# IJAE

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of Anatomy and Histology

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**della Società Italiana di Anatomia e Istologia**
Ravenna, 18-20 settembre 2025

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# Letture

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# Minimally invasive strategies for modulating congenital malformations in utero

Bruna Corradetti, PhD MSc

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Congenital malformations occur in approximately 1.9 per 1,000 live births worldwide, with neural tube defects (NTDs) being among the most severe. NTDs, which include conditions such as anencephaly, craniorachischisis, and spina bifida (SB), result from the incomplete closure of the neural tube during early fetal development. The etiology of NTDs is complex and multifactorial, with the "two-hit" hypothesis for SB suggesting that an initial failure in neural tube closure is exacerbated as gestation progresses, exposing the defect to a hostile intrauterine environment. While prenatal surgical repair and regenerative strategies have demonstrated potential in reducing the severity of these conditions, current *in utero* interventions remain invasive for both mother and fetus. Leveraging years of expertise in regenerative medicine and tissue engineering, we are exploring less invasive approaches to modulate the *in utero* environment, promote tissue homeostasis, and enhance repair mechanisms.

Mesenchymal stem cells (MSCs) from amniotic fluid (AF-MSCs) offer a promising source for these therapeutic strategies due to their roles in embryo-maternal communication and their remarkable regenerative properties. In particular, we are focusing on extracellular vesicles (EVs) derived from AF-MSCs as a stable, reproducible, and scalable alternative to cell-based therapies. These EVs show significant therapeutic potential, overcoming many of the challenges associated with stem cell-based treatments while retaining efficacy. In biodistribution studies in female mice, EVs were delivered specifically to the uterus and yolk sac, without any observed toxicity to the dams or significant changes in fetal viability. Using a well-established SB mouse model (Fkbp8 knockout), we administered continuous doses of EVs (10^9/dose) during neurulation, resulting in a marked reduction in lesion size (from 5–8mm to 3mm) and visible vertebral closure at the thoracic and lumbar levels. Furthermore, EVs maintained the stability of RNA cargo, demonstrating long-term expression of loaded mRNA both *in vitro* and in *ex vivo* whole embryo culture systems. This confirmed that EVs are capable of crossing the yolk sac, reaching the embryo, and restoring gene function in a complex biological environment.

These promising findings pave the way for developing minimally invasive, adaptable precision medicine strategies with significant potential for treating birth defects in utero. By reducing the severity of these conditions prenatally, this approach offers an innovative, lower-risk pathway that enhances therapeutic outcomes.

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# What is Life?

Paul Nurse

*Cell Cycle Laboratory, The Francis Crick Institute, London, UK and Laboratory of Yeast Genetics and Cell Biology, Rockefeller University, New York, NY, USA*

In this lecture I consider the question "What is Life?" by discussing five great ideas of biology, ranging from the 'Cell' to 'the Logic of Life'. By considering these concepts a direction of travel is set towards a definition of life.

# Presentazione orali

# *Anatomia e fisiopatologia del sistema gastrointestinale*

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# Early Parkinson's disease and REM sleep Behaviour Disorder show gut barrier impairments, NLRP3 inflammasome activation, and enteric alpha-synuclein accumulation

Pellegrini Carolina[1], Bellini Gabriele[2], Pierucci Clarissa[1], D'Antongiovanni Vanessa[1], Segnani Cristina[1], Ippolito Chiara[1], Hoxhaj Domeniko[2], Maestri Michelangelo[2], Rettura Francesco[3], Ceravolo Roberto[2], Bellini Massimo[3], Derkinderen Pascal[4], Bernardini Nunzia[1]

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**Introduction** Parkinson's disease (PD) patients show alterations of gut barrier and presence enteric inflammation that may contribute to brain pathology *via* gut-brain axis[1]. In this context, the nucleotide-binding oligomerization domain leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome complex, a bacteria-immune sensor, play a pivotal role in shaping the enteric immune/inflammatory responses in PD.[2] However, whether changes in gut mucosal-vascular barrier and NLRP3 activation are present in the early-stage PD, as well as in the idiopathic REM sleep Behavior Disorder (iRBD), an early manifestation of premotor PD which may precede the onset of prototypical motor symptoms by many years, remain unexplored. Here, we investigated the histomorphological alterations of intestinal mucosal-vascular barrier, NLRP3 inflammasome activation pathways and total alpha-synuclein (t-α-syn) accumulation in colonic biopsies from early PD and iRBD patients.

**Methods** Mucosal biopsies from the descending colon were obtained in 10 early PD patients (EPD, disease duration 0-5 years), 8 iRBD subjects and 8 healthy controls (HC). Then, colonic biopsies were processed for the evaluations of epithelial mucins, claudin-1, t-α-syn, NLRP3, apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC), caspase-1 and interleukin-1beta (IL-1β) distribution and expression by histochemistry, immunofluorescence or western blot. In order to characterize gut vascular barrier alterations and activation of inflammasome signaling in activated macrophages, double-immunofluorescences [plasmalemmal vesicle associated protein-1 (PV1)-CD31 and CD68-ASC] were assessed.

**Results** EPD patients showed altered mucins, decreased claudin-1 and increased PV-1 distribution and expression, as compared with HC and iRBD. No notable differences were found between controls and iRBD subjects. Western blot analysis of colonic samples displayed an increase in t-α-syn, NLRP3, ASC, active caspase-1 and IL-1β expression in EPD patients, as compared with HC and iRBD. Additionally, iRBD subjects displayed an increase in active caspase-1 expression. Of note, CD68positive macrophages in the colonic mucosa from EPD patients showed an increased ASC immunofluorescence, confirming the activation of mucosal NLRP3 pathways.

**Conclusions** Alterations of intestinal mucosal-vascular barrier, NLRP3 inflammasome activation and t-α-synuclein accumulation represent early events in PD, that could contribute to central disease progression thought the spread of gut-derived factors *via* vascular gut-brain axis. iRBD patients showed NLRP3-indipendent inflammation and no alterations of gut barrier and enteric t-α-syn accumulation, suggesting that iRDB could develop a PD phenotype independently from the gut-brain axis.

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**Keywords:** Parkinson's disease, Gut-brain axis, Intestinal mucosal-vascular barrier, Inflammasome.

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# Unveiling Microvascular and Extracellular Matrix Abnormalities in Chronic Intestinal Pseudo-Obstruction

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Chronic intestinal pseudo-obstruction (CIPO) is a rare and disabling condition characterized by symptoms of intestinal blockage in the absence of any mechanical cause.[1] Although certain forms of CIPO have been linked to systemic or genetic disorders, the idiopathic variant remains poorly understood.[2] Building on previous evidence of vascular abnormalities in mitochondrial neurogastrointestinal encephalomyopathy (MNGIE),[3] this study aimed to investigate whether similar mechanisms underlie non-MNGIE CIPO. Full-thickness jejunal biopsies were collected from 22 patients with non-MNGIE CIPO and compared with samples from 10 control individuals. A combination of whole-exome sequencing, mitochondrial DNA (mtDNA) analysis, and haplogroup determination was used to explore genetic contributions. Tissue analysis included morphometric assessment of submucosal vessels using orcein staining, immunofluorescence localization and quantification of thymidine phosphorylase (TP) and vascular endothelial growth factor (VEGF), and evaluation of key structural and functional parameters such as fibrosis, hypoxia, ganglionic density and spacing, and muscle layer thickness. In vitro angiogenesis assays were performed using TP-silenced human endothelial cells. The results highlighted a distinctive microvascular phenotype in CIPO patients, with a 45% reduction in total vascularized area, a 54% increase in the number of small-caliber vessels, and a striking loss of medium and large vessels. These abnormalities were accompanied by severe tissue hypoxia, a 50% reduction in myenteric neurons, thinner longitudinal muscle layers, and increased inter-ganglionic distances. Genetic analysis showed no mtDNA depletion or rearrangements, but a significant prevalence of mtDNA haplogroup J was noted, suggesting a potential genetic predisposition. TP and VEGF, both critical regulators of vascular function previously implicated in MNGIE, were downregulated by approximately 60% in CIPO tissues, and TP silencing significantly impaired angiogenesis in vitro. These findings indicate that idiopathic CIPO involves profound microvascular remodeling and extracellular matrix abnormalities, contributing to neuromuscular degeneration and gastrointestinal dysmotility. The combined evidence of angiogenic impairment and potential mitochondrial genetic susceptibility offers new insights into disease pathophysiology and suggests future avenues for diagnostic and therapeutic strategies targeting the enteric vasculature in CIPO.

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**Keywords:** Chronic intestinal pseudo-obstruction, Microvascular remodeling, Thymidine phosphorylase, Enteric neuromuscular dysfunction

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# Intestinal inflammation and ketogenic diet: the combined role of β-hydroxybutyrate and MCT oil on CaCo-2 cells

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Chronic intestinal inflammation is a significant risk factor for the development of colorectal cancer, particularly in inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease. Recent studies highlight how persistent inflammation promotes a protumorigenic environment through the activation of molecular pathways such as NF-κB, STAT3 and IL-6/IL-10, which promote cell proliferation, immune evasion and metastasis. In this context, ketogenic diets (KD) – lowcarbohydrate, high-fat and adeguate protein diets – are aTracting increasing interest for their potential anti-inflammatory and protective effects at the intestinal level. These diets stimulate the hepatic production of ketone bodies, in particular acetoacetate (AcAc) and β-hydroxybutyrate (BHB), which have demonstrated beneficial effects on the health of the gastrointestinal tract: they provide a preferential energy source for enterocytes, contribute to the maintenance of the integrity of the intestinal mucosa, promote the sense of satiety and, above all, suppress inflammation and carcinogenesis. Through the use of exogenous medium-chain triglycerides (MCT) [C6:0; C8:0; C10:0; C:12:0], an increase in the ketogenesis process occurs thanks to their rapid absorption through the portal vein and direct oxidation to acetyl-CoA in the liver.

This study aimed to evaluate the synergistic anti-inflammatory effects of BHB and MCT in vitro using CaCo-2 cells, an immortalized human colorectal adenocarcinoma cell line widely used as a model of the intestinal epithelial barrier. Cell viability, wound healing and ELISA tests were performed after treating CaCo-2 cells with 5 mM BHB and 0.5 mM MCT in the presence or absence of LPS (1 μg/ml) for 24 and 48 h. BHB and MCT oil were shown to independently enhance cell viability, migratory capacity and anti-inflammatory cytokine production. In particular, the combined treatment produced a significantly greater effect, suggesting a synergistic enhancement of anti-inflammatory activity. To further investigate the impact of this combination treatment on intestinal barrier integrity, Western blot analysis was performed to assess the expression of key adhesion proteins, including E-cadherin, Integrin α5β1, and Zona Occludents -1 (ZO-1), which play a crucial role in maintaining epithelial cohesion and tight junction function. By comparing protein expression profiles between BHB/MCT-treated cells with and without LPS, we intend to determine whether the combination treatment can counteract the LPS-induced alteration in the expression of adhesion proteins. This approach will help to clarify the molecular mechanisms through which BHB and MCT contribute to the restoration of the epithelial barrier and protection from pro-inflammatory stimuli, mimicking the inflammatory process in vivo. Therefore, these data open new avenues for future clinical studies aimed at evaluating the efficacy of BHB and MCT in patients with intestinal inflammation.

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**Keywords:** ketogenic diet, β-hydroxybutyrate (BHB), Medium-chain triglycerides (MCTs), intestinal inflammation, colon carcinogenesis, gut barrier.

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# Dissecting Integrated Stress Response-driven metastatic potential and drug resistance in Colorectal Cancer

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Background The Integrated Stress Response (ISR) is a key signaling cascade that modulates the translational landscape in response to microenvironmental stress[1]. Increasing evidence suggests that ISR contributes to cancer cell aggressiveness by promoting metastasis and drug resistance[2]. Colorectal cancer (CRC), a highly metastatic malignancy with KRAS mutations present in approximately 40% of patients, remains a leading cause of cancer-related mortality worldwide[3]. This study aims to elucidate the molecular mechanisms by which ISR drives CRC aggressiveness, with the dual goals of improving therapeutic responses and contributing data to artificial intelligence–based digital modeling platforms.

Methods We established and characterized both 2D and 3D CRC models using a range of molecular and biochemical approaches. To assess the role of ISR in metastatic potential, we employed advanced imaging technologies, including time-lapse matrix invasion assays in microfluidic devices. Extracellular vesicles (EVs)[4] were isolated, and their protein and lipid content was evaluated through proteomic and lipidomic analyses.

Results Our findings reveal a bidirectional crosstalk between the ISR and KRAS pathways: ISR activation amplifies KRAS signaling, while KRAS activity is required for ISR induction. We further demonstrated that ISR promotes CRC metastasis by enhancing matrix invasion and increasing the release of EVs, which in turn contribute to the formation of the pre-metastatic niche. Notably, we identified a novel function of ISR in conferring drug resistance through upregulation of AXL expression[5] in response to standard chemotherapeutic treatments.

Conclusions This study uncovers a previously unrecognized regulatory interaction between ISR and KRAS signaling that contributes to CRC progression and aggressiveness. ISR supports metastatic dissemination and drug resistance by reshaping the protein synthesis landscape and modulating EV-mediated communication. These findings highlight ISR as a potential therapeutic target and provide a foundation for the development of AI-informed strategies to predict treatment responses and identify novel vulnerabilities in metastatic CRC.

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**Keywords:** Integrated Stress Response, colorectal cancer, metastasis, drug-resistance.

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# Spatial transcriptomics as an innovative approach for the molecular characterization of stem/progenitor cell niches in the hepato-biliarypancreatic system

Guido Carpino[1], Diletta Overi[1], Stefano Leone[1], Vincenzo Cardinale[1], Domenico Alvaro[1], Paolo Onori[1], Antonio Franchitto[2], Eugenio Gaudio[1]

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Anatomical niches of stem/progenitor cells have been described in human liver, in biliary tree, in duodenum, and in pancreatic duct systems [1]. The phenotypic profile and functional properties of these niches have been studied by immunohistochemistry and by in vitro approaches [2]. Recently, spatial molecular profiling has emerged as an extremely compelling and innovative approach for the characterization of molecular profile based on morphological landmarks, combining the highthroughput capabilities and information provided by molecular analyses with the spatial characterization of microscopic approaches. The aim of the present study was to explore the potential of spatial transcriptomics in the study of progenitor/stem cell niches within the hepato-biliarypancreatic system. Healthy organs, including livers (N=3), extrahepatic bile ducts (N=3), pancreas (N=3), and duodenum (N=3) were obtained from orthotopic liver transplantation. Formalin-fixed paraffin-embedded samples were processed for the Nanostring® GeoMx™ Digital Spatial Profiler (DSP) platform. Transcriptomic profile was obtained by the GeoMx® Whole Transcriptome Atlas (WTA) assay. At least five separate regions of interest (ROI) for each cellular compartment were selected and the epithelial and stromal components were separately sampled for each ROI based on the morphology marker Pan-cytokeratin. Both mature (i.e. hepatocyte, cholangiocyte, pancreatic acini, pancreatic islets, intestinal villi) and stem/progenitor cell compartments (bile ductule, peribiliary glands, pancreatic duct glands, and intestinal crypts) were sampled and characterized. A different transcriptomic profile of each cellular compartment emerged from our analysis; particularly, in the mature epithelial cell compartments, the expression of relevant genes was up-regulated in the specific cell population, e.g. ALB and TF genes in hepatocytes, INS and GLC in pancreatic islets. Also, stromal ROIs showed up-regulation of genes for collagen and connective tissue remodeling. Interestingly, the molecular profile from stem/progenitor cell niches corresponded to the identity of the specific stem cells, e.g. LGR5 expression in intestinal crypts and MUC6 in peribiliary glands within the biliary tree. In conclusion, our data confirms the reliability of a spatial molecular profiling approach in the study of stem/progenitor cells niches in terms of cell characterization and phenotype. Most important, this leads to build a data repository with full transcriptomic information from desired cell niches which could be interrogated in further studies to obtain a full characterization of epithelial and stromal cellular components along the hepato-biliary-pancreatic system.

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**Keywords:** liver, stem cells, spatial transcriptomics.

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# Alpha-synuclein pathology of the peripheral nervous system in Parkinson's disease: is it a systemic disease?

Aron Emmi[1,2,3], Marta Campagnolo[1,2], Aleksandar Tushevski[1,3], Francesco Paolo Russo[2,4], Miryam Carecchio[1,2], Veronica Macchi[2,3], Raffaele De Caro[2,3], Piero Parchi[5,6], Angelo Antonini[1,2], Andrea Porzionato[2,3]

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The peripheral nervous system (PNS) has recently emerged as a key contributor to Parkinson's Disease (PD) pathophysiology, yet its extent and characteristics remain underexplored. We investigated alpha-synuclein (αSyn) pathology in vivo in the enteric nervous system (ENS) and skin of PD patients, and ex vivo in peripheral tissues and the CNS of body donors with confirmed alpha-synucleinopathy, using conformationspecific antibodies and Real-Time Quaking Induced Conversion (RT-QuIC). Our in vivo study included 97 PD patients and 28 controls, while ex vivo analysis examined 10 body donors of the Reference Center of the University of Padova. Immunohistochemistry targeted aggregated αSyn (5G4), neuronal and glial markers, immune-cell markers, and nerve fiber proteins, followed by morphometric analysis. Aggregated αSyn with a thread-like pattern was detected in the skin of 65% (67/90) of PD patients and in the gut of all biopsied cases (20/20), colocalizing with neuronal markers. Significant quantitative differences between early and advanced PD were observed. Enteric glial cells showed increased size and density, suggesting reactive gliosis. PD patients exhibited increased T- and B-lymphocytes and higher HLA-DR expression in the gut. RT-QuIC accuracy was 87.7% in skin, 67.4% in the duodenum, and 80.0% in gastric biopsies, with higher sensitivity in advanced PD. Ex vivo, αSyn pathology was detected in multiple PNS sites, including the gut, heart, and carotid body. We found evidence for αSyn pathology in the PNS, effectively distinguishing PD patients from controls. Further research is needed to determine its early onset and impact on treatment efficacy in advanced PD.

**Keywords:** Alpha-synuclein, Parkinson's Disease, Peripheral Nervous System, Enteric Nervous System, RTQuIC, Immunohistochemistry.

*Metabolismo, tessuto adiposo,*
*sistema endocrino e patologie correlate*

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# Role of phosphodiesterase type 5 in murine aortic development

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Type 5 phosphodiesterase (PDE5) is an enzyme that specifically hydrolyzes cyclic guanosine monophosphate (cGMP), a second messenger involved in various physiological processes [1, 2]. PDE5 is expressed in several tissues and cells such as the lung, brain, kidney, cardiac myocytes, penile corpus cavernosum and vascular smooth muscle cells (VSMCs) [3]. In VSMCs, the NOcGMP-PKG axis induces a decrease in intracellular calcium concentration, promoting muscle relaxation and vasodilation [4]. Selective PDE5 inhibitors, including sildenafil, tadalafil and vardenafil, are widely used in the treatment of erectile dysfunction (ED) and pulmonary arterial hypertension (PAH) [5]. However, case reports have shown aortic dissection or intracranial aneurysm after abuse of sildenafil or tadalafil [6, 7]. Moreover, it was found that sildenafil treatment exacerbates the development of the aneurysm in an abdominal aortic aneurysm mice model [8].

To investigate whether PDE5 deletion contributes to morphological and structural alterations of the aorta, we utilized a Pde5 knockout (Pde5 ko) mouse model generated in our laboratory and compared it to wild-type (Pde5 wt) mouse. Ultra-high frequency ultrasound (UHFUS) imaging revealed a significant increase in the diameters of both the aortic arch and descending aorta in Pde5 ko mice compared to wt. Hematoxylin-eosin staining showed no detectable abnormalities in Pde5 wt sections, while Pde5 ko samples exhibited clear disruptions in the tunica media architecture, including disorganized VSMCs, which appeared detached from elastin lamellae and unevenly distributed. Notably, a higher density of VSMC nuclei was observed in Pde5 ko aortas compared to wt. Considering the established role of elastin degradation in aneurysm pathogenesis, particularly in abdominal aortic aneurysm (AAA), we assessed elastin structure using orcein staining. Pde5 wt aortic sections displayed intact elastic fibers, while those from Pde5 ko mice demonstrated pronounced elastin layer thickening accompanied by increased fiber fragmentation. Collagen fiber organization was assessed via Picrosirius red staining, revealing enhanced collagen deposition in the tunica media of Pde5 ko aortas compared to wt. Immunofluorescence and Western blot analyses confirmed upregulation of type I and type III collagen, in Pde5 ko samples compared to wt. To investigate the molecular alterations associated with PDE5 deletion, we performed RNA sequencing on aortas from Pde5 ko and wt mice. Among the most significantly downregulated genes in Pde5 ko aortas were Ndufs7, Cox7, and Slc25a11. Interestingly, downregulation of Ndufs7 has recently been reported in the peripheral blood of patients with AAA [9].

Collectively, these findings suggest that PDE5 plays a crucial role in maintaining aortic structural integrity and homeostasis. Its deletion leads to vascular remodeling, implicating PDE5 as a potential regulator of aortic pathology.

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**Keywords:** PDE5, aorta development, aortic aneurysms.

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# Investigating the Role of Sarcoglycan Subunits in Adipocyte Structure and Protein Expression via siRNA-Mediated Silencing

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The sarcoglycan (SG) subcomplex, comprising six isoforms (α, β, γ, δ, ε, and ζ), was initially characterized as muscle-specific. However, recent studies have revealed its ubiquitous expression across various tissues. Notably, Groh et al. (2009) identified the presence of β- and ε-sarcoglycan subunits in adipose tissue, suggesting a potential role in glucose homeostasis.

Further investigations have demonstrated that all six SG isoforms are expressed in 3T3-L1 cells, a murine preadipocyte cell line. Upon induction of the browning process in these cells, an upregulation of SG expression was observed, indicating a possible involvement in adipocyte differentiation.

In this study, we investigated the potential involvement of individual SG subunits in adipocyte biology through gene silencing approaches. The results highlight a possible functional role for the sarcoglycan subcomplex in the regulation of adipose tissue physiology.

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**Keywords:** Sarcoglycan subcomplex, adipose tissue, siRNA.

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# *Pde5a* loss rearranges mouse bone marrow vasculature and increases bone marrow adiposity

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Phosphodiesterase type 5 (PDE5A) regulates intracellular levels of cyclic guanosine monophosphate (cGMP), thereby modulating vascular tone through smooth muscle relaxation[1]. PDE5A inhibitors, commonly employed in the treatment of erectile dysfunction and pulmonary arterial hypertension, have been implicated in the modulation of bone metabolism, although very contrasting results have been reported so far[2,3]. Using a *Pde5a* knockout (KO) mouse model, we aimed to investigate the impact of *Pde5a* deficiency on bone homeostasis and bone marrow (BM) microenvironment in male and female mice at different ages.

No significant differences were observed in overall bone architecture between KO and wild-type mice, except for a selective increase in trabecular bone mass in femurs of 3-month-old KO females. Notably, KO mice exhibited consistent alterations in BM vasculature, characterized by sinusoidal vessels with reduced luminal area. These vascular changes correlated with a marked increase in BM adiposity across sexes and age groups. Transcriptomic profiling on flushed BM revealed upregulation of pathways associated with muscle fiber differentiation and downregulation of genes involved in triglycerides metabolism. The analysis of publicly available scRNA-seq data sets indicated high expression of *Pde5a* in a subset of BM stromal cells exhibiting smooth muscle-like features, supporting a role for PDE5A in regulating vascular architecture and bone marrow stromal cell differentiation.

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# Lipidomic Changes and Oxidative Stress in Cushing's Syndrome: Insights into Disease Mechanisms and Therapeutic Strategies

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Cushing's syndrome is marked by elevated circulating glucocorticoid levels, which exert widespread effects on various physiological processes, including lipid metabolism and oxidative stress (1). In this study, we performed an untargeted lipidomic analysis using high-resolution mass spectrometry (HRMS) on plasma samples collected from patients with Cushing's syndrome, both prior to and following therapeutic intervention. These findings were compared with data from healthy individuals and evaluated in relation to the patients' clinical trajectories.

The lipidomic profiling revealed notable disruptions in lipid composition, particularly involving triglycerides (TGs) and ether-linked phosphatidylethanolamines (EtherPEs). These lipid species exhibited disease-associated alterations, and although treatment partially normalized their levels, the restoration was incomplete. Instead, therapy appeared to induce a broader restructuring of the lipidome, suggesting that treatment not only attempts to correct dysregulated lipid levels but also promotes the emergence of a new lipid profile – potentially reflecting metabolic adaptation or cortisol-related regulatory shifts.

Pathway analysis highlighted several key metabolic pathways impacted by both the disease and its treatment, offering valuable insight into the lipid disturbances associated with glucocorticoid excess. Given the well-established increase in oxidative stress in Cushing's syndrome, we also evaluated plasma levels of 4-hydroxy-2-nonenal (HNE), a recognized biomarker of oxidative damage. This approach aims to further elucidate the interplay between lipidomic changes and oxidative stress in this context.

In summary, this study enhances our understanding of lipid metabolic alterations and oxidative stress in Cushing's syndrome and may aid in the discovery of novel lipid-based biomarkers for disease monitoring and evaluating therapeutic efficacy.

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**Keywords:** Cushing's syndrome, lipidomic, oxidative damage, metabolic alteration.

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# New perspectives in the pathogenesis of human visceral fat fibrosis

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Obesity, due to a state of positive energy balance, is described as a multifactorial chronic disease frequently associated with metabolic syndrome. It is considered a risk factor for the onset of other chronic pathologies[1], including different types of metabolic disorders[2-5], and other diseases that are not classified as metabolic, such as some forms of cancer[6,7], osteoarthritis[8], obstructive sleep apnea[9] and neurodegenerative diseases[10,11].

Adipocytes are the parenchymal cells of adipose organ, a complex structure recently recognized as forming a unitary structure in both mice and humans[12]. The adipose organ of patients with obesity undergoes pathological remodeling, triggering proinflammatory immune cell responses and changes in the matrix composition within tissues and in the extracellular matrix[13,14]. The final effect is a state of local fibrosis, which increases the total rigidity of the tissue itself, reducing its expandability[15,16]. Fat fibrosis is mainly due to three types of collagen: I and III (fibrillar) and VI (non-fibrillar). In this study we measured the extent of fibrosis by histochemistry in subcutaneous and visceral fat from 50 patients with obesity and 15 lean control patients. Our studies were supported by in vitro experiments on human multipotent adipose-derived stem cells (hMADS). Fibrosis was significantly increased in visceral fat (4.7%+/-0.32 in obese vs. 2.5%+/-0.28 in lean fat; P<0.0001), with high variability among patients. Gene expression of Col I and Col III (fibrillar collagen) was elevated while Col VI expression was decreased. In vitro studies with hMADS confirmed the in vivo results. Transmission and high-resolution scanning electron microscopy, along with gene expression data, suggested that mature obese adipocytes, both in vivo and in vitro, are responsible for fibrillar collagen production, showing also that this kind of collagen is a fundamental component of the normal surface anatomy of adipocytes.

We also studied the subcutaneous fat from three patients carrying homozygous (Ullrich) or heterozygous (Bethlem) mutations of the Col VI gene – leading to absent or reduced Col VI production, respectively. Their adipose tissue showed high levels of fibrosis, approximately 6.5 times higher in the patient with Ullrich congenital muscular dystrophy and 2.8 times higher in the two patients with Bethlem myopathy, compared to corresponding fat from healthy controls. These levels of fibrosis exceed those observed in obese adipose tissue, confirming the functional relevance of Col VI in the development of fat fibrosis.

Overall, our findings align with previous studies[16-18] in suggesting that fat fibrosis in humans affected by obesity has a multifactorial origin and point to obese adipocytes as a source of collagen production. Moreover, our data highlight an unexpected role for Col VI in driving the altered metabolism of obese visceral cells, ultimately leading to collagen production and fat fibrosis.

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# The effects of exposure to endocrine disruptors during pregnancy

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Endocrine disruptor chemicals (EDCs) can affect the endocrine system by interfering with hormone synthesis, bioavailability, and action, thereby altering cellular proliferation and differentiation, tissue development, and various metabolic processes.

Among the main EDCs, bisphenol A (BPA), bisphenol S (BPS), and perfluoroalkyl compounds (PFAS) have undergone widespread diffusion worldwide in recent decades. These substances are highly persistent in the environment and are readily absorbed but only slowly eliminated.

They are manufactured through industrial processes for the production of daily-use products, mainly in plastics for food packaging. Humans are then exposed to BPA/BPS and PFAS through drinking and eating food that has been in contact with plastics. BPA and PFAS, and in particular perfluorooctane sulfonate (PFOS) seem to exert detrimental effects on human health. Indeed, human population-based associations indicate that "in utero" exposure to prevalent environmental contaminants is associated with negative pregnancy or birth outcomes. A better understanding of the mechanisms by which these pollutants cause molecular disturbances, how these mechanisms interact, and whether they display sex specificities is essential for developing mitigating and sexspecific strategies against chemical-induced diseases.

The aim of this study is to evaluate the effects of exposure to several EDCs (PFOS, BPA, and BPS) in dams during the critical period of pregnancy. Mothers were treated with EDCs from E0 to PND14, and after the birth of the pups, their maternal and anxiety behaviors were analyzed.

The Pup Retrieval Test has shown that the BPS group is the slowest to complete the test overall. The PFOS-treated group shows the most definite reduction in pup-care-associated behaviors amongst all animals tested.

Moreover, especially BPA and BPS showed signs of anxious-obsessive behavior, a result further supported by other tests, such as the Open Field tests in which all treated groups exhibited anxious behavior across several parameters analyzed, and in the Elevated Plus Maze test where treated groups demonstrated anxious-like behavior and decreased curiosity to explore their surroundings. After weaning the pups at PND21, the mothers were sacrificed, and their brains were analyzed for morphological studies. In particular, we focused our attention on the hypothalamic vasopressin system (AVP). Cell counts in the Paraventricular Nucleus showed a decrease in AVP-producing cells in the treated groups. It is known that cell number reduction is a well-documented effect of stress on the vasopressin system, and in this experiment it is positively related to the results of the behavioral tests.

In conclusion, exposure to EDCs in pregnancy shows anxiety-related behaviors and alterations to maternal care, further sustained by the consistent decrease in AVP-producing cells. *This work was supported by Progetti di Rilevante Interesse Nazionale (20203AMKTW).*

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# Modeling diabetic keratopathy using organotypic corneal epithelium

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Diabetic keratopathy (DK) is a degenerative corneal disease occurring in more than 50% of diabetic patients. This ocular pathology is due to the hyperglycemic state resulting in morphological and functional changes in corneal layers. Currently, most studies related to the cornea are made on two-dimensional (2D) models, or on animal models. The first ones have the advantage to provide large amounts of data at low cost. However, 2D models poorly represent the complex pathophysiology of the human cornea. On the other hand, the in vivo studies guarantee to reproduce the complexity of the biological events occurring in humans but present ethical problems. Therefore, it is necessary to identify new avenues and models that can integrate the information validly and effectively, to ultimately reduce the number of animals used. The goal of the current study was to characterize a new in vitro organotypic human corneal epithelial tissue model for the study of DK. We cultured corneal epithelial cells in a micro fluidic device through the technology organ-on-chip. The 3D corneal epithelium was subjected to high-glucose conditions to generate a model of DK. Our model showed wellestablished molecular and cellular features of DK, such as (1) epithelial defects and delayed wound repair; (2) inflammation, with increased expression of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and nuclear factork-appa-B (NF-kB). The data provided highlight the utility of 3D corneal epithelium-on-chip in modeling DK. This offers new avenues in drug screening, as well as in precision and personalized medicine.

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**Keywords:** diabetic keratopathy, organotypic, 3D, cytokines, inflammation.

*Neuroscienze I*

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# Temporal dynamic characterization of vascular network in the peripheral and central nervous system of rats with paclitaxel-induced painful peripheral neuropathy

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Painful paclitaxel-peripheral neurotoxicity (painful-PIPN), with paresthesia, numbness, dysaesthesia, and neuropathic pain ranks among the most common dose-limiting toxicity of paclitaxel, one of the most widely employed anticancer drugs. Beside peripheral neurons, for years considered the only reasonable target for neurotoxicity, our recent evaluations in the somatosensory pathway, reveals the microvascular angiogenesis as an important actor in the neuropathic pain development. An abundant microvascular angiogenesis was described in the central (CNS) and peripheral nervous system (PNS) of rats with painful-PIPN.

In this work, we explore the time-dependent changes in the vascular network in the peripheral nervous system during the onset, development, and chronicization of painful-PIPN.

To this aim, rats were treated with paclitaxel 10 mg/kg once a week for 4 weeks or with its vehicle. Animals were tested for neurophysiological abnormalities and pain during the treatment schedule (24 hours after the first drug infusion, 24h, after 2 weeks of drug infusions, 2ws, after 4 weeks of drug infusions, end of treatment, 4ws) and after 4 weeks of follow up (8ws). Neuropathological analysis were performed at the same timepoints on PNS targets, for PIPN characterization. For quantitative analysis of vascular network, samples of PNS and CNS were analysed at synchrotron radiation sources by X-ray Phase-Contrast Tomography (XPCT) Imaging and processed for quantitative and morphological analyses of microvascular structures. Volume rendering allowed a detailed visualization of vasculature at the sub micrometric scale. Single Nuclei RNA Seq experiments were performed to understand the genetic signature of painful PIPN with particular attention to molecules involved in angiogenic processes.

We observed that paclitaxel induces a time and dose dependent irreversible morpho-functional damage in distal caudal nerve and small unmyelinated fibres in the epidermis from week 2 to 8, mechanical allodynia at the end of treatment, that is rescued at the end of follow-up. Vascular density increased in paclitaxel group at 4 and 8W while tortuosity of vessels decreased proportionally, suggesting that vascular abnormalities emerge after morpho-functional nerve damage in paclitaxel-treated animals suggesting that peripheral nerve injury provokes angiogenesis in the involved PNS and somatosensory CNS. Immunohistochemical analysis with Tomatolectin qualitatively confirm the results. Preliminary results of the transcriptomic analysis evidenced transcriptional differences in the somatosensory cortex in response to paclitaxel treatment.

These findings contribute to elucidate if, how and when the vascular component of the neuro-vascular unit can impact on the development and chronicization of painful-PIPN, opening new windows for neuroprotective strategies.

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**Keywords:** neuropathic pain, peripheral neurotoxicity, paclitaxel, vascular network, synchrotron radiation microtomograhy.

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# Maternal neuroendocrine system adaptation during and after childbirth

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Childbirth and motherhood are highly stressful physiological events that trigger fluctuations in various hormones (1). The maternal neuroendocrine system dynamically adapts to balance caregiving demands with recovery of individual homeostasis. However, few studies have longitudinally monitored hormone levels – especially those of the hypothalamic-pituitary-adrenal axis – from childbirth through the first year postpartum, and even fewer have used saliva, the least invasive biological fluid, for such assessments. Our study aimed to measure cortisol and vasopressin during pregnancy and at 1, 6, and 12 months postpartum in mothers and infants. Saliva samples were collected from pregnant women without depression or psychiatric disorders and their infants, with at least n=16 mother-infant pairs at each time point. Samples were stored at low temperatures and analyzed using competitive ELISA. A commercial kit was used for cortisol, while vasopressin was measured with an in-house assay. Results showed elevated cortisol levels during pregnancy (mean + SEM: 635 + 87 pg/mL), followed by a significant decrease at 1 month postpartum (65 + 16 pg/mL, $p<0.0001$), a small increase at 6 months (309 + 87 pg/mL), and a further decline at 12 months (62 + 27 pg/mL, $p<0.0001$). Infants exhibited a similar cortisol trend, with significantly lower levels at 12 months (461 + 167 pg/mL, mean + SEM; $p < 0.03$) compared to 1 (1580 + 286 pg/mL) and 6 months (1715 + 321 pg/mL). Vasopressin was detectable and variable in saliva: levels remained stable from pregnancy (9.8 + 1.9 ng/mL) to early postpartum (11.4 + 3.2 ng/mL), increased significantly at 6 months (20.4 + 3.2 ng/mL, $p<0.01$), then decreased by 12 months (2.3 + 0.9 ng/mL). Infant's saliva samples were not available for vasopressin measurement. These preliminary findings provide new insights into the hormonal profile of the mother-infant dyad during the first postpartum year. The cortisol pattern suggests progressive adaptation to postnatal physiological stress in both mothers and infants, aligning with developmental milestones such as the introduction of solid foods and consolidated night sleep by one year. The decrease in vasopressin after 12 months may reflect physiological adjustments in blood pressure and plasma volume. In addition, vasopressin, like cortisol, is also involved in stress regulation and social behavior modulation and has also been proposed as a biomarker for postpartum depression (2), whereas cortisol's role remains debated (1). Additionally, vasopressin may assist in monitoring exacerbations of subclinical diabetes insipidus during pregnancy (3). In conclusion, our findings highlight saliva as a noninvasive medium for assessing psychophysical well-being and supporting timely interventions for mothers and their children.

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**Keywords:** hormones, pregnancy, hypothalamus-pituitary-adrenal axis, vasopressin, oxytocin, cortisol, saliva.

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# Neuroprotective effect of amantadine in the early model of 6-OHDA induced parkinsonism in rats

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The symptomatic efficacy of amantadine in Parkinson's disease (PD) was first identified over 50 years ago. A contemporary reassessment of its therapeutic potential should extend beyond its well documented dual e icacy on parkinsonian motor symptoms and levodopa-induced dyskinesias (LID). Emerging evidence suggests that amantadine may also play a role in the management of motor fluctuations, non-motor symptoms, and other movement disorders, as well as in the early stages of PD(1). In this study, a rat model of hemi-Parkinsonism was employed, induced by unilateral stereotaxic injection of 6-hydroxydopamine (6-OHDA) (2). We investigated the modulation of the expression of specific markers, including dopamine transporter (DAT), tyrosine hydroxylase (TH), somatostatin, and α-synuclein, following treatment with Amantadine (AMA). AMA was administered intraperitoneally (i.p.) at a daily dose of 40 mg/kg to groups of rats sacrificed at 1, 2, 7, 14, and 21 days following the stereotaxic injection. In parallel, equivalent groups received the same treatment for 7 consecutive days prior to the surgical procedure. Immediately following the intraperitoneal (i.p.) administration of AMA or vehicle (saline solution), rats were subjected to behavioral assessments in the open field. Motor and exploratory behaviors were quantified in 3-minute blocks over a total duration of 20 minutes. Concurrently, the functional integrity of the nigrostriatal pathway was evaluated at all time points. In particular at 7, 14 and 21 days post-treatment, amphetamine-induced rotational testing revealed lower rotational counts in rats pretreated with AMA compared to those receiving 6-OHDA lesions without AMA pretreatment. Our findings indicate that the expression levels of both DAT and TH are significantly elevated in the AMA pretreated group, corroborating its neuroprotective efficacy at time points between 7 and 21 days postlesion, as previously documented in the literature (3). A particularly noteworthy aspect of these results is the enhanced effectiveness of AMA during the presymptomatic stages of the model (time points 1 and 2), where it more robustly preserves dopaminergic neurons and the somatostatinergic system within the caudate-putamen. No significant differences were observed between animals pretreated with AMA prior to the lesion and those receiving AMA concomitantly with 6-OHDA. Collectively, these data highlight the critical importance of early diagnosis in neurodegenerative disorders and suggest that pharmacological interventions initiated during presymptomatic phases, as in the case of AMA, may significantly delay the neurodegenerative process.

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**Keywords:** 6-OHDA, amanatadine, dopamine transporter, tyrosine hydroxylase, α-synuclein, somatostatin.

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# Autophagy is involved in the differentiation of SH-SY5Y cells induced by retinoic acid

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Retinoic acid (RA) is an active metabolite of vitamin A. RA in the all-trans conformation regulates the expression of a large number of target genes involved in cell development, growth and differentiation [1,2]. In line with this, RA is commonly used to differentiate SH-SY5Y neuroblastoma cells [3,4]. This effect is sustained by specific modulation of gene transcription, leading to marked changes in cellular proteins [5,6]. In this scenario, it is conceivable that autophagy, which contributes to cell homeostasis by balancing protein synthesis and degradation, is pivotal in RA-induced cell differentiation.

Therefore, the aim of the present study is to analyze whether modulation of autophagy influences the effects of RA on SH-SY5Y cell differentiation. SH-SY5Y cells were treated with a single dose of 10 nM RA, for 3 or 7 days, alone or in combination with the autophagy inhibitor 3-methyladenine (3-MA), at the dose of 10 mM. In other experiments, autophagy was stimulated by using rapamycin at the dose of 100 nM. The effects induced by RA were compared to those induced by starvation, a condition, which stimulates autophagy. After each treatment, cells were analyzed at light and transmission electron microscopy. Immunostaining for specific markers (nestin, βIII-tubulin, NeuN) and some autophagy-related proteins (Beclin 1, LC3) was also carried out along with phenotype analysis.

We found that both RA and starvation differentiate SH-SY5Y cells. Specifically, RA-induced cell differentiation was concomitant with an increase in autophagy proteins and autophagy-related organelles, which persisted at least for 7 days. This phenomenon was modulated in the presence of autophagy inhibitors or stimulators. Namely, RA-induced cell differentiation was suppressed by autophagy inhibition, while autophagy stimulation enhanced RA-induced differentiation. When autophagy was stimulated for long time interval, cell degeneration appears. These findings indicate that autophagy plays an essential role in sustaining RA-induced differentiation of SH-SY5Y cells.

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**Keywords:** Ultrastructural morphology, cell morphometry, immunoelectron microscopy, autophagy vacuoles, differentiation markers, Beclin 1, LC3. Tipo di presentazione Orale.å

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# Morpho-Functional and Neuroinflammatory Adaptations of the Superior Colliculus in the MPTP Model of Parkinson's Disease

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Visual and visuomotor disturbances are increasingly recognized as early and clinically relevant features of Parkinson's disease (PD). However, the superior colliculus (SC) – a midbrain structure essential for visuomotor integration – remains poorly characterized at the molecular and structural levels in this context. This study provides novel structural and neurochemical data revealing active remodeling of the SC in the MPTP rat model of PD. Adult male rats received MPTP or saline injections, and brains were processed for immunohistochemistry using markers of excitatory transmission (GluR1, GluR2), dopaminergic modulation (D2R), inflammation (IBA1, NLRP3, Caspase-1, IL-1β), and developmental regulation (Pax7). Compared to sham, MPTP-treated animals showed widespread upregulation of GluR1 across SC layers, indicating increased glutamatergic activity. Notably, GluR2 expression was selectively increased in the stratum opticum, suggesting a layer-specific compensatory mechanism to mitigate calcium-mediated excitotoxicity in retino- recipient zones. D2R was significantly elevated in layers below the stratum opticum, consistent with its known distribution in premotor domains. Inflammatory markers were robustly upregulated across all SC layers, indicating a generalized neuroinflammatory response, including NLRP3 inflammasome activation and microglial engagement. Pax7 up-regulation suggests re-activation or amplification of developmental gene programs potentially involved in stress adaptation or neuroplasticity. Although secondary to nigral degeneration, these findings suggest the SC is not a passive relay but an actively remodeling structure, potentially contributing to early visuomotor dysfunctions in PD.

**Keywords:** superior colliculus, MPTP, rat, Parkinson, inflammation.

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# Liposome-loaded Nutlin-3a as a potential candidate for the treatment of proliferative vitreoretinal diseases

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Proliferative Vitreoretinal Diseases (PVDs) are a group of eye disorders that currently represent the leading cause of vision impairment and blindness in developed countries. PVDs are characterized by the formation of avascular or fibrovascular membranes that develop in the retina and vitreous and encompass conditions with significantly different aetiology, such as proliferative vitreoretinopathy (PVR), epiretinal membranes and proliferative diabetic retinopathy (PDR). Membranes with features similar to PVDs can also be associated with neovascular age-related macular degeneration (nAMD). Retinal pigment epithelium (RPE) is deeply involved in the development of retinal proliferative membranes, especially in PVR[1]. Moreover, it contributes to retinal vascular homeostasis in the healthy eye and to uncontrolled neovascularization in pathologic conditions such as PDR and nAMD[2].

Nutlin-3, an inhibitor of p53/MDM2 interaction, demonstrated an antiproliferative effect on RPE cells[3] and inhibited endothelial cell proliferation in a tumor-like microenvironment[4]. Nutlin-3 therapeutic employment is, however, challenging due to the lipophilic nature of the molecule, hampering its administration in aqueous medium. Moreover, drug delivery to the posterior segment of the eye is further hindered by physiological barriers. To overcome these challenges, many nanotechnological delivery systems have been proposed.

In this context, we evaluated the biological effects of Nutlin-3a, the active enantiomer of Nutlin-3, delivered by microfluidic-fabricated liposomes, on RPE and retinal endothelial cell models. In preliminary experiments, aimed at comparing the effects of free Nutlin-3 and Nutlin-3a, Nutlin-3 demonstrated a dose-dependent inhibitory action on proliferation, cell cycle and migration in the ARPE-19 RPE cell line and similar effects were shown by Nutlin-3a. By treating ARPE-19 cells with liposome-delivered Nutlin-3a, we could demonstrate that the carrier system did not impair Nutlin3a's biological effects. Experiments performed on human primary RPE cells reinforced these data, confirming that the inhibitory effects on cell proliferation exerted by liposome-loaded Nutlin-3a and by the free molecule did not differ significantly.

We also evaluated the potential role of liposome-delivered Nutlin-3a as a regulator of angiogenesis. Our data suggested that encapsulated Nutlin-3a exerted an antiangiogenic effect by modulating angiogenic factors, such as VEGF-A, released by RPE cells, and, in parallel, the proliferation and the expression of VEGF-A receptor-2 (VEGFR-2) in retinal endothelial cells.

Overall, our results lay the basis for further studies on liposome-loaded Nutlin-3a as a potential treatment for retinal diseases characterized by a deep pathogenetic involvement of RPE and vascular compartment, such as PVDs, with the aim of evaluating its actual bioavailability, benefits or possible adverse effects in a complex area such as the posterior segment of the eye.

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**Keywords:** PVDs, Nutlin-3a, retinal pigment epithelium, retinal endothelium.

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# Ex-vivo mapping of nigro-thalamic dopaminergic terminals reveals regional specialization and synaptic markers in the human thalamus

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The thalamus plays a crucial role in integrating subcortical inputs and relaying them to cortical circuits, yet the extent and specificity of dopaminergic innervation to human thalamic nuclei remain incompletely understood. Recent tractography-based studies suggest the existence of direct nigrothalamic pathways, but direct histological evidence is still limited. In this study, we provide an exvivo mapping of dopaminergic projections to the human thalamus using high-resolution immunohistochemistry and confocal microscopy.

Human brain specimens were processed to detect multiple dopaminergic markers, including tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), and aromatic L-amino acid decarboxylase (AADC), across anatomically defined thalamic nuclei. Multi-label immunofluorescence and 3D reconstruction allowed precise identification of axonal arborization and synaptic varicosities. Co-staining with NeuN and GFAP enabled structural delimitation of neuronal and glial boundaries within the mediodorsal (MD), centromedian (CM), and ventral anterior (VA) nuclei.

Quantitative analysis revealed a marked enrichment of TH+ and VMAT2+ varicosities in the MD nucleus compared to CM and VA. Densitometric values and colocalization analysis support the notion of region-specific dopaminergic input.

These results provide the first immunohistological confirmation of selective dopaminergic innervation of the human thalamus. This structural evidence complements prior neuroimaging findings and suggests a potential role for the nigro-thalamic pathway in modulating thalamo-cortical circuits involved in executive and cognitive functions.

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**Keywords:** thalamus, dopamine, immunohistochemistry, substantia nigra, human brain, VMAT2, NeuN, GFAP.

*Istogenesi, funzioni e patologie*
*del sistema muscolo-scheletrico*

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# Le differenti diete modulano diversamente la salute ossea: modelli murini

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Il tessuto osseo è un connettivo specializzato che è soggetto a continuo rimodellamento al fine di preservare la sua salute/qualità. La salute dell'osso è influenzata per tutta la vita dall'interazione tra fattori genetici, ormonali, ambientali, attività motoria e diete/alimentazione. Lo scopo di questo studio è valutare l'effetto di diete con diverso contenuto in grassi sulla salute ossea, valutando in particolare attraverso micro-CT e studi istologici femori di topi alimentati per 16 o 20 settimane con dieta normale (ND16 and ND20) o western (WD16w and WD20w), e per 20 settimane combinando ND e WD con la dieta chetogenica (KD) (WD+ND20w, ND+KD20w, WD+KD20w, rispettivamente). L'analisi di micro-CT su osso trabecolare di femori ha dimostrato la diminuzione del BV/TV% e dello spessore delle trabecole (Tb.Th) nei topi trattati con WD+KD20w rispetto a quelli alimentati con WD20w ($p<0.05$).

Lo spessore dell'osso corticale risulta significativamente ridotto nei topi alimentati con WD16w e

WD20w rispetto a quelli alimentati con ND16w and ND20w ($p<0.05$), ma anche, in topi alimentati con WD20w and WD+KD20w comparati a quelli alimentati con WD+ND20w ($p<0.01$). Coerentemente, l'analisi istologica ha dimostrato che il numero di osteoclasti/perimetro osseo trabecolare incrementa notevolmente in topi alimentati con dieta WD+KD20w rispetto ai gruppi ND20w e WD20w ($p<0.05$). Il numero di osteoblasti a livello trabecolare risulta diminuito nelle diete combinate con la KD comparate ai rispettivi controlli. Diversamente, nell'osso corticale confrontando topi alimentati WD20w con WD+KD20w un decremento del numero di osteoblasti è evidente ($p<0.05$). Il numero degli osteociti non risulta significativamente variato comparando le diverse diete. Inoltre, i risultati ottenuti con la colorazione di Masson supportano i risultati ottenuti dalla micro-CT sia a livello dell'osso corticale che trabecolare. In conclusione, abbiamo dimostrato che i diversi tipi di diete con contenuto progressivo di grassi (ND, WF, WD+KD rispettivamente) influenzano la salute ossea.

**Keywords:** Diete, salute ossea, modelli murini, cellule ossee.

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# Extracorporeal Shockwave Therapy-induced myogenesis in C2C12 cells is accompanied by mitochondrial biogenesis: an ultrastructural study

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Extracorporeal shockwave therapy (ESWT) is a non-invasive treatment that has gained popularity over the past decade for treating various musculoskeletal conditions, including both bone and soft-tissue disorders. ESWT has demonstrated beneficial effects in treating muscle injuries.

In vivo studies on animal models have shown that ESWT acts at multiple tissue levels and exhibits various effects [1]. ESWT can enhance muscle regeneration by improving muscle microcirculation, stimulating tissue regeneration, and reducing inflammatory response.

However, little is known about the exact biological mechanisms elicited by shock waves (SW) that are involved in muscle repair, such as the putative myogenesis response, which remains largely unexplored.

Myogenesis is a complex process governing skeletal muscle development and homeostasis. The early steps of myogenesis include myoblast differentiation, during which this mononuclear muscle precursor expresses skeletal muscle-specific genes and fuses with nearby myoblasts to form multinucleated myotubes [2-3].

Differentiation of myoblasts into mature myotubes needs metabolic changes to meet the increased energy demands of the forming contracting muscle tissue. To supply the highest ATP requirement, myotubes can both add oxidative components and increase the mitochondrial network [4-5].

Therefore, the present study aimed to investigate the effects of SW on the C2C12 myoblast cell line at the ultrastructural level, with a particular focus on its potential impact on mitochondriogenesis.

The shockwave treatment was applied to cell flasks in a water bath using a shockwave generator Duolith® (Storz Medical AG, Tägerwilen, Switzerland), at a dose of 0.1 mJ/mm$^2$ energy level, 3 Hz, and 500 impulses.

In this study, we found that soon after SW exposure, C2C12 myoblasts upregulate autophagy. Autophagy is an early event in myogenic differentiation and precedes mitophagy and mitochondria network remodelling. Indeed, we observed a progressive increase in mitochondrial number accompanied by a concomitant reduction in mitochondrial size. These ultrastructural findings are indicative of ongoing mitochondrial biogenesis and may reflect the presence of newly formed mitochondria.

These data highlight the role of SW in promoting myogenesis, a multistep process that begins with autophagy activation, proceeds with mitochondrial clearance, and concludes with mitochondrial repopulation. The resulting myotube is enriched with newly formed mitochondria, improving the cell's capacity to generate energy more efficiently.

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**Keywords:** ESWT, myoblasts, myotubes, mitochondriogenesis, mitophagy, autophagy.

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# Desmin acts as a new and early anabolic signal for skeletogenic progenitors in response to akt-dependent inhibition of GSK3β by myo-inositol: a bioinformatic, morpho-functional, and translational study by myo-inositol: a bioinformatic, morpho-functional, and translational study

F. P. Quartulli[1], G. Di Conza[1], F. Barbaro[1], Y. Gzara[1], F. Ariodante[1], E. Gabusi[2], G. Lisignoli[2], S. Mosca[3], M. Mosca[4], S. Sprio[5], A. Tampieri[5], R. Toni[5,6,7]

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**Introduction:** Recently, we observed that synthesis of the cytoskeletal filament desmin (DES) increases in parallel with that of RUNX2 and Alkaline Phosphatase (AP) in rat, bone marrow (BM)-mesenchymal stromal cell (MSC) colonies forming osteoid nodules *in vitro* (1). Since *in silico* bioinformatic simulation of the interactome linking DES to RUNX2/AP revealed involvment of the PI3K/AKT-dependent, Rhoa GTPase and GSK3β (2), we hypothesized that PI3/AKT-mediated restraint of GSK3β would maintain DES in an active dephosphorilated state, ultimately enhancing osteogenesis. To test this, we studied the effect of the PI3K/AKT activator myo-inositol (MYO), in doses previously investigated to *in vivo* stimulate bone anabolism in insulin resistance (3).

**Materials e Methods:** Bioinformatic analysis of the DES osteogenic interactome was based on STRING12 e STITCH5 databases, whereas biological relevance of the interactions between the DES-Akt-GSK3β proteins, cytoskeletal filaments, and osteogenic trascription factors + MYO was achieved by topological analysis using the Gephi software. The embryonic murine fibroblast cell line NIH 3T3 (courtesy of Prof. Lucio Cocco) and human, primary BM-MSC were used in relation to their multidifferentiation potential. DES was labeled by immunocytochemistry using rabbit polyclonal Abs (1:50-1:800) and either the Fast Red-AP or ABC/DAB systems. Osteogenesis (2.5-100x$10^3$cc/monolayer) was induced with ascorbic acid/β−glycerophosphate/dexametazone, and studied between 48h28 days in dependance on the cell type. MYO was administered in a 320-640 μM range, and mineralization studied with Alizarin Red histochemistry, and quantitative spectrophotometry of deposited calcium. Statistically significant differences ($p<0.05$) between control and treated cells were obtained using one-way ANOVA, Turkey HSD post-hoc tests.

**Results**: Topological analysis of the DES interactome predicted that AKT acts as an "hub" and "bottleneck" for the DES signal *en route* to RUNX2 and AP through vimentin, β-actin, Rhoa, and Hspb1 (Hsp27), to finally impinge onto GSK3β, the latter accessed by MYO directly and through AKT-mTOR. In addition, the DES signal may reach RUNX2/AP through Vim-Vinc-Rhoa and Gal1-SPARC. Consistently, both mouse and human osteoprogenitors were positive for DES (83 and 85-88%), gave rise to osteoid nodules enriched with DES and, when NIH 3T3 cells were challenged with MYO increased mineral deposition at a statistically significant level.

**Conclusions**. DES represents a new and early signal in the osteogenic chain, it is conserved in rodents and humans, and involves the AKT-GSK3β system. In fact, increased osteogenesis by MYO suggests AKT activation, able to inhibit GSK3β that, in turn may preserve DES in a functional dephosphorilated state. This introduces a new perspective in bone anabolism, whose translational application to bone loss disorders is under scrutiny (4).

**Aknowledgements:** Grants PRIN P20224TAETP and P2022H74YP.

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**Keywords:** Desmin, mesenchymal stromal cells, osteogenesis.

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# Foot Placement in Upright Posture during stabilometric exam: Anthropometric and Stabilometric correlations

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Foot position is a key factor influencing the postural stability[1], defined as the capability of the Postural Control System[2] to preserve balance modulating myofascial chains of the body against gravity and providing continuous neuromuscular adaptations in response to sensory input from the internal body and the external environment [3]. Postural stability can be evaluated by pressure plate; conflicting indications have been reported for the ideal foot placement during stabilometric exams. The aims of this study were to evaluate the correlation between anthropometric measurements (AMs) and between-feet measurements (BFMs) in self-selected comfortable foot position (SCFP) and the effect of comfortable and standardized foot position (SFP) on plantar pressure and stabilometric parameters. Stabilometry was conducted on twenty healthy subjects in terms of SCFP and SFP. Correlation between AMs and BFMs in SCFP was investigated via Pearson's analysis. Data variability was assessed using the coefficient of variation, and statistical differences between SCFP and SFP were evaluated via the Wilcoxon test. No correlation was found between AMs and BFMs. Subjects placed their feet nearly parallel in SCFP with a wider inter-heel distance. The variability of plantar pressure parameters was greater in SFP. A lower foot contact area on the right side and higher plantar pressures in the left midfoot region (p-value < 0.05) were found in SFP as compensatory foot adaptations. This study revealed how healthy subjects tend to adopt a comfortable foot position during upright standing, characterized by feet placed nearly parallel to increase the feet's support surface, which was not affected by anthropometric measurements such as height, inter-ASIS, and inter-Acromion distances. Instead, when a standardized foot position was imposed, an increase in variability in plantar pressure and stabilometric parameters was observed. Additionally, a decrease in the foot contact area of the dominant foot and compensatory increases in plantar pressures on the non-dominant side, mostly in the midfoot region, were found. These findings highlight the usefulness of allowing healthy subjects to choose a comfortable foot position during stabilometric exam in order to increase the reliability of plantar pressure and posture stability parameters used for clinical assessment and for statistics in scientific research.

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**Keywords:** postural control, anthropometry, feet position, baropodometry, pressure plate, proprioception.

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# The Calcifying Turkey Tendon: Not Just Mineralized Collagen Fibrils

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The calcifying turkey tendon has been a frequent subject for the study of the collagen-mineral relationship, but larger-scale features of this tissue were hardly ever considered. We investigated the structure and ultrastructure of the mineralized portion of turkey gastrocnemius tendon using micro computed tomography (microCT) and high-resolution scanning electron microscopy (HR-SEM).

The microCT (to the right, a 3 mm-long slice of the calcified zone, approx. 4 x 1 mm in crosssection) shows that over one third of the volume of the calcified tendon is actually represented by large longitudinal or oblique vascular channels, up to 200 μm wide and highly reminiscent of the Havers and Volkmann canals of bone. In crosssection, the calcified matrix appears denser and more compact in the immediate proximity of the vascular channels, while among them it appears less mineralized and dotted by minute pores. The mineralized gastrocnemius tendon is structured into a particularly complex hierarchy of collagen fascicles, mostly 2 to 5 μm in diameter and gathered into higher-order bundles, each wrapped by abundant endotenon and cellular processes. Elongated spaces aligned with the tendon axis contain rows of approximately isometric cells, and an intricate canalicular network similar to that of the osteocytes radiates from the cellular and vascular spaces through the surrounding matrix.

High-magnification micrographs of cryo-fractured specimens reveal a great heterogeneity of the mineral component: the central portions of the collagen fascicles contain tightly packed, tiny particles whereas large curved mineral plates, exceeding hundreds of nanometers (that is, exceeding by far the size of single collagen fibrils) are visible at the periphery. This may explain the conflicting results reported in other studies. The mineral plates can grow into ribbon-like structures (left) that run orthogonal to the collagen fibrils axis for a great length, interconnecting adjoining collagen fibrils and keeping them in phase with each other. This mechanism may increase the interfibrillar coupling and may contribute to the superior mechanical strength of the calcified tendon.

**Keywords:** Tendon, Mineralization, Electron Microscopy, Extracellular matrix.

# *Anatomia e patologia del distretto odontoiatrico*

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# In vitro cytotoxic effects after exposure switching of alternative smoking devices (IQOS and electronic cigarette) in oral human cells

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In recent years, alternative devices to traditional smoking (TS) such as electronic cigarettes (E-cigs) and heated tobacco devices (IQOS), have been introduced in order to limit the harmful effects. To date, it is still unclear how and to what extent these devices can reduce cytotoxic effects, especially following the replacement of traditional smoking. The aim of this in vitro study was to investigate the biological effects of switching from TS to alternative smoking device on human gingival fibroblasts and oral keratinocytes.

TS (Gold Pocket, Marlboro), E-cig (Noir Smoke) and IQOS (Philip Morris) extracts were used at concentrations between 100% (undiluted) and 3.12%, administered for 4 hours for 2 days with TS and for the following 2 days with medium (to simulate smoking cessation), or with E-cig or IQOS (to simulate smoke switching). Cell viability (MTT), apoptosis and cell cycle (flow cytometry) and DNA damage (immunofluorescence) were evaluated on fibroblasts. Cell viability, IL-1β and IL-6 protein level (ELISA) and gene expression (real-time-PCR) and DNA damage were performed on keratinocytes.

In fibroblasts TS significantly reduces vitality, especially at low dilutions ($p<0.001$). This decrease remains if the exposure continues with the medium alone, while it is reduced by the E-cig and IQOS. All extracts (TS, E-cig, IQOS) induce increased apoptosis, weakly reduced following treatment with IQOS. DNA damage induced by TS at low dilutions is partially reduced especially by switching to IQOS.

In keratinocytes, the switch study showed a recovery of the viability induced by TS when replaced with medium alone, recovery not always detectable with E-cig and IQOS. Only low dilution TS induced an increased secretion of IL-1β ($p<0.05$), only partially reduced by the switch with medium. The transcription of both cytokines undergoes variations not always cancelled by the switch. DNA damage induced by TS is only partially recovered following the switch with medium or IQOS ($p<0.001$); the switch to E-cig causes the greatest damage.

Alternative devices to TS seem to reduce toxicity in oral cells, especially in fibroblasts and, in particular, the switch to IQOS seemed to be more effective than the E-cig in reducing the cytotoxic effects of smoking. In keratinocytes the effects were more marked and the switch to the E-cig enhance the toxic effects. Further studies with long-term models are needed to fully understand the impact of the switch on oral cells.

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**Keywords:** switch, fibroblast, keratinocyte, toxicity.

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# New Insights into the Morphological Aspects of Unilateral Condylar Hyperplasia

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Unilateral condylar hyperplasia (UCH) is a rare benign condition characterized by abnormal growth of the mandibular condyle, often resulting in facial asymmetry and temporomandibular joint (TMJ) dysfunction. UCH is typically classified into "active" and "inactive" forms based on growth dynamics and Single-Photon Emission Computed Tomography (SPECT) imaging findings: active UCH shows continued condylar growth with positive scintigraphy, while inactive forms are considered stabilized. However, the histological characteristics of inactive UCH remain insufficiently explored, and its potential for reactivation is not well understood. This study aims to investigate the morphological and molecular features of inactive UCH and to evaluate its distinction from the active form, with the goal of identifying possible signs of residual activity that could indicate slow progression or potential reactivation.

Biopsy specimens from patients with inactive UCH undergoing proportional condylectomy were evaluated using conventional histological staining and immunofluorescence analyses targeting cartilage matrix and bone remodeling markers, such as collagen types I and II, Matrix Metalloproteinase 2 (MMP-2), Matrix Metalloproteinase 9 (MMP-9), Receptor Activator of Nuclear Factor κ B (RANK), and osteocalcin. Preliminary findings reveal structural alterations in condylar architecture that suggest deviations from a fully quiescent state. These include modifications at the cartilage–bone interface and changes in the organization of the hypertrophic cartilage layer. Observations also indicate varying levels of expression of markers related to matrix turnover and osteoclastic activity, providing further insight into the biological state of the tissue.

These results suggest that inactive UCH may exhibit histological and molecular features indicative of low-grade or residual activity, despite negative imaging. This supports the hypothesis that UCH may exist along a dynamic pathological spectrum rather than as a strictly binary condition. Proportional condylectomy remains a reliable and conservative surgical approach in the management of UCH, including inactive cases, with positive outcomes in terms of function and aesthetics. Further studies involving additional structural analyses are necessary to understand the underlying pathogenetic mechanisms better and refine histological diagnostic criteria.

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**Keywords:** unilateral condylar hyperplasia, histology, cartilage, immunofluorescence.

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# In vitro evaluation of the neuroprotective effect of natural bioactives, garcinoic acid and genistein, targeting nuclear receptor pathways administered via the nose-to-brain delivery system

Ina Varfaj[1], Gabriele Di Sante[2], Anna Maria Stabile[2], Anna Migni[1], Gina Cavaliere[1], Alessandra Pistilli[2], Vincenzo Falcone[1], Bruno Cerra[1], Giovanna Traina[1], Aurélie M. Schoubben[1], Antimo Gioiello[1], Andrea Temperini[1], Roccaldo Sardella[1], Claudia Zadra[1], Francesco Galli[1], Mario Rende[2], Desirée Bartolini[1]

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Neurodegenerative diseases are characterized by a progressive alteration of the structure and functionality of the nervous system due, among other things, to oxidative stress, mitochondrial dysfunction and neuroinflammation (1).

Delivering neuroprotective agents to the brain is hindered by the blood-brain barrier (BBB). Intranasal administration offers a promising route by bypassing the BBB via the olfactory region, enabling direct brain access (2).

Our research aims to test natural bioactives, garcinoic acid (GA) or genistein (GE) for intranasal delivery to overcome the challenges posed by the BBB. Intranasal administration offers a noninvasive route to the brain, though it faces barriers. GA, selected for its interaction with the Pregnane X Receptor (PXR) (3) and GE for its activity on Peroxisome Proliferator-Activated Receptor gamma (PPARγ) (4). These bioactives were tested in *in vitro* co-culture model simulating nasal mucosa (nasal/glial cells).

Preliminary data were obtained using glial cells and we observed that GE and especially GA showed reduced toxicity both in glial cells (HMC-3 cells) and in nasal epithelial cells (RPMI 2650 cell line). Furthermore, GA and GE exhibited redox 'scavenger' and anti-inflammatory activity on glial cells inflamed with LPS.

In co-culture model, GA and GE were able to reduce intracellular ROS, $H_2O_2$ release, SOD2 levels increasing the level of detoxifying enzyme Catalase (CAT) in glial cells inflamed with LPS through the nasal epithelium. In addition, GA and partially also GE crossed the nasal epithelium and, in glial cells inflamed with LPS stimulated the activation of PXR and its downstream genes promoting neuroprotection processes.

Additionally, GE crossed the nasal epithelium and stimulated the activation of PPAR-γ in glial cells inflamed with LPS, confirming its agonist activity on this receptor in human glial cells and in addition this bioactive reduced the levels of pro-inflammatory cytokines (IL-6 and TNF-α) in this co-culture model.

In conclusion, in the in vitro co-culture model of nasal/glial cells, it was demonstrated GA and GE could be directly delivered to glial cells through the nasal epithelium and these molecules were able to modulate two key signaling pathways involved in neuroprotection in glial cells: the PPARγ and PXR pathways, exerting both anti-inflammatory and redox scavenger effects.

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**Keywords:** Neuroprotection, co-culture model, nuclear receptors, garcinoic acid, Genistein, nasal mucosa, PXR, PPARγ.

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# Micro- and Nanoplastic release from orthodontic aligners: In-Vivo inflammatory and oxidative responses in a Hirudo verbana model

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Orthodontic aligners, increasingly preferred for their aesthetic and functional advantages, are fabricated from polymer-based materials through thermoforming (TFA) or 3D printing (DPA). Recent studies have raised concerns about the release of microplastics (MPs) and nanoplastics (NPs) from these devices due to the mechanical stress they are subjected to during use. Although the environmental and health risks of plastic particles are gaining attention, the biological effects of particles specifically released from dental aligners remain largely unexplored.

To address this knowledge gap, we employed the medicinal leech *Hirudo verbana* as an *in vivo* model to investigate the short-term biological impact of MPs and NPs released from polymeric aligners under simulated mechanical oral conditions. Our multidisciplinary approach combined molecular biology and microscopy techniques to assess inflammatory and oxidative stress responses. Gene expression analyses revealed significant upregulation of *HmAIF-1* and *HvRNASET2* (inflammation markers), as well as superoxide dismutase (SOD) and glutathione-S-transferase (GST) (oxidative stress markers) within 24 hours of exposure. Histological and transmission electron microscopy further confirmed pronounced tissue remodeling, including neovascularization and recruitment of macrophage-like cells.

These findings show that even short-term exposure to plastic particles from aligners can disrupt tissue homeostasis by triggering inflammatory and oxidative responses. This highlights the need for usebased testing and improved material design to minimize plastic particle release and safeguard patient health.

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**Keywords:** 3D-printed aligners, micro-nanoplastics, biocompatibility, oxidative stress, immune response.

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# Micro and Nanoplastic Release from Orthodontic Appliance: In Vitro Cytotoxicity on Human Dental Pulp Stem Cells

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Clear aligners are widely used in orthodontics, yet concerns persist regarding the biological effects of micro- and nanoplastic particles potentially released during wear. This study subjected various aligner materials to mechanical friction to simulate intraoral stress. Plastic residues were collected directly into the cell culture medium and tested on human dental pulp stem cells (hDPSCs), isolated from healthy young non-smoking donors to ensure biological reliability.

Cells were exposed to plastic-containing media for 24, 48, 72, and 96 hours at 500, 1000, and 5000 ng/100 µl concentrations. Preliminary morphological evaluation under an inverted microscope confirmed cell-plastic interaction and maintained metabolic activity in selected samples. We then conducted the CyQUANT™ MTT Cell Proliferation Assay to assess viability and proliferation based on the mitochondrial reduction of MTT into formazan.

Our results demonstrated markedly fewer plastic residues and improved cell viability in cultures exposed to IPE and thermoformed materials, in contrast to the higher levels of debris and significant cytotoxicity observed with 3d printed materials. Under the latter conditions, cells exhibited detachment and morphological signs of damage, indicating a material-dependent biological impact. These findings underscore significant differences among aligner materials regarding their biological safety under mechanical stress, indicacng that the choice of material may affect mechanical performance and cytocompacbility.

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**Keywords:** cytotoxicity, microplasccs, nanoplasccs, orthodoncc devices.

*Neuroscienze II*

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# Translational validation of molecular pathways involved in dystonia by DYT1 mouse model and accessible biofluids of affected patients

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*Introduction:* Dystonia is an hyperkinetic movement disorder characterized by sustained muscle contractions causing abnormal postures. No effective therapies are currently available for dystonia. Synaptic plasticity and alterations of the perineural network represent promising targets that can be explored in animal models and validated in peripheral biofluids of patients. Among them, saliva is an easily accessible biofluid which has demonstrated a promising and consolidated potential in the detection of biomarkers for Parkinson's Disease[1,2].

*Objectives:* Morphological investigation of synaptic plasticity, *TGFbeta* pathway and perineuronal network in the brain of DYT1 mice and translation to dystonic patients by the analysis of easily accessible biofluids including saliva and serum.

*Methods:* DYT1 and control mice were used for immunohistochemistry (IHC) and immunofluorescence (IF) for synaptic and SNARE complex proteins. Gomori staining, along with IHC and IF for Collagen type I, III and IV and *TGFbeta* were used to investigate changes in the peri-neuronal matrix. 35 cervical dystonia patients and 22 age and sex-matched healthy subject were submitted to ELISA for total and oligomeric a-syn, synaptophysin, CamK2beta, synaptobrevin, *TGfbeta* and *TNFalpha* in both saliva and serum.

*Results:* Morphological alterations of synaptic and SNARE complex staining were detected in different brain areas of DYT1 mice, including: striatum, cerebellum and pontine nuclei. A remodelling of the peri-neuronal network was detected, with a different balance between the collagen isoforms. TGFbeta pathway was altered with intracellular accumulation in medium spiny neurons and Purkinje cells. Molecular changes detected in animal models were substantially reproduced in the patient's biofluids, where ELISA analysis showed: increased levels of Tor1A ($p<0.01$) and synaprophysin ($p<0.01$), as well as decreased levels of CamK2beta ($p<0.01$) in the saliva of dystonic patients compared to HS. *TGFbeta* and *TNFalpha* were increased in the serum of dystonic patients and positively correlated with the levels of CamK2beta in saliva ($p<0.01$).

*Conclusions:* Our study provides the first translational analysis of Dyt1 models in relation with dystonic patients and support an altered cross-talk between SNARE complex/synaptic plasticity *TGFbeta* pathway and peri-neuronal matrix organization in dystonia.

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# Predictive biomarker of Parkinson's Disease in intranasal rotenone-treated mice

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Parkinson's disease (PD) is a neurodegenerative disorder characterised by progressive neuronal dysfunction, which is difficult to detect in its early stages. The *VGF* gene [1] encodes the proVGF protein, which is cleaved into multiple peptides named according to their initial four amino acids [2]. We previously observed a selective decrease in peptides cleaved from the C-terminus of proVGF in plasma from drug-naive PD patients and in animal models mimicking the advanced PD disease [3,4]. However, no studies to date have evaluated VGF peptides in progressive PD animal models or assessed VGF levels in both brain and plasma from the same animals. In this study, we used a progressive and early PD mouse model based on intranasal rotenone treatment. The model follows Braak's hypothesis [5], in which PD pathology originates in the olfactory bulb (OB) and progresses further to the midbrain time-dependently. The aim was to validate VGF C-terminal peptides as early biomarkers of PD assessing their changes in brain and blood in an intranasal rotenone model. Male BALB/c mice were divided into three cohorts based on the time of rotenone exposure, i.e., at 2, 3, and 6 months (at least 6 per group). Rotenone microemulsion was prepared as previously described [6]. Behavioral tests included the butyric acid avoidance [7], inverted grid [8], and pole tests [9] to assess olfactory capacity function, neuromuscular deficits, and basal ganglia-related motor dysfunction, respectively. After sacrifice, the brain was dissected from the skull, and total RNA was extracted from the olfactory bulb (OB) and striatum to perform qPCR for *Vgf*, *Th* (tyrosine hydroxylase), and *Snca* (alpha-synuclein). Blood samples were collected in EDTA tubes for quantification of the following VGF C-terminal peptides: VGF485–502 (NAPP), VGF554–563 (TLQP), VGF586–595 (AQEE), and VGF609–617 (the nonapeptide at the C-terminal end of proVGF), using an in-house competitive ELISA. Results showed significant olfactory deficits starting at 3 months and persisting through the study, whereas motor impairments appeared only after 6 months of treatment. qRT-PCR showed increased *SNCA* and decreased *TH* expression in the OB and striatum only at 6 months. *VGF* mRNA levels remained unchanged in all brain regions across cohorts. In contrast, ELISA analysis revealed that three VGF peptides as NAPP, TLQP, and C-terminus were significantly reduced in plasma at 3 months, persisting decreased at 6 months. As motor dysfunction and gene expression changes (*Th* and *Snca*) emerged only at 6 months, the model recapitulates a progressive PD-like phenotype, including early-stage pathology. Notably, reductions in VGF C-terminal peptides were detectable in plasma at 3 months, concurrent with olfactory deficits but before motor impairments or central *VGF* expression changes, suggesting early post-translational processing of VGF. These findings support the potential utility of VGF C-terminal peptides as early biomarkers for PD diagnosis at the premotor stage.

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# Decoding the functional architecture of the inferior fronto-occipital fasciculus: a multimodal neuroimaging approach

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The inferior fronto-occipital fasciculus (IFOF) is a prominent long-range association pathway connecting occipital cortex with prefrontal regions, including dorsolateral, ventrolateral, and orbitofrontal cortices. Growing clinical and cognitive neuroscience evidence implicates the IFOF in a variety of functions requiring integration of visual information with higher-order processing, such as semantic retrieval, emotion recognition, and reading. Emerging models suggest that the IFOF exhibits a functional gradient along its ventral-to-dorsal extent, spanning from perceptual to conceptual processing domains. However, the internal functional organization of the IFOF remains scarcely characterized.

In this study, we systematically investigated the functional topography of the IFOF using an novel multimodal neuroimaging framework. We integrated tractography from diffusion MRI with dynamic connectivity analyses derived from resting-state fMRI, using data from two independent, high-quality datasets that include repeated scanning sessions. Additionally, we introduce a novel method to project large-scale, meta-analytic task-fMRI activation maps onto white matter tracts, allowing inference of cognitive domains associated with specific subregions of the IFOF. Our analyses revealed consistent non-overlapping parcellations of the IFOF into ventral and dorsal components: a ventral cluster linking medial and ventral occipital with lateral orbitofrontal and frontopolar cortices; and a dorsal cluster connecting lateral occipital to inferior, middle and superior frontal gyri. Such an anatomical separation aligned with distinct functional profiles. Ventral segments were predominantly associated with visual and socio-emotional processing – such as face perception and attentional control – while dorsal portions were more tightly linked to language and semantic tasks. These distinctions also demonstrated lateralized patterns across hemispheres, further supporting the presence of a graded functional architecture within the IFOF. Our findings provide converging evidence for a graded functional architecture within the IFOF, supporting its differential contributions to perceptual and conceptual cognitive processes. Moreover, this work complements and expands the current models of functional organization of long-range association fibers, highlighting the utility of multimodal approaches for dissecting the functional architecture of long-range white matter pathways and contributing to ongoing efforts to map structure-function relationships in the human brain.

**Keywords:** connectivity, functional MRI, tractography, behavior.

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# A white matter–based neuroimaging biomarker for anatomically-informed survival prediction in glioblastoma

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Tractography has opened exciting new avenues for understanding brain structure and pathology, including the complex interactions between gliomas and white matter pathways. Glioblastomas (GBMs), the most aggressive primary brain tumors, demonstrate invasive growth patterns and have been recently shown to form synaptic connections that further promote tumor progression[1]. Mapping how GBMs interface with the white matter architecture is therefore critical for understanding tumor spread and informing prognosis[2]. In this study, we developed a novel biomarker – the Lesion-Tract Density Index (L-TDI) – to quantify the extent to which GBMs structurally engage with white matter pathways. The white matter scaffold structurally connected with each tumor was identified by embedding tumor masks from GBM patients into large-scale normative tractography data[3] and extracting the streamlines intersecting each lesion. We then computed the L-TDI, based on the average tract density values overlapping with each lesion, providing a continuous measure of tumor-white matter interaction. We also computed a Tract Density Index (TDI) as an indirect measure of tumor infiltration[4], by averaging the tract density within the GBM mask.

We applied this approach to two independent patient cohorts (N=367 and N=496) and found that the L-TDI provides a morpho-anatomical model of overall survival. Patients with higher L-TDI values exhibited poorer outcomes across multiple statistical tests and survival models. When applying unsupervised clustering to the two-dimensional space defined by tumor volume and TDI, three distinct GBM subtypes emerged: i) small tumors with low TDI, ii) small tumors with high TDI, and iii) large tumors with intermediate TDI. Post-hoc comparisons revealed that patients with small tumors and low TDI had significantly longer survival compared to those with small tumors and high TDI. Interestingly, the first group comprised tumors that mostly involved association pathways, while the GBMs in the other two primarily engaged projection, and, to a lesser extent, association and commissural pathways. Furthermore, the L-TDI improved Cox proportional hazard models when combined with clinical variables such as age, methylation status, extent of resection, and cognitive function. Finally, a logistic model incorporating L-TDI successfully predicted 12-month mortality with promising accuracy and robustness across cohorts.

These results suggest that tractography-derived, anatomically explainable biomarkers such as LTDI can provide rapid, reproducible, and clinically actionable insights to complement existing prognostic tools. While the use of normative models enables cost-effective and scalable application in clinical settings, further validation with patient-specific diffusion imaging remains imperative. Ultimately, this approach may inform more personalized treatment strategies by identifying patterns of tumor-white matter involvement that influence both prognosis and therapeutic planning.

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# Neuroanatomical correlates of preserved reflexive but impaired voluntary inhibition in Parkinson's disease: behavioural results and multimodal imaging of oculomotor circuits

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Oculomotor impairments are an early and sensitive hallmark of Parkinson's disease (PD), reflecting progressive dysfunction in cortico-subcortical motor pathways. These deficits span a range of eye movement functions, affecting both voluntary control, governed by frontal cortical areas, and reflexive inhibition, mediated by subcortical brainstem circuits. To anatomically dissociate these control levels, we compared a classical antisaccade task – dependent on cortical structures such as the frontal eye fields (FEF) and their projections to the basal ganglia and brainstem – with a saccadic inhibition (SI) paradigm probing automatic suppression of eye movements triggered by irrelevant visual transients, largely engaging the superior colliculus and mesencephalic reticular formation. We recorded eye movements in 15 PD patients and 15 age-matched healthy controls. PD patients exhibited marked impairments in voluntary saccade suppression, with high inter-individual variability, consistent with dysfunction in frontal-striatal inhibitory control. In contrast, reflexive SI responses were preserved in PD, though with prolonged inhibitory periods, suggesting a relative sparing of subcortical automatic inhibition mechanisms and possible delay in motor reprogramming. To explore the anatomical underpinnings of these behavioral findings, we are currently acquiring multimodal neuroimaging data, including structural MRI, diffusion tensor imaging (DTI), restingstate functional MRI, and [18F]-DOPA PET. These ongoing studies aim to map the microstructural and functional integrity of oculomotor circuits and correlate anatomical alterations with behavioral performance, shedding light on the differential vulnerability of cortical and subcortical networks involved in eye movement control in PD.

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# Chrysin-Loaded Extracellular Vesicles attenuates LPS-Induced Neuroinflammation in BV2 Microglial Cells In Vitro: A novel neuroprotective strategy

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Neuroinflammation, driven by activated microglia, contributes to the progression of neurodegenerative diseases. The process is driven by the polarization of glial cells, among which microglia have distinct activation profiles, including pro-inflammatory phenotypes. Specifically, microglial activation demonstrates a highly dynamic and often overlapping spectrum, with subsets of M2 activation resulting from a wide range of inflammatory mediators. These different activation states allow microglia to respond by inducing or resolving inflammation, which can be deleterious or beneficial to neurons. Following an infectious trigger or injury, pro-inflammatory signaling cascades are activated in microglia. This activation leads to the production of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β). These cytokines are released and spread to the nearby tissues, where they trigger a positive feedback loop that induces the activation of surrounding microglia and contributes to the sustained inflammation over time. Extracellular vesicles are biologically active, nanoscale, membrane-bound particles released by all cell types. Indeed, studies have shown that under conditions of cellular stress, microglia were able to upregulate the release of EVs containing proinflammatory mediators, such as IL-1β, IL-6, TNF-α, and caspase-1, contributing to the propagation of neuroinflammation. The ability of EVs to transport bioactive material between cells is crucial to their therapeutic potential. Chrysin, a natural flavone found in fruits and

propolis, has demonstrated anti-inflammatory effects. This study explored the immunomodulatory potential of chrysin-loaded EVs (EVs-Chry) derived from BV2 microglial cells. BV2 cells were treated with chrysin for 24 hours to assess cytotoxicity and proliferation. EVs were isolated from treated and untreated cells, characterized by nanoparticle tracking analysis, and applied to naïve BV2 cells prior to LPS stimulation. Effects on cell morphology, migration, cytokine expression (IL-1β, IL-6), inflammasome activity (caspase-1), and apoptosis-related protein Bcl-xL were investigated. Our results show that EVs-Chry significantly reduced LPS-induced cell proliferation, restored resting microglial morphology, and reducing migratory capacity. Furthermore, Co-treatment with EVs-Chry and LPS reduced pro-inflammatory cytokines such as IL-1β, IL-6, and caspase-1 expression while enhancing anti-apoptotic Bcl-xL levels, in-dicating a shift toward an antiinflammatory, neuroprotective micro-glial phenotype. Together, our results demonstrated that EVs-Chry have neuroprotective effects on LPS-induced microglial activation, and modulate microglial responses to inflammatory stimuli, attenuating pro-inflammatory signaling and promoting cellular homeostasis. These findings sup-port the therapeutic potential of EVsChry in the context of neu-roinflammatory and neurodegenerative disorders.

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# Motor-Cognitive Integration: Executive Functions During Dual-Task Walking Assessed via Wireless EEG and Gait Analysis

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Executive Functions (EFs) are high-level neurocognitive processes that regulate goal-directed behaviors and adaptive responses in daily life. Among these, working memory allows temporary storage and manipulation of information, while inhibition enables the suppression of automatic responses in favor of task-relevant ones. These functions are mediated by cortical networks involving the prefrontal and temporoparietal regions. In ecological contexts, the ability to walk while performing cognitive tasks (dual-task walking) is essential for autonomy. However, competing cognitive demands may lead to cognitive-motor interference, negatively affecting gait and increasing the risk of falls, particularly in older adults or individuals with neurological disorders. Although this phenomenon is well-documented, few studies have quantitatively investigated how specific EFs, such as working memory and inhibition, interact with gait dynamics and cortical activity during walking. This study aimed to explore the cortical correlates of working memory and inhibition during locomotion using wireless EEG with wet electrodes, combined with 3D Gait Analysis to detect associated motor adaptations. Thirty-one healthy participants performed two cognitive tasks during walking: a GoNoGo task (for inhibition) and an N-Back task (for working memory). EEG signals were recorded via a 19-channel wireless device, while gait was analyzed with a 3D motion capture system. A total of 51 EEG features (relative power across frequency bands), seven spatiotemporal, and nine kinematic parameters were extracted. Riemannian geometrybased algorithms were applied to improve EEG signal quality and remove gait-related artifacts. Repeated measures ANOVA ($\alpha < 0.05$) was used to evaluate differences in gait and EEG parameters across task conditions. Results showed alpha desynchronization at T5 and high beta desynchronization at F3 during N-Back tasks, indicating working memory activation. In contrast, high beta desynchronization at Fp1 during Go-NoGo tasks reflected greater inhibitory engagement. From a motor perspective, inhibition-related tasks induced significant alterations in step length, cadence, gait speed, and single support duration. Working memory-related gait changes emerged only under higher cognitive load. The findings suggest a stronger and more immediate interplay between inhibitory control and motor planning, whereas working memory influences motor execution in more demanding contexts. EEG features – specifically relative powers in alpha T5, high beta F3, and high beta Fp1 – are proposed as potential cortical biomarkers for EF engagement during gait. This integrated EEG-gait approach provides novel insights into the anatomical and functional interaction between executive and motor systems, with implications for fall risk assessment and neurorehabilitation.

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# Improved Outcomes in Peripheral Nerve Repair Using a Human Amniotic Membrane Nerve Wrap: A Comparative Preclinical Study

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Peripheral nerve injuries without substance loss are typically treated with neurorrhaphy and regenerative outcomes can be improved by nerve wraps. However, commercial devices are costly, require suturing, and provide only passive support [1]. This study investigates the potential of human amniotic membrane (hAM), a waste tissue with regenerative properties, as an alternative to commercial wraps. Preliminarily, hAM was characterized for morphostructural/ultrastructural features, and ECM proteins content (collagen/glycosaminoglycans (GAGs)). Thereafter, 12 adult male Sprague-Dawley rats underwent sharp sciatic nerve transection and were randomized into 3 groups: neurorrhaphy (NR), NR+Reaxon®, and NR+hAM. Postoperative well-being was monitored by weight analysis up to the 6-week endpoint. After euthanasia, nerves were evaluated macroscopically for adherences (scored 1–3) and processed for histology (H&E, Masson's Trichrome staining) and immunohistochemistry (CD3, F4/80, S100, β-Tubulin). The gastrocnemius (GA) muscles from both limbs were explanted for gross evaluation, weight comparison, and histological (H&E, Azan Mallory staining), morphometric, and ultrastructural (Scanning Electron Microscopy,

SEM) analyses together with collagen content assessment. The hAM showed the typical layers (epithelium, the basement membrane, and the stroma) as confirmed by SEM. The collagen and GAGs contents were 8,235±0,0588 μg/mg and 5,714±1,759 μg/mg, respectively. As for the preclinical study, all animals recovered well, with weight gain observed throughout. Adherence scores were highest in the NR group (2±0.83), lower in the NR+Reaxon® group (1.5±0.58), and lowest in the NR+hAM group (1±0); notably, the hAM was completely resorbed, as confirmed by histology, while Reaxon® remained visible. Fibroconnective tissue was more rapresented in the NR group. Immunostaining confirmed nerve regeneration (S100, β-Tubulin) in all groups, with minimal lymphomonocytic infiltration (CD3, F4/80). GA muscle weight recovery, expressed as a percentage of the contralateral side, was greatest in the NR+hAM group (44.6%), followed by NR+Reaxon® (39.4%) and NR alone (36.1%). Collagen deposition in the treated GA muscle was most balanced in the NR+hAM group (1.87 operated/healthy ratio), moderate in NR (3.33), and highest in NR+Reaxon® (5.12), indicating increased accumulation. These results aligned with the semiquantitative analysis and SEM evaluation. Muscle fiber area was largest in the NR+hAM group (2548 μm², ratio 0.868), indicating better preservation. The NR group showed intermediate values (2186 μm², ratio 0.765), while the NR+Reaxon® group had the smallest fibers (2087 μm², ratio 0.646), suggesting more atrophy. Overall, study results showed that NR+hAM promoted superior muscle preservation, lower fibrosis, and fewer adherences versus NR alone and NR+Reaxon®. These findings support hAM's potential as a cost-effective alternative to enhance outcomes in peripheral nerve repair.

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**Keywords:** Peripheral nerve repair, neurorrhaphy, nerve wrap, human amniotic membrane.

*Meccanismi cellulari e molecolari*
*nella trasformazione neoplastica*

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# Aberrant CCR2 expression in hematopoietic stem cells and megakaryocytes is associated with GATA-1 deficiency in myelofibrosis

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Myelofibrosis is the end-stage of Philadelphia-negative myeloproliferative neoplasms (MPN) and is characterized by progressive bone marrow fibrosis, extramedullary hematopoiesis, and uncontrolled production of inflammatory cytokines.

CD34+ cell-derived aberrant megakaryocytopoiesis is a distinctive feature in MF. BM megakaryocytes (MKs) are markedly hyperplastic, display peculiar morphological abnormalities and represent a major source of pro-fibrotic cytokines (1).

In addition to the well-established hyperactivation of the JAK/STAT signalling due to the presence of acquired somatic driver mutations, a very recent body of literature is intriguingly pinpointing the central role of specific Cytokine/Receptor Axes in MF pathogenesis (2). Among them, CCL2/CCR2 axis, has emerged as a key player in MF-associated inflammation and fibrosis. Our group first described the expression and function of CCL2/CCR2 axis in MF, demonstrating that (i) CCR2 is selectively expressed on MF $CD34^+$ neoplastic hematopoietic progenitors, (ii) its expression parallels the grading of bone marrow fibrosis and (iii) its engagement by CCL2 leads to the activation of a pro-proliferative signal via Akt in PMF CD34+ cells (3,4).

The promoter region of the CCR2 gene harbours putative binding sites for GATA-1, a key transcription factor in hematopoietic and MK differentiation (5,6). Notably, GATA-1 expression is markedly reduced in the BM of MF patients (7), and mice harboring the GATA-1low mutation (causing reduced GATA-1 expression) are a well-established MF murine model (8).

Based on this, we tested the hypothesis that GATA-1 may act as a transcriptional repressor of CCR2. We investigated CCR2 and GATA-1 expression in $CD34^+$ cells and MKs from MF patients and healthy control subjects. Our data revealed mutually exclusive expression patterns: $CD34^+$ cells from MF patients selectively expressing CCR2 were negative for GATA-1, whereas $CD34^+$ cells from healthy donors were GATA-$1^+$ and $CCR2^-$. We further analysed CCR2 expression in BM biopsies from MF patients with varying grades of fibrosis, compared to healthy controls. The number of $cKit^+CCR2^+$ cells was significantly higher in MF samples, and their frequency correlated with the degree of BM fibrosis. Immunofluorescence analysis also revealed several hypomorphic GATA-1 MKs expressing CCR2 in MF BM.

Finally, we assessed CCR2 expression during in vitro megakaryocytic differentiation of $CD34^+$ cells from MF patients and healthy donors cultured up to 15 days in the presence of TPO, SCF, and IL-3. Although MF-derived $CD34^+$ cells showed reduced expression of MK markers (CD41, CD61), the percentage of $CD41^+CCR2^+$ and $CD61^+CCR2^+$ MKs increased significantly during differentiation compared to controls.

These findings support a model in which MKs and progenitors in MF aberrantly express CCR2 in a GATA–1–dependent manner, suggesting that inflammatory signaling through CCR2 may contribute to MK dysfunction and fibrotic progression. Targeting this axis could offer novel insights into MF pathophysiology and therapeutic strategies.

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**Keywords:** CCR2, GATA-1, myelofibrosis, Inflammation, megakaryocytes.

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# Basal Cell Adhesion Molecule (BCAM): a possible therapeutic target in prostate cancer

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Prostate cancer (PCa) is the fourth most common cancer worldwide [1] but its molecular biology basis are still a matter of debate. Basal cell adhesion molecule (BCAM) is a member of the immunoglobulin superfamily and is composed of five glycosylated extracellular immunoglobulin domains, a transmembrane domain, and a C-terminal cytoplasmic tail [2]. BCAM was identified as a receptor for laminins in the extracellular matrix specifically interacting with the laminin α5 [3]. Alterations of BCAM expression have been linked to the progression of several malignancies. The aim of this study was to evaluate BCAM expression in normal prostate tissue, Intraductal carcinoma of the prostate (IDC-P) and in different Gleason grades of androgen-dependent PCa and castration-resistant PCa (CRPCa) in order to define the role of this glycoprotein in cancer aggressiveness prediction. We evaluated BCAM expression in normal (PWR1E) and prostate cancer (22Rv1 and LNCaP) cell lines. Moreover, we silenced and overexpressed BCAM with siRNA and expression vectors, respectively, in prostate cancer 22Rv1 and LNCaP cell lines in order to evaluate the role of this protein on cell proliferation and invasion. BCAM expression was increased in PCa tissues, especially those at advanced stage of disease such as grade group > 1 and CRPCa. BCAM expression was also increased in prostate cancer (22Rv1 and LNCaP) cell lines compared to normal PWR1E cell line. Moreover, BCAM overexpression in prostate cancer 22Rv1 and LNCaP cell lines significantly increased cell proliferation and invasion. Opposite effects were obtained when

BCAM was silenced. In conclusion, our results suggest an key role of BCAM in prostate cancer cell proliferation and invasion, favoring prostate cancer progression. Thus, BCAM-targeted therapy in prostate cancer may significantly improve the outcome of this malignancy.

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**Keywords:** BCAM, Prostate cancer, Cell proliferation, Cell invasion, PC3, LNCaP.

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# Ultrastructural characterization of radiotherapy-induced tissue damage in preclinical murine model

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Radiotherapy (RT), either alone or in combination with chemotherapy or immunotherapy, is widely used in cancer treatment. The ionization of cellular components by RT causes DNA strand breaks as well as the formation of highly reactive free radicals. Free radicals, in turn, give rise to reactive oxygen species which can damage DNA, lipids and proteins. Hyperproliferating tumor cells have reduced capacity for DNA repair mechanisms thus displaying enhanced radiosensitivity in comparison with normal tissues. However, radiotherapy is associated with both acute and late toxicities. Acute RT damage to normal tissues include skin reactions, mucositis and fatigue. Late RT effects can include fibrosis, secondary malignancies, organ-specific complications. Among these, it has been observed that childhood cancer survivors exposed to abdominal RT during treatment have an increased risk of developing chronic subcutaneous adipose tissue dysfunction and metabolic syndrome (1). To better understand RT-induced adipose tissue damage we performed morphological and ultrastructural analyses in a murine preclinical model of RT exposure at 6 weeks after treatment. RT-treated dermal and subcutaneous white adipocytes showed intra-cytoplasmic clear vacuoles and electron-transparent areas, which indicate loss of lipid content. Peri-adipocyte "crown-like" structures were also observed, resulting from macrophages surrounding and engulfing dead or dying cells (2). Molecular profiling by means of RNA-seq showed marked downregulation of key genes and pathways involved in adipocyte functions and metabolism. Our study reveals characteristic morphological alterations in RTexposed WAT that could mark the development of late-onset adipose tissue disfunctions.

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**Keywords:** Radiotherapy, tissue damage, ultrastructure, RNA-seq, white adipose tissue.

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# Immunihistochemical expression of stathmin in asbestiform fibers induced malignant mesothelioma

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Malignant mesothelioma is causally correlated with exposure to asbestos fibers. However, recent studies have also verified the correlation between exposure to 'naturally occurring asbestos' fibers and this neoplasm. Among these, there is the fluoro-edenite, a silicate mineral identified in the rock cavities inside the quarry of Monte Calvario located in the southeast of Biancavilla (Sicily, Italy). Malignant mesothelioma carries poor outcomes, and it is often diagnosed at an advanced stage due to the lack of diagnostic and prognostic biomarkers. To date, the most relevant prognostic parameters for malignant mesothelioma are represented by the histological subtype, gender, and age at diagnosis. In this context, several studies have already demonstrated how stathmin, a cytosolic protein that regulates cell growth and migration, is overexpressed across a broad range of human malignancies. However, no studies have correlated the expression of stathmin with the survival of malignant mesothelioma patients or with the clinical-pathological variables of the patients. The aim of the study is to investigate the immunoexpression of stathmin in a subset of patients affected by malignant mesothelioma induced by environmental exposure of fluoro-edenite fibers, to verify if stathmin may represent a prognostic biomarker for malignant mesothelioma, and to evaluate the capacity of identifying promptly the patients' prognosis. Ten MPM tissue samples, from patients with available clinical and followup data, were included in paraffin and processed for histological and immunohistochemical analyses. Our results showed a trend of shorter overall survival in malignant mesothelioma patients with stathmin overexpression. Furthermore, there was a significant correlation between stathmin expression and the survival time of malignant mesothelioma cases. Immunohistochemical expression of stathmin may represent a potential prognostic biomarker for malignant mesothelioma, and could serve to evaluate the capacity of identifying promptly the patients' prognosis to give indications to clinicians in the choice of therapeutic approach.

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**Keywords:** Malignant Pleural Mesothelioma, Asbestos-like Fibers, Stathmin, Prognostic biomarker.

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# Antitumor effects of *Moringa oleifera* on *in vitro* cultures of PC3 prostate cancer cells

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Prostate cancer is one of the most frequently diagnosed malignancies in men, and the development of androgen-independent forms represents a critical challenge due to their aggressiveness, resistance to conventional therapies, and high tendency to metastasize, particularly to bone[1]. In this context, the identification of natural compounds with anticancer activity is of considerable interest. *Moringa oleifera*, a plant widely studied for its pharmacological properties, has demonstrated anti-lipidemic[2], anti-diabetic[2], antioxidant[3], and antitumor effects[4], attributed to bioactive compounds such as glucosinolates, isothiocyanates, and omega-3 fatty acids.

This study aimed to evaluate the potential antitumor effects of ethanol extracts from M. oleifera leaves on the androgen-independent prostate cancer cell line PC3, with the ultimate goal of exploring its possible application in future therapeutic strategies.

PC3 cells were treated *in vitro* with *M. oleifera* extracts at different concentrations, and the cytotoxic effects were assessed using the MTT assay. Apoptosis induction and cell cycle changes were analysed by flow cytometry with Annexin V/PI and PI staining, respectively. The impact on cell migration was evaluated using the "Scratch test". To investigate the underlying molecular mechanisms, we performed Western blot analyses to determine the phosphorylation status of key signaling molecules (ERK1/2, AKT, NF-κB), and examined the expression of epithelial-mesenchymal transition (EMT) and invasion-related markers. Gene expression profiling was carried out using real-time PCR. The extract exhibited a dose-depending manner cytotoxic activity, significantly reducing PC3 cell viability, inducing apoptosis, and impairing cell proliferation and migration. Mechanistically, the treatment led to a reduction in the phosphorylation levels of ERK1/2 and AKT, two pathways associated with tumor growth and resistance to apoptosis. A significant decrease in NF-κB phosphorylation, which is activated downstream of ERK1/2 and involved in promoting inflammation and survival, was also observed. Regarding EMT, *M. oleifera* treatment reduced both the gene and protein expression of mesenchymal markers such as Vimentin, N-cadherin, and Fibronectin, while increasing the expression of the epithelial marker Occludin. Additionally, the gene expression of Integrin α2 and β1, important for adhesion and migration, was downregulated. The extract also modulated genes involved in tumor invasiveness: Cystatin A, Cystatin B, and cathepsin D were upregulated, whereas MMP-2, MMP-9, MMP-13, and Cathepsin B were downregulated. Finally, we observed a reduction in the expression of Alkaline Phosphatase (ALP), a marker associated with osteoblast activity and bone tropism of prostate cancer metastases.

These results suggest that *M. oleifera* exerts a broad-spectrum antitumor effect on PC3 cells by targeting multiple processes, including apoptosis, cell proliferation, EMT, invasion, and bone metastasis-related gene expression. The biological effects may be mediated by the synergistic action of bioactive compounds known for their anti-proliferative, pro-apoptotic, and epigenetic modulatory properties. Overall, the findings support the potential translational application of *M. oleifera* as a natural therapeutic agent for the treatment or prevention of advanced, androgen-independent prostate cancer. Further investigations are ongoing to clarify the molecular mechanisms involved and to validate these findings in preclinical models.

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# The niche megakaryocytes are the phenotypical target to track the changes in the Myelofibrotic bone marrow niche

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Myelofibrosis (MF) is a disease of the elderly associated with bone marrow (BM) failure, as consequence of abnormal production of TGF-β by corrupted megakaryocytes (MK).

MF is the end stage of the Ph-negative myeloproliferative neoplasms driven by mutations of the MPL/JAK2 axis induced by aberrant MKs with reduced GATA1 content (1). We exploited recent insights into MKs differentiation and their roles in MF. It has been recently shown that there are four functionally distinct types of megakaryocytes: the most prevalent are Platelets producing MK, the second are HSC-supportive MK, the third are classified as immune-MK, and the fourth, present in the developing embryos but not in healthy bone marrow are the niche-poised MKs, characterized by a TGF-β signature and are responsible to synthesize the extracellular matrix during the organogenesis. The MK subpopulations were recognized according to the published markers (2) by confocal microscopy observations. At first, we identified the MK subpopulations in the *Gata1low* mouse model for MF. The MKs from the BM of *Gata1low* mice were mostly immature and express reduced levels of markers of platelet-poised cells, as confirmed by ploidy determinations of CD42bpositive cells and according to the low GATA1 content.

Otherwise morphological analyses revealed the increase in frequency of the Niche-hypo lobulated MKs, expressing Collagen III (by tree fold) and Collagen I (more than ten-fold) compared to age matched controls, that positively correlates with the degree of fibrosis, due to the increased TGF-β expression in BM.

We then compared the GATA1 content and the frequency of MK subpopulations in the BM from MF patients in respect to normal controls. The increased fraction of Niche MKs was demonstrated in BM from MF patients, the majority of which negative for GATA1, than that of patients with Essential Thrombocythemia (ET) or with lymphoma but without fibrosis used as control (p=0.04 MF vs ET/CTR). We then confirmed the MKs heterogeneity in the BM microenvironment from MF patients, mostly of which were immature and characterized by higher Collagen I (p=0.01 MF vs CTR) and Collagen III (p=0.03 MF vs CTR) expression. Lastly, we studied the effects of MF therapies on MK lineages. After six cycle of pharmacological treatment, the TGF-β 1/3 inhibitor AVID200 in overt-MF patients promoted the MKs maturation, restoring the GATA1 content and platelets production, by interrupting the TGF-β effect. The TGF-β inhibition reduced the expression of Collagen I positive MKs resulting in a change of the fibrosis degree in platelet responsive patients (p=0.05). These results support that great numbers of MF MKs, express Collagen I/III may represent niche-

MKs the differentiation of which are reactivated by the high TGF-β bioavailability in the MF BM. In conclusion, the changes in the frequency of the niche-MK subpopulation can be used to evaluate the effects of specific molecules to treat and restore the MF phenotype.

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**Keywords:** Myelofibrosis, Megakaryocytes, GATA1, Bone marrow niche, Fibrosis

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# Targeting IRE1α-XBP1s Pathway to enhance cell death in Acute Myeloid Leukemia blast

Orsola di Martino[1], Giulia Pozzi[1], Samuele Cortellazzi[1], Prisco Mirandola[1], Giuliana Gobbi[1], Stephen M. Sykes[2], Francesca Ferraro[3], Marco Vitale[4], Elena Masselli[1], Cecilia Carubbi[1]

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The unfolded protein response (UPR) is a signal transduction network that acts to help cells cope with ER stress, which can be induced by various factors including chemotherapy, and it has been implicated in multiple human cancers[1]. We previously reported that UPR components support the pathogenesis of acute myeloid leukemia (AML)[1-2], showing that inhibition of the UPR transcription factor XBP1s reduces AML cell survival and colony formation and significantly delays disease onset in a genetically engineered mouse model of AML driven by MLL-AF9[3].

This current study explores the functional role and therapeutic potential of targeting XBP1s and its upstream activator, IRE1α, in human AML.

We first performed a retrospective analysis of XBP1 expression and activation across gene expression profiles of bone marrow (BM) or peripheral blood (PB) samples of AML patients and healthy donors (HD), revealing significantly higher XBP1 expression and activation in genetic subtypes as MLL-rearranged and FLT3-ITD AML compared to healthy donors.

To assess the functional role of XBP1s in AML patient cell survival, patient-derived AML (PD-AML) cells were engineered to express control or human XBP1s-targeting shRNAs and tested in co-culture with HS-27 stromal cells. XBP1s inhibition significantly reduced AML cell survival in 5 of 8 patient samples tested and reduced colony formation in 3 additional samples in cytokine-enriched methylcellulose assay. Notably, 6 of 8 responsive samples were FLT3-ITD positive, whereas non-responsive ones were FLT3-ITD negative.

While direct XBP1s inhibitors are unavailable, compounds targeting IRE1α are commercially available. Therefore, we demonstrated that genetic IRE1α depletion impaired AML cell growth and colony formation, similar to XBP1s inhibition. Additionally, given that IRE1α is both a kinase and an RNase, small molecule inhibitors of IRE1α, including 4μ8C (RNase inhibitor) and KIRA6 (dual kinase/RNase inhibitor), were tested on PD-AML samples at diagnosis. Both compounds showed anti-leukemia activity in 8 of 18 samples with no effect on healthy donor cell viability. Finally, testing drug synergy strategy, we assessed IRE1α inhibitors with FDA-approved AML drugs as cytarabine, anthracyclines, and venetoclax (VEN). We observed that IRE1α inhibitors selectively cooperated with VEN in an additive (n=2) or synergistic (n=8) manner to eliminate blasts. Remarkably, IRE1α inhibitors rendered VEN-resistant PD-AML samples sensitive to VEN treatment (n=4). No correlations between IRE1α sensitivity and AML genetic sub-type or lesion were observed, likely due to the small sample size, which is currently expanding.

These data suggest that targeting the IRE1a-XBP1s pathway may influence VEN efficacy, representing an effective strategy to overcome VEN resistance in AML.

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# Blackthorn juice selectively targets gastric cancer cells: a morpho-functional study

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Blackthorn (Prunus spinosa L.) juice, a polyphenol-rich blend from the Marche region of Italy, was chemically characterized and evaluated for its selective effects against gastric cancer cells. The juice contained a mix of polyphenols, including neochlorogenic acid, cyanidin-3glucoside, caffeic acid, and gallic acid. The juice significantly reduced the viability of AGS and KATO III gastric cancer cells while sparing non-tumorigenic GES-1 gastric cell line. Morphofunctional and molecular analyses (Micucci et al., 2023), highlighting its preferred signaling pathway, demonstrated that apoptosis is the major death response induced by Blackthorn (Prunus spinosa L.) juice in gastric cancer cells. Caspase-3 activation, bax upregulation, cytochrome c release as well as chromatin condensation, blebbing and micronuclei formation have been observed (Salucci S, 2018). Autophagy modulation is also involved in juice-induced gastric cancer death, beclin-1 downregulation and increase of LC3 activity led to autophagosomes accumulation and death.

These findings support the potential of Prunus spinosa L. juice as a selective, innovative functional juice for gastric cancer treatment acting through the modulation of biological pathways involved in tumor suppression with minimal toxicity for healthy cells.

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**Keywords:** polyphenols, gastric cancer, selective cytotoxicity, apoptosis, autophagy.

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# ERBB2 signaling and organelle remodeling drive cellular and immune responses to trastuzumab-deruxtecan in HER2/ERBB2+ breast cancer cells

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Breast cancers (BC) positive for human epidermal growth factor receptor 2 (HER2+) are aggressive and associated with poor survival rates. While ERBB2/HER2-targeted therapies such as trastuzumab and tyrosine kinase inhibitors have significantly improved patient outcomes, trastuzumab-deruxtecan (T-DXd) is now revolutionizing treatment for both HER2+ and HER2-low BC. Despite its remarkable clinical success, the subcellular and molecular mechanisms underlying T-DXd activity remain largely unexplored. Using high-resolution imaging and functional assays, we show that within 2 hours of treatment, T-DXd induces a transient activation of ERBB2 downstream signaling, accompanied by increased mitochondrial metabolism. Notably, this metabolic activation occurs without a concomitant increase in reactive oxygen species (ROS), indicating a ROS-decoupled metabolic shift. At later time points (48-72 hours), we observed the accumulation of T-DXd-loaded lysosomes near extensively fused mitochondria and the nuclear envelope, forming distinct lysosome-mitochondria-nucleus triads. These structural changes were accompanied by significant nuclear envelope alterations and increased H2AX activation, indicative of DNA damage following payload release. Concurrently, electron microscopy revealed a novel population of extracellular vesicles, termed sphereosomes, which emerged following T-DXd treatment. Immunolabeling confirmed T-DXd bound to sphereosomes, suggesting a potential mechanism for extracellular drug trafficking. Furthermore, cytokine analysis of conditioned medium (CM) from T-DXd-treated BC cells revealed an enrichment in pro-tumor cytokines (IL-6, IL-8, and TNF-α), which was accompanied by a shift toward pro-tumorigenic (M2-like) polarization. These findings highlight previously uncharacterized cellular responses to T-DXd, including mitochondrial metabolic adaptation, lysosome-mitochondria-nucleus triad formation, and sphereosome release. The presence of sphereosomes carrying T-DXd/ERBB2, along with cytokine secretion, suggests a potential role in modulating the tumor microenvironment, warranting further investigation into their functional impact.

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**Keywords:** Trastuzumab-deruxtecan (T-DXd), HER2+ breast cancer, organelle crosstalk and trafficking, extracellular vesicles and sphereosomes, tumor microenvironment modulation.

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# T- and preB-ALL cells respond to the synergistic combination of Hedgehog and PI3K/AKT/mTOR inhibitors and shares autophagic response

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**Background**. Acute lymphoblastic leukemia (ALL) T- (T-ALL) and preB- (preB-ALL) subtypes has been described to depend on aberrant activation of PI3K/AKT/mTOR and Hedgehog (Hh), that are key intracellular signaling pathways with critical roles in promoting leukemic cell growth, survival, and resistance to therapy [1-2]. While the PI3K/AKT/mTOR axis has been extensively studied, the contribution of GLI1 a key downstream transcriptional effector of the Hh pathway remains largely unexplored in hematologic malignancies. Therefore, we wanted to explore if the single or combined inhibition of the PI3K/AKT/mTOR and Hh/GLI1 pathways in T-ALL and preB-ALL cell lines may exert a synergistic effect and may influence cell growth or death, to assess the cross talk between the two pathways and therefore the influence of the two drugs on both cell lines.

**Materials and Methods.** T-ALL (Jurkat, Molt-4) and preB-ALL (NALM-6, KOPN8 and SEM) cell lines were treated with GANT-61 (a GLI1 inhibitor) for 72 hours and MK-2206 (an AKT inhibitor), alone and in combination the latter one administered for the last 24 hours. Cell viability was assessed using the CCK-8 assay. Apoptosis, cell cycle distribution and autophagy were analyzed by flow cytometry. Protein expression and localization of Gli1 and Akt was evaluated by Western blotting and immunofluorescence, respectively. The expression of total and phosphorylated downstream proteins of the two pathways (such as Akt, GSK3αβ, p70S6K, Gli1) was analyzed by Western Blotting and the localization of phosphorylated Akt and Gli1 was observed also by immunofluorescence.

**Results and Discussion.** The results showed that Gant-61 had a significant cytotoxicity in combination with MK-2206, Gant-61 the two drugs displayed a relevant synergy. In all the ALL-cell lines, both Gli1 protein expression and the phosphorylation of Akt and of its downstream targets were reduced by Gant-61 or MK-2206 when administered alone, but the combination of the drugs further increased the reduction of protein expression or phosphorylation of the targets analyzed. Images obtained by fluorescence microscope confirmed that the phosphorylation of Akt was decreased by MK-2206 or Gant-61 alone and further decreased after the combination treatment. Moreover, double administration had more relevant role in inducing cell cycle arrest in G0/G1 phase, increasing apoptosis, and autophagy to a different degree depending on the cell line. In conclusion, our data suggest that the mixture combined, and sequential administration of drugs targeted to Hh and PI3K/Akt/mTOR represents a potential strategy for T- and preB-ALL treatment supporting a promising therapeutic strategy to overcome resistance to treatments in leukemia.

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**Keywords:** T-ALL, B-ALL, PI3K/AKT/mTOR, Hedgehog, GLI1, apoptosis and autophagy.

*Anatomia settoria.*
*Anatomia clinica e forense*

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# Andrea Cesalpino in the fifth centenary of his birth

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Andrea Cesalpino was born in Arezzo in 1525. While a medical student at the University of Pisa, he studied under Vesalius and Colombo, and became appointed director of the Botanical Garden, distinguishing as a pioneer botanist for his taxonomic research. In 1592 he moved to Rome where he served as physician to Pope Clement VIII and taught medicine. He published several books, the most important of which is *Peripateticarum quaestionum* (1571), a masterpiece that reflected his admiration for Aristotle. He died in 1603. The most debated results of Cesalpino's anatomical research include studies on the heart and the motion of the blood. Cesalpino provided observations that can be considered pioneer concepts about blood circulation. He stated that the blood flowed from the heart to the periphery through the arteries and from the periphery to the heart through the veins, always inside blood vessels. He mentioned capillaries (*capillamenta*) as final small vessels, although he never claimed that they represented the connection between arteries and veins. Alternatively, he assumed the presence of small mouths (*oscula*) to indicate the communication between arteries and veins at the periphery. He recognized that the blood in the great veins flowed toward the heart, but he did not appreciate that blood in all of the veins returned only to the heart, assuming that some arterial blood was drawn into the veins. His conception was based on crucial observations, such as the distal bulging of ligated veins. Furthermore, he confirmed the evidence of the blood circulation from the right to the left ventricle through the lungs. He established the concept of artery, made up of two layers and characterized by pulsation, and vein, made up of one layer, and the role of the heart valves (*membranulae*), which allow the unidirectionality of the blood flow. Finally, the most convincing novelty was the use of the term circulation (*circulatio*) applied for the first time to the blood motion. Despite these promising notions, Cesalpino suffered from his Aristotelian vision and ancient anatomical concepts. His doctrine contains remnants of old beliefs and faulty conclusions. It includes the following notions: 1) the blood was propelled from the heart into the arteries by the heat (*spiritus*) generated inside the heart and the only purpose of the pulmonary transit of the blood was to cool it; 2) the terminal processes of the blood vessels were connected to the nerves; 3) when the arteries of the neck were occluded, the veins received blood from the arteries through anastomoses and, by retrograde flow, brought blood to the brain; 4) more blood (and heat) went from the heart to the periphery during wakefulness and more went from the periphery to the centre during sleep; 5) the vena cava supplied the kidneys with material to be excreted, without explaining how this would fit into the scheme of the blood circulation. The meaning of the word *circulatio* has been also debated. It has been hypothesized that Cesalpino meant chemical distillation. In fact, distillation was also called circulation to denote the rhythmical repetition of the chemical process. Finally, Cesalpino wrote his works without figures in a verbose Latin, full of philosophical comments, and often obscure, and spread his notions about the blood circulation one here one there. In conclusion, despite several obscure concepts, Cesalpino indeed had a clear general idea of the systemic circulation, paving the way to Harvey's *Exercitatio de motu cordis et sanguinis*.

**Keywords:** Andrea Cesalpino, blood circulation, William Harvey, University of Pisa.

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# Preliminary study on needle/fascicle mechanical interaction in sciatic nerve iatrogenic transfixion using the TruePass® technique

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Post-block neurological dysfunction is a rare complication of peripheral nerve blocks (Lemke et al.). There is conflicting evidence in the literature that fascicular injury follows needle transfixion of the nerve. The event appears infrequent even in benchtop trials, but studies on the subject do not use a standardised and reproducible technique to highlight possible iatrogenic injury, potentially underestimating the frequency of the adverse event (McLeod et al., Varela et al.). The primary objective of this study is to investigate whether iatrogenic needle transfixion causes mechanical fascicular injury in a sciatic nerve model.

A sciatic nerve sample was harvested from a fresh frozen 84-year-old male cadaver within the Body Donation Programme at the University of Padua, which serves as both the Regional and National Reference Centre for the Preservation and Use of Donated Bodies. The donor had no diseases that could interfere with the study. The length of the harvested sciatic nerve was 11 cm. On the bench, the accidental transfixion of the sciatic nerve during the anesthesiological procedure was simulated. For this purpose, 100 transfixions were performed using the TruePass® procedure with a 22 Ch straight needle (3 cm) and Polypropylene monofilament 3/0 suture (PROLENE by ETHICON). Each transfixion passed through the full thickness of the sciatic nerve, side by side. Once the procedure was completed, the sciatic nerve was preserved in formalin at room temperature and then processed for the selected stains. Overall, in 30 out of 100 transfixions, lesions were clearly demonstrated to the nerve fascicles of the sciatic nerve, while in 4 transfixions the needle passed close to the nerve fascicles, deviating them in a peculiar way without damage.

The use of the innovative TruePass® technique has allowed us to estimate for the first time the stochastic probability of iatrogenic injury to nerve fascicles in the case of transfixion while performing an anaesthetic block of the sciatic nerve. However, since it is a composite statistical probability, these results are consistent with the known clinical reality at low detection frequencies. Moreover, an interesting dynamic of the mechanical needle-fascicle interaction was highlighted in case of no nerve lesion. However, it is necessary to standardize the experimental technique in scientific studies focused on this topic, and for this reason, the diffusion of the TruePass® procedure is encouraged.

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**Keywords:** Cadaver lab, Body donation, Simulation, Iatrogenic damage, TruePass® procedure.

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# Geographic variability of anatomical variants of the cranium: are there differences in prevalence between Northern and Southern Italy?

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Anatomical variants of the skeleton (also known as "non-metric traits") have a great importance in different fields of physical and forensic anthropology, including diagnosis of ancestry and identification[1]. However, very few information is available about their origin and even less is known about the geographical variability of their frequency which represents a crucial characteristic for the possible application to personal identification[2].

This presentation aims at verifying if differences exist in prevalence of skeletal anatomical variants of the cranium between two groups from Northern Italy and Southern Italy, respectively. From the databases of the radiology units of Fatebenefratelli Hospital in Milan and University Hospital "Luigi Vanvitelli" in Naples, 200 (109 males, 91 females) and 555 head CT-scans (315 males, 240 females) were randomly extracted, respectively. Cases of acquired or congenital pathologies affecting the cranium were excluded.

In total, 17 anatomical variants of the cranium were assessed on each CT-scan and the prevalence of each variant in the two populations was calculated. Possible statistically significant differences in prevalence of each variant were assessed throughout Chi-square test ($p<0.01$).

Results showed that both in male and female population pneumatization of dorsum sellae, and right and left supraorbital foramen are significant more prevalent in the Neapolitan group than in the Milanese one, whereas spine of the nasal septum is more frequent in Milanese group than in the Neapolitan group ($p<0.01$). Moreover, in females right and left foramen of Vesalius, and pneumatization of crista galli were more frequent in Southern Italy than in Northern Italy ($p<0.01$).

Results show that most of analyzed variants do not show appreciable differences between the two populations: however, the few variants with significant discrepancies demonstrate that some non-metric traits are affected by geographical variability, even between relatively close populations, and suggest caution in their use for personal identification, especially for what concerns the preliminary calculation of the compound probability of specific profiles of anatomical variants.

The study received funding from the European Union – Next-GenerationEU – National Recovery and Resilience Plan (NRRP) – CALL PRIN 2022 PNRR D.D. 1409 14.09.22 – FRANTIC – FRequency of ANatomical Traits for personal IdentifiCation – CUP: 53D23004890006

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**Keywords:** anatomical variants, non-metric traits, geographic variations.

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# A Curvilinear Pattern-Based Method for Investigating Auricular Uniqueness in Personal Identification

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Personal identification relies on the description and comparison of individualizing characteristics. Recently,'secondary identifiers', particularly anatomical features that are visible in 2D and 3D images, have received increasing attention as constitute the primary source of available antemortem data in some contexts. Forensic research is intensifying efforts to develop methods that leverage these features and provide quantifiable, probabilistic results. In this context, the ear auricle is a valuable anatomical structure to investigate: visible in facial images, stable over time, and unaffected by expressions or weight changes.

This study aims to verify the anatomical uniqueness of the ear auricle and introduce an objective morphological method for personal identification. The method involves tracing a continuous curve termed the 'ear curvilinear morphological pattern'- by outlining the inner margin of the helix, outer margin of the concha, antitragus, intertragic notch, and tragus, between two fixed anatomical landmarks: the *posterohelixa interna* and the *tragion*.

Both ears of 41 subjects (21 males, 20 females) were photographed twice in standardized lateral view. For each ear, a curvilinear pattern was manually traced using Photopea and compared both within subjects (first vs. second image, representing the match group; N = 82) and between different subjects (mismatch group; N = 800). Each ear curvilinear pattern was discretized into 1000 equidistant points and aligned using Generalized Procrustes Analysis (GPA). Pairwise similarity was evaluated using discrete Fréchet distances. Differences by sex and group (match vs. mismatch) were assessed using the Mann–Whitney test ($p < 0.05$). The intra- and inter-operator reliability of the curvilinear pattern was tested on a subsample using Intraclass Correlation Coefficients (ICC) for the x and y coordinates of the 1.000 points.

The method showed excellent repeatability and reproducibility (ICC > 0.99). The average Fréchet distance was significantly lower in matches ($1.32 \pm 0.49$ mm) than in mismatches ($6.83 \pm 2.95$ mm) ($p < 0.05$), with no significant differences by sex or ear side ($p > 0.05$). A threshold value of 2.43 mm yielded a sensitivity of 100% and specificity of 99,6%.

This study proposes a new method for personal identification to use on 2D or 3D images of the ear, potentially valuable in forensic contexts where other approaches may not be applicable or effective. Further research is needed to validate the method in non-ideal conditions and assess its performance in real-cases scenarios.

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**Keywords:** Forensic Anatomy, Personal Identification, Ear Anatomical Uniqueness, Morphological similarity, Fréchet Distance.

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# From Textbooks to Cadavers: The Impact of the Mazzotti Anatomy Conference on Anatomy Education of Italian Medical Students

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Since 2010, over 1500 medical students from 40 Italian universities have participated in Giovanni Mazzotti Anatomy Conferences (MACs). Students perform all dissections uncovering several variations of anatomy, witness pathology, and simultaneously mature as a person making lifelong collegial relationships.

Hard work is rewarded with a certificate of completion. Pre- and post-conference exams are used to assess learning[1]. Written exams are multiple choice in both English and Italian, practical exams are fill in the blank, answers in English and/or Italian are accepted. The exams and the answers are not reviewed with the students, and instructors do not have access to the written exam questions.

Due to their own elevated interest in anatomy, and some selection and/or competitive scholarships, participants are generally strong in anatomical knowledge. Before attending, professors are contacted to be certain that they are a good fit for the program and will have completed their anatomy coursework. Some professors require anatomy exam completion, however due to logistics and better preparation for their anatomy exams, this is no longer mandatory for most. Participants hail from all years of education.

In 2024, 173 students from 26 different universities participated in the three traditional two-week MAC dissection conferences followed by a pilot conference, a Survey of Anatomy with Clinical Applications (SA). Due to a unique opportunity, scholarships ranging from 25-100% were provided to 9 of the dissection participants and 6 new students with no dissection experience. The SA is a hands-on experience utilizing the many cadavers from the dissection conferences, teams worked through clinical questions with some limited dissection and suture practice as well.

Participants of all four groups formally prepared and presented anatomical topics to their entire conference and researched suggesting causes of death before records were shared. Some students earning the highest achievements receive certificates of distinction. If warranted, awards are also given for top scores, greatest improvement and excellence in dissection.

From all aspects (participants, faculty and staff through outcomes surveys, and the community including scholarship donors) data indicate that conferences were highly successful.

Graph represents the three dissection groups (G1, G2 and G3) of 58 followed by the Survey of Anatomy students (SA1), (9 having just finished the dissection conference G3 and 6 new students).

All participants of the in Mazzotti Anatomy Conference showed increases in their overall anatomical knowledge. The participants were happy with the MAC experience, with positive comments related to dissection/lab time, staff, cultural activities, and newly developed friendships. 99.5% indicated that they would highly recommend the conference to a friend/colleague. 97% reported their improvement in anatomical knowledge ALONE was worth the expense.

Data from the MAC 2025 dissection and the more subscribed survey conference will be presented.

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**Keywords:** Anatomical Education, Dissection.

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# Ultrasound-guided core needle biopsy of deep fascia: A cadaveric study evaluating feasibility, accuracy and reliability

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Over recent decades, increasing attention has been directed toward the anatomical and pathophysiological characterization of fascial tissues. Innovations in imaging modalities, particularly ultrasound (US) and magnetic resonance imaging (MRI), have substantially enhanced our ability to explore fascial dynamics and structural changes.

However, a microscopic perspective remains essential for the accurate assessment of fascial pathologies.

This cadaveric study aimed to evaluate: (1) the visibility of anatomical landmarks for US-guided fascial core needle biopsy (CNB); (2) the precision and reproducibility of needle placement within fascial layers, verified by histological analysis; and (3) inter-rater reliability across different fascial regions.

US-guided CNB was performed on three anatomical sites: thoracolumbar fascia (TLF), fascia lata (FL), and crural fascia (CF). Histological confirmation revealed no significant differences in needle placement accuracy between longaxis and short-axis in-plane approaches across all regions (long axis: 91.88%; short axis: 96.22%; $p > 0.05$).

Our results demonstrate that US-guided in-plane CNB is a feasible, reliable, and anatomically consistent technique for fascial tissue sampling. This approach enables the acquisition of high-quality specimens for histopathological analysis and may facilitate the detection of localized fascial abnormalities, particularly in disorders with patchy distribution such as eosinophilic fasciitis.

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**Keywords:** biopsy, core needle biopsy, deep fascia, diagnosis, eosinophilic fasciitis, fascia, fascial blocks, injections, rheumatology, thoracolumbar fascia, ultrasonography, ultrasound imaging.

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# The (ProteUS) Anisotropy Effect in Deep Fascia Ultrasonography: The Impact of Probe Angulation on Echogenicity and Thickness Assessments

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This study explores the impact of ultrasound probe angulation on the assessment of deep fascia echogenicity and thickness, with the aim of overcoming key methodological limitations in musculoskeletal ultrasound (US). Given the inherently anisotropic properties of connective tissues, even slight deviations in probe orientation can introduce artifacts that compromise the accuracy and diagnostic validity of US imaging.

To investigate these effects, echogenicity and thickness were systematically evaluated under varying probe inclinations – specifically at -5°, 0°, and +5° – in both transverse and longitudinal planes, using a 3D-printed support to ensure controlled angulation. Statistically significant changes in echogenicity were observed, particularly in the transverse orientation at 0°, which showed marked deviations at -5° (mean difference = 55.14, $p < 0.0001$) and +5° (mean difference = 43.75, $p = 0.0024$). Similarly, thickness measurements were found to fluctuate across angulations, underscoring the distorting effects of non-perpendicular probe positioning. These findings were consistent in both transverse and longitudinal acquisitions. The results emphasize the critical importance of adhering to standardized scanning protocols to ensure measurement reliability. The deep fascia's structural anisotropy – highly responsive to minimal angular variation – demands meticulous control of probe alignment to accurately depict its morphology. Thus, optimizing transducer orientation is not merely a technical detail, but a fundamental requirement for improving the diagnostic precision of fascial ultrasound.

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**Keywords:** anisotropy, deep fascia, clinical anatomy, echogenicity, fascia lata, imaging, tendon, thickness, ultrasound.

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# The integration of neurosurgery and bioengineering with neuroanatomy in the High Technology degree course in medicine and surgery at Sapienza University of Rome: knowledge acquired and perception of effectiveness by medical students

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In 2020, Sapienza University of Rome was the first Italian public university to pioneer a master's degree program in High Technology Medicine and Surgery (MCHT), a program closely integrated with engineering disciplines (Basili et al., 2021). The Neuroanatomy module, a key component of the first and second years of the MCHT course, has been enriched with content from neurosurgery and bioengineering. These additions, delivered through lectures closely integrated with neuroanatomy content by neurosurgeons and engineers, mark a significant leap in medical education. This study aims to examine the educational value and students' perceptions of the effectiveness of these two types of integration, comparing neurosurgical integration with engineering integration.

The anatomical knowledge and opinions of students who attended the neuroanatomy course of the MCHT course (n = 60; academic years 2023-2024 and 2024-2025) were compared with those of students (n = 64; academic years 20232024 and 2024-2025) who attended another medicine and surgery course at Sapienza, which did not include this type of integration (MC) and which served as a control group. Students were given a previously validated Likert scale satisfaction questionnaire and a written test to assess their knowledge of neuroanatomy, divided into two sections: neuroanatomy of the brain and neuroanatomy of the spine. The data were analyzed using IBM SPSS 27 software, employing the ANOVA test and the Pearson correlation coefficient, with significance values set at $p \leq 0.05$. The internal consistency coefficient (Cronbach's alpha) was also calculated, with a value of < 0.70.

The data reveal that MCHT students hold a higher appreciation for integration with neurosurgery than with engineering. However, it's important to note that those who appreciate neurosurgical integration also value engineering integration (Cronbach's alpha = 0.876). This finding reassures us about the overall positive perception of the integrated approach. Significant differences were found in neuroanatomical knowledge between the two groups in the two sections of the test. No significant correlation was found between test scores in the two sections and levels of appreciation. In contrast, a significant correlation was found between students who appreciate neurosurgical integration and those who appreciate engineering integration.

The results obtained confirm the importance of neurosurgical integration, which serves as a valuable contextual tool, capable of enhancing students' neuroanatomical knowledge well before the clinical training period in medical and surgical courses. The results obtained are also crucial for optimizing the integration levels of neurosurgery and engineering, especially within the new master's degree courses in medicine and surgery with a strong technological focus, which are now well-established in Italy.

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**Keywords:** anatomical sciences education, neurosurgical sciences education, neurosurgical integration, bioengineering integration, technical medicine.

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# Unveiling the Anatomical Machines of the Sansevero Chapel: a clinical anatomy perspective and a multidisciplinary investigation through digital twins of structure, technique, and paleopathology

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The Anatomical Machines of the Cappella Sansevero in Naples are among the most intriguing anatomical artifacts in European history, closely associated with the Chapel and its patron, Raimondo di Sangro, VII Prince of Sansevero. Created in the mid-18th century and attributed to the Sicilian anatomist Giuseppe Salerno (1728–1792), these skeletal models consist of two skeletons: one representing a man and the other a pregnant woman, which preserve intricate representations of the human circulatory system.

Only a few anatomical and medical studies have been conducted, but their findings have been contentious and incomplete, failing to provide a comprehensive analysis. This study aims to address these controversies through a multidisciplinary approach that employs non-invasive diagnostic techniques, historical analysis, and paleopathological interpretations. By combining morphological, archaeo-anthropological, taphonomical, and photogrammetric analyses, this study clarifies the origin, structure, and medical significance of the two models while fully contextualizing their historical background and reviewing existing literature.

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**Keywords:** clinical anatomy, palaeopathology, photogrammetry, digital twins, anatomical waxes, 18th-century anatomy, Sansevero Chapel, Raimondo di Sangro.

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# Microscopic modification in cremated bones: a case report from the roman necropolis of Monte Carru (Alghero-Italy)

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**Introduction.** Cremation is a ritual involving the burning of a body, which results in characteristic changes to the bones. Previous studies have highlighted macroscopic changes in bone fragment size, weight, shape, and color. This study aimed to identify significant relationships among microscopic and macroscopic modifications, thereby correlating them with temperature and the position of the fire.

**Materials and methods.** The case study (T.27) is a multiple burial containing the remains of a young adult female and a fetus (estimated gestational age: 38 weeks to 1 month post-birth). Modifications were observed in different anatomical regions of the young female, and also between the female and the infant. Macroscopic changes, including color, weight, and shrinkage, were analyzed, and classified (according to the literature's S, U, and LD forms) for each fragment. The association of these macroscopic changes with microscopic alterations, analyzed using a Scanning Electron Microscope, was subsequently examined.

**Results.** The position of the bodies relative to the fire during cremation was inferred from all detected macroscopic features. Based on the macroscopic modifications of the bones, it is hypothesized that the fire was positioned near the woman's chest, and the infant was located on or within the woman's body during cremation.

Guided by various macroscopic modifications, samples were observed from different anatomical regions of the woman's body, as well as from the outer and inner edges of the same bone.

It is noteworthy that, during initial exposure to low fire temperatures, the bone structure of burned remains is indistinguishable from that of unburned bone. However, prolonged exposure to fire progressively degrades the bone structure, leading to modifications in crystal shape and size. These modifications are macroscopically evident, as demonstrated by the distinct differences between black and white bone fragments.

**Conclusion.** This study demonstrates a correlation between macroscopic and microscopic changes in cremated remains, suggesting a relationship with fire temperature.

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*Invecchiamento e patologie degenerative*

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# Defective autophagy in retinal pigment epithelium is a key determinant in producing some pathological hallmarks of AMD

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Multiple retinal disorders, including acute injuries and chronic diseases, feature a number of alterations in the autophagy machinery [1]. Among them, increasing evidence indicates that dysfunctional autophagy occurs in age-related macular degeneration (AMD), which is characterized by progressive retinal degeneration that eventually leads to blindness. Apart from photoreceptors AMD produces a damage to the retinal pigment epithelium (RPE), which is placed between the outer retina and the inner choroid [2,3]. In particular, RPE cells are characterized by a powerful clearing system, which relies on autophagy pathway, providing the removal and turnover of organelles and various chemical species, including proteins, sugars, and lipids [4]. Accumulation of glycogen granules and lipid droplets along with altered mitochondria is typical of RPE cells in the course of AMD. In the present study, we treated human RPE cells (ARPE) with 3-methyladenine (3MA), a strong autophagy inhibitor, to assess whether this mimics AMD phenotype. 3-MA-induced cell pathology was challenged with autophagy activators. RPE cells were analyzed through various histochemical and immunohistochemical procedure, in combination with ultrastructural studies by plain transmission electron microscopy and in situ stoichiometry of immunogold staining. We found that autophagy inhibition profoundly affects mitochondrial ultrastructure and produces accumulation of various chemical cell components, including proteins and lipids. In contrast, autophagy activators reverse all these effects, confirming the involvement of autophagy in AMD and suggesting that autophagy stimulation is a promising therapeutic strategy.

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**Keywords:** Retinal pigment epithelium, autophagy, retinal degeneration, 3-methyladenine, age-related macular degeneration (AMD), mitochondria, lipids.

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# Long-Term Noradrenergic Denervation Induces Aging-Related Brain Pathology in Mice

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**Introduction:** Early degeneration of the Locus Coeruleus (LC) may significantly contribute to the pathogenesis of Alzheimer's Disease (AD) [2]. Experimental models indicate that acute loss of noradrenergic (NA) terminals can promote amyloid deposition and intensify neuroinflammatory and neurovascular alterations in middle-aged transgenic AD mice [3–5]. However, the impact of chronic LC dysfunction on normal brain aging – potentially a more accurate model of sporadic AD – remains unexplored. This study investigates whether prolonged NA denervation alone can elicit AD-like pathological features in wild-type mice.

**Methods:** C57Bl/6J mice were observed up to 18 months of age. LC degeneration was induced by administering the selective neurotoxin DSP-4 [5] every four months. Cognitive performance was evaluated with the Open Field and Novel Object Recognition tests, conducted before and after each DSP-4 injection, as well as prior to sacrifice. Brain tissues were prepared for optical microscopy, while prefrontal cortex samples were processed for transmission electron microscopy (TEM). Immunohistochemical staining for GFAP, IBA1, and 4G8 was used to assess neuroinflammation and amyloid deposition. TEM was also used to examine neurovascular integrity. Stereological analysis quantified tyrosine hydroxylase (TH)-positive neurons in the LC and in hippocampal regions CA1, CA2, CA3, and the dentate gyrus (DG).

**Results:** DSP-4-treated mice displayed enhanced astrogliosis, microglial activation, and ultrastructural abnormalities in brain capillaries. Chronic NA denervation led to a pronounced loss of NA terminals and a significant reduction in TH+ neurons within the LC, as confirmed by stereological analysis. Moreover, reduced neuronal density was observed in hippocampal areas CA1, CA2, and DG in DSP-4-treated animals.

**Conclusion:** This study establishes a viable model of chronic NA denervation and LC degeneration. Mice with LC lesions exhibited pathological changes indicative of accelerated brain aging, even in the absence of genetic or external insults. These findings provide preliminary support for the hypothesis that LC dysfunction may be a driving factor in the early stages of AD pathogenesis.

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**Keywords:** Locus Coeruleus, Alzheimer's Disease, Brain ageing, Noradrenaline, Neuroinflammation, Blood-Brain Barrier.

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# Blood-Brain Barrier Dysfunction in Age-Related Neurodegeneration

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The blood-brain barrier (BBB) is a key component of the neurovascular unit, formed by microvascular endothelial cells sealed by tight junctions (TJs), which are primarily composed of the transmembrane proteins claudin-5 and occludin. The ECs are closely associated with pericytes and astrocytic endfeet, forming a complex multicellular architecture essential for maintaining central nervous system homeostasis. Disruption of BBB integrity, particularly through alterations in TJ composition and function, is increasingly recognized as a cause of chronic neuroinflammation, which is involved as an early pathomechanism of age-related neurodegenerative diseases, including Alzheimer's disease (Sweeney et al., 2018).

Using a senescence mouse model, SAMP8, we investigated the expression of the TJ protein occludin and the inflammasome component NLRP3 to explore structural and immune-mediated alterations associated with BBB dysfunction. Confocal analysis of cortical microvessels revealed a marked downregulation of occludin in SAMP8 mice compared to age-matched SAMR1 controls, accompanied by discontinuous and fragmented staining patterns indicative of junctional instability. Parallel double-labeling immunofluorescence for GFAP and NLRP3 demonstrated increased NLRP3 expression in hypertrophic GFAP-positive astrocytes, pointing to astroglial reactivity and inflammasome activation.

These findings reveal a close relationship between astrocyte activation and inflammasome signaling in the aging brain, suggesting a potential link between BBB disruption and neuroinflammation mediated by NLRP3 (Ising et al., 2019). Together, the results highlight both structural and inflammatory responses in the SAMP8 model that may contribute to understanding the pathomechanisms of BBB breakdown associated with cognitive decline and support the exploration of NLRP3 as a therapeutic target in age-related neurodegeneration (Heneka et al., 2018).

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**Keywords:** Blood brain barrier, Occludin, Neuroinflammation.

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# Ultrastructural Characterization of the Blood-Brain Barrier in Neonatal, Adult and Aged Mice

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The Blood-Brain Barrier (BBB) plays an important role in maintaining Central Nervous System (CNS) homeostasis by regulating molecular trafficking between the bloodstream and the brain and protecting it from harmful compounds [1]. However, BBB integrity and function can vary significantly during the lifetime, from an immature stage during neurodevelopment to progressive decline during aging. In this study, we performed a comparative ultrastructural analysis of the BBB at the level of the frontal cortex in C57BL/6 murine model, comparing neonatal mice at postnatal day 7 , with young adults and aged mice (18 months) in order to assess specific BBB features along lifespan. We utilized Transmission Electron Microscopy (TEM) to investigate ultrastructural features in the frontal cortex, focusing our attention on some parameters described below, to provide a comprehensive morphological characterization of the BBB. In detail, only crosssectioned intraparenchymal capillaries with a lumen of less than 10 μm were considered in this analysis [2,3] and considered ultrastructural parameters were: i) basement membrane thickness [2,3]; ii) abundance of transcytosis vesicles [2,4]; iii) length and width of the tight junction cleft [2]; iv) Tight junction complexity. Inter-endothelial junction complexity was classified into four types, reflecting the degree of maturation, from the less mature (type 1) to the most mature (type 4), according to [5]. By these approaches we were able to detect age -related differences in the BBB structure. This type of analysis may help to detect and quantify also the onset and extent of alterations associated with age-related degenerative processes.

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**Keywords:** Brain ageing, Neurodevelopment, Blood-Brain Barrier, Transmission Electron Microscopy.

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# Alarmins and Emerging Cytokines in COPD: Histopathological Insights and Novel Therapeutic Targets

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Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory lung disorder characterized by persistent airflow obstruction and structural remodeling (1). Recent research has highlighted the involvement of alarmins and newly identified cytokines – such as interleukin-33 (IL33), interleukin-40 (IL-40), and interleukin-41 (IL-41) – in regulating local immune responses and contributing to tissue damage (2,3,4). Among these, IL-33 has been the most extensively studied in animal and in vitro models, consistently demonstrating a pro-inflammatory role in COPD (5). Nevertheless, evidence regarding its expression in human lung tissue remains limited. This study aims to assess the tissue expression of these cytokines to better understand their potential role in the complex inflammatory processes underlying COPD, which affect the entire lung microenvironment.

We performed histological and immunohistochemical analyses on lung tissue samples from patients with COPD (n=8) and smoking controls without COPD (n=10) undergoing lung surgery. COPD diagnosis was confirmed by pulmonary function tests according to American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines. Expression of IL-33, IL-40, and IL-41 was quantified across different lung compartments, including bronchiolar epithelium, alveolar pneumocytes, macrophages, and vascular endothelial cells.

Our findings revealed a significant increase in IL-33 expression in COPD patients, particularly in bronchiolar epithelial cells and alveolar pneumocytes ($p < 0.05$), with an upward trend in macrophages and endothelium. IL-40 was also upregulated in COPD tissues, consistent with its emerging role in chronic inflammation. In contrast, IL-41 – known for its anti-inflammatory and immunoregulatory properties – was downregulated in the COPD group, suggesting a possible loss of protective immune balance. The altered expression of these interleukins highlights a disrupted cytokine network in COPD, with an imbalance favoring pro-inflammatory mediators and reduced anti-inflammatory modulation. These findings, obtained from human tissue samples, provide translational relevance and fill a critical gap in the current literature dominated by preclinical models.

In conclusion, our study demonstrates that IL-33 and IL-40 are upregulated, while IL-41 is downregulated in COPD lung tissue. These interleukins represent promising biomarkers and therapeutic targets for the development of future cytokine-based treatments, both for disease control and management of exacerbations.

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**Keywords:** COPD, Alarmins, IL-33, IL-40, IL-41, Lung Inflammation, Cytokine Dysregulation, Therapeutic Targets, Histochemical study.

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# Organoidi Cardiaci Umani come Modello Tridimensionale per lo Studio della Senescenza Cellulare

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La senescenza cellulare rappresenta un evento biologico cruciale nell'invecchiamento dei tessuti e nella progressione di numerose patologie cronico-degenerative, inclusa la cardiomiopatia dell'anziano. Tuttavia, lo studio dei meccanismi cellulari e molecolari alla base della senescenza miocardica è stato finora limitato dalla mancanza di modelli sperimentali sufficientemente affidabili e fisiologicamente rilevanti. Gli organoidi cardiaci umani (hCOs), generati a partire da cellule staminali pluripotenti indotte (iPSCs), costituiscono un modello tridimensionale innovativo, capace di replicare fedelmente l'architettura, la composizione cellulare e la funzionalità contrattile del tessuto miocardico. Tali caratteristiche rendono gli hCOs un'alternativa avanzata rispetto ai modelli animali o bidimensionali tradizionali convenzionalmente utilizzati. Nel presente studio, abbiamo generato e caratterizzato hCOs a partire da iPSCs umane. Al 22° giorno di differenziazione, per generare un fenotipo senescente del tessuto cardiaco, gli hCOs sono stati trattati con Doxorubicina (DOXO), agente chemioterapico in grado di indurre senescenza cellulare attraverso meccanismi di stress ossidativo e danno al DNA. Gli organoidi trattati con DOXO (aged-hCO), mostravano alterazioni sia funzionali che trascrizionali coerenti con uno stato senescente, tra cui un incremento significativo dell'espressione di marcatori tipici della senescenza cellulare, come $p16^{INK4A}$ e p21 e del fenotipo secretorio associato alla senescenza (SASP), un aumento dei marker di stress ossidativo e danno al DNA, accompagnati da una riduzione della proliferazione cellulare e della funzionalità contrattile. Inoltre, DOXO-aged-hCOs presentavano una ridotta espressione dei geni chiave per l'identità e la funzione cardiaca, oltre a una marcata down-regolazione dei marcatori di contrattilità miocardica rispetto ai hCOs di controllo. In maniera interessante, il trattamento combinato con senolitici (Dasatinib e Quercetina, D+Q), ha determinato una riduzione della produzione del SASP negli hCOs senescenti trattati (D+Q-aged-hCOs) rispetto agli aged-hCOs di controllo. L'eliminazione delle cellule senescenti all'interno dei D+Q-aged-hCOs è stata associata a una riduzione della fibrosi rispetto agli aged-hCOs, suggerendo quindi un potenziale terapeutico per il ripristino della vitalità e della funzionalità del tessuto cardiaco. Questo approccio sperimentale rappresenta un modello innovativo e altamente predittivo per lo studio dei meccanismi della senescenza nella cardiomiopatia dell'anziano e per la valutazione di nuove strategie rigenerative con potenziali applicazioni in biomedico.

**Keywords:** Organoidi cardiaci, senescenza cellulare, cellule staminali, biologia cellulare, senolitici.

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# BET Protein Inhibition Restores Frataxin Expression and Redox Homeostasis in Friedreich's Ataxia Models

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Friedreich's Ataxia (FA) is a neurodegenerative disease caused by homozygous expansion of GAA repeats within the first intron of the *FXN* gene (1), leading to a significant decrease in frataxin, a mitochondrial protein involved in iron-sulphur cluster (ISC) biosynthesis and redox balance in the cell (2). Frataxin deficiency causes iron accumulation (3), impairment of ISC-containing enzymes (2), mitochondrial dysfunction, and increased oxidative stress, as evidenced by elevated reactive oxygen species (ROS) levels and lipid peroxidation, in both FA patients and model systems (3,4). Despite the expected activation of compensatory antioxidant pathways, such as NRF2 signalling, this axis appears dysfunctional in FA (5), exacerbating ROS cellular vulnerability. We investigated whether targeting BET proteins – epigenetic readers involved in transcriptional regulation (6) – could restore *FXN* expression and redox balance in FA, as this protein family directly interact with NRF2, inhibiting its activity, and control the transcription of KEAP1, the main negative regulator of NRF2 stability (7,8). To this, we treated FA patient-derived fibroblasts with JQ1, a pan-BET inhibitor, after having established sublethal concentrations through viability and proliferation assays. JQ1 treatment for 24 hrs significantly reduced ROS levels and partially restored antioxidant enzyme activity, with a notable increase in glutathione peroxidases (GPx). Although JQ1 does not affects *NRF2* mRNA levels, it increases NRF2 stability, promoting its nuclear localization and transcriptional activity. This was paralleled by a significant downregulation of *KEAP1* mRNA. These data confirm that BET inhibition promotes NRF2 activation, by rescuing the redox homeostasis in FA cells. Treatments with JQ1 upregulated *FXN* mRNA as early as 3 hrs post-treatment, while frataxin protein accumulation was observed after 24 hrs. Importantly, frataxin colocalized with the mitochondrial marker TOM20, indicating that BET inhibition promote frataxin native localization. Functional reduction of cellular ROS, due to increased NRF2 activity and frataxin expression, restored mitochondrial network integrity that is severely unpaired in FA fibroblasts (9), and conferred protection against ferroptosis, a cell death pathway triggered by iron accumulation, recently associated to the pathology (4,9,10). In particular, our results demonstrate that 24 hrs of JQ1 pretreatment abolished the heightened susceptibility of FA cells to ferroptosis, induced by the GPX4 inhibitor RLS3. Collectively, our data demonstrate that BET protein inhibition via JQ1 rescues *FXN* transcription, enhances NRF2-mediated antioxidant responses, and restores mitochondrial health. These results support BET proteins as therapeutic targets for FA and lay the groundwork for future studies aimed at optimizing BET-based or combinatorial interventions to treat this disorder.

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**Keywords:** Friedreich's Ataxia, Oxidative stress, NRF2, Neurodegeneration, BET proteins.

*Modelli 3D, organoidi,*
*scaffold biologici, rigenerazione tissutale*

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# Melanoma Cancer Stem Cells and Tumor Microenvironment Remodeling: a Three-Dimensional Cell Culture Perspective

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The intricate relationship between melanoma cancer stem cells (mCSCs), a subpopulation of cancer cells with self-renewal and differentiation capabilities, and the tumor microenvironment (TME) is a critical determinant of tumor initiation, progression, metastasis, and therapeutic resistance. mCSCs and the cellular and acellular components of the TME are inextricably linked through a bidirectional interplay, wherein each critically modulates the characteristics and functions of the other[1]. Three-dimensional (3D) cell culture systems, such as spheroids, offer a highly suitable and increasingly adopted strategy to study these complex interactions. 3D *in vitro* models more accurately recapitulate the physiological architecture, cell-cell contacts and cell-ECM interactions found within tumors, effectively bridging the gap between traditional 2D cultures and animal models[2].

We established 3D culture models of human mCSCs, fibroblasts and keratinocytes, generating spheroids using low attachment 96-well plates or agarose molds. Cells within the spheroids were characterized through immunofluorescence and confocal microscopy analysis, followed by quantitative analyses and nuclei segmentation with a deep-learning-based algorithm.

In both experimental settings, we observed that mCSCs altered keratinocyte stratification, with basal keratinocyte-like cells unexpectedly populating the outermost layers of the spheroids, where they remained in direct contact with mCSCs. In keratinocytes, mCSCs induced a reduction in Involucrin expression and a concomitant increase in Cytokeratin 14 levels. In line, single-cell analysis of nuclear morphology showed a pronounced nuclear eccentricity and reduced alignment in keratinocytes adjacent to mCSCs, suggesting the idea of a loss of polarity and structural organization consistent with dedifferentiation and epithelial remodeling. Fibroblasts were also impacted by the presence of mCSCs, as revealed by their enhanced expression of Collagen I.

Using Cellpose algorithm[3], we trained an AI-based model to identify mCSCs and assess their organization within the spheroids. This demonstrated that mCSCs were differently distributed depending on the plating method used. For instance, in the agarose mold, mCSCs maintained closer contact with stromal cells, exerting a stronger influence on their phenotype. However, in both cases, mCSCs were consistently unable to penetrate deep in the spheroid.

Collectively, our findings highlight the advantages of 3D culture systems as versatile platforms for dissecting the complex interactions between mCSCs and the TME, providing insights overcoming the inherent limitations of conventional 2D methodologies.

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**Keywords:** Melanoma, Cancer stem cells, Keratinocytes, Fibroblasts.

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# Production of Decellularized Composite Biological Scaffolds With Preserved 3D Architecture and Vascular Network

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Face transplantation is a promising reconstructive procedure with the potential to revolutionize reconstructive surgery in patients with extensive facial defects. However, complete aesthetic, morphological and functional recovery is rarely achieved with facial reconstructive methods such as autografts, allografts, and synthetic implants. Furthermore, the long-term post-operative course is burdened by the need for lifelong immunosuppression and, more importantly, limited graft survival [1]. The production of bioengineered facial grafts by decellularization of faces from cadaveric donors offers a comprehensive and attractive solution. The aim of the present study is to evaluate the possibility of obtaining facial grafts with preserved 3D architecture and composition of dermal and muscular extracellular matrix (ECM) and patent supplying vasculature by decellularization of composite facial specimens. For this purpose, full-thickness rat faces (n=4) including both ears and harvested with the vascular pedicle consisting of the external carotid artery (ECA) and the external jugular vein (EJV), were obtained from animals sacrificed following other experiments performed in accordance with European Union directive for animal experiments. Specimens were decellularized in flasks containing Triton, SDS and antibiotics [2]. Macroscopic observation of decellularized rat faces showed preservation of the overall facial architecture, accompanied by evident whitening of the muscle and cartilage structure, indicating complete decellularization. Hematoxylin and Eosin (H&E) staining and residual dsDNA content of 12.13 + 2.94 ng/mg of dry tissue confirmed that the decellularized facial scaffolds met the criteria established to fulfil the intent of decellularization. Furthermore, Sirius Red, Masson's and Mallory's staining, specific quantitative assays for glycosaminoglycans (GAGs), collagen and elastin, and immunohistochemistry for laminin, fibronectin and tenascin revealed that the 3D architecture and composition of the ECM were not significantly affected by decellularization. Finally, antegrade injections through the ECA and retrograde injections through the EJV followed by homogeneous distribution of the Blu Patent V dye demonstrated the patency of the vascular network through the decellularized facial graft.

In this study, we demonstrated that the decellularization procedure can be effectively applied to a whole rat face model to produce decellularized ECM composite scaffolds with preserved 3D architecture and vascular network for facial bioengineering applications.

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**Keywords:** Decellularization, Extracellular matrix, Vascular Network.

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# The cell as integrated mechanosensor

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Cellular mechanotransduction is a fundamental informational system by which cells read the structural features of their environment to control their own form and function. The YAP/TAZ transcriptional regulators are universal transducers of physical signals into gene-expression programs. Yet, how mechanotransduction is orchestrated at the whole cell level remains largely unknown. Through live imaging and AI-assisted 3D reconstructions of cells subjected to diverse mechanical stimulations we show the workings of a continuum of interconnected subcellular systems, ultimately converging on the nuclear envelope hub, that allows the cell to dynamically restructure itself in response to mechanical cues and to work as a mechanical rheostat controlling YAP/TAZ mechanosensing. Our findings also provide a unifying model that mechanistically merges mechanosignaling with the Hippo cascade. The current model by which YAP/TAZ are regulated by Hippo kinases is through direct YAP/TAZ phosphorylation. Our data in fact provide a more integrated perspective on this model, showing that, at least in the context of mechanotransduction, Hippo signaling inhibits YAP/TAZ indirectly.

**Keywords:** Mechanotransduction**,** YAP/TAZ, 3D cell reconstruction.

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# Impact of Antenatal Exposure to Bisphenol S and PFOS on Brain Organoids: Morphological and Molecular Insights

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Human brain development relies on a finely tuned interplay between genetic programming and environmental cues. Disruptions during this critical period can significantly increase the risk of both neurodevelopmental and neurodegenerative disorders. Among the environmental threats, endocrinedisrupting chemicals (EDCs), such as bisphenol S (BPS) and perfluoro-octane sulfonate (PFOS), are widespread contaminants known to interfere with hormone signaling[1]. Critically, many EDCs can cross the placental barrier, directly exposing the developing fetal brain during gestation. Although increasing evidence links EDC exposure to adverse neurological outcomes, their effects on human brain development remain poorly understood, particularly under chronic, low-dose conditions that better reflect real-life exposure scenarios. In this study, we examined how prolonged exposure to environmentally relevant concentrations of BPS and PFOS (administered individually or in combination) affects the development, maturation, and functional architecture of human brain organoids derived from induced pluripotent stem cells (hiPSCs)[2]. Cerebral organoids were generated and exposed to the compounds from day 10 to day 40 of differentiation, encompassing a critical maturation window. Morphological and molecular analyses were performed to investigate the impact of these exposures on key developmental processes. Chronic BPS exposure reduced organoid growth and altered estrogenrelated signaling. Both BPS and PFOS disrupted internal cytoarchitecture and impaired the proliferation of neural progenitors. In addition, they interfered with synaptogenesis, cortical layer formation, and the organization of the choroid plexus. Notably, BPS treatment enhanced glutamatergic maturation. Mitochondrial oxidative phosphorylation complexes were also differentially affected by the two compounds in a compound- and complex-specific manner. Overall, chronic low-dose exposure to BPS and PFOS alters multiple aspects of human brain organoid development, including neurogenesis, cortical patterning, hormone signaling, and mitochondrial metabolism. These findings underscore the neurodevelopmental risks posed by environmental endocrine disruptors and highlight the relevance of brain organoids as a platform for human-specific toxicological modeling.

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**Keywords:**Brain Development, Morphology, Brain Organoids, Synaptogenesis, Endocrine Disrupting Chemicals.

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# Bioactive Hybrid Scaffolds based on Oxidized Polyvinyl Alcohol and Human Decellularized Nerve Matrix for Peripheral Nerve Regeneration Across Critical Length Defects

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Peripheral nerve injuries (PNIs) involving segmental substance loss (gap > 10 mm) remain a significant clinical challenge due to the limited capacity for spontaneous regeneration and the suboptimal outcomes associated with current surgical interventions. Autologous nerve grafting, the gold standard treatment, is constrained by donor site morbidity, limited availability of donor tissue, and potential neuroma formation [1]. In this context, tissue engineering offers promising alternatives by combining mechanically supportive biomaterials with bioactive components capable of guiding axonal regrowth and promoting functional recovery.

In this study, bio-hybrid scaffolds were fabricated by using synthetic oxidized polyvinyl alcohol (OxPVA), selected for its tunable mechanical properties and biocompatibility, in combination with decellularized human nerve matrix (dNM), which provides a source of preserved extracellular matrix (ECM) proteins and neuroinductive cues. These scaffolds were in vitro characterized to evaluate their structural and biological properties before their pre-clinical application as guidance conduits for innovative PNI therapy.

Human peripheral nerves were decellularized using a detergent-enzymatic protocol. The efficacy of the decellularization process was assessed through DNA quantification, and histological/colorimetric analyses, which confirmed the effective removal of immunogenic cellular components while preserving the ultrastructure and key ECM molecules (e.g., collagen, glycosaminoglycans).

Two fabrication approaches were employed to generate OxPVA/dNM hybrid scaffolds: (i) incorporation of homogenized dNM within the OxPVA hydrogel matrix to create a composite blend scaffold, and (ii) formation of a bilayer scaffold with a freeze-dried dNM coating applied to the surface of the hydrogel. Scanning electron microscopy revealed that each method produced distinct porosity profiles and surface morphologies, potentially influencing cellular behavior and axonal guidance.

Scaffold cytocompatibility was evaluated by seeding them with multipotent stem cells, demonstrating cell adhesion, proliferation and potential for neural differentiation.

To assess in vivo regenerative potential, bilayer and blend OxPVA/dNM conduits were implanted in a rat model of sciatic nerve transection to bridge a 10 mm defect. After 6 weeks, histological and immunohistochemical analyses demonstrated axonal regrowth and myelin formation across the gap, with no evidence of severe immune or inflammatory reactions associated with the implanted scaffolds.

Taken together, these findings highlight the potential of OxPVA/dNM hybrid scaffolds as bioactive and structurally supportive platforms for the repair of critical-length peripheral nerve injuries, providing a compelling alternative to autografts for clinical translation.

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**Keywords:** Bio-hybrid scaffolds, decellularized nerve matrix, oxidized polyvinyl alcohol, critical gap defect, peripheral nerve regeneration.

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# Advanced medical devices for peripheral nerve regeneration using resorbable scaffolds

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The peripheral nerve injuries, which represent the most common types of traumatic lesions affecting the nervous system, are highly invalidating for the patients, besides being a huge social burden [1]. This study aims to develop and evaluate potential advanced scaffolds for peripheral nerve regeneration by combining natural and synthetic materials with stem cells. For this purpose, Neural Crest-Derived Dental Follicle Stem Cells (FENCs) were used, due to their remarkable neurogenic differentiation capabilities [2, 3, 4]. Both commercially available and newly synthesized scaffolds were tested, and two types of culture media were used: proliferative and differentiative. Extensive *in vitro* analyses were conducted to evaluate the differentiation potential of FENCs both on their own and in combination with the various scaffold types. These analyses included XTT Cell Viability Assay, quantitative Reverse Transcription Polymerase Chain Reaction (qRTPCR), immunofluorescence and Scanning Electron Microscopy (SEM) imaging. The natural biomaterials tested were Neuragen (Integra® LifeSciences), Endoform (Aroa® Biosurgery), and Hyaluronic Acid (HA)-based material, while the synthetic biomaterials were Tisseos (Biomedical Tissues) and a Poly(lactic-co-glycolic acid) (PLGA)-based material. According to the collected data, the PLGA-based material emerged as the most promising candidate for promoting peripheral neural differentiation among the tested biomaterials. Among the materials analyzed, the PLGA-Based material consistently showed the highest potential for inducing early neural differentiation and fostering progression toward mature neuronal and neuroepithelial-like phenotypes. Its performance, across both proliferative and differentiative conditions, underscores its suitability for further development in peripheral nerve regeneration. In contrast, other materials, such as Endoform and Tisseos showed some promise, but mechanical properties or inconsistent differentiation results limited them. Despite these encouraging results, the peripheral nerve regeneration device is still in the research phase. Further optimization is required to refine the differentiation protocols, enhance the homogeneity of the cell populations, and validate the findings through extended biological evaluations, including proteomic analyses. This study underscores the potential of FENCs in regenerative medicine and highlights the importance of integrating multidisciplinary strategies for the development of advanced medical devices in tissue engineering approaches.

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**Keywords:** Neural Crest-Derived Dental Follicle Stem Cells, peripheral nerve regeneration, scaffolds, neuroepitheliallike phenotype.

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# Crafting brain's microvasculature: innovative approaches with silk and biofluidic platforms

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A core objective in developing durable engineered tissues is establishing a working microvasculature. This is critical for nutrient and oxygen delivery, preventing cell death and ensuring tissue viability. In neuroscience, a significant challenge is creating a microvascular network that accurately mirrors the neurovascular unit, which is essential for CNS vitality. Naturally, vessels invade the CNS extracellular matrix (ECM) during embryogenesis. This intricate process can be partially replicated with biomaterials that provide both structural support and signaling cues.

The THOR project (European Innovation Council-Pathfinder Programme, Grant Agreement number 101099719) tackles this by using high-performance functionalized silk fibers. An innovative spinning technique allows for the precise deposition of individual functionalized fibers (10-20 micrometers) in tailored configurations. We conducted experiments where C57BL/6 Mouse Embryonic Brain Endothelial Cells were seeded on silk scaffolds. These scaffolds were modified with either vascular endothelial growth factor (VEGF) or the IKVAV peptide from the laminin alpha-1 chain. Cells were also cultured on silk meshes and tubes to establish a three-dimensional endothelial network for hippocampal vascularization.

We observed successful vascular invasion of both scaffold types and the formation of tube-like structures. These structures expressed markers for the blood-brain barrier (claudin-5) and pericytes (CD13, $PDGFR_{\beta}$), indicating the development of key components of the neurovascular unit. The resulting endothelium secreted basal lamina proteins (collagen IV, laminin), aligning parallel, perpendicular, or anchored to the silk fibers. This demonstrates the scaffold's ability to guide the organization of the vascular matrix.

The engineered biohybrid endothelium has been integrated into a sophisticated microfluidic system. This system features a tightly sealed, pressurized lid with four parallel channels, each containing four sequential wells engineered to maintain a stable air-liquid interface. Flow and oxygen sensors are integrated for precise monitoring of experimental conditions.

Our findings indicate that diverse peptides linked to the silk and the microfluidics can direct embryonic cell self-organization within the engineered framework, supporting hippocampal tissue. This work is a significant step towards constructing intricate microvascular networks and advancing regenerative therapies for the CNS. The ability to precisely engineer these vital vascular components holds immense promise for treating various neurological conditions.

**Keywords:** Microvasculature, Neurovascular unit, Silk fibers, Hippocampal vascularization, Microfluidic system.

*Sessioni giovani*

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# Micro- and Nanoplastic Release from Orthodontic Appliances: Quantitative Characterisation of 3D-Printed vs Thermoformed Devices

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The oral cavity is the initial interaction site for materials released from dental devices. This study focuses on characterising the release of microplastics (MPs) and nanoplastics (NPs) from two distinct types of polymeric orthodontic appliances: directly 3D-printed aligners (DPA) and thermoformed aligners (TFA), simulating conditions relevant to the oral environment. For this comparison, samples representing DPA (fabricated from a photopolymer resin) and TFA (manufactured from a thermoplastic sheet material) underwent controlled friction cycles in an ultrapure water bath to simulate mechanical wear. The resultant particulate matter was meticulously analysed using a suite of microscopic techniques, including optical microscopy (OM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), complemented by gravimetric analysis to quantify the released plastic residues.

The findings revealed significant differences attributable to the appliance manufacturing process. Gravimetric analysis showed that DPA samples released a substantially greater mass of MPs and NPs (0.004±0.0001 g per 200 µl of eluent) than TFA samples (0.001 g per 200 µl of eluent). Optical microscopy further demonstrated that particles from DPA were more numerous and significantly larger, covering a microscopic field area of 32.34% compared to 1.07% for TFA. TEM analysis corroborated these findings, indicating that DPA samples yielded particles with an average grain size approximately 1000 times larger than those from TFA, measuring 216.23±44.5µm² for DPA versus 0.24±0.09µm² for TFA, and their concentration was about 10 times greater. While particle aggregation presented challenges for precise individual sizing, the data consistently indicate that these appliances' 3d printing manufacturing process results in a higher propensity for plastic particle generation during simulated oral function. This highlights a potential differential exposure to MPs and NPs within the oral cavity based on orthodontic appliance type and manufacturing method.

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**Keywords:** microplastics, nanoplastics, 3D printing, particle release, quantitative analysis, dental materials.

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# Rejuvenation of intestinal microbiota via microbiota transfer in aged mice: effects on local expression of proteins and function

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We have previously shown that microbiota transfer from aged donors into young recipients triggered a series of event leading to changes at systemic level, including the nervous central system[1]. Here we sought to evaluate the effects of the rejuvenation of intestinal microbiota via microbial transfer from young donors into aged recipient mice on the intestinal expression of proteins and function.

Aged (20 month) mice were colonized with microbiota from young (3-4 month) syngeneic donors and protein expression of the colon were evaluated by proteomic approach that enabled us to assess the expression of 2453 proteins. We observed that 24 proteins were up regulates while 14 were down regulated. Interestingly, these proteins were associated to a variety of signalling pathways including Integrin-linked Kinase (ILK), Integrin, Leukocyte extravasation and IL-1 pathways and others with an overall anti-inflammatory effect.

Also, the rejuvenation of the microbiota led to a significant increase in bacterial species (such as *Lachnospiraceae,* and *Ruminococcaceae*) involved in the production of short-chain fatty acid (SCFAs) the decline of which is considered highly detrimental for the aging organism. Further, histological analyses showed that at least some aspects of innate immunity were affected. Indeed, we observed that aged recipients showed an increased expression and production on angiogenin IV, an important mediator of immunity to pathogens produced by Goblet cells. Colonization of the ageing gut with "young-like" microbiota also triggered local changes at morphological level. Importantly, these included significant changes in the number of mucus producing cells, another critical component contributing to the integrity of the intestinal barrier.

Currently, the effects of the rejuvenation of intestinal microbiota on innate immunity (IgA to orally and nasally delivered antigens) is under evaluation. Taken together, these data show that maintaining a young-like microbiota late in life might contribute to achieve and healthy ageing.

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**Keywords:** intestinal microbiota, microbiota transfer, ageing.

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# Preclinical Evaluation of a Small Molecule Inhibitor of WDR5 in Facioscapulohumeral Muscular Dystrophy (FSHD)

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular disorder, and no cure or treatment is available for the patients. FSHD is caused by aberrant reactivation of the transcription factor DUX4, which is toxic to skeletal muscle [1]. Because its pivotal role in the FSHD pathology, targeting DUX4 expression using small molecules is an attractive solution. We previously demonstrated that WDR5, a chromatin remodeling protein, regulates DUX4 expression in FSHD [2]. Interestingly, we identify a novel WDR5 inhibitor (OICRX) with high potency and favorable pharmacokinetic properties compared to the commercial OICR9429 [3], making it a strong candidate for preclinical evaluation.

We tested OICRX in FSHD patient-derived muscle cells and confirmed that it effectively suppresses DUX4 and its downstream targets. Importantly, the intermittent treatment was sufficient to achieve sustained repression of DUX4 without compromising muscle cell proliferation or differentiation, indicating a promising safety profile and a feasible in vivo treatment.

To extend these findings in vivo, we developed a humanized mouse model of FSHD in which, after OICRX treatment, we observed an improvement in muscle cell engraftment compared to vehicle control mice using both gene expression and immunostained muscle sections. Remarkably, we observe a decrease of DUX4 expression coupled with significant reduction of DUX4 target genes without affecting differentiation markers, thus strengthening the potential of WDR5 as a druggable target.

Our results lays the foundation for potential future clinical applications and a cure which is still missing for FSHD patients [4].

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**Keywords:** Facioscapulohumeral muscular dystrophy (FSHD), WDR5, Xenograft, Small molecule.

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# Analisi Ultrastrutturale 3D delle Interazioni Reticolo Endoplasmatico Assonale-Mitocondri e Scaling Morfometrico negli Assoni Neuronali

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Il reticolo endoplasmatico (RE) e i mitocondri sono organelli indispensabili nei neuroni, cruciali rispettivamente per la sintesi proteica, l'omeostasi del calcio e la produzione di energia [1-3]. La loro complessa organizzazione tridimensionale (3D) e i contatti inter-organello, in particolare a livello delle membrane associate ai mitocondri (MAM), sono vitali per la funzione neuronale [4]. Questo studio sfrutta la microscopia elettronica con la tecnica Serial Block Face-SEM avanzata, accoppiata a sofisticati metodi di ricostruzione 3D e segmentazione con deep learning, per caratterizzare in modo completo l'ultrastruttura e le relazioni morfometriche del RE e dei mitocondri all'interno degli assoni neuronali mielinizzati del nervo periferico.

L'analisi morfometrica quantitativa di 35 assoni ricostruiti ha rivelato un forte scaling intraorganello: l'area superficiale mitocondriale era altamente correlata con il volume (r di Pearson = 0.97, $p < 0.001$), così come l'area superficiale del RE con il volume ($r = 0.96$, $p < 0.001$). Ciò indica una robusta espansione volumetrica mantenendo la complessità superficiale per entrambi gli organelli, con relazioni cubiche non lineari ($R2>0.94$) che suggeriscono complesse adattazioni di forma con l'aumento delle dimensioni. È interessante notare che i singoli parametri morfometrici (ad es., area superficiale o volume) dei mitocondri e del RE non erano significativamente correlati tra loro ($r \approx 0.04$), implicando una regolazione indipendente delle dimensioni a livello del singolo organello. Al contrario, il numero di mitocondri e di elementi del RE era fortemente correlato tra gli assoni ($r = 0.79$, $p < 0.001$). Inoltre, sia il numero di mitocondri che di elementi del RE erano significativamente correlati con il calibro assonale (ad es., area della sezione trasversale, $r \approx 0.60$-$0.68$), suggerendo che gli assoni più grandi ospitano un maggior numero di organelli. Ricostruzioni 3D dettagliate hanno evidenziato punti di interazione dinamica tra il RE assonale e i mitocondri, formando notevoli strutture "a bolla", indicando cambiamenti morfologici localizzati a livello delle MAM.

Questi risultati forniscono nuove informazioni sulle adattazioni strutturali specifiche del compartimento del RE e dei mitocondri assonali. Mentre le dimensioni individuali degli organelli sembrano regolate indipendentemente, la loro popolazione complessiva all'interno degli assoni è coordinata e scala con le dimensioni assonali. Questa abbondanza coordinata di organelli probabilmente è alla base delle diverse richieste metaboliche e di segnalazione cruciali per l'integrità e la funzione assonale. Questo studio affina significativamente la nostra comprensione dell'ultrastruttura neuronale e fornisce una base per investigare come la disregolazione RE-mitocondriale contribuisca ai disturbi neurodegenerativi.

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**Keywords:** Reticolo Endoplasmatico, Mitocondri, Assoni, Ricostruzione 3D, SBF-SEM, Morfometria, Siti di Contatto Organello, Ultrastruttura Neuronale.

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# Circadian dependent motility: the role for the perinuclear actin cap

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The circadian rhythm is responsible for the regulation of the day-night cycle by release of several factors, including the master regulator glucocorticoids (GCs). Previous studies reveal that the GCs dampen the transcriptional response to EGFR activation, by leading to the hypothesis of a nocturnal activation of the pathway [1]. In line, a recent study proved that the metastatic dissemination occurs preferentially during the rest phase and is controlled also by GCs [2]. In this work, we aim to analyze the circadian modulation of the *quasi*-normal epithelial cell line (MCF10A), upon dexamethasone (DEX) synchronization. We confirmed the oscillation of clock-related genes such as CLOCK and PER1 by mRNA analysis and BMAL1 by protein analysis. Interestingly, we observed a rhythmic production of EGFR, with an asynchronous upregulation of its negative feedback regulator ERRFI1. Notably, this interaction leads to decreased phosphorylation of EGFR, along with downstream signalling.

The actin cap architecture has been described as a driver of cell dissemination [4]. Thus, we aim to mechanistically link nighttime spreading to circadian modulation of the actin cap via SUN1 regulation. Notably, we detected rhythmic oscillation of the *SUN1* gene, which encodes a component of the LINC complex essential for anchoring the perinuclear actin cap [3]. So far, our data suggest that a circadian regulation of EGFR may influence *SUN1* expression. Indeed, by employing MCF10A HER2+ cells, which lack EGFR oscillation, we observed a marked disruption of both clock-related genes and SUN1 expression compared to normal cells.

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# Extracorporeal Shockwave Therapy Stimulates Myogenic Differentiation in C2C12 Myoblasts: Ultrastructural Insights into Muscle Regeneration

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Extracorporeal shockwave therapy (ESWT) is a non-invasive and safe treatment modality used in different medical specialties, including orthopedics and rheumatology, to manage musculoskeletal conditions [1]. Currently, there is great interest in the use of ESWT on various bone and soft-tissue pathologies for its potential therapeutic effects, ranging from pain relief to functional recovery and reduced recurrence rate. However, its application to muscle injuries and disorders remains underexplored [2]. The essential principle behind ESWT revolves around the action of shockwaves (SW), which are short-duration, high-energy acoustic waves capable of propagating through tissues. Acting as a mechanical stimulus, it is believed that SW promotes various molecular and biological effects on bone and soft tissues via mechanotransduction, stimulating tissue repair and regeneration mechanisms [3]. While there is a growing body of clinical and biochemical evidence suggesting the clinical effectiveness of SW, literature regarding the ultrastructural and immunocytochemical evaluation of SW-induced effects is lacking. Therefore, this study aims to investigate the in vitro effects of SW on C2C12 myoblast cells, focusing on cell proliferation, morphology, and ultrastructural integrity. Furthermore, the impact of SW on myogenic differentiation will be evaluated to assess its potential role in muscle regeneration. The shockwave treatment was applied via a water bath [4] using a shockwave generator Duolith® (Storz Medical AG, Tägerwilen, Switzerland), at a dose of 0.1 mJ/mm$^2$ energy level, 3 Hz, and 500 impulses, whereas the control group was maintained at the same culture conditions, without previous SW exposure. These parameters were chosen according to preliminary experiments to maximize the effect of the SW while concurrently minimizing possible negative effects. Treated and untreated cells were incubated at 37°C and assessed for cell viability, western blot, and histomorphometry from day 1 to day 7. Ultrastructural analysis was also performed at both plane transmission electron microscopy and after immunoelectron-microscopy technique to detect changes in protein subcellular distribution. By advancing our understanding of the cellular and molecular mechanisms triggered by SW application, this study will contribute to its development as a regenerative therapy for musculoskeletal injuries. These findings may pave the way for broader clinical use of SW, especially for those conditions where conventional treatments have proven less effective or where patients seek non-surgical alternatives.

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**Keywords:** shockwave therapy, muscle injury, myoblasts, myogenic markers, transmission electron microscopy, immunoelectron-microscopy.

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# The Digital Transformation of Anatomy: Connecting Body Donation, Imaging, and Biomedical Innovation

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The enactment of Law 10/2020 signified a pivotal moment in the regulation of body donation to science in Italy, establishing a national legal framework that enables more structured, transparent, and ethically grounded practices. In this context, the Anatomy Center of the Alma Mater Studiorum – University of Bologna has been officially recognised as a National Reference Centre, playing a key role in coordinating and implementing educational and research activities involving donated bodies and tissues (De Caro et al., 2021). The Anatomy Center comprises two dedicated spaces: the "Giovanni Mazzotti" Anatomical Room, which supports traditional dissection-based undergraduate training, and the "Marcella Mengoli" High-Tech Anatomical Room, which is designed for advanced postgraduate education and simulation-based clinical practice. This dual infrastructure is designed to ensure continuity in anatomical learning, from foundational knowledge to specialised application. The need for a dedicated CT scanner arose from the growing importance of personalised and precision medicine, as well as the requirement to enrich anatomical simulation with tomographic imaging. To address this need, and with the financial support of the EU-backed National Recovery and Resilience Plan (PNRR) under the HEAL Italia Project (PE6 – Spoke 6 CUP J33C22002920006, Mission 4, Component 2, Investment 1.3), in 2024 the Center acquired the first CT scanner in Italy that is dedicated exclusively to research. Installation of the scanner was completed in autumn 2024 with its official inauguration scheduled for 24 June 2025. The scanner is designed to significantly enhance anatomical imaging and teaching by supporting clinical reasoning, improving spatial understanding, and integrating advanced imaging techniques into educational and research activities. Indeed, a substantial body of research has demonstrated that CT-based studies on cadavers enhance the ability to interpret anatomy in multiple planes and promote the integration of anatomical and clinical knowledge (Chytas et al., 2022; Lufler et al., 2010; Paech et al., 2017). The CT scanner will be employed in several key domains. Firstly, it will be used in the personalisation and improvement of specialist training through image-guided surgery. Secondly, it will be used in the testing, optimisation and validation of medical devices. Thirdly, it will be used in the creation of an anatomical atlas. These digital models ease immersive, reproducible, and customisable exploration of the human anatomy, with applications in education, clinical planning, and biomedical engineering. Additionally, the system facilitates the acquisition of a substantial archive of non-pathological imaging data, which will function as a high-quality reference database for research and educational purposes.

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**Keywords:** Body donation, Digital Twins, CT scan, Precision Medicine, Anatomical education, Biomedical Research.

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# Dopaminergic differentiation from the novel pluripotent cell line hGMSCs-derived iPS: a new tool for personalized regenerative medicine

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Parkinson's disease (PD) is an intractable disease that causes localized neurodegeneration of dopaminergic neurons in the pars compacta of the substantia nigra. Despite several drugs on the market to treat Parkinson's, there are currently no curative therapies registered. This underscores the urgency of identifying new alternative approaches. Stem cells have emerged as a promising emerging therapy for brain regeneration in Parkinson's disease. Human embryonic stem cells (hESCs) would be the best candidate for regenerative medicine in this field, but there are ethical issues associated with their use for research purposes. For this reason, techniques have been developed to obtain dopaminergic neurons by differentiating induced pluripotent stem cells (iPSCs). The aim of this work is to evaluate dopaminergic differentiation starting from a new iPS cell line (hGMSCs-derived iPS) obtained by reprogramming for the first time gingival mesenchymal stem cells using a non-integrating method. The new dopaminergic cell line is characterized through cytofluorimetry, real-time PCR, and immunofluorescence analyses. The results demonstrated the differentiation of hGMSCs-derived iPS into dopaminergic neurons both in dopaminergic genes expression and membrane receptors. Furthermore, the analysis of dopamine production in the supernatants demonstrated that the neurons obtained are also functionally dopaminergic. This new dopaminergic cell line, allows to identify new promising approaches for the personalized regeneration of functional dopaminergic neurons.

**Keywords:** hGMSCs-derived iPS, dopaminergic differentiation, personalized regenerative medicine, Parkinson's disease.

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# Targeting Neuroinflammation and Aging: Milmed Yeast as a Novel Probiotic Activating Autophagy and SKN1/Nrf2 Signaling

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Microglial cells, when polarized toward a pro-inflammatory phenotype, are key players in neuroinflammation, a mechanism underlying many neurodegenerative diseases. Alterations in gut microbiota composition have been shown to increase the production of pro-inflammatory cytokines and oxidative stress, contributing to neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. The importance of the gut-brain axis and the role of the microbiota in regulating brain inflammation are increasingly recognized. Probiotics, by restoring intestinal microbial balance, can help reduce systemic and cerebral inflammation. In this context, we demonstrate that Milmed – a modified strain of *Saccharomyces cerevisiae* obtained through exposure to millimeter-wave electromagnetic radiation – is capable of reprogramming LPS-activated microglial cells (M1 phenotype) toward an anti-inflammatory M2 phenotype. This effect is evident from the morphological recovery of microglia to a resting state, the decreased expression of IL-1β, IL-6, TNF-α, and iNOS, and the increased expression of IL-10 and Arginase-1. This study further explored the effects of Milmed on autophagy and oxidative stress regulation using BV-2 microglial cells and the *Caenorhabditis elegans* animal model. In BV-2 cells, treatment with Milmed increased the expression of autophagyrelated genes (Beclin-1, ATG7, LC3, and p62) and inhibited mTOR, suggesting reactivation of autophagic flux. Simultaneously, enhanced expression of NRF2, SOD1, and GPX was observed, along with increased levels of phosphorylated NRF2, indicative of an activated antioxidant response. In *C. elegans*, dietary supplementation with Milmed extended lifespan, reduced age-related ROS accumulation, and improved resistance to oxidative stress, without altering gst-4 expression. These benefits were not observed in skn-1 mutants, confirming the involvement of the SKN-1/Nrf2 pathway. Overall, Milmed emerges as a probiotic with marked immunomodulatory, pro-autophagic, and antioxidant activities, capable of modulating microglial polarization, enhancing cellular homeostasis, and promoting healthy aging, with potential relevance in the prevention and treatment of neurodegenerative disorders.

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**Keywords:** Microglia, Autophagy, Neuroinflammation, Probiotics, Oxidative Stress, Longevity, Caenorhabditis elegans.

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# *Pde5a* deficiency promotes *white-to-beige* adipocyte conversion via cAMP-PKA activation.

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Cyclic nucleotides are critical regulators of adaptive thermogenesis and adipogenesis, with their intracellular levels finely tuned by phosphodiesterases[1,2]. Phosphodiesterase type 5 (PDE5A) modulates cyclic guanosine monophosphate (cGMP) levels in adipocytes. While PDE5A inhibition has shown promise in patients with diabetes[3], its role in metabolism remains unclear. Using different *Pde5a* knockout mouse models, we demonstrated that mice lacking *Pde5a* exhibit enhanced browning of white adipose tissue and reduced hepatic fat content. Following high-fat diet, *Pde5a* deficient mice are resistant to obesity, displaying improved glucose metabolism and enhanced thermogenesis. These protective effects stem from an early developmental knockdown of *Pde5a*, leading to a metabolic reprogramming driven by cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) pathway activation. The convergence of cGMP and cAMP signalings orchestrates thermogenic and systemic metabolic adaptations. Our findings establish PDE5A as a novel regulator of energy homeostasis, suggesting its inhibition as a valuable adjuvant therapy for metabolic disorders.

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# A probiotic mixture standardized with maltodextrin prevents cognitive decline and AD-related pathology in a mouse model of accelerated senescence by maintaining the integrity of the gut mucosal-vascular barrier

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**Introduction** Growing evidence highlights the crucial role of the microbiota-gut-brain axis in cognitive disorders related to ageing, including subjective, mild cognitive impairment (MCI), and Alzheimer's disease (AD)[1]. In this context, gut mucosal-vascular barrier (GMVB) impairments can contribute to spread gut-derived factors to the brain, thus highlighting the GMVB integrity as a promising therapeutic target.[2]. In this context, connexin (Cx)-43 is essential for preserving blood barrier function by supporting the reassembly of tight junctions[3]. Here, we investigated the effect of a new probiotic mixture (PM), containing *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, and *Bifidobacterium adolescentis*, standardized with maltodextrin, on GMVB integrity in a spontaneous model of accelerated senescence with features similar to those observed in developing AD.

**Methods** SAMP8 mice and control SAMR1 mice were treated orally with PM daily or placebo for two months, to evaluate the effects of the probiotic at 6 months of age, in which SAMP8 mice shows clinical and pathophysiological features similar to those observed in the brain of MCI patients, the phase that precede AD. Cognitive impairment, astrocyte and microglia activation, brain and colonic amyloid-β1-42 (Aβ1-42) and interleukin-1β levels and claudin-5 expression, were assessed. In addition, GMVB alterations, including circulating lipopolysaccharide-binding protein (LBP) levels (marker of indirect intestinal permeability) and alcian blue staining (marker of acidic mucins), were evaluated. Expression of glial markers

(GFAP) near blood vessels was also analyzed. Finally, serum from MCI and asymptomatic patients was used to evaluate intestinal fatty acid binding protein (FABP2) and zonulin as markers of GMVB disruption.

**Results** PM administration significantly reduced cognitive impairment and glial activation along with brain claudin-5 expression, Aβ1-42 and IL-1β in brain and colon tissues. PM preserved GMVB integrity by restoring colonic acidic mucins and reducing plasma LBP. In addition, PM reduced activated GFAP+ glial cells expression near vessels. Of note, MCI patients showed reduced levels of circulating FABP2 and zonulin.

**Conclusions** PM supplementation attenuated cognitive decline, neuroinflammation, and ADrelated pathology in SAMP8 mice, probably through preservation of GMVB integrity. These results suggest that PM represents a promising dietary intervention for the prevention of cognitive decline in patients with prodromal AD characterized by increased intestinal permeability.

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**Keywords:** Microbiota-gut-brain axis, Alzheimer's disease, Gut mucosal-vascular barrier, Neuroinflammation, Probiotics.

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# Anatomical and Functional Characterization of Norbormide-Induced Rattus-Selective Cardiovascular Toxicity: Novel Morphological Insights into Species-Specific Vascular Injury

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Norbormide (NRB) is a Rattus-selective toxicant, discovered in 1964 and marketed until 2003 as an environmentally friendly rodenticide. Although its use has declined due to inconsistent effectiveness and the advent of second-generation anticoagulants rodenticides, its unique species-selective cardiovascular toxicity remains of significant scientific interest.

While NRB's lethal effect is attributed to irreversible vasoconstriction of rat peripheral arteries, potentially resulting in cardiac damage, the exact sequence of pathological events and the identity of the target organs remain unclear.

Elucidating NRB's mechanism of action is essential for the development of species-selective toxicants that could serve as alternatives to the broad-spectrum agents currently in use (1).

This study aimed to evaluate the cardiovascular effects of NRB by combining functional assays with morphological analysis (2-4).

In spontaneously beating Langendorff-perfused rat hearts, NRB significantly reduced left ventricular pressure and increased coronary perfusion pressure ($IC_{50}$ = 0.91 μM and 0.34 μM, respectively). At the highest concentration tested (5 μM), NRB caused a 23% decrease in heart rate compared to control values, prolongation of atrioventricular conduction, and severe arrhythmias such as atrioventricular block, ventricular tachycardia, and fibrillation, sometimes progressing to cardiac arrest.

At the end of each Langendorff experiment, hearts were processed and stained with hematoxylin and eosin for morphological analysis. Consistent with the observed functional alterations, the morphometric analysis revealed a significant increase in coronary artery wall thickness in NRBtreated hearts, particularly in small arteries (22,5 %) compared to control preparations. Structural alterations included marked endothelium folding indicative of vascular wall contraction in both small and large vessels. Additionally, cytoplasmic vacuolization was evident in several smooth muscle and endothelial cells, with greater severity in smooth muscle cells, characterized by large areas of empty cytoplasm around the nuclei.

Moreover, vein morphometry revealed a reduction in luminal perimeter, diameter, and area in NRBtreated hearts compared to controls.

Taken together, these findings add new insights into the cardiovascular toxicity of NRB. Morphological analysis, complemented by functional experiments, provided critical insights into NRB's species-specific toxicity, thereby advancing the elucidation of NRB's mechanism of action.

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**Keywords:** cardiovascular toxicity coronary arteries morphological analysis.

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# Mild chronic colitis worsens cognitive impairment in a spontaneous mouse model of Alzheimer's disease *via* gut-inflammasome-brain axis

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**Introduction**. Patients with intestinal bowel diseases (IBDs), including ulcerative colitis and Chron's disease, have a higher risk of developing dementia or Alzheimer's disease (AD)[1]. Indeed, enteric inflammation can spread to the brain, *via* gut-brain axis, thus triggering central neuroinflammation and degeneration[2,3]. In this context, the nucleotide-binding oligomerization domain leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome plays an important role in shaping intestinal and brain immune/inflammatory responses in AD[4,5]. In the present study, we investigated the role of enteric NLRP3 activation pathways in exacerbating cognitive dysfunctions in a spontaneous model of AD.

**Methods.** Senescence-accelerated mouse prone 8 (SAMP8) mice (4 months old, prodromal phase of AD) and SAMR1 (respective control) were employed. In order to evaluate the impact of gut inflammation on central impairments associated with AD, mice were treated with dextran sodium sulphate (DSS) 0.5% added in their drinking water for two months. In addition, groups of animals were treated orally with INF176 (a gut-restricted, no systemically absorbed NLRP3 inhibitor) 50 mg/kg/day orally to characterize the effects of a gut-directed anti-inflammatory therapy on AD progression. At 6 months, after cognitive tests by Morris Water Maze, animals were euthanized to evaluate the following parameters: 1) NLRP3 inflammasome activation in colon and brain [western blot for NLRP3 ASC, caspase-1 and ELISA for IL-1β)]; 2) intestinal and brain barrier integrity (western blot analysis for zonulin-1 and claudin-1/5).

**Results**. SAMP8 mice displayed cognitive dysfunctions, as compared to SAMR1, which were worsened in the presence of DSS administration. In addition, SAMP8+DSS animals showed activation of NLRP3 inflammasome pathways and a decrease in tight junction expression in colon and brain. Of interest, treatment with INF176 induced an improvement in cognitive functions, a reduction in NLRP3 inflammasome activation in both colon and brain and a restoration of gut and brain barrier integrity in SAMP8+DSS mice.

**Conclusions**. In the SAMP8 AD model, the induction of gut inflammation worsened the cognitive functions and boosted central neuroinflammation. Treatment with a gut-restricted NLRP3 blockade counteracted such alterations, thus corroborating the involvement of NLRP3 pathway in the gut-brain axis unsettlement associated with AD.

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**Keywords:** Alzheimer's disease, Colitis, Gut-brain axis, NLRP3 inflammasome.

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# Optimized Lentiviral Gene Delivery and Functional Targeting of PLCβ1 in Acute Promyelocytic Leukemia Models

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Efficient gene transduction and preservation of cell viability are essential for dissecting gene function in hematological malignancies. In this study, we optimized lentiviral transduction protocols in NB-4 cells, a human promyelocytic leukemia line widely used as a model of acute promyelocytic leukemia (APL), aiming to enhance genetic manipulation tools applicable to APL research.

Although an initial transduction efficiency of about 30% was achieved, a marked reduction in cell viability was observed – an effect not observed in other leukemia lines such as THP-1. To address this issue, we found that the hPGK (human PhosphoGlycerate Kinase) promoter, widely used in lentiviral vectors, is prone to silencing in NB-4 cells, preventing expression of the puromycin resistance gene. By replacing it with other promoters, i.e. EF1α promoter, we obtained stable, high-level transgene expression and efficient antibiotic selection, thus increasing the reliability of gene manipulation in NB-4 cells.

Using this improved system, we successfully silenced PLCβ1 in NB-4 and ATRA-resistant R4 cells to explore its role in leukemic growth and differentiation. PLCβ1 knockdown led to reduced proliferation, increased treatment sensitivity, and enhanced differentiation capacity, supporting its involvement in epigenetic regulation through H3K9me3 modulation. These findings suggest that targeting PLCβ1 may offer new therapeutic avenues for relapsed or ATRA-resistant APL cases.

In conclusion, this optimized gene delivery and selection strategy enables robust functional studies in APL models and highlights PLCβ1 as a potential target in overcoming therapy resistance in high-risk APL subgroups.

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**Keywords:** Acute Promyelocytic Leukemia (APL), PLCβ1, Gene Silencing, Therapy Resistance.

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# Endothelial-derived thymidine phosphorylase deficiency triggers enteric neurodegeneration: unraveling the neurovascular crosstalk in intestinal dysmotility

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The enteric nervous system (ENS) orchestrates gastrointestinal (GI) motility through a dynamic interplay with various specialized cells, including the vascular network.[1] Impairments in the neurovascular axis are hypothesized to contribute to neurodegeneration in severe motility disorders. One such condition is mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), a rare multisystemic disorder caused by mutations in thymidine phosphorylase (TP). TP is predominantly expressed in endothelial cells and plays a pivotal role in vascular homeostasis.[2] MNGIE is clinically characterized by chronic intestinal pseudo-obstruction (CIPO), with histological evidence of neuronal loss, fibrosis, and microvascular abnormalities.[3] Notably, in CIPO-MNGIE TP depletion correlates with enteric neuronal loss, suggesting a potential neurovascular pathogenic mechanism. This study investigates whether endothelial-derived TP deficiency directly affects enteric neurons, leading to neurodegeneration. Primary myenteric neuron cultures, isolated through a standardized protocol,[4] were exposed to conditioned medium from TP-silenced endothelial cells. Morphological, electrophysiological, and molecular analyses were performed over 72 hours. Neuronal network complexity, glial and fibroblast proliferation, and markers of cellular stress were evaluated through immunofluorescence and live imaging. Electrophysiological activity was assessed using microelectrode array recordings, and cytokine profiling was conducted to examine inflammatory changes. Exposure to TP-deficient conditioned medium induced a progressive disorganization of the neural network, characterized by neurite fragmentation and increased glial and fibroblast proliferation. Neuronal stress responses emerged early and progressed over time.

Electrophysiological recordings revealed an initial hyperactivation of neuronal cultures, followed by an irreversible loss of neuronal activity after prolonged exposure. Cytokine profiling indicated an inflammatory shift, with elevated levels of pro-inflammatory mediators and a reduction in antiinflammatory factors. These findings provide evidence that TP deficiency in endothelial cells disrupts ENS homeostasis, leading to neurodegenerative changes. The results suggest that vascular-derived TP is a critical regulator of enteric neuronal integrity. This study underscores the importance of the neurovascular interplay in GI dysmotility disorders and paves the way for novel therapeutic strategies aimed at preserving enteric neuronal function in severe gut dysmotility, including CIPO.

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**Keywords:** Enteric Nervous System, Thymidine Phosphorylase, Neurovascular Axis, Chronic Intestinal Pseudo- Obstruction.

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# Pesticides exposure in HUVEC cells: Telomerase dysfunction, Morphological modification and mtDNA variation.

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Pesticides pose a significant threat to human health due to chronic exposure. Telomerase, regulated by its catalytic subunit TERT, is crucial for maintaining telomere length and vascular endothelial integrity. This study aimed to investigate the effects on Human Umbilical Vein Endothelial Cells (HUVECs), a representative model for vascular endothelium, treated with both single and combinations of pesticides, trough MTS, TERT expression, mitochondrial DNA (mtDNA) copy number, telomere length, and endothelial tube formation evaluation. The results show time-dependent reductions in HUVEC viability, and significant telomere shortening in pesticide-treated HUVEC cells, with Lambda-cyhalothrin showing the strongest effects. A downregulation of TERT expression is correlated with telomere attrition, indicating an impairment of telomerase function. Pesticides also altered mtDNA copy number, causing both increases and decreases. Moreover, morphological analysis of the tube test, conducted via Scanning Electron Microscopy (SEM), evaluates alterations in structural changes. The current study underscores the urgent need to evaluate environmental toxicants' long-term impacts and develop protective strategies to mitigate their adverse effects on human health.

**Keywords:** Pesticides, HUVECs, Telomerase (TERT), Telomere length, Mitochondrial DNA, SEM analysis on tube formation assay.

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# Multi-Modal Imaging for Comprehensive Analysis of Muscle-Tendon Unit Injury

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Muscle-tendon units, interconnected via Muscle-Tendon Junctions (MTJs), are vital for bodily support and movement, but they are vulnerable to damage from trauma, sports injuries, aging, and chronic diseases (1). Our study employed a multi-modal imaging approach on mouse muscle-tendon tissues to analyze the structural characteristics across hierarchical scales, ranging from secondary structure to tissue micro-architecture. This approach enabled the detection and quantification of changes induced by injury.

The research focused on the mouse Extensor Digitorum Longus (EDL) muscle-tendon unit, exposing post-mortem to forced eccentric contraction (EC) through stretching and a series of contractures in a high-$K^+$ solution. The contralateral EDL served as control (CTR) (2). EDL were then processed as formalin-fixed paraTin-embedded tissues (FFPE). The entire block was investigated by Synchrotron-based Phase-Contrast microtomography (PhC-microCT), supported by artificial intelligence tools for data analysis. Then, slices were cut and examined for histological and histochemical staining, and Fourier Transform Infrared Imaging (FTIRI) spectroscopy.

The combination of these complementary methods provided a comprehensive exploration of the tissues' diverse aspects and hierarchical dimensions. By synchrotron-based imaging, it was possible to explore the three-dimensional microstructure of the myotendinous tissue by evaluating the morphometric properties at an 890-nanometer pixel resolution. A quantitative analysis of the tendon density was conducted to assess the impact of stimulation on the alignment of the tendon fibers in proximity to the MTJs. This analysis detected the grey tone variation in the histogram of the brightness extracted from synchrotron images, at both distal and proximal levels. Moreover, the percentage of muscle fibers at the junctions was analyzed in both the controls and the stimulated samples to assess the structural modification of the muscle and grade eventual damage. FTIRI revealed a higher intensity of the infrared absorption within injured tendon and MTJ tissues, at both distal and proximal levels, mainly ascribable to the relative amount of collagen fibers. Histological and histochemical examinations detected the damage mainly in the muscle tissue, evidencing that the eccentric contraction altered the fiber diameter and damaged the contractile units.

Overall, we demonstrated that our approach consistently supported traditional histological methods, including Synchrotron PhC-microCT and FTIRI, in detecting morphological and structural features in tissues. Furthermore, we were able to detect damages induced by EC in muscle and tendon units, providing a deeper analysis of the muscle-tendon tissues.

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**Keywords:** Muscle, Tendon, Muscle-Tendon Junctions (MTJs), Synchrotron-based Phase Contrast Microtomography, Fourier Transform InfraRed Imaging spectroscopy, Histology.

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# *Lactiplantibacillus plantarum* IMC 510 supplementation promotes colonic well-being in animal models of cafeteria-induced obesity

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Obesity is a persistent pathological condition characterized by excessive accumulation of adipose tissue, associated with a high risk of several morbidities and increased mortality. The link between excess calories and gut microbiota imbalance is well established, and alterations in this balance contribute to the promotion of chronic low-grade inflammation. Our research aimed to examine the impact of a 12-week supplementation of Lactiplantibacillus (L.) plantarum IMC 510 (Lp) in rats fed a cafeteria diet (CAF), compared to those fed a standard diet (CHOW). To explore this aspect, the alterations in the gut microbiota were characterized, and the effects linked to the state of the colonic mucosa and enteric neuroplasticity were pointed out. Previously published data have shown that in the CAF Lp group, the probiotic maintained the predominant bacterial phyla in the composition of the intestinal microbiota compared to the CAF-fed rats [1]. Furthermore, Lp effectively reduced food intake and weight gain, with subsequent beneficial effects highlighted by serological, biochemical, and histological analyses. The CAF Lp group shows a significant reduction in blood glucose and blood lipid profile, particularly lowering LDL [1]. Using various morphological staining techniques across the different experimental groups, no alterations in the architecture and structure of the colonic crypts were observed. In the CAF Lp group compared to the CAF-fed rats, a marked reduction in mucus secretion and composition, especially in the acid profile, was noted through staining with Alcian Blue pH 2.5. A significant reduction of Mucin-2, the major protein of the polymer network composed of gel-forming mucins, was associated with the administration of Lp. Concerning the enteric nervous system, no morpho-structural alterations of the myenteric plexuses were observed with the administration of Lp. A pan-neuronal marker was tested, revealing a decrease in immunoreaction that could be related to an alteration of intestinal motility [2]. By analyzing samples incubated with glial markers, the results indicate glial rearrangement in obesityrelated inflammatory conditions. No recognizable differences were found between experimental groups when discriminating cholinergic neurons within the myenteric plexus. In contrast, the nitrergic neuronal network underwent neurodegeneration in obese groups, possibly related, in accordance with other studies, to oxidative stress and inflammatory status. In conclusion, the Lp intervention could represent a new strategy to counteract the negative effects induced by obesity-related dysbiosis, making this approach both novel and safe for the prevention and management of obesity.

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**Keywords:** Obesity, cafeteria (CAF) diet, *Lactiplantibacillus (L.) plantarum* IMC 510, Dysbiosis, Enteric Nervous System, Obesity, Cafeteria diet**.**

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# DYRK3 regola la motilità e l'invasione delle cellule tumorali tramite fosforilazione di Liprin-α1

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Le piattaforme associate alla membrana plasmatica (PMAPs) sono complessi molecolari dinamici che si organizzano in aree specifiche delle cellule, comprese cellule tumorali metastatiche, in particolare vicino alle adesioni focali, dove giocano un ruolo fondamentale nel favorire la motilità cellulare[1,2]. Sono costituite da proteine strutturali come ERC1, LL5 e Liprina-α1. Nei lamellopodi delle cellule tumorali con fenotipo invasivo, le PMAPs mostrano proprietà simili a quelle dei condensati biomolecolari (BCs), strutture con comportamento liquido formate tramite separazione di fase liquido-liquido (LLPS), processo che dipende da interazioni deboli e multivalenti tra regioni intrinsecamente disordinate (IDRs). È stato dimostrato che l'assemblaggio di questi BCs può essere regolato da eventi di fosforilazione in grado di modificare le proprietà delle proteine coinvolte. In particolare, la chinasi Ser/Thr DYRK3 è nota per disassemblare i BCs fosforilando residui amminoacidici nelle IDRs[3,4]. Per valutare se DYRK3 abbia un ruolo analogo nelle PMAPs e chiarirne l'impatto sulla motilità tumorale, abbiamo combinato microscopia confocale e saggi funzionali per valutare alterazioni della motilità cellulare. Analisi immunochimiche e mutazionali hanno permesso di mappare i bersagli di fosforilazione di DYRK3. Sono stati generati mutanti non fosforilabili (Ser/Thr → Ala) nelle IDRs al fine di identificare i siti funzionali coinvolti nella regolazione delle PMAPs mediata da DYRK3. Abbiamo osservato un'elevata espressione di DYRK3 nelle cellule di carcinoma mammario invasivo, in associazione a un ruolo chiave nella regolazione della motilità cellulare. L'inibizione dell'espressione di DYRK3 compromette significativamente la migrazione e l'invasione cellulare, mentre la sua sovraespressione porta al disassemblaggio delle PMAPs endogene, con conseguente destabilizzazione delle protrusioni cellulari e alterazione del *turnover* delle adesioni focali. Questi risultati evidenziano l'importanza di DYRK3 nel controllo del fronte migratorio e sottolineano la necessità di una sua regolazione precisa per mantenere l'equilibrio della motilità cellulare. Considerando l'effetto di DYRK3 sulle PMAPs abbiamo esplorato se le proteine chiave di tali strutture fossero substrato dell'enzima. Saggi di immunoblot ci hanno confermato che la Liprina-α1 è substrato e interattore di DYRK3. Mediante analisi mutazionali, è stato identificato il residuo Thr-701 come sito di fosforilazione fondamentale: l'espressione del mutante T701A riduce significativamente la motilità, evidenziando l'importanza funzionale di questo sito. In conclusione, DYRK3 emerge come un nuovo regolatore della dinamica delle PMAPs e della motilità cellulare, agendo attraverso la fosforilazione di Thr-701 in Liprina-α1. Questi risultati rivelano un nuovo meccanismo molecolare di regolazione del fronte cellulare e suggeriscono potenziali bersagli terapeutici per limitare la migrazione e invasione delle cellule tumorali.

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# Selenoprotein T Mitigates Cardiomyocyte Senescence via CD36 Receptor Modulation

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Ageing is a primary risk factor in the pathogenesis of cardiovascular diseases (CVDs)[1]. During aging, the myocardium undergoes maladaptive changes that contribute to the progressive deterioration of cardiac structure and function[2]. Emerging evidence highlights oxidative stress (OS) as a key driver of cellular senescence. OS arises from a disruption in redox homeostasis, which is further exacerbated during senescence due to a decline in antioxidant defences[3,4]. In this context, selenoproteins, as selenoenzymes, play a fundamental role in safeguarding cells against oxidative damage[5]. Selenoprotein T (SELENOT), an endoplasmic reticulum (ER)-resident protein, is essential for maintaining ER homeostasis, protein glycosylation, and calcium mobilisation[6,7]. This selenoprotein is highly expressed during cardiogenesis and is re-expressed in the adult heart under stressful conditions, where it exerts protective effects against OS, inflammation, hypertrophy, and apoptosis[8-11]. Given the emerging role of SELENOT in heart function and stress responses, we hypothesise that it may exert a protective action against cardiac senescence in human cardiomyocytes. Here, we employed a SELENOT-mimetic peptide, named PSELT, able to recapitulate the activity of the full-length protein through the redox-active CVSU motif and we investigated its role in counteracting Doxorubicin (DOX)-induced cardiomyocyte senescence. Our results showed that PSELT counteracted the increased β-galactosidase activity, p53/p21 upregulation, and the increase of senescenceassociated secretory phenotype (SASP) markers such as MMP3, IL-6, and TNF-α. Moreover, PSELT prevented cytosolic and mitochondrial oxidative imbalance also restoring the activity of endogenous antioxidant enzymes. Crucially, PSELT reduced DNA damage, both decreasing p-γH2AX and maintaining lamin B1 levels, and alleviated ER stress downregulating BIP, calnexin, IRE1α, and ERO1α. Notably, SELENOT was upregulated in response to senescence, suggesting a redox-sensitive stress-sensing function, and was restored by PSELT. Loss-of-function studies showed that SELENOT deficiency led to cardiomyocyte death/DNA damage, only partially rescued by PSELT, indicating a regulatory SELENOT/PSELT axis. Mechanistically, PSELT, similar to the CD36 inhibitor (SSO), suppressed senescence-induced CD36 upregulation. Interestingly, co-immunoprecipitation analysis demonstrated SELENOT-CD36 interaction, supporting the hypothesis that SELENOT physiologically engages with CD36 under both normal and stress conditions. In conclusion, this study underscores the crucial role of SELENOT in preserving the viability and genomic integrity of senescent human cardiomyocytes, functioning as an "ageing-sensing redoxin". Furthermore, our findings suggest that the PSELT/SELENOT-mediated inhibition of CD36 – a key contributor to the pathogenesis of age-related cardiac dysfunction – may represent a promising therapeutic strategy for mitigating age-associated cardiac decline.

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# Effect of type 5 Phosphodiesterase (Pde5) deletion on neurogenesis

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Type 5 phosphodiesterase (PDE5) is an enzyme that specifically controls cGMP levels by breaking the phosphodiester bond, regulating signal transduction (1). Several PDE inhibitors, among which sildenafil is the best known, have been developed and used as therapeutic agents to increase cGMP levels and modulate cellular activities (2, 3, 4). In the brain, PDE5 is expressed in the cytoplasm of pyramidal neurons in cortex and hippocampus, and, within the cerebellum, in Purkinje neurons (5, 6). Nitric oxide, cGMP, and Pde5, indirectly, are involved in a particular phenomenon of synaptic plasticity, the long-term potentiation, altered in Alzheimer's disease (AD) (7).

In our lab, we developed a PDE5 knock-out (KO) mouse model, mimicking the effect of a constant PDE5 inhibition. To evaluate the role of PDE5 in mouse brain postnatal development, we focused on studying cortical neurogenesis on wild-type (WT) and KO brain sections from one-month-old mice. Histological analysis of isolated brains revealed a general thinning of the hippocampal region of KO mice with a reduction in the size of the dentate gyrus and the pyramidal layer and a slightly reduction in the migration pattern of neuroblast precursors along the rostral migratory stream.

These observations suggest that PDE5 might support adult neurogenesis promoting neuroblasts differentiation towards GABAergic and pyramidal fate. Moreover, since Pde5 could be involved in the maintenance of the balance between the excitatory and inhibitory activities of GABAergic and glutamatergic neurons, its absence has the potential of interrupting this balance. To verify these hypotheses, we performed analysis on neural stem cells (NSCs) isolated from adult PDE5 KO and WT mice, revealing that KO NSCs proliferate and migrate at higher rates compared to WT cells, and they preferentially differentiate towards the neuronal fate at the expense of glial lineage. Several other analyses are currently ongoing to better characterize the physiological roles of PDE5 in adult neurogenesis.

Since it has been previously observed that PDE5 inhibition rescues memory impairment in a mouse model of AD (7), our model will be useful to better understand the role of PDE5 in controlling postnatal neurogenesis in health and diseases.

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**Keywords:** PDE5, neurogenesis, Alzheimer's disease.

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# Phenotyping Knee Osteoarthritis Through Gait and Balance Assessment Using Machine Learning

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Knee osteoarthritis (OA) is a major contributor to pain and mobility limitations in older adults, frequently accompanied by gait abnormalities and balance impairments. This study aimed to determine whether distinct functional subgroups could be identified within the knee OA population by analyzing gait and balance data collected via wearable sensors using unsupervised machine learning techniques. Recent advances in artificial intelligence, particularly Principal Component Analysis (PCA) and Self-Organizing Maps (SOM), offer promising tools for uncovering hidden biomechanical patterns. Applied to sensorbased movement data, these methods may support the development of more personalized physical activity interventions.

This cross-sectional study involved 50 participants (25 with knee OA and 25 healthy controls), aged 45 to 80 years. Gait and postural stability data were collected using the BTS G-Walk® inertial sensor. Gait parameters were recorded during level-ground walking, while balance was assessed in static stance under both eyes-open (EO) and eyes-closed (EC) conditions. After statistical group comparisons, a fourstage machine learning pipeline – comprising PCA, SOM, and K-means clustering – was applied to identify latent functional subtypes within the OA group.

Compared to healthy controls, OA participants showed significantly slower gait speed (1.05 vs. 1.30 m/s, $p = 0.025$), reduced cadence (101.98 vs. 110.04 steps/min, $p = 0.015$), and a shorter propulsion phase (5.3% vs. 7.6%, $p = 0.007$), alongside greater sway ellipse areas in EO (1656 vs. 427 $mm^2$, $p < 0.001$) and EC (2011 vs. 443 $mm^2$, $p < 0.001$) conditions. PCA extracted four principal components representing gait performance, asymmetry, cadence, and postural control. Clustering analysis revealed three functional subgroups among OA participants: (C1) individuals with severe functional limitations (80% with Kellgren–Lawrence grade 2–3), (C2) high-functioning individuals (50% with KL grade 1), and (C3) individuals with marked postural instability (40% with KL grade 2).

These findings highlight the functional heterogeneity within the knee OA population and emphasize the limitations of radiographic severity alone in characterizing patient profiles. The identification of distinct motor phenotypes through wearable sensor data and unsupervised learning offers a foundation for personalized movement-based interventions tailored to patients' biomechanical profiles.

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**Keywords:** Gait analysis, balance, knee osteoarthritis, machine learning, functional movements.

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# Anatomical Impact of Neurocranial Deformation on Stomatognathic Asymmetry

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Human craniofacial anatomy results from complex genetic, epigenetic, and environmental interactions, with the cranium and palatal arch being fundamental for brain protection and oral and respiratory functions. However, the influence of these factors on palatal arch morphology and asymmetry – especially in the presence of mechanical alterations of the cranium during growth – remains poorly understood, particularly with regard to the stomatognathic system. Artificial cranial modification (ACM) applied to the cranium, a common practice among pre-Hispanic Andean populations[1] and used as a marker of group identity[2,3], provides a model for studying non-physiological mechanical alterations in the developing stomatognathic system. The comparative analysis of cranial and palatal morphology between adult individuals with ACM (applied during the prepubertal period of high bone plasticity) and unmodified individuals allowed us to clarify the long-term effects of mechanical forces on craniofacial development.

We analyzed 20 adult Peruvian skulls (19th–20th century) preserved at the Museum of Anthropology and Ethnology, University of Florence (Italy), divided into two groups: 10 with clear evidence of artificial cranial modification (ACM) and 10 belonging to the unmodified group. The skulls were digitized using high-resolution 3D scanning and analyzed with threedimensional geometric morphometric methods, applying 41 semi-landmarks to the palatal surface[4] and 286 semi-landmarks to the cranium[5], in order to observe morphological variation. Subsequently, directional asymmetry of the palatal arch[4] was assessed to compare individuals with and without cranial modification.

Statistically significant cranial differences between modified and unmodified skulls confirm the effectiveness of ACM, while palatal arch morphology showed no significant differences. Most of the variance in palatal shape was due to individual variability, but directional asymmetry was greater in modified individuals (24% vs. 16%), indicating an increase in asymmetry rather than a consistent directional bias. The absence of differences in the palatal arch suggests that unfused cranial synchondroses during puberty help dissipate the mechanical stresses of ACM by providing growth centers and flexibility. However, the greater asymmetry in modified individuals indicates that the biomechanical effects of ACM can persist beyond skeletal maturation, inducing compensatory remodeling within the stomatognathic system to maintain functional balance.

In conclusion, the persistence of unfused cranial synchondroses during growth[6,7] buffers mechanical forces on adjacent structures such as the stomatognathic apparatus. Nevertheless, the directional asymmetry observed in adulthood highlights the lasting impact of early cranial modifications and the compensatory capacity of the masticatory system. These findings underscore the need to consider the craniofacial complex as an integrated unit in clinical practice.

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**Keywords:** Craniofacial adaptation, Neuroanatomy, Directional asymmetry, Artificial cranial modification, geometric morphometrics, palatal arch, Evolutionary Anatomy.

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# Morphometric Evaluation of Enteric Ganglia as a Diagnostic Tool in Severe Gut Dysmotility

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Severe gut dysmotility (SD) is a debilitating clinical condition characterized by a marked impairment of intestinal propulsion. Recent histopathological investigations[1] have revealed specific features associated with SD, including a reduction in the number of neurons per ganglion in both the myenteric and submucosal plexuses, as well as an increase in the inter-ganglionic distance within the myenteric plexus. Despite these insights, the quantification of neuronal populations remains labor-intensive and technically demanding, even when employing simplified and validated protocols. This study aimed to explore whether the number of ganglia, rather than neurons, within the submucosal and myenteric plexuses could serve as a more accessible, quantitative, and reliable morphometric index for the diagnosis of SD.

We analyzed jejunal samples from 39 patients with clinically and histologically confirmed SD and from 8 controls. Patients were evaluated both as a whole and after stratification according to histochemical phenotype in apparently normal (AN), inflammatory neuromyopathy (INF), and degenerative neuromyopathy (DEG). Formalin fixed paraffin embedded tissue sections underwent immunohistochemical staining for Neuron Specific Enolase (NSE) as a pan-neuronal marker. Following the neuromuscular ridge from right to left, all consecutive fields containing the myenteric plexus (≥8 fields) were analyzed and normalized to the plexus length (in mm), while all fields including the submucosal plexus were analyzed and normalized to the area of submucosa examined. Our findings show a statistically significant reduction in the number of myenteric ganglia per mm in SD patients compared to controls ($P = 0.0002$), with 87% of patients falling below the threshold defined by the lowest control value. This reduction was consistent across all histological subtypes (AN: $P = 0.0032$; INF: $P = 0.0145$; DEG: $P = 0.0004$). Submucosal ganglia analysis also revealed a significant decrease in SD patients ($P = 0.0410$), but only 26% of cases fell below the threshold observed in controls. The reduction in submucosal ganglia was statistically evident only in the AN and DEG subgroups.

These results suggest that the quantification of ganglia, particularly in the myenteric plexus, may represent a practical and discriminative parameter for supporting the histological diagnosis of SD. While further validation is needed, this work provides proof of concept for the development of a simplified diagnostic algorithm based on morphometric criteria.

Future research will explore the volume and size of ganglia, potential sex-related differences, and extend the morphometric assessment beyond the jejunum to distal intestinal regions such as the colon, to refine diagnostic accuracy and broaden clinical applicability.

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**Keywords:** Severe gut dysmotility, myenteric ganglia, neurons.

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# Implementation of gamification, flipped-classroom approach and 3D virtual tools in neuroanatomy teachings: a pilot phase

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In our University an International degree in Medicine and Surgery (IMS, delivered in English language) is also available. IMS students are given a basic course on human anatomy during the first year, *Fundamentals of Human Morphology*, and receive further human anatomy teachings in the subsequent years (vertical tracks). Given that the class is rather small (n=36) it is rather easy to implement innovative teaching approaches. During the 24/25 academic year we implemented all these strategies in the advanced *Neuroanatomy* course (IV year), given positive feedback for a peer-to-peer experience we organised the previous year (Alberti P., 2024).

At the start of the course, students (n=36) were asked to complete a multiple-choice questionnaire that was retaken at the end of the course to intercept the amelioration. *Anatomage Table* and the companion platform *Anatomage Lessons* were used to facilitate learning (each student was assigned an individual license). The class was divided into 6 teams to implement a gamification, as well as an interactive approach. At the start of each period, each team had a session at the *Table* exploiting the quiz mode to revise topics revised the previous period: the score of each test was recorded to generate a ranking of all teams. In the meantime, other teams completed an assignment on *Anatomage Lessons*. Then, the professor delivered the frontal part of the lesson relying mostly on live dissections on the *Table*, rather than using a slide deck (it was kept to a minimum). At the end of each lesson, once again, teams came to the Table to consolidate what presented that day, while the other teams continued to work on an assignment via *Anatomage Lessons* and/or paper copy exercises. During the last lessons, the winning team at the tournament was identified. Moreover, a satisfactory questionnaire (0, worst – 10, best score) was collected to verify students' perceptions of this novel format.

At the start of the course, the mean of correct replies to the preliminary multiple-choice questionnaire was 64% overall, with ¼ of the questions which were answered correctly by less than 45% of students. After completing the course, more than 85% of students were able to reply correctly to all questions; moreover, for 25% of the questions students were able to give a correct answer in 100% of the cases and the question with the lowest performance received 83% of correct answers. Concerning students' satisfaction, these were the median scores for the most relevant items: a) *Table* impact respect to a regular textbook: 9.5 (Q1:8.25; Q3:10); b) relevance of direct *Table* use by students: 9 (Q1:8; Q3:10); *Anatomage Lessons* use: 9 (Q1:8; Q3:10); paper copy exercise: 6.5 (Q1:4; Q3:8); *Anatomage tournament:* 10 (Q1:8.25; Q3:10).

Results of this pilot phase are encouraging and promising: next academic year we are even further implementing this approach for this course as well as in all other vertical tracks.

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**Keywords:** Virtual dissections, innovative teaching, gamification, flipped-classroom.

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# Unravelling axonal damage mechanisms in peripheral nerves: the role of sodium-calcium (NCX) exchanger

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Oxaliplatin(OHP)-induced peripheral Neurotoxicity (OIPN) is a common and potentially long-lasting adverse event of the most used regimens to treat gastrointestinal neoplasms. OIPN encompass an acute and chronic form. Transient axonal hyperexcitability characterises acute OIPN, as a consequence of OHP-induced functional alterations of Voltage-gated Sodium Channels (NaV). Chronic sensory OIPN, instead, is characterised by persistent axonal damage (AxD). The sodium(Na+)-calcium(Ca2+) exchanger 2 (NCX2) might be pivotal in AxD onset following this potential chain of events as we already demonstrated (*Alberti et al., 2020; Ballarini et al., 2022*): OHP-induced NaV unbalance, in fact, causes excessive Na+ intracellular levels leading to NCX switching to reverse-mode. Reverse mode results in Ca2+ abundant intake, thus leading to AxD via Ca2+-related neurotoxicity. We relied on *in vitro* OIPN mouse models to further explore this hypothesis.

Primary mouse dorsal root ganglia (DRG) cultures were used to undertake a series of multiple neurotoxicological studies (24- or 48-hours treatment, with scaling OHP dosages) exploiting the Nanolive CX-A 3D holotomographic microscope for an automated label-free live cell imaging, on top of classical neurite elongation analysis. Moreover, western blot (Wb) and immunofluorescence (IF) to test NCX2 protein expression and localisation were performed. SEA0400, a potent and selective NCX inhibitor, was used in a proof-of-concept setting to verify neuroprotection inhibiting the NCX family before OHP administration (3h pre-treatment, 1 μM).

Key morphological alterations in OHP-treated neurons, at holotomographic microscope, were neurite fragmentation and a high-reduced viability, preceded by autophagic stress and necroptosis. NCX2 protein expression after 24-hours treatment was significantly increased, while it decreased after a 48-hours treatment; its localisation in OHPtreated neurons was more pronounced at the level of the plasma membrane, with a pattern in correspondence of satellite cells. SEA0400 pre-treatment showed neuroprotection potential in case of exposure to different OHP concentrations (7.5, 15, 25, 50 μM).

We were able to further characterise the morphological changes over time that lead to AxD thanks to live-cell imaging and we were also able to explore the role of NCX2 in AxD development. Observed Wb findings might mirror a well-known, endogenous, despite inefficient, auto-protective mechanisms: after 24 hours, to compensate Na+ increased levels, NCX2 was overexpressed, thus boosting the reserve mode activation, whereas, after 48 hours, it was downregulated to prevent Ca2+ toxicity. In line with NCX2 pivotal role in AxD development, its pharmacological inhibition with SEA0400 was shown as an efficacious neuroprotection strategy, paving the way for novel treatments.

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**Keywords:** Axonal damage, peripheral nerves, neuropathy, NCX2, neuronal hyperexcitability, live-cell imaging.

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# Epiretinal membranes: cell receptors involved in cell migration and their interaction with the extracellular matrix

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Epiretinal membranes (ERMs) are fibrocellular structures that form at the vitreoretinal interface, sometimes asymptomatically but often leading to visual disturbances. They could be secondary to ocular or systemic diseases or idiopathic and they are composed of different cell types and extracellular matrix (ECM). In our previous works we highlighted a central role of cluster of differentiation 44 (CD44) and of one of its partner, podoplanin (PDPN) in ERM developmental dynamics[1,2]. Given that CD44 is a hyaluronic acid (HA) receptor and HA is a glycosaminoglycan (GAG) abundantly present in ECM, we focused our attention on ECM proteoglycans (PGs) and GAGs investigating their distribution on ERM samples. Our analysis revealed the presence of PGs such as decorin (DCN), prolargin (PRELP), perlecan (PLC), and collagen XVIII, alongside a widespread distribution of HA. Considering that HA is also a major constituent of the vitreous, we examined its influence on Müller cell behaviour by using the MIO-M1 human immortalized Müller cell line. With this approach we assessed the effects of HA and transforming growth factor-beta 2 (TGFβ2), a cytokine implicated in ERM formation, on cell migration, glial-to-mesenchymal transition, contractility, and ECM production. We observed that HA and TGFβ2 have different effects: HA is able to enhance MIO-M1 cell migration in scratch assay, whereas TGFβ2 significantly reduced this phenomenon. However, TGFβ2 induces a glia-to-mesenchymal transition, leading to the production of ECM components and down-regulation of glial markers. Finally, we performed immunofluorescence experiments to elucidate the localization of PDPN and CD44 in stimulated and unstimulated MIOM1 cells scratch tested. Results showed that CD44 and PDPN are co-localized in plasma membrane of cells facing the scratch while they appeared diffuse in the other cells. Taken together our results support the involvement of CD44 and PDPN expression in ERM formation and point to a possible role of HA in favouring Müller cells migration to the vitreoretinal interface.

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**Keywords:** Retina, vitreoretinal interface, podoplanin, hyaluronic acid, CD44, Müller cells.

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# Postural adaptations in upright standing due to virtual reality in healthy subjects

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Posture is the position of the human-body segments in the space. Posture stability is regulated by the Central Nervous System (CNS), consisting of a set of anatomical structures (brainstem, cerebellum, basal ganglia, thalamus, and several cortical regions) and peripheral receptors (visual, stomatognathic, vestibular, and somatosensory) [1]. Postural Control System continuously processes sensory inputs to generate motor responses stabilizing whole-body posture. Among postural receptors, vision plays a dominant role in postural adaptations; in fact, different environmental conditions and visual target distances can affect postural stability parameters [2]. The lack of a standardized testing environment affects the reliability of many scientific and clinical results. Virtual reality (VR) could be a promising tool for standardizing clinical assessment [3]. The aim of this study is to analyze the effects of a standardized virtual room and the weight of the Head Mounted Display (HMD) influences stabilometric exam. Fifty healthy young adults underwent postural analysis in upright standing under four conditions: (a) Open Eyes (OE), viewing a physical target at 0.70 m; (b) Closed Eyes (CE); (c) Open Eyes viewing a virtual target through a Head-Mounted Display (HMD) in Virtual Reality (HMD-OE); (d) Closed

Eyes wearing HMD (HMD-CE). Intra-subject variability of each parameter was evaluated by Coefficient of Variation (CV). Repeated measure ANOVA or non-parametric Friedman Test with post hoc ($\alpha < 0.05$) were used to test the effects of different conditions on postural control. The variability analysis showed that CoPsa and LSF had the highest variability (median CV: 30–50%), unlike CP-speed (median CV: 10-30 %). No significant differences emerged in the CVs of stabilometric parameters across the four conditions. Moreover, a significant increase of CoPsa and decrease of LSF were found both in CE vs OE condition and in CE vs HMD-OE ones; while no significant differences were found both in OE vs HMD-OE, CE vs HMD-CE, OE vs HDM-CE and HMD-OE vs HMD-CE. The results of variability analysis show a low reliability of stabilometric parameters, regardless of the experimental condition; in particular, Copsa and LSF showed the highest CVs, while CPspeed exhibited the lowest. Regarding the effect of different conditions on the stabilometric exam, a visual impact was observed, with postural stability worsening in CE vs OE; moreover, the comparison between natural and virtual environments reveals that VR and HMD weight did not influence postural stability (OE vs HMD-OE; CE vs HMD-CE). Eventually, the significant differences, found for CE vs HMD-OE, associated with the other nonsignificant comparisons (HMD-OE vs HMD-CE and OE vs HMD-CE) seem to suggest an additional proprioceptive stimulation due to wearing HMD, reducing the subject's sway.

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**Keywords:** posture, postural control, virtual reality, head mounted display, stabilometry.

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# Fratture di Le Fort I e II su Donatore: la preparazione anatomica ad alta fedeltà clinica finalizzata al training avanzato per la gestione in emergenza delle vie aeree del Paziente con Trauma Complesso del Distretto testa-collo

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**Introduzione.** Le fratture di Le Fort di tipo I e II rappresentano una sfida rilevante nei contesti di emergenza, per l'instabilità del massiccio facciale e l'elevato rischio di ostruzione acuta delle vie aeree. La formazione del personale medico in scenari ad alta complessità è spesso ostacolata dalla mancanza di modelli simulativi realistici e fedeli dal punto di vista anatomico.

**Obiettivo.** Con questo lavoro descriviamo la preparazione anatomica eseguita presso la sala settoria del Dipartimento di Anatomia dell'Università di Bologna, finalizzata alla riproduzione su Donatore delle fratture di Le Fort I e II, preparate per il successivo training avanzato ad alta fedeltà clinica del personale medico dei dipartimenti di emergenza-urgenza ed extraospedaliero nella gestione avanzata delle vie aeree nei Pazienti con trauma facciale complesso.

**Materiali e Metodi.** Le fratture Le Fort I e II sono state riprodotte chirurgicamente su donatore tramite l'applicazione controllata di forze meccaniche, seguendo protocolli chirurgici validati e descritti in letteratura. La riproduzione anatomica è stata valutata e confermata clinicamente da un team di chirurghi del Distretto Testa-Collo. È stato quindi allestito uno scenario anatomico realistico e fedele, con instabilità del palato duro, mobilità mascellare e collasso oronasale, grazie al quale il personale medico ha potuto esercitarsi nella gestione avanzata delle vie aeree nei traumi facciali complessi.

**Risultati.** La simulazione ha permesso una riproduzione fedele delle dinamiche cliniche associate alle fratture Le Fort I e II, offrendo un'esperienza immersiva nella gestione delle vie aeree in presenza di instabilità scheletrica del massiccio facciale. I partecipanti, provenienti da contesti di emergenza e terapia intensiva, hanno potuto esercitarsi in tecniche di intubazione difficile, cricotirotomia e accessi alternativi alle vie aeree.

**Conclusioni.** La riproduzione anatomica delle fratture Le Fort I e II su Donatore, realizzata presso il Centro Anatomico dell'Università di Bologna, si è dimostrata un valido strumento per il training avanzato pensato per ottimizzare la preparazione degli operatori dell'emergenza nella gestione delle vie aeree dei Pazienti con trauma facciale complesso. Inoltre, La presenza di figure cliniche multidisciplinari all'interno del Dipartimento di Anatomia ha consentito la costruzione di scenari altamente complessi e realistici, rendendo il training avanzato non solo fedele dal punto di vista anatomico ma anche estremamente concreto clinicamente.

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**Keywords:** Fratture di Le Fort, Gestione complessa vie aeree, Training medico, Donor Lab.

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# Stimulation of soluble guanylate cyclase: A novel approach to treat conjunctival fibrosis

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Conjunctival fibrosis is a serious clinical concern implicated in a wide spectrum of eye diseases, including outcomes of surgery for pterygium and glaucoma. It is mainly driven by chronic inflammation that stimulates conjunctival fibroblasts to differentiate into myofibroblasts over time, leading to abnormal wound healing and scar formation. Soluble guanylate cyclase (sGC) stimulation was found to suppress transforming growth factor β (TGFβ)-induced myofibroblastic differentiation in various stromal cells such as skin and pulmonary fibroblasts, as well as corneal keratocytes. Here, we evaluated the in vitro effects of stimulation of the sGC enzyme with the cell-permeable pyrazolopyridinylpyrimidine compound BAY 41-2272 in modulating the TGFβ1-mediated profibrotic activation of human conjunctival fibroblasts. Cells were pretreated with the sGC stimulator before challenging with recombinant human TGFβ1, and subsequently assayed for viability, proliferation, morphology, migration, invasiveness, myofibroblast marker expression, and contractile properties. Treatment with BAY 41-2272 effectively increased the intracellular levels of cyclic guanosine monophosphate in human conjunctival fibroblasts. Stimulation of sGC significantly counteracted TGFβ1-induced cell proliferation, migration, invasiveness, and acquisition of myofibroblast-like morphology and phenotype, as shown by a significant downregulation of *FAP*, *ACTA2*, *COL1A1*, *COL1A2*, *FN1*, *MMP2*, *TIMP1*, and *TIMP2* mRNA levels, as well as by a significant reduction in α-smooth muscle actin, N-cadherin, COL1A1, and FN-EDA protein expression. In addition, pretreatment with the sGC stimulator was capable of significantly dampening TGFβ1-induced acquisition of a contractile phenotype by conjunctival fibroblasts, as well as phosphorylation of Smad3 and release of the pro-inflammatory cytokines IL-1β and IL-6. Taken together, our in vitro findings are the first to demonstrate the effectiveness of pharmacological sGC stimulation in counteracting conjunctival fibroblast-to-myofibroblast transition, thus providing a promising scientific background to further explore the feasibility of sGC stimulators as potential new adjuvant therapeutic compounds to treat conjunctival fibrotic conditions.

**Keywords:** conjunctival fibroblasts, myofibroblasts, conjunctival fibrosis, TGFβ1, soluble guanylate cyclase stimulation.

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# Environmental pollutants impair human GnRH neuron migration: mechanistic insights from an in vitro model

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Environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, are well-known endocrine-disrupting chemicals (EDCs) capable of interfering with physiological functions and developmental processes. Increasing evidence highlighted the vulnerability of the hypothalamic gonadotropin-releasing hormone (GnRH) system to EDC exposure during fetal development, potentially impairing reproductive function.

Using human fetal GnRH neuroblasts (FNCB4), we previously showed that benzo[a]pyrene (BaP), a prototypical PAH, impairs cell migration [1], a crucial step in GnRH neuron maturation. In this study, we further investigated the molecular mechanisms causing migratory alterations in FNCB4 cells exposed to Bap (10μM, 24h). Transcriptomic analysis (RNAseq) identified 585 differentially expressed genes (DEGs) in FNCB4 cells treated with BaP, with enrichment in pathways related to cell adhesion, motility and extracellular matrix organization. Notably, BaP downregulated syndecan-2, syndecan-4, and CD44, implicating disruption of the RhoA signaling pathway, essential for actin remodeling and motility. Consistently, STRING network analysis revealed that several DEGs interacted directly or indirectly with RhoA. Moreover, immunofluorescence analysis showed that BaP significantly reduced RhoA colocalization with the membrane marker WGA, confirming the inhibitory effect on RhoA membrane translocation and activation responsible for defective downstream signaling.

To compare EDC mechanisms, we exposed FNCB4 cells to the heavy metal Cadmium (Cd, 10μM, 24h). We demonstrated that Cd also reduced migration, inducing F-actin disassembly and affecting genes crucially involved in this process during fetal development. Importantly, unlike BaP, Cd did not alter RhoA localization, indicating that the two toxicants act through distinct pathways. These findings highlight how diverse EDCs can differentially disrupt GnRH neuron migration and development, potentially compromising reproductive function.

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**Keywords:** Environmental pollution, GnRH neuron development, RhoA, Benzo[a]pyrene, Cadmium.

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# Autosomal Dominant Leukodystrophy: Dissecting astrocyte dysfunction through in vitro 2D and 3D models and ex vivo cerebrospinal fluid metabolomics

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Autosomal Dominant Leukodystrophy (ADLD) is a rare, fatal neurodegenerative disorder caused by *LMNB1* gene overexpression, leading to progressive central nervous system demyelination with no effective therapy. While oligodendrocytes are responsible for myelination, increasing evidence suggests that astrocytes play a crucial role in ADLD[1,2]. Astrocytes from ADLD patients and *LMNB1*-overexpressing cells exhibit nuclear alterations, inflammatory activation, and oxidative stress, absent in oligodendrocytes. This study investigated astrocyte dysfunction in ADLD through two complementary approaches. First, human primary astrocytes (HA) overexpressing *LMNB1* were analyzed for inflammatory cytokines and myelination support. Immunocytochemical analysis revealed nuclear localization of NFAT4 and NF-κB, suggesting astrocyte activation, while secretome analysis with proteome arrays confirmed elevated inflammatory cytokines. Transmission electron microscopy of the *LMNB1*-HA showed anomalous chromatin condensation in ADLD nuclei. When co-cultured with oligodendrocyte precursor cells (OPC) on a 3D microfiber scaffold, confocal microscopy revealed that *LMNB1*-HA impaired OPC myelin basic protein production, highlighting astrocytes' crucial role in supporting myelination. Second, patient-derived human induced pluripotent stem cells (hiPSCs) were differentiated into astrocytes and neurons, revealing significant nuclear abnormalities exclusively in astrocytes. Additionally, astrocytes in ADLD cortical organoids exhibited increased nuclear abnormalities, further indicating astrocyte dysfunction. NMR metabolomics of cerebrospinal fluid from ADLD patients revealed elevated lactate levels and disrupted alanine-lactate cycling, indicating metabolic dysfunction. The absence of glutamate and GABA, presence of glutamine, and lack of N-acetylaspartate further suggest impaired neuron–astrocyte interactions and deficits in metabolic support for myelination. These findings highlight astrocytes as key contributors to ADLD pathology, emphasizing their role in inflammation, metabolic dysfunction, and demyelination. Identifying specific biomarkers may enhance diagnostics and guide therapeutic strategies.

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**Keywords:** Lamin B1, ADLD, Astrocytes, Neurodegeneration, hiPSC models, metabolomics.

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# Targeting Diabetic Keratopathy: Evaluation of dECM Hydrogel on Organotypic Corneal Epithelium

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Diabetes mellitus, a leading cause of death and disability worldwide, is characterized by high blood sugar, leading to various complications that affect multiple organs and systems in the body, including the cornea. Diabetic keratopathy (DK) is a common eye condition, affecting over half of all diabetic patients. Currently, most research on the cornea uses either two-dimensional (2D) models or animal models. The 2D models are inexpensive and generate a lot of data, but do not accurately reflect the complex nature of the human cornea. In contrast, animal studies reproduce the complexity of the biological events occurring in humans but present ethical concerns.

Hence, this study aimed to (1) characterize a new in vitro organotypic human corneal epithelial tissue model for the study of DK; (2) validate an innovative hydrogel derived from the decellularized extracellular matrix of bovine pericardium (dECM hydrogel) embedded with NAP, an 8 amino acid peptide derived from activity–dependent neuroprotective protein (ADNP), for the treatment of DK. Results showed that our model recapitulates wellestablished molecular and cellular features of DK, including epithelial defects and inflammation. Moreover, the dECM hydrogel-NAP preserves epithelial morphology and thickness of 3D-organotypic corneal epithelium, counteracting apoptotic cell death. Overall, the data provided highlight the validity of 3D organotypic corneal epithelium in modeling DK, and the ability of dECM hydrogel-NAP to support corneal epithelial regeneration under DK.

**Keyword:** Cornea, Diabetic keratopathy, dECM hydrogel, NAP.

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# SINEUP non-coding RNAs increase RAI1 protein level in a cellular model of Smith-Magenis syndrome

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Haploinsufficiency disorders (HDs) are a class of genetic diseases associated with intellectual disability, developmental neuropsychiatric anomalies, metabolic dysfunctions or tumorigenesis. The pathogenic mechanism involves the insufficient expression of one or more genes, resulting in transcript levels reduced to approximately half of the normal amount. Smith-Magenis syndrome (SMS OMIM #182290 del17p11.2) and Potocky-Lupsky syndrome (PTLS OMIM #610883 dup17p11.2) are rare genomic diseases, highlighting the paramount role of the dosage sensitive gene RAI1. Intellectual disability, obesity and abnormal circadian rhythm are hallmarks of SMS. Currently, there is no cure [1]. SINEUP is a new technology designed to slightly boost target protein levels by 1.5-3-fold in trans. They basically are lncRNAs, naturally able to activate translation of it sense protein coding gene. This activity is the combination of two domains: an embedded mouse inverted SINE (Short Interspersed Nuclear Element) B2 repeat enhancing (Effector Domain, EF) mRNA translation and an overlapping AS region providing specificity for the target sense transcript (Binding Domain, BD). Drawbacks such as overexpression and off-targets are reduced, as SINEUP works only on cells that express the target of interest [2].

Testing SINEUP efficiency on RAI1, at several time points (samples harvested 24-48 hours posttransfection) and under different pcDNA_miniSINEUP transfection conditions (2,5; 3; 5 μg/μl and mixed) Analyzing gene transcription levels of CLOCK, ARNTL2, [3] mTOR (a RAI1's target) [4] and the MAPK/ERK and PI3K/AKT pathways.

Fibroblasts GM24309 (control cell line) and GM24310 (SMS) were purchased by Coriell Institute.

Four different SINEUPs have been designed and synthetized. SINEUPs are able to bind RAI1 mRNA with high specificity. They are labeled SINEUP 1,2,3, and 4. The initial RAI1 haploinsufficiency condition in GM24310 is evaluated and confirmed by western-blotting, RT-qPCR and immune fluorescence assays. WB (biological and technical replicates) highlights that RAI1 protein production increases in GM24310 treated with SINEUP 3 and 4 both separately and in combination. RT-qPCR confirms that the enhancement occurs at the post-transcriptional level. To evaluate the short-term effect, we test whether there are any phosphorylation disruptions related to ERK and AKT which are absent. Despite this, expression of circadian genes is boosted, as expected, while mTOR remains constant.

Synthetic miniSINUEPs increase endogenous RAI1 protein levels within physiological ranges. Unchanged RAI1 mRNA expression levels suggest that SINEUP acts properly at the post transcriptional level. The enhanced expression of circadian genes CLOCK and ARNTL2 indicates that the cellular model reacts to RAI1 protein increase.

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**Keywords:** Haploinsufficiency, SINEUP technology, RAI1 upregulation.

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# Leukaemia-Induced Microglial Activation and Neuroinflammation in an Acute Myeloid Leukemia Mouse Mode

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Acute myeloid leukemia (AML) is an aggressive hematological malignancy characterized by somatic mutations affecting genes involved in the differentiation and survival of myeloid cells (1). NPM1 mutations, the most common, require the cooperation of other genetic alterations, such as those in FLT3 (2), to induce full-blown leukemia. AML is characterized by chronic systemic inflammation, supported by elevated production of pro-inflammatory cytokines, which promote the proliferation and survival of leukemic cells (3,4). Although the role of inflammation in the pathogenesis of AML is well documented, its impact on extra-hematopoietic systems, particularly in the CNS, remains poorly explored. Systemic inflammation is involved in the pathophysiology of several psychiatric disorders frequently observed in AML patients (5). However, the possible contribution of AML-derived cytokines to microglial activation and neuroinflammation has not been studied.

In this context, we hypothesize that AML-associated inflammation may induce neuroinflammatory responses, contributing to the development of neurological complications in patients. To test our hypothesis, we used a mouse model of AML with Npm1c$^+$ and Flt3-ITD mutations, which faithfully reproduces the clinical features of the human disease.

A significant increase in total leukocyte count was detected in the brains of leukemic mice compared to controls. In particular, T helper cells (CD4$^+$), cytotoxic T lymphocytes (CD8$^+$), and macrophages (CD11b$^+$CD45$^+$^hi) were significantly elevated compared to controls. The number of microglial cells did not appear to be altered. These results suggest that leukemic progression is associated with increased immune cell infiltration into the brain, potentially contributing to neuroinflammation. Accordingly, we observed a significant reduction in the protein occludin and increased Mmp9 expression, suggesting an alteration of the blood–brain barrier. To assess neuroinflammation in leukemic mice, we measured brain mRNA levels of key cytokines. Il1b and Tnfa were significantly upregulated in the brains of leukemic mice compared to controls. Tgfb remained unchanged, indicating a selective pro-inflammatory response linked to leukemia progression.

To determine whether glial activation accompanies the neuroinflammatory state, we examined astrogliosis and microglial activation in the cortex and hippocampus. In leukemic mice (Npm1c$^+$/Flt3ITD), we observed increased numbers of GFAP-positive astrocytes with hypertrophic morphology and Iba1-positive microglia with an activated, amoeboid shape. Similar changes were seen in the hippocampus, indicating a widespread pro-inflammatory glial response.

This glial activation, together with elevated pro-inflammatory cytokines, suggests a disrupted neuroimmune environment. These neuroinflammatory alterations may contribute to the neurological complications seen in leukemia patients. *References*

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**Keywords:** Acute myeloid leukemia, Inflammation, Neuroinflammation, Microglia.

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# Spatial molecular analysis for the study of epithelial-immune crosstalk in primary sclerosing cholangitis and cholangiocarcinoma

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Primary sclerosing cholangitis (PSC) is an inflammatory disease of the biliary tree characterized by immuno-mediated injury of large intrahepatic (i.e. zonal and segmental) and extrahepatic bile ducts. Inflammatory response also involves biliary epithelial cells (BECs), which have been shown to harbor immune modulation activity in vitro [1]. Cholangiocarcinoma (CCA) is an aggressive tumour arising from the biliary tree. It is characterized by dismal prognosis and poor response to therapy, which is partly due to its immune-escape capabilities [2]. In this light, we aimed to characterize the immune landscape of PSC and CCA by spatial molecular analysis to determine relevant activated pathways involved in immune-mediated injury of the bile duct and tumour progression. Samples from PSC patients (N=10) were obtained from individuals undergoing orthotopic liver transplantation for the disease. CCA samples (N=10) were obtained from surgical resection specimens collected from different European centres joining the European Network for the Study of CCA. Bile duct samples with normal histology (N=5) were obtained from donor organs discarded for transplantation and were used as controls. Formalin-fixed, paraffin-embedded (FFPE) sections were stained for conventional histology and immunohistochemistry. FFPE slides were processed for spatial proteomic analysis on the GeoMx Digital Spatial Profiler. BECs and immune cells were segmented based on immunofluorescence for PanCK and CD45, respectively, and separately collected. Obtained probes were quantified on the nCounter Sprint. Samples with PSC were characterized by the presence of significant inflammation within the bile ducts. Immune infiltrate was typically constituted of CD3+ and CD20+ lymphocytes. When the molecular profile of BECs was studied, PSC patients were characterized by activation of proliferative pathways and by the expression of immune checkpoint elements, including PD-L1. In parallel, CD45+ cells were characterized by the expression of a proapoptotic balance based on the activation of immunomodulatory pathways. In CCA, inflammatory cells were mostly located at the interface between the tumour and the liver parenchyma. At spatial molecular analysis, tumour epithelial cells are characterized by positivity for immune checkpoint elements (e.g. PD-L1) and by activation of AKT pathway (involved in tumour progression and immunotherapy resistance). Moreover, CD45+ cells within the tumour display pro-apoptotic markers and immune-silencing elements. In conclusion, our spatial molecular approach identifies the immunemodulatory role of biliary epithelium both in chronic inflammatory biliary diseases and in cholangiocarcinoma. The PD-1/PD-L1 axis results at the basis of the crosstalk between epithelial and inflammatory cells, which could be of interest for refining therapeutic strategies.

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**Keywords:** Spatial molecular analysis, cholangiopathies, tumour.

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# Regulation of Apoptosis and Phosphoinostitide-related Pathways in Hematopoietic Cells during Azacitidine and Venetoclax Treatment

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The regulation of cell proliferation and differentiation is essential in hematopoiesis. Azacitidine (AZA) and Venetoclax (VEN) therapy is effective in Myelodysplastic Neoplasms (MDS) at higher risk of evolution to Acute Myeloid Leukemia (AML), but the molecular mechanisms remain unclear. To further analyze the regulation of these processes, this study analyzes the effect of AZA+VEN on apoptosis, proliferation, and differentiation of leukemic cell lines and mononuclear cells obtained from MDS samples.

VEN-sensitive leukemic MV4-11 and VEN-resistant monocytic THP-1 cells were treated for 24 hours with AZA, VEN, or both. Additionally, samples obtained from 9 MDS patients at higher risk of AML evolution and treated with AZA (n=5) or AZA+VEN (n=4) were analyzed for gene mutations and expression.

In both leukemic cell lines, AZA+VEN induced cell cycle arrest and a significant increase in the sub-G0 population, although with a more pronounced effect in VEN-sensitive MV4-11 cells. Apoptosis was confirmed by Annexin V and caspase-3 activation with PARP cleavage, and up-regulation of pro-apoptotic genes *BIM* and *BAK1*. Even the expression of Phospholipase C genes (*PLCB1*, *PLCG1*) increased after treatment in both cell lines, while AKT was reduced. Interestingly, in MV4-11 cells, *BCL2* mRNA increased, but protein levels decreased, suggesting a post-transcriptional regulation. In these cells, even PLCγ2 and phospho-GSK3β were reduced. As for differentiation markers, they were modulated in both cell lines, with MV4-11 cells showing upregulation of *CD33* and *CD14*, indicative of early myeloid and monocytic commitment, and THP-1 cells exhibiting increased *CD33*, *CD11b*, and *CD14*, consistent with enhanced terminal differentiation.

Stemming from these data, we analyzed MDS samples: gene expression during therapy in AZA+VEN responders paralleled MV4-11 behavior, showing downregulation of *PLCG1* and *PLCG2*, increased *CD33*, and upregulation of *BIM* and *BAK1* after 4 cycles of therapy, compared to baseline. At the same time, mutation analyses showed that 7/9 patients harbored a new BCL2 frameshift mutation (p.Ala82Glyfs*14). Although this mutation produces a truncated protein lacking the BH3 domain, hinting at a lack of response to VEN, all mutated patients treated with AZA+VEN resulted clinically responsive.

All in all, AZA+VEN promotes apoptosis, reduces proliferation, and influences myeloid differentiation in leukemic cells, through the modulation of apoptosis and phosphoinositide-related pathways. The presence of a new BCL2 mutation may even enhance this effect, potentially leading to an increased therapeutic response to the combination treatment. These findings support the need for further mechanistic studies on hematopoietic regulation, ideally involving genome-edited models and larger patient cohorts, including leukemic samples and other hematologic malignancies.

**Keywords:** Myelodysplastic Neoplasms, Venetoclax, Signalling, Apoptosis, Phosphoinositide-related pathways, Hematopoiesis, Proliferation, Differentiation.

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# From Laminopathies to Glioblastoma: Exploiting Nuclear Dysfunction to Target Tumor Resistance

Maria Vittoria Marvi[1], Camilla Evangelisti[1], Isabella Rusciano[1], Camilla Bruna Cerchier[2,3], Irene Neri[1], Lucio Ildebrando Cocco[1], Lucia Manzoli[1], Cristina Capanni[2,3], Stefano Ratti[1]

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Laminopathies are a group of rare genetic disorders caused by defective processing of lamin A, a key nuclear envelope protein. These conditions, including progeroid syndromes, are characterized by the pathological accumulation of unprocessed prelamin A, which leads to nuclear architecture disruption and increased cellular vulnerability to oxidative stress (1). Building on these molecular features, it was investigated whether a similar nuclear destabilization could be deliberately induced in glioblastoma – the most aggressive and therapyresistant tumor of the central nervous system – as a novel therapeutic strategy. Using the farnesyltransferase inhibitor SCH66336 (Lonafarnib), it was pharmacologically blocked lamin A maturation, inducing nuclear accumulation of prelamin A in glioblastoma cells. This "induced laminopathic state" was highly selective for tumor cells, which express elevated levels of lamin A compared to healthy brain tissue (2). Nuclear dysfunction sensitized glioblastoma cells to oxidative stress triggered by Menadione, while normal human astrocytes remained largely unaffected, suggesting a tumor-specific vulnerability. In this study, it was demonstrated that combined treatment with SCH66336 and Menadione significantly impaired the viability and self-renewal capacity of patient-derived glioblastoma stem cells (GSCs), reduced their proliferation, and altered the expression of key stemness-associated markers (3). As GSCs are considered central drivers of tumor progression and therapeutic resistance, these findings support the rationale for targeting nuclear structure and redox homeostasis as a dual-hit strategy to destabilize glioblastoma.

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**Keywords:** Prelamin A, Lamin A/C, Glioblastoma, Laminopathies, Oxidative Stress Sensitization.

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# Face Off: Exploring How Sex Shapes Facial Variation in Italian Adults

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Morphological differences between males and females are relevant across various fields, such as anatomy, surgery, and forensics. Understanding sex- and age-specific variation in facial soft tissues is crucial for clinical planning and outcome evaluation, and personal identification.

Sexual dimorphism of facial soft tissues has been studied using both traditional anthropometry and geometric morphometrics (GMM). However, many studies are limited by small sample sizes, broad age ranges, or admixed populations, which may confound population-specific patterns. In the Italian population, most research still relies on traditional methods with limited use of advanced GMM approaches.

This study aims to investigate sexual dimorphism facial morphology in Italian adults using a spatially dense GMM approach, offering a comprehensive analysis beyond the limitations of traditional landmark-based methods. 3D stereophotogrammetric facial images of 342 healthy Italian individuals (172 males, 23.2 ± 5.8 years; 170 females, 25.7 ± 7.2 years) were retrospectively selected and each was represented by a dense configuration of homologous quasi-landmarks by non-rigidly aligning a synthetic template mesh to the individual facial surfaces. A Generalized Procrustes Analysis (GPA) was applied to remove non-shape or non-form variation, ensuring standardized shape and form comparisons. 'Shape' refers to the geometric information after removing differences in position, translation, and size, whereas 'form' only removes differences in position and orientation retaining the size information. Principal Component Analysis (PCA) was performed to explore the main axes of shape and form variation, while Partial Least Squares Regression (PLSR) was used to model and quantify the association between facial morphology and sex.

PLSR revealed a statistically significant relationship between sex and facial shape ($p < 0.001$, $R^2 = 0.1035$) as well as form ($p < 0.001$, $R^2 = 0.2416$). Color-coded maps, averaging Italian male and female faces and highlighting the location and extent of sex-related facial differences across the entire face, are presented.

Overall, males showed larger facial dimensions, with greater height, width, and mid-facial projection (nose and perioral region), while females exhibited more anterior projection of the orbits, malar area, and upper forehead. These patterns are consistent with findings from other European populations, suggesting shared trends in sexual dimorphism. However, whether such similarities reflect the same structural configurations across populations remains unclear, as comparable phenotypic traits may result from divergent anatomical configurations. This 3D approach offers a detailed representation of sex-related facial variation in the Italian population and provides useful reference data for anthropological, clinical, and forensic applications.

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**Keywords:** Facial morphology, Morphological variation, Sexual dimorphism, Anthropometry, Geometric Morphometrics, Stereophotogrammetry.

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# Characterization of the glial dynamics within the organotypic brain to hijack glioblastoma

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Glioblastoma multiforme (GBM) is the most aggressive primary malignant tumor of the central nervous system (CNS), characterized by high molecular heterogeneity, rapid growth, invasiveness, and resistance to conventional therapies. Patients with glioblastoma often face poor prognoses. At present, the main treatment of GBM is resection, followed by radiotherapy and chemotherapy. However, postoperative recurrence is common along with epilepsy, due to the heterogeneity of the mass, the unbounded combination of the tumor and normal tissue, and the high invasiveness of glioma. Increasing evidence shows that CNS remodeling serves the GBM progression and related epilepsy, and would be successful in counteracting the tumor if driven for re-education. However, the spatio-temporal dynamic of the GBM microenvironment requires further knowledge.

In the present study, we exploited the cellular and molecular dynamics of the tumor microenvironment, focusing on astrocytic and microglial responses. We prepared acute and organotypic brain slice cultures from adult C57BL6/J mice and injected GL261-GBM cells into the cortex. Tissues were collected at day 0 or 3, and 7 post-injection for analysis of the GBM infiltration, the morphological and molecular profile of the glia, along with the neuroglial network electrophysiology.

We followed the GBM growth using the proliferation marker Ki67. We performed multi-electrode arrays, histology, confocal immunofluorescence, simulation modelling, and western blotting analysis to evaluate the brain electrical behaviour, inflammation (NfkB, the purinergic receptor P2X4R), the glial reactivity and its prediction (GFAP, Cx43; Iba1, Il-6), respectively.

Implanted glioblastoma cells integrated into the neuroglial network and invaded the brain tissue, inducing molecular and functional modifications. GBM cells grow in a concentric-shaped area by DIV3. GBM migrated in fascicle-like patterns in the subcortical areas and even into the contralateral hemisphere. This dynamic infiltration is accompanied by peritumoral astrocyte activation (GFAP+) and a marked microglial response, evidenced by morphological changes in Iba1-positive cells. Cx43 expression is affected by the tumor infiltration, indicating altered intercellular communication, while P2X4R and NfkB levels revealed inflammatory waves. Notably, Ki67 levels suggested a high proliferation rate, confirming the aggressive nature of this tumor.

Organotypic brain slice cultures offer a rapid and effective platform for studying GBM invasiveness, migration, and neuroglial interactions, revealing that glia contribute to the spread and recurrence of the GBM. Region-specific and time-dependent activation of glia highlights the complex dynamics within the tumor microenvironment. These findings could help identify potential therapeutic windows and targets for glia re-education, specific to the GBM phase of the disease.

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**Keywords:** Astrocytes, microglia, tumor microenvironment, morphology, molecular, electrophysiology, engineering simulation, mouse.

# Poster

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# Early antioxidant response and pro-inflammatory shift in cadmium-exposed human microglia

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Many neurological disorders affect the central nervous system (CNS), yet their underlying mechanisms are not fully understood. Once overlooked, microglia are now recognized as essential sentinel that maintain CNS homeostasis and play active roles in neuroprotection, neuroinflammation, and neurodegeneration [1]. Ubiquitous environmental pollutants such as cadmium (Cd), a toxic heavy metal with a long biological half- life, pose significant risks to CNS health by altering blood-brain barrier permeability and inducing oxidative stress [2]. However, the specific effects of Cd on microglial function remain poorly understood.

This preliminary study evaluates the impact of Cd exposure on human microglial cells (C13NJ), focusing on cell viability, oxidative stress responses, and pro-inflammatory activation.

The human C13NJ microglial cell line was exposed to increasing concentrations of Cd acetate (CdAc 0–100 μM) for 1 to 24 hours. Cell viability was assessed by MTT assay (10,000 cells/well), while immunofluorescence (30,000 cells/coverslip) was used to evaluate oxidative stress markers and pro- inflammatory cytokine expression.

Exposure to CdAc up to 5 μM for 24 hours did not significantly affect cell viability. However, immunofluorescence revealed early NRF2 nuclear translocation at 2 hours, indicating activation of the antioxidant defense system. Persistent cadmium exposure led to mitochondrial dysfunction, evidenced by cytochrome c release into the cytoplasm from 4 hours onword. Subsequently, oxidative stress induced increased expression of the pro-inflammatory microglial marker CD11b, detectable from 8 hours onward.

These preliminary findings show that low-dose Cd exposure triggers an early protective antioxidant response in human microglia; however, prolonged exposure impairs this defense, causing mitochondrial damage and a shift towards a pro-inflammatory phenotype, thus highlighting microglia's pivotal role in mediating CNS toxicity induced by environmental heavy metals.

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**Keywords:** microglia, cadmium, oxidative stress, pro-inflammatory cytokines.

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# Novel nanocarriers as promising therapeutic strategy for myotonic dystrophy

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Myotonic dystrophy type 1 (DM1) is a multisystemic disorder of genetic origin that represents the most common form of adult-onset muscular dystrophy. DM1 has widespread effects on human tissues and organs, and the most severely affected is skeletal muscle. At present, no therapy is available for DM1, and treatments are only made to manage the symptoms. Pharmaceutical research is screening small molecules such as pentamidine (PTM) able to mitigate the DM-associated splicing defects; however, the therapeutic application is compromised due to systemic toxicity. In this context, nanocarriers (NCs) represent a promising approach to improve the administration of therapeutic agents while decreasing adverse systemic side effects (1, 2). The present study aims to investigate the suitability of different NCs, whose biocompatibility has already been validated (3), to deliver PTM to skeletal muscle cells. In detail, liposomes (LIPO-PEG), polymeric nanoparticles (NPs), and nanohydrogels (NHs) were chosen as biologically degradable and well-tolerated nanosystems for PTM loading. The sulfate form of PTM was encapsulated in the aqueous core of LIPO-PEG, whereas the free base form was incorporated into pegylated poly(lactic- co-glycolic acid) (PLGA) (PLGA-PEG) NPs and hyaluronic acid (HA)-based NHs via electrostatic interactions. These PTM-loaded NCs were tested at various concentrations in C2C12 mouse muscle cells. Cell viability test (MTT) indicated the most suitable concentration for each NCs, while transmission electron microscopy (TEM) revealed their cellular uptake mechanisms as well as the possible occurrence of cell damage. Based on these findings, we selected LIPO-PEG (30 μM PTM), PLGA-PEG NPs (50 μM PTM), and NHs (10 μM PTM) to evaluate their therapeutic efficacy on C2C12 cells transfected with human (CTG)n expansion expressing nuclear foci (the pathological hallmark of DM1). We compared the efficacy of PTM-loaded NCs with PTM alone solubilized in the culture medium. All the loaded NCs were able to significantly reduce the formation of nuclear foci more effectively than free PTM and for a longer time. These results highlight the potential of these NCs as excellent candidates for delivering therapeutic agents for treating muscle cells in DM1.

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**Keywords:** Nanocarriers, Drug delivery systems, Muscular dystrophies, DM1 cell model.

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# Description of the neutrophil extracellular traps in the gut epithelium of young and aged mice

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Neutrophils are the most abundant immune cells in mammals, constituting the first defense against microorganisms. During inflammation, neutrophils firstly contrast pathogens releasing antimicrobial peptides (AMPs), proteolytic enzymes, such as elastase (NE) and myeloperoxidase (MPO) and reactive oepxygen species (ROS), then removing the invaders via phagocytosis (1). Another antimicrobial mechanism of neutrophils is the formation of neutrophil extracellular traps (NETs), composed of DNA, histones, granular proteins and used to capture or kill pathogenic microorganisms determining the so called NETosis (2). It has been reported as in the intestinal disorders there is an excessive release of NETs, probably correlated both to the immune response and to the clearance of the neutrophils

(3). The aim of our study has been to evaluate the presence of NETs in the intestinal epithelium of young and old mice. We evaluated the morphology of the intestinal wall through hematoxylin & eosin (H&E), and the position of neutrophils with May Grunwald - Giemsa stain. In addition, we studied the presence of DNA traps using SYTO-13 as selective fluorogenic DNA staining and the expression of Ly6g, NE and MPO as specific neutrophilic markers. After the description of the intestinal morphology and neutrophil location between young and aged mice, we found the transepithelial migration of neutrophils and the localization of NETs at the level of the goblet cells in younger mice. The first step of the migration mechanism is determined by the adhesion of the neutrophils to the basolateral membrane of the epithelium, followed by detachment, transmigration and passage in the goblet cells. These cells are not only simple and fast producer of mucus, but they can regulate several functions in the gastrointestinal (GI) barrier. Among them, we showed for the first time the possibility to act as vehicle of NETs from neutrophils to the intestinal lumen. Overall, NETs have become a therapeutic target in critical diseases and a fundamental aim is to explore their signaling pathways to find a directional guidance for possible interventions in the intestinal disorders.

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**Keywords:** Neutrophil extracellular traps (NETs), Goblet cells, Gastrointestinal (GI) barrier.

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# Anatomical Insights on Rare Case of Ileum–Ileum–Colon Intussusception in an Adult Caused by a Lipoma

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Adult ileum–ileum–colon intussusception is a rare clinical entity, typically associated with a pathological lead point. We present a case in which the intussusception was caused by a lipoma. Adult ileum–ileum–colon intussusception is anatomically characterized by the telescoping of an ileal segment, along with its mesentery, first into the distal ileum and subsequently across the ileocecal valve into the colon. The mesentery, which provides vascular supply and structural support to the intestine, is drawn into the intussuscepted segment, resulting in compression of mesenteric vessels, venous congestion, and ischemic injury. Preoperative imaging typically reveals the "target sign", with central mesenteric fat and vessels, and may identify the lipoma as a well-circumscribed, fat-density lesion serving as the lead point. Intraoperatively, the anatomical pathway is confirmed, with the ileum invaginating into the terminal ileum and colon, led by the lipoma. Histopathological analysis demonstrates benign adipose tissue consistent with lipoma, alongside evidence of transmural congestion and mucosal necrosis in the affected bowel, reflecting vascular compromise. This case highlights the critical role of mesenteric anatomy and vascular dynamics in the pathogenesis of adult intussusception, as well as the importance of considering benign lesions such as lipomas as potential lead points. Prompt recognition and surgical management, typically via segmental resection and primary anastomosis, are essential to prevent further ischemic complications and to preserve intestinal function. The rarity of ileum–ileum–colon intussusception caused by a lipoma underscores the need for awareness of diverse etiologies and the anatomical factors that predispose to this condition in adults.

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**Keywords:** Mesentery, Lipoma, Colon, Intussusception, Ileum, Ileocecal Valve.

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# Mapping Macrophage Diversity in Murine Adipose Tissue: Connecting Immune Phenotypes to Lipid-Induced Metabolic Dysfunction and Type 2 Diabetes

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Adipose tissue (AT), including both brown (BAT) and white (WAT) types, is not a static structure but exhibits notable morphological and functional plasticity [1-3]. In fact, these tissues react to metabolic alteration such as obesity and type 2 diabetes (T2D) [1-3]. Also, lipid-associated macrophages (LAMs) play a key role in these pathologies and their roles are highly dependent on their anatomical location and phenotype [2, 4]. The anatomical distribution and function of BAT, visceral WAT (eWAT) and LAMs in murine models mimicking aspects of T2D were analyzed by a morphological point of view. Diet-induced obesity (HFD), genetic obesity (db/db), and Friedreich's Ataxia (FA) knockin/knock-out (KIKO) murine models were utilized. Lipid droplet (LD) size and macrophages into BAT and eWAT were analyzed to characterize and identify LAM subset and changing in microenvironment BAT and eWAT tissue. In BAT of HFD and db/db mice, a distinct population of LAMs, characterized by high expression of PPARγ and GDF15, was identified. These subset LAMs were detected in number significative higher in HFD and db/db mice than control mice ($p<0.05$). Moreover, PPARγ$^{HIGH}$/GDF15$^{HIGH}$ LAMs anatomically localized within BAT and were associated with significant alterations in BAT adipocyte identity (*whitening* features). Specifically, TREM2$^{+}$ macrophage numbers were significantly increased in HFD BAT ($p<0.05$). Conversely, eWAT from mice under metabolic stress (HFD, db/db and FA) consistently showed a significative increased macrophage infiltration ($p<0.01$), contributing to a pro-inflammatory tissue environment. Interestingly, a significative increase of lipid vacuole size ($p<0.001$) and mitochondrial alteration ($p<0.001$) in BAT ko KIKO mice were observed when compared to control mice. Also, a reduced VEGFA expression, increased S100A9$^{+}$ cells and collagen deposition were observed in KIKO eWAT compared to control mice. This was accompanied by significantly larger adipocytes, increased lactate production ($p<0.01$), and a significant increase in CD45$^{+}$ leukocyte content ($p<0.001$), including CD68$^{+}$ macrophage infiltrates ($p<0.01$) compared to wild-type controls. Metabolic changing in adipose tissue profoundly and significantly dictates macrophage phenotype and function, and, at the same time, macrophages reshape the adipose tissue milieu. In BAT, PPARγ$^{HIGH}$/GDF-15$^{HIGH}$ LAMs represent a specialized population whose accumulation is statistically significant in obese models, correlating with changes in BAT's thermogenic capacity. In eWAT of the FA model, significant increase in macrophage infiltration and inflammatory markers allow to identify a specific pathological response. These statistically validated, site-specific anatomical and functional characteristics of macrophages are crucial for understanding metabolic dysfunction related to lipid overload and T2D occurrence in order to develop tissue-tailored target therapies.

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**Keywords:** Brown Adipose Tissue, White Adipose Tissue, Lipid-Associated Macrophages, Type 2 Diabetes.

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# In vitro mechanical, biological and microbiological properties of an Ag-OMD enhanced composite resin

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The introduction of advanced materials in conservative dentistry has allowed the use of composite resins characterized by excellent mechanical, adhesive and biological properties. Secondary caries due to marginal infiltrations remain among the main causes of clinical failure[1].

The aim of this study is to evaluate the in vitro mechanical, biological and microbiological properties of silver nanoparticles (Ag) paired to an inorganic support (OMD) and added to composite resin[2]. AgNPs were synthesized using calcium carbonate functionalized with hydroxyapatite (OMD), subsequently silanized with γ-MPS (3-methacryloxypropyl-trimethoxysilane) to improve their affinity with the resin matrix. OMD was then reacted with silver acetate to obtain OMD-Ag, which was characterized by XRPD (X-ray Powder Diffraction), TEM (Transmission Electron Microscopy), ICP-OES (Inductively Coupled Plasma - Optical Emission Spectroscopy), TGA (Thermogravimetric Analysis) and FE-SEM (Field Emission Scanning Electron Microscopy). The resulting material was incorporated at 1% by weight into the Tetric EvoCeram composite resin.

Sample groups for mechanical analysis: 1%AgOMD, 1%, 2% and 4%OMD and composite resin; for biological and microbiological evaluation 1%AgOMD, 1%OMD and composite resin. The mechanical properties were evaluated by three-point compression and bending tests (ISO standard) and morphological analysis by SEM. Biocompatibility and inflammatory response were investigated by MTT assay and ELISA test respectively (IL-1β and IL-6) on human gingival fibroblasts (BSCL138, IZSLER) at 24, 48 and 72h. The antibacterial activity was evaluated against Streptococcus mutans and Streptococcus oralis by crystal violet biofilm formation assays.

The silanization was confirmed by TGA, while XRPD highlighted the presence of crystalline phases and ICP-OES determined Ag content. Mechanical tests showed an improvement in compressive strength at 1% and 2%OMD, while a reduction was observed at 4%. In bending tests a reduction in elastic modulus was observed. FE-SEM analyses revealed spherical structures referable to AgNPs, irregular surfaces with fractures and voids attributed to the increased viscosity.

Cell viability was decreased in all samples, less marked in 1%AgOMD ($p < 0.001$ vs 1% OMD; $p < 0.05$ vs composite resin), also IL-1β and IL-6 secretion was significantly reduced, with 1%AgOMD and 1% OMD having the best performance. Microbiological tests demonstrated a significant reduction ($p < 0.05$) of biofilm biomass in 1%AgOMD.

The addition of AgNPs, despite a slight reduction in mechanical resistance, ensures good elastic behavior, adequate tolerance to stress and an inhibitory effect both on bacteria and inflammatory cytokines release. Further studies will be necessary to optimize the polymerization process, improving mechanical performance without compromising antibacterial efficacy.

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**Keywords:** composite resin, AgOMD, antibacterial.

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# Benralizumab Modulates Key Signaling Pathways in IL-5R–Positive Acute Promyelocytic Leukemia Cells: A Preliminary In Vitro Study

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**Background:** Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) characterized by the t(15;17) chromosomal translocation and a block in myeloid differentiation. While frontline therapies such as all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) have significantly improved survival, treatment resistance and disease relapse remain major clinical challenges. Benralizumab is a monoclonal antibody targeting the interleukin-5 receptor alpha (IL-5Rα), currently approved for severe eosinophilic asthma. Given recent evidence of IL-5R expression in APL-derived HL-60 cells, we hypothesized that Benralizumab may exert anti-leukemic effects through IL-5R–mediated pathways. **Methods:** In this preliminary in vitro study, HL-60 cells – known to express IL-5R – were treated with Benralizumab. We assessed changes in key intracellular signaling molecules by Western blotting. Specific focus was given to STAT-3, pERK, NF-κB, and apoptotic markers. Cell cycle progression was evaluated using flow cytometry, while apoptosis was measured through caspase-8 activation and morphological changes. Expression of p21 and p27 was quantified to evaluate cell cycle inhibition. **Results:** Benralizumab treatment led to a downregulation of STAT-3, a transcription factor associated with leukemia cell proliferation and survival. Concurrently, the activation of pERK and NF-κB was observed, suggesting a potential role in promoting myeloid differentiation. Cell cycle analysis revealed increased expression of p21 and p27, resulting in a G0/G1 phase arrest. Moreover, Benralizumab induced apoptosis via upregulation of caspase-8, indicating engagement of the extrinsic apoptotic pathway. **Conclusion:** Our findings demonstrate that Benralizumab significantly modulates leukemic signaling in IL-5R–positive HL-60 cells, promoting differentiation, inhibiting proliferation, and inducing apoptosis. These preliminary results support the rationale for further investigation of Benralizumab as a repurposed therapeutic agent in APL. Given its established safety profile in humans, Benralizumab may represent a promising adjunct or alternative for APL patients, especially those resistant to conventional therapies.

**Keywords:** Benralizumab, Acute Promyelocytic Leukemia, IL-5R, STAT-3, ERK, NFkB, Apoptosis, Cell Cycle Arrest.

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# FTIR Imaging: A new probe to characterize human uterine lesions' tissues

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Uterine leiomyoma or fibroid is a benign tumor derived from smooth muscle cells. The malignant counterpart of leiomyoma is leiomyosarcoma, an infrequent entity among malignant gynecologic tumors with a very unfavorable prognosis. Leiomyomas present different histotypes, such as usual, cellular, bizarre and apoplectic, some of which may share characteristics with malignant lesions, then complicating the differential diagnosis (1, 2). Due to the challenge this represents, recent studies have focused on the analysis of the extracellular matrix (ECM) components for specific macromolecular changes related to malignancy. In this context, Fourier Transform Infrared Imaging (FTIRI) spectroscopy constitutes an innovative technique for this type of evaluation, offering an accurate morpho- chemical classification of tissue samples (3). The present study employed a multidisciplinary approach combining FTIRI and histological analysis to investigate the ECM and cellular components of both malignant and benign uterine tumors. Leiomyosarcoma samples as well as leiomyoma samples with cellular, usual, apoplectic and bizarre histotypes were studied. Myometrial tissue was included as a healthy control. Three tissue sections were prepared for each sample: two were mounted on glass slides for histological analysis, and the third was mounted on CaF2 optical windows for FTIR analysis. To investigate the consistency and quantity of ECM, particularly collagen, a Masson's trichrome staining kit with aniline blue was used. The FTIRI analysis was carried out by an INVENIO-R interferometer, coupled with a Hyperion 3000 Vis-IR microscope and equipped with a Focal Plane Array (FPA) detector operating at liquid nitrogen temperature. Statistical analysis was performed with Prism 8.0 software, using one-way ANOVA multiple comparison and a significance level of $p<0.05$. Histological analysis showed a low collagen content in cellular and usual histotypes, also associated with spot distribution. In contrast, in the bizarre and apoplectic histotypes, the amount of collagen was higher. As regards IR analysis, well evident differences emerge at spectral level between the leiomyosarcoma and leiomyomas, both in terms of spectral profiles and in the case of specific spectral parameters related to collagen. In particular, IR analysis allowed us to assess not only the amount of collagen present in the different experimental groups, but also its structural organization. Specific spectral parameters related to collagen (such as FOLDED/UNFOLDED, ALPHA HELIX and TRIPLE HELIX band area ratios) were also identified, to improve the differential diagnosis between leiomyosarcoma and the different leiomyoma histotypes. Their statistical analysis suggests a better organized collagen in cellular and usual leiomyoma histotypes, whereas bizarre and apoplectic ones looked to have a more disorganized protein component. In conclusion, the results demonstrate that FTIRI technique together with histological analysis can be considered as a valid tool to improve and make more exact the differential diagnosis between benign and malignant uterine lesions.

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**Keywords:** Uterine fibroids, leiomyosarcoma, collagen, Fourier Transformed Infrared (FTIR) imaging spectroscopy.

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# Dual role of garlic derivatives on invasive potential and β-catenin dynamics in breast tumor cells overexpressing HER2

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Breast cancer includes tumor subtypes with distinct morphological, molecular, and clinical profiles. The intrinsic heterogeneity, combined with the toxicity of chemotherapeutics, often impacts therapeutic efficacy and contributes to the development of resistance. In recent years, natural compounds and their bioactive molecules have attracted increasing interest as potential adjuvants to conventional drugs in the development of oncological treatment strategies, due to their broad biological activities and relatively low toxicity. Garlic (*Allium sativum*) has been extensively studied for its anticancer properties, with several reports indicating its ability to counteract breast tumor aggressiveness [1, 2]. It has been widely demonstrated that garlic derivatives induce apoptosis and reduce the invasive potential of ER-positive and triple-negative breast tumor cells [3]. However, current literature lacks studies investigating their effects on HER2-positive breast cancers. To address this gap, we explored the effects of a garlic extract and of diallyl disulfide (DADS), one of its most bioactive organosulfur compounds, on breast tumor cells with a HER2+ phenotype, revealing a dual role depending on the duration of treatment. We found that while acute administration induced a decrease in invasive potential, prolonged treatment paradoxically promoted the invasiveness of HER2+ cells. These effects were directly correlated with the reorganization of actin cytoskeleton and modulation of β-catenin dynamic, both related to the activation of Akt signaling. The Akt/GSK3β/β- catenin axis culminated in the nuclear accumulation of β-catenin, which is known to induce the expression of genes associated with tumor malignancy [4].

Although further investigations are needed to establish whether the *in vitro* phenomena observed are reproducible in *in vivo* models, our results raised the attention on an often-overlooked aspect: the safety of using natural compounds in the context of complex diseases such as cancer.

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**Keywords:** HER2-positive breast tumor cells, Garlic derivatives, Actin cytoskeleton, β-catenin dynamic, Akt signaling.

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# Enhancement of Nasal Epithelial Integrity in Chronic Rhinosinusitis with Nasal Polyps: The Role of Dupilumab in Promoting Spheroid Organization and Epithelial Cell Motility

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**Introduction:** Chronic rhinosinusitis with nasal polyps (CRSwNP) is a persistent inflammatory disorder affecting the nasal mucosa and paranasal sinuses, clinically manifesting with symptoms such as nasal discharge, reduced sense of smell, and facial discomfort. It predominantly occurs in middle-aged individuals, particularly males, and is characterized by the presence of bilateral polyps. Despite the widespread use of corticosteroids and endoscopic sinus surgery, disease recurrence is frequent. Recently, biologic therapies like Dupilumab – an antibody that blocks interleukin (IL)-4 and IL-13 signaling – have shown promise by targeting central drivers of type 2 inflammation. **Methods:** To investigate the biological effects of Dupilumab on nasal epithelial repair mechanisms, we employed a three-dimensional (3D) spheroid culture model using epithelial cells derived from CRSwNP patients. This model more accurately replicates in vivo cellular architecture compared to traditional two-dimensional (2D) cultures. Cells were harvested from three groups: patients with untreated CRSwNP, patients treated with Dupilumab for 16 weeks, and a control group with inferior turbinate hypertrophy. Spheroid formation capacity and cellular motility were evaluated through phase-contrast microscopy. **Results:** Control group cells formed cohesive and non-motile spheroids, consistent with intact epithelial barrier function under non-inflammatory conditions. In contrast, epithelial cells from untreated CRSwNP patients exhibited a marked inability to form spheroids, reflecting epithelial damage and persistent inflammation. Notably, Dupilumab-treated CRSwNP cells demonstrated restored capacity to form organized, motile spheroids, indicating improved epithelial integrity and functional recovery. **Conclusion:** This study underscores the therapeutic potential of Dupilumab in restoring epithelial barrier function in CRSwNP by enhancing structural organization and cellular dynamics. The use of a 3D spheroid culture system offers a physiologically relevant model for assessing nasal epithelial responses and supports the continued development of biologic strategies for managing CRSwNP.

**Keywords:** Chronic Rhinosinusitis with Nasal Polyps, Dupilumab, Type 2 Inflammation, 3D Cell Culture, Nasal Epithelium, Biologic Therapy.

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# Integrating Machine Learning and Deep Learning for 3D Imaging in Confocal Microscopy

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Recent advances in digital data processing are transforming microscopy imaging from qualitative observation to scalable quantitative analysis, enabling the handling of large volumetric datasets and supporting the development of tools for multiscale analyses of biological structures. The goal is to obtain detailed and reliable quantitative analysis of cell shape, size, and marker expression, from the tissue level down to sub-cellular resolution, through segmentation of fluorescent signals and extraction of morphometrically consistent regions and volumes of interest (ROIs and VOIs).

Two major computational approaches are commonly used in confocal imaging: Machine Learning (ML) and Deep Learning (DL). ML methods, such as Random Forests, rely on predictive algorithms that learn decision rules from a set of predefined features. In contrast, DL methods, such as convolutional neural networks (CNNs), use layered neural network models to extract features. While ML is fast, interpretable, and data-efficient, DL excels in complex tasks but requires large annotated datasets and high computational power, often functioning as a "black box"[1]. Although ML and DL are often considered competing approaches, hybrid pipelines that combine DL's feature learning with ML's efficient classification are increasingly used in bio-image analysis.

Here, we present an innovative hybrid pipeline tailored for 3D imaging in confocal stacks, suitable for high-fidelity quantitative anatomical studies. The method comprises four key modules:

(i) Denoising: a Python-based filtering that combines Difference of Gaussians and TV-Chambolle with slice-wise normalization to suppress background noise and enhance structural integrity in volumetric data. T

(ii) Feature extraction: a 3D autoencoder, an unsupervised DL architecture, is trained on the denoised volume to capture the distribution of fluorescence throughout the stack. These encoded features are fused with outputs from traditional filters (Hessian for tubular structures, Sobel for edge enhancement, and a normalized Z-map), yielding composite feature maps with both semantic depth and structural detail.

(iii) ML classification: the enriched feature channels are input into a RF classifier, enabling accurate segmentation of cell morphology and marker expression. This step is immediately applicable to common quantitative tasks such as volumetric shape analysis and intracellular intensity measurements.

(iv) DL-based segmentation: a 3D U-Net convolutional neural network has been implemented for volumetric signal segmentation. Instead of relying on manually drawn labels, it uses probability maps from the ML model as input annotations, reducing the need for time-consuming human labeling.

This open-source hybrid framework enables anatomists and microscopists to perform reproducible, high-throughput analyses of complex experimental models, minimizing manual workload and maximizing the robustness of results.

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**Keywords:** Bioimage analysis, Confocal microscopy, Machine Learning, Deep Learning, Autoencoder, Random Forest, VL-3D segmentation, Hybrid pipeline, U-NET, Anatomical quantification.

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# Exploring Age-related impact: Morphofunctional Analysisof aged mouse explaining the link between aging, inflammation and Parkinson's disease

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As life expectancy increases, aging has been identified as a leading risk factor for many diseases, including neurodegenerative ones [1]. Prolonged life stress exposure could elevate the risk of developing neurological disorders [2]. Neuroinflammation is a central aspect in most neurological dysfunctions. Chronic stress induces the release of pro-inflammatory cytokines into the blood, which can cross the blood-brain barrier and reach the brain, activating the inflammatory response [3]. In this study we evaluated in different brain areas, represented by caudate-putamen (CP) and hippocampus (hyp) of aged mice subjected to MPTP treatment and to chronic stress factors, the expression of pro-inflammatory and anti-inflammatory markers as well as the activation of microglia and astroglia. Results demonstrated that in MPTP mice, as well as in stressed animals, both in CP that in hyp, the IL-1β as well as iNOS expression, evaluated by qPCR, was significantly increased in comparison to controls. Conversely, IL-10 mRNA expression resulted significantly reduced in both CP and hyp not only in MPTP treated mice but also in stressed animals. In all brain areas tested we detected a significant increase of Iba-1 and GFAP expression, markers of microglia and astroglia activation, respectively, in comparison to controls both in MPTP-treated mice and in stressed animals. Moreover, a number of different parameters of gait have been analyzed using footprint patterns. Following MPTP treatment, stride length decreased in both stressed and unstressed animals; also, front footprint/hind footprint overlap increased, suggesting that MPTP altered motor behavior. Besides, stride length of stressed animals resulted decreased when compared to unstressed ones, suggesting that stress exposure determined an alteration of normal gait similar to the one observed after MPTP treatment. Overall, these data suggest that chronic stress could be a potential risk factor for the development of neuroinflammatory status, thus constituting a possible condition promoting the age-related neurodegeneration.

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**Keywords:** aging, neuroinflammation, neurodegeneration, stress, central nervous system, glial cells.

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# Novel insights into the molecular mechanisms of LGMDD2: role of TNPO3 in experimental cell and Zebrafish models

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Limb Girdle Muscular Dystrophy D2 (LGMD D2) is a rare neuromuscular disorder with recognizable clinical features such as marked atrophy and muscle weakness. A heterozygous mutation in the termination codon of the *TNPO3* gene resulting in a mutated protein that is 15-aminoacids longer in its C-terminal domain has been described as causative of LGMD D2. This gene encodes for Transportin-3 (TNPO3), a nuclear carrier for serine/arginine-rich proteins which are essential for mRNA splicing and metabolism. To investigate the role of TNPO3 in skeletal muscle and the pathogenic mechanism underlying LGMDD2, we developed both *in vitro* and *in vivo* models of the disease. The *in vitro* model was established using the C2C12 cell line, transfected with plasmids encoding either the wild-type (WT) or mutated (MUT) human *TNPO3* (hTNPO3). For the *in vivo* model, we microinjected zebrafish (*Danio rerio*) embryos with mRNAs encoding WT or MUT hTNPO3. We analyzed the expression patterns of myogenesis-related genes, muscle-specific genes, myomiRNA and genes strictly related to the disease. These analyses showed an altered profile of Myogenic Regulatory Factors (MRFs) and muscle-specific proteins, and phenotypical studies, conducted through immunofluorescence and transmission electron microscopy, displayed an aberrant organization of the muscle fibers, in Zebrafish embryos microinjected with the human mutated TNPO3 mRNA. Functional tests seem to confirm that embryos microinjected with the mutant TNPO3 exhibited reduced activity levels during open field tests and displayed diminished responses to changes in light. These were complemented by protein expression studies, morphological assessments in both models, and behavioral assays to validate the LGMDD2 zebrafish model. Our results demonstrate a key role for TNPO3 in regulating myogenesis in both models and reveal that the TNPO3 mutation disrupts normal myogenic commitment, supporting its contribution to LGMDD2 pathogenesis. Overall, this study represents a significant advance in understanding the role of TNPO3 in skeletal muscle biology and the molecular basis of LGMDD2.

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**Keywords:** LGMDD2, Transportin 3, myogenesis, C2C12, zebrafish, myomiRNA.

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# Intrasciatic injection may cause fascicle compression, suggesting a mechanism for iatrogenic injury? Morphological findings on a sciatic nerve model from cadaver

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Post-Block Neurological Dysfunction (PBND) is a rare complication of peripheral nerve blocks (Lemke et al.). Research suggests that fascicular injury is infrequent following needle transfixion (McLeod et al.). We hypothesize that an alternative mechanism, such as compression of the fascicles by the injected solution, may lead to a direct mechanical damage. The primary objective of this study is to investigate whether the injection of a solution into the perineurium causes mechanical fascicular injury in a sciatic nerve model.

Per the Body Donation Program of the Institute of Anatomy of the University of Padova, 16 sciatic nerves were harvested from eight fresh frozen cadavers (aged 71-85). The donors had no diseases potentially interfering with the study. The mean length of the harvested sciatic nerves was 8.9 cm (IQR 8-9.5). Following a direct visual inspection, both nerve ends were sutured at their extremities. A 20 mL mixture of ink and saline solution (1:5) was then manually injected through a 22G needle. To ensure reproducibility of the results, we continuously monitored the injection pressure using the pressure-measuring device COMPASS Lumbar® system (Centurion Medical Products, Williamston, MI). Video recordings of each injection were made, and the pressure at five-second intervals was annotated.

Additionally, the diameters of the nerve at the midpoint before and after the injection were measured. Once the procedure was completed, we preserved the sciatic nerves in formalin at room temperature and then processed it for the selected stains. The initial nerve diameter at the midpoint was 3.5 cm (IQR 3.2-3.7), increasing to 4.4 cm (IQR 4.2-4.6) after injection, with a median increase of 0.9 cm (IQR 0.7-1.3). The injection duration was 190 seconds (IQR 145-231). Intrasciatic injection in the form of ink within the perineurium was confirmed through microscopic examination in all samples. However, no mechanical damage or external compression of fascicles was observed in any of the examined nerve bundle sections.

Our study shows that injecting a large volume of solution at low pressure does not cause direct mechanical fascicle- damage due to compression of the fascicles by the injected solution (Boscolo-Berto et al.). Given these findings, we should consider other mechanisms to explain PBND such as ischemic injury, neurotoxicity, and inflammatory responses.

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**Keywords:** Cadaver lab, Body donation, Simulation, Iatrogenic damage, Dye test.

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# Anti-inflammatory effect of acute administration of Kaempferol in the rat cerebral cortex mediated by changes in lipid homeostasis, endocannabinoid system, and PPARα receptor during transient common carotid artery occlusion and reperfusion

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Bilateral Common Carotid Artery Occlusion followed by Reperfusion (BCCAO/R) models early pro-inflammatory responses in brain tissue due to the hypoperfusion/reperfusion. To evaluate the neuroprotective potential of a single dose of kaempferol (KAM) on the BCCAO/R-challenged cerebral tissue in adult Wistar rats. We examined frontal and temporal- occipital cortices in two groups of rats, sham-operated and subjected to BCCAO/R. Six hours before surgery, half the rats were gavage-fed with a dose of KAM (40 mg/per rat in 300 µL of sunflower oil as the vehicle), while the other half received the vehicle alone. In the frontal cortex, KAM pre-treatment altered specific signalling lipids in both sham and BCCAO/R groups. Regarding the BCCAO/R rat group, KAM reduced arachidonic acid peroxides (AAx), reduced levels of endocannabiboids (eCBs), and elevated levels of palmitoylethanolamide (PEA), oleoylethaloamide (OEA), and docohexanoyl-ethanolamine (DHEA). Plasma analyses reflected with some exceptions, these lipid profile changes. Additionally, KAM increased levels of peroxisome-proliferator-activated-receptor (PPAR)-α, mirroring the increased levels of PEA and OEA, and endocannabinoids receptors CB1R and CB2R, and reduced the cyclooxygenase-2 (COX-2). No significant changes occurred in the temporal-occipital cortex. Our results suggest that, in the BCCAO/R condition, KAM activates the PPAR-α and endocannabinoid systems, and reduces AAx and COX-2. At the same time, KAM enhanced lipid metabolism further indicating its potential as a dietary supplement to protect nervous tissue during the hypoperfusion/reperfusion challenge.

**Keywords:** bilateral-common carotid artery occlusion, cerebral hypoperfusion/reperfusion, frontal cortex, neuroinflammation, signalling lipids, CB receptors, PPAR-α, COX2, GFAP, Iba1.

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# Ferroptosis as a key player in biliary epithelium during liver fibrosis

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Ferroptosis is an iron-dependent form of non-apoptotic cell death with the accumulation of toxic lipid reactive oxygen species (ROS) (1). It is implicated in various hepatic diseases including cancer, ischemia-reperfusion and liver fibrosis (2). The liver normally stores excess of iron and make it available when necessary, while iron overload in the liver induces damage increasing oxidative stress, activating Kupffer cells (KCs) and hepatic stellate cells (HSCs), and causing ferroptosis (3-4). In particular, the role of iron metabolism in the process of ferroptosis during liver fibrosis remains obscure. For that reason, we aimed to evaluate this possible role at the level of biliary epithelium. *In vivo*, we used the experimental model of bile duct ligated (BDL) rat evaluating the iron deposits through Perls Prussian blue and the presence of iron regulator proteins through immunohistochemstry. *In vitro*, we utilized a cell line of normal primary murine cholangiocytes treated with inducers and inhibitors of ferroptosis to evaluate the toxicity and growth inhibition, the changes in cellular morphology, the lipid peroxidation by specific sensor probe and the cell cycle perturbation. We found an alteration in the expression of protein regulators such as hepcidin, and transferrin receptor 1 (TFR1). We confirmed erastin as a specific inducer of ferroptosis. Whereas, sorafenib and ellagic acid fails to trigger ferroptosis in our cholangiocytes. Among the inhibitors, we found that ferrostatin-1 (Fer-1) is unable to block ferroptosis, while β-Mercaptoethanol (βME) or Lactoferrin are able to protect cholangiocytes in ferroptosis-induced cell death. We establish an *in vitro* pro-fibrotic model treating the murine cholangiocytes with recombinant IL-13 and evaluating the changes in the ferroptosis process in absence or presence of Lactoferrin. In summary, these findings could be very important to discover and understand the mechanism at the base of ferroptosis in liver fibrogenesis.

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**Keywords:** Cell death, ferroptosis, cholangiocytes, fibrosis Tipo presentazione POSTER

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# *In Vitro* Fabrication of Endothelialized Human Vascular Grafts

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Ischemic heart disease remains the leading cause of mortality worldwide, with coronary artery disease (CAD) being the most prevalent condition. Coronary artery bypass grafting (CABG) is one of the most effective surgical interventions for patients with severe CAD. However, the success of CABG largely depends on the availability of optimal vascular grafts, which remains a major challenge [1]. Aiming to develop potentially autologous and CABG-suitable bioengineered blood vessels, we tested the hypothesis of obtaining them from decellularized human blood vessels (Hd-BV) recellularized with human adipose- derived mesenchymal stromal cells (hADMSCs). Blood vessels were collected from patients undergoing limb amputation surgery (n = 20) and decellularized in SDS, Triton and antibiotics [2]. Histological analyses employing H&E, Masson's, Mallory's and Gomori's stainings demonstrated the preservation of vascular ECM architecture and composition and the effectiveness of decellularization, which was corroborated by an extremely low residual dsDNA content (13.07 + 4.662 ng/mg of dry tissue). hADMSCs were isolated from subcutaneous adipose tissue of patients undergoing abdominoplasty (n = 16), characterized before and after having induced endothelial differentiation *in vitro* by microscopic observation of cell morphology and by evaluating the expression of typical mesenchymal or endothelial cell markers by real-time PCR and immunocytochemistry. hADMSCs had a typical spindle-shaped morphology and expressed MSC markers, such as CD105, CD71, CD166, CD44 and CD90, but when induced to endothelial differentiation they acquired a cobblestone-like morphology and expression of endothelial-specific markers, such as VEGFR2, CD31, CD102, Tie-1 and Tie-2. Hd-BV scaffolds were repopulated with hADMSCs and cultured for up to four weeks. Scaffold cytocompatibility and cell viability were evaluated using trypan blue exclusion and CellTiter-Glo luminescence assays, while the endothelization of Hd-BV scaffolds was investigated by immunofluorescence and real-time PCR. Interestingly, more than 90% of hADMSCs in Hd-BV scaffolds remained viable, they layered the tunica intima at the Hd-BV vascular lumen and expressed endothelial markers. This study provides compelling evidence supporting the endothelization of decellularized human blood vessels by hADMSCs, paving the way for the construction of tailored autologous bioengineered vascular grafts for CABG.

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# Rewiring NFAT signaling: toward novel treatments in hematologic and solid tumors

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The Nuclear Factor of Activated T cells (NFAT) family consists of five transcription factors, four of which (NFATc1–c4) are activated through the $Ca^{2+}$/calmodulin-dependent phosphatase calcineurin. Under resting conditions, NFAT proteins remain in the cytoplasm in a highly phosphorylated inactive state. Upon T cell receptor engagement – or stimulation of other transmembrane receptors – intracellular $Ca^{2+}$ levels rise, activating calcineurin. This phosphatase dephosphorylates NFAT proteins, enabling their translocation into the nucleus where they initiate the transcription of target genes (1). Although initially characterized by their central role in immune activation and T cell development, NFAT proteins have been increasingly recognized as key players in disease pathogenesis. In leukemias, NFAT supports malignant cell survival and proliferation, while in solid cancers such as osteosarcoma, melanoma, glioblastoma, and breast cancer, aberrant NFAT signaling contributes to tumor progression, immune evasion, and metastasis (2). NFAT also plays a pathogenic role in graft-versus-host disease, where it sustains harmful T cell activation (3).

With this project, we aim to systematically target the NFAT family to evaluate their therapeutic relevance across diverse oncologic and immune-mediated settings. To this end, first we profiled NFATs expressions at both mRNA and protein levels in leukemia and solid tumor models, confirming widespread dysregulation. We then assessed sensitivity to FDA-approved NFAT inhibitors, Cyclosporin A (CsA), and observed notable cytotoxic effects in leukemia, osteosarcoma, melanoma, glioblastoma, and in breast cancer, where response varied with mutational background.

However, due to the off-target effects and toxicity of these classical inhibitors, we also explored alternative strategies (4-5). To overcome the limitations of classical NFAT inhibitors, we adopted a transcriptomic approach to identify novel compounds capable of modulating NFAT activity. Specifically, we performed gene expression profiling of leukemic cells following *NFATc1* or *NFATc2* knockdown and used these signatures to query the Connectivity Map (CMap) database (6) – a large-scale resource that links gene expression changes with small molecule drugs. This analysis yielded a shortlist of 45 candidate compounds predicted to mimic the transcriptional effects of *NFAT* gene silencing. These drugs were subsequently tested in a High- Throughput Drug Synergy Screening, both as single agents and in combination with standard

chemotherapeutics, uncovering several promising hits with synergistic activity – particularly in leukemia models.

Altogether, our findings position NFAT as a compelling therapeutic target, whose direct or indirect inhibition may unlock new treatment avenues for cancer and, potentially, immune- driven diseases.

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# Engineering 3D models to investigate LGMDD2 transportin 3 related

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Limb girdle muscular dystrophies (LGMD) are a group of rare inherited myopathies characterized by muscle fibers degeneration and progressive muscle weakness. Our study focuses on LGMDD2, an autosomal dominant form linked to transportin 3 (TNPO3) mutation (1). We applied tissue engineering to create 3D tissue-like structure by combining myoblast cells, biocompatible materials and biophysical elements (2, 3). We employed immortalized human myoblasts derived from LGMDD2 patients, cultured in two different 3D scaffolds both recapitulating the native tissue. The first one is a 3D collagen bio-printed hydrogel proved valuable for investigating myogenesis and exploring TNPO3 role in disease development. The second is a 3D micropillar system that facilitated the real-time monitoring of cell contractility (4). We performed analysis of genes and proteins related to myogenesis and to the disease. We found a dysregulation of the myogenic process in LGMDD2 compared to control samples, with an increased expression of Murf-1 and p62, markers of atrophy and autophagy, respectively. Dysregulation in LGMDD2 was confirmed by morphological studies on LGMDD2 myoblasts, characterized by reduced maturation, few striations, and α-actinin aggregates. To evaluate cell contractility, electrical stimulation was applied on the micropillars 3D model under live imaging to monitor micropillars deflections. LGMDD2 cells exhibited greater contractile force compared to control. However, their contractile force was irregular in frequency and amplitude suggesting functional abnormalities, possibly due to abnormal calcium release. Overall, our research introduces innovative 3D *in vitro* systems for modelling muscular dystrophies, that could serve as versatile, ethical and cost-effective alternative tool to conventional 2D cell culture systems and animal models for preclinical investigations and drug repurposing.

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**Keywords:** Skeletal muscle, Limb Girdle Muscular Dystrophy, Transportin 3, Tissue engineering.

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# Role of the somatic mutations in the Thrombopoietin/JAK2 axis differently predispose the pathological Neutrophil-Megakaryocytes emperipolesis in Myelofibrosis

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Myeloproliferative Neoplasms (MPNs) are clonal disorders driven by the somatic mutations in $JAK2^{V617F}$, $MPL^{W515L}$ and $CALR^{del}$ genes at the level of the hematopoietic stem cell clone (1).

The emperipolesis between megakaryocytes (MKs) and neutrophils is one of the pathological interactions triggered by the increased IL-8 availability in the bone marrow (BM) observed both in Myelofibrotic (MF) animal models and patients (2) (3).

The Interleukin-8 (IL-8) is a proinflammatory cytokine, which mediates neutrophil chemotaxis and promote fibrosis, a resulted process of the pathological emperipolesis (4).

In this study we analyzed the BM microenvironment from MF patients harboring the different somatic mutations, at the Pre- and Overt- MF stage.

Histomorphological analyses revealed a variegation in the IL-8 expression: in $JAK2^{V617F}$ from 85.28

A.U. in pre-MF to 95.97 A.U. in MF stage, in the MPL the levels change from 35.83 A.U. in pre-MF to 42.72 A.U. at the MF stage, in CALR from 73.83 A.U. in pre-MF to 85.72 A.U. in the MF stage. At same, a change in the frequencies of IL-8 positive MKs were observed in the bone marrow from Pre-and Overt-MF patients. According with the disease progression, the IL-8 levels directly correlate with the total number of neutrophils in the BM microenvironment ($p=0.004$; $p=0.002$; $p=0.004$ Pre- vs Overt-JAK2, MPL and CALR MF respectively); as the chemotaxis of neutrophil surrounding the IL-8 positive MKs ($p=0.0049$; $p=0.0025$; $p=0.05$ in Pre- vs. Overt- JAK2, MPL and CALR MF respectively).

The neutrophil chemotaxis observed in MF patients precedes the emperipolesis. We identified in overt MF patients the $JAK2^{V617F}$ and CALR the mutations with an increased proportion of emperipolesis, compared to MPL ($p<0.001$ JAK2 vs MPL, $P<0.001$ CALR vs MPL).

The neutrophil/megakaryocyte emperipolesis is the cellular interaction that links IL-8 to TGF-β abnormalities in the pathobiology of marrow fibrosis. The direct correlation between the degree of fibrosis and the neu-MK emperipolesis reflected the progression and the severity of the disease ($p<0.0001$; $p=0.05$; $p=0.005$in Pre- vs. Overt- MF JAK2, MPL and CALR respectively).

In conclusion, these data provide a spectrum of severity of the MF pathology according to the several somatic mutations described in MPNs and the supportive role of IL-8 as the driving pro-inflammatory cytokine of Myelofibrosis.

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**Keywords:** Emperipolesis, IL-8, myelofibrosis, Inflammation, megakaryocytes, fibrosis.

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# Extracellular Vesicle Crosstalk in Fibroids: Fibronectin Downregulation by Omega-3s

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Extracellular vesicles (EVs), comprising exosomes and microvesicles, are critical mediators of intercellular communication and are implicated in uterine fibroid (leiomyoma) pathogenesis through extracellular matrix (ECM) modulation 1, 2. Omega-3 fatty acids (EPA, DHA) have shown anti-fibrotic 3, 4, but their influence on EV-mediated signaling remains unexplored. Our aim was to characterize EVs derived from myometrial and leiomyoma primary and cell lines, to evaluate the effects of omega-3 pre-treatment on EV cargo, and to assess the influence of these EVs on fibronectin expression in recipient myometrial cells *in vitro*. Primary and cell lines myometrial and leiomyoma were treated with 50 μM EPA + DHA or vehicle (≤0.1% ethanol) for 48 h. EVs were isolated from culture supernatants by differential centrifugation and ultracentrifugation (110,000 × g). Nanoparticle tracking analysis (NTA) was used to characterize the size (30–150 nm for exosomes, 100–1000 nm for microvesicles) and concentration of EVs [5, 6]. Recipient myometrial cells were incubated with EVs for 48 h; fibronectin protein levels were quantified by Western blot analysis. Statistical analysis was performed using Kruskal–Wallis ANOVA followed by Dunn's post hoc test; significance threshold: $p < 0.05$. EVs from leiomyoma cells significantly upregulated fibronectin expression in recipient myometrial cells compared to untreated controls. Pre-treatment of leiomyoma cells with EPA/DHA attenuated these effects: exosome- mediated fibronectin elevation was significantly lower, $p < 0.05$ to untreated EVs, while microvesicles effects showed a moderate attenuation $p < 0.05$. In contrast, EVs derived from myometrial cells (with or without omega-3) did not alter fibronectin expression in recipient cells ($p > 0.05$). In conclusion, leiomyoma-derived EVs promote fibronectin deposition in myometrial cells, contributing to a fibrotic uterine microenvironment. Treatment with EPA/DHA alters EV cargo, significantly mitigating especially exosome-mediated fibronectin upregulation. These data suggest a potential therapeutic avenue whereby omega-3 fatty acids modulate EV signaling in uterine fibroids, offering novel anti-fibrotic strategies. Further mechanistic studies on EV cargo modulation and *in vivo* validation are warranted.

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**Keywords:** Uterine fibroids, Extracellular vesicles, Exosomes, Microvesicles, Fibronectin, Omega-3 fatty acids, EPA, DHA, ECM remodelling, Intercellular communication.

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# Role of anatomy in dentistry: a journey through centuries

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The evolution of dentistry and medicine has always been closely linked to the development of anatomical science. From ancient civilizations to modern times, the investigation of the human

body has served as a cornerstone for both scientific discovery and clinical practice. Anatomists have historically played a vital role in shaping dental medicine, contributing to its emergence as both a

specialized and autonomous branch within the broader medical field. This concise overview of the literature traced how anatomical studies – from Galen's animal dissections to the microscopic

analysis of dental hard tissues – have significantly influenced the advancement of dental science.

Topics covered include the anatomical history of teeth due to Eustachius work, cranial bones

examined by Valverde De Amusco, maxillary sinus studied by Highmore and Schneider, salivary glands widely treated by Stensen, Wharton and Bartholin, tongue and related innervation, labial muscles, and the microstructure of dental tissues possible due to the microscope introduction.

Understanding the historical trajectory of these elements remains highly relevant to contemporary clinical approaches and informs the direction of future research. Many foundational discoveries, particularly those from earlier periods, were made by prominent figures in the morphological

sciences and have laid the groundwork for modern dental practice. By continuously linking

historical knowledge to current research, we can reconstruct the anatomical evolution that underpins our current understanding of the oral cavity and its associated structures.

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**Keyword:** anatomy, dentistry, history, history of dentistry.

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# Morpho-Functional Assessment of Skeletal Muscle in a Rat Model of Fibromyalgia

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Fibromyalgia is a complex syndrome characterized by widespread musculoskeletal pain, fatigue, and hypersensitivity to pain, with an etiology that remains largely unclear (1). This study aims to investigate the potential involvement of skeletal muscle tissue in the pathogenesis of fibromyalgia, with a focus on specific protein complexes associated with muscle stability and function. Although muscle involvement in fibromyalgia is well recognized, as suggested by the widespread muscle pain reported by patients in what are known as "tender spots" (2), the underlying mechanisms and the specific molecular components involved remain poorly understood (3).

In this study, we analyzed markers representative of various pathophysiological aspects of skeletal muscle tissue. Inflammation was investigated through the expression of the NLR family pyrin domain-containing 3 (NLRP3) inflammasome complex and associated proteins, while oxidative stress response was assessed via the transcription factor Nuclear Factor Erythroid 2-related factor 2 (NRF2). Structural stability of muscle tissue was evaluated by analyzing proteins belonging to the dystrophin-associated glycoprotein complex (DGC), the sarcoglycan subcomplex, and the vinculin– talin–integrin system, along with cytoskeletal elements such as Alpha Smooth Muscle Actin (α-SMA) and vimentin. Vascular components were assessed using Cluster of differentiation 31 (CD31), while tissue remodeling was investigated by examining matrix metalloproteinases 3 (MMP-3) and 9 (MMP- 9). Finally, myogenic markers involved in muscle regeneration processes, including Paired Box 7 (PAX7), Myogenic factor 5 (MYF5), and Myogenic Differentiation 1 (MyoD1), were also evaluated. Analyses were performed using immunofluorescence and immunoenzymatically techniques on skeletal muscle samples obtained from healthy rats and from a reserpine-induced fibromyalgia model.

Results showed a reduction in structural proteins and an increase in markers of inflammation and oxidative stress, suggesting a potential compromise of muscle tissue integrity. Moreover, an overall variation in factors involved in the NLRP3 inflammasome and in myogenic markers was observed, indicating a potential role of these elements in the local inflammatory response. Overall, the study supports the hypothesis of a direct involvement of the analyzed muscle proteins in the pathophysiology of fibromyalgia, offering new insights into the molecular basis of the disease and the potential identification of diagnostic and therapeutic targets.

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**Keywords:** Fibromyalgia, Skeletal muscle, NLRP3, NRF2, DGC, Myogenesis.

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# The receptor RAGE is re-expressed at myofiber level and sustains the onset and progression of cachexia in cancer conditions

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Cancer cachexia (CC) is a highly debilitating multifactorial syndrome affecting most patients with advanced cancer, and it is characterized by loss of body weight and muscle atrophy, leading to severe weakness and progressive functional impairment, reduced responsiveness to anti-cancer treatments, and reduced survival [1]. The receptor for advanced glycation end-products (RAGE) is a multiligand receptor with pleiotropic activities. RAGE is involved in inflammation and cancer growth, and has a role in muscle development and regeneration, but its expression is repressed in adult healthy muscles [2]. RAGE-null (*Ager*$^{-/-}$) mice showed reduced systemic inflammation, delayed body and skeletal muscle weight loss, and dramatically increased survival in the presence of cancer [3]. Interestingly, tumor-bearing mice re-express RAGE at myofiber level, but the specific contribution of muscular RAGE to CC was unknown. Using an HSA/Cre-Lox system, we generated a tamoxifen-inducible conditional *Ager*$^{mKO}$ mouse model in which RAGE is selectively ablated in myofibers. Tamoxifen-treated *Ager*$^{-/-}$, *Ager*$^{mKO}$, and control (*Ager*$^{flox}$) mice were subcutaneously injected with Lewis lung carcinoma (LLC) cells or vehicle, and histological, molecular, and proteomic analyses were performed at 25 days post-injection. We found that, compared with LLC- *Ager*$^{flox}$ mice, LLC-*Ager*$^{mKO}$ mice showed reduced body weight loss, no significant reduction of hindlimb muscle mass and strength, and myofiber cross-sectional areas, restrained muscle and systemic inflammation, and increased survival. However, the highest protection against CC was observed in LLC/*Ager*–/– mice. Mechanistically, *Ager*$^{mKO}$ muscles resist cancer-induced atrophy by maintaining an active Akt-GSK-3β-PGC-1α pathway and promoting a fast-to-slow myofiber transition. Distinct proteomic signatures characterized muscles of tumor-bearing mice in dependence on RAGE expression, supporting a protective effect of muscle or total ablation of RAGE. Thus, RAGE re-expression and engagement at myofiber level drive muscle wasting and inflammation in tumor-bearing mice. Interestingly, we found strongly increased amounts of RAGE in the *rectus abdominis* muscles derived from clinically diagnosed pancreas carcinoma pre- cachectic and cachectic patients, compared with healthy control subjects, suggesting that the overexpression of RAGE is an early event in muscles of cancer patients, and highlighting an involvement of this receptor in the onset of CC. Altogether, these data suggest that the molecular targeting of RAGE might be used to counteract the cachectic syndrome and prolong the survival of cancer patients.

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**Keywords:** Cancer cachexia, RAGE, muscle wasting, myofibers.

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# Sarcoglycans as Regulators of Actin Cytoskeleton and Focal Adhesion Integrity in Human Articular Chondrocytes

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Chondrocytes play a key role in preserving cartilage stability through coordinated regulation of extracellular matrix (ECM) synthesis and remodeling. These processes depend on ECM dynamic interactions, mediated by integrin-based focal adhesions and associated cytoskeletal components. While the roles of core adhesion proteins are well described, the involvement of sarcoglycans (SGs) remains unclear in chondrocytes. Drawing parallels from striated muscle, where the SG subcomplex stabilizes the sarcolemma, we hypothesized that SGs similarly integrate into chondrocyte adhesion complexes. This study investigates the expression and functional role of SG isoforms (α, β, γ, δ) in human articular chondrocytes. Utilizing immunofluorescence, quantitative PCR, and siRNA- mediated gene silencing, we observed that all four SG isoforms localize to both cytoplasmic and membrane domains, with pronounced enrichment at focal adhesion sites. Co-localization studies revealed that SGs associate with F-actin stress fibers and vinculin, suggesting their integration into the core adhesion complex. Silencing individual SGs disrupted actin stress fiber organization, led to a diffuse distribution of vinculin, reduced the number of focal adhesion plaques, and altered cell morphology. These findings underscore the role of SGs in modulating actin cytoskeleton dynamics and stabilizing focal adhesions, highlighting their significance in maintaining chondrocyte shape and adhesion beyond muscle tissue contexts.

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**Keywords:** sarcoglycan, focal adhesions, chondrocyte, immunofluorescence.

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# Role of adrenomedullin in leukemic endosteal/vascular niches (protocol gimema AML2220)

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**Introduction.** In the past, several attempts have been made to improve the management of acute myeloid leukemia (AML)1. To tailor treatment as much as possible, fitness assessment for intensive chemotherapy should be mandatory at diagnosis giving attention to the patient's profile and prognostic factors. Recent findings highlight that leukemic cells remodel the bone marrow (BM) microenvironment to support pathological functions, primarily disrupting normal hematopoiesis and promoting aberrant angiogenesis, increased vascular permeability, and vessel loss. Among vascular regulatory peptides, adrenomedullin (ADM)4, a 52 amino acid protein belonging to the calcitonin gene-related peptide family, is known to contribute through CRLR and RAMP2/3 to the survival of drug-tolerant/resistant leukemic stem cells. Furthermore, the overexpression of CRLRL and/or ADM has been demonstrated to correlate with adverse outcomes in AML. Due to their impact on disease progression and treatment response, BM stromal factors, such as ADM, and adhesion molecules mediating the physical interaction of leukemic cells with endothelial cells5, 6 (CD44/E-selectin) of type H (CD31high), or type L (CD31low) could represent an intriguing source of potential predictors of outcomes in AML patients.

**Methods.** We performed preclinical flow cytometric screening of BM and peripheral blood (PB) from eligible and newly diagnosed AML patients enrolled in the Protocol GIMEMA AML2220 for the expression analysis of ADM (intracellular, iADM), RAMP2/3, CRLR, and adhesion molecules (CD31, CD38, CD44s, CD44v6). Data correlated with BM/PB blasts, mutation status, and patient risk assessment. Samples from patients under complete remission were used as references.

**Results.** An altered expression of iADM was observed in 22.7% (iADM low) and 54,6% (iADM high) of BM samples. The decreased ($P<0.05$) percentage of RAMP2-positive cells or RAMP2/RAMP3 ratio correlated with increased blasts in PB. A significant upregulation of adhesion molecules (CD31, CD44, CD44v6) and CRLR was observed in ADM-highly expressing samples.

Interestingly, iADM-low profile correlated mostly with refractory response while iADM high profile with relapse condition.

**Conclusions.** Our data suggests that the expression of iADM could be a useful marker of structural/functional BM alterations (iADMlow) or increased microvessel density (iADMhigh), which, in turn, could be potentially related to the refractory/relapse response of AML patients.

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**Keywords:** Adrenomedullin, bone marrow, acute myeloid leukemia.

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# Potential contribution of TPC2 to the Aggressive Traits of Malignant Melanoma

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Two-pore channel 2 (TPC2) is a Na+/Ca2+ ion channel located on the membrane of intracellular acidic organelles, including endo-lysosomes and melanosomes. It plays a crucial role in several cancers, including melanoma[1,2], one of the most aggressive and treatment-resistant malignancies, frequently driven by mutations in the serine/threonine kinase BRAF[3]. This project aims to explore the involvement of TPC2 in melanoma progression by comparing its role in primary and metastatic disease, with the goal of evaluating its potential as a therapeutic target for personalized treatment approaches. On this purpose, two pairs of human melanoma cell lines (IGR and WM) were analysed, both BRAF-mutated and derived from the same patient at different stages of tumor progression. TPC2 expression levels were compared between the primary (IGR39 and WM115) and metastatic (IGR37 and WM266-4) cell lines. The expression of epithelial-mesenchymal transition (EMT) markers, among which N-cadherin and E-cadherin, was assessed to examine the EMT transition. Additionally, cellsmigration was measured using a wound healing assay, and their adhesion to type I collagen was tested.To examine the role of TPC2, its selective inhibitor SG-094 was used to treat the upper listed melanoma cell lines.

Results indicated that the four human melanoma cell lines exhibit heterogeneous expression of EMT markers and display different behaviours in terms of migratory capability and adhesion to type 1 collagen. Notably, cells derived from the primary tumor(IGR39 and WM115) present lower expression of TPC2 than metastatic cells (IGR37 and WM266-4). Moreover, the pharmacological inhibition of this channel using SG-094 significantly reduced migration capacity of IGR and WM cell lines, as well as IGR cells ability to adhere to type 1 collagen, highlighting the involvement of TPC2-dependent intracellular $Ca^{2+}$ signaling in these processes.Moreover, SG-094 treatment also impaired the proliferative potential of these cells, suggesting an additional impact of TPC2 on tumor progression. Notably, our study revealed that the effect of SG-094 may involve the Microphthalmia-associated transcription factor (MITF), a master regulator of melanocytes implied in tumor aggressiveness and metastatic potential.

Overall, the findings demonstrate that TPC2 activity gives a key contribution to melanoma malignant traits, highlighting the potential of its inhibition as part of combination strategies to overcome therapeutic resistance in melanoma treatment. Given the complex regulation of TPC2 in disease progression, further studies are warranted.

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# Testing the efficacy of two different plasma indirect treatments on 2D and 3D cell cultures of glioblastoma

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**Background:** Glioblastoma (GBM), a grade IV malignant astrocytoma, represents the most prevalent type of malignancy in the central nervous system (CNS), comprising 47.1% of all diagnosed CNS tumours[1]. Plasma medicine is an interdisciplinary field that has emerged over the last two decades, with the ultimate aim of utilizing cold atmospheric plasma (CAP) in clinical settings for various biomedical applications, including oncology. While 2D cell cultures have traditionally been used to evaluate the efficacy of anticancer treatments, they lack the structural and physiological complexity of *in vivo* tumours. In contrast, 3D tumour spheroids better represent the tumour microenvironment by incorporating cell-cell and cell-matrix interactions, gradients of nutrients and oxygen, and enhanced resistance to therapies. This study aimed to compare the anti-proliferative effects of two different indirect CAP treatments in 2D vs. 3D spheroid cultures, using U-251 MG and U-87 GBM cell lines, to better understand how culture dimensionality influences treatment efficacy.

**Material and methods:** A pin-to-plate device (air gas at 1000 Hz, 200 V, and a 72 µs duty cycle) was used to treat Dulbecco's Modified Eagle's Medium (DMEM) without pyruvate for 1 to 30 minutes to generate plasma activated medium (PAM). An open-air hybrid mode discharge, which employs either glow discharge or spark discharge, was used to treat water for 5 to 30 minutes to generate plasma-activated water (PAW). Glioblastoma U-251 MG and U-87 in 2D and 3D culture were exposed to different concentrations of PAM or PAW. GBM cells were also co-cultured with monocytes CRL-9955 at a ratio of 2:1 and exposed to PAM/PAW.

**Results:** In 2D models, a significant toxicity, as well as a perturbation of mitochondrial membrane potential, was found when exposed to DMEM treated for 3 and 5 minutes. Furthermore, the co-culture with monocytes, aimed at better understanding the crosstalk between the immune system and GBM, maintains the toxic properties of the PAM after 3 and 5 minutes of treatment. On the other hand, the 3D tumour spheroids exhibited resistance to the same experimental conditions, even when cells were treated with ultrasound in combination with plasma. As for the PAW, when spheroids were exposed to the same experimental conditions found effective for 2D cultures, i.e., spark 5 min 10% and glow 15 min 20%, no significant cytotoxic effects were found. Raising the water percentage at least to 30% and doubling the treatment times results in anti-tumoural effects on 3D cell cultures.

**Conclusions:** These preliminary data suggest that studying the anti-tumoural potential of this innovative approach towards solid tumours, such as GBM, requires a thorough evaluation in 3D systems that better mirror the *in vivo* environment.

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**Keywords:** CAP, indirect treatment, glioblastoma, cell viability.

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# Recent innovations in endodontic irrigation and effects on smear layer removal: an ex-vivo study

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To evaluate the cleaning efficacy of different irrigation activation techniques in removing smear layers from root canals. Ninety lower premolars with straight root canals were assigned to six experimental groups (n=15 each): control group, conventional irrigation, passive ultrasonic activation (PUI), distilled water laser-activated irrigation (LAI), PulpSucker irrigation, and iVac irrigation. Each canal was shaped to size 30/04 and irrigated with 5% NaOCl. The final rinse was performed according to the experimental group. After chemo-mechanical procedures, the teeth were split longitudinally and subjected to scanning electron microscopic (SEM) analysis (1) for each root canal third (coronal, middle, and apical). The presence of smear layer was evaluated using a 5-grade scoring system at 500× and 1000× magnification(2). Following the Shapiro-Wilk test, data were statistically analyzed using the nonparametric Kruskal-Wallis test, followed by the post-hoc Dunn test with Bonferroni correction (α=5%), to compare the effectiveness of smear layer removal(3). The Friedman test and post-hoc Wilcoxon signed-rank test with Bonferroni correction (α=5%) were performed to assess significant differences in smear layer removal among the different tooth thirds. Activated irrigation techniques significantly outperformed conventional irrigation ($p<.05$),

with the iVac technique demonstrating the best results in smear layer removal in the apical third. LAI and PUI showed comparable results across all tooth thirds. Significant differences in cleaning efficacy were observed among the different tooth thirds within each experimental group, with the apical third exhibiting the highest presence of smear layer. Within the limitations of the study, irrigant activation demonstrated higher efficiency in smear layer removal from root canal systems compared to conventional irrigation techniques. iVac showed the best cleaning performance in each third, particularly in the apical third. IVac technology offers significant potential for improving clinical outcomes.

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**Keywords:** Endodontics, Irrigation, iVac, Laser, SEM.

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# NLRP3 Inflammasome and EndoMT in the Heart of HOCL Murine Model of Systemic Sclerosis

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Systemic sclerosis (SSc) is an autoimmune disease characterized by progressive fibrosis in multiple organs, including the heart. Cardiac involvement is a major determinant of prognosis, yet the underlying mechanisms remain poorly understood. This study explores myocardial alterations in murine model of SSc induced by subcutaneous injection of hypochlorous acid (HOCl), focusing on changes in structural proteins, inflammation, oxidative stress, and vascular remodeling. After six weeks, hearts were collected from SSc mice and analyzed using Hematoxylin and Eosin (H&E) staining, Masson's Trichrome, and immunohistochemistry. Vascular expression of vimentin and α- SMA was markedly increased, indicating endothelial dysfunction and myofibroblast activation, while reduced CD31 expression suggested endothelial-to-mesenchymal transition (EndMT). In parallel, there was a substantial increase in macrophage markers (CD68, F4/80, EP29, EPR1) and inflammasome components (Caspase-1, IL-1β, NLRP3), accompanied by elevated levels of matrix metalloproteinases MMP3 and MMP9. These findings support a strong link between inflammation, matrix remodeling, and endothelial impairment. Additionally, increased NRF2 expression may reflect a compensatory antioxidant response to oxidative stress. Overall, these results suggest that immune and inflammasome-driven signaling contribute to myocardial injury in SSc, promoting endothelial dysfunction and disrupting the balance between fibrosis and vascular repair.

**Keywords:** systemic sclerosis, heart, EndoMT, NLRP3.
Poster presentation.

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# miR-29a-3p regulates mitochondrial metabolism and redox status in cellular models of Fanconi Anemia

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Fanconi anemia (FA) is a genetic disease, typically with pediatric onset, characterized by aplastic anemia as well as a heightened susceptibility to several types of cancer[1]. It is well known that cells mutated in Fanconi genes display defective DNA double-strand break repair[2]. However, emerging evidence indicates a defect in aerobic metabolism in FA cells, which is associated with alterations in the mitochondrial network that promote a pro-oxidant and pro-inflammatory cellular environment[3]. Lymphoblasts and fibroblasts with *FANC-A* mutations exhibit aberrant mitochondrial biogenesis, dynamics, and metabolism. This condition, parallel to compromised antioxidant defense mechanisms, resulted in a redox imbalance and an increased secretion of pro-inflammatory cytokines[4-5]. Despite these findings, the molecular mechanisms underlying the interplay between genomic instability, metabolic perturbations, and chronic inflammation in FA remain poorly understood.

To address this gap, we evaluated the altered microRNA (miRNA) profile in FA cells, focusing on miR-29a-3p, which plays a crucial role in multiple cellular processes, including mitochondrial function and the differentiation, maturation, and survival of hematopoietic stem and progenitor cells[6].

To this purpose, we used lymphoblasts and primary fibroblasts derived from patients with the *FANC- A* gene mutation to evaluate: i) mitochondrial respiration, oxidative phosphorylation efficiency, and cellular energy status, by luminometric, oximetric, and spectrophotometric assays; ii) redox balance and oxidative damage accumulation by biochemical assays; and iii) expression of miR-29a-3p target proteins by western blot analysis,

*FANC-A* mutated lymphoblasts and fibroblasts exhibited significant downregulation of miR-29a-3p, which led to hyperactivation of the PI3K/AKT signaling pathway, as indicated by the overexpression of its target genes, specifically FOXO3, SGK1, and IGF1. This hyperactivation resulted in alterations in mitochondrial metabolism and a deficient antioxidant response. Furthermore, downregulation of miR-29a-3p was associated with hyperactivation of the TGF-β signaling axis. In contrast, miR-29a- 3p transfection in *FANC-A* mutated cells improved mitochondrial metabolism, enhanced antioxidant responses, and reduced DNA damage accumulation. These outcomes were achieved by inhibiting the PI3K/AKT pathway and modulating TGF-β signaling through a regulatory feedback mechanism.

In conclusion, miR-29a-3p represents a promising therapeutic target for addressing the multifactorial pathophysiological processes underlying Fanconi anemia, including oxidative stress, metabolic dysfunction, and chronic inflammation.

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**Keywords:** Fanconi Anemia, cell metabolism, microRNA.

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# Biological evaluation of dihydroartemisinin-hybrids as potential anticancer agents for colon carcinoma

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Colorectal cancer (CRC) is one of the most severe neoplasms in terms of incidence and mortality in Italy and worldwide. According to Global Cancer Observatory (GLOBOCAN) data, in 2022 there were more than 1.93 million new cases and about 935.000 deaths globally, ranking it third in terms of incidence and second in terms of mortality among all forms of cancer[1].

Current treatments for CRC include surgery, immunotherapy and chemotherapy. However, a significant proportion of tumors develop resistance to conventional chemotherapeutic agents[2]. In recent decades, novel therapeutic strategies, which involve the use of biological compounds with different mechanisms of action, have been developed, with the aim of combining them with current treatments to prevent tumor cells from developing resistance. Natural products play a crucial role in drug discovery, serving as direct pharmaceuticals or scaffolds for structural modification to enhance therapeutic efficacy[3].

Artemisinin, an antimalarial compound extracted from *Artemisia annua*, and its derivatives have been shown to possess selective anticancer properties. Recent studies demonstrated that Dihydroartemisinin (DHA), an active metabolite of Artemisinin, exhibits a stronger anti-tumor effect when used in combination with various chemotherapeutic agents. DHA has shown also the ability to reverse drug resistance in certain cancer cell lines. In this context, emerging technologies, such as molecular hybridization, have been employed in cancer therapy to combine DHA with other pharmacophores[4].

In our study, eight DHA derivatives were synthesized through molecular hybridization with bile acids, specifically ursodeoxycholic and chenodeoxycholic acids, known for their anti-tumor properties. These hybrids were evaluated for their potential anti-cancer effects on two *in vitro* models

of human colon carcinoma (HCT116 and RKO cell lines) and for their potential antiproliferative activity on endothelial cells (HUVEC). All derivatives demonstrated a stronger cytostatic activity with respect to DHA in both colon carcinoma cell lines. Hybrids demonstrated also to reduce HCT116 cell migration capability more effectively than DHA, when analyzed in real-time using an xCELLigence RTCA DP Instrument. On the other hand, they showed a lower effectiveness on a non- tumor cells' model with respect to colon carcinoma cells, demonstrating also lower toxicity compared to DHA.

Moreover, DHA hybrids seemed to interfere with angiogenesis, which has a fundamental role in tumor progression, by decreasing proliferation of human endothelial cells more effectively than DHA. In conclusion, our DHA-bile acid hybrids have been shown to significantly enhance the cytostatic and anti-angiogenic activity of DHA, making them promising candidates for further therapeutic development.

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**Keywords:** Dihydroartemisinin, bile acids, hybrids.

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# Altered Mitochondrial Function and Turnover: a Mechanism for Long-Term Aging Effects in Survivors of Childhood Cancer

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Over the past forty years, survival rates for children diagnosed with cancer have significantly improved [1]. However, Childhood Cancer Survivors (CCS) remain at risk for developing late- onset health complications, often resembling features of accelerated aging [2]. Despite this, the cellular and molecular mechanisms underlying these effects are still not fully understood. Among the hallmarks of aging, alterations in mitochondrial metabolism and increased oxidative stress play a central role.

Thus, this study aims to investigate mitochondrial functional alterations in mononuclear cells (MNCs) from CCS, focusing on oxidative phosphorylation (OxPhos) function, cellular energy status, mitochondrial dynamics, and mitophagy.

Analyses were conducted on MNCs isolated from the peripheral blood of CCS (n = 96), age- matched healthy donors (n = 67), and elderly subjects (n = 80).

Luminometric, oximetric, and spectrophotometric analyses were performed to evaluate oxygen consumption rate, aerobic ATP synthesis, and intracellular levels of ATP and AMP. Western blot analyses were used to assess the expression of proteins involved in mitochondrial dynamics and mitophagy. Statistical analyses included Student's t-test and one-way ANOVA with Tukey's post hoc test.

The results revealed that OxPhos function and efficiency in CCS were diminished relative to healthy peers and closely resembled the profiles seen in elderly subjects, as reflected by a lowered ATP/AMP ratio [3,4]. While expression levels of core mitochondrial fusion and fission proteins appeared normal, CCS samples showed increased DRP1 phosphorylation on Ser916, promoting excessive fission driven by MTFP1 and mTOR signaling.

Additionally, reduced expression of mitophagy markers and mitochondrial biogenesis regulators pointed to inefficient mitochondrial turnover and accumulation of damaged organelles.

In summary, these findings suggest that mitochondrial dysfunction may contribute to the early aging phenotype observed in CCS, offering valuable insights into the biological basis of their long-term health challenges.

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**Keywords:** Aging, Cancer Survivors, Mitochondria, Fusion/Fission, Mitophagy.

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# Neuroactive steroids allopregnanolone and pregnenolone efficiently counteract bortezomib-evoked painful/sensory symptoms in a rat model

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Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling condition resulting from antineoplastic treatment which may diminish following dose reduction or discontinuation of chemotherapeutics administration. The proteasome inhibitor bortezomib (BTZ) can cause painful peripheral neuropathy (BIPN), a set of sensory-related symptoms with a negative impact on cancer survivors' quality of life. Although reducing pain is often a main focus of BIPN treatment, remarkably few analgesics have been tested. A growing number of reports suggests that CIPN can be attenuated by the concomitant use of neuroactive steroids (NAS), cholesterol derivatives with proven neuroprotective effects in different *in vivo* models of peripheral neuropathy. Therefore, here we tested the analgesic effect of two NAS, allopregnanolone (ALLO) and pregnenolone (PREG), in a rodent model of BIPN. Female Wistar rats were intravenously treated with BTZ (0.2 mg/kg, 3qwx4) then a co-administration of BTZ with subcutaneous administration of ALLO (3mg/kg/every 2 days) or PREG (6mg/kg/every 2 days) were performed for another 4 weeks. Moreover, we tested the effect of ALLO and PREG on BTZ-induced neurotoxicity taking advantage of a battery of behavioral and neurophysiological tests, tracking their progress for 4 additional weeks of follow-up. In addition, we assessed the severity of peripheral axonopathy performing a morphological evaluation of myelinated peripheral nerves and intraepidermal small unmyelinated fibers.

As expected, treatment with BTZ for 8 weeks resulted in a clear manifestation of neuropathic symptoms, with a significant development of mechanical allodynia and thermal hyperalgesia. Therefore, a significant decrease in both sensory action potential (SAP) amplitude and sensory conduction velocity of caudal nerve, as well as a reduction in SAP of digital nerve were reported.

At the end of treatment, although neither drug showed recovery from the damage observed in the caudal nerve, a significant protection of ALLO and PREG was observed in SAP of the digital nerve. Interestingly, ALLO ameliorated both mechanical allodynia and hyperalgesia exhibited by BTZ- treated animals after 8 weeks of treatment, while PREG showed a protective effect only on thermal sensitivity thresholds. In addition, loss of IENF and significant degeneration of myelinated axons in BTZ-treated distal caudal nerves were also observed. Finally, most nerve abnormalities observed during the treatment with BTZ spontaneously recovered after drug withdrawal. Taken together, our results suggest that since NAS counteracted painful symptoms including allodynia and hyperalgesia induced by BTZ, they could be used to alleviate BIPN neurotoxic manifestations, as well as partially contrast the neurotoxic effects on digital nerves. Further studies are still needed to shed more light on the specific mechanisms of action of NAS.

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**Keywords:** CIPN, bortezomib, axonopathy, allopregnanolone, pregnenolone, mechanical allodynia, thermal hyperalgesia.

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# Cell instructive Liquid Crystalline Networks and red Photobiomodulation for myotube formation: a morphological study

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Skeletal muscle, accounting for approximately 40% of adult body weight, exhibits remarkable plasticity and exceptional regenerative capacity. The mediators of muscle regeneration are a small population of resident adult stem cells, termed satellite cells (SCs), located in a sublaminar position in close association with muscle fibers. SCs are mitotically quiescent under normal physiological conditions but can rapidly activate and proliferate in response to injury, ultimately differentiating and fusing to form new muscle fibers or repair damaged ones. Although skeletal muscle is generally stable under normal conditions, with minimal daily SCs activation, it can fully recover within weeks following major injury due to the dynamic interplay between SCs and their microenvironment. However, in case of severe injury or pathological conditions, SCs' regenerative capacity may be impaired, leading to incomplete repair and increased fibrotic tissue deposition. In this context, identifying new therapeutic strategies that improve SC-mediated regeneration while minimizing the fibrotic response is essential. To this purpose, the availability of effective cellular models on which to possibly test potential therapies is important. Our research investigated the combined effect of Liquid-Crystalline Networks (LCNs) and photobiomodulation (PBM) on the differentiation of myoblastic cells. LCNs, known for their properties to mimic the extracellular matrix mechanics and influence cell alignment and behavior, were used with varying degrees of cross-linking (10%, 20% and 40%) to assess their influence on promoting the myogenic differentiation. Simultaneously, we applied a PBM treatment using red light with an energy density of 4 J/cm$^2$. PBM has been shown in prior study to have promyogenic potential and antifibrotic effects. It consists in the application of light with a wavelength of 400-1100 nm using different laser or LED devices, with a power density lower than 100 mW/cm$^2$ and an energy density lower than 10 J/cm$^2$ at the target. However, PBM clinical efficacy remains also conditioned by the lack of standardized protocols. Myoblasts were cultured on LCN substrates induced to differentiate in a specific pro-myogenic medium (DMEM + 2% horse serum) for different times exposed or not to PBM. Myogenic differentiation outcomes were evaluated through morphological analyses. Our preliminary results demonstrate that LCNs with 20% cross-linking (LCN20) in combination with PBM treatment, provide the most favorable environment for myogenic differentiation, leading to mature myotube structures and increased expression of differentiation markers. These findings suggest a synergistic effect between LCN biomaterials and PBM in enhancing myogenic differentiation process. Further research into the molecular mechanisms underlying these effects will provide the essential experimental groundwork to support the development of optimized protocols for clinical applications.

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**Keywords:** Biomaterials, cell scaffolds, tissue engineering, photobiomodulation, myoblasts differentiation, skeletal muscle injury, regenerative medicine.

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# Cardiomyocyte Lateral Disconnection and Ephrin-B1 Reduction Characterize Left Ventricular Non-Compaction Cardiomyopathy

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Left ventricular non-compaction cardiomyopathy (LVNC), also known as trabecular, spongy, or non-compaction myocardium, is a heterogeneous condition with varied clinical presentations and outcomes, including progression to hypertrophic cardiomyopathy, dilated cardiomyopathy, heart failure, or even recovery [1]. These different phenotypes suggest distinct underlying pathogenetic mechanisms. LVNC can manifest from early development through all life stages. Its classification remains complex due to its polymorphic nature [2]. Ephrin-B1 (Eph) is a major component of lateral junctions (LJs) in cardiomyocytes, conferring essential mechanical and molecular signalling interactions between cells, and its deficiencies are believed to play a crucial role in the development of the pathology. In this work, we aimed to investigate the structure of LJs as well as the Eph expression in human LVNC. Left ventricular endomyocardial biopsies were obtained from 13 patients diagnosed with LVNC (8 males, 5 females; mean age 48 ± 12.8 years), confirmed by cardiac magnetic resonance (non-compaction/compaction ratio >2.3) and LV angiography (presence of intramural trabeculae from the endocardium). Reduced contractility was observed in 6 cases. Samples were analyzed by histology, transmission electron microscopy (TEM), and western blot (WB) to assess Eph levels [3]. Histological analysis revealed that intramural invaginations and trabeculae originating from the endocardium were partially or completely lacking endothelial lining and exhibited platelet activation, progressing in some cases to organized thrombus formation. Cardiomyocytes diffusely showed various degrees of disconnection up to complete detachment. TEM showed lateral detachment of cardiomyocytes, associated with cell shape alterations, subcellular changes, myofibrillar disarray, causing also de-anchoring of myofilaments from Z-discs and the cell membrane. Furthermore, the ultrastructural analysis identified myocyte disconnection at the lateral gap junctions, while the structure of the intercalated disk remained preserved. WB evaluation of Eph was 3.4-fold lower than controls. Our findings highlight a structural disruption of the cardiomyocyte lateral adhesive system, including electrical gap junctions in LVNC, despite preserved intercalated disks. The significant down-regulation of Eph suggests its key role in maintaining lateral cell-cell cohesion. These alterations may contribute to impaired mechanical and electrical coupling in LVNC. Targeting Ephrin-B1 pathways could offer novel insights for therapeutic intervention.

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**Keywords:** Left ventricular non-compaction cardiomyopathy, Ephrin-B1, Lateral junctions, Electron microscopy

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# Activation of the NALP3-CASP1-IL-1 β Inflammatory Pathway by Pesticide Exposure in Human Umbilical Vein Endothelial Cells

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Barrier function regulation, angiogenic potential, and immune response modulation are only few of the many roles of the vascular system that represents nowadays one of the main targets of the environmental pollutants, in particular of pesticides. We have used human umbilical vein endothelial cells (HUVECs) as an *in vitro* model to investigate the effects of pesticides on the activation of the NALP3-CASP1-IL-1β inflammatory pathway using immunofluorescence and RT-PCR investigations, reactive oxygen species (ROS) generation, and morphological alterations with SEM analysis. Our findings offer a comprehensive evaluation of the cellular and molecular damage induced by pesticide exposure and show strong inflammasome activation. They indicate that these chemicals may initiate necroptosis and drive prolonged inflammation in endothelial cells. This study provides crucial insights into how pesticides contribute to endothelial dysfunction, highlighting the need for further investigation into their inflammatory and immunemodulatory effects on vascular health.

**Keywords:** HUVECs, Pesticides, Inflammatory Pathway, Immunofluorescence, RT-PCR, SEM, ROS.

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# Ultrastructural Evaluation of Mouse-Derived Airway Organoids: Preliminary Insights from Light and Transmission Electron Microscopy

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*Background:* Mouse-derived airway organoids represent a significant advancement in respiratory biology, offering anatomically relevant in vitro models that closely replicate the cellular organization, architecture, and function of the native airway epithelium [1; 2]. These three-dimensional structures are generated from airway basal stem cells – multipotent progenitors capable of differentiating into various specialized epithelial cell types, including ciliated and secretory cells. As such, airway organoids provide an invaluable platform for investigating epithelial repair, differentiation dynamics, and the pathophysiology of respiratory diseases such as asthma, cystic fibrosis, and viral infections.

*Aim:* To assess the morphological and ultrastructural characteristics of mouse-derived airway organoids using light microscopy (LM) and transmission electron microscopy (TEM) and evaluate their suitability as anatomical models of the airway epithelium.

*Materials and Methods:* Thirty airway organoids cultured from mouse epithelial cells were fixed and processed for standard TEM. Following epoxy resin embedding, semithin sections were cut and stained with toluidine blue for preliminary analysis by LM to evaluate global architecture and identify regions of interest.

Ultrathin sections were then prepared and examined by TEM to assess epithelial polarity, cellular differentiation, and ciliary ultrastructure.

*Results:* LM analysis revealed that 87% of the organoids presented a central lumen surrounded by a thin epithelial wall. Organoid diameters ranged from 60 to 270 μm (mean ± SEM: 119.8 ± 3.5 μm). Cilia were detected in 94% of the organoids, predominantly on the external epithelial surface. TEM confirmed epithelial polarization, with apical membranes facing outward. Ciliated cells exhibited correctly docked basal bodies and canonical "9+2" axonemal structures, including dynein arms and radial spokes.

*Discussion:* These findings demonstrate that mouse-derived airway organoids recapitulate key morphological and ultrastructural features of native respiratory epithelium. This supports their use as reliable in vitro models for anatomical and morpho-functional studies. Ongoing efforts aim to develop standardized ultrastructural scoring systems to enhance the reproducibility and quality assessment of organoid-based research.

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**Keywords:** Airway Organoids, Respiratory Epithelium, Ultrastructure, Transmission Electron Microscopy (TEM), Morphological Analysis.

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# Long-term Melatonin treatment in BTBR mice: A Hippocampal Perspective in Autism Spectrum Disorder

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Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that affects communication abilities, social interaction, and is characterized by repetitive behaviors and restricted interests. Some evidence suggests that circadian rhythm disturbances, which are frequently linked to abnormal neurodevelopmental phenotypes in ASD patients, may contribute to the disruption of synaptic plasticity [1]. For this reason, it has been proposed to use melatonin, an indolamine endogenously produced by the pineal gland and present in fruits and vegetables, to alleviate the sleep disturbances often observed in patients with ASD. However, current literature provides limited data on the effects of this indolamine on the pathways underlying ASD. Recent research has highlighted the involvement of the hippocampus in this neurodevelopmental disorder, identifying it as an important contributor to social cognition and a key organizer of information; moreover, alterations in this region have been described in ASD patients [2]. Our study aims to evaluate the potential role of melatonin in synaptic modulation and glial morphology of BTBR mice, a well-known mouse model of ASD. For this aim we treated daily C57BL/6, control strain, and BTBR mice with melatonin at a dosage of 10 mg/Kg or vehicle for 16 weeks. At the end of the treatment the brains were collected, and the hippocampus was morphologically evaluated. In particular, we performed immunostaining to evaluate glutamate decarboxylase (GAD67) expression, an enzyme responsible for the synthesis of GABA, and glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed in astrocytes often used as a marker of astrocyte activation in response to injury, inflammation, or neurodevelopmental alterations. In addition, in the same region, we analyzed the potential of melatonin to regulate iron metabolism in the hippocampal CA3 and dentate gyrus, using the Diaminobenzidine (DAB)-enhanced Perls' reaction assay. Our results suggest a potential role of melatonin treatment in synaptogenesis regulation and glial activation in the hippocampus of BTBR mice. Moreover, the morphological staining with DAB-enhanced Perl's reaction indicates a possible impairment in iron homeostasis in the ASD hippocampus, which may be ameliorated with melatonin treatment. Altogether, these data support the hypothesis that long-term melatonin administration could represent an effective therapeutic strategy for ASD subjects.

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**Keywords:** Autism spectrum disorder, hippocampus, antioxidant, immunohistochemical study.

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# Therapeutic Potential of a novel GPR120 Agonist (GprA) in Experimental Bile Duct Ligation- Associated Hepatic Fibrosis

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Free fatty acids (FFAs) may exert a direct protective role in inflammation and metabolism through their interaction with a subset of G protein-coupled receptors known as free fatty acid receptors (FFARs), including FFAR4 (GPR120) (1). Administration of FFAs has been reported to improve various liver diseases, including cholestatic conditions, which are characterized by impaired bile flow due to hepatocyte and cholangiocyte dysfunction (2). Building on this evidence, we investigated the effects of a GPR120 agonist (GprA) on liver fibrosis using a surgically induced cholestasis model via bile duct ligation (BDL). In this study, animals were administered with GprA orally at a dose of 60 mg/kg daily for three weeks. The effects of GprA were compared to those of obeticholic acid (OCA, 30 mg/kg daily), an approved treatment for primary biliary cirrhosis (3). GprA was well tolerated, resulting in a significant 40% improvement in survival compared to the untreated BDL group. Although GprA did not significantly affect inflammatory markers, including interleukins -1α, -1β, - 4, -5, -10, and -6, it effectively reduced the expression of key pro-fibrotic markers such as collagen types I and III, connective tissue growth factor (CTGF), and vimentin. Additionally, GprA modulated enzymes involved in extracellular matrix remodeling, including several matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), leading to a marked attenuation of the fibrogenic process. Moreover, GprA significantly decreased the expression of critical fibrogenesis-related proteins, including alpha-smooth muscle actin (α-SMA), a marker of hepatic stellate cell activation responsible for extracellular matrix deposition, and cytokeratin 19, indicative of cholangiocyte proliferation. Unexpectedly, OCA treatment was entirely ineffective in this experimental model, with treated animals exhibiting approximately 90% mortality. In conclusion, GprA demonstrates promising efficacy in mitigating fibrosis and cirrhosis severity in the BDL mouse model. Further investigations in additional experimental models of liver fibrosis are warranted and may pave the way for the development of effective therapeutic agents for various currently unresolved liver diseases.

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**Keywords:** Fibrosis, liver, free fatty acids.

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# p53 pathway activation in bioreactor-generated breast cancer 3D spheroids

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Breast cancer is the most diagnosed malignancy among women worldwide. Despite advances in screening, early detection, and personalized treatment strategies, it remains a leading cause of cancer-related mortality. A deeper understanding of the molecular and biological mechanisms underlying the disease is essential for the development of more effective therapeutic approaches. For this reason, there is growing interest in three-dimensional (3D) models that better recapitulate the *in vivo* tumor microenvironment, such as 3D spheroid models, which offer more physiologically relevant platforms for studying tumor behavior and drug response, with respect to traditional two-dimensional (2D) culture systems [1]. Nutlin-3a, a small-molecule MDM2 antagonist, is a promising therapeutic agent that activates the p53 tumor suppressor pathway which is often functionally inactivated in breast cancer [2]. In this context, we developed MCF-7 ($ER^+$, $p53^{wt}$) breast cancer spheroids using two different methods: the hanging drop technique and the dynamic agitation through a bioreactor system (CERO 3D incubator, OLS, Bremen, Germany). Matrigel at 4% was used as the matrix to support spheroid formation for both protocols. Morphological differences between the two protocols were evaluated in time course comparing the spheroid size and the uniformity of their structure. Spheroids generated with the bioreactor yielded a higher quality and quantity of the 3D cultures, permitting further morphological characterization and drug assays. Bioreactor-made 3D spheroids increased in size until day 7 to 400 μm diameter and were cultivated until day 14. At this latter time point, pellets of spheroids were prepared, glutaraldehyde fixed and processed for transmission electronic microscopy (TEM) evaluation. At 7 and 14 days of culture they were treated with nutlin-3a (1, 10, and 25 μM) for 24 hours and collected for viability and western blotting analysis. TEM analysis revealed that spheroids are highly heterogeneous and complex, with distinct cellular morphologies depending on their spatial localization within the 3D architecture. Drug treatments showed that nutlin-3a induced a dose-dependent increase in the expression of p53 and p21 at both time points, indicating activation of the p53 pathway and subsequent cell cycle block. These data support the use of MCF-7 bioreactor-made 3D spheroids as a sustainable biomimetic preclinical model for breast cancer.

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**Keywords:** Breast cancer, spheroids, 3D models, nutlin-3a, p53 pathway, cell cycle, apoptosis.

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# The HER2 Regulatory Network: Elucidating Heterogeneity, Key Nodes and Interactions

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**Background:** The classification of HER2 status in breast cancer (BC) has traditionally relied on a binary model (positive vs. negative)[1], yet emerging evidence reveals a more nuanced and heterogeneous landscape, particularly for HER2-low tumors[2]. The goal of this study is to evaluate the concept of HER2-low BC, moving beyond simplistic classifications to explore its complex molecular characteristics and therapeutic implications.

HER2-low BC is a distinct classification for tumors with low levels of HER2 protein expression. This is typically identified through an immunohistochemistry (IHC) score of 1+ or 2+, and importantly, these tumors do not show gene amplification on in situ hybridization (ISH). This category is significant because it accounts for a substantial portion of BCs, approximately 45-55% of all cases. Historically, these tumors were grouped with HER2-negative cancers, meaning they often missed out on targeted therapies. However, recent research has revealed that HER2-low BCs have unique biological features and respond differently to treatments[3-4]. This new understanding emphasizes the need for specialized therapeutic strategies tailored to this specific subtype.

**Methods:** An in silico bulk RNA-seq cohort was built using TCGAbiolinks R package, that was selected from TCGA-BRCA cohort and including BC cases with HER2 IHC 0 or 1+ and 2+ with negative FISH. The count matrix was normalized to perform differential expression analysis with DeSeq2 R package. EnrichR R package was used for Gene ontology geneset enrichment analyses. Moreover, RNAseq data underwent coexpression analysis through WGCNA algorithm.

**Results:** Through in-silico analysis of TCGA-BRCA dataset, we identified key molecular pathways and networks. We observed 76 differential expressed genes with an enrichment in RNA processing pathways in Her2 2+ vs 0 and Her2 2+ vs 1+ by Pathway enrichment analysis. A similar enrichment has been found in Her2 1+ vs 0 and Her2 2+ vs 0 by Gene Ontology enrichment analysis. Multiple long non-coding RNAs (lncRNAs) were identified among the cohort of differentially expressed genes, with an enrichment of pathways implicated in cellular proliferation. Weighted Gene Co- expression Network Analysis (WGCNA) identified the overexpression of a "white module." Analysis of this module revealed that (Nuclear factor erythroid 2-related factor 2) NRF2 pathway was differentially regulated across HER2 status subgroups. NRF2 is a pathway close related to BC proliferation via Her2 regulation.

**Conclusions:** These findings challenge the oversimplified binary view of HER2 and advocate for a more holistic understanding that accounts for the continuous spectrum of HER2 expression and its multifaceted regulatory mechanisms. Further, they emphasize that the HER2-low category represents a heterogeneous group of tumors rather than a distinct biological subtype.

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**Keywords:** Her2 signaling, Breast cancer, In silico analysis, long non coding RNA.

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# Evaluating the Efficacy of Virtual Dissection Tables for Teaching Anatomy to Junior Urology Residents: Insights from a Randomized Trial

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**Background and Objective:** Technology integration might be helpful to address deficiencies in current teaching and training methods. Therefore, we aimed to assess the efficacy of Virtual Dissection Tables (VDT) in teaching genitourinary anatomy to junior urology residents compared to traditional lessons.

**Methods:** This is a prospective multicenter randomized study, involving 96 residents from eight Italian urology residency programs. Participants were randomized into two groups: Group A received traditional anatomy lessons, while Group B attended VDT-based practical sessions. A third comparison group (Group C) consisted of senior residents.

Evaluation tests and satisfaction surveys were conducted. Mann-Whitney U test was used to compare the scores obtained by different groups.

**Key Findings and Limitations:** Group B had a higher median (interquartile range [IQR]) score at the Final Evaluation Test compared to Group A, with 17 (13-19) and 19 (16-22) points for Group A and Group B, respectively (p=0.034). The difference was mainly due to better recognition of anatomical structures. With a median (IQR) score of 24.5 (21.5-26), Group C participants outperformed both Group A and B (p<0.001). The satisfaction survey indicated high relevance and

usefulness of the VDT-based course. The limitations include a restricted sample of Italian urology residents and a specific anatomical focus.

**Conclusions and Clinical Implications:** VDT-based sessions seem to be more efficacious than traditional lessons in teaching genitourinary anatomy to junior urology residents. This underscores the potential of VDTs in enhancing medical education and subsequently enhancing patient care, though challenges such as accessibility and standardization remain.

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**Keywords:** Medical Education, Urology Resident Training, Virtual Dissection Table, Genitourinary anatomy, Randomized Trial.

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# Obesity induced renal inflammation in high-fat-diet obese rats: possible effects of anthocyanin rich fruits

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Excess weight gain related to essential hypertension and diabetes is recognized as a major medical challenge and it is associated with the development of end-stage renal disease such as chronic kidney disease (CKD). Accumulating evidence suggests that obesity contributes to CKD through multiple mechanisms, including hemodynamic alterations, chronic inflammation, insulin resistance, and lipid accumulation [1]. Increased expression of renal injury markers and pro-inflammatory factors may be associated with downregulation of dopamine D2 receptors. Furthermore, recent findings suggest that transient receptor potential (TRP) ion channel dysfunction has a significant impact on the pathophysiology of various diseases. In this contest, the intake of dietary supplements containing natural compounds can reduce oxidative stress and inflammatory processes in animal models of obesity [2].

The present study evaluated the risk of diet-induced obesity (DIO) in rats, potentially associated with renal damage, oxidative stress, and inflammatory processes related to the regulation of the dopaminergic system and the interaction with TRP channels. The effect of tart cherry supplementation on renal morphological changes in DIO rats was investigated. Different groups of DIO rats were fed with either tart cherry seed powder (DS) or seed powder plus tart cherry juice (DJS). DIO rats were compared with control rats that were fed a standard diet (CHOW).

In our model, obesity was associated with renal cortical damage, including glomerular sclerosis and tubular atrophy. Analysis of inflammatory cytokines in DIO rats showed increased expression in glomeruli and the base of proximal and convoluted tubular cells. The expression of D5 receptors and D2-like receptors was altered in DIO rats compared with CHOW. TRPC1 and TRPM2 were upregulated, whereas TRPC6 slightly decreased, in the kidneys of DIO rats. Immunohistochemical analysis showed that TRPC6 was expressed at the glomerular and tubular segmental levels, whereas TRPC1 and TRPM2 were mainly expressed at the tubular level. While inflammation was improved in the obese group, tart cherry supplementation reduced renal IL-1β and IL-6 levels and TRP over-expression.

In conclusion, the modulation of TRP channels and dopamine receptors in rats supplemented with tart cherries may play a significant role in explaining the protective effects linked to their anti-inflammatory and antioxidant properties. Further research is needed to clarify the potential benefits of these compounds as bioactive products in preventing obesity-related kidney disease.

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**Keywords:** High-Fat Diet, Obesity related kidney damage, Anthocyanin rich fruits.

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# Restoring Tendon Cell Function After Corticosteroid Exposure: A Therapeutic Role for Vitamin D and Photobiomodulation

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Corticosteroid treatment is a widely adopted treatment for tendinopathies, due to its well-established efficacy in reducing inflammation and pain. Repeated and/or high-dose administration of glucocorticoids has been associated with alterations in tendon tissue, such as reduced tenocyte viability and proliferation and impaired collagen synthesis and extracellular matrix (ECM) organisation. Furthermore, a dose-dependent reduction in tendon stiffness and ultimate tensile strength has been detected, with negative effects that can persist for weeks after exposure [1]. In some cases, these consequences on the tissue lead to tendon rupture. Among new strategies in tissue preservation and regeneration, Vitamin D supplementation and Photobiomodulation (PBM) have captured the interest of researchers. Vitamin D is known to modulate inflammatory pathways, increase collagen production, and support matrix turnover in musculoskeletal tissues [2]. PBM has been shown to promote cellular bioenergetics, reduce oxidative stress, and stimulate tissue repair [3].

In the present study, we first investigated in vitro the effects of two commonly used corticosteroids, triamcinolone acetonide (TCA) and dexamethasone (DEXA), on immortalized human tenocytes (hAT1-tert). Then, considering any detrimental effects, we explored the capability of Vitamin D supplementation and PBM in preserving/restoring tenocyte functionality and ECM integrity.

Our results revealed a marked reduction in cell viability following TCA and DEXA administration, accompanied by an upregulation of inflammatory and oxidative stress markers, a downregulation of those related to tenocytic phenotype, and the remodelling of ECM. We also observed treatment- specific morphological alterations.

Our results suggest that the application of vitamin D and PBM may provide a valid alternative to mitigate the cytotoxic and degenerative effects of corticosteroids on tendon tissue. By enhancing tenocyte resilience, modulating inflammation, and preserving ECM organisation, this therapeutic strategy promises to improve clinical outcomes in tendinopathy management and reduce tendon degeneration over the long term.

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# Impact of uremic proteins on immune proteostasis: molecular mechanisms and apoptosis signaling in peripheral blood leukocytes

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We have recently shown that uremic plasma proteins are internalized via endocytosis by peripheral blood mononuclear leukocytes (PBML), both *in vivo* and *in vitro* (1). These proteins exhibit diverse post-translational modifications, which arise from reactive uremic solutes and free radical-mediated reactions. They are identified by membrane receptors such as RAGE, which facilitate their endocytosis and retrograde trafficking to the intracellular proteostasis machinery, where they are either repaired or targeted for degradation and recycling.

The endoplasmic reticulum (ER) and lysosomes respond to the accumulation of these modified proteins by initiating an aberrant unfolded protein response (UPR), triggering ER stress and the activation of autophagy. This dysregulated proteostasis results in elevated intracellular reactive oxygen species (ROS), particularly hydrogen peroxide ($H_2O_2$), and culminates in the activation of pro-apoptotic stress-related kinases, such as SAPK-JNK. Collectively, these events contribute to an increased apoptotic rate in uremic PBML. We have termed this process the *immunoproteostasis response* (IPR) of PBML to uremic solutes (2).

Glutathione S-transferase P1-1 (GSTP) is an inducible protein thought to play a key role in the regulation of UPR signaling, ER stress responses, and JNK activation via cysteine glutathionylation. Our earlier work first identified elevated expression and enzymatic activity of GSTP in uremic blood cells (3).

In the current study, we investigated the function of leukocyte GSTP in mediating the IPR to uremic retention solutes using both pharmacological inhibitors and gene silencing approaches. Biochemical analyses support the idea that GSTP participates in the IPR through redox-sensitive mechanisms, potentially involving its oxidative inactivation and function as a redox chaperone (GSH-dependent binary interaction protein) in uremic PBML.

In summary, uremic solutes impair GSTP expression, oxidative stability, and its role in stress signaling within PBML, underscoring the importance of this GSH-dependent enzyme in the immunoproteostasis response to uremic proteins.

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**Keywords:** Uremic solutes, proteostasis, ER stress, GSTP, oxidative stress.

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# Blood-derived NK cells modulate the calcification process in primary cultured human fibroblasts

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Natural killer (NK) cells are lymphoid cells that exert cytotoxic activity against virally infected or malignant cells. Although NK cells have been identified within mineralized tissues,[1,2] their potential pathophysiological or compensatory role in calcification remains largely unclear. Using validated pro-calcific models,[3] peripheral blood-derived human NK cells were previously found neither to undergo calcification, as demonstrated by ultrastructural analyses, nor to exhibit significant phenotypic changes, as revealed by flow cytometric assays, even when exposed to high, metastatic-like concentrations of inorganic phosphate. To assess whether NK cells might influence cell calcification, primary co-cultures of blood-derived human NK cells and human fibroblasts isolated from abdominal connective tissue were established and treated for up to 8 days with a pro-calcific medium simulating metastatic calcification. Two different co-culture conditions were tested: one in which NK cells were added only at the beginning of stimulation, and another in which NK cells were added every three days, coinciding with medium renewal. Additional pro-calcific cultures of fibroblasts alone, as well as untreated cultures of both fibroblasts and NK cells alone, were also prepared. Cultures were analysed by transmission electron microscopy to evaluate the extent of calcification, alongside flow cytometric analyses to assess the phenotypic features of NK cells. Compared to fibroblasts cultured alone in pro-calcific conditions, those co-cultured with NK cells exhibited fewer degenerative changes, with some cells showing features similar to untreated fibroblasts. The protective effects of co-culturing were more pronounced when NK cells were added repeatedly throughout the treatment period. Flow cytometric analyses revealed that, in pro-calcific co-cultures, a higher proportion of NK cells retained elevated expression of the activating receptor CD16 and upregulated NKp46, one of the main receptors triggering NK cell- mediated cytotoxicity, compared to control conditions. These preliminary data suggest that, in vivo, blood NK cells might exert a protective effect against cell calcification, as previously observed for mineralized vascular smooth muscle cells in a mouse model of hypoxic pulmonary hypertension.[4]

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**Keywords:** Natural killer cells, human fibroblasts, pro-calcific cultures, co-culture, flow cytometry, electron microscopy.

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# Cortical dendritic alterations induced by repeated oxaliplatin administration

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**Background.** Oxaliplatin (OHP), a commonly used chemotherapy agent, is well known for inducing peripheral neurotoxicity. This condition manifests as acute, cold-triggered symptoms like paresthesias and muscle cramps, along with long-term sensory disturbances, particularly in the hands and feet. Although OHP does not penetrate the blood-brain barrier, many patients undergoing treatment report experiencing cognitive difficulties – often referred to as "chemo-fog." These cognitive issues are usually temporary but can persist in more severe cases. Until now, no animal model has effectively mirrored both the peripheral and central nervous system effects of OHP-induced neurotoxicity. This study aims to fill that gap.

**Methods.** Adult male Balb/c mice (10 weeks old) received intravenous injections of oxaliplatin (7 mg/kg) once a week for eight weeks, while control animals were administered saline. After the treatment period, behavioral and neurophysiological evaluations were performed. Tissue samples were collected from the caudal nerves, footpad skin, and brain. Histological examination of the caudal nerves and skin biopsies was conducted to confirm peripheral neuropathy. Brain tissue was processed using Golgi-Cox staining, and morphometric analysis of layer V pyramidal neurons of the somatosensory cortex and the pre-frontal cortex was carried out with Neurolucida software (Microbrightfield Inc.).

**Results.** As anticipated, OHP treatment resulted in mild axonopathy within the caudal nerves and a marked reduction in intraepidermal nerve fiber density, both of which correlated with consistent behavioral and neurophysiological alterations. In the cortex, pyramidal neurons in both investigated areas from OHP-treated mice showed a significant decrease in the average length and branching (number of nodes) of basal dendrites. Moreover, the morphological distribution of dendritic spine in different types – mushroom, thin, filopodia, and stubby – was significantly altered in OHP-treated animals compared to controls, with a shift towards more immature types than mushroom.

**Conclusions.** Our findings reveal that chronic OHP administration, using a regimen known to induce peripheral neurotoxicity, also leads to pronounced morphological alterations in cortical dendritic structures. Although OHP does not directly interact with central neurons, the mechanisms driving these changes remain unclear and merit further investigation. Notably, similar dendritic abnormalities are observed in various neurodegenerative conditions, such as AD, suggesting that these structural disruptions may contribute to the cognitive deficits commonly referred to as chemo-fog. Further studies are needed to assess whether such changes occur in other brain regions and to better understand their broader implications.

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**Keywords:** Golgi staining, oxaliplatin, cortical changes.

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# Alterations of Cytoskeletal and Sarcoglycan Complex Proteins in the Cremaster Muscle in Patients with Retractile Testis and Undescended Testis

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Testicular descent is a crucial process during male fetal development, and its alteration leads to cryptorchidism (undescended testis). The cremaster muscle, derived from the gubernaculum, plays an active role in the inguino-scrotal phase of testicular descent, contributing with rhythmic propulsive contractions that guide the testis through the inguinal canal into the scrotum. Despite its relevance, the molecular mechanisms underlying cremasteric dysfunctions in these pathologies remain partially elusive. In particular, cytoskeletal proteins and those involved in muscle membrane stabilization, such as the sarcoglycan complex, talin, and vinculin, are essential for skeletal muscle integrity and function. The present study aims to investigate the expressions and distributions of these proteins in the cremaster muscle under different clinical conditions of testicular position, in order to elucidate the molecular pathogenesis of cremasteric dysfunctions.

Three groups of pediatric patients undergoing surgery were enrolled: a control group (n=10, with hydrocele or uncomplicated inguinal hernia), a group with retractile testis (n=10), and a group with undescended testis (n=10). Cremaster muscle biopsies were collected during surgery and analyzed by immunofluorescence using specific antibodies for alpha (α), beta (β), gamma (γ), delta (δ), and epsilon (ε) sarcoglycan subunits, as well as for talin and vinculin.

Immunofluorescence analysis revealed significant differences ($p < 0.05$) in the expression of the studied proteins among the groups. Compared to the control group, the cremasters of patients with undescended testis showed a marked decrease in the distribution pattern of alpha, beta, gamma, delta, and epsilon sarcoglycan subunits, as well as talin and vinculin. In contrast, in the cremasters of patients with retractile testis, an increase in the distribution pattern of all examined proteins was observed compared to the control group. Protein distribution in the undescended cremaster also appeared more disorganized, suggesting structural alterations at the cytoskeletal and membrane complex level.

The decrease of these proteins in the undescended testis could indicate an impairment of structural integrity and sarcolemma stability, leading to reduced contractile capacity and muscle disorganization. This intrinsic deficit of the cremaster muscle could prevent a proper and sufficient propulsive force necessary for testicular descent. Conversely, the increase in these proteins in the retractile testis could reflect a compensatory mechanism or exacerbated contractile activity. These findings contribute to a better molecular understanding of cremaster dysfunctions and could open new perspectives for diagnosis and potential therapeutic development.

**Keywords:** Sarcoglycans, Cremaster Muscle, Cryptorchidism.

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# Comparing differentiation protocols for pancreatic endocrine commitment of Amniotic Epithelial Cells

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Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease caused by the destruction of insulin- producing beta cells in the pancreas. While current treatments rely on exogenous insulin administration, they fail to restore endogenous insulin secretion or address the underlying immune dysregulation. Stem cell-based strategies aimed at generating functional beta-like cells represent a promising avenue for regenerative therapies [1].

Among the various cell sources under investigation, human amniotic epithelial cells (AECs) have emerged as a valuable candidate due to their pluripotency, immune-privileged profile, and non- tumorigenic potential[2,3]. In this study, we evaluated the endocrine differentiation capacity of AECs cultured in 2D using three distinct protocols: one based on a commercially available differentiation kit and two protocols developed in-house and refined in our laboratory.

AECs were isolated from term placentas and expanded under standard conditions. Upon reaching confluence, cells were induced to differentiate following the three protocols. Immunofluorescence analyses were performed to assess the expression of key pancreatic markers, including PDX1, Neurogenin3, C-Peptide and insulin, as indicators of progressive commitment toward the beta-cell lineage.

All protocols induced varying degrees of endocrine commitment, but the commercial kit demonstrated the most robust and consistent induction of pancreatic markers, including higher expression levels of insulin. Conversely, the in-house protocols, while effective in initiating early differentiation, showed less efficiency in achieving full endocrine maturation, indicating significant room for optimization and refinement.

This work lays the groundwork for the integration of 2D-differentiated AECs in more complex three- dimensional culture systems and cell therapy strategies for T1DM [4]. Further functional assays and transcriptomic analyses are ongoing to deepen the characterization of these differentiated populations.

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**Keywords:** Amniotic epithelial cells, Perinatal stem cells, Type 1 Diabetes Mellitus, pancreatic differentiation, beta cells, regenerative medicine.

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# Sorcin regulates alveolarization and airway tissue remodeling during lung morphogenesis

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Lung development occurs through two processes: i) branching morphogenesis, occurring during the pseudoglandular stage and involving the formation of bronchial tree through epithelial-mesenchymal cell interactions; and ii) alveolarization, happening later during the alveolar stage and continuing postnatally, leading to maturation of alveoli and differentiation of epithelial cellular in Type I (ATI) and Type II (ATII) pneumocytes. ATII cells are crucial for producing and recycling pulmonary surfactant, a mix of lipids and proteins (such as SP-B and SP-C), which reduces surface tension and prevents alveolar collapse (1). The epidermal growth factor receptor (EGFR) is a critical factor in lung development; its absence in mice results in collapsed, poorly air-filled alveoli, thickened alveolar walls, and reduced expression of surfactant-related genes (2)(3). Alterations in alveolar homeostasis can lead to respiratory diseases (4). Our previous study on non-small cell lung adenocarcinoma demonstrated that the EGFR signaling pathway is regulated by Sorcin (5), an important calcium sensor protein that controls the concentration of calcium in the endoplasmic reticulum (ER), conferring resistance to apoptosis and preventing ER stress.

Here, we elucidate Sorcin's role in lung development and surfactants homeostasis during lung morphogenesis using a *Sorcin-null* (*SRI*$^{-/-}$) mouse model compared to wild-type controls. We observed that *SRI* $^{-/-}$ mice, compared to wild-type controls, exhibited: 1) a decrease in EGFR protein and its downstream RAS signaling pathway, confirming the relationship between Sorcin and EGFR in lung development; 2) reduced expression of branching morphogenesis markers (e.g., Fgf10) and surfactant proteins (e.g., Sp-B, Sp-C and Abca3), indicating impaired branching, alveolarization, and surfactant secretion; 3) increased glycogen content and decreased lipid droplets, suggesting Type II pneumocyte immaturity and impaired lipid surfactant; and 4) increased phalloidin staining around bronchioles and high fat accumulation in airway tissue at 3 months of age, implying airway tissue remodeling and bronchial contractility defects.

Altogether, these data reveal a novel role of Sorcin in mammalian lung development, specifically in regulating alveolarization and maturation of pulmonary surfactant. Alterations in these processes are associated with many human diseases, including respiratory distress syndrome (RDS).

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**Keywords:** Lung Development, Sorcin, Alveolarization, Branching Morphogenesis, Airway Remodeling.

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# Mutual interaction between Schwann cells and hepatocellular carcinoma: a potential role in neoplastic progression

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The role of peripheral nerves in cancer research has been extensively studied over the past few decades, with increasing attention recently focusing on Schwann cells (SCs), which are the most abundant glial cells of the peripheral nervous system (1). Tumour-activated SCs share strong similarities with repair SCs that appear after nerve injury. A large body of research indicates that SCs significantly contribute to cancer progression modulating the tumour microenvironment by paracrine signalling and direct physical contact (2). Regarding hepatocellular carcinoma (HCC), the most common primary liver tumour, several studies have emphasised the potential significance of autonomic innervation in its development and progression. Conversely, the contribution of SCs in this area remains largely unexplored. To address this issue, we investigated the biological effects and molecular mechanisms underlying communication between SCs and cancer cells in HCC. We focused on the paracrine crosstalk between SCs and HCC cells using an *in vitro* approach to explore its potential impact on HCC cell aggressiveness and SC reprogramming.

*Methods.* Human Cell cultures of hepatoblastoma and hepatocarcinoma cells (HepG2 and Hep3B) and primary SCs. Proliferation assays (cell counting, MTT, BrdU incorporation), wound healing tests, migration and invasion assays *via* transwell chambers, WB analyses, proteomic analyses via mass spectrometry.

We used the human hepatocarcinoma Hep3B cell line and human primary SCs, treating each cell type with either control culture medium (CM) (derived from the same cell type) or CM derived from the other cell type. We assessed the effects of these treatments through functional assays and analysis of molecular and morphological profiles. Our results show that, Hep3B cells treated with SCs-CM exhibit more aggressive features associated with higher tumour spread compared to control. These features include enhanced migration and a doubled capacity for Matrigel invasion, as well as changes at the protein level of epithelial-to-mesenchymal transition markers (N-cadherin, E-cadherin and Vimentin). Similar results were confirmed using the human hepatoblastoma HepG2 cell line. In addition, paracrine signals from Hep3B cells induce a chemotactic response in SCs, promoting processes such as proliferation, migration, Matrigel invasion and the upregulation of repair-related markers (GFAP and N-cadherin), thereby fostering SCs activation. Overall, our data indicate a possible bidirectional interaction between SCs and HCCs that could have a significant impact on tumour progression, thereby highlighting the importance of a deeper understanding of the glial component of the HCC tumour microenvironment.

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**Keywords:** cancer cells, glial cells, microenvironment

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# Hypopressive abdominal gymnastics influences sleep quality: a pilot study

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**Introduction.** Hypopressive Abdominal Gymnastics (HAG) is a postural technique designed to strengthen the abdominal and pelvic floor muscles. It is primarily practiced by pregnant and postpartum women, as well as individuals with rectus abdominis diastasis, prolapse, urinary incontinence, pelvic floor dysfunction, and chronic low back pain. The technique involves combining expiratory apnoea with abdominal contraction manoeuvres, promoting diaphragm relaxation and reducing intra-abdominal pressure while activating the abdominal and pelvic floor muscles (1). As no previous studies have examined the relationship between HAG and sleep, this study aims to evaluate whether HAG could have positive effects on sleep quality.

**Methods Methods.** Twenty-eight women (mean age 43.23 ± 7.03 years) with rectus abdominis diastasis were randomly assigned to either an intervention group (INT, 17 women) or a control group (CTRL, 11 women). The INT group participated in a 2-month HAG program, consisting of one supervised session per week, while the CTRL group was advised to follow a 2-month HAG program at home. Both groups completed the Mini Sleep Questionnaire (MSQ) to subjectively evaluate sleep and wore an actigraph to obtain objective sleep measurements. MSQ and actigraphy assessments were conducted one week before and one week after the intervention. Differences in delta values between the INT and CTRL groups were analyzed using ANCOVA, adjusted for age.

**Results.** The delta values for the total MSQ score (INT: -2.67 ± 1.19 a.u.; CTRL: -0.94 ± 0.24 a.u.), the sleep component of the MSQ (INT: -1.7 ± 0.92 a.u.; CTRL: -0.99 ± 0.74 a.u.), and the wake component of the MSQ (INT: -0.97 ± 0.46 a.u.; CTRL: 0.05 ± 0.03 a.u.) showed a greater reduction in the INT group compared to the CTRL group, suggesting a more pronounced improvement in sleep. However, these differences were not statistically significant ($p = 0.67$, $p = 0.82$, and $p = 0.63$, respectively).

Regarding the actigraphic data, the delta percentages of sleep time and immobile time were positive in the INT group (sleep time: 0.62 ± 0.35%; immobile time: 0.43 ± 0.14%) and negative in the CTRL group (sleep time: -0.38 ± 0.22%; immobile time: -0.32 ± 0.25%), indicating a tendency toward sleep improvement in the INT group relative to the CTRL group. Again, these differences did not reach statistical significance ($p = 0.49$ and $p = 0.23$). No between-group differences were observed for the percentage of sleep efficiency (INT: 1.48 ± 1.02%; CTRL: 2.01 ± 1.49%).

**Conclusion.** This pilot study suggests that a 2-month HAG program may have some beneficial effects on sleep quality, with positive trends particularly evident in subjective sleep measures. However, no substantial or statistically significant outcomes were observed, likely due to the limited duration of the intervention. Extending the intervention period and increasing the sample size may lead to more pronounced and conclusive results.

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**Keywords:** hypopressive exercise, abdominal muscles, pelvic floor muscles, sleep quality.

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# Controlled Delivery of a Membrane-Targeted Photoswitch for Long-Term Neuronal Reactivation in Retinal Degeneration

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Degenerative retinal diseases, such as *Retinitis pigmentosa* (RP) and age-related macular degeneration, lead to the loss of photoreceptors and disrupt the retinal circuitry, resulting in blindness. Traditional therapies, including optogenetics and retinal prosthetics, often fail to restore physiological visual processing due to the indiscriminate activation of retinal neurons and disruption of ON/OFF pathways. Our laboratory has developed Ziapin2, a membrane-targeted photochromic compound that modulates neuronal excitability by altering membrane capacitance in response to light [1]. Ziapin2 selectively reinstates ON-OFF responses in retinal ganglion cells of blind rodents, restoring visually guided behaviors [2]. However, its clinical application is limited by the need for effective, sustained, and biocompatible delivery systems.

To address this, we adopted two strategies: fusogenic liposomes for an efficient delivery of incorporated Ziapin2 to the target neuronal membrane in the absence of organic solvents and engineered biodegradable polymeric μ-particles that encapsulate Ziapin2 and enable its prolonged release. Scanning electron micrographs of microparticles incubated with neurons under physiological conditions showed that their structure remained intact for extended periods (up to 2 months). Neither type of particle affected the viability of primary neuronal cultures, confirming their biocompatibility. *In vitro* fluorescence experiments showed that liposomes incubated with primary neurons brought about an efficient delivery of the compound, endowing neurons with photosensitivity. Similarly, neurons exposed to Ziapin2-loaded particles responded to 470 nm light stimulation with action potential firing, mirroring the response formerly seen with acute Ziapin2 application. Notably, this light-induced excitation was maintained for up to 28 days post-treatment, indicating the sustained bioavailability and functionality of the encapsulated molecule over time. Preliminary *in vivo* studies involving local injection of both types of Ziapin2-loaded particles into rodent models of RP revealed subretinal localization of the particles, suggesting efficient delivery to the target retinal regions.

These findings suggest that the designed delivery systems may enable a long-term modulation of neuronal activity by photochromic compounds like Ziapin2, thus boosting the translational potential of light-controlled therapeutic strategies for restoring physiological activity in neural tissues affected by degeneration. Further studies are currently underway to investigate the potential of these systems for long-term vision restoration in preclinical models of blindness.

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**Keywords:** Controlled Delivery systems, Membrane-Targeted Photoswitch, Retinal Degeneration, Neurons.

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# Potential anti-inflammatory effect on the intestinal barrier of carbonic anhydrase modulators targeting host-gut microbiota interactions

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Gut dysbiosis represents the disruption in composition and function of the intestinal microbiota. This altered state reflects an imbalance in the host homeostasis, and it is linked to tissue inflammation and oxidative stress, potentially leading to gastrointestinal, neurological, metabolic and immune diseases, and cancer [1]. Modulation of metabolic pathways essential for bacteria represents a valid approach in treating dysbiosis and inflammatory diseases [2,3]. In this context, carbonic anhydrases (CAs) inhibitors and activators may represent a therapeutic strategy for diseases related to microbial dysbiosis [4]. Here we explore the anti-inflammatory role of a new class of bacterial CAs activators, designed from serotonin and its precursor tryptamine, and 2-picolylamine. These activators possess higher selectivity towards probiotic isoforms of *Bifidobacterium longum* (BilCAβ), *Lactobacillus rhamnosus* (LrhCAα) and *Lactobacillus reuteri* (LreCAγ) over the human isoforms hCA I and II. The biocompatibility of compounds was assessed using *in vitro* models based on unstimulated macrophages and primary human intestinal epithelial cells (HIEC-6) under basal conditions after 24 h of exposure to increasing concentrations of compounds. Selected newly synthesized CA modulators, namely CA-19 and CA-20, were administered on LPS-stimulated macrophages and HIEC-6 cells under pro-inflammatory conditions (conditioned medium). Cell metabolic activity was measured, and imaging analyses were performed using the Operetta CLS™ system. Data reveal that compounds counteract inflammation in LPS-stimulated macrophages and highlight the modulation profile of occludins involved in the intestinal barrier integrity (TJP1). These preliminary findings pave the way for future investigations into the role of CA- 19 and CA-20 in the context of gut dysbiosis.

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**Keywords:** Inflammation, carbonic anhydrase, macrophages, microbiota, gut.

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# Preservation of Intestinal Permability by Hazelnut Film in Cellular Model of Inflammatory and Radiation-Induced Mucosal Injury

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Radiation therapy is a cornerstone in cancer treatment, used in approximately 50% of cases globally, with around 3 million patients treated annually in Europe [1]. It serves various clinical purposes: curative (to eradicate disease), neoadjuvant (to reduce tumor size pre-surgery), adjuvant (to eliminate residual disease post-surgery), and palliative (to alleviate symptoms such as pain or bleeding). However, its efficacy is often limited by side effects such as radiation enteritis in which radiation injury compromise mucosal integrity [1]. The intestinal epithelium plays a critical role in maintaining gut homeostasis by acting as a selective barrier against harmful substances. A disrupted barrier allows the passage of microbial components like lipopolysaccharide (LPS) into systemic circulation, triggering inflammatory responses and contributing to disease progression [2, 3]. Some natural compounds are known to enhance tight junction (TJ) integrity, reduce oxidative stress, and downregulate pro-inflammatory cytokine production. Among these, hazelnut film (Hz), an agricultural byproduct typically considered waste, has emerged as a promising candidate due to its bioactive properties [4]. However, the therapeutic potential of Hz in protecting intestinal epithelial cells under radiation injury conditions has not yet been fully explored. This study investigated the protective effects of Hz on gut barrier integrity under conditions of LPS-induced injury and radiation exposure. Human epithelial colon tissues cells (Caco-2) were cultured on Transwell membranes for 21 days to form a differentiated monolayer. Cells were exposed on the apical side to Hz, LPS, or Hz + LPS for 24 hours and then were subjected to radiation therapy (6 MV X-rays, 2 Gy/day for 7 days). Barrier permeability (via FD-4 probe), TJ protein expression by immunofluorescence analysis, and reactive

oxygen species (ROS) production were assessed daily during radiation exposure. Our findings indicate that LPS significantly disrupts mucosal barrier function, while Hz administration – both alone and in combination with LPS – partially rescues the barrier integrity detected by decreased FD-4 probe permeability and enhanced TJ expression. We observed a significant difference between cells treated with LPS and the other groups ($p<0.01$), while Hz treatment was associated with a significant decrease in ROS levels compared to untreated cells ($p<0.001$) since the first day of radiation exposure. In conclusion, Hz demonstrates a protective effect on the intestinal epithelial barrier following LPS injury and radiation exposure. These results support the potential use of Hz as a co- adjuvant therapy to mitigate gut barrier damage in patients undergoing radiotherapy.

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**Keywords:** Tight Junction, Mucosal Barrier, Nutraceutical Product, X Ray injury.

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# Expression of CD34 and α-SMA in obese mice

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Obesity is a major public health challenge that affects almost every country in the world.

The transformation of modern lifestyles and population ageing are among the main causes that have led to a rapid spread of obesity, which seriously threatens human health[1].

Obesity is defined as a chronic metabolic disease, due to excessive fat accumulation in the body, and is closely related to inflammatory diseases [2,3]. White adipose tissue is formed by unilocular adipocytes and several other cell types, such as immune cells, endothelial cells, mesenchymal cells, and Telocytes, which are a type of stromal cell.

Telocytes show a small somatic body and two or several long, slender, moniliform cytoplasmic processes (telopodes); these cells express CD34 [4,5] and are involved in microenvironment homeostasis by cell-to-cell contacts and extracellular shedding vesicles [6].

α-Smooth Muscle Actin (α-SMA) labels myofibroblastic cells, which are regarded as important effector cells of tissue fibrogenesis [7].

In this preliminary study, we characterized telocytes in adipose tissue of obese mice with the following antibodies: CD34 and α-SMA.

Our results showed in control mice (non-obese) numerous CD34-positive telocytes and few α-SMA- positive cells. In contrast, in obese mice, we observed a few CD34-positive cells and numerous cells expressing α-SMA. Furthermore, some stromal cells showed co-expression of CD34 and α-SMA, demonstrating that these same cells can perform different functions in the same tissue depending on their state of health.

Numerous studies have been conducted on telocytes in white adipose tissue related to various pathologies [8]. It would also be interesting to investigate the relationship between telocytes, myofibroblasts, and immune cells in obesity, given its relevance in causing significant health problems such as cardiovascular disease, diabetes, and cancer [9,10].

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**Keywords:** CD34, α-SMA, obesity, inflammation, mice Tipo di presentazione: Presentazione poster.

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# Dissecting Isoform-Specific Functions of AKT in Acute Myeloid Leukemia (AML)

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Acute Myeloid Leukemia (AML) is a hematological malignancy characterized by the uncontrolled proliferation and impaired differentiation of myeloid progenitor cells, accompanied by a plethora of genetic alterations.

AKT is a serine/threonine kinase that plays a key role in intracellular signaling pathways. Although AKT is rarely mutated in AML, it is located at the intersection of various signaling pathways, where different components can be activated by gene mutations. However, there are three distinct AKT isoforms in humans: AKT1, AKT2, and AKT3, each exhibiting a certain degree of tissue-specific distribution (1). Recent studies indicate that each isoform may exhibit different affinities for various partners, thereby specifically affecting one pathway over another (2).

In this study, we focused on AKT1 and AKT2 to investigate their isoform-specific roles, if any, in AML progression. Using the CRISPR-Cas9 technique on the U937 cell line, we selected clones in which either AKT1 or AKT2 were silenced. While silencing of AKT1 cells was complete, we observed only partial silencing of AKT2. To unravel this issue, we compared the colony formation ratio between the two cell clones.

AKT2 knockout (KO) cells displayed a significantly reduced colony-forming efficiency compared to wild-type cells and to AKT1 KO cells. Therefore, we asked which pathways were differentially altered in these samples by means of a proteomic analysis.

Among the numerous pathways altered by either AKT1 or AKT2 KO, we are currently focusing on the RAP1 pathway, which is strongly downregulated in AKT2 KO cells only. Given this pathway's role in actively modulating adhesion, survival, and proliferation of hematopoietic cells (3), all functions that are central to their clonogenic potential, as well as its well-known interplay with AKT2, it is tempting to expect an involvement of RAP1 in AKT2-mediated regulation of cell growth in U937 cells as well as in AML cells in general. Experiments are ongoing to confirm this hypothesis.

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**Keywords:** Acute Myeloid Leukemia (AML), AKT, RAP1
Poster presentation

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# AI-Powered Morphological Screening of the Skin: A Useful Tool in Aesthetic Medicine

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It's crucial that a clinician can assess the health of the skin accurately before and after cosmetic procedures if they want safe and effective results [1]. Conditions like hyperpigmentation, sun damage, dermatitis, and early inflammatory changes can affect not only the suitability and timing of interventions, but also the quality of results [1], [2]. With the demand for customized, minimally invasive treatments growing, it's more important than ever to have smart tools that support accurate, real-time dermatological assessments [3]. Using artificial intelligence, we have developed a desktop application for aesthetic medicine professionals that analyses and classifies common skin conditions based on images. Convolutional neural networks (CNN) were used to process high-resolution images of the skin, enabling automatic detection and categorization of abnormalities with 93% accuracy. The model can distinguish between inflammatory disorders, pigmentary changes, acneiform lesions, and post-treatment reactions using an extensive dataset of dermatological images.

As a pre-assessment tool, the app helps practitioners, namely aesthetic doctors, to identify potential contraindications before starting laser resurfacing, chemical peels, dermal fillers, botulinum toxin injections, microneedling or other aesthetic medicine treatments. The app enhances procedural planning, as well as post-treatment monitoring, detecting things like post-inflammatory hyperpigmentation, localized infections, delayed erythema, and scarring factors that can affect follow-up care. In addition to its diagnostic utility, the software has an intuitive interface that gives you actionable insights in seconds.

Clinical decisions can be made based on evidence at the point of care, and patient communication can be enhanced with visual aids. Incorporating AI-based skin morphological analysis into the aesthetic workflow can reduce diagnostic uncertainty, streamline treatment customization, and improve results. Combining aesthetic medicine with artificial intelligence means more personalized, preventative care. With this application, clinicians can deliver safer, more tailored treatments, elevate the standard of aesthetic practice and support long-term skin health.

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**Keywords:** Skin morphology, Skin Diagnostics, Artificial Intelligence, Deep Learning, Safety, Image Analysis, Precision Medicine, Personalized Medicine.

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# Curcuma caesia Roxb. A possible Blue elixir of youth and wellness

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Aging is a complex biological process influenced by genetic, environmental, and lifestyle factors, including diet and exercise. This process not only leads to visible signs of skin aging, such as wrinkles and elasticity loss but also predisposes individuals to a spectrum of age-related diseases. Oxidative stress and inflammation are key events driving this phenomenon. This study aims to delve into the antioxidative and protective potential of a rhizome extract from *Curcuma caesia* Roxb., a less explored species within the turmeric family, focusing on its effects on HaCaT cells, subjected to UV radiation. *Curcuma Caesia* Roxb. rhizome was extracted for a phytocomplex (CCRE). Through HPLC- ESI-MS/MS, the chemical analysis identified key phenolic compounds, including (-)-epicatechin, procyanidin B2, and p-coumaric acid. HaCaT cells were treated with CCRE in healthy and UVB- induced conditions to assess their impact on oxidative stress and aging. Results demonstrated a significant reduction in mitochondrial superoxide anion levels without affecting mitochondrial membrane potential, indicating enhanced cellular resilience to oxidative stress. Additionally, CCRE decreased UVB-induced IL-6 expression and IKK phosphorylation, which play a crucial role in inflammaging. Notably, CCRE treatment also improved cell viability upon UVB exposure and mitigated UVB-induced cell apoptosis, further underscoring its potential to preserve cellular integrity and function in relation to environmental stressors.

The experiments conducted on HaCaT cells have provided valuable insights into the cellular mechanisms influenced by CCRE. The extract showed a protective effect against UVB-induced apoptosis, a significant reduction in mitochondrial superoxide anion levels, and attenuation of the pro-inflammatory cascade. These findings emphasize the extract's capability to reduce oxidative stress, a key factor in skin aging, while maintaining cell viability under environmental stress. In conclusion, CCRE emerges as a promising tool for anti-aging strategies, with its array of bioactive compounds providing a multifaceted strategy to controlling cellular aging. This study paves the way for further research into the potential applications of *Curcuma caesia* and its compounds in skincare formulations and nutraceutical products, such as functional foods and food supplements,

potentially contributing to healthier, more resilient skin and an overall improved quality of life for aging populations.

**Keywords:** Nutraceuticals, Cosmeceuticals, Aging, Blue Turmeric, Polyphenols, HaCaT cells.

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# Immunohistochemical evaluation of nerves and muscles in two tetraplegic patients undergoing upper limb nerve transfer surgery

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Nerve transfer surgery is increasingly employed in selected tetraplegic patients to improve upper limb function and autonomy [1]. This technique involves the redirection of a functional donor nerve to reinnervate muscles affected by spinal cord injury. While its clinical efficacy is supported by growing evidence, limited data are available on the molecular mechanisms underlying neuromuscular recovery.

The aim of this study is to analyze the immunohistochemical profiles of the donor/recipient nerves and muscles in two patients undergoing upper limb nerve transfer surgery.

Tissue samples were collected intraoperatively, fixed in formalin, embedded in paraffin, and sectioned using a microtome. General morphology was assessed with hematoxylin-eosin staining, and immunohistochemical analysis was performed using markers specific for nerve or muscle tissue: PGP9.5 for nerve fibers, S100 for the myelin sheath, and musclespecific actin for muscle tissue.

The donor nerves and muscles exhibited well-preserved structures; the nerves displayed intact myelin and organized nerve fibers, while the muscles showed positive and diffuse actin staining. In contrast, the recipient nerves generally exhibited disorganized nerve fibers and myelin fragmentation, while the recipient muscles showed signs of denervation and a decreased actin positivity, suggesting ongoing remodeling.

These preliminary findings provide insights into the morphological and molecular changes occurring after nerve transfer and may help guide strategies to support and enhance personalized nerve regeneration.

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**Keywords:** nerve transfer, human patients, immunohistochemical study.

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# An Antioxidant and Neuroprotective effect of Ubiquinol Diacetate in In Vitro Models of Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disease characterized by the degeneration of dopaminergic neurons in the substantia nigra and the presence of misfolded α-synuclein in the brain. Mitochondrial dysfunction and oxidative stress are factors contributing to the death of these neurons. Coenzyme Q10 (CoQ10) serves as an antioxidant and cofactor for mitochondrial enzymes, and its deficiency can exacerbate neurodegenerative processes in PD. However, the clinical efficacy of CoQ10 is limited by its low bioavailability and instability. Ubiquinol diacetate (CoQ10Ac), an esterified form of CoQ10, shows improved pharmacokinetic properties and potential as a prodrug, converting into the reduced antioxidant form of CoQ10 by esterases in the body. This study aimed to investigate the antioxidant and neuroprotective effects of CoQ10Ac compared to CoQ10 in in vitro PD models using dopaminergic SH-SY5Y cells, TagGFP2-α-synuclein SH-SY5Y cells, and the neurotoxin 6-hydroxydopamine (6-OHDA). CoQ10Ac showed higher antioxidant activity than CoQ10 at both extracellular and intracellular levels, particularly in the membrane and cytosolic compartments. It exhibited superior neuroprotection against 6-OHDA toxicity, showing a greater ability to reduce the activation of caspase-3, a key executor of neuronal death, compared to CoQ10. Both coenzymes decreased the increased ratio of DRP1 to OPA1 induced by 6-OHDA in SH-SY5Y cells, enhancing OPA1 levels and promoting antiapoptotic death. However, CoQ10Ac was more effective than CoQ10 in preserving mitochondrial structural integrity and mass. Additionally, both coenzymes significantly inhibited the aggregation of α-synuclein induced by 6-OHDA.

Our study shows that CoQ10 Ac, an esterified form of CoQ10, has superior antioxidant and neuroprotective properties compared to CoQ10 in neuronal SH-SY5Y cells. CoQ10 Ac increased cellular TAA and provided stronger protection against mitochondrial dysfunction and neuronal death from 6-OHDA. It was more effective in maintaining mitochondrial integrity and mass, supporting its potential as both an antioxidant and mitochondrial modulator. CoQ10 Ac is converted more efficiently to the active antioxidant form, CoQ10H2, through esterase activity at neuronal levels. This enhanced conversion may overcome limitations of current CoQ10 supplementation, particularly in individuals with low reducing enzyme activity and neurodegenerative diseases, such as PD. Given its promising profile, CoQ10 Ac is a compelling prodrug candidate for further preclinical and clinical investigation as a therapeutic strategy for PD.

**Keywords:** Antioxidant, Neuroprotection, Ubiquinol Diacetate, Ubiquinone, Parkinson's Disease.

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# Anatomical Variability of Dental Structures in Northwestern Italy: A CBCT- Based preliminary Study

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**Introduction:** Cone Beam Computed Tomography (CBCT, Soredex Cranex 3D) has significantly advanced endodontic diagnostics by providing high-resolution, three-dimensional imaging of dental anatomy[1]. However, concerns regarding cumulative ionizing radiation exposure remain a critical issue in clinical practice. This study investigates the anatomical variability of dental structures in the Northwestern Italian population, with the aim of identifying reliable morphological markers that could guide diagnostic protocols and reduce the need for repeated CBCT scans.

**Materials and Methods:** A retrospective analysis was conducted on CBCT files from 61 patients aged between 10 and 75 years (mean age: 52.58 ± 43.4). The study sample included: 32 patients for incisors and canines (20 females, 12 males), 29 for molars (19 females, 11 males), and 34 for premolars (23 females, 11 males). A total of 398 teeth have been analyzed to date using Radiant Dicom Viewer software. Assessments were performed based on Vertucci's classification[2], considering root number, canal morphology, and anatomical variability, stratified by sex and tooth group[3].

**Results:** Distinct anatomical trends emerged. In incisors and canines, females showed a greater tendency to have two canals (85.71%), predominantly in mandibular teeth (86%), with a 6:1 ratio compared to males. Vertucci type I was the most prevalent in both sexes (91.13% in females; 98.39% in males), but types III (7.26%) and V (1.61%) appeared only in females. Premolars revealed a higher frequency of two-rooted configurations, especially in maxillary premolars. Overall, premolars showed a tendency toward having two roots rather than one. Molars presented significant variation: 97.67% of mandibular molars had two roots, while only 2.23% had three. Conversely, 91.67% of maxillary molars exhibited three roots, and 8.23% had two. Notably, the mesial root in two-rooted molars showed considerable variability in canal configuration, with Vertucci type II being the most common (33%).

**Conclusion:** These preliminary findings highlight recurring anatomical features that may serve as reliable diagnostic indicators in specific populations. Incorporating such data into clinical protocols could limit unnecessary CBCT usage and support a more personalized, safe, and targeted approach to endodontic care.

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**Keywords:** CBCT, dental anatomy, anatomical variability, radiation safety, root canal morphology, Northwestern Italy.

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# The role of mesenchymal stromal cells in regulating cellular senescence in alveolar lung cells: implications in the pathogenesis of idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPS) is one of the most aggressive forms of idiopathic interstitial pneumonia, characterized by chronic and progressive fibrosis, whose causes and molecular mechanisms are still unknown. A growing body of evidence suggests that the progressive accumulation of senescent fibroblasts and alveolar epithelial cells contribute to the pathogenesis of IPF and represent a potentially targetable mechanism. Cellular senescence is a condition in which cells react to diverse forms of age-related damage and stress, and it is characterized by permanent growth arrest, resistance to apoptosis, and acquisition of a senescence-associated secretory phenotype (SASP). Repeated injuries to the alveolar epithelium induce a senescent condition which could impair lung resident mesenchymal stem cell (MSCs) and compromise alveolar repair, predisposing to lung fibrosis. Although the cellular senescence of alveolar cells (ACs) is a critical cause of MSCs failure a clear demonstration of the relation between alveolar cells and lung resident MSCs is missing. Therefore, the aim of the study was to investigate the role of cellular senescence in ACs and the relation with MSCs with the final goal to clarify the role of cellular senescence in regulating the alveolar repair.

A 3D model of alveolospheres was established by culturing A549 lung cells with Geltrex™ and medium supplemented with alveolar factors. 3D alveolospheres were cultured for 3, 6, and 8 days. Cell viability assays and morphological analyses were performed to assess the absence of cell death. The expression of the alveolar markers keratin 8/18 and AQP5 was evaluated to confirm the alveolar phenotype. To simulate cellular senescence, 3D alveolospheres were exposed to doxorubicin for 24 hours. Senescent 3D samples were subsequently co-cultured with MSCs in Transwell systems for 3, 6, and 8 days. Cell viability assays, along with the expression of senescence markers p21^Waf1/Cip1 and p16^INK4, the fibrotic marker collagen type I, and inflammatory markers, were analyzed in senescent 3D alveolospheres co-cultured with MSCs.

Results showed high cell viability in 3D alveolospheres after 3, 6, and 8 days of culture, supported by morphological analysis. The expression of keratin 8/18 and AQP5 in the 3D model clearly demonstrated the alveolar phenotype. Senescent 3D alveolospheres showed high expression levels of senescence and fibrotic markers, which were significantly reduced when senescent 3D samples were co-cultured with MSCs.

These data demonstrate the successful establishment of an in vitro 3D alveolosphere model for investigating alveolar senescence, a condition that may predispose to lung fibrosis. Co-culture of senescent 3D alveolospheres with MSCs significantly reduced the fibrotic phenotype, suggesting a key role for MSCs in supporting alveolar repair and preventing lung fibrosis.

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# Inter-cellular communications between Mesenchymal-Stromal Cells and MDS hematopoietic cells during Luspatercept response

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Mesenchymal stromal cells (MSCs) within the bone marrow microenvironment (BMM) critically regulate hematopoietic stem cell (HSC) behavior and influence disease progression. Myelodysplastic neoplasms (MDS) are hematologic malignancies characterized by bone marrow dysfunction leading to cytopenias and dysplastic cell morphology[1]. Luspatercept, a TGF-β pathway inhibitor, is effective on MDS at lower risk of evolution into Acute Myeloid Leukemia by enhancing erythropoiesis and modulating the BMM. This study examines the relationship between MSC/HSC dynamics and Luspatercept therapeutic response.

MSC-leukemic cell interactions were studied using MSCs from two sources (HS-5 cell line and dental pulp- derived MSCs) in both direct contact and transwell co-culture systems. We performed 24-hour co-cultures with MNCs obtained from 12 MDS samples (before and after Luspatercept treatment) and a healthy subject, while THP-1 leukemic cell line was co-cultured for up to 7 days. The expression of 15 markers, including CD11b and CD14, was analyzed by a flow cytometry approach, while morphological analysis, employing optical and scanning electron microscopy (SEM), was applied to THP-1 cells only.

Among the 12 low-risk MDS patients analyzed, ten cases achieved a favourable response (PR/HI, CR) while two of them remained non-responsive.

UMAP analysis showed a separation of monocytic populations between responders and non-responders, with CD11b/CD14 expression as primary discriminating factors, hinting at a Luspatercept impact on monocytic lineages.

Interestingly, MSCs enhanced monocytic differentiation in THP-1 cells, as well as in MNCs obtained from a representative Luspatercept-responder patient after treatment. Conversely, co-culture with baseline MNCs from the same patient reduced monocytic differentiation compared to single cultures. This suggests that Luspatercept may modulate MSC-myeloid cell crosstalk.

SEM analysis of THP-1 cells revealed distinct morphological phenotypes: single-cultured cells showed rounded morphology with pseudopodia, while direct co-culture cells lost these characteristic features and transwell-cultured cells displayed intermediate morphology with elongated projections. Ongoing immunolabeling and transmission electron microscopy will provide detailed differentiation marker localization and intracellular ultrastructural analysis.

Our findings identify monocytic signatures associated with Luspatercept response and demonstrate MSC- mediated enhancement of monocytic differentiation post-treatment, suggesting that Luspatercept may influence BMM cellular interactions. Future investigations should elucidate the molecular mechanisms underlying MSC-induced differentiation and identify signaling pathways mediating Luspatercept effects on MSC-HSC communication networks.

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**Keywords:** Myelodysplastic neoplasms, Luspatercept, Mesenchymal stromal cells, Flow cytometry, Scanning electron microscopy.

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# Study of the development of new bio-sustainable food packaging technologies to prevent chronic inflammatory conditions in disease

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Chronic inflammatory bowel diseases, including ulcerative colitis and Crohn's disease, are chronic relapsing-remitting disorders of multifactorial aetiology, mainly affecting the gastrointestinal tract. A growing body of experimental evidence shows that constant exposure of the gastrointestinal tract to risk factors generates oxidative stress, that is an overproduction of reactive oxygen species (ROS) and free radicals. These interact with each other and are triggered and maintained by the overproduced ROS within the pro-inflammatory microenvironment. This results in a vicious cycle that amplifies and sustains inflammation, promoting the progression of intestinal diseases and also predisposing to cancer. The goal of our in vitro study is to investigate how the utilize of bioplastic may reduce gastrointestinal pathologies related to microplastic impact, with consequent contingent socio-economic and health costs, and to identify factors that reduce the toxic/tumour effects of food contact packaging. In this research we investigate the effects of different bioplastics composed of plant-extracted microgranules on two cell types, CaCo-2 and HT29, which are human-derived colorectal adenocarcinoma cell culture lines. In this comparative study, we analyse the anti-inflammatory and antioxidant properties of the different types of bioplastics in order to define risk factors in the food/packaging paradigm affecting upstream food industry packaging systems and downstream welfare.

**Keywords:** Crohn's disease, inflammatory bowel diseases, bioplastic, microplastic, CaCo-2, HT29.

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# Evaluating the expression and the subcellular localization of phosphoinositide-specific Phospholipase C enzymes in different human osteosarcoma cell lines

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The involvement of signal transduction in cancer progression is well documented for several types of cancer, including osteosarcoma (OS), and it still remains an active area of research. Among the signaling pathways under investigation, those regulating calcium homeostasis – particularly those mediated by phosphoinositide-specific phospholipase C (PLC) enzymes – deserve growing interest. This enzyme family plays a crucial role in calcium signaling and interacts with various components from other signaling cascades [1]. Notably, differences in the expression levels and subcellular distribution of PLC isoforms have been observed comparing normal with respect to cancerous cells in different tumor types [2-4]. In this study, we examined the subcellular localization of PLC isoforms in four human OS cell lines with distinct origins and malignancy grade (MG63, U2OS, HOS, and 143B). We identified cell line–specific variations, suggesting putative biological significance and implications in tumor progression. A thorough characterization of the cellular models is essential to provide a robust interpretative framework for future experimental investigations. In particular, we focused our attention on the 143B OS cell line, as a representative and valuable model for studying the proliferative and invasive capabilities of human OS *in-vitro* condition.

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**Keywords:** osteosarcoma, cancer, migration, calcium-signaling, phospholipaseC.

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# Glioblastoma-on-Chip: An Innovative Microfluidic System to Investigate the Secretome and Tumor Microenvironment Interplay

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Glioblastoma (GBM) is a highly aggressive and treatment-resistant brain tumor, accounting for 60% of primary brain cancers and associated with poor survival rates. This therapeutic resistance is partly attributed to glioblastoma stem cells (GSCs), which reside in specialized tumor niches characterized by hypoxic microenvironments that promote GSC quiescence [1]. These niches are sustained by the secretion of growth factors and cytokines, collectively altering the tumor secretome. The modified secretome plays a critical role in maintaining cancer cell pluripotency, driving invasiveness, facilitating intra-brain tumor dissemination, and conferring resistance to chemotherapy [2].

In this study, we developed a novel *in vitro* microfluidic dynamic system to investigate the interplay between the GBM secretome and the cancer mass. This *in vitro* microfluidic system replicates the tumor microcirculation and provides a single-organ platform for culturing immortalized GBM cell lines (e.g., U87MG) or patient-derived GBM organoids. The design includes a compartmentalized structure with a porous, permeable membrane separating the GBM culture chamber from a fluid flow compartment, thereby mimicking the circulatory system's role in disseminating the secretome.

Preliminary experiments demonstrated that immortalized GBM cells exposed to deferoxamine, a hypoxia-mimetic agent, exhibited increased levels of vascular endothelial growth factor (VEGF) and interleukin-1β (IL-1β) in their secretome compared to controls. These findings highlight the dynamic changes in secretome composition under hypoxic conditions.

By simulating these microenvironmental dynamics, this microfluidic platform offers a valuable tool for studying GSC behavior during therapeutic interventions. Furthermore, it holds promise for use in preliminary screening of therapeutic agents targeting GBM, potentially accelerating drug discovery and improving treatment strategies.

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**Keywords:** Glioblastoma, microfluidic system, tumor microenvironment.

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# Histological evaluation of peri-implant dehiscence treated with Sub-Periosteal Peri-implant Augmented Layer (SPAL) technique combined with deproteinized bovine bone mineral: a retrospective proof of concept study

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**Background and aim:** Prosthetically-driven implant placement in a reduced horizontal bone width often results in peri-implant bone dehiscence (PIBD), a defect needing for correction to improve the stability and health of peri-implant tissue over time. The Sub- Periosteal Peri-implant Augmented Layer (SPAL) technique, which involves surgical isolation of a periosteal layer through a split-thickness flap, has been employed to address PIBD (1,2). The isolated periosteum acts as a mechanical, osteogenic, and angiogenic membrane (3). SPAL technique was successfully used in combination with a particulate deproteinized bovine bone mineral (pDBBM) or deproteinized bovine mineral block (bDBBM), achieving correction rates of up to 90,9%. However, previous studies focused primarily on clinical and radiographic outcomes. The present study aims to histologically evaluate the newly formed bone tissue, combining SPAL technique with either pDBBM or bDBBM.

**Methods:** Four patients, after providing written informed consent, were treated with SPAL technique in combination with either pDBBM or bDBBM, due to the presence of a PIBD larger than 2 mm at the time of implant placement in the posterior mandible. After six months, a cylindrical bone biopsy, oriented perpendicular to the bone crest, was harvested from an area involved in the augmentation procedure. Samples were immediately fixed in 10% formalin for 10 days, washed in phosphate buffer saline, dehydrated in increasing alcoholic scale, infiltrated and finally embedded in acrylic resin. Resin blocks were sectioned in two parts with a diamond blade, glued on plastic slides, grounded to 80-100 µm thickness, stained with Toluidine Blue and counterstained with Pyronin Yellow. Slides were observed under bright-field light microscopy for histological evaluation. Stereological analysis was performed to quantify the percentage of lamellar bone, woven bone, osteoid, medullary spaced and blood vessels.

**Results:** At 6 months, complete resolution of the PIBD was observed in all patients, with newly formed tissue up to the polished implant collar. Histological analysis revealed well-organized newly formed bone in close contact with the residual graft material in all samples, with the proportion of lamellar bone ranging from 19.29% to 69.67%. Furthermore, samples from patients treated with bDBBM presented a higher percentage of residual biomaterial compared to those treated with pDBBM. Medullary spaces were populated by osteoblast-like cells and blood vessels running adjacent to both the residual graft and newly formed bone. No inflammatory infiltrates were observed.

**Conclusion:** Based on the histological findings, SPAL technique combined with either pDBBM or bDBBM appears to be an effective surgical approach for the correction of PIBD. The results of this study suggest that creating a secluded subperiosteal space, bordered by an intact periosteal layer, can promote the formation of new supracortical bone.

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**Keywords:** peri-implant bone dehiscence, SPAL technique, newly formed bone, regenerative dentistry.

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# 3D facial morphometric analysis in two sets of mosaic twins with Cri-du-Chat syndrome

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Background and aim of the study. Distal 5p deletion syndrome, also known as Cri-du-Chat syndrome (CdCs), is a rare genetic condition with an incidence of approximately 1 in 50,000 live births. Clinical manifestations include a typical cat-like cry at birth, intellectual disability and craniofacial dysmorphisms, with phenotypic variability. In presence of genetic mosaicism (3% of cases), clinical expressivity can vary significantly, even among genetically identical individuals [1]. A comprehensive three-dimensional (3D) facial morphometric investigation on disease-discordant mosaic monozygotic twins can help understand how the phenotype varies in the presence of the same genetic alteration, providing valuable information both in clinical practice and in genetic and morphological research.

Subjects and methods. Two pairs of male disease-discordant monozygotic twins with a cytogenetically confirmed diagnosis of mosaicism for a distal 5p deletion on peripheral blood lymphocytes (twins A, 19 yrs; twins B, 6 yrs) were recruited for the study. From facial 3D stereophotogrammetric images, inter-landmark linear distances and angles were quantified and compared to those of age- and sex-matched healthy controls [2]. Furthermore, geometric morphometric (GMM) analyses of facial shape (allometry, general Procrustes analysis, Principal Component Analysis, Procrustes distance) were applied to the entire facial surface of both CdCs and control subjects to identify the facial regions most affected by the syndrome and to assess the direction and extent of any dysmorphism [3].

Results. *Twins A*: Only the twin with a severe phenotype displayed the typical facial morphometric characteristics associated with CdCs. These included reduced facial widths, decreased lower facial depth (indicative of retrognathia), shortened philtrum length, and increased facial asymmetry. *Twins B*: Both twins exhibited similar mild dysmorphic features, except for a marked cheek retrusion in the twin with the more severe phenotype. No concordance was observed between the percentage of mosaicism and the extent of facial dysmorphism in either of the two twin pairs. Notably, prominent nasal and oral regions were observed in all four CdCs subjects and appear to be independent of mosaicism levels.

Conclusions. The morphometric analysis of two pairs of monozygotic twins with varying degrees of mosaicism did not reveal a concordance between the proportion of affected cells and the extent or distribution of facial dysmorphisms. Despite the small sample size, the findings suggest that other factors, such as tissue-specific mosaicism during craniofacial development and growth and epigenetic mechanisms, may play a crucial role in determining the facial phenotype. Further research is needed to explore the underlying causes of phenotypic discordance in twins with CdCs.

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**Keywords:** face, facial anthropometry, geometric morphometrics, stereophotogrammetry, Cri-du- Chat syndrome (CdCs), mosaicism, clinical anatomy.

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# Erythrogram Indicators as Prognostic Biomarkers in Patients with Coronavirus Disease 2019

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Despite the decrease in acute Coronavirus disease 2019 (COVID-19) cases, the impact of SARS-CoV-2 pandemic remains significant in the fields of respiratory medicine and the management of infectious diseases. COVID-19 has posed unprecedented challenges to global public health, highlighting the importance of prognostic biomarkers in critically ill patients. The oxidative stress in COVID-19 is associated with impairment in various organs and systems, leading to erythrocyte injury which, in turn, promotes the elevation of red cell distribution width (RDW) and systemic inflammation [1-3]. The present study examined the prognostic value of erythrogram indicators in intensive care unit (ICU) patients affected by COVID-19, emphasizing their role in predicting severe clinical outcomes. This study involved 91 ICU patients, categorized into survivor patients and non-survivor patients. The non- survivor patients showed higher RDW and lower values of red blood cell count, hemoglobin, and hematocrit respect survivor group. The RDW increase was also correlated with the rise of C-reactive protein (CRP) levels, another important clinical outcome for these patients. In conclusion, elevated RDW and CRP levels at hospital admission may be reliable prognostic biomarkers of unfavorable outcomes. These preliminary data can contribute to more comprehensive laboratory monitoring during hospitalization.

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**Keywords:** Erythrogram indicators, Inflammation, Oxidative Stress, Red cell distribution.

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# Soluble α-Klotho protects dermal microvascular endothelial cells against endothelial-to-myofibroblast transition

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Endothelial-to-myofibroblast transition (EndMT) is a key contributor to dermal fibrosis. The soluble form of the α-Klotho (sKL) hormone has been shown to counteract fibrotic processes in multiple organs, but its role in dermal fibrosis and EndMT remains unexplored. To investigate whether sKL may inhibit transforming growth factor β1 (TGFβ1)-induced EndMT in human dermal microvascular endothelial cells (H-dMVECs), cells pretreated with recombinant human sKL and subsequently stimulated with recombinant human TGFβ1 were assessed for morphological changes, gene and protein expression of both endothelial and myofibroblast markers, and functional contractility through qPCR, Western blotting, immunofluorescence, and collagen gel contraction assays, respectively. TGFβ1-treated H-dMVECs underwent significant changes in cell morphology, with loss of endothelial markers (i.e., CD31 and VE-cadherin) and a concomitant increase in the expression of myofibroblast markers (i.e., α-SMA, type I collagen, and S100A4/fibroblast-specific protein 1) and of EndMT-associated transcription factors (Snail1, Twist1, and Zeb1). Moreover, TGFβ1-treated H-dMVECs acquired the ability to contract collagen gel matrices. Pretreatment with sKL significantly attenuated all the aforementioned morphological, molecular, and functional changes, preserving the endothelial phenotype and mitigating myofibroblast- like contractile activity. In conclusion, sKL effectively prevented TGFβ1-induced EndMT in H-dMVECs, highlighting its potential as a novel therapeutic agent against dermal fibrosis.

**Keywords:** α-Klotho, dermal fibrosis, microvascular endothelial cells, endothelial-to-myofibroblast transition.

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# A study on the co-detection of a neuromelanin pigment and neurotransmitters in the human dentate nucleus

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Neuromelanin (NM), is a black brown pigment mainly distributed in the neuronal cytoplasm of catecholaminergic neurons of the locus coeruleus (LC) and of the substantia nigra pars compacta (SNpc). Several studies have outlined to NM a neuroprotective role in the SNpc and in the LC as a potent antioxidant. Furthermore, although, NM is a bioactive compound in these brain regions, its functions and properties are not fully known. In addition, NM play a significant role in mitochondrial oxidative dysfunction related to neurodegenerative disorders (e.g. Parkinson's disease, Alzheimer's disease), and to some psychiatric disorders (e.g. schizophrenia). This is due to mainly a dysregulation of NM precursors, and NM accumulation. Furthermore, recent studies have demonstrated the existence of a cerebellar dopaminergic system, however the presence of NM in the cerebellum is essentially denied. Currently, the presence of NM in the human dentate nucleus in a rarely condition known as neuromelanosis has been demonstrated. Therefore, the aim of this study was to evaluate in the neurons of human dentate nucleus the presence of NM and to identify their neurotransmitter phenotype. The study was carried out on fragments of postmortem human dentate nucleus 36-48h after death. Each fragment was fixed in an aldehyde and picric acid solution or in neutral buffered formalin, embedded in paraffin, cut into 5μm sections, and subjected to light microscopy depigmentation histological protocol and to immunohistochemical procedures with polyclonal antisera to serotonin (5-HT), dopamine transporter (DAT), dopamine receptor type 2 ($DRD_2$), neurotensin (NT). Immunoreaction were revealed by streptavidin-biotin technique and 3, 3'-diaminobenzidine (DAB) or DAB-nickel. For positive control of the depigmentation procedure, section of midbrain containing SNpc has been used. Depigmentation protocol proves the presence of NM in large neuron types of the human dentate nucleus. Moreover, Immunohistochemical results demonstrate immunoreactivity for all the antigens in neuronal cell bodies and processes of small and large neuron types; in addition, in a subpopulation of large neuron types the co-presence of NM and of positivity to 5-HT, DAT, $DRD_2$ NT has been also observed. These results demonstrate in the human dentate nucleus the presence of NM in monoaminergic and neuropeptidergic large neuron types. Finally, we suggest a possible involvement of these large neuron types neuromelanin-containing of dentate nucleus in neurodegenerative disorders such as spinocerebellar ataxias and Parkinson's disease.

**Keywords:** Human cerebellum, dentate nucleus, neuromelanin, dopamine, serotonin, neurotensin, immunohistochemistry.

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# Photobiomodulation Wavelength-Dependently Regulates Cellular Activities in Epidermal Regenerative Processes

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Photobiomodulation (PBM) is a non-invasive therapeutic approach that employs light energy to induce photochemical changes in tissues, offering potential benefits for a variety of clinical applications, including wound healing. This study explores the impact of PBM on cellular mechanobiology using an *in vitro* epidermal model composed of HaCaT keratinocytes and human dermal fibroblasts (HDFs) [1].

Key cellular functions relevant to wound repair, namely cell viability, migration, and extracellular matrix (ECM) remodeling, were investigated. Cells were exposed to diode laser irradiation at four wavelengths (530, 625, 780, and 850 nm) and two energy densities (1.5 and 3 J/cm$^2$) [2, 3]. To assess the biological responses, scratch wound assays, MTT assays, viability tests, and collagen quantification were performed, enabling evaluation of wound closure, cellular proliferation, and ECM modulation following irradiation.

The results demonstrated that the effects of PBM are both wavelength-dependent and cell-type specific. In keratinocytes, irradiation at 850 nm and 3 J/cm$^2$ significantly enhanced cell viability and wound closure. In contrast, fibroblasts responded most effectively to 780 nm at 1.5 J/cm$^2$, with notable improvements in wound healing and collagen synthesis. These differences suggest that distinct photobiological pathways are activated depending on the wavelength, likely due to differential interactions with endogenous chromophores.

Overall, the findings underscore the importance of optimizing PBM parameters, particularly wavelength and energy density, to achieve targeted therapeutic effects in specific cell populations. This study contributes to the growing evidence supporting PBM as a promising tool in tissue regeneration and wound healing, and highlights the necessity of precise protocol standardization to maximize clinical efficacy.

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**Keywords:** Mechaobiology, color light radiation, remodeling ECM, collagen type I, keratinocyte cell line (HaCaT), fibroblast cell line (HDF).

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# Hemp Extracts Mitigate LPS-Induced Renal Inflammation: Evidence from a Rat Model

Giorgia Pia Lombardo[1], Anthea Miller[2], Magdalena Kotańska[3], Marek Bednarski[3], Laura Spiccia[1], Valentina Natale[1], Alba Migliorato[4], Eugenia Rita Lauriano[1], Giuseppina Rizzo[5], Simona Pergolizzi[1]

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The general histological structure of the rat kidney is comparable to that of other mammalian species, indeed, rats serve as a valuable animal model for investigating inflammation, toxicity, and renal immunology. The kidney is an immune-active organ, integrating functions of immune surveillance, response, and modulation, especially in contexts of inflammation, infection, or damage. In particular, it can modulate the immune response through the crosstalk between tubular and immune cells and the release of anti-inflammatory factors [1]. It is well established that lipopolysaccharide (LPS), a bacterial cell wall component, activates the TLR-4 signaling pathway, which plays a key role in stimulating macrophages to secrete pro-inflammatory mediators such as IL-6 [2]. Therefore, in this study, TLR-4 and IL-6 expression levels have been used as indicators of renal inflammatory status, allowing comparison between untreated LPS-induced inflammation and the modulatory effects of hemp extract administration**.** Several studies indicate that hemp may exert its biological effects by interacting with diverse intracellular molecular targets associated with antioxidant, anti- inflammatory, and antiapoptotic pathways [3,4,5]. Our results showed a significant difference between control, LPS-infected samples, and extract-treated samples. In LPS-treated samples, a considerable number of antibody-positive immune cells were detected, particularly macrophages. These samples also showed marked inflammatory features, including increased vascularization, immune cell infiltration, and morphological alterations, most notably, the distortion of renal tubules. In contrast, extract-treated samples exhibited a significant reduction in immune cell presence and a restoration of normal renal morphology. These findings suggest that the antioxidant and anti-inflammatory properties of hemp extracts help attenuate tissue damage and support tissue repair. Consequently, hemp extracts may represent a promising alternative to conventional antiseptics, offering antimicrobial efficacy and inflammation control with minimal side effects

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**Keywords:** Hemp extracts, LPS, renal inflammation, IL-6, TLR-4, rat model Tipo di presentazione: Presentazione poster.

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# Osteo-dental assessment of Imperial Roman Skulls from an Urban Necropolis in Milan: a Preliminary Observational Study

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**Background and aim:** Bioarchaeology is the study of human remains within an archaeological context. The evaluation of osteo-dental parameters allows to infer about changes in morphology and how these were influenced by the environmental habits of the time. The present study aimed to assess the osteo-dental conditions and morphology of a sample of skulls from the Roman era.

**Methods:** Skulls from the Imperial Roman era (2nd–5th century AD) were obtained from the osteological collection CAL of the Laboratory of Forensic Anthropology and Odontology (LABANOF)[1]. The remains were recovered from the urban necropolis of the Università Cattolica del Sacro Cuore. To ensure sample homogeneity, only skulls of adult females of similar age, with over 90% dental completeness and anatomically intact teeth, were included. Teeth were examined morphologically, and the disarticulated ones underwent X-ray imaging and root length measurement. The dental formula and the following scoring systems were evaluated: the Oral Hygiene Index (OHI) for calculus assessment, ICDAS and Black's Classification for carious lesions, the Tooth Wear Index (TWI) for dental wear, and bone loss assessment as the distance from the cementoenamel junction (CEJ) to the alveolar bone crest for each tooth.

**Results:** Six female individuals (16-50 years of age) were analyzed. Post-mortem analysis suggested that causes of death may have included ear and thoracic infections, as well as anemia. Dental completeness ranged from 90% to 100%. Third molar agenesis was observed in 4 individuals. The average root length was 11.61-14.07 mm for incisors, approximately 15 mm for canines, 14.22 to 17.00 mm for premolars, and 11.40 to 12.72 mm for molars. No morphological anomalies were observed, except for the presence of two-rooted mandibular canines in one individual (teeth 3.3 and 4.3). Calculus deposits ranged from 0.8 to 3, mostly located below the CEJ, mainly in the V sextant, followed by the I and III. Dental wear (TWI 0.9-3.2) was generalized but more pronounced in the anterior teeth. Carious lesions were primarily Class I and II, with an average of 6 per individual (range 2 to 14). Of these, 58.3% were superficial, 25.1% involved the dentin, and 16.6% extended to the pulp. The average bone loss was approximately 3.2 mm.

**Conclusion:** The dento-skeletal morphology was consistent with contemporary standards. Variability in calculus deposition and caries distribution suggests different levels of oral hygiene in the analyzed sample, while dental wear ranged from moderate to severe, likely reflecting a diet consisting of hard and abrasive foods. Bone loss suggests evidence of periodontal inflammation. Due to the inherent limitations of bioarchaeological interpretation particularly concerning the reconstruction of individual age, lifestyle habits, and contextual factors and the variability observed among the specimens, further investigations supported by larger sample sizes are necessary.

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**Keywords:** bioarcheology, morphology, caries, bone resorption, dental wear, dental calculus.

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# Immunohistochemical evaluation of immune cells in rat lung parenchyma after LPS-induced inflammation

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Systemic inflammation is a complex response that can involve multiple tissues and organs. It is a physiological process triggered by various factors, including infection and tissue injury. To induce inflammation, rats were exposed to lipopolysaccharide (LPS), a component of the cell wall of Gram- negative bacteria.

LPS acts as an activator of the innate immune system, and its interaction with cells such as macrophages and dendritic cells leads to the formation and release of inflammatory mediators essential for innate antibacterial defense.[1]

In this study, we evaluated immune cells in the lung parenchyma following inflammation. To characterize the immune cells, antibodies against major histocompatibility complex II (MHCII) and CD86 were used.[2] Our results showed a significant difference between control and LPS-infected samples. Several immune cells were positive for the tested antibodies in the inflamed lung samples, especially in the alveolar walls, compared to the control samples, where few cells were found to be antibody positive.

In conclusion, this study indicates that bacterial LPS can activate various cell populations of the lung parenchyma immune cells.

**Keywords:** Lung, MHCII, CD86, LPS Tipo di presentazione: Presentazione poster.

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# Low Bifurcation of the Superficial Temporal Artery: A Rare Variant Anatomical and Clinical Insights from a Cadaveric Dissection Introduction

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The superficial temporal artery (STA), a terminal branch of the external carotid artery, is a critical landmark in both surgical and diagnostic procedures. Typically, the STA bifurcates into frontal and parietal branches approximately 3–4 cm superior to the zygomatic arch, with the frontal branch following an oblique upward and forward trajectory. However, anatomical variants of this bifurcation may have significant clinical implications. In this dissection performed on a 78-year-old male cadaver, we observed an early and low bifurcation of the STA's frontal branch, along with precise measurements of the main arterial segments. This study expands our understanding of vascular variants and their practical relevance.

**Materials and Methods:** A detailed dissection of the temporal region was conducted on a 78-year-old male cadaver. The main arterial and venous branches of the region were identified and isolated. Millimeter-scale measuring tools and high- resolution photography were used to document the pathways and bifurcations of the STA branches, with particular attention to the frontal branch morphology and its variants. Measurements were compared with data from the literature (Nakajima et al., 1995; Kim et al., 2011; Lee & Jang, 2014; Nash & Burchiel, 1999).

**Results and Discussion:** Dissection revealed an early bifurcation of the STA, with the frontal branch dividing into two distinct branches at a lower level than usual. The superior frontal branch measured approximately **2.53 cm**, while the inferior/accessory frontal branch measured approximately **1.61 cm**. The common STA trunk before bifurcation measured approximately **2.0 cm**. These branches ran parallel along the superior orbital margin, deviating from the typical oblique trajectory. Such a configuration, reported in the literature with an incidence of up to 20–30%, has important clinical implications:

- **Aesthetic and reconstructive surgery**: This prominent variant may increase the risk of vascular injury during temporal lift procedures or hair transplant surgeries.
- **Regional anaesthesia**: Nerve blocks may be less effective due to the atypical vascular distribution.
- **Imaging and vascular pathology**: The presence of variants may complicate angiographic interpretation and the management of conditions such as temporal arteritis (Horton's disease).

**Conclusions:** The observed variant, characterized by an early and low frontal bifurcation of the STA, underscores the importance of detailed knowledge of vascular variants in the temporal region. Awareness of such anatomical variations is essential for ensuring safety and precision in surgical, anaesthetic, and diagnostic procedures. Future studies, supported by dissections of larger samples, may further quantify the incidence and clinical impact of such variants.

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# Stem cell derived extracellular vesicles ameliorate the neuron mitochondrial damage induced by ROS-LPS-exposure: *in vitro* model of neuron, microglia, and astrocyte triple co-culture

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Brain damage is closely related to both the increase in reactive oxygen species (ROS) and the presence of lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria. Both play a crucial role in neuroinflammatory and neurodegenerative mechanisms. LPS is a powerful immune activator, and it is often used in experimental models to induce acute or chronic neuroinflammation, mimicking conditions such as neurodegeneration associated with systemic inflammation. LPS can trigger ROS through activation of NADPH oxidase. ROS play a central role in brain damage during stroke, especially in the ischemia-reperfusion phase. Oxidative damage can lead to neuronal apoptosis, chronic inflammation, and synaptic dysfunction, contributing to diseases such as Alzheimer's (AD), Parkinson's, and stroke. Significantly increased oxidative stress is observed in the brains of AD patients, where mitochondrial damage is both a cause and a consequence of the accumulation of Aβ and hyperphosphorylated tau. Moreover, ischemic events can occur in patients with AD. In fact, there is a link between vascular pathology and AD, especially in the elderly.

To investigate perturbations in brain cells occurring in mixed dementia (AD plus vascular dementia components), we used a model a triple culture system containing neurons, astrocytes, and microglia, which better recapitulate the human disease pathology. Thus, we induced neuronal injury by 48h LPS (1 μg/μL) and 3h H2O2 (250 μM)-exposure. Cell viability test showed that neuronal death occurred mainly through apoptosis and DNA damage. Moreover, we observed increased expression of NADPH oxidase isoform 2, as source of ROS, as well as the levels of FOXO3 and SOD2, as mitochondrial ROS scavengers. Indeed, neurite thickness decreased, and mitochondria changed in their morphology through a fission process.

The treatment with extracellular vesicles (EVs) derived from amniotic fluid stem cells was tested due to their rich content of antioxidant molecules. Interestingly, EVs reversed the negative effects of LPS/ H2O2 suggesting a protective role on neuronal injury *in vitro* associated with the EV- cargo, such as miR-125b, miR-181b, miR-34a and miR-82b, targeting NOX2, and miR-21, as an antiapoptotic factor.

**Keywords:** neurons, LPS, ROS, mitochondria.

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# Characterizing Biliary Compartment Remodeling in MASLD: A Molecular and Histopathological Analysis

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Metabolic dysfunction-associated Steatotic Liver Disease (MASLD) is characterized by accumulation of lipids into the hepatocyte [1]. In our previous study, we identified dysregulated lysosomal acid lipase activity in lipid metabolism, correlating with histomorphological features of MASLD and impaired autophagy [2, 3]. We also observed a correlation between ductular reaction (DR) and autophagy. Furthermore, autophagy impairment was associated with altered expression of liver X receptor alpha (LXRα) and farnesoid X receptor (FXR) along with their downstream targets involved in bile efflux, including ATP-binding cassette subfamily G members 5 and 8 (ABCG5/8), which are directly regulated by LXRα and FXR [4]. These transporters were associated with increased liver injury in MASLD patients. This study aimed to further investigate cholestasis-related alterations in the biliary compartment in MASLD using both histological and molecular approaches. Liver biopsies and clinical data from 48 MASLD patients were collected. Based on serological R ratio profiles (R=[ALT/ALT_ULN] / [ALP/ALP_ULN]), patients were classified into cholestatic, mixed, or hepatocellular patterns. Morphological analysis of biliary markers was performed on FFPE liver tissues. In parallel, a 58-gene panel was analyzed using NanoString technology, and the results were correlated with clinical data. Key histomorphological features, such as steatosis, fibrosis, DR, and inflammation, were evaluated in relation to structural changes in the biliary compartment. Our data revealed distinct patterns of biliary compartment remodeling across the hepatic lobule, significantly associated with steatosis ($p<0.05$), fibrosis ($p<0.05$), and DR ($p<0.05$). A trend toward statistical significance was also observed in association with increasing NAS score. Gene expression analyses supported the classification of patients into cholestatic, mixed, or hepatocellular patterns, also allowing a more refined stratification, further dividing the mixed group into predominantly cholestatic or hepatocellular subtypes. This molecular classification revealed distinct patterns of pathway dysregulation between the two groups. Our findings highlight a compelling relationship between biliary compartment remodeling and MASLD features such as steatosis, necro-inflammation, DR and fibrosis, alongside clinical serum profiles. Moreover, gene panel analysis provided clearer differentiation between cholestatic and hepatocellular patterns than serum biomarkers alone. Taken together, these findings may facilitate the earlier identification of MASLD patients with a cholestatic phenotype, a subgroup known to be associated with poorer clinical outcomes.

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**Keywords:** MASLD, Cholestasis disease, Biliary Compartment.

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# Wool waste-derived keratin patches infused with grape pomace extracts differently impact adhesion molecules and cell functions in human cancer and normal epithelial cell lines: finding biomaterial for possible medical applications

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Keratin is a global class of biological material representing a group of cysteine-rich filament-forming proteins [1]. Keratin materials have been extensively used in tissue engineering and regenerative medicine owing to their biological function, structural support, excellent biocompatibility, and favorable biodegradability characteristics. Here, the high molecular weight keratin has been spun for the first time by solution blow spinning [2]. Furthermore, patches were employed singularly or loaded with a freeze-dried powder of grape pomace extracts (GPE) in an infused format. GPE represents an essential portion of winery by-products with well-documented health benefits [3]. Experiments were conducted with human HaCaT healthy keratinocytes and HT-29 colon adenocarcinoma cells. Keratin samples were washed with cell medium (RPMI 1640 or high glucose DMEM) using the following procedure: the patch (about 5 mm length vs width) was placed in a volume of 1 mL of RPMI or DMEM for about 90 min, then mild centrifugation (1500 rpm × 10 min) was performed to discard the supernatant and collect the keratin samples. The patches, soaked and with a gelatinous appearance, were recovered and added to HT-29 and HaCaT cells. Results highlight a different behaviour for cancer and normal epithelial cells: in fact, high doses (100 μg/ml) of GPE did not improve wound healing in HaCaT cells, but the combination of patch and GPE induced a mild increase in monolayer repair. Indeed, the adhesion molecule CD54 (ICAM-1) reveals a significant peak after 24 h, remaining statistically relevant after 48 h. Intriguingly, CD54 on HT-29 cells mildly increases only after 24 h, restoring the lower level of control samples after 48 h. Notably, GPE demonstrates to mitigate the mitochondrial membrane hyperpolarization induced by keratin patches after 24 h in normal HaCaT cells, whereas after 48 h, the mitochondrial membrane potential (MMP) does not appear perturbed by any treatments. In the HT-29 epithelial cancer cell line, keratin patches do not significantly increase MMP. However, GPE and GPE/keratin patches decrease basal MMP as compared to control samples. Finally, ROS behaviour appears to be a valid target of this newly assembled biomaterial: in fact, HaCaT cells do not highlight, during time, a significant peak in percentages of mitoSOX-positive cells, a probe mainly detecting superoxide anion, which is generally considered more reactive and potentially more dangerous than hydrogen peroxide within mitochondria. On the contrary, in HT-29 cells, percentages of mitoSOX-positive cells appear higher in patch- and GPE/keratin patch-treated cells, particularly after 24 h. In conclusion, grape pomace-infused wool keratin patches differently impact cell adhesion and function in cancer and normal epithelial cells, prompting the consideration of this newly combined biomaterial for possible medical applications.

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**Keywords:** Wool waste-derived keratin patches, grape pomace extracts, normal and cancer epithelial cells, biomaterial for possible medical applications.

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# Effects of a 5-day fasting-mimicking diet on body composition, performance, and hormonal responses in trained males

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The fasting-mimicking diet (FMD), developed and published by Dr. Valter Longo in 2015 [1], is a plant-based, low-calorie nutritional protocol designed to replicate fasting's physiological effects while providing essential nutrients. Initially studied for anti-aging and regenerative benefits, FMD has demonstrated potential in improving metabolic health, reducing inflammation, and promoting cellular renewal [1, 2]. However, its effects on physically active populations remain underexplored, particularly regarding performance and hormonal responses. This study evaluated a 5-day FMD (1,100 kcal on Day 1; 800 kcal on Days 2–5) in ten trained males, assessing body composition, neuromuscular performance, and salivary hormones. Pre- and post-intervention measurements included bioelectrical impedance analysis (BIA), skinfold thickness, and anthropometrics. Performance was tested via flexibility (sit-and-reach), maximal strength (push-ups, pull-ups, squats), and explosive power (broad jump, seated medicine ball throw). Salivary cortisol and testosterone were analyzed to assess stress and anabolic states. Results revealed significant reductions in body weight (–3.7%, $p<0.0001$), fat mass (–17.2%, $p=0.003$), and waist circumference (–3.4%, $p=0.014$), while lean mass and cellular integrity (phase angle, % body cell mass) were preserved. Performance metrics remained unchanged ($p>0.05$), indicating no strength or power deficits. Extracellular water decreased slightly (–2.7%, $p = 0.058$), suggesting a trend toward reduced inflammation. Salivary cortisol and testosterone levels did not indicate a stress-related endocrine response, potentially reflecting preserved anabolic capacity and consistent with the observed fat loss. These findings align with the hypothesis that FMD enhances metabolic efficiency without impairing muscle function [3]. In conclusion, short-term FMD appears safe for trained individuals, promoting favourable body composition changes while preserving neuromuscular performance. These results support FMD as a potential tool in sports nutrition, though long- term applications require further study.

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**Keywords:** Fasting-mimicking diet (FMD), body composition, performance, bioelectrical impedance (BIA), metabolic health, trained subjects.

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# Antonio Pacchioni: an illustrious italian physician and anatomist

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Antonio Pacchioni was an italian physician and anatomist who identified the nodular formations involved in the reabsorption of CSF. He was born on June 24th, 1664 in Reggio Emilia, Italy, and died on November 5th, 1726 in Rome, Italy. The physician Antonio Pacchioni (1664–1726) devoted many years to the study of anatomy. Three centuries after his death, his contributions remain essential to the field of neuroanatomy and neurology. His pioneering studies on the structure of the meninges and the identification of nodular formations involved in the reabsorption of CSF, now eponymous in his honour (the Pacchionian granulations). Pacchioni's pioneering research substantially advanced the understanding of the dura mater, the outermost membrane enveloping the brain and spinal cord. His meticulous studies led to the first detailed descriptions of structures now known as arachnoid granulations or Pacchionian granulations, which play a crucial role in CSF dynamics. Another fundamental neuroanatomical discovery of Pacchioni is the Pacchionian foramen (incisura tentorii or tentorial notch) which allows the communication between two closed spaces: the larger anterior space includes the anterior and middle cranial fossas and lodges the cerebrum; the small posterior space, the posterior cranial fossa, contains the cerebellum, the pons, and the medulla.

Pacchioni was the son of Giambattista, a modest craftsman, and Leonora Dugoni. He received his early education in the humanities which, in late 17th-century Italy, was a common route for those who wanted to become physicians, because classical studies were seen essential to the development of moral character and intellectual rigor. He completed his medical studies on April 25th, 1688, at the Studio Pubblico of Reggio Emilia, where he obtained the degree in medicine and philosophy. The famous anatomist Antonio Vallisneri taught in the Studio Pubblico. In 1689, Pacchioni moved to Rome to improve his medical qualifications. There, under the guidance of Antonio Vallisneri, he collaborated before at the hospital of Santo Spirito in Sassia. Afterwards, he worked at the Ospedale di San Giovanni in Laterano and at the Ospedale della Consolazione, where he gained extensive clinical experience. In the late 1690s, he collaborated extensively with Marcello Malpighi (1628– 1694), another leading anatomist, who profoundly influenced his scientific pursuits. Being close to Malpighi enabled him to refine his anatomical techniques, which he applied both to human and animal samples, with the aid of a basic microscope. Pacchioni's primary research focus was the structure and function of the dura mater. Employing innovative techniques, such as the maceration of anatomical specimens in various fluids, like vinegar and alcohol, he meticulously studied this layer of the meninges. By softening the tissue, this procedure made it possible to properly separate and examine its fiber layers and related features. To improve this technique, he used microscopy, influenced by Malpighi's scientific processes. In 1705, Pacchioni published his seminal work, "Dissertatio epistolaris de glandulis conglobatis durae meningis humanae," wherein he provided the first comprehensive description of small protrusions of the arachnoid membrane penetrating the dura mater. These structures, later termed Pacchionian bodies or granulations, are vital for the reabsorption of CSF into the venous system. Pacchioni hypothesized that the dura mater possessed muscular properties similar to those of the heart, and postulated that the dura mater plays an active role in brain function. He thought that lymph could be secreted from the "glandulae" of the dura mater to lubricate

the sliding movement between the meninges and the brain during contractions. Although this hypothesis was later contested, notably by Giorgio Baglivi (1668–1707), it underscores Pacchioni's commitment to integrating knowledge of an anatomical structure with its physiological function. Another seminal discovery was the description of the tentorial notch (also known as "Pacchioni's oval foramen"), an anatomical opening that allows the passage of cerebral peduncles and arteries between supratentorial and infratentorial compartments. In Rome Pacchioni collaborated with other prominent scientists, including Giovanni Maria Lancisi (1654-1720), who was pontifical archiatry of two popes (Innocenzo XI and Clemente XI), and founder of the Lancisi Academy of Rome. Lancisi helped Pacchioni to access clinical and anatomical resources, especially at the Ospedale di Santo Spirito, one of Rome's top teaching hospitals at that time, and encouraged his anatomical studies. Lancisi's backing had a crucial impact on Pacchioni's capacity to disseminate his "Dissertationes anatomicae de durae meningis fabricâ et usu" (1705), a publication that was printed with papal authority and dedicated to Cardinal Girolamo Casanate. On november 5th, 1705 Pacchioni was appointed head physician at the Ospedale di San Giovanni in Laterano, a position that allowed him to continue his clinical practice alongside his research endeavors until his death, on 1726. Pacchioni's meticulous studies laid the groundwork for future research into the meninges and CSF dynamics, leaving behind a legacy of scientific inquiry that continues to influence modern neuroscience and neuroanatomy.

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**Keywords:** Antonio Pacchioni, Pacchionian granulations, Pacchionian foramen, Incisura tentoria.

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# Bone regeneration triggered by new bioactive glass scaffolds in a rabbit model

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When bone defects develop, in many cases the body's natural healing ability is neither sufficient nor complete. Surgical interventions involving grafts or synthetic biomaterials are therefore necessary to restore the defect. Among the biomaterials used as bone substitutes, bioactive glasses (BGs) hold a prominent position due to their ability to enhance osteointegration. BGs are also used in tissue engineering to construct three-dimensional scaffolds, while providing not only temporary mechanical support but also the release of bioactive substances that promote *in situ* tissue regeneration. Two new BGs (Bio_MS and BGMS10) with different ionic concentrations were recently studied1 in the form of granules and demonstrated good biocompatibility and osteoconductivity. The purpose of this study is to evaluate in adult rabbits the regenerative and osteoconductive potential of these new BGs in the form of scaffolds, compared with the 45S5 gold standard, in two different bone architectures (trabecular bone at the femoral proximal epiphysis and compact bone at the tibial proximal diaphysis). BG scaffolds were implanted into cylindrical defects drilled bilaterally in the hindlimb of animals. After 60 days from surgery bone specimens containing the scaffold were retrieved and processed for histomorphometric and histological analyses. The histomorphometric results concerning the Affinity Index (AI) – *viz,* the surface of the scaffold in contact with newly formed bone - showed that it is always higher in the Bio_MS group with respect to the 45S5 and BGMS10 ones, regardless of bone architecture. On the other hand, the BGMS10 group always showed the lowest AI values. The histological results showed that the surface of all BG scaffolds appears surrounded partly by osteoclasts, connective tissue and newly formed bone characterized by the presence of large lacunae containing osteocytes. It is possible to observe numerous osteoblast laminae lining the newly formed bone mostly in the Bio_MS samples compared to the other BG scaffolds; the abundance of osteoblastic laminae could be correlated in Bio_MS specimens to the higher AI, and consequently to the higher osteoconductivity. On the contrary, BGMS10 scaffold surfaces were lined with many osteoclasts and their presence might prevent the formation of bone directly in contact with the BGs; this fact could explain the low AI values recorded in this group. Moreover, the abundance of osteoclasts could be correlated to the higher release of Mg from BGMS10 BGs, whose ion concentration is double with respect to Bio_MS ones. Interestingly, several others studies2,3 indicate that the increase in Mg ionic concentration enhances osteoclast differentiation and reduces osteoblast differentiation. In conclusion, these our preliminary results suggest that Bio_MS scaffolds seem to display a higher regenerative and osteoconductive potential compared to other BGs.

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**Keywords:** bioactive glasses, scaffolds, Bio_MS, BGMS10, bone defect, bone regeneration, osteoconduction.

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# More CAMs - One Goal: Building a Living Platform For Bone Regeneration

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The chorioallantoic membrane (CAM) represents a flexible and cost-effective *in vivo* model widely used for biomedical research due to its biocompatibility, accessibility, and ability to support various experimental conditions [1]. It enables the evaluation of biological processes such as angiogenesis, tumor invasiveness, and biocompatibility of materials. Notably, experiments completed before embryonic day 17 (ED17) do not require ethical approval from animal experimentation committees, making it a convenient platform for short-term studies. However, its use for longer protocols, such as those needed in preclinical testing of regenerative strategies, remains limited due to this time restriction. This study aims to develop a prolonged organotypic culture system using the CAM model, intended as a preclinical tool to evaluate bone regeneration strategies in cases of critically sized bone defects. The proposed model involves the preparation of femur explants from chick embryos, which are then cultured, in temporal succession, on more host CAMs. On embryonic day 15 (ED15), femurs are harvested from donor embryos. Following removal of the condyle and femoral head, bone marrow is extracted, and a controlled critical lesion is induced at the mid-diaphyseal region. These bone samples are then incubated on CAMs for up to six days to assess viability and suitability for regenerative studies.

The study successfully established a method to maintain organotypic femur cultures *in ovo* for extended periods. A first key aspect of the setup was determining the optimal embryonic stage at which to perform the femoral harvest, that is at which femur diaphysis is sufficiently ossified to allow for standardized bone lesion creation. Throughout the incubation period, bone sample viability was evaluated using the Alamar Blue assay, which measures cellular metabolic activity, and supported by histological analysis to verify bone tissue integrity. The other crucial step is to define the times and methods of transfer of the organotypic cultures from a host CAM to one or more CAMs in succession, as suggested by other authors [2], to extend the experimentation time without derogating the ethical limits of use of the CAM model (phase still being developed).

At the moment, this preliminary work demonstrates the feasibility of creating a reproducible organotypic culture model with critically sized bone defects, to host on more CAMs in succession. The model holds promise for assessing bone repair strategies in a preclinical setting, potentially serving as an alternative to early-phase animal testing. Future work will focus on refining and standardizing the preparation of host CAMs to extend the experimental window beyond the current limitations.

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**Keywords:** CAM model, organotypic culture, critical-size bone defects, bone regeneration, preclinical setting.

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# Evaluation of a gel based on Hyaluronic acid and Spermidine for oral regenerative purposes: an *in vitro* and *ex vivo* study

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**Background and aim:** Oral mucosal barrier restoration following surgery, trauma, or inflammatory lesions is essential to prevent local bacterial superinfections, pain, patient discomfort, and potential systemic complications. In conditions such as diabetes and immunosuppression, where wound healing is impaired and tissue regeneration delayed, there is a critical need for topical agents that can effectively promote oral repair. Among bioactive agents with regenerative effects, hyaluronic acid (HA) is widely used for its viscoelastic, anti-inflammatory, and hydrating properties (1), while spermidine (SP), a natural polyamine, has gained attention for its capacity to modulate inflammation, oxidative stress, and cellular proliferation (2). The combination of HA and SP has been proven to be effective in vivo in a peri-implant model (3), however its use has never been employed on oral wound healing. This study aimed to investigate the biological effects of a gel formulation combining HA and SP, focusing on both connective and epithelial compartments of gingival tissue, to further explore its regenerative potential.

**Methods:** A dual experimental strategy was adopted: (i) *in vitro*, Human Gingival Primary Fibroblasts (HGF) were tested with HA 0.2% and SP 0.1% (HA-SP1) or left untreated (CT) for 48h. Slot blot analysis was performed to assess collagen type I and III (COL-I, COL-III), matrix metalloproteinase (MMP-1) protein and tissue inhibitor of MMP-1 (TIMP-1) in both groups (4). Cell migration was evaluated on both groups using a wound healing assay.

*(ii) Ex vivo* analyses on organotypic cultures (OC) were performed. OCs were treated with HA 0.2% alone or in combination with SP at the concentrations 0.1% and 1% (HA-SP2). OCs were collected at 24h, 48h and 72h after treatment and stained with Hematoxylin and Eosin and immuno-stained for Ki-67. A semi-quantitative analysis of Ki-67 expression was carried out to assess cell proliferation.

**Results:** *In vitro*: HA-SP1 did not influence significantly HGF concerning COL-I or COL-III protein levels but significantly affected COL-III/ COL-I ratio. MMP-1 and TIMP-1 levels were not significantly altered. Notably, HGF migration was significantly enhanced in the treated group. *Ex vivo*: samples treated with both HA-SP1 and HA-SP2 presented preserved epithelial architecture, especially in the HA-SP1 group. Treated OCs maintained intact epithelial layers up to 48 hours, in respect to untreated control and HA 0.2% formulation. Ki-67 staining revealed stable proliferation in all treated samples at 48h, with an increase at 72h in HA-SP2 group.

**Conclusion:** The combination of HA and SP promotes HGF migration and modulates extracellular matrix remodelling while preserving epithelial integrity and proliferation in OC. Its dual effect on connective and epithelial tissue suggests promising clinical potential for oral wound healing applications, particularly in scenarios where enhanced soft tissue regeneration is required.

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**Keywords:** Hyaluronic acid, Spermidine, Oral wound healing, Gingival fibroblasts, Organotypic cul-tures, ECM remodeling.

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# The Role of GATA3 and TGF-β in Trophoblast Development: Molecular Interactions in Normal Pregnancy versus Preeclampsia

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GATA3 is a key transcription factor involved in the development and function of various tissues, with robust expression in both hematopoietic and non-hematopoietic compartments, particularly during embryogenesis and placental development [1]. Similarly, transforming growth factor-beta (TGF-β) signaling is essential for proper placental formation and has been shown to influence GATA3 expression [2]. Understanding the interplay between these two factors is critical for elucidating the mechanisms underlying both normal and pathological developmental processes.

In this study, the roles of GATA3 and TGF-β in trophoblast development were investigated using immunohistochemistry to assess their expression and localization in term placentas from both normal pregnancies and those complicated by preeclampsia. The findings revealed increased expression of both GATA3 and TGF-β in preeclamptic placentas, with distinct localization patterns in the villous epithelium, villous stroma, and decidua.

These observations suggest that GATA3 and TGF-β contribute to the defective trophoblast development characteristic of preeclampsia, a condition marked by dysregulation of trophoblast proliferation, differentiation, and invasion [3]. It has been demonstrated that GATA3 can inhibit GCM1 activity by binding to its regulatory regions. Moreover, GATA3 deficiency has been associated with increased expression of HtrA4, a GCM1 target gene encoding a serine protease involved in trophoblast invasion. This highlights GATA3's regulatory role in modulating trophoblast invasiveness, a function that parallels that of TGF-β [4].

A logical direction for future research would be to investigate the molecular crosstalk between TGF-β, GATA3, GCM1, and downstream effectors such as HtrA4. This line of inquiry is particularly compelling in the context of the well-characterized TGF-β–HtrA1 axis, known to play roles in the development of multiple cell types [5].

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**Keywords:** GATA 3, TGF-β, placenta, pre-eclampsia, trophoblast development.

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# Detection of neurodegenerative and neuroinflammatory biosignatures characterizing Mild Cognitive Impairment condition

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Alzheimer's disease (AD) is a neurodegenerative disorder leading to cognitive decline and memory loss, often preceded by Mild Cognitive Impairment (MCI). MCI represents a transitional state between normal aging and dementia, and not all MCI individuals progress to AD.

The aim of this study was to identify peripheral biomarkers to generate a biological profile useful both to characterise the MCI condition and to uncover potential targets for the development of effective strategies to counteract, limit, slow down or prevent this syndrome. We enrolled a total of 45 individuals subdivided into three groups: MCI patients (N=26, MCI), aged 65 years or older; elderly healthy subjects (N=19, OH), aged 65 years or older; young healthy subjects (N=10, YH), aged between 18 and 35 years.

The comparison of the molecular signatures obtained from the MCI group with the ones from elderly and young healthy subjects resulted in: i) significantly modified plasmatic levels of NGF and proNGF (ELISA assays); ii) significant deregulation of expression levels 152 genes involved to 13 different pathways related to preclinical pathological condition, (functional transcriptomic and bioinformatic approaches); iii) correlations between certain deregulated genes and cognitive performance, assessed using the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA).

In conclusion, our study highlights the complex alteration of the molecular pattern of MCI in the context of ageing. The combination of peripheral protein and gene determinants, occurring with this condition, will be useful to generate neurodegenerative and neuroinflammatory biosignatures and will uncover novel potential therapeutic targets for the improvement of management of MCI patients.

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**Keywords:** Alzheimer's disease, Mild Cognitive Impairment, biomarkers, NGF, proNGF, gene expression, cytokines.

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# Exploring the regenerative effects of mesenchymal stromal cell-derived extracellular vesicles on myofibers

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Traumatic injuries can severely impair skeletal muscles, resulting in limited regeneration, muscle loss, fibrosis, and compromised function. Despite various therapeutic strategies, including cell transplantation, effective treatments remain elusive. Mesenchymal stem cells (MSCs) have emerged as a promising tool for muscle repair, primarily due to the paracrine and immunomodulatory effects mediated by their extracellular vesicles (EVs). While the cellular and molecular mechanisms of muscle regeneration are well-characterized, the specific role of EVs in mediating intercellular communication during myofiber repair is still under investigation. In particular, the influence of MSC-derived EVs on myogenic regeneration remains largely unexplored. This study aims to elucidate the role of MSC-derived EVs in promoting muscle repair and regeneration.

EVs were isolated from murine myoblasts using a polyethylene glycol-based liquid exchange precipitation protocol. EVs obtained from control myoblasts, differentiated myoblasts, and MSCs were analyzed for size, surface markers, and myokine content via electron microscopy, western blotting, and ProQuantum immunoassays. These EVs were then applied to damaged differentiated myoblast cultures to assess their reparative effects. Myotube formation was monitored using inverted light microscopy, and expression of myogenic markers (MyoD and myogenin) was evaluated via western blotting.

Results demonstrated the presence of Evs in myoblasts and MSCs. The expression of EV markers was related to the different conditions used to simulate a muscle damage. Injured myoblasts release inflammatory EVs, while MSC-derived EVs mitigate this inflammatory milieu, enhance myogenic repair, and facilitate muscle regeneration.

In conclusion, our findings underscore the pivotal role of MSC-derived extracellular vesicles in modulating myogenic differentiation and supporting skeletal muscle regeneration following injury.

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# Human Fibroblast Migration Across the Wound Bed is Enhanced by the Novel Nucleopeptide NP3

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There is a significant unmet clinical need for therapies that promote the healing of chronic wounds, accelerate the repair of acute wounds, and reduce scar formation [1]. To address this challenge, we developed a small molecule capable of non-covalent polymerization to form a supramolecular network that may enhance the wound healing process. Specifically, we synthesized a novel nucleopeptide, designated *NP3*, using Fmoc-based solid-phase peptide synthesis combined with nucleoside chemistry. The compound was characterized by mass spectrometry and circular dichroism (CD) spectroscopy. Based on its chemical structure, we hypothesize that the aromatic rings in *NP3* enable π–π stacking interactions, promoting non-covalent polymerization and network formation. To evaluate the biological effects of *NP3*, in vitro studies were conducted using IMR90 human lung fibroblasts. Cytotoxicity was assessed via the MTT assay, which confirmed that *NP3* is non-toxic at the tested concentrations. Immunofluorescence microscopy was used to analyze changes in cytoskeletal morphology and the distribution of late endosomes/lysosomes within the cytoplasm, with no signs of cytotoxicity observed. A wound healing (scratch) assay was subsequently performed. IMR90 cell monolayers were scratched and treated with 0.5 or 1 μM *NP3*, and cell migration into the wound area was monitored at 24, 48, and 72 hours. Preliminary results indicate that *NP3* enhances fibroblast migration compared to untreated controls, suggesting its potential to accelerate wound closure.

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**Keywords:** Wound healing, nucleopeptide synthesis, cytoskeletal morphology, lysosome distribution.

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# Exploring a multidimensional perspective on intestinal permeability in inflammatory bowel disease: the interplay of microbiota, nutrition, morphological and molecular biomarkers

Laura Restaneo[1,2,3], Lorenzo Nevi[4], Giovanni De Carlo[1], Simone Filardo[5], Chiara Ceriani[2,3,6], Martina Nespolì[2], Sofia Remedia[2,3,6], Alessia Lo Dico[2,3], Rosa Sessa[5], Michele Cicala[7,8], Michele Pier Luca Guarino[7,8], Gloria Bertoli[2,3], Annamaria Altomare[1,7,8], Simone Carotti[4,9]

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Emerging evidence highlights the role of environmental factors[1], collectively referred to as the exposome, including diet, lifestyle, and pollutant exposure, in shaping the intestinal microbiota[2] and affecting gut barrier function[3,4]. Disruption of this barrier, characterized by increased intestinal permeability, is implicated in the pathogenesis and symptoms of chronic gastrointestinal diseases, including Inflammatory Bowel Disease (IBD)[5]. This study investigated the relationship between lifestyle habits and colonic microbiota composition in patients with IBD versus healthy controls (HCs), with a focus on implications for intestinal permeability. A total of 38 IBD patients and 48 HCs were enrolled. Fecal microbiota composition was assessed via 16S rDNA V3/V4 region sequencing (Illumina). Mucosal barrier integrity was evaluated by IHC. Dietary habits and physical activity were evaluated using a 7-day food diary, a Food Frequency Questionnaire, and the IPAQ. Fecal zonulin levels were assessed using ELISA, and selected circulating microRNAs (miRNAs) were analyzed in plasma and serum by TaqMan-based quantitative Real-Time PCR. IBD patients had significantly reduced dietary fiber intake compared to HCs ($p<0.05$), correlating with decreased microbial alpha diversity ($p<0.01$), particularly in phylogenetic diversity and number of observed features ($p=0.007$). Beta diversity analysis (weighted/unweighted UniFrac) showed distinct clustering between groups ($p=0.007$). LEfSe analysis revealed higher numbers of differentially abundant taxa in HCs, reflecting greater microbial richness. Notably, no overtly pathogenic taxa were enriched in IBD samples, suggesting dysbiosis was driven by loss of beneficial microbes rather than pathogenic overgrowth. Microbial diversity was also significantly higher in rural versus urban residents ($p=0.03$), highlighting the influence of environmental context. Fecal zonulin levels were markedly elevated in IBD patients with low adherence to the Mediterranean Diet compared to adherent individuals ($p<0.001$), indicating a potential link between dietary patterns and gut barrier dysfunction. Additionally, inflammation- related circulating miRNAs were differentially expressed in IBD. MiR-24, involved in epithelial apoptosis and renewal, was downregulated ($p=0.01$), suggesting impaired regenerative capacity. MiR-923, associated with tight junction regulation and paracellular permeability, was also reduced ($p<0.05$), supporting a role in barrier compromise. This study highlights a significant association between environmental factors and gut microbiota alterations in IBD. Reduced microbial diversity, elevated fecal zonulin in patients with low adherence to the Mediterranean Diet, and differential expression of circulating microRNAs suggest a link between the exposome and intestinal barrier dysfunction. These findings support the potential of microbial and molecular markers to refine disease characterization and inform personalized approaches.

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**Keywords:** Exposome, Gut microbiota, Inflammatory Bowel Disease (IBD), Intestinal permeability, Biomarkers.

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# Morphological Characterization and Origin of Vernix Caseosa

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In full-term newborns the epidermis is thicker and transepidermal water loss is lower than in adults, so the skin plays an important protective role [1]. In the first two days after birth, the epidermal stratum corneum is still adapting to extrauterine life and appears to be involved in the absorption of skincare products and topical medications [1,2]. Vernix caseosa is a physiological white barrier found on the skin of newborns that gradually covers the skin in a cephalocaudal manner during the final trimester of pregnancy [3]. Maternal and placental hormones control vernix caseosa formation and coverage [4]. Vernix caseosa is uniquely human and consists of waxes, sterol esters, squalene, cholesterol, triglycerides, and free sterols, as well as cellular elements [5]. To our knowledge, the cellular elements present in vernix caseosa have not been fully elucidated. Visscher et al. [4] suggested that the cells in vernix caseosa may originate from the hair follicles, which has a recognised local hypothalamic–pituitary–adrenal axis. Therefore, this study aimed to morphologically characterize the vernix caseosa, with a primary focus on its formation and the role of the hair infundibulum in this process. Vernix caseosa was gently scraped from the skin of 9 newborn infants and appropriately processed for morphological and ultrastructural analyses. Morphological evaluations revealed the presence of highly hydrated corneocytes embedded in lipids that were larger than those from the intact stratum corneum, suggesting they may be swollen with imbibed amniotic fluid. Transmission electron microscopy revealed flattened structures indicative of differentiated corneocytes, devoid of nuclei and intercellular lipids. Furthermore, the ultrastructural analyses indicated that vernix caseosa originates from the hair infundibulum. Although vernix caseosa is usually removed from the skin surface at birth, but it is not a "waste product" of human development. The present study supports the World Health Organization's recommendations to avoid wiping vernix off newborn infants' skin at birth and to delay the first bath for at least six hours [4].

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**Keywords:** Newborns skin, Vernix caseosa, Stratum corneum, Hair follicles.

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# Modulation of S100B in experimental models of multiple sclerosis: effect on different glial cell types

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It has been demonstrated that S100B actively participates in neuroinflammatory processes of different diseases of the central nervous system (CNS), such as Experimental Autoimmune Encephalomyelitis (EAE), a recognized animal model for Multiple Sclerosis (MS). The inhibition of S100B activity using pentamidine and of S100B synthesis using arundic acid are able to determine an amelioration of clinical and pathologic parameters of MS with milder and delayed symptoms. This study further goes in detail on the role of S100B, and in particular of astrocytic S100B, in these neuroinflammatory processes. To this aim we used a model of S100B knockout (KO) mice. As expected, S100B protein levels were significantly reduced in the S100B KO mouse strain resulting in an amelioration of clinical and pathological parameters (clinical and morphological analyses). To dissect the potential mechanisms that could explain the role of S100B in the development of EAE we sorted, cultured and compared glial subpopulations (astrocytes, oligodendrocytes and microglia) deriving from S100B KO and wild type mice, through flow cytometric panels and ELISA. Glial cells were analyzed for proinflammatory molecules showing a signifi-cant reduction of TNFa protein in mice where S100B was silenced. To dissect the role of S100B in MS we cultured astrocytes and microglial cells magnetically sorted and enriched from the brains of EAE affected animals, both from KO and wild type animals. Both ge-netic silencing of S100B and pharmacological inhibition with S100B-targeting compounds demonstrated a direct impact on specific subpopulations of astrocytes (mainly), oligodendrocytes and microglia. The present results further individuate astrocytic S100B as a key factor and as a potential therapeutic target for EAE neuroinflammatory processes.

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**Keywords:** S100B, arundic acid, pentamidine lsethionate, Experimental Autoimmune En-cephalomyelitis, Multiple Sclerosis.

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# Pathogenic Effects of the AP1G1 c.196G>A Variant in Usmani-Riazuddin Syndrome: Functional and Neuroanatomical Evidence from Zebrafish

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Usmani-Riazuddin Syndrome (USRIS, OMIM 619467) (1) is a rare neurodevelopmental disorder characterized by intellectual disability, speech impairment, hypotonia, epilepsy, behavioral abnormalities, and facial dysmorphism. Dominant and recessive mutations in the *AP1G1* gene, encoding the γ1 subunit of the AP-1 vesicular transport complex, have recently been implicated in the disease.

In this study, we report the identification of a novel *de novo* heterozygous missense variant in *AP1G1* (c.196G>A, p.Gly66Arg) in a patient presenting with intellectual disability and behavioral disturbances. The variant affects a highly conserved residue and is absent from population databases. *In silico* analyses predict a destabilizing effect on protein structure, suggesting a potential dominant-negative pathogenic mechanism.

Immunofluorescent analysis on patient-derived fibroblasts revealed altered intracellular localization of the mutant AP1G1 protein, which was excluded from membranes and accumulated in cytoplasmic aggregates. Disorganization of the trans-Golgi network (TGN) was observed, consistent with impaired vesicular trafficking.

To assess the in vivo effects of the variant, we used the zebrafish model. Overexpression of mutant mRNA (p.Gly66Arg) caused dose-dependent mortality and morphological abnormalities. At a sublethal dose (50 pg/embryo), 40% of embryos exhibited developmental delay, growth retardation, and central nervous system defects. In contrast, wild-type (WT) mRNA induced a largely normal phenotype. Rescue experiments in *ap1g1*$^{-/-}$ embryos showed that human WT mRNA restores viability, while the mutant variant fails to do so. Co-injection of WT and mutant mRNAs resulted in only partial rescue, supporting a dominant-negative effect. Behavioral assays revealed profound impairment of locomotion, sensory response, and motor maturation in mutant embryos. At 6 days post-fertilization, they showed reduced swimming distance, decreased speed, and altered responses to stimuli, as measured by automated motion tracking. Through *in situ* hybridization for *ngn1* and confocal imaging in the Tg (*neurod1*:EGFP) transgenic line, we documented severe malformations at the mesencephalon-metencephalon boundary and a marked reduction in neurogenic expression in the brain and spinal cord.

Altogether, our data provide strong functional evidence for the pathogenicity of the *AP1G1*

p.Gly66Arg variant, confirming its causal role in USRIS. This study highlights the power of integrated in vitro and in vivo approaches in elucidating the molecular mechanisms of rare genetic neurodevelopmental disorders.

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**Keywords:** Usmani-Riazzudin syndrome, Intellectual disability, Neurodevelopmental delay, AP1G1.

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# Role of SOD-1/Nrf2 in the molecular events triggered by Complex Magnetic Fields application in an *in vitro* $H_2O_2$-stimulated endothelial cell model

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One of the compelling topics for the scientific community is to promote tissue regeneration when damaging stimuli occur, an event that necessarily relies on the proper functioning of the underlying endothelial system. Oxidative stress and inflammation, primarily driven by the production of Reactive Oxygen Species (ROS), are major contributors to endothelial dysfunction after noxious stimuli exposure, thereby compromising the regenerative capacity of surrounding tissues (1, 2). Among the tools to stimulate tissue healing and regeneration, Complex Magnetic Fields (CMFs) are included. Their application, through a non-invasive electronic device, emerged for the treatment of several pathologic alterations, such as diabetic foot, edema, muscular lesions, and many others, demonstrating also a valid pro-angiogenic effect (3). In the present study, an *in vitro* model of oxidative stress-stimulated EA.hy926 endothelial cells (ECs) was established through 3 h of pretreatment with 150 μM $H_2O_2$ in order to stimulate ROS production. Then, 3 CMF programs were applied (Anti-inflammatory, Anti-stress, Regenerative Tissue) in two cycles spaced 24 h apart (T0+T24 condition) in order to investigate the antioxidant potential of the system. Results demonstrate that CMF applications substantially reduce ROS production, leading to an increased cell metabolic activity and a reduction of $H_2O_2$-induced cytotoxicity. Additionally, T0+T24 application promotes cytosolic SOD-1 and Akt/Nrf2 proteins expression, together with an increased total EC antioxidant capacity, an increased number of live cells, and a decrease of some pro-apoptotic proteins. These results suggest that T0+T24 modality of CMF applications can mitigate oxidative stress ROSinduced through the synthesis of antioxidant molecules: from one side, SOD-1 and Nrf2 can act as transcription factors stimulating the synthesis and the release of other antioxidant elements. On the other side, SOD-1 and Nrf2 can protect the cells from apoptotic events, delaying or suppressing them, and promoting cellular survival. In conclusion, CMF applications can ameliorate EC dysfunction caused by ROS production, finding applications when an antioxidant and anti-inflammatory effect is needed, such as in edema, muscular lesions, tendon lesions, and many others.

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**Keywords:** endothelial cells, Complex Magnetic Fields, ROS, antioxidants, SOD-1, Nrf2.

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# 3D spheroids for morphological analysis of prostate carcinoma cells with different EMT-related phenotype: tips and tricks

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3D-spheroids maintain a 3D cell arrangement to mimic the tissue architecture, thus retaining most of the biological characteristics that are lost when culturing cells in 2D and represent an excellent experimental setting to study cancer cells biology.

Since our previous reports demonstrated that 3D spheroids allow the characterization of carcinoma and melanoma cell phenotype, in this study we compared different 3D experimental settings for the morphological analysis of prostate cancer cells having a different EMT-related phenotype.

For this purpose, DU145 and PC3 prostate cancer cells spheroids were obtained using three different technical approaches: 1% agarose-coated 24-multiwell plates, Apricell 3-in-1 plates and Ibidi μ-Slide Spheroid Perfusion.

Our results show that DU145 cells, exhibiting a more epithelial phenotype, formed 3D spheroids containing tightly packed cells when cultured in agarose-coated wells. By contrast, PC3 cells, characterized by mesenchymal features, formed loosely aggregates that do not maintain their integrity when manipulated for morphological analysis processing.

When DU145 and PC3 cells were cultured in Apricell 3-in-1 plates, densely packed spheroids with regular morphology were rapidly generated, and spheroid integrity was preserved in both cell types during the procedures to prepare the samples for morphological analysis.

Similar to Apricell 3-in-1 plates, Ibidi μ-Slides facilitated the rapid formation of 3D spheroids that retained their integrity during the manipulation. However, the small amount of cell culture medium in each well did not allow to culture the spheroids for long periods of time without the perfusion.

Overall, our results show that different technical approaches to culture prostate cancer cells in 3D arrangement offer different advantages during the manipulation of spheroids depending on cell phenotype. However, each of the experimental settings considered confirms that 3D spheroids represent a suitable pre-clinical model for identifying and validating tumor markers and to studying new therapeutic tools.

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**Keywords:** 3D spheroids, EMT, prostate cancer.

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# Molecular mechanisms underlying inflammatory and remodeling events differently regulated by cruelty-free method-extracted snail slime and glycolic acid

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Snail slime (SS), traditionally used for treating skin disorders, has gained scientific attention for its potential in cosmetics, wound healing, and anti-inflammatory applications (1). This study investigates the effects of SS extracted by a cruelty free method on human keratinocytes (HaCat) and on endothelial cells (ECs), comparing for the first time its properties to glycolic acid (GA), a key component of SS (2). On HaCat cells, viability was measured by MTT assay, cytotoxicity by lactate dehydrogenase (LDH) assay, and an evaluation of a putative inflammatory pathway was investigated by measuring the protein expression of phosphoinositide 3-kinase (PI3K)/Akt/NF-κB pathway and through cyclooxygenase-2 (COX-2) gene expression. Additionally, extracellular matrix (ECM) remodeling was assessed by measuring some Matrix metalloproteinases (MMPs) gene expression by real-time polymerase chain reaction (RT-PCR). On EA.hy926 endothelial cell lineage a preliminary evaluation of SS effect was assessed in terms of cell viability and cell migration. Results show that SS is well-tolerated by keratinocytes at dilutins of 1:40, 1:60 and 1:80, whereas GA alone exhibits cytotoxicity over prolonged exposure, suggesting that the complex composition of SS can mitigates GA's adverse effects. Moreover, SS induces a controlled, brief inflammatory response via the PI3K/Akt/NF-κB pathway unlike GA, which triggers stronger and sustained pro-inflammatory events. In parallel, SS capability, through an upregulation of MMP-2 and MMP-9 gene expression, to positively contribute to ECM remodeling, a crucial event for skin repair, is detected. Additionally, in ECs, SS sustains cell viability and migration, thus supporting processes of wound healing and ultimately skin repair.

These findings evidence that SS, due to its unique blend of multiple beneficial macromolecules, appears tolerated by skin cells, differently from what recorded for GA. Furthermore, SS discloses the capability to induce a brief, well-controlled and regulated inflammatory process, thus representing the starting point for cellular renewal and ECM remodeling events. Conversely, the inflammatory effect triggered by GA, being sustained, prolonged and requiring a longer time to get a restoration, appears not able to trigger, within the cells, further beneficial events. To conclude, this study underscores SS's potential in modulating cellular signaling pathways thus highlighting its future therapeutic potential use in the challenging fields of skin regeneration and wound healing.

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**Keywords:** snail slime, glycolic acid, inflammation, extracellular matrix remodeling, keratinocytes.

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# Morphostructural and Biomechanical Characterization of the Human Large Bowel: Toward Computational Modeling for Device Design in Colostomy

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Colostomy, a procedure that redirects fecal matter to an external bag, often causes physical and psychological challenges [1]. Emerging biomedical technologies aim to develop continence devices that enable bag-free periods, improving patients' quality of life. To this purpose, this study focused on morphostructural (19 patients)/mechanical (8 patients) characterization of human large bowel (LB) obtained from patients undergoing abdominal surgery for benign conditions at Padova University Hospital (Ethics Approval: CESC Code AOP3212). These analyses are required to support the development of realistic computational models that are essential for accurately simulating tissue- device interaction, which is critical for optimizing device design. The mean thicknesses of different tissue layers were: 669.7 ± 331.5 μm, mucosa; 18.5 ± 8.5 μm, muscularis mucosae; 1173.1 ± 705.7 μm, submucosa; 1813.5 ± 433.4 μm, circular muscle layer; and 1209.2 ± 617.8 μm, longitudinal muscle layer. In parallel, collagen content in the submucosa was evaluated using semi- quantitative analysis of Azan-Mallory–stained sections via ImageJ software. Collagen quantification was expressed as the percentage of image area occupied by collagen relative to the total image area (% = collagen area / total area × 100), yielding an average of 51.16% ± 7.91. Mechanical testing was performed (Mach-1 system, ©Biomomentum) on samples oriented along three directions: longitudinal (L, n=8), circumferential (C, n=20), and radial (R, n=12). After preconditioning, L and C samples underwent tensile testing either to failure (strain rate: 100%/s) or under stress-relaxation (SR) conditions (six 30% strain ramps; strain rate: 100%/s; 600 s rest). R samples were subjected to compression protocols, including loading-unloading (LU: 60% strain, 1%/s, 10 cycles) and SR (six 10% strain ramps; 100%/s; 600 s rest). For tensile tests, key parameters were calculated from stress- strain curves: ultimate tensile strength (UTS), failure strain (FS), and both initial (Ei) and final elastic moduli (Ef), corresponding to the toe and quasi-linear regions, respectively. From SR tests, equilibrium relative stiffness ($\gamma\infty$) was determined as the final normalized stress. LU tests yielded values for Ei, Ef, and peak stress (PS). Results highlighted the mechanical behavior of LB tissue as anisotropic, non-linearly elastic, and time-dependent. Key parameters in each direction were: UTS [kPa]: 427 ± 173 (L), 588±290 (C) and – (R); FS [-]: 2.46 ± 0.72 (L), 3.30 ± 1.50 (C) and - (R); PS [kPa]: - (L), - (C) and 12.13±1.98; $E_i$ [kPa]: 20±17 (L), 18±13 (C) and 0.85±0.34 (R); $E_f$ [kPa]: 485±259 (L), 278±93 (C) and 147±74 (R); $\gamma\infty$ [-]: - (L), 0.12±0.02 (C), 0.07±0.02 (R). The study provided the basis for a visco-hyperelastic constitutive model of fiber-reinforced materials, enabling accurate simulation of LB tissue mechanics for the design of advanced continence devices.

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**Keywords:** Large bowel, colostomy, morphostructural analysis, mechanical testing, computational modeling, continence devices.

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# TEM-based characterization of a hybrid lipid nanoparticle system for the intracellular gene delivery

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To date, a wide range of nanomaterials with gene delivery capabilities or photothermal effects has been developed in the field of biomedicine (Kim et al., 2016; Cui et al., 2022). In this study, we aimed to design a hybrid lipid nanoparticle (LNP) system, composed of a lipid shell coated with gold nanorods (AuNRs), hereafter referred to as LNP/AuNRs, to enable light-responsive gene delivery. To confirm the successful integration of AuNRs into the LNP platform, we carried out a comprehensive physicochemical characterization of the hybrid system using UV–Vis spectroscopy, transmission electron microscopy (TEM), dynamic light scattering (DLS), and microelectrophoresis. To assess the transfection efficiency and cytocompatibility of the LNP/AuNR system, we performed transfection and viability assays in HEK293 cells, followed by ultrastructural analysis using TEM. Untreated HEK293 cells displayed typical morphological features of adherent, epithelial-like human embryonic kidney cells. In contrast, cells treated with LNPs or LNP/AuNRs showed specific ultrastructural features relative to untreated controls. Although the overall cellular architecture remained largely intact, changes in specific organelles were observed, indicating active cellular responses to nanoparticle uptake and the transfection process. Electron-dense material corresponding to internalized nanoparticles was frequently detected within intracellular compartments, suggesting active endocytosis and intracellular trafficking via the endolysosomal pathway. LNPs and LNP/AuNRs were predominantly localized within vesicular compartments, such as early and late endosomes and lysosomes, and were rarely observed free in the cytoplasm. In control cells, mitochondria exhibited the expected elongated or ovoid morphology with well-preserved double membranes and densely packed, lamellar cristae. In nanoparticle-treated and transfected cells, however, mitochondrial alterations were frequently observed, including cristae dilation and widened intercristae spaces. In some cases, the inner mitochondrial membrane appeared partially disorganized or fragmented. These changes may indicate early metabolic stress or oxidative damage, although further biochemical validation is warranted. Our findings demonstrate that LNP/AuNRs achieve efficient cellular uptake and functional delivery while maintaining excellent cytocompatibility. Cell viability remained comparable to that of untreated controls, and TEM analysis revealed a preserved overall ultrastructure, supporting the safety of the system for further development. These results establish a solid foundation for future studies involving laser activation to leverage the photothermal properties of AuNRs and to enhance transfection efficiency through controlled release mechanisms.

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**Keywords:** Lipid nanoparticles, Au nanoparticles, transmission electron microscopy.

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# Adipose-derived mesenchymal stem cells extracellular vesicles mitigate neurotoxicity induced by antineoplastic drugs in sensory neurons

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Chemotherapy-induced peripheral neuropathy (CIPN) constitutes a major clinical side effect, manifesting as paraesthesia, numbness, and dysesthesia, that often necessitates dose reduction and/or discontinuation of therapy with platinum-based compounds and proteasome inhibitors [1]. Despite decades of investigation, no neuroprotective modality has achieved definitive clinical efficacy. Nevertheless, in vitro studies have highlighted the therapeutic promise of extracellular vesicles derived from mesenchymal stem cells (ASC-EVs). EVs are emerging as important mediators of intercellular communication and have been shown to carry a variety of bioactive molecules, including proteins, RNAs, and lipids, and in particular ASC-EVs contribute to cellular repair processes.

This study aimed to assess the neuroprotective potential of ASC-EVs towards the neurotoxic effects of cisplatin (CDDP) or bortezomib (BTZ) on sensory neuron primary cultures derived from embryonic (E15) Dorsal Root Ganglion (DRG).

Sensory neurons were treated with CDDP (6 μM) or BTZ (20 nM), either alone or in combination with EVs (1 μg/mL), for 24-48 hours. Neuronal viability was evaluated using bright-field microscopy, based on the count of viable cells identified by the birefringent neuronal soma.

Consistent with established neurotoxicity profiles, CDDP induced a pronounced, time-dependent decrease in neuronal viability at both 24 h and 48 h time points. However, co-treatment with ASC-EVs significantly rescued neuronal survival, indicating a potential neuroprotective effect.

In parallel, BTZ induced a distinct neurotoxic profile, with milder effects observed at earlier time points, likely reflecting differences in its action mechanism with respect CDDP[3]. Nevertheless, co-treatment with ASC-EVs also mitigated BTZ-induced toxicity, suggesting a potentially neuroprotective capacity.

These results support the hypothesis that the neuroprotective efficacy of ASC-EVs is dependent upon both the pharmacological profile of the chemotherapeutic agent used and the treatment duration, suggesting a time-dependent interplay between ASC-EVs activity and drug-induced neurotoxicity.

ASC-EVs appear to counteract the detrimental effects of chemotherapeutic agents through mechanisms that may likely involve oxidative stress and apoptotic molecular pathways, which are now under investigation. Moreover, further studies are required to optimize this therapeutic approach by precisely defining the most effective time window for ASC-EVs administration, identifying the specific molecular components responsible for the observed neuroprotective effects.

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**Keywords:** Chemotherapy-induced peripheral neuropathy (CIPN), Extracellular Vesicles (EVs), Neuroprotection.

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# Assessing Ancient Disability: An Integrated Osteo-imaging Analysis of Congenital Hip Dysplasia

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This study presents a detailed morphological analysis of a skeletal individual excavated from a burial context in ancient Rome, whose pathological features shed light on the lived experience of congenital disability in the past.

The focal point of this research is the individual's left hip joint, which demonstrates marked anatomical abnormalities consistent with a diagnosis of severe developmental dysplasia of the hip (DDH), a congenital condition affecting the formation and stability of the hip joint. Anatomical and morphological evaluation played a central role in identifying and characterizing the condition. Careful examination of the hip bones revealed a shallow and misshapen acetabulum, lacking the depth and curvature required to properly house the femoral head. This malformation was accompanied by a displaced and irregularly shaped femoral head, showing evidence of flattening, superior migration, and disrupted joint articulation.

The morphological features of the proximal femur further supported the diagnosis: the femoral neck appeared shortened and thickened, with an altered angle of inclination suggesting compensatory remodeling in response to abnormal mechanical loading. Signs of cortical bone hypertrophy and asymmetrical bone development point to long-standing biomechanical stress, consistent with chronic joint instability and abnormal gait patterns. These skeletal markers, discernible through meticulous anatomical analysis, enabled a confident diagnosis of congenital hip dysplasia, despite the absence of soft tissue indicators.

In addition to traditional osteological methods, advanced imaging techniques, particularly computed tomography (CT), were employed to complement and enhance the morphological findings. CT scans provided detailed cross-sectional and 3D visualizations of internal bone architecture, revealing secondary degenerative changes, including subchondral cysts, osteophyte formation, and joint space narrowing. Nevertheless, it was the initial anatomical and morphological assessments that laid the groundwork for diagnostic accuracy, enabling a nuanced understanding of the structural deformities prior to radiological confirmation.

The rarity of DDH in archaeological contexts from Italy – and the apparent severity of this case – makes it a significant paleopathological finding, possibly representing the earliest documented example of this condition in the region. The skeletal evidence not only allows for refined diagnostic classification but also raises compelling questions about the individual's social context, including their access to care, assistance, and the broader community's response to visible physical disability. That this person reached adulthood suggests a level of accommodation or social support, highlighting the complex interplay between health, impairment, and society in Late Antiquity.

This case study demonstrates the value of an integrated methodological approach, combining thorough anatomical and morphological evaluation with noninvasive imaging to increase diagnostic resolution. By doing so, it exemplifies how congenital disorders – which may present with subtle or multifaceted skeletal manifestations – can be more accurately identified and interpreted, contributing to broader discussions in historical bioethics, particularly concerning disability, resilience, and care in ancient societies.

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**Keywords:** Developmental Dysplasia of the Hip (DDH), Congenital anomalies, Osteological analysis, Anatomical morphology, Paleopathology.

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# From Wax to Science: The Pioneering Work of Paolo Mascagni in Anatomical Research

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The "Cattaneo" Anatomical Wax Collection is part of the University of Bologna Museum Network. It is a heterogeneous collection recalling the 17th century cabinet of curiosities and tracing the 18th and 19th centuries period by bringing together the sections of normal and pathological anatomy [1]. The normal anatomy section is predominantly comprised of waxes modeled by Clemente Susini according to the Florentine school. Among them, a head, hosted in a horizontal gold-decorated case, stands out for its magnificence. It is a life-sized wax model, polychromatic and polymateric, displayed on a wooden platform. It reproduces with extraordinary truthfulness and accuracy a head and neck dissection performed on cadavers by the anatomist and illustrator Paolo Mascagni. The pose, emulating those found in renowned anatomical textbooks of the era, portrays a young man with a slightly recumbent head.

The most revolutionary and intriguing anatomical feature is the presence, within the central nervous system, of an intricate network of lymph vessels and nodes, produced by means of cotton or silk threads soaked in wax, colored and then placed on the surface of the model. This feature is extremely surprising because the lack of lymphatic system in the encephalic region has been an anatomy dogma until 2015 when dural lymphatic vessels were "re-discovered" and linked to brain fluid clearance [2,3]. Such structures' topography, lining the meningeal sinuses and connected to the profound cervical lymph nodes, may have rendered their detection challenging due to their depth. Therefore, Mascagni's discovery holds immense significance as it validates the rigor and efficacy of his scientific methodology. The experimental model for Mascagni's research, which began in 1777 and culminated in the 1787 publication of the monography "*Vasorum lymphaticorum corporis humani historia et ichonographia*", involved the injection of *hydrargyrum* into lymphatic vessels followed by dehydration so creating a cast and thereby achieving the first three-dimensional morphological depiction of the cerebral lymphatic system [4-5].

This pioneering work provided the first systematic description of the lymphatic system, enhanced by remarkable tables crafted by the Bolognese engraver Ciro Santi. These preparations were dispatched to the esteemed La Specola, where they were magnificently reproduced by means of wax. At that time, La Specola had already gained international recognition as a ceroplastic workshop of the highest caliber and the University of Bologna, as other European universities, acquired the waxes between the years 1782 and 1787.

Anatomical waxes can thus be regarded as a component of an organic system of "visual culture of science", fundamentally rooted in human body dissection which was, and continues to be at the Anatomy Center of the University of Bologna, a powerful experimental tool in teaching and research of the "*alphabetum medicorum*" (Berengario da Carpi).

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**Keywords:** Anatomical wax models, Dissection, Paolo Mascagni, Cerebral lymphatic system, Historical anatomy.

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# Somatostatin and Analogs: Biological and Molecular Insights from *In Vitro* Breast Cancer

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**Background:** Somatostatin is a neuropeptide with antiproliferative, pro-apoptotic, and anti-angiogenic properties, whose activity is mediated by five G protein-coupled receptors (SSTR1–5). Its synthetic analogs, octreotide and pasireotide, are already used clinically for the treatment of neuroendocrine tumors, but their therapeutic potential in breast cancer is a subject of growing interest. This study investigates the ability of these compounds to modulate key processes involved in tumor progression, including cell proliferation, apoptosis, autophagy, and the regulation of gene expression mediated by microRNAs, in two breast cancer cell lines MDA-MB231 and MCF-7.

**Materials and Methods:** Two human breast cancer cell lines (MDA-MB231 and MCF-7), representative of distinct molecular subtypes (triple-negative and luminal A, respectively), were used to evaluate the effects of somatostatin, octreotide, and pasireotide, administered alone or in combination at different time points. The expression of somatostatin receptors (SSTR1–5) were assessed in both cell lines by Western blot analysis. Following treatment, functional assays were performed to evaluate apoptosis (annexin V staining), while autophagy and cell cycle distribution were analyzed by flow cytometry. Total RNA was extracted for microRNA expression profiling using RNA sequencing (RNA-seq).

**Results and Discussion:** Treatment with somatostatin and its analogs in combination effectively altered cell cycle distribution in both breast cancer cell lines examined. Moreover, both models exhibited modulation of LC3 expression levels an indicator of autophagy activation although with different timing. This effect was especially pronounced following combined treatment with somatostatin–octreotide (SO) and somatostatin–pasireotide (PO). Additionally, microRNA expression profiling in MDA-MB231 cells revealed differential regulation of specific miRNAs involved in MAP kinase signaling pathways, suggesting a potential regulatory role for these compounds within critical oncogenic networks. This study demonstrate the potential of somatostatin and its analogs, particularly when used in combination, effectively modulate key cellular processes involved in breast cancer progression, including cell cycle regulation and autophagy. The observed differential regulation of microRNAs related to MAP kinase signaling in triple-negative breast cancer cells further supports the potential of these compounds to interfere with oncogenic pathways. These results highlight the therapeutic promise of somatostatin analog combinations as modulators of tumor biology in distinct breast cancer subtypes.

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**Keywords:** Somatostatin, Octreotide and Pasireotide, Breast Cancer, Autophagy, miRNA expression.

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# BeING-FrESH: Benefits Induced by NGF against Risks derived from Environmental Contaminants for Sperm

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Male infertility in recent decades shows a global increasing trend. A key driver is the decline of sperm quality, a fundamental parameter for assessing male fertility potential[1-2]. Various elements can compromise sperm health, including environmental factors, related to the release of chemical pollutants and behavioural factors such as cigarette smoking[3-4].

Recent studies, initially conducted on animal models and humans, have identified the expression of Nerve Growth Factor (NGF) and its receptors (p75NTR and TrkA) in the testes, epididymis, and seminal fluid and its implication in distinct pathological conditions[5], suggesting a potential role of NGF in sperm physiology.

The aims of this study were: (i) to analyze the expression of NGF and its receptors (p75NTR and TrkA) as potential biomarkers of male fertility, and (ii) to evaluate the potential protective role of NGF under exposure to environmental contaminants.

Semen samples were collected from 29 individuals aged 31–40 years, divided into two groups: oligozoospermic patients (n=12) and normozoospermic patients (n=17). Samples underwent microbiological screening and were analysed for standard semen parameters: concentration, motility, morphology, and viability. NGF levels were quantified in seminal plasma, and gene (qPCR) and protein (flow cytometry) expression of NGF receptors were assessed on spermatozoa. Sperm samples were also exposed to environmental contaminants (cadmium, zinc, Bisphenol A, and Perfluorooctanesulfonic acid), both with and without NGF treatment, to compare the expression of the p75NTR and TrkA receptors.

The results showed that NGF levels in seminal plasma do not directly correlate with sperm parameters. However, NGF's interaction with its receptors plays a critical role in sperm health. High expression of TrkA in normozoospermic samples and its positive correlation with key sperm parameters suggest that TrkA may serve as a biomarker of sperm quality. Conversely, increased p75NTR levels in oligozoospermic samples, along with elevated pro-inflammatory cytokines, support the idea that p75NTR acts as a sensor of compromised sperm quality. Exposure to environmental contaminants further emphasized p75NTR's role, revealing a negative correlation between its expression and sperm concentration in polluted samples. Notably, the addition of NGF reduced p75NTR expression in these samples, indicating a potential protective effect.

In conclusion, the balance between TrkA and p75NTR expression appear to be candidate as a potential biomarker of sperm quality. NGF treatment appears to mitigate damage induced by environmental contaminants, reducing p75NTR expression and associated apoptotic processes. These findings point to possible clinical strategies to improve sperm quality and fertility outcomes, even in the presence of environmental stressors.

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**Keywords:** Spermatic paremeters, human reproduction, neurotrophins, TrKA and p75NTR.

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# Nanostructured PCL surfaces modulate the biological activity of human ureteral epithelial and muscle cells: a preliminary study

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Clinical conditions such as urolithiasis, chronic inflammation, infections and ureteral carcinoma can severely damage the ureter, making surgical intervention necessary (1). However, the widespread use of such procedures has led to a significant increase in iatrogenic ureteral injuries (2). Current surgical techniques for ureteral reconstruction are invasive and often associated with complications (3). Ureteral tissue regeneration (UTR) represents a promising and innovative approach to avoid surgery. The aim of this study was to characterize *in vitro* a resorbable scaffold for ureteral regeneration, made of synthetic polycaprolactone (PCL). This scaffold was used as a control or subjected to treatment with plasma and/or rifampicin, in order to evaluate its effects on cell adhesion, proliferation and protection against bacterial infections. The *in vitro* biological effects of PCL were evaluated on primary bladder epithelial cells (BdEC) and primary bladder smooth muscle cells (HBdSMC), both of normal human origin. Cell proliferation, cytotoxicity, inflammation and morphology were assessed on day 4 and 7 in both monocultures and co-cultures to investigate potential paracrine interactions between the two cell types. The results showed that cytotoxicity, in both cell culture conditions, was higher in the presence of rifampicin compared to control, whereas plasma treatment showed a rate of viable cells similar to the control, suggesting that plasma counteract the effect of rifampicin. PGE-2 release increased in both BdEC and HBdSMC in the presence of rifampicin only after 7 days of treatment, however, this effect was notably reduced by plasma. Gene expression analysis using RT-qPCR revealed modulation of markers related to tissue proliferation, differentiation, and homeostasis. In co- cultures, plasma treatment reduced the expression of pro-inflammatory genes, counteracting the rifampicin-induced upregulation. Cell morphology was assessed by optical microscopy: treatments did not appear to affect cell structure. In summary, preliminary data suggest that plasma treatment may significantly enhance the performance of rifampicin-loaded PCL scaffolds for ureteral regeneration, thanks to reduced cytotoxicity and inflammatory response. However, further studies are needed to clarify the underlying mechanisms.

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**Keywords:** Biomaterials, Tissue regeneration, Scaffold.

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# Patellofemoral Pain Syndrome: Focused Vibrations Plus Kinesiotaping with Insights into Radiological Influences-An Observational Study

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**Background:** This observational study investigates the efficacy of combining local muscle vibration (LMV) therapy and kinesiotaping using the McConnell method (KMcCM) in patients with patellofemoral pain syndrome (PFPS). PFPS is a prevalent knee condition characterized by anterior or medial knee pain exacerbated by activities that overload the patellofemoral joint.

**Objective:** The primary aim of this study was to evaluate the effectiveness of LMV combined with KMcCM in reducing pain and improving function in PFPS patients.

**Methods:** A total of 52 participants, aged 25-85, with PFPS were included. Participants underwent LMV and KMcCM treatments three times weekly for three weeks. Pain and function were assessed using the Visual Analog Scale (VAS) and the Knee Injury and Osteoarthritis Outcome Score (KOOS) at baseline (T0) and six months post-treatment (T1). Radiological assessments of patellar alignment and biomechanics were also conducted through dynamic MRI.

**Results:** Significant pain reduction and functional improvements were observed across all age groups. Notably, younger participants showed greater improvement compared to older participants. Among women, those in the younger age group experienced more substantial reductions in VAS scores compared to their older counterparts. KOOS scores improved significantly, indicating enhanced knee function overall. A significant decrease in VAS scores from T0 to T1 was observed across all patellar alignment groups, signifying a reduction in pain levels. However, Group 2 (Laxation and Subluxation) experienced the most substantial reduction in VAS scores at T1 compared to the other groups. These results suggest that the combination of LMV and KMcCM may be particularly effective in addressing biomechanical abnormalities associated with patellar maltracking and enhancing VMO muscle contraction, leading to more substantial improvements in these patients.

**Conclusions:** The combination of LMV and KMcCM demonstrates promising efficacy in reducing pain and improving knee function in PFPS patients, with age and gender influencing treatment outcomes. The most significant improvements were observed in younger individuals and those with specific patellar alignment issues, highlighting the potential of this combined approach for the targeted treatment of PFPS

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**Keywords:** PFPS, patellofemoral pain, patellofemoral osteoarthritis, anterior knee pain, chondromalacia, musculoskeletal disorder, prevention, MRI, US, KOOS, VAS.

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# Macular Alterations in a Cohort of Caucasian Patients Affected by Retinitis Pigmentosa

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**Objectives:** Our objective was to investigate the prevalence of macular complications detected by spectral-domain optical coherence tomography (SD-OCT) in a large Caucasian cohort of RP patients, highlight the major alterations in chorioretinal structure, and compare the macular structural changes in eyes affected by retinal dystrophies with those in healthy controls.

**Methods:** This was an observational, retrospective, and cross-sectional study. Three hundred and seven patients with RP were consecutively enrolled and underwent clinical assessment. In particular, SD-OCT images were used to ascertain the morphology of the posterior pole of patients with RP by evaluating the residual ellipsoid zone (EZ), the volume and thickness of the outer nuclear layer (ONLT), and subfoveal choroid thickness (SCT). At the same time, the pathological finding that the patients' vision was reduced under treatment was analyzed.

**Results:** A total of 436 eyes of 218 patients with RP were studied. Considering all of the eyes studied, 103 had cystoid macular edema (CME) (23.62%), 123 (28.21%) had vitreomacular traction (VMT), and 199 (45.75%) had epiretinal membranes (ERMs). There were also 12 (2.75%) cases of lamellar macular holes (LMHs), of which 3 (1.38% of all patients) cases were bilateral. Only 137 eyes (31.42%) did not have the above-mentioned alterations. SCT was significantly reduced compared to that of the control group (193.03 μm ± 67.90 SD vs. 295 μm ± 69.04 SD), while the foveal central macular thickness (FCMT) was greater (270.91 μm ± 74.04 SD vs. 221 μm ± 37.25 SD).

**Conclusions:** This research highlights the high incidence of macular complications. The results of our study indicate the importance of regular monitoring of RP patients and early intervention to avoid further complications in this group of subjects with severe visual field impairment to avoid further central vision loss.

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**Keywords:** cystoid macular edema, epiretinal membranes, hereditary retinal dystrophies, lamellar macular hole, retinitis pigmentosa, spectral-domain optical coherence tomography (SD-OCT), subfoveal choroid thickness.

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# Anatomical Distribution and Phenotypic Specialization of Macrophages in Murine Adipose Tissues: Implications for Type 2 Diabetes Models

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Adipose tissue (AT), brown (BAT) and white (WAT), are not statical tissue but showed a morphological and functional plasticity [1-3]. In fact, these tissues react to metabolic alteration such as obesity and type 2 diabetes (T2D) [1-3]. Also, lipid-associated macrophages (LAMs) play a key role in these pathologies and their roles are highly dependent on their anatomical location and phenotype [2, 4]. The anatomical distribution and function of BAT, visceral WAT (eWAT) and LAMs in murine models mimicking aspects of T2D were analyzed by a morphological point of view. Diet-induced obesity (HFD), genetic obesity (db/db), and Friedreich's Ataxia (FA) knock-in/knock-out (KIKO) murine models were utilized. Lipid droplet (LD) size and macrophages into BAT and eWAT were analyzed to characterize and identify LAM subset and changing in microenvironment BAT and eWAT tissue. In BAT of HFD and db/db mice, a distinct population of LAMs, characterized by high expression of PPARγ and GDF15, was identified. These subset LAMs were detected in number significative higher in HFD and db/db mice than control mice ($p<0.05$). Moreover, PPARγ$^{HIGH}$/GDF15$^{HIGH}$ LAMs anatomically localized within BAT and were associated with significant alterations in BAT adipocyte identity (*whitening* features). Specifically, TREM2$^{+}$ macrophage numbers were significantly increased in HFD BAT ($p<0.05$). Conversely, eWAT from mice under metabolic stress (HFD, db/db and FA) consistently showed a significative increased macrophage infiltration ($p<0.01$), contributing to a pro-inflammatory tissue environment. Interestingly, a significative increase of lipid vacuole size ($p<0.001$) and mitochondrial alteration ($p<0.001$) in BAT ko KIKO mice were observed when compared to control mice. Also, a reduced VEGFA expression, increased S100A9$^{+}$ cells and collagen deposition were observed in KIKO eWAT compared to control mice. This was accompanied by significantly larger adipocytes, increased lactate production ($p<0.01$), and a significant increase in CD45$^{+}$ leukocyte content ($p<0.001$), including CD68$^{+}$ macrophage infiltrates ($p<0.01$) compared to wild-type controls. Metabolic changing in adipose tissue profoundly and significantly dictates macrophage phenotype and function, and, at the same time, macrophages reshape the adipose tissue milieu. In BAT, PPARγ$^{HIGH}$/GDF15$^{HIGH}$ LAMs represent a specialized population whose accumulation is statistically significant in obese models, correlating with changes in BAT's thermogenic capacity. In eWAT of the FA model, significant increase in macrophage infiltration and inflammatory markers allow to identify a specific pathological response. These statistically validated, site-specific anatomical and functional characteristics of macrophages are crucial for understanding metabolic dysfunction related to lipid overload and T2D occurrence in order to develop tissue-tailored target therapies.

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**Keywords:** Brown Adipose Tissue, White Adipose Tissue, Lipid-Associated Macrophages, Type 2 Diabetes.

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# Human Lung d-ECM Scaffolds as an In Vitro Model of the Pre-Metastatic Niche in Breast Cancer

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**Introduction:** Breast cancer frequently metastasizes to distant organs, with the lungs being one of the most common sites of secondary tumor growth. The establishment of a favorable microenvironment, known as the pre-metastatic niche, is a critical step that precedes the arrival of circulating tumor cells and facilitates their colonization and outgrowth. Despite its importance, the cellular and molecular mechanisms governing pre-metastatic niche formation in the lung remain incompletely understood, largely due to the complexity of tumor–host interactions and the lack of physiologically relevant models. The study proposed aimed to develop an effective and reproducible in vitro model that recapitulates the dynamic interactions between breast cancer cells and lung stromal components during the early stages of metastasis.

**Materials and Methods:** Decellularized extracellular matrix (d-ECM) scaffolds were obtained from healthy human lung tissue, sectioned into 100 μm slices and decellularized over 24 hours using a solution containing equal concentrations of SDS and Triton X-100, supplemented with antibiotics. Decellularization was confirmed by Hematoxylin-eosin staining (absence of nuclei) and residual DNA quantification (<50 ng/mg dry weight d-ECM). Scaffolds were repopulated with human lung fibroblasts and cultured for one week under standard conditions, then exposed to conditioned media from estrogen receptor-positive and triple- negative breast cancer cell lines. Morphological evaluation was performed using confocal immunofluorescence: Phalloidin was used to visualize F-actin and assess cytoskeletal organization, while Vimentin staining highlighted the intermediate filament network to characterize fibroblast morphology on the matrix prior to conditioned media exposure. Fibroblast activation into cancer-associated fibroblasts (CAFs) after conditioned medium administration was assessed by western blot for specific markers like FAP, α-SMA, and COX-2.

**Results:** Decellularized scaffolds showed effective fibroblast colonization and preserved matrix integrity. Hematoxylin-eosin staining confirmed complete removal of nuclear material while maintaining the overall lung matrix architecture. Phase-contrast microscopy demonstrated a well-preserved 3D scaffold, and fibroblasts exhibited efficient colonization after one week, adhering closely to the matrix structure. Confocal immunofluorescence revealed no morphological differences between fibroblasts cultured on standard plates and those on decellularized matrices, indicating preserved cell viability and organization. Conditioned media from breast cancer cells induced up-regulation of CAF markers, confirming tumor-responsiveness.

**Conclusions:** This three-dimensional human lung d-ECM-based system offers a robust in vitro platform to study tumor–stroma interactions during breast cancer metastasis and represents a promising tool for preclinical research and therapeutic exploration.

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**Keywords:** Breast Cancer, Pre-metastatic Niche, Decellularized Matrix, Cancer Associated Fibroblasts, In vitro Model.

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# Alteration of collagen deposition in cardiac muscle tissue of mice following antibiotic-induced gut dysbiosis

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The human gut microbiota (GM) contains more than 100 trillion microbial cells that are involved in the host's biological processes via several mechanisms, devoted to nutrient absorption, metabolism, and immunity. Gut dysbiosis, defined as an alteration of the composition of the GM, including number and diversity of microbes, is known to be correlated with disease in different body districts. Specifically, recent studies have underlined a key role of GM dysbiosis in the onset of cardiovascular diseases (CVDs).

To investigate the effects of GM homeostasis alteration on cardiac muscle tissue, we induced dysbiosis in an in vivo mouse model through oral administration of vancomycin dissolved in drinking water. Untreated mice (CT) and mice fed with a high-fiber diet (HFiber) following antibiotic administration were also evaluated to investigate the eventual protective effect of dietary intervention.

In the same experimental setting, we previously evaluated the cardiac tissue at the structural and ultrastructural level following antibiotic-induced gut dysbiosis using light and transmission electron microscopy (TEM). Although histological evaluation of heart tissue showed no evident structural differences, TEM analysis showed significant alterations in the shape and dimension of interfibrillar mitochondria and their dynamics in treated mice compared to controls, and an imbalance of oxidative stress pathways as well. Interestingly, proteomic analysis suggested a possible role of dysbiosis in favoring cardiac fibrosis.

Considering our previous findings, in the present work we investigated cardiac fibrosis in the same experimental setting by evaluating interstitial collagen content in paraffin embedded sections of the heart of CT and vancomycin-treated mice through quantitative analysis of Sirius Red stained sections. To assay the potential effect of a high-fiber diet, also the heart of HFiber mice was analyzed. Collagen content was expressed as a fibrosis index.

Our preliminary results suggest an increased fibrosis index in treated mice compared to CT, leading to the hypothesis that dysbiosis induced by vancomycin could be involved in cardiac fibrosis. Interestingly, the fibrosis index was similar to CT in HF, suggesting that a high-fiber diet could be effective in reducing the adverse cardiac effects triggered by gut dysbiosis, showing a protective effect and potentially lowering the risk of CVDs.

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**Keywords:** gut dysbiosis, cardiovascular disease, heart fibrosis.

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# Modulation of miR-145 targets in response to Akt inhibitor AZD5363 (capivasertib) in a mouse xenograft model of prostate cancer

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Prostate cancer (PC) is the second leading cause of cancer-related death in men worldwide [1]. Current therapies for PC, including surgical prostatectomy, chemotherapy, radiation therapy and androgen deprivation therapy (ADT) improve survival rate, but most patients develop resistance and progress to a form of the disease referred to as castration-resistant prostate cancer (CRPC).

Many studies have focused on characterizing the molecular landscape of advanced prostate cancer to identify networks with potentially druggable targets that might aid in the development of better treatment strategies [2]. MicroRNAs (miRNAs) are small endogenous non-coding molecules, that regulate gene expression by degrading messenger RNAs (mRNAs), repressing protein synthesis or interacting with long non coding RNAs [3]. Each miRNA targets a wide range of molecules resulting in the alteration of cellular processes.

MiR-145 is a key tumor suppressor miRNA, downregulated in many types of tumors including prostate cancer [4], that controls the expression of oncogenes such as Ras. Our recent *in vitro* studies identified for the first time an adaptive resistance mechanism to Akt inhibitor capivasertib in which the downregulation of miR-145 triggers a dramatic increase of Ras. Concurrently, we observed reactivation of PI3K/Akt signaling, possibly due to Ras which is a well known upstream activator of PI3K, and a main cause of resistance to therapy with PI3K/Akt inhibitors.

The objective of the present study was to investigate *in vivo* the effect of capivasertib in a xenograft mouse model of PC to confirm compensatory activation of Ras and reactivation of Akt signaling, ultimately limiting the efficacy of the drug. The xenograft mouse model of PC was represented by NOD/SCID mice injected with PC3 cells to reproduce the neoplastic lesion. After 3 weeks of tumor engraftment, mice were treated with capivasertib up to 4 weeks. Starting from the first week of treatment, capivasertib significantly reduced tumor growth in comparison to vehicle-treated control group. However, the efficacy of the drug to reduce the tumor size decreased over time. At the end of the 4$^{th}$ week indeed the percentage of mice not responding to the drug in terms of reduction of the tumor size reached 60%.

Immunoistochemical analysis revealed that capivasertib increased the expression of Ras and other targets negatively regulated by miR-145 such as SENP1, MTDH, vimentin, MMP9 and Twist both at 1 and 4 weeks of treatments, although the effect was particularly evident at 1 week. These results were confirmed by real-time PCR. In conclusion, our study identified a previously undescribed mechanism of adaptive resistance to therapy with Akt inhibitors *in vivo*, in a PTEN *null* xenograft model of PC. This result is particularly important as the association of capivasertib to ADT has been the focus of a number of recent clinical studies with promising results. The previously undescribed vulnerabilities identified here in the molecular mechanisms that drive resistance to Akt inhibitors in PC, may thus be targeted to improve therapy efficacy. In the next future it will be to identify reliable biomarkers enabling the clinicians to pick out sensitive from unsensitive patients.

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**Keywords:** prostate cancer, Ras, capivasertib, xenograft mouse model.

# VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI SIAI, 2024

SIAI Società Italiana di Anatomia e Istologia

**VERBALE DELLA SEDUTA AMMINISTRATIVA E DELL'ASSEMBLEA GENERALE DEI SOCI DELLA SOCIETÀ ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI) TENUTASI VENERDÌ 13 SETTEMBRE 2024 ALLE ORE 16:30 PRESSO L'AULA MAGNA DELL'UNIVERSITÀ UNIVERSITA' DEGLI STUDI DI GENOVA.**

In data 13 Settembre 2024, alle ore 16:30, in seconda convocazione, ha avuto luogo, presso l'Aula Magna dell'Università di Genova, l'Assemblea Generale dei Soci della Società Italiana di Anatomia e Istologia per discutere il seguente Ordine del Giorno:

1) Comunicazioni del Presidente.
2) Rinnovo cariche sociali.
3) Relazione del Tesoriere sul rendiconto finanziario dell'anno 2023 e sulla previsione finanziaria per l'anno 2025. Relazione dei Revisori dei Conti.
4) Attività dei Collegi di Anatomia Umana e di Istologia ed Embriologia Umana. Relazioni dei Presidenti o dei loro Delegati.
5) Aggiornamento sull'Italian Journal of Anatomy and Embryology.
6) Aggiornamento sul sito web della SIAI.
7) Assegnazione Premio alla Carriera.
8) Assegnazione Premi Ricercatori under 40.
9) Assegnazione Premio Migliore Comunicazione Orale.
10) Assegnazione Premi Poster.
11) Prossimi Congressi Nazionali SIAI: proposte temi di relazione.
12) Borsa di Studio SIAI intitolata ai Proff. Carlo Grossi e Alessandro Moretta.
13) Proposte di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.
14) Commemorazione Soci Scomparsi.
15) Varie ed eventuali.

Presiede la riunione il Presidente della SIAI, Prof. Lucio Ildebrando Maria Cocco; funge da Segretario Verbalizzante la Prof.ssa Monica Mattioli Belmonte Cima.
Sono presenti i Soci riportati nell'allegato "Presenze" (all.1).
Il Presidente dichiara aperta l'Assemblea e procede alla discussione dell'Ordine del Giorno.

1) Comunicazioni del Presidente
Il Presidente conferma la avvenuta iscrizione della SIAI nell'Elenco Ufficiale delle Società Scientifiche in ambito sanitario del Ministero della Salute.

Si congratula per i successi ottenuti dai Soci SIAI nel Congresso mondiale IFAA tenutosi in Corea e in particolare la dr.ssa Ryskalin (Pisa) per il Best Paper Award e il Prof. Alberto Cacciola (Messina) per l'attribuzione di relazione orale. Ringrazia La Prof.ssa Manzoli e il Prof. Caggiati per la loro opera quali Delegate / Representative SIAI nell'ambito dell'IFAA.

2) Rinnovo cariche sociali

Il Presidente invita il Prof. Franco Fais, Presidente della Commissione elettorale a illustrare i risultati della votazione. Il Prof: Fais illustra quanto segue:

Votazione: **Candidati Presidente, Segretario e Tesoriere per il Consiglio Direttivo della Società Italiana di Anatomia e Istologia**
Descrizione:
Tipologia della votazione: **Su candidato**
Tipo di scrutinio: **Segreto**

DATI DI AFFLUENZA

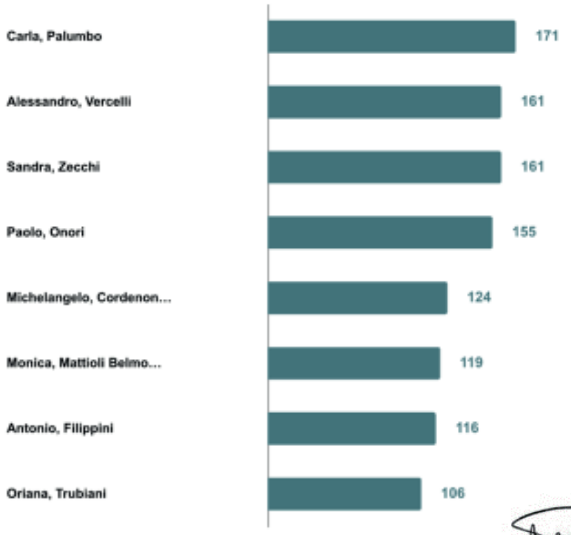
| Aventi diritto | Hanno votato | Non hanno votato | Affluenza totale |
|---|---|---|---|
| 272 | 227 | 45 | 83,46% |




Votazione: **Candidati Consiglieri per il Consiglio Direttivo della Società Italiana di Anatomia e Istologia**
Descrizione:
Tipologia della votazione: **Su candidato**
Tipo di scrutinio: **Segreto**

DATI DI AFFLUENZA

| Aventi diritto | Hanno votato | Non hanno votato | Affluenza totale |
|---|---|---|---|
| 272 | 230 | 42 | 84,56% |



Votazione: **Candidati per il Collegio dei Probiviri**
Descrizione:
Tipologia della votazione: **Su candidato**
Tipo di scrutinio: **Segreto**

DATI DI AFFLUENZA

| 272 | 225 | 47 | 82,72% |
|---|---|---|---|
| Aventi diritto | Hanno votato | Non hanno votato | Affluenza totale |




L'Assemblea unanime proclama eletti i candidati sopra riportati.

3) Relazione del Tesoriere sul rendiconto finanziario dell'anno 2023 e sulla previsione finanziaria per l'anno 2025. Relazione dei Revisori dei Conti
Il Presidente invita il Tesoriere Prof. Papaccio a prendere la parola sull'argomento. Il Prof. Papaccio Illustra i bilanci e le relazioni e l'approvazione dei revisori dei conti e legge i nominativi dei Soci decaduti in base all'art. 15 dello Statuto (all.2)
L'Assemblea approva unanime.

4) Attività dei Collegi di Anatomia Umana e di Istologia ed Embriologia Umana. Relazioni dei Presidenti o dei loro Delegati.
Il Prof. Papaccio illustra l'attività del Collegio dei Docenti di Istologia ed Embriologia (all.3) Il Prof. Montella illustra l'attività del Collegio dei Docenti di Anatomia Umana (all.4). L'Assemblea recepisce e si congratula per l'attività svolta.

5) Aggiornamento sull'Italian Journal of Anatomy and Embryology.
Il Presidente chiede all'Editor-in-Chief Prof. Domenico Ribatti di riferire. Il Prof. Ribatti riferisce che tutti volumi sono on-line e che per il 2024 sono stati pubblicati sia il 1° che il 2° volume. Ringrazia il Prof. Familiari, il Prof. Paternostro e il Prof. Papaccio per la fattiva collaborazione sia editoriale che gestionale della rivista. L'Assemblea ringrazia a sua volta il Prof. Ribatti.

6) Aggiornamento sul sito web della SIAI.
il Presidente ricorda l'importanza della preparazione della pagina "Home" in Inglese. La Prof ssa Zecchi si impegna a sollecitare il gruppo di lavoro "giovani" a predisporre l'aggiornamento.

7) Assegnazione Premio alla Carriera.

Il Presidente comunica che il Direttivo SIAI in data 12.09.2024 ha approvato il verbale della Commissione composta dalle Proff.sse Monica Mattioli Belmonte, Carla Palumbo e Oriana Trubiani (all.5). Il Premio per l'anno 2024 viene assegnato al Prof. Raffaele De Caro. Il Presidente consegna la targa e il Prof. De Caro ringrazia.

8) Assegnazione Premi Ricercatori under 40.

Il Presidente comunica che il Direttivo SIAI in data 12.09.2024 ha approvato il verbale del Comitato Scientifico (all. 6) per l'assegnazione dei due premi Ricercatori under 40. I premi vengono assegnati alla Dott.ssa Carolina Pellegrini (Pisa) e alla Dott.ssa Celeste Caruso Bavisotto (Palermo). Le Dott.sse ricevono la pergamena e il premio in denaro sarà loro accreditato dal Tesoriere via bonifico bancario. Le Dott.sse ringraziano.

9) Assegnazione Premio Migliore Comunicazione Orale.

Il Presidente fa presente che il Direttivo ha nominato i Membri della Commissione per l'assegnazione del Premio alla Migliore Comunicazione Orale. Essa sarà formata dai Moderatori delle due Sessioni di Comunicazioni Orali in cui ci sono più presentazioni e cioè

1."Sistema immunitario e sue modalità di organizzazione morfo-funzionale: dai meccanismi molecolari alle applicazioni terapeutiche", Moderatori: Antonio Filippini, Roberto Di Primio, Domenico Ribatti, Giuseppina Rizzo

2." Modelli 3D e organoidi, ingegneria tessutale e medicina rigenerativa", Moderatori: Virginia Tirino, Francesca Boccafoschi, Carla Palumbo, Mario Raspanti.

La premiazione, poiché le sessioni termineranno il giorno successivo all'Assemblea, avverrà nella cerimonia di chiusura.

10) Assegnazione Premi Poster.

Il Presidente fa presente che il Direttivo ha nominato la Commissione per i Premi Poster (2 Sessioni). Essa sarà composta dai Proff. Bernardini, Filippini, Marchisio, Onori, Sorci e Trubiani. La premiazione, poiché le sessioni termineranno il giorno successivo all'Assemblea, avverrà nella cerimonia di chiusura.

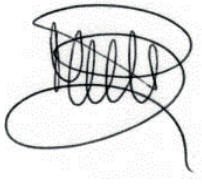
11) Prossimi Congressi Nazionali SIAI: proposte temi di relazione.

Il Presidente comunica che ad oggi non sono pervenute candidature. Sollecita la loro presentazione e l'Assemblea stabilisce che sarà il prossimo Direttivo a valutarle, qualora pervenute. Riferisce inoltre che per il 2026 il Prof. Macchiarelli candida la sede di L'Aquila.

12) Borsa di Studio SIAI intitolata ai Proff. Carlo Grossi e Alessandro Moretta.

il Presidente dà lettura del Bando per la Borsa di Studio SIAI, intitolata ai Proff. Carlo Enrico Grossi e Alessandro Moretta, già deliberata dal Direttivo (all. n.7). L'Assemblea conferma e rivolge un sentito pensiero alla memoria dei due illustri Colleghi e Amici della Morfologia Genovese prematuramente scomparsi.

Viene anche presentata la relazione sull'attività svolta dalla Dott.ssa Irene Neri, vincitrice della Borsa di studio 2024 intitolata al Prof. Giovanni Orlandi e usufruita presso la Hochschule Kaiserslautern, Campus Zweibrücken, Germania (all.8)



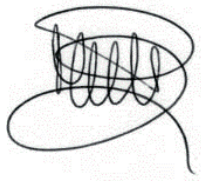
13) Proposte di ammissione nuovi Soci e proposte per Soci Emeriti ed Onorari.
Il Presidente presenta l'elenco dei nominativi di coloro che hanno presentato la domanda in qualità di Soci Ordinari durante l'anno e che sono stati approvati dai Direttivi durante il 2024:

1. BELLI MANUEL
2. BAGNARA DAVIDE
3. BIANCHI ENRICA
4. CENERI ELEONORA
5. DE NUCCIO FRANCESCO
6. DEL VECCHIO VITALE
7. DI MARTINO ORSOLA
8. DI VINCENZO MARIANGELA
9. DURANTE MIRIAM
10. FULCERI FEDERICA
11. HENIN DOLAJI
12. LICINI CATERINA
13. LIMANAQI FIONA
14. MARINO FABIOLA
15. MARRAZZO PASQUALE
16. MARROCCO ILARIA
17. MAZZARELLO ANDREA NICOLA
18. MAZZONE ANTONELLA
19. MERINGOLO MARIA
20. MILLER ANTHEA
21. PACCA PAOLO
22. PAGLIARINI VITTORIA
23. PAGANELLI FRANCESCA
24. PRESTA VALENTINA
25. RICCIOLI ANNA
26. RIZZI MANUELA
27. SAPONARO CONCETTA
28. SAVERINO DANIELE
29. SCOTTO MARCO
30. SERAFIN VALENTINA
31. SORRENTINO GIOVANNI
32. TORGE DIANA
33. VERSARI ILARIA



L'Assemblea approva i nominativi che saranno inseriti nell'elenco dei Soci a partire dal 2025.

14) Commemorazione Soci Scomparsi.
Vengono commemorati
Prof. Vincenzo Mitolo (da Prof. Panaro)
Prof. Elena Pompili (da Prof. Fumagalli)
Prof. Elio Raviola (da Prof. Ribatti)
Prof. Alessandro Ruggeri (da Prof. Raspanti)
Prof. Mario Stefanini (da Prof. Filippini)
Prof.ssa Lia Guidotti (da Prof. Di Primio)



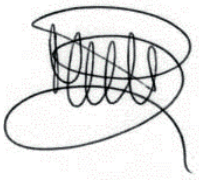
15) Varie ed eventuali.
Nulla da discutere

Essendo esaurito l'OdG, il Presidente dichiara chiusa la seduta alle ore 18:30. Il presente verbale viene, letto approvato e sottoscritto seduta stante.

F.to Il Presidente
Lucio Ildebrando Maria Cocco

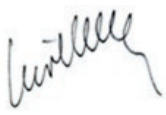


F.to Il Segretario Verbalizzante
Monica Mattioli Belmonte Cima



Allegato N.1

| Nome | Cognome |
|---|---|
| Paola | Alberti |
| Alida | Amadeo |
| Francesco | Amenta |
| Giuseppe | Anastasi |
| Francesca | Arnaboldi |
| Marco | Artico |
| Pasquale | Bandiera |
| Federica | Barbagallo |
| Fulvio | Barbaro |
| Silvia | Barbon |
| Marchi | Barchi |
| Virginia | Barone |
| Desirée | Bartolini |
| Michela | Battistelli |
| Immacolata | Belviso |
| Sara | Bernardi |
| Nunzia | Bernardini |
| Valeria | Bertagnolo |
| Eugenio | Bertelli |
| Francesca | Bianchi |
| Serena | Bianchi |
| Pamela | Bielli |
| Francesca | Boccafoscchi |
| Marina | Boido |
| Antonella | Bonetti |
| Elisa | Borsani |





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| Elisa | Boschetti |
| Rafael | Boscolo |
| Iacopo Junio Valerio | Branca |
| Paola | Brun |
| Silvia | Bruno |
| Alberto | Cacciola |
| Alberto | Caggiati |
| Antonella | Camaioni |
| Elena | Canciani |
| Barbara | Canonico |
| Alfredo | Cappariello |
| Annalisa | Cappella |
| Graziella | Cappelletti |
| Alessandra | Cappellini |
| Francesco | Cappello |
| Gianluca | Carnevale |
| Simone | Carotti |
| Guido | Carpino |
| Cecilia | Carubbi |
| Caruso Bavisotto | Celeste |
| Arianna | Casini |
| Clotilde | Castaldo |
| Sergio | Castorina |
| Roberta | Castriconi |
| Amelia | Cataldi |
| Guido | Cavaletti |
| Gabriele | Ceccarelli |
| Maria Paola | Cecchini |
| Ludovica | Ceci |
| Eleonora | Ceneri |





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| Antonio | Centofanti |
| Flaminia | Chellini |
| Sara | Chiappalupi |
| Antonia | Cianciulli |
| Pasquapina | Ciarmela |
| Giovanni | Cirillo |
| Lucio Ildebrando Maria | Cocco |
| Michelangelo | Cordenonsi |
| Katia | Cortese |
| Vanessa | Cossu |
| Gabriella | Cusella |
| Giuseppina | Cutroneo |
| Velia Maria | D'Agata |
| Agata Grazia | D'Amico |
| Paolo | De Blasiis |
| Raffaele | De Caro |
| Antonio | De Luca |
| Ciro | De Luca |
| Elena | De Santis |
| Alessia | De Stefano |
| Mariella | Della Chiesa |
| Ylenia | Della Rocca |
| Claudia Paola Bruna | Dellavia |
| Silvia | Di Agostino |
| Valentina | Di Felice |
| Viviana | Di Giacomo |
| Orsola | Di Martino |
| Franca | Di Meglio |
| Roberta | Di Pietro |
| Roberto | Di Primio |





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|---|---|
| Gabriele | Di Sante |
| Anna | Di Vito |
| Gabriella | Dobrowolny |
| Claudia | Dolci |
| Susanna | Dolci |
| Alessandra | Dondero |
| Elena Bianca | Donetti |
| Orlando | Donfrancesco |
| Miriam | Durante |
| Mariella | Errede |
| Camilla | Evangelisti |
| Cinzia | Fabrizi |
| Irene | Faenza |
| Franco | Fais |
| Elisabetta | Falcieri |
| Mirella | Falconi |
| Paola | Falletta |
| Giuseppe | Familiari |
| Angelo | Favaloro |
| Gaia | Favero |
| Francesco | Fazi |
| Antonietta | Fazio |
| Michela | Ferrucci |
| Antonio | Filippini |
| Roberta | Fiume |
| Matilde Y. | Follo |
| Antonio | Franchitto |
| Nicoletta | Gagliano |
| Francesco Maria | Galassi |
| Alessandro | Galgani |





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| Marta | Gatti |
| Eugenio | Gaudio |
| Marco | Gesi |
| Claudia | Giampietri |
| Daniele Maria | Gibelli |
| Matteo | Giovarelli |
| Giuliana | Gobbi |
| Silvia | Grassilli |
| Paola | Grimaldi |
| Giulia | Guarnieri |
| Germano | Guerra |
| Massimo | Gulisano |
| Dolaji | Henim |
| Foteini Dionysia | Koufi |
| Paola | Lenzi |
| Dario Domenico | Lofrumento |
| Claudia | Lombardo |
| Carla | Loreto |
| Angela | Lucariello |
| Veronica | Macchi |
| Guido | Macchiarelli |
| Angela Bruna | Maffione |
| Romina | Mancinelli |
| Francesca | Mancuso |
| Mirko | Manetti |
| Lucia | Manzoli |
| Tullia | Maraldi |
| Emanuela | Marcenaro |
| Saverio | Marchi |
| Marco | Marchisio |





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|---|---|
| Giulia Adalgisa | Mariani |
| Marianna | Marino |
| Antonella | Marino Gammazza |
| Paola Lorena | Marmiroli |
| Sandra | Marmiroli |
| Maria Vittoria | Marvi |
| Daniela | Marzioni |
| Silvia | Masciarelli |
| Elena | Masselli |
| Letizia | Mattii |
| Monica | Mattioli Belmonte |
| Grazia | Maugeri |
| Cristina | Meregalli |
| Fabrizio | Michetti |
| Selenia | Miglietta |
| Alba | Migliorato |
| Demetrio | Milardi |
| Anthea | Miller |
| Prisco | Mirandola |
| Stefania | Montagnani |
| Andrea | Montella |
| Manuela | Monti |
| Annamaria | Morelli |
| Gabriele | Morucci |
| Claudia | Moscheni |
| Giuseppe | Musumeci |
| Luca Maria | Neri |
| Irene | Neri |
| Vanessa | Nicolin |
| Giulio | Nittari |





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| Stefania Annarita | Nottola |
| Paolo | Onori |
| Monia | Orciani |
| Francesca | Orofino |
| Fulvia | Ortolani |
| Diletta | Overi |
| Paolo | Pacca |
| Alessandra | Pacini |
| Francesca | Paganelli |
| Carla | Palumbo |
| Maria Antonietta | Panaro |
| Luigi | Pannarale |
| Stefano | Papa |
| Veronica | Papa |
| Federica | Papaccio |
| Martina | Parigi |
| Ferdinando | Paternostro |
| Carolina | Pellegrini |
| Gaia | Pellegrini |
| Simona | Pergolizzi |
| Angelica | Perna |
| Laura | Pierdomenico |
| Carmelo | Pirri |
| Simona | Pompili |
| Chiara | Porro |
| Andrea | Porzionato |
| Giulia | Pozzi |
| Gianpaolo | Papaccio |
| Marina | Protasoni |
| Marina | Quartu |





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|---|---|
| Fabio | Quondamatteo |
| Vittoria | Rago |
| Giulia | Ramazzotti |
| Rosa Alba | Rana |
| Francesca | Rappa |
| Mario | Raspanti |
| Stefano | Ratti |
| Silvia | Ravera |
| Michela | Relucenti |
| Mario | Rende |
| Filippo | Reno |
| Rita | Rezzani |
| Domenico | Ribatti |
| Giulia | Ricci |
| Francesca | Riuzzi |
| Federica | Riva |
| Manuela | Rizzi |
| Giuseppina | Rizzo |
| Arianna | Romani |
| Veronica | Romano |
| Irene | Rosa |
| Alessandra | Ruggeri |
| Larisa | Ryskalin |
| Anna Maria | Sacco |
| Sara | Salucci |
| Silvia | Salucci |
| Silvia | Sancilio |
| Antonietta | Santoro |
| Chiara | Sassoli |
| Daniele | Saverino |





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|---|---|
| Federica | Scalia |
| Roberta | Schellino |
| Arianna | Scuteri |
| Valentina | Serafin |
| Maria Pina | Serra |
| Claudio | Sette |
| Roberta | Sferra |
| Chiarella | Sforza |
| Mariaconcetta | Sicurella |
| Pasquale | Simeone |
| Simona | Sivori |
| Valeria | Sogos |
| Paola | Soldani |
| Michele | Sommariva |
| Guglielmo | Sorci |
| Maria Alessandra | Sotgiu |
| Alessandra | Stacchiotti |
| Serena | Stanga |
| Carla | Stecco |
| Elena | Stocco |
| Marta Anna | Szychlinska |
| Carlo | Tacchetti |
| Luca | Tamagnone |
| Samanta | Taurone |
| Seyed khosrow | Tayebati |
| Gabriella | Teti |
| Virginia | Tirino |
| Daniele | Tomassoni |
| Diana | Torge |
| Enea | Traini |



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|---|---|
| Oriana | Trubiani |
| Marcello | Trucas |
| Rosa | Vaccaro |
| Alessia | Ventura |
| Alessandro | Vercelli |
| Giovanna | Vermiglio |
| Ilaria | Versari |
| Assunta | Virtuoso |
| Maria Teresa | Viscomi |
| Giorgio | Vivacqua |
| Rebecca | Voltan |
| Piero Antonio | Zecca |
| Sandra | Zecchi |
| Maria | Zingariello |





BILANCIO CONSUNTIVO 2023



**Bilancio consuntivo anno 2023 ALLEGATO N.2**

| CAUSALE DELLE ENTRATE | ENTRATE Euro | CAUSALE DELLE USCITE | USCITE Euro |
|---|---|---|---|
| Quote sociali incassate nel corso dell'anno 2023 (n°377) incluse le quote arretrate, le quote incassate non al netto e in attesa di integrazioni e le quote non riconducibili allo stato di alcun Socio | € 22.860,00 | **Elenco spese per attività statutarie** | |
| Incasso Contributi per stampa articoli (.14) su IJAE | € 3.500,00 | | |
| | | Quota di Iscrizione al Congresso SIAI 2023 di n.1 Socio vincitore del premio poster | € 265,00 |
| | | Premio (2) Ricercatori under 40, anno 2023 | € 4.000,00 |
| | | Rimborso Spese Dott.ssa A. Virtuoso, anno 2023 | € 318,68 |
| | | Contributo IFAA, anno 2023 | € 300,00 |
| | | Spese varie (mantenimento conto corrente bancario, spese bollo e commissioni bancarie ecc.), anno 2022 | € 975,57 |
| | | Contributo liberale società SII | € 500,00 |
| | | Contributo Congresso Modena-Reggio Emilia, anno 2023 | € 5.000,00 |
| | | Quota associativa EFEM, anno 2023 | € 450,00 |
| | | Spese sito web (FILARETE), anno 2023 | € 175,69 |
| | | Borsa di Studio Dr. I. Neri | € 10.000,00 |
| | | **Elenco spese di funzionamento** | |
| | | Spese per il funzionamento del Consiglio Direttivo, anno 2023 | € 1.830,00 |
| **TOTALE DELLE ENTRATE** | **€ 26.360,00** | **TOTALE DELLE USCITE** | **€ 23.814,94** |

| | | | |
|---|---|---|---|
| **AVANZO DELL'ESERCIZIO FINANZIARIO 2023** | **€ 2.545,06** | | |

| CAUSALE DELLE ENTRATE | ENTRATE Euro | CAUSALE DELLE USCITE | USCITE Euro |
|---|---|---|---|
| Saldo Conto Corrente Bancario al 31/12/2022 | € 39.385,04 | | |
| TOTALE SALDO FINANZIARIO AL 31/12/2022 | € 39.385,04 | | |
| AVANZO DELL'ESERCIZIO FINANZIARIO 2023 | € 2.545,06 | | |
| SALDO FINANZIARIO AL 31/12/2023 | € 41.930,10 | | |
| STANZIAMENTI IMPEGNATI AL 31/12/2023 | | | Euro |
| Spese per il funzionamento della Segreteria, Tesoreria e Presidenza, anno 2023 | | | € 6.000,00 |
| TOTALE IMPEGNO DI SPESA | | | 6.000,00 |
| SALDO DISPONIBILE al 31.12.2023 | € 35.930,10 | | |

**Relazione di accompagnamento al rendiconto economico e finanziario per l'anno 2023**

Come risulta dal bilancio consuntivo, il saldo finanziario al 31/12/ 2023 è pari ad **€ 41.930,10**

A tale importo devono essere sottratti **€ 6.000,00** impegnati nel bilancio previsionale del 2023, ma non ancora effettivamente utilizzati alla data del 31/12/2023, per le seguenti voci di spesa:

- **Spese per il funzionamento della Segreteria, Tesoreria e Presidenza, anno 2023: € 6.000,00;**

Pertanto l'anno 2023 si chiude con un **saldo disponibile di € 35.930,10**

Durante il 2023, le quote associative incassate sono state soltanto 377 comprese alcune quote arretrate ed integrazioni di versamenti di quote non corretti, per un totale di **€ 22.860,00**, che sommate sia all'avanzo (incasso) delle quote liberali (dei contributi) per la stampa degli articoli pubblicati sull'IJAE pari ad **€ 3.500,00** sia al saldo finanziario al 31/12/2022 pari ad **€ 39.385,04**, hanno dato la disponibilità di **€ 65.745,04.**

Le entrate hanno permesso di coprire sia le spese previste che non previste, includendo i fondi impegnati e non erogati.

La rispondenza dei Soci in merito alla regolarizzazione dei pagamenti delle quote associative è stata buona tenuto anche conto che la maggioranza dei soci sono in regola con i versamenti delle quote arretrate tutte raccolte durantegli anni precedenti. Rimane ancora un piccolo numero di Soci che debbono regolarizzare la loro posizione, che è stabilmente bassa grazie alla regolarizzazione delle quote. Il Tesoriere sottolinea che il pagamento delle quote da parte dei Soci deve essere puntuale, ad inizio di ciascun anno solare, in modo da consentire alla SIAI di effettuare una adeguata programmazione delle attività statutarie e di intraprendere nuove iniziative.

Va anche messo in conto che nell'anno 2024 la quota era ancora pari a 60,00 EUR mentre attualmente è stata portata ad 80,00 EUR, cosa che permetterà di ope[illegible] ancora meglio in favore dei soci.

rare

Il Tesoriere
Prof. Gianpaolo Papaccio

Firmato digitalmente da Gianpaolo Papaccio
Data: 29.07.2024 12:29:24 CEST

PREVISIONE FINANZIARIA ANNO 2025



| | | |
|---|---|---|
| Quota adesione all'European Federation for Experimental Morphology, EFEM, anno 2025 | € | 500,00 |
| Quota adesione all'International Federation of Anatomical Associations, IFAA, anno 2025 | € | 400,00 |
| Spese varie (bancarie, necrologi, etc.), anno 2025 | € | 1.200,00 |
| Spese impreviste, anno 2025 | € | 2.000,00 |
| ***Totale spese per attività statutarie*** | ***€*** | ***35.800,00*** |

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|---|---|---|
| Spese per il funzionamento della Segreteria, Tesoreria, Presidenza e Consiglio Direttivo | € | 6.000,00 |
| ***Totale spese di funzionamento*** | ***€*** | ***6.000,00*** |
| **Totale Uscite** | **€** | **41.800,00** |

## Previsione finanziaria anno 2025

| | |
|---|---|
| **SOCI NEL 2023:** | **441** |
| **SOCI NEL 2024:** | **465** |
| **SOCI ORDINARI 2024:** | **447*** |
| *compresi n. 35 nuovi Soci ratificati all' Assemblea di settembre 2023 | |
| **SOCI DIMISSIONARI/CANCELLATI/DECEDUTI 2024:** | **11** |
| **SOCI EMERITI/ONORARI:** | **18** |

| | | | |
|---|---|---|---|
| Quote Sociali anno 2024 | 447 | € | 35.760,00 |
| Quote Sociali arretrate 2021 – 2024 | | € | 1.040,00 |
| Contributi liberali per pubblicazione lavori scientifici su Italian Journal of Anatomy and Embryology | | € | 5.000,00 |
| **Totale Entrate** | | € | **41.800,00** |

**USCITE**

| | | |
|---|---|---|
| Contributo al 78° Convegno Nazionale 2025, atti di convegni, altri contributi a convegni, partecipazione a convegni, organizzazione eventi scientifici, borse di studio, etc. | € | 15.000,00 |
| Accantonamento per premi poster dell'anno 2025 e per premio alla migliore comunicazione orale assegnati nell'anno 2025 | € | 3.000,00 |
| Accantonamento per premi SIAI (Premio alla Carriera e n. 2 Premi Ricercatori under 40), anno 2025 | € | 4.200,00 |
| Contributo alla Firenze University Press per pubblicazione lavori scientifici su Italian Journal of Anatomy and Embryology, anno 2025 | € | 6.000,00 |
| Spese per sito web della Società, anno 2025 | € | 3.000,00 |
| Contributo GISN anno 2025 | € | 500,00 |

**Relazione di accompagnamento alla previsione finanziaria per l'anno 2024**

La chiusura del bilancio consuntivo del 2023 con un saldo disponibile di **€ 53.653,22** ha permesso al Tesoriere di sostenere alcune spese indicate nella Previsione Finanziaria del 2024.

Al 31 agosto 2024, sono state incassate 361 quote sociali comprensive di quelle relative all'anno in corso e arretrate (dal 1° settembre 2023 al 31 agosto 2024).

Al 31 agosto 2024, il totale delle entrate è pari a **€ 67.766,47** e comprende le quote riscosse finora. Il piano previsionale del 2024 prevedeva entrate pari a **€ 41.000,00**, dovute alla riscossione delle quote dell'anno in corso, più una cifra forfettaria concernente il recupero delle quote arretrate. In particolare in tale previsione, è stata indicat- questa cifra sulla base dell'esperienza degli anni precedenti, tenuto anche conto del fatto che, grazie al monitoraggio costante ed alla decadenza dei soci non in regola per più di 2 anni consecutivi, vi è sostanziale stabilità finanziaria.

La Società conta attualmente 465 Soci, di cui 447 Soci Ordinari e 18 Soci Emeriti o Onorari (esonerati dal pagamento della quota Sociale). Nel corso del 2023 ad oggi (31 agosto 2024), n. 11 Soci sono stati cancellati poiché decaduti o hanno espresso la volontà di rassegnare le dimissioni dalla Società.

Allo stato attuale, dei 447 Soci Ordinari che sono tenuti a pagare la quota associativa:

- 10 Soci sono in regola fino al 2025;
- 313 Soci sono in regola fino al 2024;
- 63 Soci sono in regola fino al 2023, devono la quota 2024;
- 16 Soci sono in regola fino al 2022, devono le quote 2023 e 2024;
- 45 Soci sono in regola fino al 2021, devono le quote 2022, 2023 e 2024.

Il Tesoriere fa presente che la parità di bilancio potrà essere raggiunta con spese contenute e che le previsioni non si discostano dalla realtà.

Il Tesoriere fa inoltre presente che, essendo di molto diminuiti i Soci morosi e che l'ammontare delle quote arretrate si è considerevolmente ristretto, per cui la Società, è in grado di raggiungere gli scopi sociali ed aumentare le liberalità in favore dei giovani.

Il Tesoriere

Prof. Gianpaolo Papaccio

Firmato digitalmente da Gianpaolo Papaccio
Data: 03.09.2024 13:31:38 CEST

**VERBALE DELLA RIUNIONE DEI REVISORI DEI CONTI DEI BILANCI DELLA SIAI**

Il giorno 13.09.2024 alle ore 13.00, presso i locali dell'Albergo dei Poveri, Università di Genova, Piazza Emanuele Brignole, Genova, i Revisori dei Conti dei Bilanci della SIAI, nelle persone dei proff. Marina Quartu, Virginia Tirino e Angelo Favaloro, si sono riuniti per esaminare i documenti ricevuti in data 09.09.2024 via mail. Dopo attento esame, i proff. Quartu, Tirino e Favaloro hanno ritenuto approvare senza riserve i documenti così come inviati.

La riunione termina alle ore 14.

Genova, 13.09.2024

Prof.ssa Marina Quartu _____

Prof.ssa Virginia Tirino _____

Prof. Angelo Favaloro _____

SOCI DECADUTI n. 33

- **SULLA BASE DELL'ART 15 DELLO STATUTO**





| Numero | Cognome | Nome | Sede | E-Mail |
|---|---|---|---|---|
| 1 | Barbatelli | Giorgio | Dip. di Medicina Sperimentale e Clinica, Sez. Neuroscienze e Biologia Cellulare | g.barbatelli@univpm.it |
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| 9 | Barbiera | Alessandra | UniCatt. | alessandra.barbiera@unicatt.itt |
| 10 | Dell'Orbo | Carlo | DIP. DI MEDICINA E CHIRURGIA. Lab. Morfol. Umana "L. Cattaneo" | carlo.dellorbo@gmail.com |
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| 13 | Ferri | Gianluca | Dip. Citomorfologia, Cittadella Universitaria, Università degli Studi di Cagliari | ferri@unica.it |
| 14 | Follenzi | Antonia | Dipartimento di Scienze della Salute dell'Università del Piemonte Orientale | antonia.follenzi@uniupo.it |
| 15 | Galli | Carlo | Dipartimento di Medicina e Chirurgia - Università di Parma | carlo.galli1@unipr.it |
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| 17 | Leonardi | Luisa | Dipartimento di Scienze Anatomiche Umane e Fisiopatologia dell'Apparato Locomotore | Luisa.Leonardi@unibo.it |
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| 30 | Spinoso | Giulio | Dipartimento di Biomedicina, Neuroscienze e Diagnostica Avanzata | giulio.spinoso@gmail.com |
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All. 3
Attività Coll. Istol.Embriol.

Collegio dei Docenti di Istologia ed Embriologia Umana

Società Scientifica riconosciuta dal Ministero della Salute

**22 e 23.2.2024** — **XXIII Convegno Scientifico ed soci- Roma**

**Assemblea** — **IV Edizione Premio Monesi-Rizzoli+ aggiuntivi (3)**

**premi** — **Riconferma cariche sociali 24/27**

**Nomina Collegio Probiviri**

# SURVEY

**BASI PRE CLINICHE DELLA DISCIPLINA ED IMPATTO**





**7.11.24**

**SEMINARI SCIENTIFICI NUOVI PO ED**
**ASSEMBLEA STRAORDINARIA**



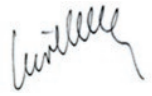


**N. 4 riunioni Giunta**



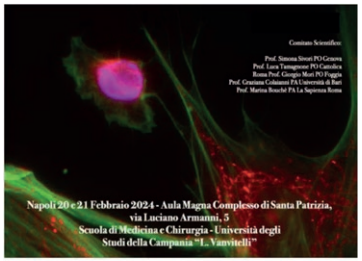


**Organizzazione XXIV Convegno**
**Napoli 20 e 21.2.2024**





XXIV CONVEGNO ED ASSEMBLEA
COLLEGIO DEI DOCENTI DI ISTOLOGIA ED EMBRIOLOGIA UMANA
Società Scientifica riconosciuta dal Ministero della Salute



# 20/21.2.24 Napoli

- **MAIN TOPICS:**
- Tissue homeostasis and regeneration: understanding normal tissues to prevent and treat degenerative diseases
- Modeling normal and pathological tissues in vitro: organoids and biomaterials
- The histology of Cancer: novel investigation approaches and therapeutic opportunities
- Imaging in the era of deep molecular analyses at single cell level
- Understanding the unfolding of Human development: from the knowledge of basic processes to developmental disorders.





- **ATTIVITA' DEL COLLEGIO**





**ALLEGATO 4**

**Intervento di Andrea Montella Presidente del Collegio dei Docenti di Anatomia Umana occasione della Assemblea SIAI di Genova 2024**

Nel corso dell'ultimo anno, Il Collegio dei Docenti di Anatomia Umana ha svolto un'intensa attività nell'ambito delle proprie competenze istituzionali, continuando ad affrontare le tematiche relative a:

1) Aggiornamenti Legislativi d'interesse per il settore;
2) Numerosità Docenti BIOS/12A e ulteriori sondaggi;
3) Ambiti del corpus dottrinale proprio dell'Anatomia Umana;
4) ASN;
5) Aggiornamenti dai gruppi di lavoro;
6) Aggiornamenti dall'Intercollegio di area medica;
7) Accesso al Corso di Laurea i Medicina e Chirurgia;
8) Aggiornamenti attività sale settorie e Legge 10/2020:
9) Programmazione attività future.

In particolare, con riferimento alle attività parlamentari in corso, relative all'accesso al Corso di Laurea in Medicina e Chirurgia e alla possibilità che la Anatomia Umana possa essere ricompresa tra le discipline propedeutiche per l'accesso ai corsi di area di scienze della salute, tutti gli interventi succedutisi in assemblea hanno manifestato importanti perplessità e hanno sottolineato, anche in base agli elementi presenti nella declaratoria, la necessità di operare affinché questa evenienza non si realizzi.

In questo senso il presidente ha chiesto e ottenuto dalla Assemblea l'autorizzazione a poter rappresentare questa volontà del Collegio nelle sedi opportune se necessario e in relazione alla evoluzione dei lavori parlamentari.

Infine, si sottolinea che la partecipazione straordinariamente numerosa delle Colleghe e dei Colleghi nell'assemblea di Roma del 20 aprile 2024 e nell'ultima assemblea del Collegio in occasione del Congresso SIAI del 12 Settembre 2024, testimonia il gradimento del lavoro finora svolto dal Collegio.





**Intervento scritto di Andrea Montella Presidente del Collegio dei Docenti di Anatomia Umana al Direttivo SIAI del 30 novembre 2024**

Successivamente all'assemblea del Collegio in occasione del Congresso SIAI del 12 Settembre 2024, l'attività del Collegio è proseguita informando tutti i docenti di I e II fascia sui seguenti punti di interesse:

Questionario per assistenza e odontoiatria

DM costituzione gruppo di lavoro per riforma 240

Atti commissione senato per accesso CdL Medicina e Chirurgia

DM per proroga commissione ASN e concorsi ex art. 24

DM piano straordinario RU

DM gruppo di lavoro accesso ai Corsi di Scienze della Salute

Con riferimento a quest'ultimo punto, in relazione alla evoluzione delle attività parlamentare e in considerazione della nomina da parte del Ministro dell'Università e della Ricerca, Sen. Anna Maria Bernini, del Gruppo di Lavoro per lo svolgimento di attività di supporto per l'elaborazione di proposte, il direttivo del Collegio ha ritenuto opportuno formalizzare per iscritto le volontà espresse a tal proposito dall'assemblea del Collegio nella riunione di Genova.
Il 25 novembre 2024 si è riunito il direttivo del Collegio dei Docenti di
Anatomia Umana ed è stata redatta la lettera sotto riportata, inviata al Prof Andrea Lenzi.





# Collegio dei Docenti di Anatomia Umana

Sassari, 25.11.2024

Al Chiar.mo
Prof. Andrea Lenzi
Presidente del Gruppo di Lavoro
per l'accesso e formazione ai corsi di studio area Scienze della Salute

Caro Presidente,

Oggi 25 novembre 2024 si è riunito il direttivo del Collegio dei Docenti di Anatomia Umana (Gruppo Scientifico Disciplinare 05/BIOS-12) per discutere la posizione degli Anatomici italiani relativamente alle prospettive aperte dal decreto delega sulle modifiche dell'accesso ai corsi di laurea di area di Scienze della Salute.

Con riferimento anche a quanto già discusso e concordato nell'ultima assemblea del Collegio tenuta a Genova nel settembre 2024, il direttivo del Collegio ha rivalutato tutta la documentazione relativa ai lavori parlamentari della 7ª Commissione Istruzione del Senato su questo specifico argomento, a partire dall'audizione del 30 gennaio c.a. fino all'ultima seduta del 16 ottobre.

In considerazione della nomina da parte del Ministro dell'Università e della Ricerca, Sen. Anna Maria Bernini, del Gruppo di Lavoro per lo svolgimento di attività di supporto per l'elaborazione di proposte, il direttivo del Collegio intende fornire il suo contributo in relazione al coinvolgimento del proprio settore nel semestre propedeutico all'accesso ai corsi di laurea di area di Scienze della Salute.

Dopo approfondita discussione, considerati gli atti parlamentari, considerato quanto presente nella declaratoria del GSD Anatomia Umana, considerato il pronunciamento dei colleghi anatomici nell'ultima assemblea nazionale di Genova, sentito anche il Prof. Lucio I. Cocco, Presidente della Società Italiana di Anatomia e Istologia, il Collegio dei Docenti di Anatomia Umana dichiara il proprio parere contrario al coinvolgimento del GSD Anatomia Umana negli insegnamenti propedeutici del primo semestre.

Infatti, la complessità del proprio corpus dottrinale non permette una sua frammentazione senza determinarne una riduzione delle potenzialità formative proprie del settore. In aggiunta, la sua specifica identificazione con le scienze della salute umana non permette compatibilità con corsi di area veterinaria e, conseguentemente, non può fornire crediti in quell'area.

Per quanto sopra, il direttivo del Collegio dei Docenti di Anatomia Umana, chiede al Gruppo di lavoro di tenere adeguatamente conto di questo parere, maturato con grande attenzione e con lo scopo di contribuire costruttivamente ad una migliore e più funzionale riforma dell'accesso ai corsi di laurea di Scienze della Salute e in particolare a quello di Medicina e Chirurgia, nell'interesse primario degli studenti che intendono intraprendere questo percorso di studi universitari, capace di formare professionisti, qualitativamente e quantitativamente adeguati, idonei ad assicurare le indispensabili risposte ai fabbisogni di salute dei cittadini della nostra Nazione, coerentemente con gli obiettivi delle riforme avviate dal Governo.

Prof. Andrea Montella
Presidente del Collegio dei Docenti di Anatomia Umana

**ALLEGATO 5**

**COMMISSIONE PER L'ATTRIBUZIONE DEL PREMIO ALLA CARRIERA**

**DELLA SOCIETA DI ANATOMIA E ISTOLOGIA (SIAI)**

Il giorno 3 settembre 2024 alle ore 14.30 in modalità telematica (https://teams.microsoft.com/l/meetup-join/19%3ameeting_Zjk2NmI2MzItODdhNy00MmZhLWI2NzctYzAyNjZiMzZjNjc5%40thread.v2/0?context=%7b%22Tid%22%3a%22117b418d-fb21-416f-a85f-1e9ff725bf2c%22%2c%22Oid%22%3a%22e0cb2fad-9ff6-4f39-80ba-0bab7874ee88%22%7d) si è riunita la Commissione preposta per l'attribuzione del Premio alla Carriera della Società di Anatomia e Istologia (SIAI) 2024, nominata nel Consiglio Direttivo del 12 giugno u.s.

La Commissione risulta composta dalle Proff.sse Monica Mattioli Belmonte, Carla Palumbo e Oriana Trubiani.

Assume la Presidenza la Prof. Oriana Trubiani. Svolge le funzioni di Segretario la Prof. Monica Mattioli Belmonte.

La Commissione prende atto che entro il termine prefissato sono giunte due proposte: Prof. Francesco Amenta e Prof. Raffaele De Caro, entrambi del GSD 05/BIOS 12A (SSD BIO/16) - Anatomia Umana

La commissione prende visione dei CV presentati che vengono ritenuti entrambi di notevole valore scientifico didattico nell'ambito delle discipline morfologiche.

In considerazione dei ruoli di rappresentanza ricoperti nell'ambito della Società di Anatomia e Istologia, la Commissione, unanime, propone l'assegnazione del Premio alla Carriera della Società al Prof. Raffaele De Caro.

La Commissione conclude i lavori alle ore 15.00 e redatto il presente verbale lo trasmette al Presidente della SIAI.

Letto, approvato e sottoscritto seduta stante

03/09/2024

Il Segretario

Prof. Monica Mattioli Belmonte

Firmato digitalmente da Monica Mattioli Belmonte Cima
Data: 03.09.2024 15:02:08 CEST
Organizzazione: UNIVERSITA' POLITECNICA DELLE MARCHE/00382520427

**ALLEGATO 6**

**VERBALE DELLE RIUNIONI DEL COMITATO SCIENTIFICO PER L'ATTRIBUZIONE DI NUMERO 2 PREMI AI RICERCATORI "UNDER 40"**

Il Comitato Scientifico, nelle persone di:

Prof.ssa Emanuela Marcenaro (in qualità di Presidente)
Prof.ssa Bianca Maria Scicchitano (in qualità di Componente)
Prof.ssa Matilde Yung Follo (in qualità di Componente)
Prof. Antonio De Luca (in qualità di Componente)
Prof. Guido Carpino (in qualità di Segretario)

si è riunito telematicamente (via TEAMS) giovedì 25 luglio 2024 per valutare le Candidature per l'assegnazione dei 2 Premi ai ricercatori "under 40".

Sono pervenute n. 4 candidature, nelle persone di:

Dott.ssa Carolina Pellegrini (presentata dalla Prof.ssa Nunzia Bernardini)
Dott.ssa Caterina Franco (presentata dalla Prof.ssa Rita Rezzani)
Dott.ssa Celeste Caruso Bavisotto (presentata dal Prof. Francesco Cappello)
Dott.ssa Cristina Meregalli (presentata dalla Prof.ssa Marina Quartu)

Il Comitato ha dapprima valutato la posizione dei Candidati rispetto al pagamento delle quote di iscrizione alla SIAI e ha rilevato che tutte le Candidate sono in regola.

Successivamente, il Comitato ha valutato attentamente i curricula delle Candidate, le loro pubblicazioni selezionate, gli indici bibliometrici specificati nel Bando, le attività in qualità di PI in progetti di ricerca nazionali e/o internazionali che prevedano la revisione tra pari; gli eventuali brevetti accettati almeno in EUROPA (PCT); ed eventuali altri titoli di qualità e non di quantità.

Alla fine di tale valutazione, il Comitato propone di assegnare all'unanimità i 2 Premi a:

**Dott.ssa Carolina Pellegrini**
La Dott.ssa Carolina Pellegrini svolge la sua attività di ricerca focalizzandosi sulla caratterizzazione morfologica e molecolare dei sistemi di regolazione neuro-immunitari e del sistema purinergico. Il suo lavoro esplora come questi sistemi influenzino le funzioni neuro-muscolari intestinali, sia in condizioni normali che patologiche.

**Dott.ssa Celeste Caruso Bavisotto**
La Dott.ssa Celeste Caruso Bavisotto concentra la sua attività di ricerca sull'isolamento e la caratterizzazione di nanovescicole derivate da cellule e tessuti di mammiferi. I suoi studi comprendono vari modelli sperimentali, tra cui quello carcinogenetico, mirati a comprendere meglio il ruolo e il potenziale terapeutico di queste nanovescicole.

Addì, 25 luglio 2024

Prof.ssa Emanuela Marcenaro

Prof.ssa Bianca Maria Scicchitano

Prof.ssa Matilde Yung Follo

Prof. Antonio De Luca

Prof. Guido Carpino

ALLEGATO 7

Bando Borsa di Studio SIAI intitolata ai Proff. Carlo Enrico Grossi e Alessandro Moretta

BANDO PER BORSA DI STUDIO DELLA SOCIETA' ITALIANA DI ANATOMIA E ISTOLOGIA (SIAI)

Articolo 1

Tema dell'iniziativa

La Società Italiana di Anatomia ed Istologia (SIAI) ha istituito nel 2022 una Borsa di Studio del valore di Euro 10.000,00 di durata semestrale, da svolgersi presso prestigiose Istituzioni estere. La SIAI garantirà inoltre una indennità di viaggio di importo non superiore ad Euro 1.000,00 a fronte della esibizione dei titoli di viaggio. La prima edizione della Borsa è stata intitolata al Prof. Francesco Antonio Manzoli e la seconda al Prof. Giovanni Orlandini. La terza, a partire dal 1° Febbraio 2025, sarà intitolata ai Proff. Carlo Enrico Grossi e Alessandro Moretta. Possono partecipare candidati che posseggano i requisiti esposti nell'Articolo 2.

Le tematiche di ricerca, che faranno parte del progetto presentato da ciascun candidato, dovranno essere coerenti con quelle delle discipline che costituiscono la Società Scientifica (Anatomia ed Istologia). Qualora vengano prodotte pubblicazioni scientifiche nell'ambito del progetto, sarà obbligatorio citare la SIAI nei ringraziamenti.

Articolo 2

Requisiti per la partecipazione

Possono partecipare alla selezione:

– i dottorandi di ricerca iscritti a Dottorati con settori riconducibili alla Società Italiana di Anatomia e Istologia;

-i dottori di ricerca con titolo conseguito in Dottorati con settori riconducibili alla Società Italiana di Anatomia e Istologia

-gli assegnisti di ricerca;

-i borsisti;

-i ricercatori di tipologia RTD-A, RTT

I candidati debbono inoltre possedere i seguenti requisiti:

-essere iscritti alla SIAI ed essere in regola con i pagamenti della quota sociale sino all'anno di emissione del bando;

-essere stati presentati come nuovi Soci SIAI e approvati dall'Assemblea Generale dei Soci -avere una età non superiore ai 40 anni al momento della scadenza del Bando

-frequentare una struttura universitaria ed afferente alla Anatomia o alla Istologia.

Ciascun candidato deve inoltrare formale lettera di richiesta, con allegato CV in formato europeo, corredato dalle pubblicazioni.

All.8

# REPORT FINALE PER LA BORSA DI STUDIO

# "PROF. GIOVANNI ORLANDINI"

# SIAI - SOCIETÀ ITALIANA DI ANATOMIA E ISTOLOGIA

# PRESENTATO DA: IRENE NERI

**Titolo del progetto: "Effetti della carenza di timidina fosforilasi (TP) sull'anatomia e sulla funzione del sistema vascolare e nervoso enterico"**

**Periodo di svolgimento:** 8 Gennaio 2024 – 3 Agosto 2024
**Luogo:** Zweibrücken, Germania
**Università:** Hochschule Kaiserslautern, Campus Zweibrücken
**Gruppo di lavoro:** ENS Workgroup guidato dal Prof. Karl-Herbert Schäfer

## OBIETTIVO DEL PROGETTO

Il progetto si è concentrato sullo studio degli effetti della carenza di timidina fosforilasi (TP) sui neuroni enterici, con particolare attenzione all'analisi dello sviluppo morfologico dei rami neuronali e alla successiva indagine sui cambiamenti nella comunicazione cellulare. TP è un enzima coinvolto nel metabolismo dei nucleotidi e ha un ruolo significativo nell'angiogenesi, potenzialmente influenzando il sistema nervoso enterico (ENS). Questa ipotesi è stata formulata in seguito ai dati ottenuti sull'Encefalomiopatia NeuroGastroIntestinale Mitocondriale (MNGIE), ma soprattutto sulla Pseudo-Ostruzione Intestinale Cronica (CIPO), condizioni in cui la carenza di TP è stata associata a danni vascolari e neuronali intestinali, con rilevanti cambiamenti micro-anatomici e perdita neuronale.

## ATTIVITÀ SVOLTE E METODOLOGIE UTILIZZATE

Durante il progetto sono state svolte le seguenti attività:

1. **Silenziamento di TP tramite siRNA** in cellule endoteliali murine (bEND3) e valutazione dell'efficacia di silenziamento tramite qPCR e western blot

2. **Isolamento di colture primarie di cellule nervose enteriche (ENC)** dal plesso mienterico di topi post-natali e trattamento per 72 ore con il secretoma delle cellule endoteliali silenziate.
3. **Analisi morfologica e funzionale delle ENC** tramite live imaging, immunofluorescenza e misurazioni dell'attività neuronale acuta e cronica.
4. **Creazione di organoidi epiteliali intestinali (enteroidi)** per studiare l'impatto del secretoma delle cellule endoteliali silenziate sulla proliferazione cellulare, analizzando l'espressione di Ki-67.

## RISULTATI

1. **Disgregazione della rete neuronale nelle colture ENC:** L'esposizione delle colture ENC al microambiente carente di TP ha portato alla disgregazione della rete neuronale e all'aumento del numero di cellule non neuronali, evidenziato dalla presenza di nuclei al di fuori della rete neurale.
2. **Proliferazione di cellule gliali e fibroblasti:** Nelle ENC esposte al microambiente carente di TP per 72 ore, è stato osservato un aumento del numero di cellule gliali (+735%) e fibroblasti (+96%), mentre il numero di neuroni non è stato significativamente influenzato. Ciò suggerisce che la carenza di TP promuove la proliferazione di componenti non neuronali nelle colture ENC.
3. **Gonfiore neuronale e aumento della produzione di nNOS nelle ENC:** Le ENC esposte al microambiente carente di TP hanno mostrato un gonfiore neuronale (+89% nelle dimensioni medie dei corpi cellulari) e un aumento significativo dei livelli di nNOS (+565%), marcatori associati a stress neuronale. I livelli di GFAP, invece, non sono risultati significativamente influenzati.
4. **Effetto progressivo nel tempo:** L'analisi dei marcatori a 24 e 48 ore dopo l'esposizione ha rivelato una modulazione progressiva degli effetti. Ad esempio, l'aumento delle dimensioni dei corpi neuronali è progredito da +29% a 24 ore, +49% a 48 ore fino a +89% a 72 ore. Ciò suggerisce che l'esposizione a lungo termine a un microambiente carente di TP potrebbe portare a cambiamenti significativi nella morfologia neuronale.
5. **Attività neuronale aberrante nelle ENC:** Le registrazioni dell'attività neuronale tramite MEA hanno mostrato che il trattamento acuto delle ENC con il secretoma delle cellule endoteliali silenziate ha portato a un aumento significativo dell'attività neuronale (+200%), mentre l'esposizione cronica per 24 ore ha causato una perdita completa dell'attività (burnout). Questo suggerisce che una prolungata esposizione a TP-silenziamento può portare a una disfunzione irreversibile dell'attività neuronale.





6. **Profilo infiammatorio nel supernatante delle ENC trattate:** È stata rilevata una modulazione significativa di 11 fattori infiammatori nel supernatante delle ENC esposte al microambiente carente di TP, con un aumento di citochine pro-infiammatorie come IFNγ, IL6, IL7, e una riduzione di TIMP-1, una molecola anti-infiammatoria. Questo indica che la carenza di TP potrebbe indurre uno stato infiammatorio nelle colture ENC.
7. **Riduzione della proliferazione negli enteroidi:** Gli enteroidi esposti per 5 giorni al microambiente carente di TP hanno mostrato una riduzione del 47% nella proliferazione, evidenziata dalla diminuzione dell'espressione di Ki-67. Ciò suggerisce che la carenza di segnali derivati dalle cellule endoteliali con TP-silenziamento può compromettere la rigenerazione e la funzionalità dell'epitelio intestinale.

## CONCLUSIONI

Il progetto ha dimostrato che la carenza di TP nelle cellule endoteliali influenza profondamente la morfologia e la funzione del sistema nervoso enterico. In particolare, i risultati ottenuti suggeriscono per la prima volta un possibile meccanismo di azione per spiegare la perdita neuronale osservata in MNGIE e CIPO, attraverso l'induzione di stress neuronale, infiammazione e alterazioni nella comunicazione cellulare. Questi risultati forniscono una base per ulteriori studi sul ruolo della TP nelle interazioni neuro-vascolari e sui meccanismi di stress e infiammazione associati alla carenza di TP. I risultati ottenuti hanno inoltre consentito la futura stesura di un articolo scientifico, la cui sottomissione è prevista entro l'anno solare.

## CONSUNTIVO DELLE SPESE SOSTENUTE

- **Spese di viaggio:** €500
- **Spese di affitto:** €4.900
- **Spese di costo della vita:** €2.800





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