device and the associated app) that acquires high-resolution multi-object images and 2) a “remote part” or “recognition system” (ISS server with deep-learning model) which is responsible for processing multi-object images and generating predictions of classification. The results obtained in the initial two years of doctoral, have generally confirmed the correctness of the AI model prediction for the seven “target” species (Aedes albopictus, Culex pipiens, Anopheles maculipennis s.l., Aedes caspius, Aedes vexans, Aedes koreicus, Aedes aegypti) on which the algoritmh was trained, providing a solid base for use of the device in the field. Consequently, a workshop (8-9 May 2025) was held at ISS with partners of the MOSAICO network (18 collaborators in local health authorities/research institutes from 16 regions) to practice the use of the device prototype (hardware and software) before validating the device in field tests across multiple sites nationwide. During the entomological survey in the summer 2025 (from june to october), the network partners were provided with copies of the prototype, which they utilised to assess its capacity, accuracy, and efficiency in classifying mosquitoes on specimens captured in the field. To date, a total of 24 multi-object images (1.452 mosquito

specimens) have been uploaded and processed; 92% of mosquitoes were correctly classified, 5% were not classified (due to being highly damaged or not being included in the training set) and only 3% were incorrectly classified. The findings of this study are encouraging and pave the way for the exploration of novel application scenarios for the MOSAICO prototype: 1) routine national surveillance (e.g. the WNV network) to reduce the workload of entomologists and/or non-expert personnel, 2) surveillance at points of entry (airports, ports and geographical borders) to issue a warning regarding the presence of invasive species and 3) surveillance in regions and areas where no data is yet available.

P METHODOLOGICAL OPTIMIZATION FOR MICROPLASTIC ANALYSIS IN SEAWATER: APPLICATION AND PRELIMINARY RESULTS FROM THE SEA CARE PROJECT

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Plastic is one of the most widely used materials due to its favorable properties, including low weight, high durability, corrosion resistance, and relatively low production costs. These characteristics have promoted its use across various sectors, such as packaging, construction, industry, the medical sector, and consumer goods. Despite the economic and industrial disruptions caused by the COVID-19 pandemic, global plastic production did not experience a significant decline; however, shifts in production and application patterns were observed, reflecting changes in industrial demand. Microplastic (MP) pollution, defined as plastic particles with diameters smaller than 5 mm, represents an increasing environmental and public health concern. MPs are ubiquitous in marine ecosystems and are ingested by a wide range of organisms, from plankton to fish and invertebrates. Once incorporated into the food web, MPs may be transferred along trophic chains through bioaccumulation and biomagnification processes, potentially affecting human health. The analysis of microplastics in seawater is particularly challenging due to the high concentrations of organic matter and inorganic salts, which can interfere with the identification and quantification of MP particles. A major analytical challenge lies in the removal of organic matter without compromising the integrity of target polymers, as overly aggressive digestion procedures may lead to polymer degradation and biased results. To date, no standardized protocol exists for the pretreatment of seawater samples, and a wide range of solvents, digestion methods, and analytical conditions are currently employed in MP research. In this study, preliminary experiments were conducted to identify optimal solvents and analytical conditions capable of minimizing matrix-related interferences while preserving microplastic integrity, with the aim of unifying pretreatment methodologies. Various solvents were evaluated in combination with separation techniques, including filtration and density-based separation, and recovery tests were performed to assess method efficiency. MPs were quantified using fluorescence microscopy and Raman spectroscopy. The optimized method was subsequently applied to real samples collected within the framework of the Sea Care project, a collaborative research initiative involving multiple scientific institutions, including the Italian National Institute of Health, in cooperation with the Italian Navy. Seawater samples were collected from different oceanic regions worldwide. Preliminary results from sampling campaigns conducted between 2022 and 2023 indicate that the most frequently detected polymers were commonly used materials, namely LLDPE (41%), PE (30%), and PP (15%), primarily employed in packaging and consumer products, confirming their widespread prevalence in the marine environment.

P ANALYSIS OF THE IMMUNOMODULATORY PROFILE OF THE HIV TAT PROTEIN

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T cells are functionally compromised during HIV infection, exhibiting increased activation and proliferation. While T cell hyperactivation is among the strongest predictors of disease progression, the mechanisms driving it remain poorly understood. The HIV Tat protein plays a crucial role in viral replication and spread. Furthermore, Tat is released extracellularly and taken up by neighbouring cells, where it can modulate their function. Given that the presence or induction of anti-Tat immune responses is associated with reduced T cell dysfunction and preservation of $CD4^+$ T cells, we explored whether Tat affects both resting and activated human $CD4^+$ and $CD8^+$ T cells. We analysed peripheral blood samples collected from healthy donors from different age groups, including young donors under 40 years of age and elderly donors over 65. We examined the effect of the Tat protein on $CD4^+$ and $CD8^+$ T cell activation, both under resting conditions and following activation mediated by anti-CD3 engagement of the TCR as well as antigen-specific stimulation through in an *in vitro* priming process of $CD8^+$ T lymphocytes directed toward an unrelated MHC-I presented epitope. $CD4^+$ and $CD8^+$ T cells activated in the presence of the Tat protein showed increased cytokine release, suggesting that Tat can contribute to T cell hyperactivation. We further observed that this immunomodulatory effect is also evident in an *in vitro* priming process of $CD8^+$ T lymphocytes directed toward an unrelated MHC class I-presented epitope, in which administration of the Tat protein leads to increased antigen-specific $CD8^+$ clonal expansion. By contrast, when cells are in a resting state, the HIV Tat protein exhibits an opposite immunomodulatory activity, promoting the maintenance of cellular quiescence. Moreover, for some of these functions, the relevant Tat domains have been mapped. This dual immunomodulatory capacity of the Tat protein suggests multiple roles in HIV immunopathogenesis, indicating a potential contribution both to the lymphocyte hyperactivation characteristic of HIV infection and to the establishment of viral latency and reservoirs. This could provide new insights for developing therapeutic approaches aimed at reducing both the immune activation typical of people living with HIV (PLWH) and the formation of reservoirs containing latent virus, which is the main reason HIV cannot be fully eradicated by current antiretroviral therapies targeting only actively replicating virus.

P IMPACT OF GENDER ON THE FIVE-YEAR OUTCOMES AFTER SURGICAL VERSUS TRANSCATHETER AORTIC VALVE REPLACEMENT WITH NEW GENERATION DEVICES FROM THE PROSPECTIVE OBSERVANT STUDIES

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The Propensity Score (PS) has been used to reduce bias in observational studies. When treatment assignment is strongly ignorable, methods that adjust for the PS can yield unbiased estimates of treatment effects. The objective of the present study is to evaluate the impact of gender on the Five-year outcomes after surgical versus transcatheter aortic valve replacement with new generation devices from the prospective OBSERVANT studies. The cohorts displayed several differences in baseline characteristics. To address this imbalance and reduce treatment-selection bias, stabilized inverse probability of treatment weighting (SW) based on the PS was applied. After weighting, covariate balance was assessed using standardized mean differences (<0.10). Weighted Cox proportional hazards models with robust variance estimators were used. To evaluate whether the effect of treatment differed by sex, an interaction term between treatment and sex was included in the weighted Cox model. Additionally, sex-stratified weighted Cox model was fitted to provide sex-specific hazard ratios. The original dataset included 7,676 patients (69.7% SAVR, 30.3% TAVR). After considering inclusion criteria, 5,747 patients were analyzed (64.0% SAVR, 36.0% TAVR), including 57.1% women and 42.9% men in the TAVR group. At 5 years, TAVR was associated with higher mortality compared with SAVR in men (HR_{adj}1.57, 95% CI 1.22–2.00; p <0.001) but not in women (HR_{adj} 1.13, 95% CI 0.91–1.40; p=0.27), with a significant sex–treatment interaction (p=0.04). TAVR was associated with higher risks of major adverse cardiac and cerebrovascular events (MACCE), heart failure, cardiac causes, and pacemaker implantation in both sexes, with a significant sex–treatment interaction for MACCE only. Valve-related events were rare and similar between treatments, while reintervention were less frequent after TAVR in women. Finally, TAVR was associated with worse 5-year outcomes compared with SAVR, particularly in men. Men undergoing TAVR experienced higher long-term mortality. These findings highlight important sex-specific differences in long-term outcomes and underscore the need to consider sex in treatment selection and risk stratification.

P EXTRA-VIRGIN OLIVE OIL POLYPHENOLS EXTRACTED BY A "GREEN CHEMISTRY" APPROACH HOLD PROMISING ANTITUMOR EFFECTS AGAINST COLORECTAL CANCER

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Despite advances in early detection and treatment, Colorectal Cancer (CRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide, highlighting the urgent need for novel preventive and therapeutic strategies. Emerging evidence underscores the potent biological effects of Extra-Virgin Olive Oil (EVOO) polyphenols, including their anti-inflammatory and antitumor properties (ref.). Recently, an eco-compatible extraction method based on Natural Deep Eutectic Solvents (NaDES) has been developed to isolate these bioactive compounds from EVOO (Poly-NaDES). The present study aims to investigate the antitumor effects of Poly-NaDES and the underlying mechanisms *in vitro* and in a highly aggressive murine CRC model, in which the Escherichia coli toxin CNF1 is added to the AOM/DSS carcinogenesis protocol to accelerate tumor development. *In vitro*, we demonstrated that Poly-NaDES significantly reduce CNF1-induced reactive oxygen species production in intestinal epithelial cells (IEC-6) and immune cells (THP1) and, consequently, attenuate CNF1-induced phosphorylation of histone H2AX in IEC-6 cells, a marker of the DNA damage response. We further showed that Poly-NaDES exert a marked anti-inflammatory activity by inhibiting CNF1-induced NF-κB nuclear translocation in IEC-6 cells and by reducing the release of pro-inflammatory cytokines in both epithelial and immune cells. *In vivo*, daily supplementation with Poly-NaDES for 60 days reduced inflammatory infiltrates in colonic tissue and delayed tumor development. Fecal microbiota analysis revealed that daily Poly-NaDES intake increased microbial diversity. Moreover, micronucleus

analysis in bone marrow cells demonstrated a protective effect of polyphenols against CNF1-induced genotoxic damage. In conclusion, Poly-NaDES appear to counteract CNF1-driven tumorigenesis through combined antioxidant and anti-inflammatory effects, thereby preserving intestinal homeostasis.

POLYSEMY OF CRISES IN THE COMPLEXITY OF MODERN SOCIETIES: A QUANTITATIVE ANALYSIS FOR MONITORING AND PREDICTING THE WELL-BEING AND HEALTH OF TERRITORIES

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This dissertation is grounded in the observation that the contemporary socio-economic context is characterized by strong interconnections among the various domains that constitute a modern society. Such interconnections play an active and decisive role, particularly in situations of social and economic crises, and significantly affect the well-being and wealth of a territory. The main objective of this thesis is to analyze, through stochastic methods, the temporal evolution of indicators associated with specific domains of population well-being, using as an informational basis the official data provided by the BES (Equitable and Sustainable Well-Being) and BesT (Equitable and Sustainable Well-Being of Territories) frameworks. The analysis aims to construct forecasts of the considered indicators that are as accurate as possible, in order to support the design and implementation of public policies consistent with the emerging predictive framework. In particular, the study focuses on the Economic Well-Being domain at the national level and on the Health domain with reference to the territory of Taranto. A central and innovative contribution of this work lies in the interpretation of the BES and BesT domains as latent variables that can be traced back to the concept of the correct data-generating process underlying cointegrated stochastic processes. The thesis argues that the indicators belonging to each domain are generated by a common latent process, represented by the domain itself, which induces a high probability of structural cointegration among the observed time series. This perspective establishes a one-to-one correspondence between the conceptual domain and the underlying data-generating process, thereby strengthening the theoretical foundation for the use of cointegration in this context. Alongside cointegrated models, artificial neural networks are employed in an ancillary manner to enhance the overall predictive performance of the framework. Initial empirical results are coherent and sufficiently robust to support the proposed theoretical hypothesis. Moreover, a statistical methodology is developed to simulate increased temporal granularity of the data while preserving their underlying dynamics and reproducing a level of information quality that is realistically attainable in practice. This approach leads to a further improvement in the effectiveness of cointegrationbased models. Finally, the methodological framework is integrated with René Thom's Catastrophe Theory, with the aim of providing an interpretative perspective on the crucial role of cointegration in the predictability of complex phenomena. The conceptual structure identified within the BES and BesT domains is considered extendable to other phenomena characterized by similar latent properties, suggesting a broader applicability of the proposed approach and its predictive potential.

P MEASURING COMMUNITY DEPRIVATION AND FRAGILITY: KEY DIMENSIONS AND INSIGHTS FROM A NARRATIVE REVIEW OF INDICATORS AT AREA LEVEL

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This narrative review explores conceptual and methodological frameworks for measuring community-level fragility and deprivation. It pursues two main objectives: first, to analyze existing indicators used in area-level epidemiological monitoring and Health Impact Assessments (HIAs) of local interventions through a detailed examination of their construction; second, to establish a conceptual and methodological foundation for developing an indicator tailored to the Italian context. Fragility is increasingly understood as a multidimensional latent construct emerging from the interaction between exposure to risks and limited capacities to prevent, cope with, or recover from them. Closely linked is deprivation, defined as a measurable state of socioeconomic and contextual disadvantage experienced by individuals and communities. As highlighted in multiple studies, contextual deprivation can influence health outcomes independently of individual-level socioeconomic status. The review integrates a narrative synthesis of scientific literature with insights from qualitative interviews conducted with experts in environmental epidemiology, sociology, statistics and public health. This dual approach enabled the identification of three domains: individual socioeconomic conditions, social context and environmental/territorial features. These domains encompass dimensions such as employment, education, housing quality, social capital, access to essential services, availability of green and blue spaces and exposure to environmental hazards, aligning with contemporary frameworks on social and environmental determinants of health. Seven European deprivation or fragility indices were examined by analyzing their domains, variable selection criteria, statistical synthesis methods, data sources and territorial granularity. Methodologically, the indices employ techniques ranging from Principal Component Analysis (PCA), widely used to synthesize correlated socioeconomic variables, to aggregative approaches based on the standardized combination of basic indicators, such as those used in the Italian Deprivation Index. The English Index of Multiple Deprivation (IMD) stands out for its multi-domain structure and weighting strategy based on empirical evidence and expert validation, while the European Deprivation Index (EDI) adopts a hybrid approach combining EU-SILC microdata with census-level variables. The analysis reveals a strong convergence in the domains and basic variables used, suggesting broad agreement on what should be included to measure deprivation and fragility. However, many indices lack adequate spatial granularity and fail to incorporate the concept of “community” as a socially and territorially cohesive unit. Additionally, although diverse statistical methodologies are applied, the choice of variables is rarely theory-driven and is often constrained by data availability. The review underscores

the importance of concept-driven indicator development, emphasizing the integration of socioeconomic, environmental and contextual dimensions. It also highlights the value of participatory approaches to ensure alignment with community realities and policy needs. A conceptual framework is proposed for future indicator development, adaptable across spatial scales and suited to support HIAs, environmental epidemiological monitoring programmes and strategies promoting equity in environmental public health.

P THE ROLE OF UNCONVENTIONAL T CELLS IN HOST DEFENSE AGAINST BACTERIAL INFECTIONS

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Introduction. Unconventional T cells are a special group of T lymphocytes which recognize a large variety of non-peptide antigens, lipids or metabolites presented by non-classical MHC-like molecules and have a conserved TCR repertoire. They include different cell subsets like Mucosal-Associated Invariant T (MAIT) cells, Natural Killer T (NKT) cells and γδ T cells which play significant roles in infections by recognizing a broad spectrum of microbial antigens. They act as a crucial bridge between innate and adaptive immunity, especially at mucosal and barrier sites, by rapidly producing a large amount of cytokines and cytotoxic molecules. Human NKT cells are characterized by the co-expression of typical receptors of T cells and Natural Killer (NK). In particular, invariant NKT (iNKT) express a semi-invariant T Cell Receptor (TCR), made up of a Vα24 chain and a Vβ11 chain, along with some NK cell markers such as CD161 and CD56. iNKT are activated by the recognition of lipid or glycolipid antigens (α-galactosylceramide) presented by the non-polymorphic MHC class I like molecules CD1d. They respond faster than conventional T cells and more diversely upon TCR stimulation. Human MAIT cells express an invariant TCR a chain Vα7.2 -Jα33 which recognizes riboflavin-derivative antigens from a wide range of microbes presented by the MHC class I-like protein MR1. Principally at mucosal surfaces they rapidly release IFN-γ, TNF-α, IL-17 by enhancing macrophage and neutrophil responses and they have cytotoxic activity against infected cells. Human γδ T cells express a TCR composed of γ and δ chains that undergo somatic recombination leading to a vast sequence diversity. These cells recognize a wide range of antigens (phosphoantigens) with an MHC independent mechanism. Also, γδ T cells provide a first line of defense against infections with innate-like responses that directly kill infected cells, recruit neutrophils and activate phagocytes.

Aims.

I. To analyse iNKT, MAIT, and γδ T cell response to different intracellular and extracellular bacteria (Mycobacterium abscessus, Mycobacterium avium, Escherichia coli and Acinetobacter baumannii) most responsible for nosocomial infections.
II. To analyse sex differences in the frequency and response of iNKT, MAIT and γδ T cells in peripheral blood of heathy donors.
III. To analyse the number, function and potential as prognostic biomarkers of unconventional T cells in patients with nosocomial infections and polymicrobial sepsis.

Methods. iNKT, MAIT and γδ T cells will be identified in PBMC isolated from men and women healthy donors by multiparametric flow cytometry with the following antibodies' panel: anti-CD3, CD4, CD8, CD161, CD56, Vα24, Vα7.2 and TCR γδ. Human monocytes CD14+ purified from healthy donors PBMC by magnetic bead isolation will be stimulated with heat killed bacteria at different ratios. The CD14- fraction will be recovered to obtain all T lymphocytes. The cocultures will be expanded with IL-2 and the unconventional T cell

populations will be analysed for antigenic specific proliferation by CFSE staining, for cytotoxic activity via the granzyme-perforin axis and for the release of cytokines such as TNF-α, IFN-γ, and IL-17.

Results. MAIT and γδ T cells but not iNKT cells proliferate in response to autologous monocytes pulsed at different ratio with the intracellular bacteria Mycobacterium avium and Mycobacterium abscessus as well as the extracellular bacteria Escherichia coli, as shown by the CFSE profiles, suggesting that these bacteria express the specific microbial antigens recognized by MAIT and γδ T cells but not by iNKT cells. Data obtained from 10 men and 10 women show that there are no sex differences in the frequency of iNKT, MAIT and γδ T cells in peripheral blood and in the specific microbial response.

Conclusions. Studying unconventional T cells in infection is of crucial importance because of their sentinel role in the early containment of infection before the beginning of the adaptive immune response and as targets of broad-spectrum vaccines or immunotherapies.

PHD Project Titles

T Study of the effects of phytocompounds present in essential oils on biofilm formation and the pathogenicity of enterohemorrhagic strain *E. coli* O157:H7

Allen Amburose Stephen
Biological Technical and Scientific Service

T Implementation of innovative regulatory tools in preclinical studies for advanced therapy products: evaluation of the transition from animal models to digital and artificial intelligence-based technologies

Alessandra Ambrosone
National Center for Drug Research and Evaluation

T Development of Computational Methodologies and Monitoring Techniques for Dose Optimization in Ionizing Radiation Treatments

Massimiliano Antonini
National Center for Radiation Protection and Computational Physics

T Development of a multidisciplinary strategy for identifying and characterizing consumption trends and the permanence of new drugs on the market: from the national to the global level

Valeria Aquilina
National Center for Addiction and Doping

T Identification of early markers of neurodevelopmental disorders

Martina Attenni
Technical and Scientific Service for the Coordination and Promotion of Research

T Evaluation of the antiviral efficacy of natural and synthetic compounds against hepatitis E virus (HEV): a One Health approach to human and animal health

Sara Barbarulo
Department of Food Safety, Nutrition, and Veterinary Public Health

T Integrated ecotoxicological assessment strategy for the management of ecosystems in urban areas subject to extreme climatic phenomena

Melissa Barra
Department of Environment and Health

T Investigatin the interpaly between Epstein-Barr virus infection and host-immune system imbalance in multiple sclerosis pathogenesis

Lucia Benincasa
Department of Neuroscience

T Exploring the role of wild animals as coronavirus reservoir

Irene Berselli
Department of Food Safety, Nutrition, and Veterinary Public Health

T A One Health approach to antibiotic resistance: the role of toxic freshwater cyanobacteria in the evolution and spread of antibiotic resistance. A study of cultures and natural communities

Gloria Bianchi
Department of Environment and Health

T Integrated analytical and ecological strategies for assessing the risk of emerging contaminants in marine ecosystems

Alessandro Bolella
National Center for Water Safety

T Genomic analysis and identification of clinical and environmental Legionella pneumophila isolates

Enza Bruna Bonazza
Department of Infectious Diseases

T Investigating neuronal mechanisms and novel pharmacological treatments in preclinical models of neurodevelopmental disorders

Annalisa Canonico
Reference Centre for Behavioural Sciences and Mental Health

T WIDMApp - Wearable Individual Dose Monitoring Apparatus

Marina Carruezzo
National Centre for Radiation Protection and Computational Physics

T Isolation and characterization of new bacteriophages targeting drug-resistant bacteria

Alice Casata
Department of Infectious Diseases

T Criteria for the application of new methodologies in the regulatory process, aimed at a next-generation assessment of human health risk

Cristina Catinari
Department of Environment and Health

Modeling of patient-specific drug response in cystic fibrosis via nasal organoids

Aurora Ceci
Department of Oncology and Molecular Medicine

Computational And Cellular Screening of Sars-CoV-2 NSP13 Inhibitors to Counteract Viral Evasion of Innate Immune responses

Chiara Cencini
Department of Infectious Diseases

Engineering, Development and Validation of an EIS-Mediated Exosomal System for the Targeted Delivery of Heterologous miR-25: A Novel Therapeutic Strategy to Promote Endogenous Neurogenesis After Stroke

Martina Cese
Department of Neurosciences

Implementation of *in vitro* porcine intestinal epithelium models to study host–parasite interactions between the intestine and Giardia duodenalis, and for the investigation and evaluation of novel antiparasitic therapeutic treatments for veterinary use

Natalya Chervyakova
Technical and Scientific Service for Large-Scale Instrumentation and Core Facilities

Indoor monitoring of food service environments

Alessandra Ciccozzi
Center for Chemical Substances, Cosmetic Products and Consumer Protection

T Genomic sequencing of HBV and HCV using Next Generation Sequencing technology in blood donations positive for infection markers of these viruses

Giulia Costanzi
Department of Infectious Diseases

T Generative Artificial Intelligence in the Field of Medicine

Gabriele D'Andrea
National Centre for Radiation Protection and Computational Physics

T Use of diagnostic imaging in oncological, endocrinological, and cardiological preclinical research to support the implementation of the 3Rs principle in applied research

Antonio D'Ermo
Centre for Animal Experimentation and Welfare

T Understanding Emotional Labor Through a Twin Study: Genetic and Environmental Contributions to Surface and Deep Acting in Public-Facing Occupations

Matteo D'Onofrio
Reference Centre for Behavioural Sciences and Mental Health

T Redefining Regulatory Toxicology: New Approach Methodologies, High-Content Methods, and Integrated Test Batteries as tools towards Next Generation Risk Assessment

Teresa D'Amore
Department of Environment and Health

T Assessment of human exposure to nano- and microplastics as a central component in the protection and promotion of health

Giorgia Danese
Department of Environment and Health

T Development of molecular and biochemical methods for evaluating quality and safety of live biotherapeutic products for human use

Luca Del Pio
National Centre for Drug Control and Evaluation

T Biomonitoring of environmental contaminants in bovine milk and their *in vitro* toxicological evaluation on human cell lines

Laura Di Benedetto
Department of Food Safety, Nutrition and Veterinary Public Health

T Deep Underground Radiobiology: Cellular and Molecular Effects on the Immune System

Daniele Di Chio
National Center for Artificial Intelligence and Innovative Technologies for Health

T Development of an innovative method to combine protein and nucleic acid detection of biomarkers in exosomes

Marisa Di Giuseppe
Department of Oncology and Molecular Medicine

T Development of a 3D multicellular melanoma model for optimization of therapeutic treatment

Alice Di Netta
National Centre for Preclinical and Clinical Research and Evaluation of Medicines

T Development and validation of a questionnaire on dietary habits for the assessment of cardiovascular risk

Annalisa Di Nucci
Department of Cardiovascular, Endocrine-Metabolic Diseases and Ageing

T Preclinical development of an innovative vaccination strategy against respiratory infections based on engineered extracellular vesicles

Micaela Donnini
National Centre for Global Health

T Neurons chemically induced from retinal fibroblasts: an innovative approach for the study of human glaucoma

Karim El Bahnsawy
Department of Neurosciences

T Exploiting PBMC/muscle cell crosstalk to dissect adjuvant mechanism of action in intramuscularly administered vaccines

Monica Fabiani
Department of Infectious Diseases

T Development of humanization indicators for patients with rare diseases

Lorenzo Facciaroni
National Centre for Rare Diseases

T Bias Correction via Bayesian Nonparametrics: An Application to Autism Spectrum Disorder Data

Lucia Gallucci
Technical and Scientific Service for Statistics

T Leaching of elements from water distribution networks into water intended for human consumption and potential formation of organometallics and organic complexes

Alice Garbini
National Centre for Water Safety

T Spatial and spatiotemporal Bayesian methods for disease risk analysis based on current data flows

Arianna Guaita
Technical and Scientific Service for Statistics

T Dissecting reactogenic and immunogenic properties of innovative RNA-based strategies for future pandemic emergency in *in vitro* human primary cell models

Matilda Hushi
Department of Infectious Diseases

T Oxidative stress as a modulator of DNA damage-induced innate immunity: insights into the cGAS-STING pathway

Federica Iris
National Center for Artificial Intelligence and Innovative Technologies for Health

T Systems Methods for Understanding the Climate-Food System-Noncommunicable Disease Pathway

Rachel Juel
Department of Environment and Health

T Identification and Analysis of the Protein–Protein Interaction Network in the Oocyst Stage of *Plasmodium berghei*

Despoina Koukouli
Technical and Scientific Service for Large-Scale Instrumentation and Core Facilities

Population-level assessment of healthcare needs, access to care, and resource utilization in the natural history of oncological diseases

Lorenzo Lasorsa
National Centre for Disease Prevention and Health Promotion

The bacterial toxin CNF1 and its potential role in colorectal cancer progression and metastasis

Ilenia Laterza
Department of Cardiovascular, Endocrine-Metabolic Diseases and Ageing

Investigating casuality with faecal microbiota transplantation in SOD mice

Alessio Lauricella
National Centre for Preclinical and Clinical Research and Evaluation of Medicines

Study of genetic and environmental factors in a cohort of patients affected by maculopathies

Alessandro Leone
Department of Oncology and Molecular Medicine

Study of Au and Ag nanoparticles on protection against oxidative stress induced by ionizing radiation

Diego Lipani
National Center for Innovative Technologies in Public Health

WRNIP1 as a new potential factor modulating chemosensitivity in BRCAness cancers

Nicola Lomurno
Department of Environment and Health

T Research, definition, and evaluation of innovative training initiatives on Health, Environment, Biodiversity, and Climate topics

Camilla Lugli
Presidency - Training Service

T Characterization and development of therapeutic mRNA-based drugs for enhancing endogenous post-stroke human neurogenesis

Francesca Maiolo
Department of Neurosciences

T Neuronal response to stress factors in space: study of protein quality control and aggregation in human neuroblastoma cells following chronic exposure to ionizing radiation and altered gravity

Enrico Mandolini
National Centre for Artificial Intelligence and Innovative Technologies for Health

T New frontiers for containing sand fly borne diseases from a One Health perspective: study of microbiota's role in pathoghens development, tools for in-depth study on vectors physiology and on insecticides resistance

Claudia Mangiapelo
Department of Infectious Diseases

T Targeting the molecular mechanisms involved in radioresistance in paediatric rhabdomyosarcoma

Valeria Manzi
Department of Oncology and Molecular Medicine

T Analysis of the impact of exposure to environmental chemical contaminants and diet on metabolic alterations, with identification of sex-specific effects using *in vivo* experimental models

Benedetta Martorano
Reference Centre for Gender Medicine

T Identification of suspended macroplastics in surface water bodies and characterization of the attached microbial communities

Martina Menichino
National Centre for Water Safety

T Use of innovative light-based methods for the control of vectors of public health interest

Isabella Mercuri
National Center for Chemical Substances, Cosmetic Products and Consumer Protection

T Role of per- and polyfluoroalkyl substances (PFAS) in hepatocyte lipid metabolism and food safety aspects

Milena Mikhail
Department of Food Safety, Nutrition and Veterinary Public Health

T Plasmodium falciparum transcriptional profiles in hemoglobinopathies

Alisia Pantanetti
Department of Infectious Diseases

T Cell-autonomous and Non-Cell-Autonomous Responses to Replication Stress in Normal and Tumor Cells

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Department of Infectious Diseases

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Department of Oncology and Molecular Medicine

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National Centre for Rare Diseases

T Evaluation of the antiviral activity of low-molecular-weight molecules inhibiting the HPV 16 E1 protein

Mara Saccoccio
Department of Infectious Diseases

T Targeting mitochondrial dysfunction to treat Niemann Pick type C disease

Marianna Samà
National Centre for Preclinical and Clinical Research and Evaluation of Medicines

Characterization of pre-symptomatic phases in mouse models of Rett syndrome: potential new diagnostic tools and early pharmacological interventions

Lisa Scansalegna
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"Baby-Friendly Community and Health Services". Pilot Project for the implementation of Good Practice for the protection, promotion, and support of breastfeeding and health promotion in the first 1000 days in Europe

Flavia Splendore
National Centre for Disease Prevention and Health Promotion

Primary astrocytes dysfunction in the pathogenesis of MLC leukodystrophy: investigating shared pathological mechanisms of myelin degeneration in astrocytopathies

Sara Sposito
Department of Neurosciences

Identification of sex-specific biomarkers for gastric and colorectal cancers associated with exposure to natural or anthropogenic asbestos

Rabia Tabassum
Reference Centre for Gender Medicine

Methodologies for measuring the activity concentration of alpha and beta emitters in drinking water

Maria Federica Teatini
National Centre for Radiation Protection and Computational Physics

T Endocrine-disrupting mechanisms induced by PFAS through the modulation of obesogenic miRNAs in hepatic, renal, prostatic, and placental cells under hyperglycemic and/or hyperlipidemic conditions.

Matteo Telesca
National Center for Chemical Substances, Cosmetic Products and Consumer Protection

T Development of serological assays to study the spread of some emerging arboviruses with a One Health approach

Roberta Tomei
Department of Food Safety, Nutrition and Veterinary Public Health

T Developmental Epileptic Encephalopathies and Childhood Movement Disorders: Pathogenic Mechanisms, Genotype-Phenotype Correlations, and Therapeutic Perspectives

Mariateresa Tondo
Department of Oncology and Molecular Medicine

T Effects of a lipid-enriched diet during periadolescence on cognitive function in mice

Cristiana Tonelli
Reference Centre for Behavioural Sciences and Mental Health

T P.I.T.E.R. Project: Management and analysis of complex data aimed at defining the epidemiological profile and studying health determinants in patients from a network of multidisciplinary clinical centers

Andrea Vaiano
National Centre for Global Health

T Integration of omics data for the characterization of potential novel biomarkers and the reconstruction of gene-/isoform-centric regulatory networks in physiological and pathological contexts

Diletta Vialardi
Technical and Scientific Service for Large-Scale Instrumentation and Core Facilities

T Study of the role of viral infections in neurodegenerative diseases

Francesco Zanzi
Department of Infectious Diseases

T Medium-throughput screen for the identification of erythrocytes factors involved in susceptibility to invasion by Plasmodium falciparum

Davy Zongo
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