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University of Padua
PhD Courses
Medical and Biomedical Sciences

PhD Course STUDENT RESEARCH PROJECT DAY
MEDICAL AND BIOMEDICAL SCIENCES
(XL Cycle)

Monday, October 5th, 2026

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PhD COURSE "ARTERIAL HYPERTENSION AND VASCULAR BIOLOGY"

COORDINATOR: PROF. TERESA MARIA SECCIA

CT-BASED BODY COMPOSITION ASSESSMENT IN PATIENTS WITH METASTATIC CASTRATION RESISTANT PROSTATE CANCER TREATED WITH ENZALUTAMIDE: A SUB-GROUP ANALYSIS OF THE EORTC 1333 AND SAKK 08/14 CLINICAL TRIALS

Ph.D. Student: Dr. Elena AGOSTINI
TUTOR: Prof. Filippo CRIMI' – CO-TUTOR: Prof.ssa Teresa Maria SECCIA
Ph.D. Course Arterial Hypertension and Vascular Biology

Background
Enzalutamide is one of the standard treatments for metastatic castration-resistant prostate cancer (mCRPC), yet its impact on body composition remains incompletely characterized. Opportunistic CT-based analysis is a validated method for body composition assessment. The primary objective of this study was to evaluate the association between enzalutamide treatments (+/- metformin and radium-223) and changes in muscle mass. Secondary objectives included association between enzalutamide-based treatments and changes of other body composition parameters.

Material and Methods
This study was conducted during a research period abroad at the Ente Ospedaliero Cantonale in Lugano (Switzerland).
CT scans from patients enrolled in the EORTC 1333 (enzalutamide±radium-223) and SAKK 08/14 (enzalutamide±metformin) trials were analyzed at baseline, cycle 4 and cycle 7. Skeletal muscle area (SMA), skeletal muscle index (SMI), visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and bone mineral density (BMD) were quantified. Changes over time and across treatment groups were assessed.

Results
102 patients (61 SAKK 08/14; 41 EORTC 1333) and 306 CT scans were evaluated. We observed a significant downward trend of SMI (median -1.9 cm^2/m^2, p=0.009) and SMA (median -5.9 cm^2, p=0.003) from baseline to cycle 7, although no significant difference in SMI or SMA change was observed between different enzalutamide treatments. VAT decreased significantly from baseline to cycle 7 (−24 cm^2, p=<0.001), with the largest reduction in the enzalutamide+metformin group (−42.8 cm^2). SAT changed significantly only when considering different treatments (p<0.01), showing a slight increase in enzalutamide alone and decrease in combination groups. SMA density, VAT density, SAT density, and BMD showed no significant changes.

Conclusions
A significant reduction in muscle mass and visceral fat was observed over 6 months of enzalutamide-based therapy. This study supports incorporating CT-based body composition assessment in mCRCP to enable early identification of patients at risk for adverse metabolic outcomes.

Opportunistic CT Imaging Biomarkers for Predicting Functional Capacity in Patients Undergoing TAVI

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Ph.D. Course Arterial Hypertension and Vascular Biology

Background: Patients undergoing transcatheter aortic valve implantation (TAVI) routinely undergo preoperative computed tomography (CT) for procedural planning. Beyond anatomical assessment, these examinations contain quantitative information that may serve as opportunistic imaging biomarkers of frailty. This exploratory study investigated whether CT-derived measures of skeletal muscle quantity, muscle quality, and bone density are associated with functional capacity and may improve preoperative risk stratification.

Material and Methods: A total of 297 consecutive patients undergoing TAVI were retrospectively analyzed. Semi-automated CT segmentation was used to extract Psoas Index**,** Hounsfield Unit Average Calculation (HUAC), and L1 trabecular bone density from routine preoperative CT examinations. Associations between CT-derived biomarkers and peak VO_2 obtained from cardiopulmonary exercise testing were assessed using multivariable regression analysis. Receiver operating characteristic (ROC) analysis was performed to evaluate the ability of these biomarkers to identify patients with reduced functional capacity.

Results: All three CT-derived biomarkers were significantly associated with peak VO_2, demonstrating their relationship with functional capacity. Among them, Psoas Index and HUAC showed the highest discriminatory performance for identifying patients with reduced exercise capacity. These findings suggest that routine preoperative CT provides clinically relevant information on frailty without requiring additional imaging or radiation exposure

Conclusions: routine preoperative CT may provide valuable opportunistic biomarkers for functional risk assessment in patients undergoing TAVI. These preliminary findings support further validation in a larger 500-patient cohort, with the ultimate goal of integrating automated CT-derived biomarkers into routine preoperative risk stratification and personalized clinical decision-making

KIDNEY SHEAR WAVE ELASTOGRAPHY FOR RENAL STIFFNESS IN SYSTEMIC HYPERTENSION: A PROSPECTIVE PILOT STUDY

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Ph.D. Course Arterial Hypertension and Vascular Biology

BACKGROUND
Arterial hypertension is a leading cause of chronic kidney disease (CKD), mediating renal damage through sustained glomerular hypertension, ischemia, and progressive interstitial fibrosis, a process collectively referred to as hypertensive nephrosclerosis. Shear wave elastography (SWE) is a non-invasive ultrasound-based technique that quantifies tissue stiffness by measuring shear wave propagation velocity, expressed in kPa. Preliminary evidence suggests that long-standing hypertension and renovascular disease may lead to increased renal parenchymal stiffness, potentially serving as a marker of kidney damage. However, the independent contribution of specific hypertension determinants, including renal perfusion, venous congestion, blood pressure control, and metabolic factors, to renal cortical stiffness remains poorly understood. No studies have systematically assessed renal SWE across different hypertensive subtypes while controlling for multiple relevant covariates. This pilot study aims to investigate the independent determinants of renal parenchymal stiffness, assessed by shear wave elastography, and to compare renal stiffness across different hypertensive subtypes and healthy controls, with the ultimate goal of evaluating its potential as a non-invasive marker of hypertension-related renal organ damage.

MATERIALS AND METHODS
This is s a single-centre, prospective, observational pilot study conducted at the Radiology Institute of the University of Padova. Adult patients (≥18 years) with arterial hypertension undergoing renal artery Doppler ultrasound (DUS) as part of their routine diagnostic workup are prospectively enrolled from May 2026 to January 2029. At the time of DUS, SWE measurements are obtained at 3 sites for each kidney. Standard liver SWE is also recorded as a technical reference. Anthropometric, clinical, and biochemical data are collected from the Hypertensive Clinic reports, including age, sex, BMI, waist circumference, blood pressure, renal function tests, electrolytes, and renin-angiotensin-aldosterone axis assessment. Within the hypertensive cohort, comparisons of renal SWE values will be performed across clinically defined subgroups: essential hypertension, renovascular hypertension (confirmed by DUS and/or CT angiography), and primary aldosteronism (biochemically confirmed). A group of healthy volunteers is taken as a control. We plan to enroll 50 individuals for each group. Associations between imaging biomarkers, clinical characteristics and long-term outcomes will be evaluated using multivariable statistical models.

RESULTS
Patient identification and enrollment are currently ongoing. Several preliminary sessions were carried out on healthy volunteers before the study in the presence of radiological colleagues to ensure reproducible of SWE measurements across the study population. Database construction and image analysis are currently in progress.

CONCLUSIONS
This study will provide a systematic characterization of renal parenchymal stiffness by shear wave elastography. By comprehensively accounting for clinical, hemodynamic, and biochemical covariates, this study will allow the independent determinants of renal cortical stiffness to be identified in a well-characterized hypertensive population. If confirmed, differences in renal SWE across hypertensive subtypes may shed new light on the distinct pathophysiological mechanisms of renal injury in each condition, and support the potential role of renal SWE as a non-invasive imaging biomarker of hypertension-related organ damage.

METABOLIC AND VASCULAR CT PHENOTYPING ACROSS SODIUM HOMEOSTASIS AND PRIMARY ALDOSTERONISM

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Ph.D. Course Arterial Hypertension and Vascular Biology

Background
High-salt diet and primary aldosteronism (PA), a model of aldosterone-driven sodium retention, promote tissue damages and organ fibrosis through oxidative stress, chronic inflammation, mineralocorticoid receptor activation, and micro- and macrovascular dysfunction. These mechanisms provide a biological rationale for a potential contribution of sodium excess and PA to the development of metabolic dysfunction-associated steatotic liver disease (MASLD) and abdominal aortic calcification (AAC). Nevertheless, the prevalence of MASLD and the burden of AAC in these patients, as well as the potential contribution of MASLD to vascular calcification, remain insufficiently characterized. Opportunistic computed tomography (CT) imaging enables the extraction of quantitative imaging biomarkers from scans acquired for unrelated clinical indications, providing additional diagnostic information without additional examinations, radiation exposure or costs. This PhD project aims to investigate the relationships between PA, sodium excess, MASLD and AAC biomarkers through opportunistic CT phenotyping.

Material and Methods
This retrospective, single-centre observational study includes consecutive adult patients with primary aldosteronism or essential hypertension referred to the Hypertension Unit who underwent chest and/or abdominal CT within five years before or after the initial clinical and biochemical assessment. Clinical, biochemical, hormonal and imaging data are retrospectively collected from electronic medical records and the institutional Picture Archiving and Communication System (PACS). CT examinations are screened for technical suitability, including availability of non-contrast axial images and absence of relevant motion artefacts. Hepatic and splenic attenuation are measured using three standardized regions of interest on non-contrast axial CT scans respectively at hepatic and splenic hilum. MASLD is defined as a the liver-to-spleen ratio (L/S ratio) < 1.0. Abdominal aortic calcification (AAC) is quantified using both the Agatston score and total calcified plaque volume (mm3) between infrarenal aorta to iliac artery bifurcation. Associations between imaging biomarkers, sodium intake, clinical characteristics and long-term outcomes will be evaluated using multivariable statistical models.

Results
The study protocol has been developed for data collection. Among 1590 patients screened, 694 had technically suitable CT examinations and were selected for further analysis Standardized methodologies for CT-derived quantification of hepatic steatosis and abdominal aortic calcification have been established to ensure reproducible measurements across the study population. Database construction and image analysis are currently in progress.

Conclusions
Opportunistic CT imaging allows existing scans to be repurposed for the assessment of hepatic and vascular biomarkers without additional examinations, radiation exposure, or costs. Its integration with clinical, biochemical, hormonal, and urinary sodium data may reveal frequently underdiagnosed comorbidities, including MASLD and abdominal aortic calcification, providing a more comprehensive characterization of the metabolic and vascular burden associated with primary aldosteronism and/or excessive sodium intake.

Digital approaches using metadata to ensure accurate laboratory exams in the territory for the management of cardiovascular risk

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Ph.D. Course Arterial Hypertension and Vascular Biology

Background
The evolution of laboratory medicine requires reliable, comparable, and interoperable results across healthcare settings, which requires highly standardized traceability of pre-analytical, analytical and post-analytical phases. At the same time, the increasing complexity of laboratory workflows and ISO 15189:2022 requirements highlight the need for innovative approaches to support documentation standardization, data management, and quality improvement. This PhD project aims to harmonize the Total Testing Process (TTP) of hormonal and metabolic tests and develop artificial intelligence (AI)-based solutions to enhance quality, efficiency, and interoperability in clinical laboratories, reducing human-related workflows.
Material and Methods
The project was developed through complementary research activities. The first activity focused on the development of a modular alphanumeric standard for the structured registration of pre-analytical, analytical and post-analytical variables within the TTP, enabling comprehensive traceability of laboratory procedures through a transferable and machine-readable format. The second activity involved the design of two structured questionnaires to investigate the entire TTP, to assess current laboratory practices for aldosterone and renin measurements. The survey aims to identify the main sources of variability and to provide the basis for future harmonization initiatives involving laboratories affiliated with the Italian Society of Hypertension (SIIA) and the Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC). The third activity focuses on the development and evaluation of AI-based workflows to support laboratory professionals by automating repetitive, time-consuming, and data-intensive activities.
Results
The research activities performed during the PhD project have led to the development of a structured alphanumeric standard for recording and sharing of pre-analytical, analytical and post-analytical conditions across labs, providing a foundation for improved standardization and data interoperability. Two questionnaires for aldosterone and renin testing were developed to collect information on current laboratory practices and to identify critical factors contributing to variability in cardiovascular and endocrine-metabolic diagnostics. In parallel, preliminary activities have been initiated for the implementation of AI-assisted workflows aimed at improving the analytical reliability of laboratory tests and optimizing laboratory processes. Further, AI-powered automated workflows are being evaluated to support the automation of repetitive tasks, reduce manual workload, improve consistency, and facilitate the management of complex quality-related activities.
Conclusions
This PhD project addresses two major challenges in modern laboratory medicine: the need to harmonize the entire TTP beyond the analytical phase and the opportunity to introduce AI as a supportive technology for laboratory professionals. The integration of structured standard systems for pre-analytical, analytical and post-analytical variables with AI-driven workflow optimization represents an innovative approach to improve laboratory result comparability, patient safety and operational efficiency through standardized data collection and technology-assisted quality management.

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PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "EMERGING TECHNOLOGY FOR THE HEALTH PROFESSIONS"

Isometric Hand-Grip Exercise for Preventing Thrombosis and Occlusion of Midline Venous Catheters: A Randomized Controlled Trial

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Ph.D. Course Clinical and Experimental Sciences
Curriculum "Emerging Technology for the Health Professions"

Background Midline venous catheters are increasingly used to provide reliable peripheral venous access for patients requiring medium-term intravenous therapy. Despite their advantages, catheter-related complications, including thrombosis and catheter occlusion, may compromise treatment and require additional interventions. Isometric hand-grip exercise may promote upper-limb muscle contraction and venous flow, potentially reducing venous stasis and the risk of catheter-related complications. This randomized controlled trial aims to evaluate the effectiveness of a structured isometric hand-grip exercise program in preventing thrombosis and occlusion in patients with a Midline venous catheter.

Material and Methods This is a prospective, randomized, controlled, parallel-group, open-label trial involving adult patients aged 18–70 years with a newly inserted Midline venous catheter. Participants are randomly allocated to either an intervention group, receiving usual care plus a structured isometric hand-grip exercise program involving the limb with the Midline catheter, or a control group receiving usual care alone. The primary outcome is the incidence of Midline-related thrombosis and/or catheter occlusion during the study period, assessed according to predefined clinical and instrumental criteria. Secondary outcomes include other catheter-related complications and relevant clinical and procedural variables. The study is being conducted at the University Hospital of Padua and was prospectively registered on ClinicalTrials.gov (NCT07654101). The planned sample size is 840 participants.

Results The study is currently ongoing and participant recruitment started in July 2026. At the time of abstract preparation, data collection is still in progress and no final outcome data are available. Therefore, the present abstract describes the study rationale and methodology rather than reporting efficacy results.

Conclusions This trial will provide evidence on the potential role of a simple, non-pharmacological and low-cost intervention for preventing Midline-related thrombosis and occlusion. If effective, isometric hand-grip exercise could represent an easily implementable nursing intervention to support Midline catheter management and potentially improve patient safety and catheter longevity.

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PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "ENDOCRINE AND METABOLIC SCIENCES AND GENDER MEDICINE"

Antiproliferative and Antimigratory Activity of Licorice Extract and ß- Glycyrrhetinic Acid in Papillary Thyroid Carcinoma Cell Cultures

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TUTOR: Prof. Caterina MIAN – CO-TUTOR: Dr. Loris BERTAZZA
Ph.D. Course Clinical and Experimental Sciences
Curriculum "Endocrine and Metabolic Sciences and Gender Medicine"

Background
Papillary thyroid carcinoma (PTC) accounts for approximately 80–85% of thyroid cancers and generally has a favorable prognosis. Current treatment of PTC includes thyroidectomy, radioactive iodine (RAI), and, in advanced cases, targeted molecular therapies. However, some patients develop metastatic or RAI-refractory disease, for which treatment options remain limited and prognosis is poorer. Therefore, there is a need for new therapeutic strategies for advanced PTC. In this context, licorice (*Glycyrrhiza* spp.) is of particular interest due to its documented antitumor properties, attributed to several bioactive compounds, including glycyrrhizin, licochalcone A, glabridin and β-glycyrrhetinic acid (ß-GA). ß-GA also inhibits 11β-HSD2 and modulates the mineralocorticoid receptor (MR), whose expression has recently been demonstrated also in PTC, suggesting a possible involvement of this pathway in the cellular response. Therefore, this study evaluates the therapeutic potential of the licorice phytocomplex (FC) and β-glycyrrhetinic acid (ß-GA).
Material and Methods
The study is conducted using the BCPAP cell line, a representative model of PTC. Cell viability assays (MTT) are performed to evaluate the cytotoxic effects of FC and β-GA and to determine the concentrations to be used in subsequent analyses. ROS levels are also quantified using the DCFDA probe, and immunofluorescence analyses (IF) are performed to identify and characterize the targets of interest (NRF2, acetylated α-tubulin, GLUT-1, and LAT-1). In the future, NGS analyses will be integrated for transcriptomic profiling, characterization of miRNAs and potential epigenetic modulations, while evaluating the possible involvement of MR.
Results
MTT assays revealed a significant dose-dependent cytotoxic effect for both compounds. FC 50 µg/mL and β-GA 5 µg/mL doses were selected for subsequent analyses, as they induced only a limited reduction in cell viability. ROS quantification showed a trend toward increased intracellular ROS levels following both treatments. Accordingly, NRF2, the main regulator of the cellular antioxidant response, was investigated and IF analysis showed a reduction in its overall signal, accompanied by increased nuclear localization. Both treatments also reduced the acetylated α-tubulin signal in IF, potentially indicating alterations in microtubule stability and intracellular communication. Finally, FC treatment decreased the overall GLUT-1 and LAT-1 IF signals, with GLUT-1 showing a possible reduction in intracellular trafficking and increased Golgi localization.
Conclusions
The increase in ROS levels may contribute to the activation of the NRF2-mediated antioxidant response and its translocation into the nucleus, while the concomitant reduction in the overall NRF2 signal may suggest that this adaptive response is insufficient to fully restore redox homeostasis. The reduction in acetylated α-tubulin and the altered GLUT1 pattern may indicate a potential functional relationship, as decreased α-tubulin acetylation could affect the proper intracellular trafficking and localization of GLUT1. The reduction in LAT1, another key nutrient transporter, may further suggest that FC could influence cellular metabolic processes in the PTC model.
During the third year of the PhD program, further investigations will be required to better elucidate the involvement of the pathways altered in PTC following treatment with FC and ß-GA.

Transgenerational effect of endocrine disruptors at subtoxic dosage on male reproductive function

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Ph.D. Course Clinical and Experimental Sciences
Curriculum "Endocrine and Metabolic Sciences and Gender Medicine"

Background: Endocrine disruptors (EDs) are compounds that have the ability to affect the endocrine system by interfering with the synthesis, bioavailability, and action of hormones. Among the main EDs we can include bisphenol A (BPA), bisphenol S (BPS) and perfluoroalkyl compounds (PFAS). The aim of this project is to evaluate the effects of the current daily human exposure to single or combined EDs (PFOS, BPA and BPS) during the highly sensitive prenatal developmental stages In this PRIN study (Grant 20203AMKTW_002) we evaluated the possible transgenerational effects of EDs on testicular function in male F1 mice born from mothers treated in pregnancy and during lactation with subtoxic levels within the recognized tolerability levels for these three substances: group 1: BPA (0,4 ng/kg/bw) N=12, group 2: BPS (0,4 ng/kg/bw.) N=14, group 3: PFOS (0.7 ng/kg/bw.) N = 15, group 4: BPA (0,4 ng/kg/bw) and PFOS (0.7 ng/kg/bw) N=15 and group 5: BPA (0.4 ng/kg/bw), BPS (0.4 ng/kg/bw).and PFOS (0.7 ng/kg/bw) N=15.
Material and Methods: Mice were sacrificed at sexual maturity (P = 120 days), and subsequently blood was collected, testes were stocked at - 80°C, and spermatozoa were retrieved from their respective epididymis to analyze the effects of different EDs by assessment of sperm count and motility, quantification of plasma and testes hormones by ELISA and Mass Spectrometry (LC/MS) method, and analysis of protein expression by western blotting (WB) technique.
Results: Compared with the unexposed controls, the group exposed to EDs showed a reduced sperm concentration ($P < 0.0001$). In parallel, the percentage of sperm with progressive motility dropped dramatically (-90% on average) in all EDs-exposed groups compared with the control group**.** Quantification of hormones in plasma showed decreased levels of testosterone and INSL3 in all groups of exposed mice compared with controls (all $p<0.05$), while FSH and LH levels were increased in exposed mice compared with control (all $p<0.05$). Quantification of steroid hormones in testicular tissues showed a reduction in testosterone ($p=0.0279$). This pattern is supported by a disrupted steroidogenic process, which was consistent with the reduced StAR expression, compared to unexposed controls, as observed by WB analysis ($P=0.0212$ vs CTRL). Testis histology and transmission electronic microscopy analysis revealed a marked alteration of seminiferous tubule architecture upon exposure to EDs, together with Leydig cells vacuolization. Ultrastructural analysis of epididymal spermatozoa further revealed membrane thickening with irregular cell surface in exposed males to EDs, consistent with compromised sperm integrity. Flow cytometry imaging by Filipin III staining, a marker of membrane-cholesterol content, showed increased fluorescence intensity in spermatozoa from-exposed males (all $p<0.0001$). Finally, bioaccumulation of BPA, BPS and PFOS was evaluated by LC-MS, showing that all three EDs were concentrated at the testicular and plasma levels, all $p<0. 05$ compared with control.
Conclusions: This study demonstrates for the first time a transgenerational effect of EDs at subtoxic dosages on testicular function, with direct damage on spermatogenesis and Leydig cell function, with intratesticular accumulation of these molecules

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PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "HEMATOLOGICAL AND GERIATRIC SCIENCES"

Vitamin D Deficiency and 12-Month Outcomes After Fragility Hip Fracture: Data from the Hip-POS Fracture Liaison Service

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Ph.D. Course Clinical and Experimental Sciences
Curriculum "Hematological and Geriatric Sciences"

Background: Fragility hip fractures are associated with substantial morbidity and mortality and require coordinated secondary prevention. Fracture Liaison Services (FLS) provide systematic post-fracture care aimed at identifying skeletal fragility and reducing the risk of subsequent fractures. In this setting, vitamin D deficiency (VDD) is highly prevalent and may represent a marker of both impaired bone health and clinical vulnerability. We investigated the prevalence, predictors, and association of VDD with 12-month clinical outcomes in patients hospitalized for fragility hip fracture within an FLS program.
Materials and Methods: Consecutive patients aged ≥50 years hospitalized for fragility hip fracture were prospectively enrolled in the Hip-Padua Osteosarcopenia (Hip-POS) FLS between March 2023 and March 2025. Patients with traumatic or pathological fractures or unavailable serum 25-hydroxyvitamin D [25(OH)D] measurements were excluded. Clinical characteristics, previous vitamin D supplementation, and laboratory parameters were assessed during hospitalization. VDD was defined as 25(OH)D <50 nmol/L. Mortality, refractures, and rehospitalizations were assessed at 12 months. Predictors of VDD were investigated using multivariable logistic regression and decision tree analysis. Outcomes were evaluated using Cox regression and Kaplan–Meier analyses, with inverse probability of treatment weighting based on age, sex, and comorbidity burden.
Results: Overall, 934 patients were included, with a mean age of 83.0 ± 8.9 years; 71.2% were women. VDD was observed in 501 patients (53.6%) with a median 25(OH)D concentration of 22 nmol/L among deficient patients. Patients with VDD had a greater comorbidity burden, higher parathyroid hormone (PTH) levels, and a biochemical profile consistent with increased bone turnover. Male sex, diabetes mellitus, dementia, and active smoking were independently associated with VDD, whereas previous vitamin D supplementation was inversely associated. A simplified decision tree based on supplementation status, sex, and age identified patient phenotypes with VDD prevalence ranging from 17.2% to 86.3%. Among the 554 patients with available 12-month follow-up, VDD was associated with higher mortality in the unadjusted analysis (HR 1.65, 95% CI 1.12–2.42; p=0.010), but this association was attenuated and no longer significant after adjustment for age, sex, and comorbidity burden (HR 1.31, 95% CI 0.88–1.96; p=0.188). No significant associations were observed between VDD and refracture or rehospitalization rates.
Conclusions: VDD is highly prevalent among older adults hospitalized for fragility hip fracture within an FLS setting and is associated with greater comorbidity burden and increased bone turnover. Although associated with higher crude 12-month mortality, this relationship appears largely explained by age and clinical complexity. VDD may therefore represent a marker of both impaired bone health and clinical vulnerability. These findings highlight the importance of recognizing VDD and implementing targeted vitamin D supplementation strategies in high-risk hip fracture populations.

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PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "HEPATOLOGY AND TRANSPLANTATION SCIENCES, RARE AND BIOTECHNOLOGICALLY HIGHLY COMPLEX DISEASE"

THE ASSESSMENT OF SERPINB3 EXPRESSION, PAR2 AND SCCA-PD POLYMORPHISM IN PATIENTS WITH ACUTE RESPIRATORY DISTRESS SYNDROME

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TUTOR: Prof. Annalisa BOSCOLO BOZZA
Ph.D. Course Clinical and Experimental Sciences
Curriculum "Hepatology and Transplantation Sciences, Rare and Biotechnologically Highly Complex Disease"

Background
SerpinB3 is a serine-protease inhibitor implicated in epithelial injury and repair, with PAR2 activation and the SCCA-PD polymorphism proposed as related mechanistic pathways in acute lung injury. This ongoing PhD project investigates SerpinB3 and PAR2 expression, and the incidence of the SCCA-PD variant, in patients with Acute Respiratory Distress Syndrome (ARDS), with the aim of identifying candidate biomarkers of disease severity and clinical course.
Methods
From May 2025 to August 2026, patients admitted to Padua University Hospital and intubated were progressively screened and enrolled patients meeting Berlin criteria for ARDS within 72 hours of diagnosis and hospitalized controls. Clinical and bioumoral variables were collected; blood, plasma, plasma-derived extracellular vesicles (EVs), and bronchoalveolar lavage (BAL) were sampled to quantify SerpinB3 in raw and protein-normalized form across compartments. Group comparisons were performed using the Mann–Whitney U test, while Spearman's rank correlation was used to assess associations between variables. Ventilator-free days at day 28 and time to successful extubation were compared between groups (Mann-Whitney, Kaplan-Meier, Cox regression), with Cox models used to test the independent, group-adjusted effect of each candidate predictor, including all SerpinB3 forms. Chest CT severity was additionally scored using the Ichikado score in a subset of patients (n=10) with available imaging.
Results
Nineteen mechanically ventilated patients (9 ARDS diagnosed by Berlin criteria, 10 controls) were enrolled within 72 hours of diagnosis. Groups were well balanced for demographics and comorbidities. BAL characteristics did not differ significantly between groups, except for a higher proportion of CD80-positive macrophages in the ARDS group. Both raw and protein-normalized serum SerpinB3 were consistently lower in ARDS after adjustment for oxygenation severity (raw: p=0.035; normalized: p=0.025–0.026), and BAL SerpinB3 remained consistently lower in ARDS independently of P/F ratio in a BAL-quality-restricted sensitivity analysis (p=0.014–0.0025). ARDS patients experienced fewer ventilator-free days at day 28 than controls (median 18.0 vs 24.5 days, p=0.017). Higher serum SerpinB3, both raw and protein-normalized, was independently associated with slower extubation. The Ichikado CT score was significantly higher in ARDS than in controls (median 115.3 vs. 103.3, p=0.019; n=10). A borderline association with fewer ventilator-free days was observed, whereas no significant correlation was found with any SerpinB3 measure.
Conclusions
In this preliminary analysis, serum SerpinB3 (raw and protein normalized) was inversely associated with ARDS status and an unfavorable ventilatory outcomes, independently of oxygenation severity, while, SerpinB3 Plasma-EV emerged as the most robust correlate of BAL cellularity. The Ichikado score corroborated greater radiological involvement and longer duration of ventilation in ARDS but did not correlate with SerpinB3. Given the limited sample size, these findings require validation in a larger cohort.

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PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "KIDNEY, PHYSICAL EXSERCISE AND NUTRITION SCIENCES"

From Model to Practice: A Translational Pathway for Exercise Prescription in Breast Cancer Care

Ph.D. Student: Dr. Federica DUREGON
TUTOR: Prof. Andrea ERMOLAO – CO-TUTOR: Dott.ssa Francesca BATTISTA
Ph.D. Course Clinical and Experimental Sciences
Curriculum "Kidney, Physical Exercise and Nutrition Sciences"

Background: Aromatase inhibitors (AIs) represent the gold-standard endocrine therapy for hormone receptor-positive breast cancer, and up to 50% of patients experience AI-associated musculoskeletal syndrome (AIMSS), a leading cause of treatment discontinuation and reduced quality of life. Structured exercise has emerged as a promising non-pharmacological strategy to counteract these side effects, but moving from general principles to real clinical benefit requires a coherent path connecting practical models, pilot evidence, and structured research. This work presents a translational pathway developed at the Sports and Exercise Medicine Division, University-Hospital of Padova, starting from a real-world implementation model for exercise prescription and moving through a pilot study in breast cancer survivors.

Material and Methods: The pathway comprises two sequential phases. First, a real-world implementation model for individualized exercise prescription in chronic disease was described, integrating clinical screening, functional assessment, and tailored programming within a multidisciplinary framework (Duregon et al., BMC Sports Sci Med Rehabil, 2025). Second, this model was applied in a pilot interventional study involving 15 breast cancer survivors undergoing AI therapy, who completed a 10-session, exercise professional-supervised program combining aerobic, resistance, and impact training, with assessments at baseline, post-intervention, and 6-month follow-up.

Results: The pilot study demonstrated significant improvements in functional capacity, with aerobic capacity increasing significantly from baseline to follow-up ($p=0.003$), lower limb strength improving significantly after the intervention ($p=0.028$), and upper limb strength progressively increasing through follow-up ($p=0.010$). Perceived physical health (SF-36 PCS) also improved significantly ($p=0.021$), supporting the feasibility and clinical relevance of a supervised, individualized exercise protocol even in a small, real-world oncological population.

Conclusions and Perspectives: These findings support the feasibility and clinical value of translating a real-world exercise prescription model into oncological practice. As a future perspective, building on this pilot evidence, a randomized controlled trial (*"Move Again in Breast Cancer"*) has been designed to compare aerobic-focused versus resistance-focused exercise prescriptions in premenopausal women with AI-induced arthralgia, assessing quality of life as the primary outcome alongside an extensive biomarker panel—including inflammatory, bone turnover, and neurotrophic/vascular markers—to elucidate the biological mechanisms underlying exercise benefits. This progressive approach, from clinical practice to structured research, aims to define personalized exercise prescriptions capable of improving quality of life and treatment adherence in breast cancer survivors.

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PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "RHEUMATOLOGY AND LABORATORY SCIENCES"

Clinical and immunobiological effects of anti-IL-5/IL-5R monoclonal antibodies in EGPA: longitudinal dynamics of type 1 and type 2 inflammation

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Ph.D. Course Clinical and Experimental Sciences
Curriculum "Rheumatology and Laboratory Sciences"

Background: Eosinophilic granulomatosis with polyangiitis (EGPA) is a rare systemic disease characterized by eosinophilic inflammation, granulomatous lesions and small-vessel necrotizing vasculitis. Its pathogenesis involves both a type 2 and eosinophilic axis and type 1 and Th1-Th17 mechanisms contributing to vasculitic inflammation. Anti-IL-5 and anti-IL-5Rα therapies, particularly mepolizumab and benralizumab, have transformed EGPA management, but their biological effects beyond eosinophil depletion and the determinants of heterogeneous treatment response remain incompletely understood. This project integrates longitudinal cellular, soluble, molecular and clinical data to clarify EGPA pathogenesis and support more personalized treatment.
Material and Methods: A prospective translational study was designed to characterize EGPA patients at different disease stages and during anti-IL-5/IL-5R treatment. Peripheral blood eosinophil morphology is assessed on May-Grünwald-Giemsa-stained smears by oil-immersion microscopy, examining eosinophils and classifying nuclear abnormalities, hypogranularity, vacuolization and cell death according to predefined consensus criteria. Plasma IL-4, IL-5, IL-13, IL-17A and CCL26/eotaxin-3 concentrations are quantified by ELISA to investigate type 2 inflammation, eosinophil trafficking and the potential contribution of the Th17 axis. A reproducible two-tube multiparametric flow-cytometry strategy was developed on fresh peripheral blood. Tube 1 characterizes eosinophil identity and viability (Siglec-8, CD45, 7-AAD), maturation (CD16, CD10), activation/adhesion and trafficking (CD11b, CD62L, CD69, CCR3) and IL-5-related/immunoregulatory phenotype (CD125, PD-L1). Tube 2 evaluates T-cell populations and differentiation using CD3, CD4, CD8, CD45RA and CCR7, and identifies circulating Th1-, Th2- and Th17-like subsets through CXCR3, CCR4 and CCR6, with PD-L1 providing additional immunoregulatory characterization. Targeted TaqMan real-time PCR will complement these analyses by assessing the IL-5/IL-5R pathway, including IL5RA, CSF2RB, JAK2, STAT5A and STAT5B, together with genes involved in eosinophil activation, differentiation, trafficking and type 2 inflammation. In parallel, retrospective and prospective longitudinal clinical data are collected to assess disease activity, glucocorticoid exposure, relapse, treatment failure, discontinuation/switching and safety.
Results: Cytological analyses showed increased activation-associated eosinophil abnormalities, particularly hypogranularity and vacuolization, together with increased cell death in eosinophilic groups compared with healthy donors and reactive hyper-eosinophilia. Preliminary cytokine analyses showed considerable inter-individual variability, with a trend toward higher IL-5 concentrations in eosinophilic groups, whereas IL-17A largely overlapped and IL-4/IL-13 remained low in most samples. The two multiparametric flow-cytometry panels have been finalized and optimized for prospective implementation. The retrospective clinical cohort currently includes 33 patients treated with benralizumab and 40 patients treated with mepolizumab, providing the clinical framework for longitudinal integration with translational biomarkers.
Conclusions: The integration of clinical, cytological, cytokine, flow cytometric and molecular data will enable a longitudinal characterization of EGPA and of the effects of anti-IL-5/IL-5R therapies. This approach aims to explore treatment-response heterogeneity and identify signatures associated with remission and relapse, supporting more personalized therapeutic strategies..

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BAFF/BAFF-R dynamics following rituximab-induced B-cell depletion in systemic sclerosis: preliminary results from a prospective study

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Ph.D. Course Clinical and Experimental Sciences
Curriculum "Rheumatology and Laboratory Sciences"

Background: B-cell dysregulation plays a central role in systemic sclerosis (SSc), and rituximab (RTX) is increasingly used for the treatment of selected disease manifestations. The B-cell activating factor (BAFF)/BAFF-receptor (BAFF-R) axis is essential for B-cell survival and maturation and may contribute to autoreactive B-cell persistence. Recent evidence showed that patients with SSc have higher serum BAFF levels and reduced B-cell BAFF-R expression compared with healthy subjects, with more pronounced abnormalities in clinically active disease and an inverse relationship between serum BAFF and BAFF-R expression. In other autoimmune diseases, B-cell depletion with RTX induces a further increase in circulating BAFF, potentially favouring the survival of autoreactive B cells during repopulation—the so-called "BAFFling effect". However, longitudinal changes in the BAFF/BAFF-R axis following RTX have not yet been characterized in SSc. We aimed to investigate B-cell and BAFF/BAFF-R dynamics following RTX treatment and to explore the biological rationale for sequential BAFF-targeted therapy.
Material and Methods: This is a prospective, single-centre study including adult patients fulfilling the 2013 ACR/EULAR classification criteria for SSc and receiving their first RTX course for a clinical indication. RTX-treated patients are evaluated at baseline (T0), approximately 3 months after treatment during B-cell depletion (T1), and after 6 months (T2), with B-cell repopulation defined as CD19+ cells >1% of total lymphocytes. Two control groups are included at baseline: patients with clinically inactive SSc without indication for RTX and healthy subjects. Clinical characteristics, disease activity, organ involvement and treatment-related outcomes are recorded. Peripheral lymphocyte populations and BAFF-R-expressing lymphocytes are assessed by flow cytometry, while serum BAFF concentrations are measured by ELISA at the different time points.
Results: So far, fifteen patients have been enrolled: median age was 56 years (IQR 50.5–59), 87% were female and 80% had diffuse cutaneous SSc. Median disease duration was 4 years (IQR 1.5–10.5) and median modified Rodnan Skin Score at RTX initiation was 18 (IQR 12–23). Interstitial lung disease was present in 53%, cardiac involvement in 33%, and gastrointestinal involvement in 73%. Median CD19+ lymphocytes decreased from 13% (IQR 9–16.5) at T0 to 0% at T1, confirming complete peripheral B-cell depletion. At T2, median CD19+ cells were 0.6% (IQR 0–1), indicating that most patients had not yet reached the predefined repopulation threshold. BAFF-R+ lymphocytes decreased from a median of 1.7% at T0 to 0.025% at T1, followed by a partial increase to 0.102% at T2. Serum BAFF measurements are ongoing.
Conclusions: These preliminary findings show that RTX induces profound B-cell depletion in SSc, accompanied by a marked reduction in circulating BAFF-R+ lymphocytes and an increase in BAFF-R positivity during subsequent B-cell recovery. These observations are particularly relevant in light of recent evidence showing that clinically active SSc is already characterized by an imbalance in serum BAFF and B-cell BAFF-R expression, suggesting an intrinsically dysregulated BAFF/BAFF-R axis. Whether RTX-induced depletion further amplifies this imbalance, through an increase in circulating BAFF during B-cell reconstitution, remains to be established. Comparison with inactive SSc and healthy controls, together with longitudinal serum BAFF measurements and evaluation at complete B-cell repopulation, will help distinguish disease-related from treatment-induced alterations of this pathway. Confirmation of a post-RTX BAFF-driven environment could provide a mechanistic rationale for sequential RTX–belimumab therapy in SSc.

Challenges in Linking Interferon Signatures to Clinical Trajectories in Juvenile Idiopathic Arthritis

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Ph.D. Course Clinical and Experimental Sciences
Curriculum "Rheumatology and Laboratory Sciences"

Background
Based on the recent finding that higher baseline expression of interferon (IFN)-driven gene signature in blood positively correlates with good response to methotrexate (MTX) in Juvenile Idiopathic Arthritis (JIA), this study tested the hypothesis that baseline IFN scores differ significantly between trajectory groups of response to MTX, defined by machine learning models enabling clinicians to predict not just the likelihood, but the specific longitudinal course of therapeutic response.

Material and Methods
We utilized longitudinal clinical data from 699 children and young people (CYP) with active JIA initiating MTX within the CHARMS and CLUSTER cohorts, analysing a nested sub-cohort of 102 CYP with available baseline PBMC transcriptomic data. Clinical disease courses were classified using multivariate group-based trajectory modelling, while baseline interferon activity was quantified using established 51-gene and 5-gene expression signatures derived from bulk RNASeq. Statistical analyses included representativeness and non-parametric tests. The associations between clinical trajectories and IFN scores were evaluated by regression modelling, complemented by post-hoc power calculations to assess study sensitivity.

Results There was no significant association between baseline 51-gene and 5-gene IFN response scores and distinct clinical disease trajectories following methotrexate initiation. Post-hoc calculations indicated that the study was underpowered (44–58%) to detect medium effects.

Conclusions clinical trajectories accurately describe patient heterogeneity, but the IFN score does not represent a marker for a specific trajectory course. Future stratification strategies require larger multi-centre cohorts to identify non-responders early and exploit the window of biological opportunity.

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PhD COURSE "DEVELOPMENTAL MEDICINE AND HEALTH PLANNING SCIENCES"

COORDINATOR: PROF. GIANNI BISOGNO

Curriculum "ONCOHEMATOLOGY, MEDICAL GENETICS, RARE DISEASES AND PREDICTIVE MEDICINE"

PREDICTING VAGUS NERVE STIMULATION OUTCOMES IN PEDIATRIC DRUG-RESISTANT EPILEPSY: A MULTIMODAL LONGITUDINAL COHORT STUDY

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Ph.D. Course Developmental Medicine and Health Planning Sciences
Curriculum "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background and Rationale. Vagus nerve stimulation (VNS) is an established treatment for drug-resistant epilepsy (DRE) and its response is still predominantly assessed through seizure frequency. Currently, predictive factors for a favorable response to this treatment are unknown. Quantitative EEG might provide additional biomarkers of treatment response, and also longitudinal data integrating electrophysiological, clinical, and neuropsychological outcomes could better assess VNS response in DRE, particularly in pediatric patients. We developed a multimodal longitudinal protocol to characterize VNS response beyond seizure reduction. The protocol was tested as a proof of concept in a child with DRE associated to Tuberous Sclerosis Complex and subsequently implemented in an ongoing retrospective and prospective pediatric cohort of DRE.
Materials and methods. A multimodal longitudinal protocol combining clinical assessment, standard EEG, 64-channel high-density EEG (HD-EEG), and neuropsychological evaluation was initially tested in an index pediatric patient with DRE before VNS implantation (T0), at four months (T4), and at one year (T12). Following this proof-of-concept application, the same framework was implemented in an ongoing retrospective and prospective pediatric VNS cohort. HD-EEG recordings included spontaneous wakefulness and sleep. Interictal epileptiform discharges were manually marked on 0.5-Hz high-pass-filtered recordings at the peak of the sharp waveform. Spike frequency was calculated separately during wakefulness and sleep. Manually marked spikes subsequently underwent automated feature extraction, including identification of a presumptive source channel and characterization of spike duration, amplitude, and propagation. Background spectral activity was analyzed on artifact- and epileptiform-discharge-free epochs of ≥10 s, separately during wakefulness and sleep. Power spectral density was assessed in the delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (14–24 Hz) bands.
Results. In the index case, seizure burden and interictal epileptiform activity showed a marked early improvement after VNS. Spike frequency decreased from 14.4 to 2.2 spikes/min during wakefulness and from 23.1 to 8.0 spikes/min during sleep at T4, alongside a reduction in the spatial extent of epileptiform discharges. At T12, a partial rebound was observed (10.7 and 18.2 spikes/min, respectively), paralleling the clinical course, although values remained below baseline. Background spectral analysis was performed on 40, 102, and 154 s of spike- and artifact-free wakeful EEG and 146, 304, and 186 s of sleep EEG at T0, T4, and T12, respectively. Background spectral organization showed a more sustained evolution, characterized by reduced abnormal delta activity during wakefulness and a more physiological wake–sleep distribution. Neuropsychological follow-up showed relatively stable standardized scores, alongside qualitative gains in attention, communicative intentionality, and interpersonal engagement.
Conclusions. The index case provided a proof of concept for the feasibility of a multidimensional longitudinal protocol assessing VNS response in pediatric patients with DRE and served as the methodological basis for an ongoing retrospective and prospective cohort study. Preliminary cohort data support the applicability of this framework across heterogeneous pediatric epilepsies and will allow investigation of whether clinical seizure burden, interictal epileptiform activity, background EEG dynamics, and neuropsychological outcomes represent complementary dimensions of VNS response that may follow partially independent trajectories. This approach may contribute to a more comprehensive characterization of treatment response than seizure frequency alone.

Overcoming treatment resistance mechanisms in ALK-driven cancers

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Background
ALK-positive anaplastic large T-cell lymphoma (ALK+ ALCL) is a lymphoma driven by the oncogenic NPM-ALK fusion protein, with approximately 30% of pediatric patients experiencing relapse despite intensive chemotherapy. ALK-targeted tyrosine kinase inhibitors (TKIs) have shown promising activity, but resistance remains a major challenge. Brigatinib, a second-generation ALK TKI, is currently being investigated in ALK+ ALCL. We performed a genome-wide CRISPR/Cas9 knockout (KO) screen in ALK+ ALCL SupM2 cell line to identify genes whose loss confers Brigatinib resistance.

Material and Methods
A genome-wide CRISPR/Cas9 screen was performed using the GeCKO v2 lentiviral library at a molteplicity of infection (MOI) of 0.3 and repeated in three independent biological replicates to ensure robustness and reproducibility of the results. Following puromycin selection, cells were treated with Brigatinib or vehicle, and single guides RNA (sgRNAs) enrichment was assessed by next-generation sequencing (NGS) and the MAGeCK bioinformatic pipeline. Candidate genes were validated by CRISPR/Cas9 KO, Sanger sequencing and Western blotting. Resistance was assessed by Brigatinib dose-response assays, with viability measured after 72 hours of treatment using CellTiter-Glo.

Results
Quality control analysis using the MAGeCK Flute pipeline showed satisfactory and consistent sgRNA library representation across replicates, with mapping rates of approximately 60% and less than 3% of missing sgRNAs. Comparison of the three screens identified four recurrent candidate resistance genes: *PTPN1*, *PTPN2*, *CAPRIN1* and *STK11*, consistently enriched across all three replicates. Notably, *PTPN1* and *PTPN2* were identified as internal positive controls, as both genes were previously reported to mediate resistance to ALK TKIs in a comparable genome-wide CRISPR/Cas9 screen in ALK+ ALCL (*Chiarle et al., 2022*), supporting the robustness and biological validity of our screening approach. Among the novel candidates, *CAPRIN1* and *STK11* were prioritized for functional validation through the generation of corresponding KO cell lines. We subsequently focused on *CAPRIN1* KO cells, which displayed a significantly higher Brigatinib IC50 compared with wild-type cells, confirming an increased drug-resistance phenotype. Furthermore, following Brigatinib treatment, *CAPRIN1* KO cells showed a less pronounced reduction in STAT3 signaling, a major downstream effector of activated ALK, compared with wild-type cells, suggesting that *CAPRIN1* loss may contribute to Brigatinib resistance through sustained ALK/STAT3 pathway activity.

Conclusions
Our findings identify *CAPRIN1* and *STK11* as candidate mediators of Brigatinib resistance in ALK+ ALCL, consistent with their known roles in cellular stress responses, metabolic regulation and survival. In particular, *CAPRIN1* loss was associated with increased Brigatinib resistance and sustained STAT3 signaling following TKI treatment. Further studies are warranted to confirm the preliminary findings on *CAPRIN1* and to elucidate the molecular mechanisms underlying the resistance phenotype conferred by *STK11* loss. Additional investigations will determine whether these mechanisms extend to other clinically relevant ALK inhibitors and will assess their clinical relevance in patient-derived and *in vivo* models.

Tissue factor expression and preclinical activity of tisotumab vedotin in ovarian and uterine clear cell carcinomas

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Background:
Ovarian and uterine clear cell carcinomas are rare gynaecologic malignancies with limited histology-specific treatment options and poor outcomes after recurrence. Tissue factor (TF) is a transmembrane protein involved in coagulation and tumor progression and is the target of the antibody-drug conjugate tisotumab vedotin (TV). We evaluated TF expression in ovarian and uterine clear cell carcinomas and investigated whether TF-directed therapy has preclinical activity in clear cell carcinoma models.

Material and Methods:
TF expression was assessed by immunohistochemistry in 182 tumors, including 116 ovarian and 66 uterine clear cell carcinomas. Two tumor cores were evaluated when available, and a patient-level H-index (0-300) was calculated. TV activity was examined in three clear cell carcinoma models and compared with a TF-low uterine carcinoma control. TF expression in selected cell lines was characterized by flow cytometry, with mean fluorescence intensity (MFI) used as a quantitative measure of relative cell-surface TF expression. Dose-response cytotoxicity, γ-H2AX phosphorylation as a marker of double-strand DNA damage, and antibody-dependent cellular cytotoxicity (ADCC) were evaluated. Group comparisons used t tests or analysis of variance, as appropriate; two-sided $P<0.05$ was considered significant.

Results
TF was evaluable in 134 clear cell tumors: 90 ovarian and 44 uterine. Median H-index was 51.5 (range, 1-300) in ovarian and 62.5 (range, 1-300) in uterine clear cell carcinomas. An H-index >100 was observed in 28/90 ovarian (31.1%) and 12/44 uterine tumors (27.3%). Flow cytometry confirmed marked TF expression in the uterine (PEM14; MFI 1753.63) and ovarian (OVA9; MFI 306.34) clear cell carcinoma models, compared with the TF-low control (UTE1; MFI 14.75). TV produced dose-dependent cytotoxicity, with IC50 values of 19.09 ng/mL in PEM14, 93.97 ng/mL in KRCH156, and 156.3 ng/mL in OVA9; IC50 was not reached in UTE1 at concentrations up to 1000 ng/mL. TV significantly increased γ-H2AX phosphorylation compared with control ADC in all three clear cell models at both tested concentrations (all $P<0.05$). Both unconjugated tisotumab and TV also induced significant ADCC at effector-to-target ratios of 5:1 and 10:1.

Conclusions
TF is expressed at intermediate-to-high levels in approximately 30% of evaluable ovarian and uterine clear cell carcinomas. TV demonstrated TF-associated direct cytotoxicity together with DNA damage and Fc-mediated immune killing, whereas the TF-low control was resistant to direct treatment within the tested concentration range. These findings support TF as a therapeutically relevant target in gynecologic clear cell carcinomas and provide a rationale for biomarker-driven clinical evaluation of tisotumab vedotin in these rare tumors.

Advanced Therapies in Ovarian Cancer Treatment: From Molecular Diagnostics to the Optimization of "Targeted Therapies" and Liquid Biopsy

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Background. Current Homologous Recombination Deficiency (HRD) tests have drawbacks, such as high costs, high turn-around times and a high number of false positives. ~80% of patients with ovarian cancer have a recurrence, recurrent ovarian cancer bears different variants from the primary tumor and current methods to diagnose the recurrence detect it later than new technologies. For these reasons, we constituted the clinical study EOC Focus, to test new methods to detect HRD, one based on the analysis of Copy Number Variations (CNVs) and via CNVs and SNPs analysis. Moreover, primary tumor omics will be analyzed to detect actionable mutations from different types of cancer and to possibly detect new targeted biomarkers. Patient's cell free DNA will be analyzed to detect whether it's possible to diagnose HRD, and to study tumoral variants evolution during the therapy by analyzing cfDNA in important time-points of the therapy. Finally, cfDNA will be analyzed during the Progression Free Interval (PFI) to confirm that Next-Generation Sequencing (NGS) methods can detect recurrence earlier than conventional methods such as CA-125

Material and Methods 25 patients with High Grade Ovarian Cancer will be recruited to the EOC Focus cohort. Plasma samples will be obtained in different timepoints, and tissue samples will be obtained after surgical resection. FFPE samples will be used to test our HRD scoring algorithm in real world scenarios via shallow whole genome sequencing (sWGS) and panel sequencing. Fresh Frozen samples will be used to analyze tumor omics via: Whole Genome Sequencing, Whole Exome Sequencing, gene panel sequencing, Whole Transcriptome Sequencing, and Whole Genome Bisulfite Sequencing. Plasma samples will be analyzed via shallow whole genome sequencing and gene panel sequencing, during PFI only sWGS and TP53 will be sequenced. The obtained data will be analyzed via bioinformatic pipelines and programs, such as: Sarek, NVIDIA Parabricks, rna-seq, methylseq, Samurai, and HRProfiler. Variants will be analyzed via Ensembl and ClinVar.

Results EOC Focus was recently approved by the Regional Ethics Committee, and we are currently in the recruitment phase. During this period, extensive testing of our bioinformatic pipelines was conducted; these pipelines were installed and tested in Amazon Web Services (AWS) HealthOmics, then tweaked to optimize their functionality in this environment. Additionally, we conducted extensive benchmarking of cfDNA extraction kits using different sample types to identify the most efficient options with the highest yield, reproducibility, and robustness. Finally, we authored and managed the entire approval process for the research protocol submitted to the Regional Ethics Committee.

ConclusionsThe EOC Focus study has recently entered its recruitment phase, built on the methodological and regulatory groundwork established during the first and second years of this PhD. The bioinformatic infrastructure has been deployed and optimized on AWS HealthOmics, the cfDNA extraction workflow has been benchmarked and selected, and full ethical approval has been obtained. These foundations will enable the systematic generation and analysis of multi-omic data from the cohort.

Uncovering Distinct Disease Severity Trajectories in Juvenile Systemic Sclerosis Through Machine Learning

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Ph.D. Course Developmental Medicine and Health Planning Sciences
Curriculum "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background: Juvenile systemic sclerosis (JSSc) often presents with overlapping features, making phenotypic classification and disease progression difficult to define. The present study aimed to describe longitudinal disease progression in a cohort of children with JSSc, to identify latent subgroups with distinct disease severity trajectories over time, and to evaluate baseline predictors associated with trajectory class membership.

Material and Methods: Retrospective cohort study using prospectively collected data from 45 patients with JSSc between January 2005 and December 2024. Children were included if fulfilled both pediatric and/or adults' classification criteria for JSSc/SSc and had a minimum follow-up of four years. According with the recent classification proposal, we considered four JSSc subtypes: limited cutaneous subtype (lcSSc), diffuse cutaneous subtype (dcSSc), systemic sclerosis sine-scleroderma subtype (ssJSSc), and the newly proposed fibrotic subtype (fJSSc). Demographic, clinical, laboratory, and disease severity data were collected. The primary outcome was progression-free survival (PFS), defined as percentage change in the Juvenile Systemic Sclerosis Severity score (J4S) over time. A change exceeding ±25% from baseline was considered clinically significant. To describe disease progression Kaplan–Meier survival analysis was performed to estimate PFS, and a Growth Mixture Model (GMM) was used to identify latent disease severity classes characterized by distinct longitudinal trajectories and their baseline predictors. For clinical validation, Kaplan–Meier survival analyses were performed to compare time-to-event outcomes among the latent trajectory classes.

Results: Thirty-eight JSSc patients (66% females), followed for ≥48 months, entered the study. PFS remained high in the fibrotic and limited cutaneous JSSc subtypes (80–100%) over the observation period, whereas the diffuse and sine scleroderma subtypes showed marked deterioration, with PFS decreasing to 54% and 25%, respectively. We identified two latent classes of disease severity: Class 1 (74% of subjects) showed low severity and good treatment response; Class 2 (26% of subjects) showed an active, progressive course with poor long-term outcomes. Baseline predictors of poor outcome (i.e, Class 2 membership) were J4S >7.5, treatment initiation >9 months from disease onset, and >1.5 organs involved. Kaplan–Meier analysis revealed distinct PFS patterns across GMM-derived classes. The comparison between the Kaplan–Meier curves of the predefined clinical subtypes to those derived from the GMM-identified latent classes showed good concordance ($\kappa = 0.660$).

Conclusions: We identified two latent classes of disease severity in JSSc with clear prognostic significance. High baseline J4S scores, delayed treatment, and greater organ involvement predicted a more severe disease course, paving the way for precision health strategies and optimized patient management.

Pediatric Hepatology: Mechanisms and Implications of Liver Disease in Children with Vascular, Drug-Induced, and Autoimmune Liver Injuries.

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TUTOR: Prof. Mara CANANZI - CO-TUTOR: Dr.ssa Paola GAIO
Ph.D. Course Developmental Medicine and Health Planning Sciences
Curriculum "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background and aims: Paediatric liver diseases encompass multiple disorders with distinct aetiology and pathogenesis. This project investigates three major forms of paediatric liver injury: **(I)** vascular, **(II)** drug-induced, and **(III)** immune-mediated. **(I)** Fontan-associated liver disease (FALD) results from altered hepatic haemodynamics in children who underwent Fontan surgery, but its natural history and risk factors remain poorly defined. **(II)** Ketamine-induced hepatobiliary toxicity is a potentially severe complication of prolonged exposure for analgosedative purpose in critically ill patients, although paediatric data are limited. **(III)** In paediatric autoimmune hepatitis (pAIH), the role of follow-up liver histology in assessing treatment response and guiding treatment withdrawal remains controversial. This study aims to **(I)** characterise the prevalence, severity, complications, and risk factors of FALD; **(II)** investigate ketamine-associated hepatobiliary toxicity during prolonged continuous infusion in children; and **(III)** assess the predictive value of biochemical, immunological, and histological parameters in pAIH.
Methods: (I) Retrospective multicenter cohort study of patients undergoing Fontan surgery at five UK pediatric centers (1977–2024), with demographic, clinical, laboratory, imaging, elastography, and outcome data collected. **(II)** Retrospective single-center study of critically ill children admitted to the PICU of the University Hospital of Padua (2017–2023). Analysis included ketamine exposure–response relationships, propensity-matched exposure groups, and the independent effect of prolonged exposure. **(III)** Retrospective multicentric study including children with pAIH diagnosis, evaluating biochemical, immunological, serological, and histological parameters at follow-up.
Results: (I) Among 1,118 patients, biochemical abnormalities increased with time since Fontan, from 29% at surgery to 65% at 20 years and 78% at 30 years ($p=0.041$). Nodular liver, heterogeneous parenchyma, portal hypertension, and splenomegaly occurred in 43%, 41%, 33%, and 26%, respectively. Parenchymal abnormalities were associated with portal-axis involvement (OR 2.89, 95% CI 1.82–4.58; $p<0.001$), while portal burden and baseline MELD-XI predicted death or transplantation. **(II)** Among 106 ketamine-exposed children, hepatotoxicity occurred in 81%, predominantly biliary (75%). Thresholds of 5 days for infusion duration and 164023 mcg/kg for cumulative dose predicted the onset of liver injury with high sensitivity and specificity. Persistent biochemical abnormalities occurred in 49%, with secondary sclerosing cholangitis in two patients and biliary cirrhosis requiring transplantation in one. Prolonged exposure was independently associated with hepatotoxicity (aOR 5.22–6.00) and biliary injury (aOR 13.0–13.5). **(III)** Among 71 patients with pAIH, biochemical and histological remission were discordant in 20% of cases. Biochemical response (BR) and complete biochemical response (CBR) predicted histological remission in 80% and 82% of cases, respectively.
Conclusions: This PhD project provides new insights into vascular, drug-induced, and immune-mediated liver injury in children. **(I)** FALD develops during childhood and may progress to advanced liver disease, supporting structured long-term surveillance. **(II)** Prolonged high-dose ketamine exposure is associated with chronic liver injury, predominantly affecting the biliary tree. **(III)** Active histological disease persists in 20% of patients with pAIH despite BR/CBR, supporting the value of follow-up liver biopsy in children.

Identification of New Biomarkers and Potential Therapeutic Targets through Proteomic Characterization of the Plasma Antibody Profile in Patients with Desmoplastic Small Round Cell Tumor

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Curriculum "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background. Desmoplastic Small Round Cell Tumor (DSRCT) is a rare highly aggressive pediatric sarcoma characterized by the chromosomal translocation t(11;22)(p13;q12), which results in the EWSR1::WT1 fusion oncogene. DSRCT predominantly affects male adolescents and young adults and typically presents as an abdominal mass, with frequent extra-abdominal metastases often already present at diagnosis. It has a relapse rate of 80% and a 5-year overall survival rate of 15%. To date, no reliable prognostic biomarkers have been identified for DSRCT, and effective therapeutic targets for chemoresistant or relapsed disease remain lacking. In this study, we investigated the ability of patients' humoral immune response, as reflected by autoantibody production, to recognize changes in tumor antigen expression, with the aim of identifying novel biomarkers associated with disease onset, progression, and survival, as well as potential targets for therapeutic intervention.
Material and Methods. In this retrospective study, patients diagnosed with DSRCT were selected for analysis. Small volumes of plasma containing circulating autoantibodies were used to probe protein microarrays (ProtoArrays) representing more than 80% of the human proteome, including the majority of known tumor-associated antigens (TAAs). Autoantibody profiles were analyzed at both the cohort level (DSRCT patients vs. controls) and the individual patient level to characterize patient-specific and shared antigenic profiles and to identify disease-associated autoantibodies. Candidate TAAs identified through statistical analysis were selected for further characterization. The expression of selected TAAs was assessed by qPCR, while their functional relevance was investigated *in vitro* using DSRCT cell models treated with targeted agents of potential clinical interest. Finally, RNA-sequencing transcriptomic analysis was performed on a larger validation cohort of DSRCT biopsies and integrated with the autoantibody profiling data to identify additional DSRCT-associated antigens and potential targets for antibody-based or pharmacological therapeutic approaches.
Results. Autoantibody screening identified patient clusters characterized by distinct but partially overlapping antibody profiles and enabled the selection of relevant tumor-associated antigens (TAAs). Patient-specific analyses revealed both antigens uniquely recognized by individual patients and antigens shared across multiple patients, highlighting the heterogeneity of the humoral immune response in DSRCT. The overexpression of selected statistically significant, group-specific TAAs was confirmed by qPCR. Functional enrichment analysis showed that these antigens were predominantly associated with chromatin organization, chromosome structure maintenance, and transcriptional regulation. Integration of autoantibody profiling with RNA-sequencing data further identified novel DSRCT-associated tumor antigens, including candidates amenable to immunologic targeting or pharmacologic inhibition.
Conclusions. Our findings demonstrate that humoral immune profiling can effectively characterize the DSRCT antigenic landscape and identify disease-associated autoantibody signatures. The identification of both shared and patient-specific tumor antigens highlights opportunities for the development of disease-directed and personalized therapeutic strategies. Integration of autoantibody profiling, gene-expression analysis, and functional studies further identified novel DSRCT-associated antigens and potential therapeutic vulnerabilities, providing a basis for the development of antibody-based and pharmacologic approaches for chemoresistant or relapsed disease.

CARDIAC REMODELLING IN CONGENITAL HEART DISEASE

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Curriculum "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background
Atrial septal defect (ASD) is the second most common congenital heart defect. Although considered a mild lesion, a persistent left-to-right shunt can lead to right ventricular volume overload, atrial arrhythmias, pulmonary hypertension, systemic embolism, and premature mortality.
The GORE® Cardioform ASD Occluder (GCA; W. L. Gore & Associates) is a transcatheter closure device with a soft nitinol framework that conforms to the atrial septum. This study evaluated the impact of transcatheter ASD closure with the GCA on atrial and ventricular remodeling in a pediatric cohort.
Materials and Methods
This prospective study evaluated pediatric patients with ASD at baseline (T0) and at 6 months (T1) and 12 months (T2) after transcatheter closure. At each time point, patients underwent clinical assessment, 12-lead electrocardiography, and transthoracic two-dimensional echocardiography with speckle-tracking analysis (STE). The assessed parameters included left ventricular (LV) end-diastolic volume (EDV, mL), LV ejection fraction (LVEF, %), tricuspid annular plane systolic excursion (TAPSE, mm), LV global longitudinal strain (LVGLS, %), LV global circumferential strain (LVGCS, %), right ventricular free-wall strain (RVFWS, %), and left and right atrial reservoir strain (LASr and RASr, %). Changes over time were analyzed using the Friedman test with pairwise comparisons.
Results
Sixteen patients were enrolled (mean age, 8.7 ± 2.6 years; 62.5% female). Twelve patients (75.0%) had a single ASD, 2 (12.5%) had a multifenestrated ASD, and 2 (12.5%) had multiple ASDs. At 12 months, 2 patients had not yet completed the follow-up assessment; therefore, preliminary echocardiographic analyses were performed in 14 patients (mean age, 9.0 ± 2.7 years; 71.4% female). No statistically significant differences were observed between baseline, 6-month, and 12-month assessments for any parameter analyzed. Similarly, no significant pairwise differences were identified after correction for multiple comparisons. Median [IQR] EDV was 41 [33–51] mL at T0, 46 [39–54] mL at T1, and 37 [36–57] mL at T2 (χ^2=3.00, p=0.223), while LVEF remained stable at 67 [64–72]%, 68 [66–71]%, and 66 [65–78]%, respectively (χ^2=1.38, p=0.500). TAPSE ranged from 25 [22–26] to 20 [16–22] and 19 [17–21] mm, without significant change (χ^2=2.57, p=0.276). Similarly, STE parameters showed no significant longitudinal differences. Median LVGLS was −21.89 [−23.37 to −21.07]% at T0, −21.35 [−21.71 to −20.94]% at T1, and −21.41 [−22.03 to −20.77]% at T2 (χ^2=1.00, p=0.607); LVGCS was −23.21 [−26.70 to −20.75]% at T0, −24.87 [−26.86 to −22.25]% at T1, and −23.73 [−24.39 to −20.53]% at T2 (χ^2=1.08, p=0.584). Median RVFWS was −31.90 [−35.33 to −30.13]% at T0, −31.13 [−32.70 to −29.30]% at T1, and −31.00 [−32.04 to −29.41]% at T2 (χ^2=1.71, p=0.607). Atrial strain analysis showed a median LASr of 53.34 [39.09–61.53]% at T0, 44.61 [33.09–50.73]% at T1, and 44.50 [37.40–55.30]% at T2 (χ^2=2.71, p=0.257); median RASr was 89.50 [65.46–93.81]% at T0, 76.99 [64.95–89.26]% at T1, and 74.60 [62.50–83.70]% at T2 (χ^2=2.00, p=0.368).
Conclusions
GCA implantation was not associated with adverse changes in atrial or ventricular mechanics during short- to mid-term follow-up in a small cohort of pediatric patients. Left and right ventricular systolic function and myocardial strain remained preserved at 6 and 12 months after ASD closure. A larger sample size and longer-term follow-up are needed to confirm these preliminary results.

DEVELOPING PATIENT-DERIVED NEUTRALIZING ANTIBODIES FOR RHABDOMYOSARCOMA TREATMENT IN CHILDREN

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Background
Alveolar rhabdomyosarcoma (ARMS) is an aggressive pediatric soft tissue sarcoma characterized by a very poor prognosis. Metastases are often present at the time of diagnosis and relapses occur soon after initial therapy. Evidences revealed that fibroblast growth factor 8 (FGF8) is an oncogenic driver of tumor progression and recurrence in several types of cancer including ARMS. Interestingly, elevated levels of endogenous anti-FGF8 autoantibodies in plasma from ARMS patients correlate with significantly improved event-free survival (EFS), suggesting a protective role for endogenous anti-FGF8 immunoglobulins. We therefore aim to generate high-affinity monoclonal antibodies targeting FGF8 to impair its oncogenic effect, by directly harvesting antigen-specific memory B cells from pediatric patients.

Material and Methods
To isolate naturally occurring protective antibodies, we optimized a single-B-cell sorting and recombinant antibody generation platform. Antigen-specific memory B cells were isolated from PBMCs via high-throughput single-cell fluorescence-activated cell sorting (FACS). Variable heavy (V_H) and light (V_L) chain immunoglobulin genes were amplified from single cells by nested PCR and cloned into expression vectors containing human IgG1 constant regions to reconstitute full-length recombinant immunoglobulins. To ensure sequence integrity, Sanger sequencing was performed prior to and following expression vector insertion. Recombinant monoclonal antibodies expressed through HEK293T transient transfection *in vitro* were evaluated for antigen-specific binding affinity using ELISA and dot blot assays. Simultaneously, a phage display approach was employed to screen and select anti-FGF8 antibodies from a broad human healthy donors-derived library. Binding affinity was evaluated through ELISA, dot blot, and immunoprecipitation assays, while neutralizing efficacy was quantified by analyzing downstream signaling kinetics in ARMS cell lines via Western blots.

Results
We have optimized a single-cell cloning platform, by successfully isolating monoclonal antibody sequences from single memory B cells. We have also demonstrated sensitivity and fidelity by accurately reconstructing fully functional immunoglobulin genes. Recombinant monoclonal antibodies expressed by transfected HEK293T displayed high binding specificity, and non-specific cross-reactivity in ELISA and dot blot panels. Having validated its efficacy, this pipeline will be employed to isolate and express rare, patient-derived human anti-FGF8 immunoglobulins from PBMCs of ARMS patients in long-term complete remission. By phage display we additionally obtained a promising specific anti-FGF8 monoclonal antibody with functional neutralizing effect on FGFR and MAPK signaling in ARMS cell lines.

Conclusions
We have successfully adapted a single-B-cell cloning technology specifically tailored to overcome the stringent limitations of scarce pediatric clinical samples, by producing full-length recombinant human antibodies with high sequence fidelity from isolated rare single memory B-cells. We furthermore successfully generated a neutralizing monoclonal anti-FGF8 antibody that represents a promising anti-tumoral candidate aimed at preventing disease progression in ARMS patients.

Combining Metabolomics and NIRS for Early Detection of Intestinal Dysfunction and Optimization of Nutrition in Preterm Neonates

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Background: Feeding intolerance (FI) is common in preterm neonates and delays the achievement of full enteral feeding (FEF), prolonging exposure to central venous catheters and increasing the risk of late-onset sepsis. A shared definition is still lacking: the criteria currently used (gastric residuals, abdominal distension, vomiting) rely on arbitrary thresholds, have low specificity and overlap widely with the early signs of necrotizing enterocolitis (NEC). Two non-invasive approaches appear promising for characterizing FI and distinguishing it from NEC: untargeted urinary metabolomics, which detects alterations of the metabolic profile already at a preclinical stage and has been applied in our center to the early prediction of NEC (Moschino et al., 2024), and near-infrared spectroscopy (NIRS), which allows continuous monitoring of splanchnic ($SrSO_2$) and cerebral ($CrSO_2$) regional oxygen saturation, of their ratio (SCOR) and of the fractional tissue oxygen extraction (FTOE). Available evidence on abdominal NIRS, however, concerns almost exclusively NEC, whereas studies on FI are few and based on limited samples (Vyas et al., 2025). The aim of the study is to identify early and reliable markers of FI in preterm neonates through an integrated clinical, NIRS and metabolomic approach, in order to promote a rapid and safe achievement of FEF and to distinguish FI from NEC at an early stage.
Material and Methods: The study was approved by the Ethics Committee (METNEC study, 5128/AO/21). Since April 2021, neonates with gestational age (GA) $\leq$ 32 weeks are enrolled after written parental consent; chromosomal abnormalities, isolated gastrointestinal malformations and known or suspected metabolic diseases are excluded. NIRS monitoring is performed within 48 hours of birth and at 7, 14, 21 and 28 days, with 30-minute pre-prandial recordings, using a forehead sensor for cerebral ($CrSO_2$) and an infra-umbilical sensor for splanchnic ($SrSO_2$) regional oxygen saturation; FTOE is calculated as $(SpO_2 - rSO_2)/rSO_2$ and SCOR as the $SrSO_2/CrSO_2$ ratio. For untargeted metabolomics, 2 mL of urine are collected at T0, 14 ± 2 and 28 ± 2 days and sent to the Mass Spectrometry Laboratory of the Istituto di Ricerca Pediatrica Città della Speranza. Neonates are divided according to whether FEF is achieved within or beyond 26 days, the cohort mean. Quantitative variables are compared by Student's t test and qualitative variables by the chi-square test; correlations with the time to FEF as Pearson's coefficient from a univariate linear model.
Results: Forty-eight neonates have been enrolled; the preliminary analysis of the first 28 is reported here (mean GA 28.3 ± 2.0 weeks; mean birth weight 967.7 ± 299.4 g), whose mean time to FEF was 26 days. In the whole cohort, between 24 hours and 14 days $CrSO_2$ remained between 72% and 75%, $SrSO_2$ rose from 65.1% to 68.2% at 7 days, splanchnic FTOE decreased from 0.58 to 0.42 and SCOR increased from 0.86 to 0.90. In neonates achieving FEF beyond versus within 26 days, splanchnic FTOE was 0.63 vs 0.58 at 24 hours, 0.30 vs 0.46 at 7 days and 0.35 vs 0.46 at 14 days, while $CrSO_2$ was comparable at 24 hours (73.0% vs 74.6%) and higher from day 7 (78.0% vs 73.6%; 75.0% vs 72.3% at 14 days). A splanchnic FTOE > 0.3 was significantly associated with FEF within or beyond 26 days at 24 h ($p = 0.03$) and at 7 days ($p = 0.01$), the association being borderline at 14 days ($p = 0.06$); a mean $CrSO_2$ < 74.5% showed borderline associations at 24 hours and 7 days ($p = 0.06$ and $p = 0.05$), while mean $SrSO_2$ and SCOR did not reach significance in this preliminary sample. Metabolomic analysis has not yet been performed.
Conclusions: Preliminary data suggest differences in NIRS parameters between neonates achieving FEF within 26 days and those achieving it later, splanchnic FTOE in the first week being the parameter most consistently associated with the time to FEF. Extending the analysis to the whole cohort (48 neonates) and starting the urinary metabolomic analysis will make it possible to confirm these findings and test the predictive value of NIRS parameters

METABOLOMIC INVESTIGATION OF CHILDREN AND YOUNG ADULT AFFECTED BY ALGODYSTROPHY

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Background
Complex Regional Pain Syndrome type 1 (CRPS-1), also known as algodystrophy, is a rare disabling pain condition whose pathobiology, particularly in paediatric patients, remains poorly understood. Objective diagnostic and prognostic biomarkers are currently lacking. This study aims to characterize the urinary metabolomic profile of children and young adults with CRPS-1, identify potential early diagnostic metabolites, investigate longitudinal metabolic changes, and explore their association with clinical phenotype and disease severity.

Material and Methods
This ongoing longitudinal observational study enrols children, adolescents and young adults with CRPS-1 fulfilling the Budapest criteria. Originally designed as a single-centre study, the project has been expanded to a multicentre collaboration to increase sample size, with the Treviso centre currently participating. Clinical and demographic data are prospectively collected together with urine and plasma samples at predefined time points from diagnosis through treatment and recovery. Clinical assessment includes pain intensity, fatigue, CRPS Severity Score (CSS), health-related quality of life (PedsQL), and anxiety/depressive symptoms assessed by TAD or DASS-21 according to age. After completion of sample collection, urine samples will undergo untargeted UPLC-MS metabolomic analysis in positive and negative electrospray ionization modes. Multivariate and longitudinal analyses will investigate metabolic patterns, their evolution over time and their relationships with clinical features and disease severity. The planned metabolomic methodology and longitudinal sampling scheme are consistent with the study protocol.

Results
Nine unique patients have currently been enrolled (8 females and 1 male), with a mean age of 19.8 years (range 11.4–35.4), contributing a total of 35 longitudinal clinical and sampling timepoints. One patient presented CRPS-1 involving two anatomical sites and was counted as a single participant. Four patients (44.4%) have reached the recovery phase, although the final recovery sample remains to be collected in one case, and one patient (11.1%) was lost to follow-up. Preliminary exploratory analyses showed no significant correlation between CSS and PedsQL or between CSS and anxiety/depression scores. A moderate inverse association between PedsQL and TAD depression scores was observed but did not reach statistical significance, likely also reflecting the limited current sample size. Metabolomic analyses will be performed once sample collection is completed and are expected to identify disease-related metabolic patterns and their longitudinal evolution.

Conclusions
Preliminary data support the feasibility of longitudinal clinical characterization and biological sampling in patients with CRPS-1. The planned untargeted metabolomic analysis may identify disease-related metabolic patterns, characterize their changes from diagnosis to recovery, and explore their association with clinical phenotype and disease severity. Given the exploratory nature and limited sample size of the present study, the findings are expected to provide preliminary evidence and generate hypotheses that may guide subsequent studies in larger patient cohorts.

MultiOmicAsphyxia Study - Unravelling early biomarkers and therapeutic targets in hypoxic-ischemic encephalopathy following perinatal asphyxia by use of multi-omic technology

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Background
Hypoxic-ischemic encephalopathy (HIE) remains a major cause of mortality and long-term neurodevelopmental impairment after perinatal asphyxia; therapeutic hypothermia (TH) is currently the only approved treatment for such condition. Using untargeted metabolomics, our group previously identified two putative markers (L-lysine and L-3-methylhistidine) and a significant derangement of the neurosteroid, lysine-degradation and carnitine-synthesis pathways in HIE newborns. Building on this background, the MultiOmicAsphyxia Study aims to validate these findings and to integrate transcriptomics, miRNA analysis, targeted and untargeted metabolomics, and lipidomics in order to identify robust prognostic biomarkers and therapeutic targets complementary to TH, ultimately translating the results into a bedside low-field NMR-based tool.
Material and Methods
Newborns with HIE undergoing TH at the Neonatal Intensive Care Unit of the University Hospital of Padova are prospectively enrolled, with blood and urine collected at three time-points (pre-TH, during TH, post-rewarming). Plasma is analysed by targeted MS-based metabolomics (among others: amino acids, polyamines, kynurenine-pathway metabolites, tyrosine/tryptophan-related neurotransmitters, steroids) and by untargeted high-field NMR and HDMS-based metabolomics and lipidomics; leukocyte RNA is profiled by Clariom S microarray, and circulating miRNA by a high-throughput plasma assay. All infants undergo brain MRI and longitudinal neurodevelopmental follow-up (Bayley-III/WPPSI-IV). Multi-omic data will be integrated to build a bedside low-field NMR fingerprinting platform coupled with machine-learning classifiers.
Results
At the end of the second project year, enrollment for the omics cohort is complete: 56 patients have been recruited, exceeding the planned target of 40, with 18 (32%) showing pathological MRI findings at the end of TH; recruitment has nonetheless been extended to June 2026 to refine the analysable sample size and complete transcriptomic sampling. A retrospective re-analysis of untargeted metabolomic data confirmed the role of neuroactive steroids and intact lysine and additionally revealed a potential neuroprotective contribution of estrogenic/progestinic steroids (β-estradiol, estriol, 17-hydroxyprogesterone), now being prepared for publication. The transcriptomic protocol was amended to include RNAlater stabilization, yielding high-quality, well-aligned RNA-seq reads; optimization of globin depletion and anticoagulant choice is ongoing to reduce the duplicate-read rate. High-to-low-field NMR transferability testing, needed for the bedside device, is currently in progress.
Conclusions
Patient recruitment and sample collection are on track and exceeding initial projections, supporting the feasibility of the multi-omic approach in this population. Preliminary ancillary findings point to novel steroid-related neuroprotective candidates alongside the previously identified markers. Completion of transcriptomic, miRNA, metabolomic and lipidomic analyses, together with MRI and neurodevelopmental follow-up data, will enable integrated biomarker discovery and the development of a bedside low-field NMR platform for personalized management of HIE.

URINARY METABOLOMIC PROFILING AND SINGLE-CELL RNA SEQUENCING OF TRACHEAL ASPIRATE IN THE RESPONSE TO SYSTEMIC CORTICOSTEROIDS IN PRETERM INFANTS WITH EVOLVING BRONCHOPULMONARY DYSPLASIA (ProMeSSA STUDY)

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Background
Bronchopulmonary dysplasia (BPD) is the main chronic complication of prematurity. Systemic postnatal corticosteroids are widely used to facilitate extubation and prevent BPD, but their efficacy varies markedly between infants, and treatment carries relevant adverse effects (intestinal perforation, hyperglycaemia, hypertension, growth impairment), particularly when started before 7 days of life. No biomarker currently identifies the infants most likely to benefit. Pharmacometabolomics—untargeted profiling of metabolic changes before and after a drug—has been applied to postnatal steroids in only two studies (Lewis 2019; Stockard 2022), which identified plasma and urinary metabolites whose pre/post-treatment change correlated with improvement of the Respiratory Severity Score at 7 days; neither assessed whether pre-treatment profiles predict response, nor whether the changes persist at BPD staging. In parallel, single-cell RNA sequencing (scRNA-seq) has begun to map the cellular landscape of the preterm airway and of BPD, but the cellular response to corticosteroids in vivo has never been described.

Material and Methods
Prospective, monocentric, non-pharmacological observational study. Population: preterm infants <32 weeks' gestation with evolving chronic lung disease receiving systemic corticosteroids for BPD prevention. Exclusion criteria: death <7 days, major congenital or genetic anomalies, metabolic disease, admission after 48 h of life, recent corticosteroids for other indications. Planned sample: 40–50 infants for metabolomics over a 3-year enrolment period; about 20 for scRNA-seq. Urine (2 mL, non-invasive collection) at ≤48 h of life, before steroids, at the end of the course and at 36 weeks' postmenstrual age (PMA); untargeted UPLC-QToF mass spectrometry at the Mass Spectrometry and Metabolomics Laboratory, IRP Città della Speranza, with QC-based normalisation and multivariate analysis. Tracheal aspirate (1 mL, during routine suctioning) at first intubation, before steroids, at extubation (or after 4 days of treatment if not extubated) and at any re-intubation; scRNA-seq at the IRP Single Cell Facility (10x Genomics; Seurat/Harmony, Leiden clustering, trajectory analysis). Response definitions: short-term, successful extubation without re-intubation within 72 h or ≥20% FiO2 reduction; medium-term, presence and severity of BPD at 36 weeks' PMA. Secondary outcomes: adverse effects, pulmonary hypertension at 36 weeks, neurological examination at term, respiratory morbidity and Bayley scores at 2 years.

Results
Study in progress. Enrolment is ongoing: 10 infants have been enrolled to date (September 2026), with serial urine and tracheal-aspirate samples being collected and stored; no analytical data are available yet.

Conclusions
Expected outputs: (i) description of the urinary metabolic and airway cellular changes induced by systemic corticosteroids in evolving BPD; (ii) identification of pre-treatment metabolomic and cellular signatures predictive of short- and medium-term response, to support the selection of infants who benefit from treatment; (iii) the first in-vivo single-cell description of the corticosteroid response in the preterm airway. Next steps: completion of enrolment, first untargeted metabolomic and single-cell analyses on the initial cohort.

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Dipartimento
di Scienze cardio-toraco-vascolari
e Sanità pubblica

PhD COURSE "DEVELOPMENTAL MEDICINE AND HEALTH PLANNING SCIENCES"

COORDINATOR: PROF. GIANNI BISOGNO

Curriculum "HEALTH PLANNING SCIENCES"

PROFUSE STUDY
(Prognosis and Follow-Up in Silicosis due to Engineered stone)

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Background
In the last 20 years there has been a re-emergence of silicosis due to the spread of the high silica content artificial stone (AS) in manufacturing of domestic benchtops. The evolution of this form of disease is unknown. In this ongoing longitudinal study, we aim to continuously evaluate the progression of AS silicosis in an actively expanding cohort of Italian workers.
Material and Methods
The cohort includes patients diagnosed with AS silicosis since 2016, with new diagnoses continuously being made and added to the study. Ongoing annual follow-up examinations include respiratory function tests. Chest HRCT is performed every 24 months and evaluated according to International Classification of High-resolution Computed Tomography for Occupational and Environmental Respiratory Diseases standards. We also administer the HADS (Hospital Anxiety and Depression Scale) questionnaire to assess symptoms of anxiety or depression.
Results
As of the end of 2025, the cohort includes 28 men whose mean ± SD age at diagnosis was 46.26 ± 8.81 years and with a duration of exposure before the diagnosis of 14.77 ± 4.74 years. At diagnosis six patients exhibited a restrictive pattern and two had a mixed pattern; 39.2% of patients had a reduction of DLCO values; 25% of patients presented with progressive massive fibrosis (PMF). Three years after the diagnosis there was a significant decline in FEV1, FVC, TLC and in DLCO values. At the last partial evaluation, 45% of patients had PMF and 58% of patients had a borderline or pathological score at HADS.
Conclusions
We observed that AS silicosis is characterized by a relatively young age at diagnosis and that the radiological, clinical and functional impairment evolves rapidly. Continuous follow-up is underway to assess long-term outcomes. Furthermore, in light of the ongoing new diagnoses, an active surveillance program targeting the exposed population across the Veneto region will start shortly to identify undiagnosed cases.

Improving Clinical Outcomes for Acutely Malnourished Children and Low-Birth-Weight Newborns in Low-Resource Settings

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Completion of two studies envisaged by the timetable for 2026

1) *Pietravalle A, Cavallin F, Putoto G, Trevisanuto D.*
Postnatal gestational age determination by ultrasonographic measurement of transverse cerebellar diameter: A simulation study.
PLoS One. 2026 Aug 24;21(8):e0356370. doi: 10.1371/journal.pone.0356370. PMID: 42636195; PMCID: PMC13502679.

Background:
Accurate determination of gestational age (GA) has an important role in clinical practice, especially prevention/anticipation of prematurity-related complications. In low- and middle-income countries, the availability and accuracy of the gold standard for GA determination is very limited. In fact, prenatal ultrasonography in the first trimester is strongly affected by high costs, lack of skills and poor maternal access to health service. Furthermore, the determination by Last Menstrual Period has a very low accuracy, as well as postnatal clinical tools such as the Ballard Score. Recent studies in high-income countries tested TCD measurement by cranial ultrasound for GA determination with reported acceptable accuracy. The development of technology over the years is leading to a progressive spread of cranial ultrasound also in LMICs. **Objective:** To assess the performance of a published equation in estimating GA using fetal TCD measurements from low-income settings.
Methods:
Data were simulated using available information from relevant studies conducted in LMICs. Estimated GA was calculated as GA in weeks = 0.470 × TCD in mm + 13.162. The performance was assessed using modified calibration plots and Bland-Altman plots. Pooled bias and 95% limits of agreement (LoA) were also calculated.
Results:
Four datasets (n = 400, 257, 450, 500) were generated based on data from relevant studies. For GA 23-32 weeks, the equation was prone to overestimate and pooled bias was 6.0 days (95% LoA -6.7 to 18.7 days). For GA 33-36 weeks, the equation was prone to underestimate in one dataset and pooled bias was -1.5 days (95% LoA -16.8 to 13.8 days). For GA 37-40 weeks, the equation showed bad calibration and pooled bias was -5.0 days (95% LoA -25.8 to 15.8 days). When extending the original interval to GA 23-40 weeks, pooled bias was 2.8 days (95% LoA -13.0 to 18.6 days).
Conclusions:
Within the limitations of a simulation study, our findings suggest that the TCD-based equation may provide some benefits in postnatal estimation of GA between 23-32 weeks in low-resource settings. Lower precision, yet acceptable for clinical use, may be expected beyond 32 weeks' gestation. Future studies may validate the equation in real newborns in low-resource settings where precise GA dating is available.

Integrated Approaches to Pediatric Infectious Disease Surveillance: Laboratory Methods, Population-Based Epidemiology and Arbovirus Research

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Background.
This PhD project investigates complementary approaches to the surveillance of pediatric infectious diseases by integrating laboratory, behavioral and population-based research. Since February 2025, activities have addressed antibody responses, maternal and infant preventive strategies, routinely collected health data, active One Health surveillance, and arbovirus research in Europe and Brazil.
Material and Methods.
Laboratory methods and vaccination research. Paired serum and volumetric absorptive microsampling (VAMS) specimens from children and adults were compared using influenza A(H1N1) and SARS-CoV-2 serology. A live-virus microneutralization assay was adapted to a 384-well high-throughput format. FluMist study enrolls healthy children receiving intranasal live attenuated or injectable inactivated influenza vaccine; serial saliva samples will be analyzed for influenza-specific and polyreactive mucosal IgA and IgG. A questionnaire was developed to assess vaccination-related knowledge and preferences among women of reproductive age, focusing on vaccination during pregnancy and maternal RSV vaccination versus Nirsevimab. *Population-based studies.* I contributed to an Italian-Norwegian study of SARS-CoV-2 infection and outcomes in immunocompromised children, children with chronic conditions, and reference children, and to a PEDIANET analysis of pertussis and pertussis-like cough incidence and antibiotic prescribing from 2010 to 2024. *One Health surveillance.* The systematic review examines active surveillance systems involving at least two One Health. *Arbovirus studies.* Activities included the narrative review "Pediatric Arboviral Infections in Europe: Epidemiology, Clinical Features, Diagnosis and Prevention"; Italian and European surveys of pediatric healthcare professionals' knowledge, perceptions, training, and educational needs; and a systematic review of WNV-associated acute cerebellar ataxia combined with a pediatric case description. The Brazilian component will include 10-year follow-up of children with congenital Zika virus exposure in the ZIKAction Pediatric Registry and prospective surveillance of acute febrile illness.
Results. *Laboratory methods and vaccination research.* The microsampling study included 163 participants. VAMS showed strong serum correlations in binding (ELISA) and functional (FRNT) antibody assays in adults and children. Integration into high-throughput microneutralization workflows was feasible, although qualitative results depended on assay stringency and pre-analytical processing. Findings were presented as a poster at ESCMID 2026. The FluMist study has enrolled approximately 50 children; a second winter of enrollment is planned, and samples have not yet been analyzed. The maternal vaccination and infant RSV prevention survey has been drafted; ethics review, survey dissemination, and participant recruitment are pending. *Population-based studies.* The COVID-19 study included 29,520 children in Italy and 851,517 in Norway. Infection risk was similar across groups; immunocompromised children in Norway had a higher hospitalization risk, while severe outcomes were rare. The study was published in *Acta Paediatrica* (Di Chiara C, Boracchini R, Trinh NTH, Giugni A, Sturniolo G, et al. 2026;115:1451–1460. DOI: 10.1111/apa.70509). The PEDIANET analysis of 456,912 children documented a marked increase in pertussis and pertussis-like cough in 2024. The manuscript is under review at *Research Connections*. *One Health systematic review.* Literature screening and data extraction were completed. *Arbovirus studies.* The review has been published in *Viruses* (Viruses 2026, 18(9), 970; https://doi.org/10.3390/v18090970). The Italian and European surveys included 160 and 157 responses respectively, analyses are ongoing. Screening is ongoing for the WNV systematic review, which will be integrated with the pediatric case description in a single manuscript. The Brazilian studies are approved and are scheduled to begin in October 2026.
Conclusions.
The first two years established an integrated program spanning laboratory, behavioral, population-based, One Health, and arbovirus research. Findings support VAMS feasibility, document the burden and management of vaccine-preventable infections, and identify gaps in arbovirus preparedness. Ongoing studies will extend this work nationally and internationally to inform pediatric infectious disease prevention and surveillance.

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PhD COURSE "MOLECULAR MEDICINE"

COORDINATOR: PROF. ARIANNA LOREGIAN

Curriculum "BIOMEDICINE"

MICROTUBULE ORGANIZATION DETERMINES CELL MECHANORESPONSIVENESS BY CONTROLLING AMOT DEGRADATION

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Ph.D. Course Molecular Medicine
Curriculum "Biomedicine"

Background
Mechanotransduction is the process through which cells perceive and actively respond to physical cues coming from their surrounding environment. The contribution of microtubules (MTs) to Mechanotransduction has been so far largely neglected. We have previously demonstrated that MTs rapidly respond to mechanical stimulation by reorganizing their architecture. In mechano-activated cells, MTs display an astral arrangement departing from a single, perinuclear area called MTs Organizing Centre (MTOC) and radiating towards the cell periphery, while mechano-inhibited cells show a cage-like structure of only short and peripheral MTs. Impairments of MTs astral architecture in mechano-ON cells exclude YAP/TAZ from the nucleus. Still, how MTs dynamics connects to cell mechano-responsiveness via YAP/TAZ is to be explored.

Material and Methods
To study MTs architecture and dynamics we employed live probes and live high resolution confocal imaging with 3D reconstructions, coupled with *loss-of-function* experiments based on RNA interference or targeted pharmacological treatments. The molecular and cellular effects of MTs perturbation were validated through Western Blot, qRT-PCR and Immunofluorescence. To derive YAP/TAZ common cytoplasmic interactors, we unbiasedly mined BioGRID4.4 dataset, together with publicly available pull-down and *in vivo* proximity labelling data.

Results
To investigate on the interplay between MTs organization and YAP/TAZ Mechanotransduction, we performed *loss-of-function* experiments addressing either MTOC formation or positioning, demonstrating that any impairment of MTs aster blunts YAP/TAZ activity. Given that the MTOC and proteasome colocalize, and MTs are essential for retrograde trafficking of proteins, we evaluated the hypothesis that a YAP/TAZ cytoplasmic inhibitor might exist, that is transported along MTs to the centrosome and degraded specifically in mechano-ON conditions, while being stabilized in mechano-OFF conditions due to the absence of such efficient and polarized transport. Among all YAP/TAZ interactors, only Angiomotin (AMOT) appeared to match this hypothesis. To validate that AMOT stability depends on a MTs and proteasome degradation, we chemically inhibited either MTs polymerization or proteasome activity in mechano-activated cells, resulting in AMOT protein stabilization. Additionally, to test dinein-mediated AMOT retrograde transport along MTs, we impaired dinein activity either pharmacologically or by knock-down of its key components, observing AMOT stabilization in treated mechano-activated cells.

Conclusions
We have demonstrated that MTs role is pivotal for cell mechano-responsiveness, since their architecture controls the level of AMOT, the long-sought YAP/TAZ cytoplasmic inhibitor, directly linking cytoskeletal remodelling to gene expression changes and cellular responses.

Nanodelivery systems of plant actives to overcome antimicrobial resistant SSTIs

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Background Skin and soft tissue infections (SSTIs) are becoming increasingly common worldwide, partly due to an aging population, a growing number of critically ill and immunocompromised patients, and increased intravenous drug use. The emergence of multidrug-resistant microorganisms has also made conventional antimicrobial treatments more challenging. As a result, plant-derived molecules with antimicrobial, anti-inflammatory, and antioxidant properties are being investigated as potential alternatives. However, their clinical use is often limited by poor stability, low absorption, and rapid degradation. These limitations may be overcome by incorporating them into suitable nanotechnology-based delivery systems. This PhD project therefore aims to develop and evaluate a nanoscale delivery system based on natural nanoparticles loaded with standardized plant-derived molecules, provided by ABResearch s.r.l., with the goal of targeting antimicrobial-resistant microorganisms responsible for SSTIs.

Materials and Methods The first year of the PhD focused on screening ABR plant extracts to determine their minimum inhibitory concentration (MIC) against MRSA and *Pseudomonas aeruginosa* (PAO1). ABR1 and ABR2, containing standardized amounts of verbascoside (VB) and plantamajoside (PMS), respectively, were identified as the most promising candidates. This year, the extracts were also tested against clinical strains of *Candida* spp. The research then examined the solubility of ABR1 and ABR2 in bacterial culture media and their persistence after exposure to MRSA and PAO1 by mass spectrometry. The bacteriostatic/fungistatic activity of the extracts against MRSA, PAO1, and *Candida albicans* was assessed by monitoring growth curves following extract addition at 7 hours of culture. The cytotoxicity of ABR1 and ABR2 toward HaCaT cells (keratinocytes) was assessed using the MTT assay, while their potential to promote wound healing *in vitro* was evaluated using the scratch assay. Possible synergistic effects between ABR1 and ABR2, and between these extracts and conventional antimicrobials, were investigated against MRSA, PAO1, and *C. albicans* using resazurin assays and bacterial growth curves. Finally, a liposomal formulation of ABR1 was evaluated against MRSA and *Escherichia coli* using the resazurin assay and by monitoring bacterial growth curves.

Results Regarding the mass spectrometry analysis, neither VB nor PMS was detected in the incubation supernatants, suggesting that these molecules interact with the bacterial cells. Possible mechanisms include binding to the bacterial membrane, cellular uptake, or bacterial metabolism. ABR2 showed a stronger bacteriostatic/fungistatic effect against MRSA and *C. albicans* compared with that of ABR1, whereas neither extract exhibited activity against PAO1. In the MTT assay, only high concentrations of ABR1 and ABR2, close to their MICs against PAO1, were tested, and these concentrations showed cytotoxic effects on HaCaT cells. In the scratch assay, both the MIC against MRSA and the MIC against PAO1 were evaluated. Only the lower concentration, corresponding to the MIC against MRSA, did not negatively affect *in vitro* wound healing, resulting in a wound closure rate comparable to that observed in untreated HaCaT cells. The synergistic assays showed that ABR1 and ABR2, either combined with each other or with several conventional antibiotics, generally produced an additive effect against MRSA and PAO1. Interestingly, both extracts showed a synergistic effect against *C. albicans* when combined with amphotericin B. Finally, the liposomal formulation of ABR1 showed a MIC against MRSA similar to that of ABR1 in solution and exerted a bacteriostatic effect at lower concentrations. Against *E. coli*, all tested concentrations allowed bacterial growth while maintaining a bacteriostatic effect compared with the untreated control.

Conclusions Since the concentrations of ABR1 and ABR2 active against PAO1 and *C. albicans* were cytotoxic to mammalian cells, combining these extracts with antibiotics appears to be the most promising strategy, allowing lower doses and potentially reducing antimicrobial resistance. Future studies should investigate the cytotoxicity of these lower doses and the mechanism of action of the extracts against pathogens.

CHARACTERIZATION OF A NOVEL INHIBITOR OF HPV E7 ONCOPROTEIN

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Ph.D. Course Molecular Medicine
Curriculum "Biomedicine"

Background High-risk human papillomaviruses (HR-HPVs) are recognized by the World Health Organization as important etiological agents of several cancers, including cervical, vulvar, vaginal, penile, anal, and head and neck cancers. HPV-induced carcinogenesis is driven by the two major oncoproteins (E6 and E7), which represent attractive targets in drug discovery. In a previous study, we screened a small-molecule library and identified Cpd20 as a promising scaffold for the inhibition of the E7/PTPN14 interaction. Within the same library, we identified CB-1 as able to disrupt the interaction between HR-HPV E7 and pRb by selectively binding to E7, and to rescue pRb levels in HPV18-positive HeLa cells. We also demonstrated the ability of CB-1 to induce pRb rescue in human tumour cell lines infected with other HPV genotypes and investigated its cellular effects, with particular focus on the activation of the intrinsic and extrinsic apoptotic pathways. Finally, since CB-1 selectively binds E7, we used it as a warhead for the synthesis of anti-E7 PROTACs (PROteolysis TArgeting Chimeras), i.e., molecules that can lead to the proteasomal degradation of a protein of interest upon recruitment of a cellular ubiquitin ligase.
Material and Methods HPV-positive cervical cancer cell lines, including HeLa (HPV18), CaSki (HPV16), ME180 (HPV68), and MS751 (HPV45) were employed. Cells were treated with CB-1 for different times and the effects of CB-1 on pRb levels were assessed by Western blotting. CB-1-induced apoptosis in HeLa cells was evaluated by flow cytometry using Annexin V/propidium iodide staining, by JC-1 staining to assess mitochondrial membrane potential ($\Delta\Psi$), as well as by Western blot analysis of full-length caspase-8. The activity of the anti-E7 PROTACs was assessed in HeLa cells by evaluating HPV18 E7 degradation and pRb rescue by Western blotting.
Results CB-1 was able to rescue pRb levels in a dose-dependent manner in HeLa (HPV18), CaSki (HPV16), ME180 (HPV68), and MS751 (HPV45) cell lines, thus showing a promising broad-spectrum activity against different HR-HPV genotypes. To identify the most appropriate time points for subsequent transcriptomic analyses, we also performed a time-course (14, 24, 48, and 72 h) analysis of pRb rescue in HeLa cells upon CB-1 treatment, and a significant pRb rescue was observed at all the considered time-points. Furthermore, as demonstrated by flow cytometry experiments performed in HeLa cells, CB-1 remarkably induced apoptosis and the collapse of $\Delta\Psi$, consistent with the involvement of intrinsic apoptotic pathway. Activation of apoptosis through the extrinsic pathway was assessed by Western blot analysis of full-length caspase-8, whose levels were not affected by CB-1. Finally, PROTACs based on CB-1 were tested in HeLa cells and some of them efficiently promoted E7 degradation and restored pRb levels.
Conclusions CB-1 effectively restored pRb levels across cervical cancer cell lines infected with different high-risk HPV genotypes, supporting its broad-spectrum activity against HR-HPV. A rapid and sustained pRb rescue observed over time along with a marked induction of apoptosis and mitochondrial membrane depolarization, consistent with involvement of the intrinsic apoptotic pathway, was observed in HeLa cells. Furthermore, CB-1-based PROTACs efficiently promoted HPV18 E7 degradation and restored pRb levels, highlighting the potential of CB-1 as a promising compound for the development of HPV-targeted therapies.

Point-of-care biosensors for Helicobacter Pylori diagnosis: a synthetic biology approach

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Background:
Helicobacter pylori is a major pathogen associated with gastritis, peptic ulcers, and gastric cancer. Although current diagnostic methods are accurate, they are limited by cost, invasiveness, specialized equipment, and the risk of false-negative results following antibiotic treatment. Therefore, there is a need for rapid, affordable, and reliable diagnostic tools. Synthetic biology offers promising solutions through the development of innovative biosensors, although challenges related to biosafety and regulatory frameworks still need to be addressed. Two approaches have been pursued: a whole-cell biosensor for the detection of urea that can complement the Urea breath test and a molecular diagnostics point-of-care for H.Pylori Urea gene identification

Material and Methods
The urea biosensor was engineered and optimised in *E. coli* through genetic circuit design, negative feedback regulation, and mathematical modelling. Circuits have been designed and simulated through MATLAB and molecular cloning. Their performance was evaluated using fluorescence assays with varioskan lux and results displayed with a normalization of fluorescence/absorbance.
RNA technology to detect acid nucleic biomarkers, especially the H.pylori UreA gene, has been studied and built with NUPACK. Their activity was assessed in a cell-free expression system and in vitro transcription.

Results
Urea MIC on bacteria chassis harboring the genetic circuits have established, the fluorescence analysis to different urea concentration was analised with Varioskan Lux spectrofotometer and repeated with higher urea concentration. Matematical model has been established and Negative feedback loop compared to the positive feedback loop. In cell free system Toehold switches were analysed and the best permored one used for RNA biomarker concentration assessment.

Conclusions
The urea biosensor showed a dynamic operating range of 0–500 mM, with fluorescence increasing proportionally to urea concentration at lower levels and decreasing above 500 mM due to urea toxicity. A basal fluorescence signal was observed even in the absence of induction, indicating biosensor leakiness. To improve sensitivity and reduce leakiness, optimization strategies including mathematical modeling and the implementation of a negative feedback circuit were investigated. Mathematical simulations enabled prediction of biosensor behavior under different genetic and experimental conditions, while the negative feedback architecture maintained the operating range and improved the expected response at lower urea concentrations, although leakiness remained unresolved. Further model-guided optimization and fine tuning is ongoing.
The NUPACK algorithm identified two 36-nucleotide regions within the *Helicobacter pylori ureA* mRNA suitable for toehold switch design. Both switches were cloned into the pUC57 backbone and evaluated in a cell-free system. Toehold Switch 1 showed higher sensitivity than Toehold Switch 0, likely because it targets a sequence located at the beginning of the *ureA* gene. Further validation with *in vitro*-transcribed RNA demonstrated that strong fluorescence was achieved only at high target RNA concentrations, whereas lower concentrations produced delayed and weaker signals. These findings indicate that an upstream isothermal nucleic acid amplification step, such as RPA or NASBA, is required for point-of-care detection

ROLES OF G-QUADRUPLEXES IN KAPOSI'S SARCOMA HERPESVIRUS LATENT REPLICATION

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic virus capable of establishing life-long latent infections in host cells, ultimately resulting in Kaposi's sarcoma, an incurable cancer affecting immunocompromised patients. Maintenance of KSHV latent infection requires the binding of the viral protein latency-associated nuclear antigen (LANA) both to the host cell chromosomes and to the viral non-coding terminal repeat (TR) sequences, highly guanine-cytosine-rich repeats that comprise about 20% of KSHV genome. Given their sequence, it has been proposed that TRs can fold into G-quadruplexes (G4s), secondary DNA structures highly conserved among herpesviruses. However, the possible function of these non-canonical DNA motifs within KSHV genome and how they are resolved by the eukaryotic host replication machinery remain unknown. In this work, putative G4-forming sequences within the TR were identified through bioinformatic analysis and their folding was experimentally confirmed by circular dichroism spectroscopy and PCR stop assay. To validate these findings in a cellular context, we applied CUT&Tag in KSHV latently infected cells, confirming G4s formation within the TR during viral latent infection. To gain insight into the molecular mechanisms underlying G4-mediated regulation of replication, we took advantage of Xenopus laevis egg extracts as a model for replication studies. In this context, replication of a TR sequence was impaired, leading to accumulation of single-stranded DNA and checkpoint activation, collectively indicating G4-mediated replication stress. Finally, we identified several eukaryotic proteins potentially involved in TR replication and likely associated with G4s, currently under study to define their role in TR G4s resolution. This work supports the propensity of G4s to fold within the TR region and their intrinsic ability to interfere with the eukaryotic DNA replication machinery in the absence of LANA binding.

An unbiased proximity proteomics method to study MIEF1 interactome

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Background
Mitochondrial dynamics play an essential role in human physiology and disease. The dynamics of mitochondria consist in continuous fusion and fission events. Mitochondrial fission is dictated by the GTPase DRP1 which is actively recruited at mitochondria fission foci and plays a pivotal role in this process. The recruitment and function of DRP1 is adjuvated by adaptor proteins such as MIEF1, MIEF2, and MFF. MIEF1, in particular, is suggested to be involved in neurodegeneration, apoptosis, and mechanotransduction. However, its exact protein interactors that mediate the above-mentioned biological processes are yet to be defined. Hence, a proximity proteomics method was set up and optimized with MIEF1 as a bait protein to reveal its protein partners.
Material and Methods
TurboID proximity-based method was implemented to study MIEF1 interactors. For that MIEF1-TurboID was expressed in MCF10A-Ras cells via a doxycycline-inducible system to obtain near-to-endogenous levels of expression, and after induction biotin labelling was performed. Cell lysates were extracted, quantified, and incubated with streptavidin magnetic beads to pull down the proteins biotinylated due to their proximity with MIEF1. Cells expressing the cytoplasmic TurboID were treated and extracted in parallel, and lysates were pulled down, as control of biotin labelling. Consequently, the streptavidin-enriched samples were eluted and run for LC-MS/MS analysis. Finally, the enriched proteins were analysed with STRING database and protein-protein interaction networks were reconstructed.
Results
The proteomics analysis resulted in a list of proteins that were significantly enriched upon MIEF1-TurboID induction and biotin labelling. After filtering this list from the background TurboID labelling, approximately 90 hits resulted as high confidence MIEF1-neighboring proteins. The list was particularly rich in outer mitochondrial membrane proteins and among them were present MIEF2 and MFF, established MIEF1-proximal proteins. DRP1 was not present in the high confidence list, due to its strong labelling by the cytoplasmic TurboID, likely because of the primarily cytosolic nature of the DRP1 protein. Moreover, the significant enrichment of Miro-2, Myosin-19, and TRAK1 may suggest an interesting role of MIEF1 in mitochondrial trafficking and distribution in the cell.
Conclusions
Our proximity proteomics study provided us with approximately 90 proteins that may constitute MIEF1 broader interactome. The presence of outer mitochondrial membrane proteins deemed as MIEF1 interactors suggests the reliability of the method. As a next step for validating putative MIEF1 interactors is performing co-immunoprecipitation and proximity ligation assays to examine the physical interaction among the proteins of interest.

Exploring the Frontiers of Lyssaviruses Diversity with *In Vitro* and *In Vivo* Approaches

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Background
Lyssaviruses are the etiological agents of rabies, a zoonotic acute and fatal encephalomyelitis. While *Lyssavirus rabies* (RABV) is the unique multi-host pathogen, non-RABV lyssaviruses are mainly associated to bat species. Among these viruses, *Lyssavirus caucasicus* (WCBV) and *Lyssavirus lleida* (LLEBV), circulating in *Miniopterus schreibersii* bat in Italy, are of particular concern due to the lack of effective immunizing countermeasures. The project aimed to investigate the pathogenicity and host tropism of WCBV and LLEBV in comparison with RABV by (i) investigating their pathogenicity in the Syrian hamster model, (ii) developing reverse genetics systems and characterizing the recombinant and recombinant mutant viruses *in vitro*, and (iii) establishing a serological assay for the detection of lyssavirus-specific antibodies in bats and accidental spillover hosts.

Material and Methods
In vivo experiments were conducted in Syrian hamsters to investigate the pathogenicity of WCBV following oral and intradermal inoculation. In addition, in light of previous experimental findings, a sequential infection experiment was performed to assess whether prior LLEBV infection could confer protection against subsequent WCBV challenge. Recombinant wild-type lyssaviruses were generated using a reverse genetics system based on transient transfection of BSR T7/5 cells with the genomic and helper plasmids, the latter encoding for the viral N, P, and L proteins. Plasmids were generated, sequence-verified and used to establish and optimize the transfection conditions required for recombinant virus rescue. Site-directed mutagenesis was initiated to generate recombinant mutant viruses. Finally, a pseudovirus-based neutralization assay based on a dual-reporter luciferase system was developed, validated and evaluated in comparison with the Rapid Fluorescent Focus Inhibition Test (RFFIT).

Results
Animal experiments showed that WCBV induced significant mortality following intradermal inoculation, whereas oral administration was not lethal. In a separate experiment, hamsters previously inoculated intramuscularly with LLEBV were subsequently challenged with WCBV, showing significantly higher survival than the WCBV-only control group and suggesting that prior LLEBV infection may confer cross-protection against WCBV. All plasmids required for the generation of wild-type recombinant viruses were successfully generated, while transfection and viral amplification protocols were optimized and site-directed mutagenesis was initiated for the generation of recombinant mutant viruses. In parallel, a pseudovirus-based neutralization assay was successfully established and evaluated against RFFIT. Preliminary results showed good agreement between the two methods; further validation analyses are still ongoing.

Conclusions
Together, these complementary models enhance our understanding of lyssavirus pathogenesis and host responses, paving the way for future research into this zoonotic group of viruses.

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PhD COURSE "MOLECULAR MEDICINE"

COORDINATOR: PROF. ARIANNA LOREGIAN

Curriculum "REGENERATIVE MEDICINE"

Human neuromuscular organoids mimic cancer-induced muscle cachexia

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Background
Cancer cachexia is a complex metabolic syndrome characterized by progressive skeletal muscle wasting, loss of muscle function and systemic metabolic alterations. Despite its major impact on patients' quality of life, effective treatments are still lacking. The limited availability of human experimental systems capable of reproducing the multicellular organization and functional properties of skeletal muscle represents a major obstacle to the identification of disease mechanisms and therapeutic targets. To address this limitation, we developed human induced pluripotent stem cell (hiPSC)-derived neuromuscular organoids (NMOs) as a human in vitro model to investigate tumor-induced muscle wasting and test therapeutic strategies.
Material and Methods
Human NMOs were exposed to conditioned medium derived from cachexia-inducing C26 colon cancer cells. Cachectic exposure was investigated by assessing myofiber morphology, muscle function, calcium homeostasis, mitochondrial function, cellular metabolism and autophagy. Transcriptomic, proteomic and metabolomic analyses were integrated to identify molecular pathways altered by tumor-derived factors and candidate mediators potentially involved in the establishment and progression of muscle wasting. To investigate therapeutic strategies, NMOs were treated with antisense oligonucleotides targeting the catabolic regulators FOXO1, FOXO3 and TRIM63/MuRF1. Finally, the translational relevance of the model was evaluated by exposing NMOs to sera obtained from patients with cancer cachexia.
Results
Exposure of NMOs to C26-derived conditioned medium reproduced multiple hallmarks of cancer cachexia. Treated organoids displayed reduction in myofiber size together with impaired contraction and altered calcium handling. These functional defects were associated with mitochondrial dysfunction, extensive metabolic remodeling and alterations in autophagy-related processes, demonstrating that the model reproduces both structural and molecular features of cachectic skeletal muscle. Multi-omic profiling further revealed transcriptional, proteomic and metabolic alterations and identified a panel of candidate mediators potentially released either by tumor cells or by the responding cachectic NMOs and capable of contributing to disease progression. Targeting the FOXO-dependent catabolic program using antisense oligonucleotides against FOXO1/3 and TRIM63 completely prevented muscle wasting and restored muscle function, providing proof-of-concept for a multitarget RNA-based therapeutic strategy. Importantly, exposure to sera from patients with cancer cachexia was sufficient to reproduce the pathological phenotype in human NMOs, inducing myotube atrophy and activation of autophagy-related processes. These results indicate that circulating factors present in patients can be functionally interrogated using the organoid platform.
Conclusions
hiPSC-derived neuromuscular organoids provide a human and functionally relevant model of cancer-induced muscle cachexia that recapitulates morphological, functional, metabolic and molecular features of the disease. The platform enables mechanistic investigation of tumor-muscle communication, identification and validation of circulating mediators, and testing of therapeutic interventions

Role of YAP/TAZ in immune coldness in breast cancer

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Curriculum "Regenerative Medicine"

Background

The general aim of this project is to investigate the role of two mechanosensitive transcriptional cofactors, Yes-associated protein 1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ), in the progression and maintenance of breast cancer (BC) using genetically engineered mouse models. Preliminary experiments revealed that conditional YAP/TAZ KO (cKO) only in cancer cells in established primary masses lead to a strong infiltration of immune cells, which was not present in control (YAP/TAZ expressing) tumours. In fact, $CD8^+$ T cell population changed in composition when YAP/TAZ were knocked out in tumour cells; in particular, there was an increase in the fraction of active cytotoxic T lymphocytes (CTLs), while YAP/TAZ wild-type tumours were immunologically cold. The presence of less or exhausted CTLs is functional to the malignancy of YAP/TAZ wild-type tumours. These preliminary results hinted at a novel, non-cell-autonomous function of YAP/TAZ in BC, where their activation blocks CTLs from infiltrating the tumour. Therefore, our aim is to understand how YAP/TAZ exert this immunosuppressive function.

Material and Methods

Single cell RNA-sequencing was performed on MMTV-PyVT YAP/TAZ wild-type and cKO tumours to deeply cover the differences in BC cells after YAP/TAZ cKO. We exploited bulk RNA sequencing in *in vitro* MMTV-PyVT culture to understand whether the differential expression was due to changes in the tumour micro-environment or a cell-autonomous consequence of YAP/TAZ KO. We performed RT-qPCR in MCF-10A, E0771.lmb, 4T1 cells receiving opposite mechanical cues (sparse vs dense, stiff vs soft) to evaluate if the differences were recapitulated also by mechanoregulation and not only with YAP/TAZ depletion.

Results

Single-cell RNA seq in YAP/TAZ wild-type and cKO tumours revealed that interferon stimulated genes (ISG) were the most significantly upregulated family upon YAP/TAZ KO. Among the upregulated genes we find secreted chemokines with well-established functions in T-cell recruitment and activation, as well as intracellular signal transducers and transcription activators that can boost the expression of ISG within YAP/TAZ KO tumor cells. The same genes are upregulated after *ex vivo* YAP/TAZ KO in purified tumor cell cultures, suggesting that the activation of this program is a direct, cell-autonomous consequence of YAP/TAZ genetic depletion. We found the inhibition of YAP/TAZ by low mechanical stimulation – rather than genetic depletion – also induced ISG upregulation. Finally, we investigated the intracellular molecular mechnaisms by which YAP/TAZ could modulate the expression of ISG.

Conclusions

These results contribute to the understanding of how YAP/TAZ regulate ISG in tumour cells. *In vitro*, we were able to show that YAP/TAZ KO and mechano-OFF states upregulate ISG, recapitulating what we observed *in vivo*. This hints at the possibility that YAP/TAZ prevent ISG expression in BC cells, while YAP/TAZ KO makes BC cells permissive to the ISG program.

Bioactive and Synthetic Nerve Wraps for Peripheral Nerve Repair: A Preclinical Study of Fibrosis, Functional Recovery, and the Regenerative Microenvironment

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Curriculum "Regenerative Medicine"

Background
Peripheral nerve injuries (PNIs) often result in incomplete functional recovery due to excessive perineural fibrosis and adhesions that obstruct axonal regeneration and impair nerve gliding. Although autologous nerve grafting remains the clinical gold standard for bridging nerve defects, the recurrence of fibrosis and lack of bioactivity in conventional synthetic barriers limit optimal outcomes. Nerve wraps offer a strategy to mechanically shield the repair site; however, synthetic barriers lack intrinsic bioactivity, while naturally-derived alternatives may provide additional regenerative cues to actively modulate the healing microenvironment.
Material and Methods
Electrospun poly (vinyl alcohol) (PVA) membranes, with or without multi-walled carbon nanotubes (MWCNTs), were fabricated, chemically crosslinked with citric acid, and characterized for ultrastructure (SEM/TEM), mechanical properties, and electrical conductivity. Cryopreserved human amniotic membrane (AM) was obtained from a tissue bank and characterized for morphostructural organization (H&E, Masson's Trichrome, Alcian Blue), immunogenicity (HLA-DR expression), and growth factor content via a 41-plex antibody array. In the preclinical study, twelve Sprague-Dawley rats underwent sciatic nerve transection and reverse autograft repair, randomized into three groups: autograft alone, autograft + PVA wrap, and autograft + AM wrap. Functional recovery was assessed by Sciatic Functional Index (SFI) at 6 and 12 weeks post-surgery. At endpoint, perineural adhesions were scored according to Petersen's classification, and explanted nerves and gastrocnemius muscles were analyzed by histology, morphometry, and SEM.
Results
The PVA wrap significantly reduced perineural adhesions compared to autograft alone (Petersen score: 1.5 ± 0.58 vs. 2.5 ± 0.58, $p<0.05$), correlated with improved SFI values and reduced epineurial area (33.94% vs. 54.86%). MWCNT incorporation enhanced electrical conductivity but was associated with a more pronounced fibrotic response. AM characterization revealed a trilaminar ECM-rich structure with absence of HLA-DR expression, confirming low immunogenicity, and a distinctive growth factor profile dominated by Hepatocyte Growth Factor (89.76%) and Epidermal Growth Factor (66.62%), alongside neurotrophins including GDNF, NT-3, and NGF. In vivo, the AM group also exhibited reduced adhesions (1.5 ± 0.58), comparable to the PVA group, with preserved muscle architecture across all treatments.
Conclusions
Electrospun PVA represents a promising synthetic barrier for reducing perineural fibrosis and improving functional recovery. Importantly, the amniotic membrane, beyond its mechanical barrier function, provides a rich reservoir of anti-fibrotic and neurotrophic factors—suggesting it can actively modulate the regenerative microenvironment. These findings highlight the potential of AM as a superior bioactive wrap for peripheral nerve repair, warranting further investigation into its long-term regenerative efficacy and the mechanisms underlying its bioactive contribution.

Understanding Treatment Response to bulevirtide in HDV Hepatitis: an In-Depth analysis

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Ph.D. Course Molecular Medicine
Curriculum "Regenerative Medicine"

Background Chronic hepatitis Delta virus (cHDV) is responsible for the most severe form of viral hepatitis that affects ~70 million individuals. HBV-HDV co-infection or superinfection rapidly leads to liver fibrosis progression and to an enhanced risk of liver decompensation and hepatocellular carcinoma development with respect to HBV monoinfection. Studies have shown that HDV infection is associated with severe depletion and functional impairment of circulating mucosal-associated invariant T (MAIT) cells, an innate-like T-cell population with potent antimicrobial and antiviral effector function. Bulevirtide (BLV), a recently approved entry inhibitor, blocks the sodium taurocholate co-transporting polypeptide (NTCP) receptor required for hepatocyte HBV and HDV entrance. BLV has shown to achieve durable virological and biochemical responses in most treated patients, regardless of cirrhosis, but the reason why most patients respond to treatment, some do have a rebound and whether virological control translates into immune reconstitution has not been characterized.
Material and Methods Eleven patients with chronic HDV initiating BLV at our outpatient clinic were prospectively enrolled and longitudinally followed, with clinical, virological and flow-cytometric assessment at baseline and during treatment. MAIT cells (CD3+CD161+Vα7.2+) were subdivided into CD8+, CD4+ and CD4−CD8− subsets and characterized on CD8+ for CD38, PD-1 and CD127 expression; natural killer cells (CD3-CD56+), another major component of the intrahepatic immune-cell infiltrate, were characterised into CD56bright(CD16+) and CD56dim subsets and for the expression of the inhibitory NKG2A. Furthermore, microbiota composition of each patient was characterised with the aim to understand if BLV therapy could affect its composition and, possibly, the restoration of beneficial bacteria.
Results From baseline to 24 months of therapy, 2 patients died because of HCC complications. Of the remaining that achieved this timepoint (8 patients) ALT and AST normalization achieved respectively 50% and 75% of our population. Virological response (> $2\log_{10}$) at 24 months was detected in all treated patients. For immune cells characterisation, HDV treated patients were compared to 10 HBV monoinfected patients and 10 healthy controls. The expression of the co-stimulatory CD127 on CD8MAIT was significantly lower at baseline for HDV patients with respect to healthy controls. NKs CD56 bright population (more immature and immunoregulatory subset) with respect to total NKs was significantly lower at baseline. Finally, one patient experienced a virological rebound at 24 months. Although he maintained a virological response to treatment, his immunological profile was with the highest expression of exhaustive marker PD-1.
Conclusions In this exploratory longitudinal cohort, BLV therapy was associated with clear biochemical and virological responses and early signs of MAIT-cell and NK-cell reconstitution/activation. Data from MYR301 clinical trial reveals that BLV monotherapy is effective and safe but late relapses after treatment discontinuation can occur even in the absence of HDV RNA detectability at the end of treatment.
Looking at the future where this population could benefit from a personalised treatment duration, response achievement and long-term HDV RNA undetectability, matching data at ScRNA sequencing may provide a more comprehensive view at the single level and in vitro cells stimulation with specific anti-viral peptides may reveal the potential activity of antigen-specific CD8+ cells. Larger cohorts and other functional assays are needed.

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PhD COURSE "ONCOLOGY AND IMMUNOLOGY"

COORDINATOR: PROF. ANTONIO ROSATO

Decoding the clinical impact of somatic mtDNA variants in high-grade serous ovarian cancer

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Background: Epithelial ovarian cancer (EOC) is characterized by a high mortality rate, largely due to late-stage diagnosis. Beyond classic genetic alterations, mitochondrial DNA (mtDNA) variants are among the most frequent genetic events in these tumors, potentially impacting cellular metabolic homeostasis. By altering the oxidative phosphorylation (OXPHOS) system and reactive oxygen species (ROS) production, mtDNA mutations can significantly shape the tumor's metabolic profile. However, their prognostic and predictive value for current therapies (chemotherapy, PARP inhibitors, bevacizumab) remains poorly defined. This is largely because they have often been overlooked or considered mere passenger events. Furthermore, while previous studies investigated mtDNA mutations, there is a lack of information specifically regarding highly represented variants: experimental models indicate that a high Variant Allele Frequency (VAF >50%) is necessary to significantly alter the tumor's metabolic phenotype. Consequently, the actual clinical impact of high-VAF mtDNA mutations in ovarian cancer is still poorly understood and requires further investigation.
Material and Methods: The discovery cohort (retrospective) included 62 fresh-frozen tumor samples of high-grade serous EOC (HGS-EOC), with corresponding clinical and pathologic data, obtained from patients diagnosed and treated at the Division of Obstetrics and Gynecology of the ASST Spedali Civili of Brescia (Italy) between January 2005 and May 2021. Patients underwent debulking surgery and adjuvant platinum-based chemotherapy; subsequently, 32 entered observation and 30 received bevacizumab maintenance. mtDNA sequencing data were correlated with progression-free survival (PFS) and overall survival (OS). The validation cohort (prospective) (IOV - Padova) comprises 144 EOC samples undergoing mtDNA sequencing, stratified into Cohort A (carboplatin/paclitaxel + bevacizumab + PARP inhibitor) and Cohort B (carboplatin/paclitaxel + PARP inhibitor).
Results: In the retrospective cohort, mtDNA sequencing identified 110 distinct variants across 62 samples (31 carrying ≥2 variants, 20 with 1, and 11 wild-type). Most variants (67.3%) mapped to OXPHOS genes, predominantly Complex I (42%) and IV (16%), followed by mt-rRNA (23.6%). Among coding variants, 89% were missense, whereas 11% were nonsense and frameshift variants. To achieve a more accurate estimation of tumor VAF and exclude potential stroma contribution, VAFs were normalized to tumor purity. High-level heteroplasmy (normalized VAF >90%) was detected in 13 patients (20.9%). Interestingly, homoplasmic mtDNA mutations strongly correlated with improved PFS (p=0.00042, HR=0.24) and OS (p=0.0053, HR=0.29). Expanding the threshold to VAF >50% (21 positive vs. 41 negative patients) confirmed this significant survival benefit in both PFS (p=0.0036, HR=0.40) and OS (p=0.0097, HR=0.42). However, interaction tests indicated no significant treatment-specific predictive value for bevacizumab; rather, mtDNA mutations emerged as strong independent prognostic factors for improved survival irrespective of treatment.
Conclusions: Somatic high-VAF mtDNA mutations act as strong prognostic biomarkers associated with significantly improved survival outcomes in HGS-EOC. To validate these findings and explore their clinical applicability, ongoing analyses on the independent prospective cohort (n=144) will assess the prognostic and predictive value of mtDNA variants for combined targeted therapies (bevacizumab and PARP inhibitors), alongside their functional interplay with the *BRCA1/2* mutational status.

UNRAVELLING THE CLINICAL AND BIOLOGICAL FEATURES OF ACCELERATED CHRONIC LYMPHOCYTIC LEUKEMIA: A RARE HISTOLOGICAL VARIANT OF CLL

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Background: Chronic lymphocytic leukemia (CLL) has an indolent course and is the most common adult leukemia. However, 2–10% of patients undergo Richter's transformation (RT), to an aggressive lymphoma, an almost invariably fatal event. Accelerated CLL (aCLL) is a rare histological variant of CLL, diagnosed on lymph node tissue by the presence of large proliferation centers, increased mitotic rate, or Ki67 >40%, that may sit in-between CLL and RT. Past published series on aCLL were small, monocentric and on patients treated with chemo-immunotherapy (CIT). Although they reported a worse survival than CLL, the behavior of aCLL under targeted agents and its biology have never been investigated.
Material and Methods: We conducted a multicentric retrospective cohort study enrolling patients with a histologically proven diagnosis of aCLL. Patients with CLL and RT followed at the Hematology Unit of the University of Padova served as controls. Clinical, radiological, histological and biological data were collected. OS was the primary endpoint, time to next treatment (TTNT) and the cumulative incidence of RT were secondary endpoints. Targeted next-generation sequencing (NGS, 73-gene panel) and single-cell RNA sequencing (scRNAseq) were performed on archived FFPE lymph node samples from the three conditions.
Results: A total of 249 patients were enrolled: 78 aCLL (31.3%), 155 CLL (62.3%) and 16 RT (6.4%). Compared with CLL, aCLL more frequently presented lymphadenopathies >5 cm (39.7% vs. 5.2%), systemic symptoms (25.6% vs. 9.7%) and extranodal involvement (23.1% vs. 6.5%; all $p \leq 0.001$), and showed metabolic and proliferative activity intermediate between CLL and RT (median SUVmax 7.9 vs. 2.6 and 15.4; median Ki67 20% vs. 6% and 70%). aCLL was enriched in unmutated *IGHV* (79.7% vs. 59%; $p<0.001$), *TP53* disruption (36.6% vs. 19.7%; p=0.033) and complex karyotype (54.3% vs. 25%; p=0.02). OS was intermediate (5-year OS 63% in aCLL vs. 77% in CLL and 0% in RT; HR vs. CLL 1.83, 95%CI 1.03–3.26, p=0.038). First-line targeted therapy (n=25) outperformed CIT (n=9), with longer TTNT (median not reached vs. 47 months; HR 0.28, 95%CI 0.08–0.98; p=0.04). The 5-year cumulative incidence of RT was 9.0% in aCLL vs. 0.65% in CLL (p=0.003). NGS identified *TP53* (38%), *SF3B1* (19%), *BRAF* (14%) and *KMT2D* (14%) as the most frequently mutated genes. In pseudo-bulk analysis aCLL occupied an intermediate transcriptional position between CLL and RT while retaining its own program. Four aCLL-enriched B-cell clusters upregulated genes of B-cell activation (*FCRL5*, *TNFRSF17*), microenvironmental responsiveness (*IL4R*, *CCR7*, *ROR1*) and proliferation (*MKI67*, *CCND2*). Along the CLL-aCLL-RT continuum, M2-primed macrophages and exhausted CD8+ T cells progressively increased.
Conclusions: aCLL is a distinct entity sitting between CLL and RT, whose aggressiveness appears driven by B-cell activation and a tumor-supporting microenvironment. It carries adverse prognostic factors, shorter survival and a higher risk of RT, yet responds to targeted agents as effectively as CLL. Identifying aCLL may therefore represent the first realistic opportunity to prevent RT rather than treat it. Ongoing work will address clonal relatedness, BCR reactivity and the microenvironment on sequential samples.

FAECAL CALPROTECTIN AND THE MICROSCOPIC PHENOTYPE OF IMMUNE CHECKPOINT INHIBITOR-INDUCED COLITIS

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Background
Immune checkpoint inhibitor colitis (ICIC) is the most frequent gastrointestinal immune-related adverse event and a leading cause of treatment discontinuation. No biomarker is validated in this setting and the evidence on faecal calprotectin (FC) is discordant: one series found that FC rises with endoscopic severity, two cohorts identified a threshold of about 620 mcg/g for corticosteroid non-response, while the largest cohort available reported a median of 273 mcg/g and no association with endoscopic severity. These studies sampled FC at different points of the disease course and answered different questions, which may explain the discrepancy but has never been formally addressed. A further gap concerns the microscopic phenotype, in which endoscopy is normal and FC is expected to be low, so that a normal endoscopy may be falsely reassuring.

Material and Methods
Patients with colitis occurring during ICI therapy and enrolled at the Gastroenterology Unit of Padua within the European ICIC-ECCO registry (REDCap, PID 829) were reviewed. Inclusion required at least one lower endoscopy showing macroscopic and/or histological colitis after starting ICI. The dataset was reconciled against departmental records and updated to January 2026, and clinical, endoscopic, histological, laboratory and treatment variables were extracted at baseline and at 3, 6, 12 and 24 months. In parallel, a systematic search of MEDLINE and Embase retrieved 1324 unique records.

Results
Thirty-eight patients were included, 18 retrospective and 20 prospective; the retrospective patients partly overlap with the published European cohort, to which this centre contributed. Baseline colitis was CTCAE grade 1 or 2 in most patients. Ulceration was present in 5 of 38, the mucosa was normal in 13 and showed non-ulcerative inflammation in 13. A microscopic pattern was reported in 30 of 38 patients (79%), 19 collagenous and 11 lymphocytic, and in 9 of these endoscopy was normal. Baseline FC was available in 26 patients, median 765 mcg/g, and among the 9 with a normal mucosa median FC was 447 mcg/g (range 84-1891).

Conclusions
The cohort shows a mild-to-moderate, predominantly non-ulcerative phenotype in which FC is often elevated despite a normal endoscopy. The second year has identified a methodological rather than a biological problem: the microscopic phenotype is not defined consistently across series, so that prevalence figures ranging from 9% to 79% may reflect classification rather than disease. Three lines of work follow. A systematic review will address the discordance between FC studies by separating the questions they answer, since thresholds for post-treatment remission and for treatment escalation are not interchangeable. All cases will undergo central histopathological re-reading with quantification of intraepithelial lymphocytes and collagen band thickness, replacing categorical labels with continuous measures, and will be compared with idiopathic microscopic colitis. Finally, the trajectory of FC will be analysed within the European registry as a predictor of remission and corticosteroid dependency.

SERUM METABOLOMIC SIGNATURES FOR CIRRHOSIS DETECTION AND PREDICTION IN PRIMARY SCLEROSING CHOLANGITIS

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Background
Primary Sclerosing Cholangitis (PSC) carries a variable progression toward advanced fibrosis and cirrhosis, contributing to its poor prognosis and complicating accurate disease staging. Radiological findings in PSC often overlap with those of cirrhosis, further limiting diagnostic accuracy. Incorporating metabolic information may offer valuable insights into disease severity. This proof-of-concept study aimed to explore serum metabolomic profiles associated with cirrhosis in PSC and identify potential biomarkers to improve cirrhosis detection and stage stratification.

Material and Methods
In this international multicenter study, serum samples were obtained from 216 PSC patients enrolled at nine centres across seven countries. Patients were randomly allocated (70:30) to a discovery cohort (n=154; 22% cirrhosis) and a validation cohort (n=62; 29% cirrhosis). Metabolomic profiling was performed using ultra-high-performance liquid chromatography–mass spectrometry (UHPLC-MS). Multivariate logistic regression adjusted for age, sex, inflammatory bowel disease, alkaline phosphatase, and bilirubin was used to identify metabolites independently associated with cirrhosis. Candidate metabolites were prioritized using bootstrap- based Elastic Net regression with cross-validation and combined into predictive models. The best-performing model was validated in the independent cohort. Stable metabolites were subsequently used to construct a model for 5-year cirrhosis prediction.

Results
Among 484 detected metabolites, 121 were significantly associated with cirrhosis, of which 27 (16 increased, 11 decreased) remained independently associated after adjustment. A seven-metabolite model differentiated cirrhotic from non-cirrhotic patients in the discovery cohort (AUC= 0.86). In the validation cohort, the model achieved an AUROC of 0.78. Among non-cirrhotic patients (n=56), 8 (14%) developed cirrhosis during five-year follow-up, despite no baseline differences in FIB-4 scores (p=0.15). A six-metabolite model derived from the original signature predicted 5-year cirrhosis development with high accuracy (AUROC 0.88), and demonstrated superior discrimination compared with existing prognostic scores Amsterdam-Oxford Score (0.66; p=0.04) and revised Mayo Risk Score (0.47; p<0.01).

Conclusions
Serum metabolomic profiles differ according to the stage of PSC, highlighting metabolomics as a promising complementary tool for disease staging and cirrhosis detection. These findings support the potential integration of metabolomic data into current patient stratification models. Exploratory analyses indicate potential utility for predicting cirrhosis, supporting further validation in larger independent cohorts and integration into patient stratification models. Future research should focus on optimizing the proposed algorithms and once refined, validating it in larger, independent cohorts.

EPIGENETIC REMODELING DURING GASTRIC CARCINOGENESIS: FUNCTIONAL CHARACTERIZATION OF METHYLATED GENES IN THE METAPLASIA–DYSPLASIA–CARCINOMA SEQUENCE. PRELIMINARY RESULTS FROM THE METHYLEPIC-PROJECT

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Background
Gastric adenocarcinoma develops through stepwise morphologic, molecular, and epigenetic alterations along the non-neoplastic gastric mucosa–intestinal metaplasia–dysplasia–carcinoma sequence. However, the timing of DNA methylation changes across these histologically defined stages remains incompletely understood. We conducted an exploratory pilot analysis to determine whether promoter methylation alterations emerge progressively during gastric carcinogenesis or are already detectable in early precursor lesions.
Materials and Methods
Matched samples of non-neoplastic gastric mucosa, intestinal metaplasia, dysplasia, and carcinoma were retrospectively selected from two Epstein–Barr virus-negative, microsatellite-stable patients. For each histologic stage, representative lesional areas were reviewed and selected by an expert pathologist. Targeted methylation profiling was performed using the Biomodal duet multiomics solution kit (Biomodal, Cambridge, UK), enriched with the Twist Alliance Pan-Cancer Methylation Panel (Twist Bioscience, South San Francisco, CA, USA). Candidate genes were prioritized according to promoter coverage, temporal methylation trajectory, inter-patient concordance, and stage-to-stage methylation changes, and classified as early remodeling/plateau, progressive, mixed/non-monotonic, or stable/weak. Pathway enrichment was assessed using g:Profiler, with promoter-associated genes interrogable by the Twist panel as the custom background and correction for multiple testing.
Results
Distinct temporal promoter hypermethylation trajectories were observed across the histologically defined stages of the Correa cascade. Twenty-one candidate genes showed an early-remodeling pattern, characterized by a marked increase in promoter methylation between non-neoplastic gastric mucosa and intestinal metaplasia**,** followed by relative stability in dysplasia and carcinoma; *CDO1* was representative of this pattern. In contrast, 12 candidate genes showed a progressive promoter hypermethylation toward carcinoma, as exemplified by *ZNF529*. Enrichment analysis of the early-remodeling candidates, using all genes interrogable by the Twist panel as the background set, showed no significant enrichment for Gene Ontology Biological Process or Reactome categories (lowest adjusted $p \approx 0.177$). Reactome pathway enrichment was detected among the 12 candidates showing progressive promoter hypermethylation toward carcinoma (adjusted $p = 0.038$); however, most terms were supported by a single overlapping gene and were functionally redundant. Integrin cell-surface interactions represented the clearest multi-gene signal, involving *COL23A1* and *JAM3*.
Conclusions
These preliminary findings suggest that epigenetic remodeling during gastric carcinogenesis may be temporally heterogeneous across morphologically recognizable disease stages. Some methylation alterations may arise as early as intestinal metaplasia, whereas others may continue to evolve through dysplasia toward invasive carcinoma. The pathway-level signal identified among progressively remodeled genes, particularly those involved in cell–cell and cell–matrix interactions, provides a preliminary biological rationale for validation in larger, independent patient cohorts.

EXTERNAL VALIDATION AND INTERIM PET INTEGRATION OF THE E-HIPI IN EARLY-STAGE CLASSICAL HODGKIN LYMPHOMA

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Background. Modern PET-guided therapeutic strategies achieve high cure rates in early-stage classical Hodgkin lymphoma (cHL), although a clinically relevant minority of patients experience relapse or primary refractory disease. Currently, therapeutic choices are informed by dichotomic risk factor that classify cHL in early favourable or early unfavourable stage. The recently proposed Early-Stage Hodgkin Lymphoma International Prognostic Index (E-HIPI) predicts patient-specific 2-year progression-free survival (PFS) from sex, maximum tumour diameter (MTD), haemoglobin and albumin. After external validation of the E-HIPI in a contemporary multicentre cohort, this project explored E-HIPI-based risk stratification and the possibility of improving predictions by using a dynamic model that incorporates the result of interim PET scans (PET2; performed after two chemotherapy cycles).

Material and Methods. Patients aged 18–65 years with early-stage cHL diagnosed between 2007 and 2025 at four Italian centres and treated with ABVD- or BEACOPP-based PET-guided approaches were retrospectively included. PFS was defined as time from diagnosis to progression, relapse or death. E-HIPI-predicted 2-year PFS was calculated using the published coefficients and original plausibility exclusions; missing predictors were handled by multiple imputation by chained equations. Discrimination was assessed using Harrell's C-index, while calibration was evaluated by calibration-in-the-large and calibration slope. PET2 was classified according to Deauville score, with scores 4–5 considered positive. Post-PET2 PFS was evaluated by landmark analysis. The dynamic risk groups combined the lowest quartile of E-HIPI-predicted progression risk with PET2 negativity, the remaining PET2-negative patients, and PET2-positive patients.

Results. A total of 271 patients were enrolled: median age was 31 years, 49% were female, 93% had stage II disease, 32% had bulky disease and 25% had B symptoms. First-line treatment was ABVD-based in 261 patients (96%); seven (3%) received an HD17-like regimen and three (1%) an unspecified regimen. Albumin and MTD were missing in 16 and 37 patients, respectively. After a median follow-up of 63 months, 38 patients experienced progression or relapse and eight died. Two-year PFS was 87.4% (95% CI, 83.6–91.7), while 2-year overall survival was 100%. After external validation, E-HIPI showed moderate discrimination (C-index, 0.654; 95% CI, 0.55–0.75), with a C-index value in keeping with that of the original publication. Predicted progression risk was systematically lower than observed at 2 years (observed–predicted difference, 6.3 percentage points; 95% CI, 2.6–10.4), while the calibration slope was 1.162 (95% CI, 0.444–1.941). The PET2 analysis included 264 patients: 242 (92%) were PET2-negative and 22 (8%) PET2-positive. Two-year post-PET2 PFS was 91.0% and 35.6%, respectively (HR, 8.8; 95% CI, 4.4–17.5; $p<0.001$). The dynamic groups had 2-year post-PET2 PFS estimates of 96.8%, 89.0% and 35.6%, respectively. Compared with all other patients, the low-risk E-HIPI/PET2-negative group had a lower risk of progression or relapse (HR, 0.20; 95% CI, 0.05–0.97; $p=0.04$).

Conclusions. We successfully performed external validation of the E-HIPI in a contemporary early-stage cHL cohort treated with PET-guided approaches. In our patient population, the model underestimated absolute progression risk, suggesting that baseline-risk recalibration may be required before clinical implementation. Combining E-HIPI with PET2 identified a subgroup with particularly favourable disease control and sharply separated PET2-positive patients. These findings further strengthen the position of PET2 as a strong prognostic marker for cHL, and suggest that a specific subset of patients is characterized by a low chance for relapse or refractoriness, and is therefore a prime candidate for less intensive therapeutic approaches.

FUNCTIONAL STUDY OF NON-CODING RNAS IN T-LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Ph.D. Course Oncology and Immunology

Background T-cell Large Granular Lymphocyte Leukemia (T-LGLL) is a rare chronic lymphoproliferative disorder characterized by the clonal expansion of cytotoxic T lymphocytes. Constitutive JAK/STAT activation, particularly through gain-of-function *STAT* mutations, plays a central role in disease progression, with *STAT3* mutations associated with neutropenia in CD8+ T-LGLL. Recent evidence implicates non-coding RNAs (ncRNAs) in T-LGLL pathogenesis, including the STAT3–miR-146b–FasL axis involved in neutropenia in *STAT3*-mutated patients. Building on these findings, we investigated circular RNAs (circRNAs), a largely unexplored class of ncRNAs, and the effects of miR-146b restoration, aiming to functionally characterize circRNAs involved in T-LGLL and define the transcriptomic and proteomic changes induced by miR-146b restoration.

Material and Methods Primary LGLs were treated *ex-vivo* with IL-6 or STATTIC (ST) to investigate STAT3-mediated regulation of circRNA expression. Cell viability was assessed by flow cytometry, while circRNA expression and STAT3 activation were evaluated by RT-qPCR and Western blot, respectively. Functional studies involved optimizing siRNA-mediated knockdown in K562 cells by lipid-based transfection. In parallel, miR-146b restoration was performed in primary T-LGLL cells to validate selected findings from previous RNA-sequencing and mass spectrometry-based proteomic analyses; miRNA expression was evaluated by RT-qPCR.

Results Previously obtained RNA-seq data identified several circRNAs differentially expressed between T-LGLL patients and healthy donors (HD), with 5 significantly altered in *STAT3*-mutated patients. Public ChIP-seq data (GSE117164) identified circSETBP1, circPVT1 and circZBTB46 as direct STAT3 targets, also confirmed *ex vivo* where IL-6 stimulation induced STAT3 activation ($p<0.05$) and symptomatic-like circRNA expression ($p<0.01$), while ST reversed these effects ($p<0.05$). These findings suggest that the expression of the selected circRNAs are STAT3-dependent. To perform circRNA functional studies, knockdown optimization was performed on K562 cells transfected with siGLO Green to assess the feasibility and toxicity of lipid-mediated transfection. Different siGLO/Lipofectamine conditions were tested, achieving ~60% transfection efficiency among viable cells with 2 pmol siGLO Green and 3 µL Lipofectamine. Experiments are currently underway using a GAPDH-targeting siRNA as a positive control to assess whether increasing siRNA concentrations result in a progressive knockdown before targeting the selected circRNAs. For what concerns miR-146b, transcriptomic analysis revealed a marked disease-specific response to miR-146b restoration, with extensive gene expression changes in leukemic cells but limited effects in HD. MiR-146b transfection reduced the expression of key factors involved in neutropenia, like ELAVL1 and FasL, and modulated inflammatory pathways, including TNFα/NF-κB and IL-6/JAK/STAT3. Proteomic analysis confirmed the modulation of apoptosis- and MAPK-related proteins, further supporting a role for miR-146b in T-LGLL pathogenesis. LGLs from neutropenic *STAT3*-mutated patients transfected with miR-146b are currently being collected to validate these findings using techniques as Western Blot and ELISA.

Conclusions These results support a role for ncRNAs in T-LGLL pathogenesis, highlighting circRNAs as potential mediators of disease activity and miR-146b as regulator of pathogenic processes, supporting the development of an RNA-based therapy for T-LGLL.

CLINICAL AND FUNCTIONAL OUTCOMES IN PATIENTS WITH SOFT TISSUE SARCOMAS: PRELIMINARY RESULTS OF A CLINICAL AND BIOLOGICAL PROSPECTIVE COHORT

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Background: Soft tissue sarcomas (STSs) are a rare and heterogeneous group of malignant tumors characterized by a wide spectrum of histological subtypes and clinical behavior. Despite advances in multidisciplinary management, prognosis remains influenced by tumor histology, stage, local aggressiveness, metastatic potential and the feasibility of complete surgical resection. The identification of reliable biomarkers for prognostic stratification and treatment monitoring remains an unmet clinical need. Recent evidence suggests that systemic inflammatory and metabolic pathways may contribute to tumor progression and dissemination. In this prospective cohort, we longitudinally evaluated clinical and functional outcomes in patients undergoing surgery for STSs and evaluated circulating inflammatory and biochemical biomarkers to investigate their association with functional recovery and oncological outcomes. This report presents preliminary results from the ongoing cohort

Materials and Methods: Consecutive patients undergoing surgical treatment for STSs at the Orthopedic Clinic of Padova were prospectively enrolled from July 2024 to June 2026. Demographic, clinical, histopathological, treatment -related data were collected together with perioperative laboratory parameters. Functional outcomes were assessed at 6, 12, 18 and 24 months after surgery using the Musculoskeletal Tumor Society (MSTS) score, Eastern Cooperative Oncology Group (ECOG) performance status and Karnofsky Performance Status (KPS). Laboratory profiling included systemic inflammatory markers (LMR, NMR and CRP, together with P-LAD, S-PTH, S-osteocalcin, S-1,25 vitamin D, S-beta-2 microglobulin, ferritin, transferrin, serum iron and albumin, when available). Descriptive statistics were used for the preliminary analysis.

Results: Twenty-three patients were enrolled (13 males, 10 females), with a mean age of 65.6 ± 19.1 years (range 16–95) and mean BMI of 25.7 ± 5.9 kg/m². Histological heterogeneity was observed, including liposarcoma, myxofibrosarcoma and synovial sarcoma. Wide-margin excision was performed in 17 patients (73,9%) and amputation in 6 (26.1%); Neoadjuvant chemotherapy and preoperative radiotherapy were administered in 10 (43.5%) and 11 (47.8%) patients, respectively. Mean MSTS score increased from 15.1 to 21.1 and mean KPS from 59.1 to 71.8, while mean ECOG Performance Status decreased from 2.27 to 1.27, indicating an overall improvement in functional and performance status. Mean hematological and inflammatory parameters were as follows: hemoglobin 111.15 g/L, platelet count 224.51 $\times 10^9$/L, neutrophils 5.36 $\times 10^9$/L, lymphocytes 1.27 $\times 10^9$/L, monocytes 0.711 $\times 10^9$/L, eosinophils 0.121 $\times 10^9$/L, and basophils 0.0265 $\times 10^9$/L. The mean inflammatory indices were 1.90 for LMR, 7.95 for NMR , 4.95 for NLR, 3.55 for SIRI, and 949.7 for SII, with mean CRP and procalcitonin levels of 28.81 mg/L and 2.97 µg/L, respectively.

Conclusions: This prospective real-world cohort enabled the integrated collection of clinical, functional and systemic inflammatory data from patients undergoing surgery for STSs.Preliminary analyses showed an overall improvement in functional status after surgical treatment, with continued recovery observed in patients with longer follow-up up to 24 months. The integration of inflammatory and biochemical profiles may provide additional information for prognostic stratification and outcome monitoring. Further follow-up and biomarker analyses are warranted to assess their association with functional recovery and oncological outcomes and to identify clinically useful prognostic indicators.

REDUCED PLASMIN-GENERATING CAPACITY ACROSS THE SPECTRUM OF CIRRHOSIS IS ASSOCIATED WITH PORTAL VEIN THROMBOSIS, DISEASE PROGRESSION, AND 90-DAY MORTALITY IN ACUTE-ON-CHRONIC LIVER FAILURE

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Background
While coagulation in cirrhosis has been extensively studied, fibrinolysis remains less understood. Plasmin generation (PG) is a novel global assay to assess fibrinolysis. We aimed to characterise PG across the spectrum of cirrhosis and determine its association with disease progression and thrombo-haemorrhagic complications.
Material and Methods
In this multicentre prospective study, 228 patients with cirrhosis (64 compensated advanced chronic liver disease (cACLD), 54 stable decompensation (SD), 52 acute decompensation (AD), and 58 ACLF) and 40 healthy controls underwent PG and thrombin generation (TG). Patients were followed for a median of 625 days. Associations between PG and liver-related outcomes were evaluated using cause-specific Cox and Fine-Gray competing-risk analyses.
Results
PG progressively declined across disease stages, with PG peak decreasing from 67.2 to 23.2 nM and endogenous plasmin potential (EPP) from 229 to 72 nM/L·min from cACLD to ACLF ($p<0.001$), accompanied by progressively higher TG/PG ratios, indicating progressive imbalance between coagulation and fibrinolysis. During follow-up, 15.6% patients with cACLD developed first decompensation, 44.8% patients with SD/AD experienced further decompensation/ACLF/liver-related mortality, 69% of patients with ACLF had liver-related mortality, 8.4% developed PVT, and 20.9% of AD/ACLF patients experienced NPH bleeding. Lower PG peak and EPP independently predicted PVT (aHR 0.52–0.55; $p\leq0.048$), first decompensation in cACLD (aHR 0.89–0.97; $p<0.001$), further decompensation/ACLF/liver-related mortality in AD (aHR 0.98–0.99; $p\leq0.023$), and 90-day mortality in ACLF (aHR 0.97–0.99; $p\leq0.017$). No associations were observed with NPH bleeding. Sepsis-associated ACLF exhibited profound hypofibrinolysis with further reductions in PG, independently of ACLF severity.
Conclusions
PG progressively declines with disease severity, most profoundly in ACLF. Sepsis in ACLF induces further reductions in PG. Reduced PG was independently associated with PVT, disease progression, and liver-related mortality, supporting a role for impaired fibrinolysis in the progression of cirrhosis.

DOES AMPUTATION IMPROVE SURVIVAL IN CHONDROSARCOMA? A SEER 2010-2022 PROPENSITY SCORE-MATCHED ANALYSIS OF CANCER-SPECIFIC SURVIVAL

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Background
To compare cancer-specific mortality (CSM), other-cause mortality (OCM), and overall survival (OS) between limb-sparing surgery and amputation in patients with primary chondrosarcoma undergoing curative-intent surgery, and to identify factors associated with surgical selection and mortality outcomes.
Material and Methods
We identified patients with first primary malignant chondrosarcoma of bone from SEER 2010-2022 who underwent limb-sparing surgery or amputation. Missing covariates were handled using multiple imputation. Propensity score matching was performed within each imputed dataset using 1:1 nearest-neighbor matching from the ATT perspective. CSM and OCM were analyzed using competing-risk methods, and OS was evaluated using Cox regression and Kaplan-Meier curves. Estimates were pooled across imputed matched datasets using Rubin's rules.
Results
A total of 1,392 patients were included, including 1,168 treated with limb-sparing surgery and 224 treated with amputation. Before matching, the amputation group had older age, larger tumors, more dedifferentiated histology, higher grade, and more advanced stage disease. In a representative imputed dataset, 1:1 matching yielded 220 pairs with improved covariate balance. Multivariable logistic regression showed that older age, larger tumor size, and distant-stage disease were associated with higher odds of amputation. After matching, amputation was not significantly associated with CSM (HR = 1.10, 95% CI 0.68-1.80; P = 0.693) or OCM (HR = 1.15, 95% CI 0.55-2.38; P = 0.716) in cause-specific Cox models. Cumulative incidence curves and OS curves also showed no significant between-group differences after matching.
Conclusions
After balancing measurable confounders, amputation did not demonstrate a cancer-specific, other-cause, or overall survival advantage over limb-sparing surgery. Prognosis appeared to be driven primarily by tumor burden and biology, including tumor size, dedifferentiated histology, grade, and stage, as well as patient age.

NANOPARTICLE-MEDIATED B2M SILENCING FOR IMMUNE EVASION IN LIVER TRANSPLANTATION

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Background Inducing immune tolerance in solid organ transplantation remains a major unresolved clinical challenge. Current immunosuppressive therapies reduce acute rejection but cause serious adverse effects, infections, malignancies, and metabolic complications, negatively impacting graft survival. The vascular endothelium serves as the central immunological interface, contributing to antigen presentation and immune activation. Silencing MHC class I expression through β-2microglobulin (B2M) downregulation reduces alloreactive T cell activation, enabling "immunological invisibility" of the graft. Lipid nanoparticles (LNPs) and polymeric carriers are effective platforms for RNA delivery, already approved for siRNA and mRNA therapeutics. This project develops a transient gene expression modulation strategy using shRNA delivered via LNPs or glycoplexes to silence B2M in human hepatic cells and in mice, aiming to engineer "stealth" grafts that evade adaptive immune recognition, enabling long-term graft survival without chronic immunosuppression.
Material and Methods In vitro: immortalized human hepatic cells and hepatic sinusoidal endothelial cells (HHSECs); three B2M-targeting shRNA constructs screened, the best formulated into MC3-based LNPs and glycoplexes, assessed by qPCR and flow cytometry against lentiviral shB2M. In vivo: C57BL/6N (LNPs, n=5) and CD1 (glycoplexes, n=3) mice received a single intravenous 10 μg shRNA dose. Liver, spleen, lung and kidney were collected at days 4, 6 and 8 for qPCR; plasma cytokines were profiled at the same time points.
Results In vitro, the lead construct reduced B2M transcript by 80–91% and HLA class I surface expression by 71–94% in both cell types, sustained for 30 days. Nanoparticle characterization confirmed optimal size (LNPs: 120 nm; glycoplexes: 130 nm) and charge stability. In vivo, hepatic B2M silencing was progressive and sustained, reaching 84.6% with LNPs and 82.9% with glycoplexes by day 8 ($p<0.01$). Silencing extended beyond the liver but was transient: kidney reached 94.7% at day 4 and lung 93.4% at day 6 under LNPs, both recovering by day 8, whereas the spleen was unaffected throughout. Plasma cytokines showed no significant treatment-related elevation at any time point after correction for the ten analytes tested. No adverse effects were observed.
Conclusions anoparticle-based anti-B2M shRNA delivery effectively reduces B2M expression in murine hepatic tissue, with LNPs and glycoplexes achieving comparable hepatic silencing in their respective strains. The absence of plasma cytokine induction indicates that this degree of knockdown is achieved without systemic immune activation. These findings provide a preclinical foundation for developing "stealth" grafts capable of evading immune recognition. This approach offers a promising strategy for inducing immunological tolerance in solid organ transplantation, potentially eliminating chronic immunosuppression and improving long-term graft survival and patient quality of life.

CLINICAL AND MOLECULAR PREDICTIVE FACTORS IN THE TREATMENT OF GLIOMAS

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Background
Glioblastoma (GBM) is an aggressive brain tumor with poor prognosis and limited therapeutic options. Circulating biomarkers may provide minimally invasive tools for patient stratification and disease monitoring. GBM is characterized by systemic immune dysregulation, including expansion of myeloid-derived suppressor cells (MDSCs), whose longitudinal dynamics and prognostic relevance remain incompletely defined. We therefore investigated circulating myeloid populations and related functional markers over time and their association with clinical outcomes in patients with GBM.

Material and Methods
Peripheral blood samples were longitudinally collected from patients with newly diagnosed GBM (n=36) and recurrent GBM (n=16), while healthy donors (n=55) served as controls. In newly diagnosed GBM, samples were obtained prior to chemoradiotherapy (T0) and one month after completion (T1). In recurrent GBM, samples were collected before initiation of systemic therapy (T0) and after two months of treatment (T1). Multiparametric flow cytometry quantified four MDSC subsets (monocytic: MDSC1 and MDSC4; granulocytic: MDSC2; early-stage: MDSC3), together with CD15 granulocytes and PD-L1–expressing $^{+}$ CD14^{+} monocytes. pSTAT3 signaling and plasma ARG1 and HO-1 levels were also measured. Overall survival was analyzed using Cox proportional hazards models adjusted for steroid use.

Results
At baseline, GBM patients showed a significantly altered systemic myeloid profile compared with healthy donors. Median MDSC2 levels were markedly increased in newly diagnosed GBM (17.1% [8.5–28.3]) and recurrent GBM (12.8% [8.3–18.5]) compared with controls (1.26% [0.24–3.2]; p<0.0001). CD15^{+} granulocytes were significantly expanded (69.6% and 68.1% vs 58%, p=0.0001), and PD-L1^{+} CD14^{+} monocytes were approximately doubled (9.3% and 9.9% vs 4.9%, p<0.0001), indicating a systemic immunosuppressive phenotype already present at diagnosis. Longitudinal analyses revealed dynamic changes in circulating myeloid populations. In primary GBM, CD15^{+} granulocytes increased from 68.4% to 76.3% (p=0.032). In recurrent GBM, MDSC2 markedly expanded over time (12.8% to 44.7%, p=0.0108), suggesting progressive expansion of immunosuppressive myeloid populations during glioblastoma progression. Survival analyses identified circulating myeloid subsets associated with clinical outcome. In primary GBM, higher baseline MDSC3 levels were associated with improved overall survival (HR 0.38, 95% CI 0.13–0.93,p=0.019), remaining significant after steroid-adjusted analysis (HR 0.37, p=0.0357). Higher baseline MDSC2showed a non-significant trend toward worse survival (HR 1.98, 95% CI 0.75–5.07, p=0.087).

Conclusions
Overall, these findings indicate systemic myeloid immune dysregulation in GBM that evolves during disease progression and may influence clinical outcome.

HO-1-targeting molecule ZnPPIX mediates transcriptional and epigenetic reprogramming in human macrophages

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Background Macrophages within the tumor microenvironment frequently acquire an immunosuppressive phenotype that limits antitumor immune responses and supports tumor progression. Increasing evidence suggests that this phenotype is tightly linked to iron metabolism. Heme oxygenase 1 (HO-1), the rate limiting enzyme in heme degradation, emerges as a potential regulator of macrophage immunosuppression. Our group previously demonstrated that HO-1 modulation with Zinc protoporphyrin IX (ZnPPIX) reduces the suppressive activity of macrophages on activated T cells, indicating that this pathway may control key functional programs.
Material and Methods Immunosuppressive macrophages were differentiated from blood monocytes isolated from healthy donors. HO-1 protein regulation and subcellular localization of HO-1, Nrf2, Bach1, and ZnPPIX were assessed by imaging flow cytometry, Western blotting, and confocal microscopy. Intracellular ROS levels and HO-1 enzymatic activity were evaluated using CellROX ROS probe and FerrOrange Live Cell Dye, respectively. Global transcriptional and chromatin accessibility changes were analysed by bulk RNA-seq and ATAC-seq. Nuclear HO-1 interactors were investigated by co-immunoprecipitation followed by mass spectrometry.
Results Although ZnPPIX is commonly regarded as a specific HO-1 inhibitor, we found that ZnPPIX treatment of *in vitro*-derived human macrophages increases HO-1 protein abundance after 24 hours from the treatment. The observation that ZnPPIX localizes to both the cytosol and the nucleus prompted investigation of potential functions beyond HO-1 enzymatic inhibition.
RNA seq analysis revealed that ZnPPIX profoundly reshaped macrophage gene expression, highlighting a broad transcriptional rewiring. Genes associated with anti-inflammatory responses, pro tumoral functions, and extracellular matrix remodeling were downregulated, whereas genes associated with cellular and iron metabolism were upregulated. In parallel, bulk ATAC-seq analysis showed that ZnPPIX treatment induced a marked global increase in chromatin accessibility, especially at the level of promoters and 5'UTR regions.
Among the induced pathways, we observed a strong activation of the NRF2 signaling axis and reduced levels of its repressor Bach1. Accordingly, ZnPPIX markedly reduced intracellular H_2O_2 levels, as measured with a fluorogenic probe, indicating activation of an antioxidant response. However, preliminary functional assays with Bach1 and Nrf2 pharmacological modulation suggest that the Bach1-NRF2 axis does not account for the ZnPPIX-induced relief in macrophage immunosuppressive activity.
Given the emerging evidence of non-canonical nuclear functions of HO-1, its potential involvement in ZnPPIX-mediated macrophage reprogramming was investigated. We found that both HO-1 and ZnPPIX are detectable in macrophage nuclei, and this led us to hypothesize that ZnPPIX action may be linked to a possible nuclear HO-1 function. Immunoprecipitation experiments of nuclear HO-1 followed by mass spectrometry identified several putative interacting proteins, mainly involved in nucleosome organization and assembly, chromatin remodeling, and regulation of protein stability.
Conclusions These results suggest that ZnPPIX modulates macrophage immunosuppression through a mechanism involving epigenetic and transcriptional rewiring, independently of the canonical enzymatic roles of HO-1 in iron metabolism and anti-oxidant responses.

TRANSLATIONAL PROGRAMME: THE EUROPEAN GAMBIT STUDY

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Background. The A-BRAVE phase III trial randomized patients with high-risk early-stage triple-negative breast cancer (TNBC) to one-year of adjuvant avelumab or observation. The translational program of A-BRAVE aims to develop immune-metabolic biomarkers of relapse in high-risk early TNBC. To secure an independent setting for downstream testing before biomarker models were finalized, we established the GAMBIT network as a real-world platform of more than 40 institutions for future external validation. As the network's first scientific output, we aimed to define a clinical-immune benchmark for residual risk and relapse patterns among patients achieving pathological complete response (pCR) after neoadjuvant treatment. While being considered generally cured within current frameworks, around 10% of patients with pCR still relapse.
Material and Methods. This multicenter retrospective study included 2,457 patients with stage I-III TNBC or estrogen receptor-low, HER2-negative disease treated with neoadjuvant therapy and surgery at 18 European centers. Overall, 1,192 patients achieved pCR and 690 had evaluable baseline stromal tumor-infiltrating lymphocytes (sTILs). Distant relapse-free survival (DRFS) and overall survival (OS) were analyzed using Cox models with robust variance estimates clustered by institution and stratified by neoadjuvant regimen. Competing-risk methods compared first relapse sites between pCR and residual-disease cohorts and across combined clinical nodal status (cN) and sTIL strata. sTILs were modeled continuously and using the predefined 30% cut-off.
Results. Within the pCR cohort, cN involvement was independently associated with worse DRFS and OS. In the pCR-TILs cohort, each 5% increase in sTILs was associated with a 14% lower risk of distant relapse or death (hazard ratio [HR] 0.86, 95% confidence interval [CI] 0.82-0.91) and a 16% lower risk of death (HR 0.84, 95% CI 0.79-0.90). A cN-positive/low-TILs subgroup, representing 29.4% of the cohort, had 5-year DRFS of 83.4% and OS of 85.8%; each other stratum exceeded 95% for DRFS and 97% for OS. Compared with residual disease, pCR was associated with lower 5-year cumulative incidence of any distant relapse (7.2% versus 29.9%) and any central nervous system (CNS) relapse (3.6% versus 7.0%), whereas isolated CNS relapse was similar (2.6% versus 2.1%, p=0.443). In the cN-positive/low-TILs subgroup, 5-year cumulative incidence reached 7.5% for any CNS relapse and 6.9% for isolated CNS relapse.
Conclusions. This first GAMBIT publication establishes a clinical-immune benchmark for residual risk after pCR and demonstrates the network's capacity to identify clinically relevant risk groups. Within my PhD project, GAMBIT provides the external real-world platform in which the A-BRAVE can subsequently be tested.

Adoptive cell therapy with Cytokine-Induced Killer cells as immunomodulatory living drugs

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Background: Cytokine-induced killer (CIK) cells are a heterogeneous population of *ex vivo* expanded lymphocytes characterized by the co-expression of CD3 and CD56 and have demonstrated cytotoxicity against a broad range of tumor types. In clinical settings, CIK cells have shown a favorable safety profile, including a low incidence of acute graft-versus-host disease (GvHD) in both autologous and allogeneic settings, supporting their potential as an off-the-shelf cellular therapy. However, although the limited alloreactivity of CIK cells has been well documented, the mechanisms underlying remain poorly understood. It remains unclear whether CIK cells can actively modulate host immune responses in addition to their direct cytotoxic function. Understanding the immunomodulatory properties of CIK cells is therefore important for developing their potential as an allogeneic cellular therapy and for expanding their application in immune-related diseases. In this study, we investigated the capacity of CIK cells to modulate T-cell functions, with the aim of elucidating the mechanisms underlying their limited alloreactivity and assessing their potential application as an allogeneic cellular therapy for autoimmune diseases.
Methods: CIK cells were generated from peripheral blood mononuclear cells (PBMCs) obtained from healthy donors using a serum-free, GMP-compliant expansion protocol. To determine whether CIK cells can modulate PBMC activation and proliferation, CIK cells were co-cultured with autologous PBMCs in anti-CD3/anti-CD28 coated plates. PBMC proliferation was assessed after 4 days by analyzing CFSE dilution. To investigate the effects of CIK cells on alloreactive T cell activation, CIK cells were co-cultured with autologous PBMCs in the presence of irradiated allogeneic PBMCs as stimulators in a mixed leukocyte reaction (MLR). In addition, the phenotypic profiles of both PBMCs and CIK cells were analyzed following co-culture.
Results: CIK cells significantly suppressed the proliferation of PBMCs activated either with anti-CD3/anti-CD28 antibodies or with allogeneic stimulator cells in a mixed leukocyte reaction (MLR). Notably, the suppressive effect observed was not rescued by exogenous IL-2, suggesting that CIK cells exert their immunomodulatory effect during the early phase of T cell activation rather than primarily through competition for IL-2. In contrast, the suppressive effect was partially attenuated by preincubating CIK cells with an anti-LFA-1 antibody, supporting the involvement of LFA-1-mediated interactions in CIK cell-mediated immunomodulation. Similarly, physical separation of CIK cells from responder PBMCs using a transwell system reduced the suppressive effect and was associated with reduced CIK cell proliferation, indicating that both direct cell-cell contact and CIK cell activation are required. Phenotypic analysis of proliferating PBMCs further revealed an increased proportion of CD4+ T cells with higher CD62L expression when co-cultured with CIK cells, suggesting that CIK cells may influence the composition and differentiation state of activated T cells.
Conclusion: Taken together, these findings demonstrate that expanded CIK cells exert immunomodulatory effects by limiting the activation and proliferation of PBMCs and alloreactive T cells. This activity appears to depend, at least in part, on direct cell-cell interactions and LFA-1-mediated mechanisms. In addition, CIK cells may shape host immune responses by altering the expansion and phenotype of activated T-cell subsets. Collectively, these findings provide new insights into the immunoregulatory properties of CIK cells and may contribute to a better understanding of their favorable safety profile in allogeneic therapeutic settings.

IMPACT OF *RAS* STATUS IN MISMATCH REPAIR DEFICIENT/MICROSATELLITE INSTABILITY HIGH (dMMR/MSI-H) METASTATIC COLORECTAL CANCER (mCRC) TREATED WITH SINGLE AGENT OR COMBINATION IMMUNE CHECKPOINT INHIBITORS (ICIs) IN A LARGE REAL-WORLD COHORT

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Background In mismatch repair deficient/microsatellite instability high (dMMR/MSI-H) metastatic colorectal cancer (mCRC), dual checkpoint blockade recently showed benefit over anti-PD-1 monotherapy. A trend toward reduced benefit with immune checkpoint inhibitors (ICIs) in *RAS* mutant (mut) patients (pts) stands out from clinical trials with anti-PD-1 monotherapy, but not with combinations. Repression of immune checkpoints and immune-sensing pathways in *RAS*-driven colorectal cancer has been observed as well.

Material and Methods This retrospective multicenter study included dMMR/MSI-H mCRC pts treated with ICIs monotherapy or combination at Veneto Institute of Oncology IOV – IRCCS and ten additional Institutions in between Europe and North America.

Primary objectives were progression-free survival (PFS) and overall survival (OS) in pts treated with monotherapy or doublet, according to *RAS* status. Kaplan-Meier method and Cox proportional hazards models were used for survival analyses. Secondary endpoint was objective response rate (ORR).

A targeted transcriptomic panel encompassing immune-related and tumor-microenvironment genes was used to investigate inflammatory infiltrate and gene expression profiles according to mutational status on archival formalin-fixed embedded tissue of 86 pts treated at Veneto Institute of Oncology with available pre-treatment specimens.

Results Among four hundred and ten pts, 88 (21%) were *RAS* mutant (mut) and 322 (79%) *RAS* wild-type (wt), with 174 (42%) both *RAS/BRAF* wt. *RAS* mut were more frequently younger and left sided, compared to the other subgroups (p <0.001).

Median follow-up was 46.5 months, with significant PFS and OS differences across subgroups stratified by *RAS* status and treatment type (log-rank p = 0.012 and p = 0.028). Combination therapy was independently associated with improved PFS (HR 0.64, 95% CI 0.44 – 0.92, p = 0.018), with signals toward OS benefit. In a multivariable model *RAS* status was not independently associated with outcomes and showed no significant interaction with treatment modality.

ORR was higher with combination therapy versus monotherapy (67 vs 49%, p <0.001). A significant interaction between *RAS* status and treatment was observed, with reduced ORR with combination therapy in *RAS* mut tumors (OR 0.26, 95% CI 0.09 – 0.78, p = 0.017).

Differential expression analyses and immune transcriptomic profiling highlighted a distinct profile according to mutational status. *RAS* mut displayed a transcriptionally colder phenotype, with significant downregulation of immunoregulatory genes such as PD-1 and IDO1, and lower Tumor Inflammation Signature scores. Hierarchical clustering performed based on immune cell type profiling and gene expression signatures showed enrichment for neutrophilic and myeloid cell populations, indicative of a more immunosuppressive microenvironment, in *RAS* mut.

Conclusions Our findings underscore survival benefit from ICIs combinations in dMMR/MSI-H mCRC, regardless the mutational status. Less pronounced ORR was observed in *RAS* mut subgroup, where heterogeneity in responsiveness and immune landscape pave the way for further functional immune profiling to refine immunotherapy strategies.

Large extracellular vesicles fingerprint of PSVD: endothelial-platelet activation and immune dysregulation

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Background: PSVD is a rare vascular liver disease that causes intrahepatic vascular injury and portal hypertension (PH). Although its pathophysiology is not fully understood, PSVD is characterized by damage to the intrahepatic portal microvasculature. In this context, large extracellular vesicles (lEVs) can play a key role. This study quantifies circulating lEVs from endothelial cells, platelets, and monocytes in PSVD, with and without PH.
Material and Methods: The study included 111 patients with PSVD: 31 without PH but histologically defined, and 80 with PH. Thirty-five healthy individuals served as controls. Platelet-poor plasma was prepared according to ISTH/ISEV guidelines. lEVs (0.2–0.9 μm) were isolated by high-speed centrifugation and analyzed by calibrated flow cytometry. lEVs were stained with calcein (a marker of vesicle integrity) and Annexin V (a marker of activation), and further characterized by immunophenotyping for endothelial markers (CD62E/CD105/CD147/TF), platelet markers (CD41/CD62P), and monocyte/macrophage markers (CD45/CD14).
Results: The PSVD cohort had a median age of 57 years (range 43–66). An identifiable cause was present in 66.7%, mainly immune disorders (30.6%) and drug or toxin exposure (20.7%); myeloproliferative disease accounted for 9.9%. Specific lesions were observed in 61.0%, notably obliterative portal venopathy (39.0%, n=39). Among those with PH, 48.8% experienced PH-related complications. PSVD patients showed reduced endothelial, platelet, and monocyte markers compared to controls. Specifically, endothelial lEV subtypes, including $Calcein^{+}CD62E^{+}$ (455.14 [288.01–721.55] vs 882.55 [634.94–1297.93]) and $Calcein^{+}CD147^{+}$ (654.52 [416.01–817.07] vs 898.48 [592.71–1427.38]), were significantly decreased ($p \leq 0.007$). Platelet lEVs also showed significant reductions: $Calcein^{+}CD41^{+}$ (753.01 [596.20–1014.25] vs 1142.40 [657.57–1692.32]) and $Calcein^{+}CD41^{+}CD62P^{+}$ (269.08 [187.70–380.49] vs 496.12 [339.90–908.21]; $p \leq 0.005$). Endothelial lEVs of the endoglin family were significantly higher in myeloproliferative-PSVD ($p=0.037$; $p=0.004$). Monocyte/macrophage lEVs were likewise reduced, as shown for $Calcein^{+}CD45^{+}CD14^{+}$ lEVs (1046.35 [799.81–1458.66] vs 1290.22 [1079.38–1641.88]; $p \leq 0.005$). PSVD patients without PH also showed significantly altered endothelial ($p<0.007$) and platelet lEVs ($p<0.008$), together with increased procoagulant $Calcein^{+}Annexin\ V^{+}$ lEVs (505.16 [416.44–593.29] vs 413.66 [325.51–460.22]; $p=0.011$), whereas monocyte/macrophage lEVs did not differ from controls. Significant differences emerged between PSVD with and without PH, with total, procoagulant, endothelial, and monocyte/macrophage lEVs all lower in patients with PH ($p \leq 0.011$). Moreover, there were no notable differences between patients with complicated versus non-complicated PH.
Conclusions: Our data reveal a distinct endothelial-platelet lEV profile in PSVD. This pattern persists even without PH, indicating it reflects core disease biology. It is further accentuated once PH develops but does not vary with the occurrence of PH-related complications. Circulating lEVs serve as accessible biomarkers for disease assessment and require further prospective validation.

DESIGN AND CHARACTERIZATION OF HUMANIZED ANTI-PSMA scFv-D2B VARIANTS

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Background Despite significant advances in precision oncology, the development of efficient and selective targeting strategies for solid tumors remains challenging, highlighting the need for novel targeting platforms with translational therapeutic potential. In prostate cancer (PCa), prostate-specific membrane antigen (PSMA) has emerged as a well-established tumor-associated antigen (TAA) and therapeutic target, owing to its marked overexpression on PCa cells and accessibility to targeting molecules. Its established clinical relevance supports the development of optimized PSMA-targeting molecules for therapeutic applications. In this context, single-chain variable fragments (scFvs) represent versatile antigen-recognition domains with potential applications in targeted and cell-based therapies, including chimeric antigen receptor (CAR)-T cells.
Here, we aimed to characterize humanized variants of a murine anti-PSMA scFv (D2B) and assess their PSMA-binding properties and cellular specificity as well as their compatibility with a CAR format.
Material and Methods Five humanized variants of murine scFv-D2B were computationally designed using the CUMAb method and screened by yeast display for expression and PSMA binding. Three PSMA-binding variants were selected for soluble expression in mammalian cells, of which hz1 and hz3 were successfully purified as monomeric proteins. Their binding to cell-surface PSMA was assessed by flow cytometry on PC-3 WT (PSMA -) and PC-3-PIP (PSMA +) cell lines. Cell-based titration was performed over a broad concentration range to estimate apparent K_D (K_D^{app}) values by non-linear regression. The variants were also evaluated in the CAR format to assess the retention of PSMA recognition and binding.
Results Among the five designed scFv-D2B variants, three showed PSMA binding in yeast display assay and were selected for further characterization. Out of these three variants, hz1 and hz3 were successfully expressed and purified as soluble monomeric proteins. Both hz1 and hz3 showed concentration-dependent and selective binding to PSMA-positive PC-3-PIP cells, while no binding was observed on PSMA-negative PC-3 WT cells. Cell-based titration confirmed nanomolar-range apparent affinity, with K_D^{app} values of 13 nM for hz1, 9 nM for hz3, and 10 nM for the parental murine scFv-D2B. Notably, both hz1 and hz3 variants retained nanomolar-range apparent affinity, comparable to that of the parental scFv-D2B. PSMA recognition was also retained when hz1 and hz3 were incorporated into the CAR format.
Conclusions Overall, hz1 and hz3 represent humanized scFv-D2B variants that retain selective recognition of PSMA-positive tumor cells and nanomolar-range apparent affinity. These results support their further development as anti-PSMA targeting molecules. SPR analysis will be performed during the third year to further characterize their binding affinity and kinetics toward PSMA.

Prognostic value of circulating $CD11b^+CD33^+$ myeloid cells in patients with localized prostate cancer

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Background
Current clinical tools inadequately identify localized prostate cancer (PCa) patients at the highest risk of recurrence. While circulating myeloid cells are associated with poor prognosis in advanced PCa, their role in primary PCa remains unclear.
Material and Methods
We prospectively enrolled 79 patients with localized PCa undergoing radical prostatectomy at the Urology Department of the Azienda Ospedale-Università Padova. An independent cohort of 24 biopsy-proven PCa cases from a separate institution (Ente Ospedaliero Cantonale - EOC, Lugano and Bellinzona) was included for RNA sequencing of $CD11b^+CD33^+$ cells. The study protocol was approved by the Padua Province Clinical Experimentation Ethics Committee (reference no. CESC 5170/AO/21) and by the Ethical Committee of Canton Ticino (IOSI-IOR 002, 2017-01631, CE TI 3269). The frequency of circulating $CD11b^+CD33^+$ myeloid cells was quantified by flow cytometry. RNA sequencing was performed on fluorescence-activated cell sorting (FACS) isolated cells from 39 patients across two independent cohorts: radical prostatectomies from Padua Hospital and biopsy-confirmed cases from Lugano and Bellinzona.
Results
Of the 79 patients enrolled, 69 constituted the study population and were included in the final analysis. Median age was 65 years (range 57-77). Patients were stratified into three risk groups according to the EAU guidelines (high, intermediate and low risk). Preoperative circulating $CD11b^+CD33^+$ frequency was significantly higher in high-risk (n = 31, median circulating frequency [MCF]: 7,3%) versus intermediate (n = 33, p=0.04, MCF: 5,7%) and low-risk patients (n = 5, p=0.009, MCF: 2,4%). A higher circulating $CD11b^+CD33^+$ frequency was associated with adverse pathological features, including Internationaly Society of Urological Pathology (ISUP) grade 4–5 (p=0.003) and perineural invasion (p=0.03). No association was observed with lymphovascular invasion (LVI), extra-prostatic extension (EPE) or pathological T stage. Transcriptomic profiling of these cells revealed distinct transcriptional programs in high-risk patients, with an enrichment of tumor-promoting and immunosuppressive gene signatures.
Conclusions
Higher frequencies of circulating CD11b + CD33 + myeloid cells are observed in high-risk localized PCa. Remarkably, this myeloid expansion occurs even in organ-confined disease, revealing an early systemic immune response previously unrecognized in localized PCa, which warrants validation in larger cohorts. Further analyses are needed to explore the impact of these cellular and molecular findings on biochemical recurrence and metastasis-free survival after primary surgical treatment.

ASSESSMENT OF PD-L1 ON MATCHED BIOPSY AND SURGICAL SPECIMEN IN RESECTABLE ESOPHAGOGASTRIC ADENOCARCINOMA

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Ph.D. Course Oncology and Immunology

Background
Programmed death-ligand 1 (PD-L1) expression defines eligibility for immunotherapy in patients with esophagogastric (EG) adenocarcinoma. Limited data exist on the agreement of PD-L1 combined positive score (CPS) between diagnostic biopsies and matched surgical specimens. We do not know the impact of therapy on PD-L1 expression. We aimed to evaluate the reliability of PD-L1 assessment on biopsies compared with matched surgical specimens.

Material and Methods
The multicentric observational study included patients with resectable esophagogastric adenocarcinoma (cTNM II–IVa) who were treated at Istituto Oncologico Veneto–IRCCS and Azienda Ospedaliero-Universitaria Pisana. The study collected and analyzed with immunohistochemistry tissue microarray sections from the biopsies and, from the matched resected specimens. PD-L1 CPS was defined as negative (<1), low (1–4), intermediate (5–9), or high (≥10).

Results
From May 2014 to November 2024 254 patients who had both a biopsy and a surgical specimen were enrolled. The median age of the patients was 62 years [Interquartile Range (IQR) 58 -76 years]. The patients were 73% male. ECOG performance status (PS) of patients was 0 in 52%. The cases of gastric or gastroesophageal junction tumors were 93%. Patients underwent surgery upfront (n=109, 43%) or were treated with neoadjuvant oncological treatment (n=126, 49.6%) or concomitant chemoradiotherapy (n=19, 7.4%).
Distribution of PD-L1 CPS categories was similar for biopsies and surgical specimens: negative (n=20 vs. n=26), low (n=72 vs. n=75), intermediate (n=46 vs. n=37), and high (n=116 vs. n=116). Agreement between biopsy and surgical tumor samples was low in patients with fewer than six biopsies ($\kappa = 0.125$; $p = 0.054$; $N = 114$) and significantly higher in those with six or more biopsies ($\kappa = 0.316$; $p < 0.001$).
The probability of PD-L1 CPS falling into different categories did not differ between the Surgery and Neoadjuvant groups (OR 0.86; 95% CI 0.46–1.61; $p = 0.6$). PD-L1 CPS values tended to be higher when assessed with the 22C3 antibody compared with SP263 (estimated difference +4.40 CPS; 95% CI −0.62 to 9.40; $p = 0.086$).

Conclusions
PD-L1 CPS demonstrates limited concordance between diagnostic biopsy and matched surgical specimens in resectable EG adenocarcinoma, reflecting substantial intratumoral and sampling-related heterogeneity. This variability may reduce the reliability of baseline PD-L1 assessment. Therefore, caution is warranted when using PD-L1 CPS as a sole biomarker to guide treatment decisions, and adequate biopsy sampling should be ensured to improve its clinical applicability.

DEVELOPMENT OF INNOVATIVE DUAL-TARGETING RADIOPHARMACEUTICALS FOR PRECISION DIAGNOSIS AND THERAPY OF PROSTATE CANCER

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Background
Prostate cancer is the fourth most common cancer worldwide and the second most frequently diagnosed in men (2022). Despite its high incidence, mortality remains relatively low for localized cases, but it increases significantly for advanced metastatic disease. Therefore, several strategies have been explored to enable early diagnosis. The theragnostic couple ^{68}Ga- and ^{177}Lu-iPSMA (approved in 2020 and in 2022, respectively) showed promising results, although this agent is not effective in all the patients. A key reported limitation is its low internalization in prostate cancer cells. The strategy in which we focused in to increase the internalization is a bifunctional compound combining iPSMA with a GRP78-targeting peptide. GRP78 is a protein overexpressed on the surface of several cancer types as cell-surface GRP78 (CS-GRP78) making it an interesting target for targeted radionuclide therapy (TRT). After promising results obtained by ININ (Mexico), we focused on the evaluation of two heterodimeric agents: one in collaboration with ININ (Mexico) and the other with Arzanya s.r.l.

Material and Methods
The heterodimeric compound developed in collaboration with ININ and its control monomers were radiolabelled with ^{99m}Tc and evaluated *in vitro* in three prostate cancer cell lines with different PSMA and CS-GRP78 expression profiles reported in the literature: PC3 (PSMA-/CS-GRP78-), PC3-PIP (PSMA+/CS-GRP78-) and LNCaP (PSMA+/CS-GRP78+). Uptake, internalization and retention were evaluated and PSMA-binding specificity was investigated through blocking studies. To better mimic the tumour microenvironment, 3D spheroid models of the three cell lines were developed and preliminary uptake studies were performed using ^{99m}Tc-HYNIC-iPSMA. Finally, the first *in vivo* evaluation of these ^{99m}Tc-radiolabelled compounds was carried out in NOD/SCID mice bearing both PC3-PIP and PC3 tumours, to assess whether the heterodimer retains PSMA affinity and specificity despite its steric hinderance.

Results
The radiochemical purity (RCP) after the radiolabelling was above 95% for all the compounds. After 4 h of incubation, the internalization values of both heterodimer and the iPSMA monomer was higher in PC3-PIP than LNCaP cells (28% vs 4%, and 27% vs 12%, respectively), as expected from the higher PSMA expression in PC3-PIP cells. Retention assays showed that approximately 70% of the initially internalized heterodimer remained intracellular after 1 h. Blocking studies after 1 hour with the heterodimer showed lower uptake inhibition in LNCaP than PC3-PIP cells (74% vs 86%, respectively) consistent with the interaction with CS-GRP78, which is expressed in LNCaP cells but not in PC3-PIP cells. The first *in vivo* study in PC3 and PC3-PIP tumour-bearing mice demonstrated specific radiotracer uptake in PC3-PIP tumours, whereas no uptake was observed in PC3 tumours. The tumour heterodimer uptake was lower than that of the iPSMA control (17% vs. 28% ID/g, respectively), which can be attributed to the steric hindrance.

Conclusions
Although these preliminary findings require further *in vitro* and *in vivo* validation, they provide a strong rationale for the further preclinical development of this dual-targeting approach.

PROTEIN KINASE CK2 DRIVES INFLAMMATION AND BET PROTEIN INHIBITORS RESISTANCE IN MANTLE CELL LYMPHOMA

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Background
Mantle Cell Lymphoma (MCL) is an aggressive B-cell malignancy characterized by frequent relapse and treatment resistance. Despite the development of targeted therapies, it remains largely incurable. Disease progression is influenced by oncogenic signaling, epigenetic regulation and a tumor microenvironment (TME) that promotes lymphoma survival and immune escape. Protein kinase CK2 is overexpressed in MCL and regulates pathways involved in proliferation, apoptosis and transcription. CK2 can also enhance c-Myc activity and phosphorylate the BET protein BRD4, a key regulator of MYC and BCL2 transcription. Since resistance limits the efficacy of BET proteins inhibitors (BETi), we investigated whether CK2 contributes to MYC-driven signaling, inflammatory cytokine production and BETi resistance in MCL.

Material and Methods
MCL cells were treated with the CK2 inhibitors CX-4945 (Silmitasertib) or SGC-CK2-1, alone or combined with the BETi JQ-1 or INCB054329. CK2 was also silenced by gene-targeting approaches. A JQ-1-resistant MCL model was generated by prolonged exposure to increasing JQ-1 concentrations. Cell viability, proliferation and apoptosis were evaluated by MTT and Annexin V/Propidium Iodide assays. Gene and protein expression were analyzed by RT-qPCR and immunoblotting. Cytokine secretion was assessed by cytokine array and validated by RT-qPCR. Public transcriptomic datasets were analyzed to investigate inflammatory signatures and CK2 expression in MCL.

Results
CK2 inhibition reduced MCL cell proliferation, induced apoptosis and markedly decreased c-Myc expression. CK2/BET co-targeting also reduced the immunosuppressive molecules IL-10 and PD-L1. CK2 inhibition or silencing significantly altered the inflammatory secretome, decreasing pro-inflammatory mediators, including IL-1β, TNFα, osteopontin and MIF, as well as immunosuppressive factors such as IL-10, CCL18 and HGF. Transcriptomic profiling confirmed increased inflammatory signatures (IFNG, CXCR4 and TGFB1) in MCL compared with healthy individuals. Relapsed patients showed higher *CSNK2B* expression than newly diagnosed patients, while IL10 expression was increased in relapsed bone marrow MCL T cells compared with newly diagnosed ones. Moreover, publicly available single-cell RNA-seq patient database analyses showed increased *CSNK2A1*, *CSNK2B* and *MYC* expression from normal B cells to BTKi-fast and BTKi-relapsed MCL cells, with the highest expression observed in relapsed cells, supporting a correlation between *CK2* and *MYC* expression.
Our developed JQ-1-resistant MCL cell model displayed increased CK2 subunits, c-Myc and anti-apoptotic proteins, while CK2 inhibition induced apoptosis and restored sensitivity to BET blockade.

Conclusions
Our data identify CK2 as a key link between oncogenic transcription, inflammatory signaling and immune suppression in MCL by regulating c-Myc and inflammatory/immunosuppressive cytokine network. Its increased expression in relapsed disease further supports its involvement in MCL progression.

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PhD COURSE "PHARMACOLOGICAL SCIENCES"

COORDINATOR: PROF. MICHELE MORARI

WES PLUS – Development of a Targeted Enrichment Panel and Integrated Bioinformatics Analysis for Variant Detection

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Ph.D. Course Pharmacological Sciences

Background
Whole-exome sequencing (WES) is a next-generation sequencing (NGS) method that targets protein-coding exons. These regions comprise ~20,000 genes (~1% of the genome) and contain an estimated 95% of pathogenic variants. WES is used in both research and clinical diagnostics for suspected genetic disorders. However, some clinically relevant variants occur outside exons (e.g., intronic and untranslated regions) or are structural variants, which are better detected by whole-genome sequencing (WGS).
The reliable analysis of WES data requires robust and reproducible bioinformatic pipelines, whose development and validation can vary across laboratories and sequencing platforms.
This study aims to extend conventional WES coverage to include intronic regions potentially associated with pathogenic variation and to get a fully validated workflow.
Material and Methods
We used the GENEQUALITY® Whole Exome Sequencing Kit as a starting point; it sequences ~42 Mb of the exome (19,441 genes) and the entire mitochondrial genome, and detects CNVs, InDels, SNPs, and SNVs.
The experiment was conducted at AB ANALITICA using the NIST reference material Coriell NA12878 at two DNA inputs (50 ng and 100 ng). Pathogenic regions were identified from updated mutation databases (ClinVar, HGMD) and targeted with a customized xGen™ Custom Hyb Panel-Accel Probe (IDT). Performance was compared with the Vazyme Expanded Panel WES V3 (20,762+ genes; target/capture: 36.79/53.55 Mb), also tested with NA12878 at 50 ng and 100 ng. Sequencing was performed on the Illumina NovaSeq X Plus (paired-end 150 bp). Read depth was set to 1M reads/sample for the IDT extension panel to match WES coverage (40M reads/sample) and increased to 2M reads/sample to account for capture efficiency and coverage variability.
Results
We identified 13300 pathogenic positions in chr 1-22+X+Y based on GRCh38 and not included in WES regions from ClinVar (2025-10-19, only pathogenic/likely_pathogenic) and HGMD (2025.2, only DM/DM?/DP) databases. Of these clinically relevant variants, 8986 probes (67,56%) were genotypable, passed the QC and could be included in the panel (1.06 Mb). The bioinformatic pipeline was implemented both in Bash and Nextflow/Docker and included 1. Preprocessing: FASTQ quality assessment, adapter/quality trimming, read filtering. 2. Alignement: alignment to hg38, sorting/indexing, duplicate removal, mapping/insert-size metrics. 3. Variant Calling: target callable-region assessment, capture metrics, region analysis, germline SNV/InDel calling.
Conclusions
We identified 13,300 clinically relevant genomic positions not included in the original WES regions; 67.6% were genotypable and passed probe QC, yielding a 1.06 Mb targeted enrichment panel. We also developed a reproducible bioinformatic pipeline for QC, alignment, coverage assessment, and variant calling, migrating it from Bash to Nextflow/Docker to improve portability and reproducibility. Overall, Extended Whole Exome Sequencing may be a cost-effective alternative to Whole Genome Sequencing for detecting pathogenic variants.
Next steps include completing kit validation, expanding sample testing, and optimizing the workflow.

Gene Silencing of ANGPTL3 Induces PCSK9: Exploring the Biological Significance in the Hepatoma Huh7 Cell Line

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Ph.D. Course Pharmacological Sciences

Background
Angiopoietin-like 3 (ANGPTL3) and proprotein convertase subtilisin/kexin type 9 (PCSK9) are liver-derived regulators of lipid homeostasis and important therapeutic targets for dyslipidemia. ANGPTL3 inhibits lipoprotein and endothelial lipases, whereas PCSK9 promotes LDL receptor degradation. Previous work showed that acute ANGPTL3 gene silencing in Huh7 hepatoma cells induces PCSK9 expression and lipid accumulation. However, the biological significance of this response remains unclear. This study aimed to characterize the transcriptional changes associated with ANGPTL3 and/or PCSK9 silencing and to explore whether ANGPTL3 modulation affects pathways beyond lipid metabolism.

Material and Methods
Huh7 cells were transfected for 48 h with 20 nM scramble siRNA, ANGPTL3-siRNA, PCSK9-siRNA, or combined ANGPTL3/PCSK9-siRNAs. RNA sequencing was performed in triplicate for each condition, generating approximately 30-40 million reads per sample. Differentially expressed genes were identified at FDR < 0.05, followed by principal component and Gene Ontology enrichment analyses. Selected findings were validated by RT-qPCR and Western blotting. Conditioned media were analyzed by ELISA for apolipoprotein B (ApoB), coagulation factor V (FV), and factor VIII (FVIII). Statistical comparisons used Student's t-test or one-way ANOVA, with $p < 0.05$ considered significant.

Results
Among 13,945 detected transcripts, 192 genes were differentially expressed after ANGPTL3 silencing, 88 after PCSK9 silencing, and 219 after combined ANGPTL3/PCSK9 silencing compared with scramble controls. ANGPTL3 silencing significantly increased PCSK9 transcripts ($p = 0.0294$), and this effect was confirmed at both mRNA and protein levels. In contrast, PCSK9 silencing caused only a partial increase in ANGPTL3 mRNA without a corresponding protein change. Gene Ontology analysis showed enrichment of lipid-metabolism pathways after ANGPTL3 silencing and blood-coagulation-related processes after combined silencing. SERPINA1 and LMAN1 were markedly downregulated after ANGPTL3 silencing, alone or combined with PCSK9 silencing, and these findings were validated experimentally. ANGPTL3 silencing also increased FV and FVIII mRNA expression and extracellular levels. FV secretion increased by 24% after ANGPTL3 silencing and 21% after double silencing, whereas FVIII increased by 11% and 21%, respectively. PCSK9 silencing alone had no significant effect on FV or FVIII, and ApoB secretion remained unchanged despite PCSK9 induction.

Conclusions
ANGPTL3 silencing induces a broader transcriptional response than PCSK9 silencing in Huh7 cells and is associated with changes in secretory- and coagulation-related genes together with increased FV/FVIII release. The similarity between ANGPTL3 silencing and combined ANGPTL3/PCSK9 silencing suggests that these effects are predominantly driven by ANGPTL3 rather than secondary PCSK9 induction. These findings extend the potential biological relevance of ANGPTL3 beyond lipid metabolism, although the relationships among ANGPTL3, LMAN1/SERPINA1, and coagulation factors remain associative and require validation in more physiologically relevant hepatic models.

INVESTIGATION OF MICROGLIAL INVOLVEMENT AND EFFECTS OF 2-PENTADECYL-2-OXAZOLINE IN A MOUSE MODEL OF FENTANYL-INDUCED HYPERALGESIA

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Ph.D. Course Pharmacological Sciences

Background
Opioid-induced hyperalgesia is a paradoxical increase in pain sensitivity following opioid exposure and represents a relevant limitation of opioid therapy. Neuroimmune processes, including microglial activation, may contribute to its development. We investigated the contribution of microglia to fentanyl-induced hyperalgesia and the effects of 2-pentadecyl-2-oxazoline (PEA-OXA), an oxazoline derivative of the endogenous lipid mediator palmitoylethanolamide.
Material and Methods
Acute fentanyl-induced analgesia and secondary hyperalgesia were evaluated in adult male and female C57BL/6J mice. Complete Freund's adjuvant (CFA; 1 mg/ml, 20 µl) was injected into the hind paw. Four days later, mice received fentanyl (4 × 60 µg/kg, s.c., at 15-min intervals). PEA-OXA (30 or 50 mg/kg, i.p.) was administered as a single injection 1 h before or after fentanyl, whereas minocycline (50 mg/kg, i.p.) and CLI-095 (3 mg/kg, i.p.) were administered 30 min before fentanyl. Nociceptive sensitivity was assessed using the tail immersion test. Baseline withdrawal latencies were measured before CFA injection and pharmacological treatments, 1 and 4 h after the last fentanyl injection, and daily for the following 4 days. All animal-related procedures were approved by the Italian Ministry of Health.
Results
Fentanyl induced a comparable acute analgesic response in male and female mice but produced distinct sex-dependent temporal profiles of subsequent hyperalgesia. In both sexes, hyperalgesia developed 4 h after fentanyl administration. In males, it peaked on day 1 and persisted through day 3, whereas in females it resolved rapidly. Minocycline produced an intermediate response between vehicle- and fentanyl-treated animals, without significant differences from either group. This pattern may be consistent with a possible involvement of microglia. CLI-095 did not significantly affect hyperalgesia, providing no evidence for a major contribution of TLR4 signaling under the experimental conditions used. The effects of PEA-OXA varied according to sex- and timing of administration. In males, PEA-OXA accelerated recovery from hyperalgesia, particularly when administered shortly after fentanyl. In females, PEA-OXA attenuated the early hyperalgesic response; however, the rapid spontaneous recovery observed in this group limited the detection of additional effects at later time points.
Conclusions
Fentanyl-induced hyperalgesia displayed distinct temporal profiles in male and female mice. PEA-OXA modulated this response according to sex and treatment timing, supporting its further investigation as a potential pharmacological strategy to modulate opioid-induced hyperalgesia. Additional studies are required to clarify the involvement of microglial and other neuroinflammatory mechanisms.

IDENTIFICATION OF A PROMISING BIOACTIVE EXTRACT FOR TAREGETING CELLULAR SENESCENCE

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Ph.D. Course Pharmacological Sciences

Background
Cellular senescence is a key contributor to age-related dysfunction and is associated with oxidative stress, chronic inflammation, and the secretion of the senescence-associated secretory phenotype (SASP). Natural extracts represent a promising source of bioactive compounds targeting these interconnected processes. Based on a literature-driven selection, two proprietary extracts, referred to as GY and ED, were identified as potential candidates for anti-senescence activity.
Material and Methods
GY and ED were initially evaluated in the same cellular model to compare their potential anti-senescence effects. Based on the preliminary screening, ED was selected as the most promising candidate and further investigated in different experimental models of cellular senescence. Its safety profile was assessed in non-senescent cells, followed by the evaluation of antioxidant and anti-inflammatory activities. Anti-senescence effects were investigated through the analysis of SA-β-gal positivity and senescence-associated markers. The effects of ED on the SASP and key cellular pathways involved in aging, including oxidative stress, apoptosis, and AKT/mTOR signaling, were also characterized.
Results
The preliminary comparison identified ED as the most promising extract for further investigation. ED showed no cytotoxic effects on non-senescent cells at the tested concentrations and exhibited significant antioxidant and anti-inflammatory activities, particularly at 1 μg/mL. In different cellular models, ED reduced SA-β-gal-positive cells and modulated several senescence-associated markers. Furthermore, ED affected the SASP and key cellular processes involved in aging, including oxidative stress, apoptosis, and AKT/mTOR signaling, supporting its multifaceted anti-senescence activity.
Conclusions
The comparative screening of two proprietary extracts led to the selection of ED as a promising anti-senescence candidate. Its favorable safety profile and effects on multiple processes associated with cellular aging support further investigation of its molecular mechanisms and *in vivo* potential.

BEYOND THE TRIP: THE METABOLIC ACTION OF SUB-PSYCHEDELIC PSILOCYBIN IN STEATOTIC LIVER DISEASE

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Ph.D. Course Pharmacological Sciences

Background
Psilocybin, a serotonergic compound with established central activity, is gaining interest beyond neuropsychiatric indications. Its active metabolite, psilocin, acts as a partial 5-HT2A/2C agonist and 5-HT2B antagonist. Hepatic serotonin signaling regulates metabolic homeostasis and fibrogenesis, with 5-HT2B activation promoting hepatic stellate cell activation. However, its role in metabolic dysfunction-associated steatotic liver disease (MASLD) and steatohepatitis (MASH) remains unclear. In parallel, clinical pharmacokinetic data on psilocybin are largely limited to immediate-release formulations at moderate–high doses in healthy subjects, with scarce evidence on low-dose strategies, modified-release formulations, and obesity-related effects.
Methods
Psilocin (10 μM) was tested in steatotic HepG2 cells, including CRISPR/Cas9 5-HT2A-mutant lines, and compared with a selective 5-HT2B inhibitor. Fibrogenic responses were assessed in LX-2 cells by immunocytochemistry and RT-PCR. In vivo, male mice on a Gubra-Amylin NASH (GAN) diet received oral sub-psychedelic psilocybin (0.05 mg/kg) from weeks 20–32, followed by histological, ultrastructural (TEM), and molecular analyses. In parallel, a Phase I randomized, double-blind, placebo-controlled single ascending dose study evaluated safety, tolerability, and pharmacokinetics of a modified-release formulation (REL-P11), designed to reduce peak plasma exposure (Cmax), in normal-weight and overweight/obese subjects (0.5–2.0 mg), with psilocin quantified by LC–MS/MS.
Results
Psilocin reduced lipid accumulation in steatotic HepG2 cells, an effect preserved in 5-HT2A-mutant cells ($p<0.05$ vs untreated steatotic cells) and replicated by 5-HT2B inhibition ($p<0.01$). In TGF-β-activated LX-2 cells, fibrogenic markers (Col1a1, α-SMA, vimentin; ACTA2 and MMP2 transcripts) were significantly downregulated, mirroring 5-HT2B blockade.
In GAN-fed mice, psilocybin attenuated early fibrotic features without worsening steatosis and restored liver ER and mitochondrial ultrastructure. .
In the phase 1 trial, REL-P11 was safe and well tolerated, with no serious or clinically relevant adverse events and no detectable psychoactive effects. Psilocin exposure was dose-proportional with low variability (CV<30%), and comparable across BMI groups, with no relevant impact of obesity.
Conclusions
Psilocybin exerts metabolic and anti-fibrotic effects *via* modulation of hepatic serotonin signaling, primarily through 5-HT2B antagonism, highlighting a novel pharmacological mechanism for MASLD and MASH resolution. Importantly, low-dose modified-release psilocybin enables controlled exposure while avoiding Cmax-related toxicity and psychoactive effects, supporting its translational potential as a safe therapeutic strategy for metabolic liver disease.

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PhD COURSE "TRANSLATIONAL SPECIALISTIC MEDICINE «G.B. MORGAGNI»"

COORDINATOR: PROF. DARIO GREGORI

Curriculum "BIOSTATISTICS AND CLINICAL EPIDEMIOLOGY"

STUDY OF RISK FACTORS IN AREAS OF HIGH PREVALENCE OF EPILEPSY IN TROPICAL COUNTRIES: CASE OF FATICK REGION BY LONGITUDINAL STUDY AND CASE-CONTROL WITH A FOCUS ON GENDER ANALYSIS

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Ph.D. Course Translational Specialistic Medicine "G.B. Morgagni"
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Background
Epilepsy carries a substantial burden in low- and middle-income countries, where underdiagnosis, limited access to care, and social stigma remain major obstacles. In Senegal, prevalence estimates are scarce and likely underestimate the true burden of the disease. The largely rural Fatick region, with limited specialized neurological care, is a relevant setting to study epilepsy prevalence and its determinants. This study aims to estimate epilepsy prevalence in Fatick and identify the socioeconomic, demographic, cultural, and environmental factors associated with it, with particular attention to gender differences in care pathways and management.

Materials and Methods
The study combines a cross-sectional household survey with longitudinal and case-control components. The first phase is a population-based survey using the 2023 Senegal Population and Housing Census (RGPH) as the sampling frame, employing a stratified two-stage random design to ensure urban and rural representation. Data are collected via a structured REDCap questionnaire, and identified cases will be cross-referenced with health facility records before a case-control study investigates potential risk factors.

Results
The 2023 census reported **a prevalence of 8/1000** in the Fatick region (mean age **31.4 years (SD=21.3)**; median **25 years (IQR: 14–47)**), **53.3%** female and **46.7%** male. Most lived in rural areas (**75.3%**), and **51.6%** had never attended school; cases were concentrated in the Fatick (**45.9%**) and Foundiougne (**40.3%**) departments. It is important to note that these were self-reported or suspected cases rather than clinically confirmed. Currently, The prevalence household survey is **55% complete** (data collection is planned among 294 individuals), despite operational challenges linked to the rainy season, including flooding and difficult road access in several localities.

Conclusions
These preliminary findings indicate a first prevalence of 8/1000-based census results in Fatick, with notable socioeconomic and geographic patterns that warrant further investigation. The household survey will refine prevalence estimates beyond those from the census self-report. At the same time, linkage with health records and the case-control component will help identify risk factors and explore gender differences in access to and management of care.

TRANSPORTABILITY OF PROGNOSTIC SURVIVAL MODELS: LLM-ASSISTED EVIDENCE SYNTHESIS AND SIMULATION, WITH AN APPLICATION TO LUNG CANCER STAGING

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Background
Prognostic models for non-communicable diseases, most often Cox proportional hazards models, are developed on one dataset and then applied to populations that differ in case-mix, baseline risk and measurement practice. Their transportability is rarely assessed, partly because the external evidence needed is scattered and costly to synthesise. This project develops a framework that combines large language model (LLM)-assisted evidence synthesis with simulation to evaluate how robust a prognostic survival model is when the target population departs from the development data. The framework is applied to the IASLC ninth-edition TNM staging of non-small cell lung cancer (NSCLC): a categorical prognostic model derived from an international database of uneven geographic composition, whose stage groups must stay ordered and well separated wherever it is used.

Material and Methods
A multi-agent LLM pipeline was developed in R (ellmer) for screening and structured data extraction, with multi-model, multi-pass screening, majority-vote extraction, audit files and a rule requiring an explicit source location for every extracted value. Validation reproduces five published systematic reviews as a gold-standard reference set, with pre-specified success criteria and contamination-risk controls. In the application, the pipeline retrieves stage-specific survival estimates from registries in regions under-represented in the IASLC database. Summarised by random-effects meta-regression, these estimates are not pooled but define plausible parameter ranges for a simulation study. Survival data are generated from flexible parametric models fitted to the IASLC data and perturbed through separate mechanisms: shifts in baseline hazard, attenuation of predictor effects (compression of stage separation), predictor misclassification (stage migration) and changes in case-mix (regional composition). A Cox model with stage group as predictor is fitted to each simulated dataset, and performance is assessed through preservation of stage ordering, adjacent-stage hazard ratios, discrimination (Harrell's and Uno's C) and calibration of predicted survival, reported as tipping points beyond which the prognostic structure fails.

Results
Evidence synthesis: the pipeline has been applied at full scale in two NSCLC reviews, on circulating tumour DNA (902 full texts assessed, 174 studies included, unanimous agreement across three independent passes for 98.2% of assessed records) and on lymph node evaluation after resection (10,275 records screened by three LLMs, 421 studies included); validation against the reference set is ongoing. Prognostic modelling: the simulation study has been designed, including the four data-generating mechanisms, the Cox analysis model, the performance measures and the rules for deriving parameter ranges from the extracted evidence. The descriptive profile of the IASLC data by region and stage and the extraction of regional survival evidence are in progress, and simulation results are expected in the third year.

Conclusions
The framework separates the mechanisms through which a prognostic survival model can lose performance in a new population, and uses automated, auditable evidence synthesis to set realistic bounds for each. In lung cancer staging it is expected to identify fragile adjacent-stage contrasts and inform the geographic coverage of the tenth edition, and it is transferable to other prognostic models in non-communicable diseases.

COST-EFFECTIVENESS ANALYSIS OF EARLY DIAGNOSIS OF CONGENITAL HEART DISEASE (CHD) AT BEIRA CENTRAL HOSPITAL (HCB), 2020–2025

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Background Congenital Heart Diseases (CHDs) are among the most common birth defects, affecting approximately 1 in 100 newborns globally. In Mozambique, a low-income country with limited healthcare resources, CHDs contribute significantly to infant morbidity and mortality. Early diagnosis and intervention are crucial for improving health outcomes, yet the implementation of diagnostic programs remains constrained by financial, infrastructural, and training limitations.
Material and Methods This study evaluates the cost-effectiveness of congenital heart disease (CHD) diagnosis in Mozambique (2020–2025) using a mixed-methods design combining quantitative data (interviews, questionnaires, document analysis, processed in Excel and Jamovi) with qualitative thematic analysis. The cost-effectiveness analysis adopts a societal perspective, integrating direct and indirect costs with QALYs (EQ-5D-3L) to calculate the ICER for early versus late diagnosis, tested through sensitivity analyses.
Results The research will assess the specificity and accuracy of diagnostic techniques, calculate direct and indirect costs, and analyze the economic benefits of early detection compared to late diagnosis. Additionally, the study intends to identify barriers and facilitators to program implementation and explore the long-term economic impacts of early intervention in Mozambique. By providing evidence-based insights, this study will seek to inform healthcare policy makers, to optimize resource allocation, and improve maternal and child health outcomes in Mozambique. The findings will contribute to global health knowledge and support the development of cost-effective strategies for managing CHDs in resource-limited countries. Ethical considerations, including informed consent and data confidentiality, will be strictly adhered to throughout the research process.
Conclusions This study is expected to yield updated estimates of the prevalence and incidence of congenital heart disease at Hospital Central da Beira (2020–2025), a cost profile of diagnosis and treatment from both the health system and household perspectives, and cost-effectiveness indicators (ICER and QALYs) for early diagnosis in this setting. It will also identify the main socioeconomic and geographic factors shaping access to diagnosis, informing evidence-based health policy recommendations at the national and regional levels**.**

Keywords: Congenital heart disease; Cost-effectiveness analysis; Early diagnosis; Mozambique; ICER; QALY; Resource-limited settings; Health policy.

AN EXPLORATORY REVIEW OF THE CONCEPTUAL DEFINITION OF THE VARIABLE "MENTAL HEALTH" IN LATIN AMERICA

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Background
The modern conceptualization of mental health has its foundational milestone in 1948, with the creation of the WHO and the first International Congress on Mental Health, at which time the term gained international recognition. In Latin America, its institutionalization began with the First Latin American Seminar on Mental Health, organized by PAHO in Mexico in 1962, followed by the spread of humanistic mental health during the 1960s, driven by PAHO and intellectuals such as Erich Fromm. Between the 1970s and 1990s, mental health was progressively integrated into public health systems, academia, and regional health policies, with the 1990 Caracas Declaration marking the most significant turning point by promoting a community-based, decentralized model of care that respects human rights.
Given the vagueness of these terms ("health" and "mental") and considering their various meanings and interdisciplinary nature, it is important to conduct a study that allows us to understand the conceptual definitions and reflect on their ethical and political relevance in the Latin American context.
Material and Methods
An exploratory review of the conceptual definition of the variable "mental health" in Latin America was conducted using the PubMed, PsycInfo, and Scielo databases through 2025. The phases described are based on the PRISMA criteria: 1) Identification of the problem and objective, 2) Literature search, 3) Inclusion and exclusion based on titles and abstracts, 4) Selection of documents, 5) Data extraction and systematization, and 6) Synthesis of knowledge.
The synthesis of the evidence is conducted using a descriptive and narrative approach. Once the data extraction process is complete, the information will be organized and analyzed to answer the research questions and map out the conceptualization of the "mental health" variable. In a second stage of synthesis, a conceptual analysis will be applied, and the results will be grouped according to the categories and emerging themes identified during data extraction.
Results
In this exploratory review aimed at identifying theoretical frameworks on mental health in Latin American countries, a total of 6,684 articles were identified, of which 473 were selected for analysis.
Preliminary results indicate that the concept of mental health is not usually mentioned explicitly, but the perspective from which the work is conducted is most frequently identified. It is common to observe that the term is used predominantly to refer to mental disorders or illnesses, and that few documents explore its meaning from a health perspective. Given the interdisciplinary nature of mental health, a variety of conceptual definitions is expected to be found in terms of ontological, epistemological, and methodological perspectives.
Conclusions
It is necessary to understand the definitions of the variable "mental health" and to reflect on its ethical and political relevance in the Latin American context. This has implications for how projects, programs, strategies, and public policies aimed at mental health intervention, prevention, and promotion are formulated and evaluated.

TRENDS IN GASTRIC CANCER INCIDENCE IN PASTO, COLOMBIA: AN AGE-PERIOD-COHORT ANALYSIS

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Background
Gastric cancer represents an important public health problem in Pasto, Colombia. Its epidemiological behavior should be examined not only through overall temporal trends, but also through differences related to age, historical periods, and generations of birth. Age-period-cohort (APC) models allow these temporal dimensions to be distinguished. This study aims to analyze trends in gastric cancer incidence in Pasto using an APC approach to identify differential patterns by age group, calendar period, and birth cohort.

Material and Methods
An ecological time-series study will be conducted using incident gastric cancer cases (ICD-10 C16) among residents of Pasto during 1998–2019. Data will be obtained from the Population-Based Cancer Registry of Pasto. Crude, age-specific, and age-standardized incidence rates per 100,000 inhabitants will be estimated. Age and calendar period will be grouped into five-year intervals, and birth cohorts will be derived from their relationship. Analyses will also be stratified by sex. Data will undergo cleaning, consistency checks, and restructuring before APC modeling. The analytical strategy includes estimation of age, period, and cohort effects, with the Intrinsic Estimator and a Bayesian APC approach considered for comparison. Statistical analyses will be performed in R.

Results
The methodological design and APC analytical strategy have been consolidated, together with the definition of the main variables and a focused review of the scientific literature. The Population-Based Cancer Registry of Pasto has formally approved the delivery of the data required for this research. Beginning in the second week of September 2026, the database will be received, reviewed, cleaned, organized, and prepared for statistical analysis. Epidemiological estimates and APC model results will be generated after completion of this data-processing stage.

Conclusions
Formal access to the registry data allows the study to move from methodological preparation to the analytical phase. The APC approach is expected to provide a more detailed understanding of gastric cancer incidence trends in Pasto by distinguishing variations associated with age, historical changes, and generational patterns, thereby contributing evidence for epidemiological research and public health planning in the territory.

ADVANCED BIOSTATISTICAL METHODS FOR PERSONALIZED AND PRECISION MEDICINE

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Background
Forecasting infectious disease incidence is a long-standing challenge in public health surveillance, with direct implications for resource allocation, outbreak preparedness, and epidemic control. Classical time-series methods (ARIMA, exponential smoothing) remain the standard for capturing trend and seasonality in surveillance data, while more flexible approaches Generalized Additive Models (GAM) and, more recently, deep-learning architectures such as Long Short-Term Memory (LSTM) networks have been increasingly proposed to capture nonlinear and long-range temporal dependencies that classical models may miss (Hyndman & Athanasopoulos, 2021; Box & Jenkins, 1970; Wood, 2017; Hochreiter & Schmidhuber, 1997). However, comparative evidence on how these families of methods perform against one another, and under what data conditions each is preferable, remains limited and is often specific to a single disease or setting.

Objectives
To develop, compare, and apply advanced biostatistical methods combining classical time-series analysis, semi-parametric regression, and Bayesian spatio-temporal modeling in order to build a methodological foundation transferable to personalized and precision-medicine applications.

Material and Methods
All analyses were performed in R, using dplyr, tidyr, and purrr for data cleaning and wrangling, ggplot2 for exploratory and diagnostic visualization, forecast for ARIMA and ETS modeling, mgcv for GAM, and keras/tensorflow for LSTM.Weekly case counts (by city, age group, and gender) were cleaned, aggregated by week and demographic group using dplyr, and missing values in the case counts were imputed using the weekly median. The aggregated series was converted into univariate (total cases) and multivariate (age/gender-stratified) time-series objects. Stationarity was assessed and the series required differencing. An ARIMA(4,0,1)(1,1,0)[52] model using the forecast package. GAM fitting (via mgcv), ETS(M,A,N) and an LSTM model (via keras/tensorflow) were planned as the semi-parametric and deep-learning comparators.

Results

Model	AIC	BIC	Training MAPE (%)	MASE
ARIMA(4,0,1)(1,1,0)[52]	3845.4	3877.7	17.8	0.61
ETS(M,A,N)	—	—	—	—

Table 1. Preliminary in-sample performance metrics for the ARIMA and ETS models.

The ARIMA(4,0,1)(1,1,0)[52] model captured both short-term autoregressive structure and a pronounced yearly seasonal cycle (AIC = 3845.4, BIC = 3877.7; training MAPE = 17.8%, MASE = 0.61, with near-zero residual autocorrelation), consistent with the strongly cyclical transmission pattern visible in the raw series. Taken together, these preliminary results indicate that classical models already capture a substantial share of the temporal structure in the data, which will serve as a benchmark against which the semi-parametric and deep-learning comparators are evaluated. A preliminary GAM fit was produced but has not yet been formally validated, and the LSTM model has not yet been implemented; consequently, the fixed-window/rolling-window validation and cross-model comparison requested by the supervisory team could not yet be completed. This is the principal limitation of the current stage: without all four models validated on a common out-of-sample scheme, no conclusion can yet be drawn about which method is best suited to this type of surveillance data, and the relative contribution of nonlinear (GAM) versus long-memory (LSTM) structure remains unquantified.

Conclusions
This first pass establishes a working preprocessing pipeline and two validated baseline models (ARIMA, ETS) with quantified, interpretable performance, providing an initial demonstration of the classical time-series competencies that underpin the broader thesis methodology. The immediate next steps are to (i) finalize and formally evaluate the GAM fit, (ii) implement and tune the LSTM model, and (iii) conduct rolling/fixed-window validation across all four models using consistent metrics (RMSE, MAE, MAPE, MASE), which will allow a principled comparison of classical, semi-parametric, and deep-learning approaches.

Statistical learning for communicable diseases and non-communicable disease

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Background
Communicable diseases (CDs) and non-communicable diseases (NCDs) increasingly co-occur, driven by shared determinants such as poverty, malnutrition, and limited health-system access (e.g., TB–diabetes, HIV–cardiovascular disease). Health systems in low- and middle-income countries face a growing double burden, yet predictive tools largely treat CDs and NCDs separately, and classical statistical methods often fail to capture the non-linear, high-dimensional, uncertain dynamics of modern health data.

Material and Methods
Multi-phase design: (1) systematic review of statistical/ML methods for CD and NCD prediction (2015–present); (2) harmonized multi-source dataset from surveillance systems, EHRs, and health surveys; (3) model development spanning regularized regression, ensemble methods, deep learning, and Bayesian hierarchical models; (4) validation via cross-validation and external testing, assessed on discrimination, calibration, and subgroup fairness.

Results
Progress to date spans five complementary outputs: a workshop paper presented (Kasereka et al., 2026), two under review or submission (Godwill et al.; Luty et al.), one in preparation (Philippe et al.), and one in press (Lupweka et al.). Together, these confirm the value of structure-preserving and uncertainty-aware methods, and surface recurring challenges high heterogeneity, limited cross-protection, and inconsistent predictive associations that the core modeling framework of this research is to be built to address these issues. Core model development and validation.

Conclusion
Advanced statistical learning is expected to improve joint CD–NCD prediction and surveillance over siloed approaches, supporting integrated, equitable health system planning. Findings will be interpreted with attention to data limitations, generalizability, and the ethics of algorithmic risk prediction.

Related Outputs

- Kasereka, S. K., **Godwill Ilunga** et al. (2026). *Nonstandard Finite Difference Schemes for Uncertain Epidemic Dynamics: From Fuzzy ODEs to AI-Enhanced Discretization.* AI2M4RI Workshop, Athens. Procedia Computer Science. *Presented on the 20th of august 2026.*
- **Godwill Ilunga**., et al. (under submission). *Vaccines Against Zaire Ebolavirus and Their Potential Cross-Protection Against Bundibugyo Ebolavirus: A Narrative Review.*
- Luty, N., **Godwill Ilunga** et al. (under review). *Screening Strategies and Prevalence of Neonatal Hearing Loss in Africa: A Systematic Review and Meta-Analysis.*
- Philipe, **Godwill**., et al. (in preparation for submission). *Animal Reservoirs of Trypanosoma brucei gambiense in Africa: A Systematic Review and Meta-Analysis to Support the WHO 2030 Elimination Goal.*
- Lupweka Kayoyo, **Godwill Ilunga** et al. (in press). *Echocardiographic Characteristics Associated with Metabolic Syndrome Among Patients Followed at the Lubumbashi Cardiology Center (January 2020–August 2024).*

Comprehensive Surveillance of SHAPU: Protocol design with REDCap tool registry for Clinical, Entomological, and Botanical Biomarkers

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Background: Seasonal Hyperacute Panuveitis (SHAPU) is a rapidly progressive, blinding intraocular inflammatory disease developing over hours to days in Nepal. Despite 50 years of clinical recognition, no standardised digital surveillance instrument exists, and its etiology and biomarkers remain unidentified. This research aims to develop a protocol-based dataset and digital data dictionary for SHAPU surveillance and to identify clinical, entomological, and botanical biomarkers, generating an analyzable dataset to advance basic, clinical, and translational research on SHAPU pathogenesis and management.

Materials and Methods: A prospective observational multicentre surveillance study was conducted at three endemic provinces in Nepal after obtaining ethical clearance from the Nepal Health Research Council. A web-based survey among registered Nepalese ophthalmologists was conducted using a 30-item validated instrument hosted on Research Electronic Data Capture (REDCap). The REDCap SHAPU Surveillance Tool (REDSuiT), a structured digital case-report platform was designed to capture the clinical records, entomological and botanical exposures, and longitudinal clinical data across participating centres in a uniform, analyzable format. Relevant statistical tools were used to determine the various biomarkers.

Results: A nationwide survey of 120 Nepalese ophthalmologists produced the first professional consensus framework for the diagnosis and management of SHAPU, where the majority of ophthalmologists endorsed formal Neglected tropical disease designation for SHAPU. Seasonal onset (94.8%; very high consensus) and unilateral panuveitis (89.5%; high consensus) were the most strongly endorsed diagnostic criteria.

A comprehensive digital data dictionary, the **RED**Cap **S**HAPU S**u**rvei**i**llance **T**ool (REDSuiT), was developed with 218 variables, enabling multicentre, longitudinal, real-time data capture during seasonal outbreaks. The instrument linked three elements of exposure, the ecological reservoir (the host plant *Alnus nepalensis*), the biological vector (the host insect *Gazalina chryosolopha*), and human host exposure, as measurable variables within the same record.

Sixty-one SHAPU cases were analyzed during the SHAPU outbreak, where the median age group was 1-18 years in 67.2%; around 73.3% of patients self-reported proximity to white-moth-endemic areas, and moths' setae were directly visualized on slit-lamp examination in 18.0% of eyes at presentation. Leukocoria was present in 65.6%, hypopyon in 21.3%, and vitreous cellular reaction in over 70% of eyes at baseline. Severe vision loss (hand motion, perception of light, or no perception of light) affected 37.7% of eyes at 1 week, falling to 29.5% at 1 month and 25.0% at 12 months. By 12 months, structural complications with phthisis bulbi were found in 15.0% and complicated cataract in 25%.

Conclusions:

Digital, protocol-driven surveillance through REDSuiT allowed detailed characterization of one-year clinical course of SHAPU across different provinces of Nepal. This consensus survey provided the first empirical basis for a standardised, stage-stratified SHAPU management protocol.

Multimodal histology and omics for non-small-cell lung cancer subtype and outcome prediction

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Background
Multimodal learning can combine tumour morphology with molecular and clinical information to classify cancer subtypes and predict prognosis or treatment response. Its comparative performance, validity, and translational limitations remain uncertain. We assessed the state of this evidence and defined priorities for clinically credible model development.
Material and Methods
We conducted a PRISMA 2020 systematic review. Searches of PubMed, Scopus, and IEEE Xplore identified 5,504 records. After removal of 1,122 duplicates, 4,382 titles and abstracts were screened; 65 reports underwent full-text assessment and 35 studies were included. Eligible original human studies focused on NSCLC, or reported NSCLC results separately, applied AI or machine learning, and integrated histological imaging with at least one genomic, transcriptomic, proteomic, or epigenomic modality. We extracted population, modality, architecture, validation, endpoint, and performance. Risk of bias was assessed using an adapted PROBAST and PROBAST-AI framework. **Results**
Findings Thirty-five studies were included. Nineteen addressed survival or prognosis, 14 subtype classification, eight mutation or molecular inference, and seven treatment response, with overlap between outcome groups. Representative results ranged from an external NSCLC subtype AUC of 0.726-0.864 to high internal discrimination in selected development cohorts. Nineteen studies had some concerns and 16 had high overall risk of bias; none met all criteria for low risk. Quantitative pooling was not feasible because outcomes, target definitions, metrics, validation settings, and recurrent public cohorts were incompatible. Pooled effects, heterogeneity statistics, publication-bias tests, and prediction intervals were therefore not estimated. Multimodal histology–omics models consistently showed technical promise, but the evidence does not establish transportable clinical benefit. External validation, calibration, prospective comparison with standard care, and transparent reporting should precede clinical use.
Conclusions
Across 33 unique studies, multimodal histology-omics models detected clinically and biologically relevant signals for cancer subtyping, molecular inference, prognosis, spatial organisation, and treatment response. The strongest studies used independent cohorts and connected predictions to interpretable tissue or molecular features. However, the evidence remains dominated by retrospective development, repeated public cohorts, heterogeneous endpoints, incomplete calibration, and limited prospective evaluation. Future studies should begin with a prespecified clinical role and use institution-level external validation, patient-level leakage controls, transparent cohort provenance, calibration, decision-curve analysis, missing-modality testing, and prospective workflow evaluation. Multimodal prediction should be considered clinically ready only when it improves decisions or outcomes relative to standard care and when its operational, economic, regulatory, and equity implications are documented.

PREVALENCE AND ASSOCIATED FACTORS OF SLEEP DISORDERS IN CHRONIC HEMODIALYSIS PATIENTS IN SENEGAL

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Background
Sleep disorders affect 49–98% of patients receiving chronic hemodialysis and are associated with impaired quality of life, cardiovascular complications, and mortality. Although a Senegalese multicenter study reported an overall prevalence of 88%, objective data on obstructive sleep apnea-hypopnea syndrome (OSAHS) and the performance of commonly used screening questionnaires remain limited. This study aims to estimate the prevalence of OSAHS and other sleep disorders, identify associated factors, assess their relationship with quality of life, and evaluate questionnaire performance against respiratory polygraphy.

Material and Methods
We are conducting a multicenter cross-sectional, descriptive and analytical study from December 2025 to December 2026 in five Senegalese dialysis centers. Adults aged >18 years, receiving maintenance hemodialysis for at least 3 months, and providing written informed consent are eligible. Sociodemographic, clinical, anthropometric, dialysis, medication, and laboratory data are collected. Sleep is assessed using the Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Berlin questionnaire, and International Restless Legs Syndrome Study Group criteria. Participants undergo home respiratory polygraphy interpreted by a pulmonologist according to American Academy of Sleep Medicine criteria. OSAHS is defined by an apnea-hypopnea index (AHI) ≥5 events/hour and categorized as mild (5–14.9), moderate (15–29.9), or severe (≥30). The target sample is 215 participants. Descriptive statistics and multivariable logistic regression will estimate prevalence and identify associated factors. Questionnaire performance will be evaluated at AHI thresholds of 5, 15, and 30 using sensitivity, specificity, positive and negative predictive values, and ROC area under the curve with 95% confidence intervals.

Results
Recruitment is ongoing. As of 2 September 2026, 55 of the planned 215 participants had been enrolled (25.6%). Data cleaning and quality control are continuing in parallel. No interim inferential analysis has been conducted; prevalence, associated factors, and diagnostic performance will be analyzed after completion of recruitment and database lock.

Conclusions
This ongoing study will provide multicenter, objectively measured estimates of sleep disorders among Senegalese hemodialysis patients. Comparing standardized questionnaires with respiratory polygraphy will help define practical screening strategies and support earlier referral and management in dialysis care.

Ergonomics Hazards and Musculoskeletal Disorders Among Long Distance Bus Drivers in Kassla State-Sudan

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Background: Work-related musculoskeletal disorders (WMSD) affect workers in many occupations including drivers of large vehicles. Urban bus drivers have been found to have high prevalence rates of back problems in overseas studies. {6} Professional drivers frequently suffer from musculoskeletal disorders (MSDs) due to prolonged sitting, poor posture, repetitive movements, and exposure to whole-body vibrations. These factors contribute to pain and discomfort, commonly affecting the shoulders, limbs, back, and neck {2}A descriptive cross_sectional study was conducted to evaluate the ergonomics hazards and musculoskeletal disorders among long distance bus drivers in Kassla state -Sudan

Material and Methods: the study was conducted among 68 long-distance bus drivers in Kassala State, Sudan. Data were collected using the Standardized Nordic Musculoskeletal Questionnaire to obtain the prevalence of MSDs in 9 region of the body, other part othe questionnaire was to obtain information about risk factors, knowledge, attitude and practices of the bus drivers through paper-based and telephone interviews. body weight and height, were measured, and Body Mass Index (BMI) was calculated. Collected data were entered and managed using REDCap and analyzed using jamovi statistical software v.2.6.44. Descriptive statistics were used to summarize participants' characteristics and the prevalence of musculoskeletal symptoms, while x2 and regression tests were used to assess associations between musculoskeletal symptoms and selected demographic and occupational factors. A p-value <0.05 was considered statistically significant.

Results: Among the 68 long-distance bus drivers included in the study, the highest prevalence of musculoskeletal disorders (MSDs) during the previous 7 days was reported in the shoulders (39.7%), followed by the lower back (38.2%), upper back (30.9%), neck (29.4%), knees (23.5%), wrists/hands (10.3%), ankles/feet (7.0%), elbows (2.9%), and hips (1.5%). The prevalence of MSDs over the previous 12 months was consistently higher than that reported over the preceding 7 days.The findings further demonstrated that 75.0% of the drivers worked without an assistant driver. A (77.9%) of participants had no knowledge of the meaning of ergonomic hazards, while 57.4% did not perceive a need to adjust their working posture. Moreover, 60.3% reported that they did not use cushions or other supportive seating devices while driving. Regarding potential risk factors, BMI was not significantly associated with most MSDs reported during the last 7 days, except for wrist/hand ($p < 0.01$). BMI was also significantly associated with knee disorders reported during the last 12 months ($p = 0.044$). Work duration showed no significant association with most MSDs during the last 7 days, with significant associations observed for knee disorders ($p = 0.045$) and wrist/hand disorders ($p < 0.001$). During the previous 12 months, work duration was significantly associated with MSDs in several body regions,ankles/feet, knees, wrists/hands, and hips with p-values< (0.002, 0.001, 0.001) respectively.

Conclusions: Long-distance bus drivers in Kassala State experience a substantial burden of musculoskeletal disorders, particularly in the shoulders and lower back, alongside considerable gaps in ergonomic knowledge and preventive practices. The findings emphasize the urgent need for practical ergonomic interventions, including driver education, promotion of appropriate posture, and improved seating support, to reduce occupational musculoskeletal health risks among long distance bus drivers.

Mortality from chronic diseases in Argentina and its relationship with demographic, socioeconomic and health service indicators, during period 2001-2023

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Background: Noncommunicable diseases (NCDs) -mainly cardiovascular diseases (CVD), cancer, diabetes (DB), chronic kidney disease (CKD) and chronic respiratory diseases (CRD)- are the leading cause of death worldwide, with a major impact on quality of life and healthcare costs. They are associated with social inequalities, including poverty and barriers to healthcare access. In Argentina, detailed analyses of their geographic distribution and relationship with socioeconomic and healthcare indicators are lacking.

Materials and Methods. Study Design: ecological study. Official Data Sources: INDEC (population, projections, population density, urban/rural population, and ageing index from the 2001, 2010, and 2021 censuses); DEIS (mortality, 2001–2023, by sex, department of residence, and ICD-10 code); REFES (healthcare facilities) and CONICET (Quality of Life Index [QLI] for 2001, 2010, and 2021). Units of analysis: 510 departments, with the Autonomous City of Buenos Aires analyzed separately. Dependent variables all-causes mortality, overall NCD mortality and mortality from five major NCD groups: CVD, cancer, DB, CKD and CRD. Independent variables: population density, urban/rural population, ageing index, QLI, proportion of ill-defined causes of death (data quality indicator) and public/private healthcare facilities by level of care. Analysis: Age-standardized mortality rates (ASMRs) by direct standardization for Argentina and standardized mortality ratios (SMRs) by indirect standardization for each department, both annually and cumulatively. The WHO standard population was used for ASMRs and the Argentine population for SMRs. Further analyses will include time trends, thematic mapping, associations with demographic, socioeconomic, and healthcare indicators, and regression models. Estimates will include 95% confidence intervals with statistical significance set at $p < 0.05$. R software was used.

Results: A 100% match between department codes and geographic boundaries was achieved. National ASMRs for all-cause, NCD, and cause-specific mortality showed patterns consistent with published data. At the department level, DM, CKD, and CRD showed a greater proportion of extreme SMRs (< 0.6 or > 1.4). Low SMRs occurred mainly in sparsely populated and arid areas, where potential under-registration or inadequate cause-of-death coding cannot be ruled out. High SMRs occurred mainly in isolated areas with dispersed populations, greater poverty, a higher proportion of Indigenous populations and geographic barriers to healthcare access. These patterns were particularly observed for DM, CKD, and CRD.

Conclusions

The main methodological challenge was data heterogeneity across 510 departments over 23 years, which complicated geographic harmonization and validation. Nevertheless, a 100% geographic match was achieved. Next steps include harmonizing and validating demographic, socioeconomic, and healthcare service data, followed by regression models to assess geographic patterns and associations between mortality, socioeconomic conditions, and healthcare resources.

Spatial and temporal patterns of malaria burden in sub-Saharan Africa: a Bayesian analysis of GBD 2021 estimates

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Background Malaria burden in sub-Saharan Africa (SSA) is geographically heterogeneous and temporally dynamic. Identifying where high burden persists, which population groups remain most affected, and how changes in rates compare with changes in absolute numbers is important for malaria control and surveillance. We examined country-level patterns in malaria incidence, mortality, and disability-adjusted life years (DALYs) across SSA using Bayesian spatiotemporal models.

Methods We analysed GBD 2021 estimates for 46 SSA countries from 2000 to 2021. Incidence, deaths, and DALYs were described by year, age, sex, and country. Bayesian spatiotemporal models were fitted separately for incidence and mortality using Poisson and negative-binomial likelihoods with alternative spatial and temporal structures. A bivariate shared-component model examined spatial and country-year patterns common to incidence and mortality. Models were compared using DIC, WAIC, mean negative log predictive score (MLS), conditional predictive ordinate (CPO), and probability integral transform (PIT) diagnostics.

Results Between 2000 and 2021, population-weighted mortality, incidence, and DALY rates declined by 47.3%, 36.7%, and 50.5%, respectively. Over the same period, the estimated number of incidence malaria cases increased by 12.1% as the study population grew by 77.1%. Children younger than five years accounted for 44.8% of incident cases, 65.9% of deaths, and 76.3% of DALYs. In 2021, the highest age-specific mortality rate was observed among adults aged 70–74 years (268.6 per 100,000), compared with 233.4 per 100,000 among children younger than five years. Eighteen countries met the persistence criterion for elevated incidence risk and 17 for mortality. The bivariate model showed a broadly similar spatial pattern for incidence and mortality, while shared country-year variation was weaker for mortality.

Conclusions Malaria rates declined markedly across SSA, but the estimated number of incident cases increased and elevated risk persisted in several countries. Children younger than five years accounted for most malaria deaths and DALYs over the study period, while high age-specific mortality among older adults indicates that severe outcomes are not limited to childhood. Malaria Programmes should consider absolute burden alongside rates and use model-based country-level risk estimates together with local surveillance when setting priorities. These findings can help direct prevention, surveillance, and control efforts towards populations and settings where malaria burden remains high.

Keywords: malaria; sub-Saharan Africa; Bayesian disease mapping; spatiotemporal analysis; INLA; BYM2; incidence; mortality; DALYs

Descriptive epidemiology of pertussis in District 17D03, Quito, Ecuador, 2014–2025: a time-series study using routinely-collected surveillance data, with person, place, and time analysis and statistical aberration detection[1].

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Note: Work in progress; not yet sent to the tutor for review.

Background
Pertussis remains a major cause of infant morbidity and mortality worldwide, with periodic resurgences despite vaccination. In Ecuador, District 17D03 (Quito) experienced a pertussis outbreak in 2024–2025 that was not formally confirmed by statistical surveillance methods at the time, as the endemic-channel method in use is unsuited to low-incidence series. This study characterizes pertussis epidemiology in the district from 2014 to 2025 and evaluates statistical aberration-detection methods appropriate for both the endemic period and the outbreak.

Material and Methods
Observational descriptive time-series study, reported following STROBE and its extension for routinely-collected health data, RECORD, using confirmed-case surveillance data (SIVE-Alerta linelist), with an endemic baseline (2014–2023) and outbreak period (2024–2025). The Flexible Farrington Model (FFM) and a negative-binomial LR-CUSUM were applied to the full weekly series; a short-term quasi-Poisson trend analysis was also evaluated as a non-historical early signal

Results
280 confirmed cases were identified (48 endemic, restricted to the 0–4 age group; 232 outbreak-period, spanning all age groups). The proportion of cases reported by private institutions rose from 2.1% to 47.0% during the outbreak. Reporting-delay distributions did not differ significantly between periods (Kolmogorov-Smirnov, p=0.54). LR-CUSUM detected the outbreak from 16 March 2025 and FFM from 30 March 2025 — both weeks before the marked case increase observed in April 2025.

Conclusions
Two independent statistical methods would have enabled early outbreak detection weeks before it became visually apparent, highlighting a concrete gap in the district's surveillance capacity. Findings support the development of reproducible analysis code applicable to other districts, provided its parameters are recalibrated to local data characteristics.

[1] This study is part of the doctoral project titled "A Comprehensive Whooping Cough Surveillance Model for Ecuador: An Integration of Multiple Epidemiological Methods."

Saliva urea nitrogen dipstick as a triage tool for kidney dysfunction in agricultural workers from northern Senegal: a prospective diagnostic accuracy study

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Background:
Kidney disease is a growing global health burden, particularly in low-resource settings where access to laboratory testing remains limited. Saliva urea nitrogen (SUN) dipsticks may provide a simple, non-invasive approach for kidney dysfunction triage. We evaluated their diagnostic performance and longitudinal repeatability among agricultural workers in northern Senegal.
Material and Methods:
In this prospective diagnostic accuracy study, 300 male sugarcane workers underwent SUN testing and serum creatinine measurement at baseline and 3 months. Kidney dysfunction was defined as eGFR <60 mL/min/1.73 m² using CKD-EPI 2009 without the Black race coefficient. Diagnostic performance was assessed using ROC analysis, and longitudinal agreement using exact agreement and weighted Cohen's κ.
Results:
The AUC was 0.77 (95% CI, 0.71–0.84) at baseline and 0.83 (95% CI, 0.77–0.90) at 3 months. The AUC difference was 0.060 (95% BCa bootstrap CI, −0.027 to 0.142). At the optimal cutoff of 14.5 mg/dL, sensitivity was 93% and 96.4%, specificity 51.6% and 51.8%, positive predictive value (PPV) 23.8% and 17.1%, and negative predictive value (NPV) 97.8% and 99.3% at baseline and 3 months, respectively. Among 206 participants with stable kidney function, exact SUN agreement was 53.9% (95% CI, 46.8–60.8), with a weighted κ of 0.227 (95% CI, 0.086–0.348)
Conclusions:
SUN showed high sensitivity and NPV but limited specificity and PPV. It may be useful as a rule-out or triage tool in resource-limited settings, while positive results require confirmation using creatinine-based kidney function assessment.
Key-words: Saliva Urea Nitrogen Dipstick; Chronic Kidney Disease; Point-of-care Testing; Low-resource settings; Agricultural workers, Senegal

SISTEMATIC REVIEW OF PRENATAL CARE IN THE INDIGENOUS POPULATION

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Background: According to the WHO, all pregnant women should have access to timely and adequate care to prevent or reduce the likelihood of perinatal deaths; with a minimum of eight prenatal care contacts, deaths related to the perinatal period can be reduced by up to eight per 1,000 births, compared to a minimum of four visits.
Therefore, this systematic review aims to understand the impact of prenatal care on indigenous women in minimizing or preventing maternal death and maternal-perinatal death.
Material and Methods: A literature search was conducted in Embase, LILACS, PubMed, SciELO, and Scopus to identify primary studies related to prenatal care in Indigenous populations. No geographical or publication date restrictions were applied. Data extraction was performed simultaneously and independently by two reviewers using Covidence. Using Cochrane and the PRISMA 2020 protocol, the quality of the data was assessed. For bias assessment, tools corresponding to each study design were applied, such as ROB-2 (Cochrane), NEWCASTLE-OTTAWA, etc. A thematic analysis was conducted, the findings were synthesized into descriptive narratives, and tables were designed to facilitate comparison and analysis. The protocol was registered in PROSPERO 2025 under CRD420251113373.
Results: The database search strategy yielded 6.055 results, of which 4.996 were removed before screening. Of these, 1.059 were assessed by title and abstract, with 949 being excluded; subsequently, 110 were assessed for potential eligibility, and finally 15 studies met the inclusion criteria. Of these, six were cohort studies, five were cross-sectional studies, one was a non-randomized experimental study, one was a case series, and one was a retrospective comparative analysis. Six studies were conducted in Australia, while the remaining studies were from countries in the Americas. The studies predominantly included rural or remote Indigenous populations with different sociodemographic characteristics. Analysis of the results identified four emerging thematic categories: effective and continuous prenatal care, barriers to access to health services, cultural adaptation of prenatal care, and prenatal care for detecting and reducing maternal and perinatal risks.
Conclusions: The analysis of the studies shows that barriers to access to health services for Indigenous populations are mainly framed by the geographical distance between their homes and the health center or hospital, logistical problems, language barriers, and ethnic discrimination. Therefore, designing and implementing culturally adapted prenatal care models through cultural safety frameworks, the use of native languages, working in synergy with Indigenous midwives, and developing active community outreach strategies is no longer optional, but has become an epidemiological and human rights imperative essential for access to and adherence to prenatal care programs for Indigenous communities. These strategies make it possible to achieve timely and continuous care, promoting the use of screening, risk detection, and referral services, with an impact on reducing prematurity, neonatal admissions, or perinatal mortality. However, due to the heterogeneity of the studies, no direct reduction in maternal mortality attributable to prenatal care was demonstrated.

AI-based Prediction Models for Recurrence, Progression, and Treatment Outcomes in Genitourinary Tumors

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Background: pT1 high-grade non-muscle-invasive bladder cancer (NMIBC) represents a biologically heterogeneous disease, with markedly different recurrence trajectories despite apparently similar clinicopathological characteristics. Conventional risk stratification models based on tumor size, multifocality, grade, and concomitant carcinoma in situ remain imperfect in identifying patients at higher risk of recurrence. Pathological features reflecting the extent of invasion and tumor–host interactions, including microstaging, lymphovascular invasion (LVI), and tumor-infiltrating lymphocytes (TILs), may provide additional prognostic information. However, their assessment on transurethral resection specimens may be affected by tissue fragmentation, suboptimal orientation, and interobserver variability. We therefore investigated the prognostic value of these pathological features and developed a computational pathology framework for quantitative characterization of tumor, stromal, and tumor–stroma interface phenotypes.
Material and Methods: We retrospectively included 117 patients with primary pT1 high-grade bladder cancer treated by transurethral resection. Recurrence-free survival (RFS) was the primary endpoint. Conventional clinicopathological variables included tumor characteristics, lymphovascular invasion (LVI), microstaging, and tumor-infiltrating lymphocytes (TILs). In parallel, a pathology-supervised computational pipeline was developed on 10 representative whole-slide images (WSIs). Regions of interest were annotated as Tumor, Stroma, or Mixed tumor–stroma interface. Tiles were extracted at 224×224 pixels, 0.50 μm/pixel, with 50% overlap and ≥80% annotation purity. All 258 tiles originating from Mixed regions underwent patch-level pathological review; 217 were confirmed as Mixed, 16 relabeled as Tumor, 15 as Stroma, and 10 excluded. The final development set included 1,069 tiles (267 Tumor, 585 Stroma, 217 Mixed). Frozen feature extraction using ImageNet-pretrained ResNet50 was compared with the pathology foundation model UNI, using identical multinomial logistic regression classifiers and strict leave-one-WSI-out validation.
Results: Among the 117 patients, recurrence risk remained heterogeneous despite conventional pathological stratification. On multivariable analysis, stromal TILs, pathological microstaging, and LVI independently predicted RFS. Higher stromal TILs were associated with a reduced risk of recurrence (HR 0.716, p=0.012), whereas increasing microstaging extent was associated with poorer RFS (HR 1.377, p=0.023). LVI showed the strongest adverse association with recurrence (HR 6.412, p<0.001), supporting the complementary prognostic relevance of invasive tumor characteristics and the local immune microenvironment. For computational tissue classification, blinded repeat quality control of pure Tumor and Stroma tiles showed 20/20 concordance. Using strict WSI-level cross-validation, frozen ResNet50 achieved a mean accuracy of 0.872, mean balanced accuracy of 0.876, and pooled macro-F1 of 0.842. Partial ResNet50 fine-tuning did not improve WSI-level generalization. Using the identical training and validation framework, UNI consistently outperformed ResNet50 across all 10 held-out WSIs, achieving a mean WSI-level accuracy of 0.932, mean balanced accuracy of 0.943, median balanced accuracy of 0.966, and pooled macro-F1 of 0.905. Pooled accuracy increased from 0.868 with ResNet50 to 0.921 with UNI. Class-specific F1 scores with UNI were 0.900 for Tumor, 0.959 for Stroma, and 0.856 for Mixed, compared with 0.847, 0.931, and 0.746, respectively, with ResNet50. No true Tumor tile was directly misclassified as Stroma by UNI. Importantly, UNI also improved classification in the most challenging WSI, with balanced accuracy increasing from 0.613 to 0.761, indicating greater robustness to inter-slide heterogeneity.
Conclusions: Microstaging, LVI, and stromal immune infiltration identify clinically relevant prognostic heterogeneity in pT1 high-grade NMIBC, but conventional pathological assessment may not fully capture the quantitative and spatial complexity of the tumor microenvironment. A frozen pathology foundation model provided robust discrimination of tumor, stroma, and tumor–stroma interface regions and consistently outperformed ImageNet-based feature extraction without requiring fine-tuning. Following model lock, the next step will be an independent technical validation on previously unseen WSIs, followed by application of the validated pipeline to the full cohort to derive quantitative tissue composition, tumor morphology, immune, and spatial interface features and assess their incremental prognostic value for RFS.

Knowledge and Practices of Health Professionals, Family Perceptions, and Clinical Profile of Pediatric Cancer in the Thiès Region: A Mixed-Methods Cross-Sectional Study Supporting the Decentralization of Pediatric Oncology Care

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Background
In Senegal, the management of childhood cancer has long been centralized in Dakar. The development of pediatric surgery services at the Thiès Regional Hospital Center (CHR) is part of a decentralization effort aimed at bringing diagnosis and initial treatment closer to populations in peripheral regions.
Material and Methods
A mixed-methods, cross-sectional study with both descriptive and analytical aims, combining three complementary components: a quantitative component among health professionals (Knowledge, Attitudes and Practices), a qualitative component among families, and a retro and prospective clinical component. Final population analyzed: 17 health professionals (7 specialists, 10 paramedical staff) from the departments that agreed to participate and children treated for a tumor condition in the pediatric surgery and neurosurgery departments of the Thiès CHR between January 2022 and April 2026, i.e., 118 usable records.
Results
Of the two level-2 health facilities in the Thiès region, only one participated fully in the survey. Only 6 departments of the first facility participated, totaling 17 professionals. None of the region's three health districts participated in data collection, despite two training sessions delivered at two of the three selected collection sites institutional participation falling well short of the study's initial target. Thematic analysis of the data collected from families revealed two converging findings:
A perceived deficit in educational communication about pediatric cancer, in particular the lack of involvement of cultural and social representatives (religious and traditional authorities, community relays) in disseminating information; Frequent initial recourse to traditional healers, explicitly justified by families as a result of this deficit in communication and awareness-raising around pediatric cancer. The population was balanced in terms of sex (50.8% girls). The median age at diagnosis was 48 months [IQR 24–105]. Nearly half of patients came from a health facility other than the Thiès CHR, reflecting the existence of a referral network to this center. A binary logistic regression was performed to identify factors associated with death, first in univariate analysis, then in adjusted multivariate analysis.
Conclusion
Taken together, the three components of this study paint a coherent picture: a marked deficit in knowledge and training among front-line health professionals regarding early diagnosis of pediatric cancer (no paramedical staff trained, fewer than a third of specialists) in Senegal.

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PhD COURSE "TRANSLATIONAL SPECIALISTIC MEDICINE «G.B. MORGAGNI»"

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TRANSLATIONAL AND CLINICAL RESEARCH IN DCD HEART TRANSPLANTATION AFTER A 20-MINUTE NO-TOUCH PERIOD: ADVANCES IN TECHNIQUES, CLINICAL OUTCOMES, AND MECHANISTIC INSIGHTS

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Background: Heart transplantation from controlled donation after circulatory death (cDCD) is challenged by myocardial injury occurring during withdrawal of life-sustaining therapy, circulatory arrest, and subsequent ischemia–reperfusion. This challenge is particularly relevant in Italy, where death determination requires a mandatory 20-minute no-touch period—the longest mandatory stand-off worldwide—resulting in a prolonged warm ischemic burden historically considered prohibitive for cardiac donation. Thoraco-abdominal normothermic regional perfusion (taNRP) allows myocardial reperfusion, reanimation, functional recovery, and in-situ graft assessment before procurement. Once myocardial function has been restored, conventional transplantation exposes the graft to a second cardioplegic arrest, cold ischemic preservation, and reperfusion. Total beating heart transplantation (TBHT) was introduced to minimize this additional ischemia–reperfusion burden by maintaining myocardial perfusion through procurement, transport, and implantation.
Material and Methods: This ongoing translational clinical programme evaluates the Padova experience under the Italian 20-minute no-touch rule within the multicenter DCD.IT study (ethics approval 5825/AO/23). Twenty-one cases with sufficiently complete transplant and postoperative data were included in the present preliminary retrospective and prospective single-center analysis. Three complementary components are being investigated: (i) clinical, assessing donor ischemic times, taNRP recovery, operative variables, primary graft dysfunction (PGD), mechanical circulatory support, mortality, intensive care course, and serial ventricular function; (ii) technical, reconstructing procurement, preservation, and implantation strategies from primary sources and operative/perfusion documentation; and (iii) mechanistic, characterizing myocardial ischemic exposure and recovery using biochemical, perfusion, and functional variables. To explore whether avoidance of an additional post-NRP ischemia–reperfusion phase is associated with subsequent graft recovery, nine TBHT procedures were compared with 12 non-TBHT procedures. TBHT was defined by the absence of a second post-NRP cardioplegic/cold-ischemic phase, cross-checked against recorded cold ischemic time and available operative and perfusion documentation. Categorical variables were compared by Fisher's exact test and continuous variables by the Mann–Whitney U test, with Benjamini–Hochberg correction for multiple comparisons and statistical power estimated by simulation.
Results: Preliminary analysis confirms that successful cDCD heart transplantation is feasible despite the prolonged ischemic burden imposed by the Italian 20-minute no-touch period, with taNRP permitting myocardial reanimation and functional assessment before procurement. Severe LV-PGD occurred in 4 of 19 evaluable cases, postoperative extracorporeal membrane oxygenation was required in 3 of 20, and no 30-day deaths occurred among recipients with available follow-up. In the exploratory TBHT versus non-TBHT comparison, no statistically significant differences were detected in severe LV-PGD (2 of 8 versus 2 of 11 evaluable cases), postoperative extracorporeal membrane oxygenation, in-hospital mortality, mechanical ventilation, or intensive care unit stay. A higher left-ventricular ejection fraction was observed at postoperative day 21 in the TBHT group [61% (IQR 59–63) vs 54% (IQR 50–57.5); unadjusted p=0.022], but this difference did not persist after correction for multiple comparisons (adjusted p=0.37). Two baseline imbalances currently favour the TBHT group—shorter asystolic time (20 vs 28 minutes, p=0.014) and shorter donor intensive care stay (9 vs 16 days, p=0.040)—whereas functional warm ischemia is equivalent (41 vs 43 minutes, p=0.94); together with an estimated power of only about 20% to detect a reduction in severe PGD from 40% to 10%, these preclude definitive interpretation at this stage. Protocol reconstruction has also identified additional quantifiable ischemic and perfusion variables not systematically captured in the existing registry.
Conclusions: These preliminary findings support the feasibility of cDCD heart transplantation despite the prolonged warm ischemic burden associated with the Italian 20-minute no-touch period. After myocardial rescue with taNRP, minimizing subsequent ischemic exposure through TBHT represents a rational evolution of the programme, although current between-group comparisons remain exploratory and are constrained by sample size and baseline imbalance rather than by evidence of absent effect. Ongoing work focuses on source-data verification, detailed reconstruction of ischemic and perfusion exposure, longitudinal assessment of myocardial recovery, and expansion of the analysis within the multicenter Italian DCD.IT cohort.

Beyond Conventional Genetic Testing: A Multi-Omics Approach to Uncover Missing Heritability in Inherited Cariomyopathies

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Background: Non-ischemic cardiomyopathies are genetically heterogeneous disorders and a major cause of heart failure and sudden cardiac death. Despite the widespread use of next-generation sequencing (NGS), approximately 50% of clinically diagnosed patients remain genetically unresolved. This project aims to investigate whether structural genomic variants and epigenetic alterations contribute to the missing heritability of inherited cardiomyopathies.
Material and Methods: A multi-omics approach was applied to patients with non-ischemic cardiomyopathies. Copy number variations (CNVs) were investigated using an NGS-based pipeline. Genome-wide DNA methylation profiling was performed on frozen myocardial tissue from explanted hearts, and chromatin accessibility was assessed by ATAC-seq in induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).
Results: An NGS-based CNV calling pipeline was optimized and applied to 320 patients (216 males and 104 females; 192 genotype-negative and 128 genotype-positive). Twenty-four candidate CNVs were identified in 23 patients (14 genotype-negative and 9 genotype-positive). Validation by MLPA and RT-PCR confirmed one CNV in a genotype-negative patient involving *KCNH2* (chr7:150947057–150947889; exons 12–13; 833 bp), whereas nine candidates were not validated. Further analyses are ongoing, including the implementation of a cardiovascular Digital MLPA panel with additional targeted probes covering more than 60 cardiomyopathy-related genes insufficiently covered by exome sequencing and conventional MLPA.
Genome-wide DNA methylation profiling was performed on myocardial tissue from 24 explanted hearts (16 arrhythmogenic cardiomyopathy [ACM] patients and 8 healthy controls). Differential methylation analysis highlighted pathways involved in fibrosis, adipogenesis, ion channel regulation and arrhythmogenesis, energy metabolism, and sarcomere organization, potentially reflecting the molecular remodeling associated with end-stage disease. In genotype-negative patients, five genes (*TIE1, CBS, PHACTR1, GNAS,* and *CHRFAM7A*) showed prominent methylation changes. RNA sequencing and functional characterization are ongoing to assess their potential contribution to disease pathogenesis.
For chromatin accessibility studies, an iPSC-derived cardiomyocyte line carrying a pathogenic *LMNA* variant was successfully generated and characterized. Preliminary analyses revealed differences in chromatin accessibility between *LMNA*-mutant and wild-type cardiomyocytes, together with morphological abnormalities and cytoplasmic granularity at terminal differentiation. CD34+ cells from a second patient carrying a different *LMNA* variant were also successfully reprogrammed into iPSCs, which are currently undergoing characterization. Further ATAC-seq, immunofluorescence, and transmission electron microscopy (TEM) studies are ongoing.
Conclusions: The identification of a *KCNH2* CNV in a previously genotype-negative patient highlights the diagnostic potential of CNV analysis beyond conventional sequencing. Methylation profiling identified differential methylation patterns involving pathways related to fibrosis, metabolism, ion channel regulation, and sarcomere organization, reflecting molecular remodeling associated with end-stage myocardial disease. The establishment of patient-specific *LMNA* iPSC-CM models further revealed preliminary molecular and morphological abnormalities. The integration of CNV, methylation, and chromatin accessibility data will further clarify the contribution of these mechanisms to cardiomyopathy pathogenesis and their potential relevance to molecular diagnosis.

LONGITUDINAL CHANGES IN PERICORONARY FAT ATTENUATION AS A NON INVASIVE MARKER OF CARDIAC ALLOGRAFT VASCULOPATHY PROGRESSION

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Background
Pericoronary adipose tissue (PCAT) density on coronary computed tomography angiography (CCTA) has emerged as a non-invasive marker of perivascular inflammation and a potential prognostic biomarker in heart transplant recipients. However, relationship between the longitudinal changes in PCAT attenuation and cardiac allograft vasculopathy (CAV) progression remains poorly defined. The aim of this study is to evaluate whether static PCAT density values and their longitudinal change predict CAV progression and adverse evolution in stable heart transplant recipients.

Material and Methods
We retrospectively enrolled 127 recipients with clinical, echocardiographic, and biopsy-proven stability undergoing paired CCTA at Padua University Hospital. Proximal, pancoronary, and right coronary artery pericoronary fat attenuation were measured at baseline and follow-up CCTA

Results
Baseline CCTA was performed a median of 7.5 years after transplantation and follow-up 2.0 years later. Radiological CAV progression occurred in 14 patients (11.0%) according to International Society for Heart and Lung Transplantation criteria; 20 patients (15.7%) met the composite radiological or clinical endpoint (worsening of New York Heart Association functional class, or hospitalization for cardiovascular causes). Cohort-level PCAT density values and in event-free patients were stable, while subjects presenting composite events showed longitudinal increases in all six attenuation metrics. A newly proposed biomarker, the dynamic PCAT ratio (baseline/follow-up) was consistently higher than 1 in patients with radiological or clinical progression, while remaining close to 1 in event-free subjects. In Firth penalized regression, dynamic PCAT ratios remained associated with the composite endpoint after multivariable and multiplicity adjustment, whereas associations with static measurements did not remain significant after correction.

Conclusions
In heart transplant recipients, temporal evolution of PCAT density, rather than single measurements, appears to capture the biological activity associated with subsequent clinical deterioration, but warrants prospective multicentre validation.

Molecular Signatures of Inflammatory Cardiomyopathies:
An Integrated Approach to Biomarker Discovery and Disease Stratification

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Background
Inflammatory cardiomyopathies (ICMs), including arrhythmogenic cardiomyopathy (ACM), dilated cardiomyopathy (DCM), and myocarditis, comprise a heterogeneous spectrum of disorders with overlapping clinical, genetic, and molecular features, complicating differential diagnosis and patient stratification. Disease- and phenotype-associated molecular signatures may improve diagnostic discrimination while providing insights into the biological mechanisms underlying ICM heterogeneity. This project aims to identify such signatures through an integrated approach combining genetic, immune, circulating, and disease-relevant cellular features.

Materials and Methods
Complementary molecular layers were investigated in a clinically and genetically characterized cohort of ICM patients. Circulating proteins were profiled using Olink® Proximity Extension Assay in 176 serum samples (ACM=30, DCM=38, Myocarditis=41, Healthy controls=35, relatives genotype positive=24), and anti-heart autoantibodies (AHA) were assessed by indirect immunofluorescence in 429 samples (ACM=154, DCM=154, Myocarditis=31, Relatives=55, other phenotype=35). HLA class II genotypes (*HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1*) were inferred from whole-exome sequencing data in 394 patients using HLAssign and HISAT2. Extracellular miRNAs were investigated in iPSC-derived cardiomyocytes carrying the *DSG2* c.797A>G variant and wild-type controls. Candidate miRNAs identified by next-generation sequencing were validated by RT-qPCR and evaluated in serum from patients with ACM (n=34), DCM (n=9), and healthy controls (n=17). Discriminatory performance was assessed by ROC analysis.

Results
More than 350 proteins were investigated in ICM serum samples, but none reached biologically and statistically significance alterations, indicating heterogeneous circulating molecular alterations in ICMs. Conversely, AHA positivity was significantly more frequent in myocarditis (21.7% vs 7.5%, p=0.012) and less frequent in DCM-related phenotypes (17.4% vs 35.2%, p=0.007). Notably, AHA positivity combined with genetic negativity discriminated myocarditis from other phenotypes (AUC=0.724; specificity=91.9%), supporting the value of integrating immune and genetic features for phenotypic stratification. In the *DSG2* cell model, 42 differentially expressed miRNAs were identified and seven candidates were subsequently validated by RT-qPCR in serum samples. Among these, hsa-miR-125a-5p emerged as a promising candidate, showing increased expression in ACM and discriminating right- from left-dominant disease (ARVC vs ALVC; AUC=0.788). Moreover, the combination of hsa-miR-125a-5p and hsa-miR-6126 demonstrated high discriminatory performance between ACM and DCM (AUC=0.900), supporting the potential of extracellular miRNA signatures as molecular markers of disease phenotype.

Conclusions
AHA and circulating miRNA signatures showed potential as biomarkers of ICM phenotypes, with AHA positivity combined with genetic negativity showing high specificity for myocarditis and miR-125a-5p emerging as a promising biomarker for ACM phenotypic discrimination. Ongoing work will combine HLA class II profiles with AHA, clinical, and genetic data and extend the miRNA approach to patient-derived iPSC cardiomyocytes carrying *TTN* variants to explore genotype-associated extracellular miRNA signatures in DCM.

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PhD COURSE "TRANSLATIONAL SPECIALISTIC MEDICINE «G.B. MORGAGNI»"

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Curriculum "CLINICAL AND TRANSLATIONAL NEUROSCIENCES"

USE OF SIMULATION-BASED WORKSHOPS TO MOBILIZE EMOTIONS AND EMPATHY DURING COMMUNICATION TRAINING IN NURSING STUDENTS (Pilot Study)

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Background
Effective healthcare communication is essential for patient satisfaction, safety, outcomes, and ethical practice, particularly by fostering empathy, patient participation, shared decision-making, and informed consent. Simulation-based training provides an emotionally engaging educational approach to developing communication skills and might promote empathy among healthcare professionals.
Objectives
(A) To assess changes in emotional perceptions (EP), emotional state (ET), and empathy among nursing students following a simulation-based workshop (SBW) on delivering bad news.
(B) To evaluate the sensitivity of the Positive and Negative Affect Schedule (PANAS), Jefferson Scale of Empathy–Healthcare Professional Version (JSE-HP), and Jefferson Scale of Patient Perceptions of Physician Empathy (JSPPPE) to detect changes in emotions and empathy.
Material and Methods
Observational, longitudinal study. The SBW was a curricular activity designed to train communication skills among nursing students. Nine fourth-year nursing students participated. The 6-hour SBW comprised three weekly sessions (S1–S3). In S1 (R0), communication protocols and a clinical case were discussed, and baseline PANAS and JSE-HP scores were obtained. In S2 (R1), participants engaged in role-playing with simulated patients (SPs), recorded their emotional perceptions (EP), and completed the PANAS, JSE-HP, and JSPPPE. Instructors (Is) and SPs also completed the JSPPPE. In S3 (R2), debriefing was conducted, and EP and all scales were reassessed.
Results
Nervousness was the most frequently reported EP during S2 and S3. From S1 to S2, PANAS scores increased for *alertness* (+23%), *nervousness* (+23%), *jitteriness* (+55%), and *fear* (+4%), while *excitement* decreased (−12%). From S2 to S3, negative affect decreased, including *alertness* (−23%), *fear* (−32%), *nervousness* (−18%), *jitteriness* (−36%), and *fearfulness* (−25%). JSE-HP scores decreased from 93.1 to 87.3 ($p<0.05$). JSPPPE scores differed significantly between student participants and SPs, with SPs assigning higher empathy ratings across all domains ($p<0.05$) and demonstrating good-to-excellent internal consistency ($\alpha \geq 0.80$).
Conclusions
The SBW brought out measurable emotional responses and changes in empathy among student participants. PANAS, JSE-HP, and JSPPPE demonstrated sensitivity to these changes, supporting their potential utility for evaluating communication training in simulation-based education.

The Pathophysiological Role of Vascularization in Auditory Damage

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Background
Age-related hearing loss (ARHL) is associated with progressive dysfunction of the cochlear microvasculature and stria vascularis.
The Kir4.1 potassium channel, expressed in the intermediate cells of the stria vascularis, is essential for maintaining the endocochlear potential and ionic homeostasis. Its downregulation has been associated with auditory dysfunction in ARHL; however, the mechanisms underlying its alteration remain poorly understood. In particular, whether endothelial dysfunction and blood-labyrinth barrier (BLB) impairment contribute to Kir4.1 dysregulation and auditory dysfunction in ARHL remains unclear. This study aims to investigate the relationship between functional and morphological strial alterations and microvascular dysfunction, while evaluating the potential geroprotective effects of quercetin.
Materials and Methods
To investigate this, an *in vivo* study was conducted using C57BL/6 mice assigned to four groups: D-galactose (D-gal)-treated mice as an accelerated aging model, D-gal + quercetin, quercetin alone, and vehicle controls. Functional hearing thresholds were assessed via auditory brainstem response (ABR) recordings. Stria vascularis morphometry (length, thickness, and area) was analyzed using custom MATLAB scripts. Immunohistochemical staining for Kir4.1 and CD31 was performed using DAB, while ZO-1 immunofluorescence was used to assess BLB tight-junction organization. Concurrently, an *in vitro* model using human umbilical vein endothelial cells (HUVECs) was established. Dose-response viability assays were performed to identify suitable concentrations of D-gal and quercetin for subsequent studies of barrier function and senescence.
Results
ABR recordings confirmed auditory threshold shifts in the D-gal-treated group, which were partially attenuated by quercetin treatment. MATLAB-based morphometric analyses revealed significant structural remodeling of the stria vascularis in D-gal-treated mice.
Furthermore, CD31 and ZO-1 staining revealed alterations in the strial microvasculature and tight-junction organization, respectively, with preliminary evidence of altered ZO-1 organization in the D-gal group and a potential protective effect of quercetin. *In vitro*, dose-response viability tests on HUVECs allowed us to identify suitable concentrations for subsequent senescence and permeability experiments.
Conclusions
By integrating functional (ABR), quantitative morphological, and molecular approaches, this study investigates the potential contribution of microvascular dysfunction to ARHL beyond downstream homeostatic alterations involving Kir4.1. The *in vivo* associations between auditory decline, strial remodeling, and altered BLB tight-junction organization provide a framework for investigating the relationship between cochlear vascular dysfunction and auditory impairment.
The ongoing *in vitro* HUVEC model will further investigate the contribution of endothelial senescence to barrier dysfunction. Ultimately, this integrated approach will help determine whether quercetin may exert senomorphic and vasoprotective effects that contribute to the preservation of cochlear vascular integrity during accelerated aging.

Longitudinal dynamics of substance use and anxiety and depression symptoms among young people in deprived urban areas of Latin America

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Background
Substance use frequently co-occurs with anxiety and depression during adolescence and early adulthood, but cross-sectional associations cannot establish how these variables influence one another over time. Longitudinal evidence from socioeconomically deprived urban settings in Latin America remains limited. This study examined between-person and within-person associations between substance use and internalizing symptoms over 24 months.
Material and Methods
Participants aged 15–16 or 20–24 years were recruited in deprived neighbourhoods of Buenos Aires, Bogotá, and Lima within the OLA project and assessed at baseline, 12 months, and 24 months. Anxiety and depressive symptoms were measured with the GAD-7 and PHQ-8, respectively, and substance use with the ASSIST. A random-intercept cross-lagged panel model separated stable between-person differences from within-person fluctuations. Full information maximum likelihood estimation was used to include participants with incomplete follow-up data.
Results
The baseline sample comprised 2,402 participants. Attrition was approximately 48% at 12 months and 58% at 24 months. At the between-person level, higher average substance use across the study period was associated with higher depressive symptoms ($r = .262$), higher anxiety symptoms ($r = .211$), and lower quality of life ($r = -.305$). Within-person analyses identified one statistically significant cross-lagged path: a higher-than-usual level of substance use at baseline predicted slightly lower-than-expected depressive symptoms at 12 months (beta = -.126, $p < .05$). No prospective within-person effects were found between substance use and anxiety, and neither age group nor gender moderated the associations.
Conclusions
The co-occurrence of substance use with anxiety and depressive symptoms was explained mainly by stable differences between individuals, with limited evidence of prospective within-person effects. The isolated negative path from substance use to subsequent depressive symptoms should be interpreted cautiously and does not indicate a beneficial effect of substance use. These findings illustrate the value of separating stable vulnerability profiles from temporary fluctuations when analysing longitudinal mental health data.

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PhD COURSE "TRANSLATIONAL SPECIALISTIC MEDICINE «G.B. MORGAGNI»"

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THE ROLE OF NON-HLA ANTIBODIES IN SOLID ORGAN TRANSPLANTATION

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Background
Kidney transplantation is the treatment of choice for children with end-stage kidney failure; however, donor organ availability remains limited, and many grafts fail within 20 years. A major cause of graft loss after the first year is antibody-mediated rejection, mainly driven by anti-HLA antibodies. Recently, non-HLA antibodies targeting molecules such as angiotensin II type 1 receptor (AT1R) and endothelin receptor type A (ETAR) have also been identified as potential contributors to graft injury. The biological effects of non-HLA antibodies were investigated in endothelial cells to characterize antibody-mediated alterations in endothelial responses.

Material and Methods
Conditionally immortalized human glomerular endothelial cells (ciGEnCs) were used as an in vitro model of endothelial cells, which represent a key cellular target of circulating recipient antibodies.
AT1R and ETAR expression was assessed by immunofluorescence staining and fluorescence microscopy.
Cells were exposed for 48 hours to purified patient-derived IgG (2 mg/mL) selected based on the levels of anti-AT1R and anti-ETAR antibodies and their previously demonstrated AT1R- and ETAR-activating properties. Angiotensin II (1 μM) and endothelin-1 (100 nM) were used as positive controls, either alone or in combination with their corresponding receptor antagonists, valsartan (10 μM) and sitaxentan (10 μM), respectively.
The molecular effects of patient-derived IgG on glomerular endothelial cells were investigated by quantitative reverse-transcription PCR (qRT-PCR), using a panel of genes associated with endothelial inflammation and pro-thrombotic activation, including E-selectin, P-selectin, ICAM-1, VCAM-1, von Willebrand factor, and tissue factor.

Results
Immunofluorescence staining confirmed the expression of both AT1R and ETAR supporting the suitability of ciGEnCs for subsequent functional studies.
qRT-PCR analysis showed that patient-derived IgG markedly modulated VCAM-1 and Tissue Factor mRNA levels following 48 hoursof exposure. The magnitude of these effects was greater than that observed following stimulation with the natural agonists angiotensin II and endothelin-1. Treatment with AT1R and ETAR antagonists partially reduced the observed effects, supporting the involvement of receptor-mediated mechanisms.

Conclusions
These findings support the use of ciGEnCs as an in vitro model for investigating the functional effects of anti-AT1R and anti-ETAR antibodies.
Patient-derived IgG modulated the expression of genes associated with inflammatory and pro-coagulant endothelial responses, potentially through receptor-dependent mechanisms. Further studies are ongoing to investigate the effects of these non-HLA antibodies on the immune cell compartment.

Assessment of Burnout and Associated Factors Among Workers in Residential Geriatric-Care Facilities in Ragusa, Italy: A Cross-Sectional Study Protocol

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Background
Burnout is a critical occupational health issue among workers providing geriatric care, significantly compromising worker well-being and the overall quality of care delivered to older adults. Despite its clinical importance, empirical evidence regarding burnout and its determinants among residential geriatric-care staff in Ragusa, Italy, remains sparse. This proposed study aims to assess the levels of personal, work-related, and client-related burnout and identify associated sociodemographic, occupational, interpersonal, and organizational factors among workers in residential geriatric-care facilities in this municipality.

Material and Methods
A quantitative cross-sectional study will be conducted across participating residential geriatric-care facilities in the Municipality of Ragusa. Given the defined size of the target workforce, a census sampling approach will be used, inviting all eligible care staff to participate. Data will be collected via a structured questionnaire evaluating individual, work-related, and organizational variables. Burnout dimensions will be measured continuously using the validated Copenhagen Burnout Inventory (CBI). Descriptive statistics will characterize burnout distribution, and multivariable regression modeling, accounting for facility-level clustering, will be performed to identify independent predictors for each CBI subscale.

Results
Expected Outcomes: As data collection has not yet commenced, this study expects to generate primary empirical data on the prevalence and severity of personal, work-related, and client-related burnout within this regional workforce. The statistical analysis is anticipated to identify research-specific individual, occupational, resident-related, and structural factors that significantly predict higher burnout scores, while highlighting facility-level variations in occupational stress.

Conclusions
This research protocol addresses a vital regional gap in occupational health literature for residential geriatric care in Southern Italy. Upon completion, the anticipated findings will offer actionable, evidence-based insights to guide workplace interventions and administrative policies aimed at mitigating caregiver burnout, improving staff retention, and elevating the quality of long-term care for older adults.

DESCRIPTIVE AND INFERENTIAL CHARACTERIZATION OF MSPAS SIGSA 3 AND SIGSA 4 RECORDS (2023–2024): diagnostic evidence and input for the IHSN case study in Sololá

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BACKGROUND: The availability of nominal health-care records provides an opportunity to generate evidence on service utilization, continuity, and organization. The SIGSA 3 and SIGSA 4 databases of the Ministry of Public Health and Social Assistance (MSPAS), corresponding to 2023–2024, were analyzed as antecedent diagnostic evidence to characterize patterns of care and provide empirical information for the design of the case study on implementation of the Integrated Health Services Networks (IHSN/RISS) model for people with diabetes mellitus and/or arterial hypertension in Sololá. The original databases were structured as service events; therefore, data cleaning, standardization, and longitudinal linkage were required to construct an analytical cohort.
MATERIALS AND METHODS: An observational, retrospective study combining descriptive and inferential analyses was conducted using SIGSA 3 and SIGSA 4 outpatient consultation records from 2023–2024. Twenty-eight variables were reviewed, and a unique patient identifier was constructed. In total, 593,943 patients and 2,751,752 consultations were identified and linked; 95.2% of patients were linked with high confidence, and all consultations were assigned to a patient identifier. The analysis estimated indicators of service utilization, longitudinal follow-up, continuity of care, intervals between consultations, productivity, pharmaceutical management, epidemiological profile, and territorial distribution. Continuity of care was defined as at least one consultation in 2023 and at least one consultation in 2024. Comparisons across the 29 DDRISS used the Kruskal–Wallis test for the number of consultations per patient and for age, followed by Dunn's post hoc tests when appropriate. Pearson's chi-square test and Cramer's V were used for categorical variables. Statistical significance was set at $\alpha = 0.05$. This analysis provides antecedent diagnostic evidence rather than a comprehensive evaluation or complete baseline measurement of IHSN implementation.
RESULTS: The cohort was predominantly female (72.9%), and health-care utilization was concentrated among people aged 40–79 years. The mean number of consultations per patient was 4.63, with a median of 2; 47.3% of patients had only one consultation. Overall, 128,739 patients (21.7%) received care in both years. Among patients with at least two consultations, 33.0% had at least one interval of six months or longer, and 8.9% had an interval of 12 months or longer. Essential hypertension and unspecified diabetes mellitus without complications accounted for 43.45% and 35.37% of consultations, respectively. In Sololá, 34.16% of consultations had no medication recorded, and 2.38% documented a stockout event. Comparisons across DDRISS showed statistically significant differences in utilization, age, sex, medication availability, and diagnostic complexity ($p < 0.001$). The effect sizes were small for both medication availability (Cramer's $V = 0.279$) and diagnostic complexity (Cramer's $V = 0.142$).
CONCLUSIONS: The characterization of longitudinal patterns and statistically significant differences across health-service territories provides an empirical basis for defining relevant indicators, identifying priorities for further investigation, and informing the design of the doctoral case study. The observed quantitative patterns cannot, by themselves, explain the organizational, managerial, governance, resource-related, territorial, and contextual mechanisms underlying differences in the implementation of Integrated Health Services Networks (IHSN). Findings concerning continuity, prolonged intervals between consultations, medication management, and territorial variation provide antecedent quantitative evidence for selecting indicators and informing qualitative sampling for the Sololá case study.

Simulation of Labor involving birth partners to reduce fear of childbirth and improve childbirth self-efficacy among primiparous women: a randomized controlled trial protocol.

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Background:
Antenatal education is a key component of prenatal care and may influence women's fear of childbirth, anxiety, perceived competence, and overall childbirth experience. However, current antenatal education programs vary widely in content and delivery and often provide limited experiential or simulation-based training. This study aims to evaluate whether a structured labor simulation, integrated into antenatal education, can reduce fear of birth, improve maternal preparedness, self-efficacy, awareness, and childbirth experience.
Material and Methods:
A randomized controlled trial will be conducted among 72 primiparous women with low-risk pregnancies. After completing standardized questionnaires assessing fear of childbirth, childbirth-related anxiety, self-efficacy, pregnancy-related anxiety, general anxiety, personality traits, and childbirth preferences, participants will be allocated randomly to either an intervention group or a control group, with a minimum of 36 women in each group. Randomization will be stratified according to baseline fear of childbirth, measured using the Wijma Delivery Expectancy/Experience Questionnaire version A (WDEQ-A). All participants will attend an antenatal education course of their choice. In addition, women in the intervention group will participate in a structured, personalized labor simulation conducted in small groups and involving birth partners. Before birth, both groups will complete the same standardized questionnaires administered prior to the antenatal education course (control group) or the simulation (intervention group). In the postpartum period, participants will be interviewed to collect birth narratives and assess perceived childbirth experience, maternal competence, awareness, and any adverse events. Data will be integrated with clinical record review. Women will also complete a questionnaire evaluating the perceived usefulness of the simulation and the antenatal course.
Discussion:
This trial aims to bridge psychological preparedness, partner activation, and childbirth self-efficacy in a real-world antenatal context, assessing whether a labor simulation can reduce fear of childbirth, increase perceived self-efficacy and enhance antenatal preparation beyond standard education. The intervention may also promote greater maternal awareness, active participation in decision-making, improved coping strategies, and a more positive childbirth experience. Potential benefits on pain management and selected obstetric outcomes will also be explored. If effective, this approach could support the integration of simulation-based methods into routine antenatal education programs

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Macrophage M1/M2 polarisation in interstitial lung diseases: an immunohistochemical evaluation

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Background:
The interstitium in interstitial lung diseases (ILD) is characterised by extracellular matrix accumulation and cytoarchitectural disruption. Macrophages recruited in response to epithelial alveolar damage are thought to play a central role in the fibrotic process. Although macrophage activation is highly heterogeneous, these cells are traditionally described into M1-like pro-inflammatory and M2-like pro-fibrotic phenotype associated with tissue remodeling and fibrotic responses. Based on previous evaluations of our research group on cultured macrophages, we evaluated, by immunohistochemistry, the M1/M2 polarization on macrophages in clinically collected bronchoalveolar lavages (BAL) samples from ILD patients.
Material and Methods:
BAL samples were obtained from 6 patients with sarcoidosis, 2 patients with idiopathic pulmonary fibrosis (IPF) and 1 patient with hypersensitivity pneumonitis (HP). Cytological slides were prepared using Cytospin 4 (Thermo Scientific) at 460 rpm for 6 min Macrophages were identified, by immunohistochemistry, as CD68+ cells, with M1 and M2 phenotype identified by HLA-DR/iNOS and CD206 expression, respectively. At least 20 non-consecutive high-power fields (HPF) and at least 100 macrophages were evaluated for each condition. Results were expressed as a percentage of HLA-DR+, iNOS+ or CD206+ among CD68+ macrophages. Negative controls for nonspecific binding were processed, either omitting the primary antibody or using isotype IgG, and revealed no signal. All analyses were performed with a Leica light microscope and a video recorder linked to a computerised image analysis system (Leica LAS w3.8). Statistical analyses were performed with GraphPad Prism v.9.5.1, with p-value < 0.05 considered statistically significant.
Results:
CD68+ macrophages were higher in sarcoidosis patients (mean ± sd: 82.27 ± 9.6% CD68+ cells,) compared to HP patient (68% CD68+ cells) and IPF patients (68.5 ± 5.2% CD68+ cells). Among CD68+ macrophages, CD206+ cells (M2- like phenotype) were also higher in sarcoidosis patients (72.8 ± 20.6%) compared to HP patient (47.8 %) and IPF (41.9 ± 47.4 %). iNOS+ macrophages (M1-like phenotype) were more frequent in sarcoidosis patients (41.17 ± 40.16% iNOS+ cells) than in IPF patients (1.35 ± 1.9 % iNOS+ cells) while no iNOS+ macrophages were detected in the 1 patient with HP (0% iNOS+ cells). HLA-DR expression (another marker of M1-like macrophages) was higher in IPF patients (90.6 ± 2.4% HLA-DR+ cells) compared to Sarcoidosis patients (88.2 ± 8.4 % HLA-DR+ cells) and HP patient (61.8 % HLA-DR+ cells).
Conclusions:
Although preliminary, these findings open an interesting landscape in the macrophage polarisation of interstitial lung diseases. Sarcoidosis patients presented a higher presence of macrophages without a clear M1/M2 polarisation trend, while IPF patients presented a more directed M2 polarisation. On the other hand, HP had a low expression of both macrophages and M1/M2 markers. Larger populations and evaluation of clinical associations with this research are warranted.

CLINICAL AND SEROLOGICAL FACTORS INFLUENCING OUTCOMES IN PLEURAL INFECTIONS

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Background:
Infectious pleural effusion (IPE) is a globally increasingly prevalent condition with a highly heterogeneous clinical course, ranging from mild infection to fatal illness. Treatment remains incompletely standardized across current guidelines, and reliable predictors of therapeutic response and overall outcomes are lacking.
Material and Methods:
We conducted a retrospective, single-center study including patients requiring chest drainage for IPE between 2023 and 2026. Clinical outcomes were categorized as "Standard" (recovery via drainage ± intrapleural fibrinolytics) or "Surgical" (requiring VATS or thoracotomy). Baseline thoracic ultrasound (TUS) findings were classified into three patterns: (1) Anechoic, (2) Septated, or (3) Hyperechoic. Clinical and serological markers were correlated with TUS patterns and final outcomes.
Results:
Of the 38 included patients, 10 (26%) required surgical intervention. The Surgical cohort was significantly younger than the Standard group (median 52 vs. 76 years; p=0.007). Upon admission, the Surgical cohort exhibited significantly higher systemic inflammation, with a median CRP of 232 vs. 146 mg/L (p=0.04). TUS analysis revealed that 100% of Surgical cases presented with a septated pattern, compared to 64% in the Standard group (p=0.04). Microbiological isolates from the pleural effusion, RAPID scores, and chest drain sizes did not differ significantly between the two cohorts.
Conclusions:
A septated TUS pattern combined with a high systemic inflammatory index (CRP >200 mg/L) at presentation may predict medical management failure. Identifying this "septated-hyperinflammatory" phenotype should trigger early surgical consultation to reduce delays in definitive care and optimize clinical outcomes.

A digital-pathology pipeline for interstitial lung disease: integrating morphometry, pulmonary function and quantitative imaging

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Background.
Interstitial lung diseases (ILDs) remain a diagnostic challenge in which histopathology retains a decisive role whenever the clinical–radiological pathway does not converge. Expert semiquantitative scoring of morphological features shows moderate inter-observer agreement, and quantitative digital-pathology readouts on whole-slide images are a natural methodological extension. A structured pipeline coupling quantitative histological descriptors with pulmonary function and quantitative imaging in ILD is not yet established.
Aim.
To develop a digital-pathology workflow supporting the morphological characterisation of ILD, contributing reproducible quantitative descriptors to the diagnostic process, and to explore its integration with pulmonary function and quantitative imaging.
Material and Methods.
Retrospective analysis. Whole-slide images from ILD surgical biopsies and explanted lungs are analysed with an open-source digital-pathology and AI workflow, producing quantitative readouts of morphological parameters relevant to the differential diagnosis of ILD, including bridging fibrosis, density of lymphoid aggregates and fibroblastic foci. Normal parenchyma from lung donors, and non-neoplastic parenchyma from tumour-bearing resections, serve as reference tissue. Alveolar-space enlargement is assessed as a separate module, using an in-house morphometric approach previously developed in a porcine transplantation-related cohort, with COPD/emphysematous explants as morphological comparator. Fibroblastic foci are further characterised by a focused immunohistochemical panel (tenascin-C, p16INK4a), complemented by exploratory markers of retrotransposon derepression and type-I interferon response (LINE-1 ORF1p, LINE-1 ORF2p, MxA/ISG15). Data integration is performed at patient level: quantitative histological readouts, immunohistochemical scores, pulmonary function tests (FVC, DLCO), 6-minute walk test, HRCT-based radiomics and 3D lung-volume reconstruction are consolidated in a single per-patient matrix and analysed jointly through correlation and multivariable modelling, with the digital histopathological signature as the anchor variable.
Expected Results.
A reproducible, WSI-based morphometric and immunophenotypic readout of ILD-relevant parameters, coherent with independent functional and quantitative imaging descriptors at patient level.
Conclusions.
Digital-pathology quantitative descriptors, coupled with a focused immunohistochemical characterisation of fibroblastic foci and exploratory retrotransposon/interferon markers, may complement expert morphological review in ILD as an adjunct to multidisciplinary interpretation.

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