UNIVERSITÀ DEGLI STUDI DI PADOVA

University of Padua
PhD Courses
Medical and Biomedical Sciences

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

Wednesday, September 3, 2025

Old University
Aula Magna - Archivio Antico
Palazzo del Bo
Via 8 Febbraio, 2 - 35122 Padova

Organized by
Prof. Dario Gregori
Department of Cardiac Thoracic Vascular Sciences and Public Health

In collaboration with:
Alessandra Cervellin
Department of Cardiac Thoracic Vascular Sciences And
Public Health
Tel 049/8272288 – Fax 049/8272294
e-mail: alessandra.cervellin@unipd.it

A.R.C.A.
Associazione Ricerche Cardiopatie Aritmiche
Via A. Gabelli, 86
35121 Padova
sito web: http://www.arca-cuore.it/

INDEX

PhD COURSE
"DEVELOPMENTAL MEDICINE AND HEALTH PLANNING SCIENCES"

PhD COURSE
"MOLECULAR MEDICINE"

PhD COURSE "ARTERIAL HYPERTENSION AND VASCULAR BIOLOGY"

COORDINATOR: PROF. TERESA MARIA SECCIA

Investigating the Role of NFAT in Mediating Endothelin-1 Induced Epithelial to Mesenchymal Transition in Human Kidney Proximal Tubular Epithelial Cells (HK2)

Ph.D. Student: Mustafa ATTA UL
TUTOR: Prof. Teresa Maria SECCIA – CO-TUTOR: Dr. Brasilina CAROCCIA
Ph.D. Course in Arterial Hypertension and Vascular Biology (ARHYVAB)

Background: Epithelial to mesenchymal transition (EMT) is a biological process characterized by the down regulation of epithelial markers such as E-cadherin and up regulation of mesenchymal markers like α-smooth muscle actin (α-SMA), resulting in enhanced cell motility and fibrosis. Endothelin-1 (ET-1) elevates intracellular calcium levels and activates multiple signaling pathways that promote EMT in renal cells. The calcium-dependent transcription factor NFAT5 may serve as a key mediator linking ET-1 signaling to the regulation of EMT-associated genes.

Materials and Methods: Human proximal tubular epithelial (HK2) cells were cultured to approximately 70% confluence in 6-well plates and serum-starved (1% FBS) for 48 hours to achieve synchronization. Cells were treated with 100 nM ET-1 or left untreated as controls, then harvested at 6, 24, 48, 72, and 96 hours post-treatment. Total RNA was extracted and reverse-transcribed to cDNA for quantitative real-time PCR (qRT-PCR) analysis targeting NFAT5, E-cadherin (epithelial marker), and α-SMA (mesenchymal marker), using GAPDH and PBGD as housekeeping controls. Subsequent phases include protein analysis via Western blot and immunofluorescence to assess NFAT5 nuclear translocation and EMT marker localization. Functional assays, such as wound healing and migration, will evaluate EMT-associated phenotypic changes. NFAT activity will be inhibited pharmacologically (e.g., VIVIT peptide) to elucidate its role in ET-1–induced EMT. Data will be analyzed to define the relationship between ET-1 treatment, NFAT5 activation, and EMT progression.

Results: Preliminary qRT-PCR analysis of HK2 cells treated with ET-1 at 6, 24, 48, 72, and 96 hours showed variable expression patterns of EMT markers and NFAT5. While some time points exhibited expected trends, such as decreased E-cadherin and increased α-SMA and NFAT5 levels, other time points displayed inconsistent or opposite expression changes. These mixed results suggest a complex, time-dependent regulation of EMT by ET-1 and NFAT5, warranting further detailed investigation. Additional protein-level analyses and functional assays are underway to clarify these observations.

Conclusions: These initial findings highlight a potential role for NFAT5 in ET-1–induced EMT in renal tubular cells, though the temporal dynamics require further elucidation. Ongoing studies focusing on protein expression, NFAT5 nuclear localization, and functional assays will provide a more comprehensive understanding of NFAT5's involvement. Ultimately, this research aims to identify novel therapeutic targets to prevent or slow renal fibrosis and chronic kidney disease progression.

Modulation of Neurogenesis Biomarkers by Repetitive Transcranial Magnetic Stimulation in Post-Stroke Patients: Preliminary Findings

Ph.D. Student: Dr. Nicoletta Giuseppa CARACCIOLO
TUTOR: Prof. Claudio LETIZIA – CO-TUTOR: Prof. Danilo TONI
Ph.D. Course in Arterial Hypertension and Vascular Biology (ARHYVAB)

Background
Stroke is a leading cause of long-term neurological disability and enhancing post-stroke recovery remains a major therapeutic challenge.
Ischemic stroke acts as a trigger for the proliferation, migration toward the ischemic lesion, and differentiation into mature neurons of neural progenitor cells (NPCs).
Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique known to induce brain plasticity.
The miRNA 17~92 cluster consists of non-coding RNA molecules involved in brain development; this effect is thought to be mainly mediated by miR-25, which is highly expressed in the post-ischemic brain and overexpressed following rTMS in mice. Other factors that play a role in neuroplasticity include: brain-derived neurotrophic factor (BDNF), Netrin-1, and Semaphorin-3A.
This study aimed to evaluate biomarkers related to neurogenesis and axonogenesis — namely semaphorin-3A (Sema3A), Netrin-1, miR-25, miR-106, and BDNF — in plasma samples from post-stroke patients undergoing rTMS.

Material and Methods
Adults over 18 years of age with a diagnosis of ischemic stroke were enrolled. Patients were randomly assigned to one of two groups: 7-days of active rTMS or sham stimulation.
Biomarker levels were measured at baseline (T0), immediately before the stimulation period (T7), and after 7 days of stimulation (T14).

Results: a total of 16 patients were enrolled (10 males, mean age 69.4 ±10.1 years).
Preliminary data showed that rTMS was associated with a significant increase in miR-25, miR-106, and Sema3A levels compared to sham. Changes in Netrin-1 and BDNF were less pronounced.
These variations suggest a stimulation-induced modulation of pathways involved in neuroplasticity and axonal remodeling.

Conclusions The observed molecular changes in our preliminary data suggest that rTMS may influence key mediators of post-stroke recovery, supporting its potential role in promoting neurogenesis and axonogenesis. Futher analysis on a larger patient samples is needed to confirm the role of the selected biomarkers, as well as the potiential of rTMS in modulating neuroplasticity after stroke.

Exploring the Molecular Mechanism of Primary Aldosteronism: A Study of Secondary Hypertension

Ph.D. Student: Dr. Narjis KHATOON
TUTOR: Prof. Giulio CEOLOTTO – CO-TUTOR: Prof. Brasilina CAROCCIA
Ph.D. Course in Arterial Hypertension and Vascular Biology (ARHYVAB)

Background
Primary aldosteronism (PA) is the most common form of secondary hypertension and is frequently caused by aldosterone-producing adenomas (APAs). These benign adrenal tumors often carry somatic mutations in ion channel-related genes such as **KCNJ5**, **CLCN2**, **CACNA1D**. These mutations lead to dysregulated intracellular calcium homeostasis inducing the overexpression of **the aldosterone synthase (CYP11B2) and excessive aldosterone production**. CD56 also known as neural adhesion molecule (NCAM1), is a membrane marker of aldosterone-producing cells in the adrenal zona glomerulosa and APAs. Previous studies have demonstrated that **CD56 positive cells** express significantly higher levels of **CYP11B2** and produce more aldosterone as compared to CD56 negative cells. However, limited data exist on their mutational landscape, co-expression with steroidogenic enzymes, and contribution to tumor heterogeneity.
Methodology
APA tissue samples were collected from patients and were processed. CD56 positive and CD56 negative cells were isolated using immunomagnetic beads method. DNA was extracted from the APA tissues, CD56 positive cells and CD56 negative cells for targeted next-generation sequencing (NGS) to assess mutational status, particularly focusing on the **KCNJ5** gene and other aldosterone-driver mutations. Immunohistochemistry (IHC) was performed to assess the localization and co-expression of **CD56**, **CYP11B1**, and **CYP11B2** in tissue sections. The presence of the **KCNJ5** mutation in CD56 positive and CD56 negative cells was also studied.
Results
We confirmed the presence of pathogenic **KCNJ5 mutations** in APA tissues and demonstrated that this mutation is also detectable in **CD56 positive cells**, with a higher allelic frequency than in CD56 negative cells. This suggests that CD56 positive cells are enriched for the mutational burden linked to aldosterone overproduction. Additionally, we observed **co-expression of CD56 with both CYP11B2 and CYP11B1** in the same histological regions, indicating potential overlap in cellular function. Early immunohistochemical and genetic findings support a distinct molecular profile for CD56 positive cells. Further analyses with additional APA samples are ongoing to confirm these trends and to perform transcriptomic comparisons.
Conclusions
Our findings support the hypothesis that CD56 positive cells represent the main aldosterone-producing population within APAs, exhibiting a higher mutational burden especially in KCNJ5 and co-expressing CYP11B2. The identification of novel mutations and differential expression patterns highlights the cellular heterogeneity of APAs and its role in disease pathophysiology. The integration of IHC, mutational, and transcriptomic data will provide deeper insights into aldosterone biosynthesis and may guide future targeted diagnostics or therapies.

Organ damage and novel molecular biomarkers in patients affected by atrial fibrillation

Ph.D. Student: Dr. Luca MARINO
TUTOR: Prof. Claudio LETIZIA – CO-TUTOR: Prof. Luigi PETRAMALA
Ph.D. Course in Arterial Hypertension and Vascular Biology (ARHYVAB)

Background
Atrial fibrillation (AF) is the most common cardiac arrhythmia with a significant impact on the health system that is progressively increasing due to the aging of the world population, progressively higher prevalence of obesity, and improved attention to its early detection. AF is indeed associated with several clinical adverse events, such as acute heart failure, ischaemic stroke, ischaemic heart disease, thromboembolic events, all presenting with high rates of hospitalization, morbidity and mortality.
In recent years many efforts have been made to fully understand the pathophysiology of AF and its complications. Several epidemiological studies have been conducted to early identification of AF, and pay more attention to AF risk factors, including arterial hypertension, dyslipidemia, type 2 diabetes mellitus, obstructive sleep apnea syndrome (OSAS), physical inactivity, and alcohol excess. Furthermore, while the traditional view attributes the primary cause of AF onset to myocardial remodelling (i.e. atrial dilatation, ventricular hypertrophy), cutting-edge research has increasingly highlighted a pivotal role for systemic and myocardial inflammation in the etiology of this arrhythmia.
Material and Methods
Since May 2024 to April 2025, at the Emergency Medicine Department - Azienda Ospedaliera Universitaria Policlinico Umberto I (Rome, Italy) 73 subjects (35 males, 38 females) with atrial fibrillation, were enrolled. 38 subjects presented new-onset AF while 35 patients had chronic AF. All patients had been admitted to the ED for diseases on symptoms not directly related to atrial fibrillation. Plasma concentrations of interferon-γ (IFN-γ), thromboxane-B2 and TNF-α were measured.
Results
Demographic, anthropometric and biochemical parameters were obtained for all the enrolled patients.
Furthermore, the prevalence of cardiac remodeling in the two groups was evaluated through ultrasonographic indicators including the reduction of the left ventricle ejection fraction, the atrial enlargement and the eventual pericardial effusion. Regarding the inflammatory biomarkers, the new-onset AF group showed higher levels of IFN-γ and thromboxane-B2 compared to chronic AF group while no differences were observed on TNFα and other coagulation parameters. The new-onset AF group showed higher prevalence of stroke and palpitations compared to chronic AF.
On the other hand, new-onset AF group exhibited higher prevalence of dyslipidemia respect to chronic AF group and usage of antiplatelets medication. The chronic AF patients showed higher prevalence of chronic kidney disease, treatment with diuretics, β-blockers (68%), uric acid-lowering medications and oral anti-coagulants with respect to the new-onset AF.
Conclusions
The results showed that the higher concentration of plasma IFN-γ and TXB_2 - together with the other markers such as CRP, WBC and PLT- evidences the impact of systemic inflammation as substrate for the onset of AF. And confirms the new-onset AF as a significant condition of high-risk cardiovascular complications. Further markers will be investigated to elucidate the molecular mechanism of organ damage associated to Af.

Non-Invasive Evaluation of Chorioretinal Microcirculation with Advanced Imaging in Primary Aldosteronism

Ph.D. Student: Dr. Federico Bernardo ROSSI
TUTOR: Prof. Teresa Maria SECCIA– CO-TUTOR: Prof. Michel PAQUES
Ph.D. Course in Arterial Hypertension and Vascular Biology (ARHYVAB)

Background

Microvascular Remodelling and capillary rarefaction represent the earliest detectable alterations in human arterial hypertension (HT), moreover they anticipate the development of Hypertension-Mediated-Organ-Damage (HMOD) and hold prognostic value for cardiovascular events and mortality. Newly developed advanced non-invasive imaging technologies allow the direct quantitative assessment of the retinal microcirculation in-vivo, in a reproducible and depth-resolved manner. Primary aldosteronism (PA), the most common cause of secondary hypertension, is associated with excess cardiovascular damage and, when unilateral, is amenable to surgical cure.
Our objective was to determine whether the chorioretinal microvasculature is also affected by this condition.

Material and Methods

Optical Coherence Tomography (OCT) and OCT-Angiography (OCT-A) scans of the superficial and deep retinal macular plexi were obtained in PA patients at diagnosis, in wash-out from RAAS-confounding drugs (WO), and again during mineralocorticoid receptor antagonist (MRA) therapy and 4-6 months after surgical cure (PS).
The images were analysed to measure choroidal thickness and, upon automated ImageJ processing, to compute indexes of total vascular area density (VAD) and length fraction (VLF), a surrogate for the number of perfused vessels.

Results

We enrolled 11 patients with PA (51±10 years; 27% Female); of these, 10 had unilateral PA and underwent curative surgery ($p<0.05$ for decrease in Blood Pressure and Aldosterone-Renin-Ratio, increase in serum K^+). We found that medical therapy with MRA had no effect on any of the investigated parameters. At variance, curative adrenalectomy determined a reduction of VAD in both the superficial and deep plexus (-6.4% retinal area, $p=0.017$ and -3.5%, $p=0.023$, respectively) and of VLF in the superficial plexus (-2.2%, $p=0.033$). Moreover, choroidal thickness (on average: -21μm, $p=0.003$ at 2-way ANOVA) decreased after adrenalectomy.

Conclusions

OCT and OCT-A allow to accurately track changes in choroid- retinal vascularization,
highlighting a decrease of retinal microvascular density and choroidal thickness in patients with unilateral PA, who were cured from PA. Further studies are required to establish whether these biomarkers could improve risk stratification and predict response to therapy in PA patients.

PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "ENDOCRINE-METABOLIC SCIENCES AND GENDER MEDICINE"

PROPOSAL FOR A NEW DIAGNOSTIC-THERAPEUTIC CLASSIFICATION OF MALE FACTOR INFERTILITY: PRELIMINARY ANALYSIS

Ph.D. Student: Dr. Andrea GRAZIANI
TUTOR: Prof. Alberto FERLIN – CO-TUTOR: Dott. Giuseppe GRANDE
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Endocrine-Metabolic Sciences and Gender Medicine"

Background
Couple infertility is a clinical condition defined as the inability to conceive after at least 12 months of regular and unprotected sexual intercourse. It is estimated that the male factor infertility (MFI) is present, alone or in combination with the female factor, in about 2/3 of cases of couple infertility. The complete diagnostic pathway of MFI (medical history, physical examination, hormonal tests, scrotal ultrasound, complete semen analysis, semen microbiological evaluation, transrectal prostate ultrasound and, when needed, genetic tests and testicular cytology) allows to categorize the causes of MFI into specific categories that help the clinician to orient towards the most appropriate therapeutic choice. These categories have recently been proposed by a group of authors (new diagnostic-therapeutic classification of MFI). The aim of the study was to carry out a preliminary evaluation of this new classification of MFI in a large cohort of infertile patients referring to the Unit of Andrology and Reproductive Medicine (University Hospital of Padua).
Material and Methods
We therefore retrospectively evaluated infertile male patients attending our Unit, collecting data in order to categorize the patients in the most appropriate category of MFI. For this preliminary analysis, we excluded patients with the presence of known female problems, enrolling only patients with known isolated MFI as a main cause of couple infertility.
Results
We eventually enrolled 500 male patients (mean age 37.5 years, mean age of the partner 35.5 years, mean time of couple infertility 2.5 years).
The enrolled patients were divided into the diagnostic-therapeutic categories of MFI as follows: IA (semen infection): 72/500 (14,4%); IB (semen inflammation): 89/500 (17,8%); II (congenital or acquired total obstruction): 19/500 (3,8%); IIIA (primary testicular disease with increased FSH concentrations): 143/500 (28,6%); IIIB (primary testicular disease with normal FSH concentrations): 116/500 (23,2%); IV (hypogonadotropic hypogonadism) 13/500 (2,6%); V (idiopathic seminal alterations): 14/500 (2,8%); VI (idiopathic couple infertility with normal semen analysis): 21/500 (4,2%). As for clinically relevant varicocele, considered as a cause itself (namely, category VII), it was present in 13/500 (2,6%) patients.
Conclusions
This preliminary evaluation showed that it is almost always possible to find a cause for MFI; this data collection, however, is affected by the fact that it refers to a case history of patients attending a third-level referral Unit, justifying the high prevalence of patients in category IIIA. This new diagnostic-therapeutic categorization of MFI is simple and rapid, also providing an indication of the possible therapeutic proposal for the male infertile patient.

Application of the PANOMEN-3 grade to specific pituitary adenoma phenotypes

Ph.D. Student: Dr. Alessandro MONDIN
TUTOR: Prof. Filippo CECCATO – CO-TUTOR: Dr. Mattia BARBOT
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Endocrine-Metabolic Sciences and Gender Medicine"

Background: We already assessed the role of the newly developed Pituitary Society grading system (PANOMEN-3) in a retrospective cohort of pituitary adenomas (PAs) of any type. Patients were followed at our center over a long follow-up period (median 100 months [57.5; 154]). Grade could not predict the outcome of initial surgery, but was related to the risk of relapse after surgical success and to the need for further treatments following surgical failure. Moreover, grade 3 disease predicted persisting disease on the long-term after multimodal treatments. Early downgrade in medically treated cases predicted long-term biochemical response in secreting PAs and prevented additional interventions. Following these promising results, we analysed the potential applications of the PANOMEN-3 grade in the setting of specific PA phenotypes.

Material and Methods: We reassessed the retrospective cohort of 401 patients with PAs (120 ACTH-secreting PAs, 78 GH-secreting PAs, 9 TSH-secreting PAs, 111 prolactinomas, 83 non-functioning PAs) followed at our center over the last two decades focusing on specific PA phenotypes.

Results: Considering Cushing's Disease (CD), first-line pituitary surgery achieved disease remission at one year in 65% (73/112) of cases; urinary free cortisol normalized with preoperative medical treatment in 15 patients. Considering a median follow-up of 71 months [40; 105], 29 out of 67 (43%) cured cases recurred: a higher baseline grade and uncontrolled hypercortisolism before surgery increased the risk of recurrence ($p<0.01$ and $p=0.04$, respectively). Higher post-operative grade in CD trended to further interventions ($p=0.08$). Contrarily to the whole cohort analysis, initial grade could predict surgical outcome in non-functioning PAs (grade 2 OR 0.13, 95%CI [0.03; 0.49], $p < 0.01$ and grade 3 OR 0.18, 95%CI [0.04; 0.75], $p < 0.01$, compared to grade 1) and acromegaly (grade 3 OR 0.21, 95%CI [0.05; 0.83], $p = 0.04$, compared to grade 2). Early downgrade in medically treated prolactinomas favoured long-term biochemical control. CD cases were ad more frequently referred to surgery ab initio and were more prone to receive additional interventions in case of persistent disease compared to acromegaly and prolactinomas; on the long term they also presented higher remission rate (54% for CD, 29% for acromegaly, 26% for prolactinomas).

Conclusions: The PANOMEN-3 grade could be useful even in the setting of specific PA phenotypes. Especially for CD, a high initial grade could encourage clinicians to institute pre-operative medical treatment and to adopt a closer follow-up schedule in cured cases. Multicenter prospective studies are needed to corroborate our findings.

DOI: 10.1007/s12020-025-04292-x.

PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "HEMATOLOGICAL AND GERIATRIC SCIENCES"

Cognitive Training in Neurocognitive Disorders: impact on Cognition, Salivary Cortisol, and Heart Rate Variability across the spectrum of cognitive decline

Ph.D. Student: Dr. Adele RAVELLI
TUTOR: Dr. Maria DEVITA – CO-TUTOR: Prof. Giuseppe SERGI
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Hematological and Geriatric Sciences"

Background
The rising prevalence of neurocognitive disorders (NCDs), driven by population aging, poses a major public health challenge with significant socio-economic and caregiving implications. In the absence of disease-modifying therapies, non-pharmacological interventions aimed at preserving cognitive functioning and autonomy are gaining increasing attention.
Among these, cognitive training (CT), shows potential in enhancing neuroplasticity, compensatory resource use, and emotional well-being. Yet, evidence on their long-term efficacy and interaction with pharmacological treatments remains limited. Moreover, the underlying mechanisms, whether cognitive, affective, or neuroendocrine, are still poorly understood. Identifying early, accessible biomarkers of physiological dysregulation could support risk stratification and help monitor treatment response. Two promising candidates are salivary cortisol, reflecting hypothalamic–pituitary–adrenal (HPA) axis function, and heart rate variability (HRV), indexing autonomic regulation. Both systems are implicated in cognitive and emotional processing and interact with central brain networks such as the central autonomic network (CAN).
This doctoral research investigates the clinical efficacy and psychophysiological correlates of CT across the continuum of cognitive decline, from Subjective Cognitive Impairment (SCI) and Mild Cognitive Impairment (MCI) to mild major NCD, through a series of integrated studies.
Materials and Methods
Participants were recruited at Geriatrics Unit of Padua University Hospital.
Study 1: 108 older adults with mild major NCD received repeated CT cycles, alone or combined with acetylcholinesterase inhibitors (AChEI), over 30 months. Cognitive outcomes (MMSE, ENB-2) were analyzed in relation to treatment, cognitive reserve (CR), and sex.
Study 2: included 62 patients with NCD (CT vs. AChEI) and 43 healthy controls. Salivary cortisol was collected at six timepoints across the day (baseline, 3 and 6 months), and a subsample underwent resting HRV recording via Empatica E4 before and after CT intervention.
Study 3: An ongoing study targeting SCI and MCI individuals includes comprehensive cognitive, affective, and physiological (HRV, cortisol) assessments before and after a 12-week CT.
Results
Study 1 demonstrated the long-term efficacy of CT, with cognitive performance either stable or improved over 30 months. CT alone outperformed the combined CT+AChEI approach.
Study 2: CT significantly reduced cortisol levels (AUC: –17.5%) and improved circadian secretion patterns, unlike pharmacological treatment. Preliminary HRV data suggest associations with affective symptoms; further analyses are ongoing.
Study 3 has so far recruited 11 SCI, 19 MCI, and 10 healthy controls. The intervention is proving feasible, with very positive participant engagement. Data collection is ongoing.
Conclusions
This research highlights the clinical relevance of CT across the NCD continuum. CT appears effective in modulating not only cognitive performance but also stress-related physiological systems such as the HPA axis and autonomic regulation. Integrating cognitive, emotional, and physiological measures may enhance early detection and guide more tailored, stage-specific approaches in cognitive care.
Cognitive training in preclinical and early stages of neurocognitive disorders: clinical impact and physiological correlates

PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "KIDNEY, PHYSICAL EXSERCISE AND NUTRITION SCIENCES"

Development and validation of novel clinical-instrumental investigations in mountain medicine

Ph.D. Student: Dr. Nicola BORASIO
TUTOR: Prof. Andrea ERMOLAO - CO-TUTOR: Dr. Giacomo STRAPAZZON
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Kidney, Physical Exercise and Nutrition Sciences"

Background
Hypoxia, acute and chronic, presents complex physiological challenges that vary across different populations. This Ph.D research focuses on investigating adaptive responses to high altitude in three groups: elite mountaineers, healthy individuals, and patients with congenital heart disease (CHD). The overarching goal is to identify clinical-instrumental markers that support risk stratification in mountain medicine, with hypoxia as the common thread.

Methods
Elite Mountaineers (K2-70 operation study): Three male climbers who summited K2 without supplemental oxygen and acclimatized above 5000 m for four weeks were re-exposed to 8043 m in the TerraXcube hypobaric chamber. Cardiopulmonary exercise testing and arterial blood gas analysis was performed at altitude and repeated at sea level for comparison. Healthy Subjects (ScreenX Study): 54 healthy volunteers underwent an 18-hour exposure to simulated altitude (4500 m) in the TerraXcube. Multimodal monitoring included transcranial Doppler of the middle cerebral artery, cerebral NIRS, optic nerve sheath diameter (ONSD) ultrasound and non-invasive intracranial pressure estimation using the Brain4care device. CHD Patients: A systematic review and meta-analysis were conducted to compare cardiopulmonary responses to exercise at low (<2500 m) and high altitude (≥2500 m) in patients with CHD versus healthy controls. Additionally, a case-control study protocol has been developed to investigate cardiovascular and ventilatory responses to altitude (2500–3000 m) in patients with aortic coarctation, which will be conducted in the TerraXcube chamber in 2026.

Results
Elite Mountaineers: $\dot{V}O_2max$ decreased to 33% of sea level values. Significant increases were observed in alveolar-arterial oxygen gradient and lactate levels during maximal exertion. Arterial oxygen saturation was lower at rest and exercise, yet higher than predicted for this altitude**.** Healthy Subjects: 34 out of 54 participants developed acute mountain sickness (AMS). Doppler measurements showed increased cerebral blood flow velocities and cerebral perfusion pressure, without significant changes in estimated intracranial pressure. ONSD showed a trend toward enlargement with prolonged hypoxia. CHD Patients: findings revealed that CHD patients had overall lower cardiorespiratory fitness but experienced a smaller decline in peak workload and oxygen saturation when transitioning from low to high altitude compared to healthy controls. No adverse events were reported in the included studies.

Conclusions
This project demonstrates the value of innovative, non-invasive tools for assessing hypoxia-related physiological responses across diverse populations. The K2-70 Operation is the first to measure breath-by-breath gas exchange during exercise in acclimatized climbers acutely re-exposed to 8043 m, with results consistent with predictive models. In healthy subjects, cerebral hemodynamic changes may help to identify AMS. Meta-analysis suggest that short-term exposure to moderate altitude is likely safe for stable, low-risk CHD patients. The upcoming hypobaric trial will further support personalized evaluation and risk stratification in mountain medicine.

Gitelman Syndrome's patients show higher expression of miR-103 and miR-155: implications for fibrosis and oxidative stress-related renal and cardiovascular damage

Ph.D. Student: Dr.ssa Martina CACCIAPUOTI
TUTOR: Prof. Federico NALESSO
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Kidney, Physical Exercise and Nutrition Sciences"

Background. Gitelman's Syndrome (GS) is a genetic tubulopathy characterized by hypokalemic metabolic alkalosis with hypomagnesemia. Despite showing hyperreninemia and hyperaldosteronism, these patients present with normo/hypotension and do not develop the Ang-II-induced cardiovascular and renal damage, mostly due to the blunted activation of AT1R intracellular signaling pathways. More specifically, the intracellular mediator RhoA/RhoKinase is less expressed in GS compared to healthy controls, therefore determining protection from remodeling, atherogenesis induced by oxidative stress, vasoconstriction and fibrosis. Therefore, GS are considered an opposite model to that of hypertension-mediated damage. MiRNAs are non-coding nucleotides of 18-21 bp that target – and lead to degradation - specific mRNAs. MiR-103 was found to inhibit the production of ROS in HUVECs exposed to H2O2. In mice cells, overexpression of miR-155 was demonstrated to counteract the function of RhoA, that is also an intracellular mediator of the TGF-β intracellular cascade. The aim of our comparative case/control study was to evaluate the miR-155 and miR-103 expression in GS patients vs healthy subjects.
Patients and Methods. GS patients were recruited from the cohort in active follow up at the Nephrology Unit of Padua University Hospital, while healthy subjects, matched by age and sex, volunteered for the study. Exclusion criteria were current infection and treatment with antihypertensive drugs or corticosteroids. Blood samples were drawn from both groups and total RNA was isolated from plasma using "miRNeasy Serum/Plasma Kit" (Qiagen). The specific miRNA population was then reverse-transcribed to cDNA. miR-155 and miR-103 were analysed using digital droplet PCR technology combined with the TaqMan Advanced miRNA Assays (Thermofisher) which provided preformulated primer and probe sets. The U-Mann-Whitney test was used to compare the results of the two groups. Statistical significance was set at $p<0.05$.
Results. All the GS patients (N=16, 7 males; mean age 43.22±14.28 years) were homozygotes or compound heterozygotes for pathogenic mutations of *SLC12A3*. The control group included 20 volunteers (8 males; mean age 45.03±11.91 years) matched by age and sex with the cases. Excluding outliers, in the GS group (N=16) the median number of copies per sample of miR-103 was 80.25 copies/sample, with an IQR of 94.43, while in the control group (N=20) the median expression was 22.51 copies/sample with an IQR of 36.35 copies/sample. In the GS group the expression of miR-103, was statistically higher than in the control group. ($p=0.0011$). Regarding miR-155, the median number of copies/sample in the GS group (N =16) was 528.7 with an IQR of 573.2, while in the control group (N=16) it was 6.05 number of copies/sample with an IQR of 123.0. Also for miR-155, the number of copies/sample was higher in the GS group compared to the control group, with a statistically significant difference. ($p<0.0001$).
Conclusions. This is the first study investigating miRNAs' profile in a GS population. The striking difference in the expression of miR-103 and miR-155 in these patients compared to healthy controls suggests that these miRNAs might contribute to the well-established protection from cardiovascular and kidney damage. As GS serves as a model opposite to hypertension, these findings are relevant from a therapeutic perspective aimed at preventing damage in hypertensive patients.

DIGITIZATION IN ERGONOMIC AND HUMAN MOVEMENT ASSESSMENT TO REDUCE WORK-RELATED MUSCULOSKELETAL DISORDERS

Ph.D. Student: Dr. Beatrice DORO
TUTOR: Prof. Marco BERGAMIN – CO-TUTOR: Prof. Stefano GOBBO
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Kidney, Physical Exercise and Nutrition Sciences"

Background: The application of artificial intelligence (AI) in assessing the risk of musculoskeletal disorders (MSDs) is rapidly evolving, particularly in fine-grained human action recognition, which plays a fundamental role in workplace safety, productivity, and injury prevention. A key emerging approach involves markerless techniques, which do not rely on external sensors or markers. These methods enable continuous monitoring of workers' posture and movement, allowing the early identification of high-risk tasks and postures, thereby supporting proactive interventions and reducing injury incidence. Notably, real-time recognition of incorrect posture facilitates immediate correction, potentially decreasing the long-term risk of MSD development.

Material and Methods: The research project *"Digitization in ergonomic and human movement assessment to reduce work-related musculoskeletal disorders"* aimed to develop a tool for collecting, digitizing, and interpreting AI algorithms to predict work-related musculoskeletal disorder (WRMSD) risk. The study focused on real-world industrial scenarios, unlike prior works relying on controlled conditions or wearable sensors. Markerless motion analysis was conducted on video data from 37 different manufacturing environments, exhibiting high variability in subjects, backgrounds, and viewpoints. For 2D pose estimation, OpenPose was initially used, followed by MMPose for more detailed hand keypoints. Due to the limitations of keypoint-only analysis, the project transitioned to MMAction2, a PyTorch-based toolbox for more comprehensive video understanding. The dataset was restructured by reducing the number of activity classes and setting a consistent temporal unit (8 frames per second). Original videos were segmented into 978 short clips (1–4 seconds), each labeled with one of 12 human-object interaction actions (e.g., "wiping a surface with the right hand" or "stacking objects"). Processing was performed on Google Cloud Platform (GCP) using an NVIDIA L4 GPU.

Results: For action recognition, several deep learning architectures were tested, including the SlowFast network and Graph Convolutional Networks (GCNs), in particular the Spatial-Temporal Graph Convolutional Network (ST-GCN) and the Two-Stream Adaptive Graph Convolutional Network (2S-AGCN). These models were evaluated for their ability to capture spatial and temporal patterns in 2D skeletal data. Despite challenges related to data variability and limited video duration, the models achieved promising results. The 2S-AGCN achieved the best overall performance with an accuracy of 62.44%, significantly exceeding baseline models. ST-GCN also demonstrated efficient execution times, making it suitable for real-time applications.

Conclusions: The outcomes of this project demonstrate the feasibility and effectiveness of AI-driven, markerless systems for real-time recognition of occupational actions. These tools can be integrated into MSD risk assessment frameworks to support ergonomic evaluations and proactive injury prevention strategies. The results open opportunity for the application of such approaches to more complex industrial scenarios, contributing to improved health and safety in the workplace.

Exoskeleton at the workplace: myth or fact? The EXO-LIFT study

Ph.D. Student: Dr. Francesco FAVRO
TUTOR: Prof. Marco BERGAMIN – CO-TUTOR: Prof. Stefano GOBBO
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Kidney, Physical Exercise and Nutrition Sciences"

Background
Work-related musculoskeletal (WR-MSD) disorders majorly affect manual workers, and low-back pain is generally self-reported as the most prevalent affection. Ergonomic interventions are aimed at reducing biomechanical risk, and can include the reorganization of the workflow, the adoption of safe lifting principles and the use of lifting aids. In recent years, exoskeletons, wearable devices that support the user during manual handling of loads and holding static postures, have emerged as a possible solution to mitigate the risk of developing WR-MSDs in this population, but most of the research in this field focuses on acute interventions performed in a laboratory settings. Therefore, the aim of this project is to implement a longitudinal study in which manual workers will use a passive back-support exoskeleton for 4 weeks on their workplaces.

Material and Methods
Participants were workers involved in manual handling of loads or holding awkward trunk postures for long periods of time. They were administered the Quality of Life questionnaire (WHOQOL), Nordic Musculoskeletal Questionnaire (NMQ), and the Brief Pain Inventory (BPI). Afterwards, their physical efficiency was tested with 6 tests: tapping test, handgrip, one leg balance, YMCA step test, 5 times sit-to-stand, and toes reach. The exoskeleton was then fitted to the participants, and a short familiarization session was performed.
A battery of six functional tests was then conducted while wearing the exoskeleton: 5 times sit-to-stand, toes reach, load lift, load carry, forward bent posture hold and YMCA step test. Perceived difficulty of the task was assessed using a visual analogue scale (0-10) after each test. Lastly, the participants completed an Exoskeleton Usability Questionnaire (EUQ). The intervention consisted of 4 weeks of exoskeleton use in the workplace. Post-intervention, the participants completed the modified NMQ, BPI, the battery of 6 functional tests and the EUQ. Data are presented as mean ± sd.

Results
So far, 5 participants (5 males, age: 44.6 ± 14.3 years) have completed the protocol. In the intervention period, they used the exoskeleton for a total of 2624 ± 1354 minutes. Pre-post intervention, NMQ scores remained stable, while functional tests performance and VAS scores showed an improving trend, without reaching the significance threshold. Regarding the EUQ, there was a small with all decrease in the score of all questions with two exceptions: the score assigned to "lumbar offload", which increased by 0.8 ± 4.6, and "work interference", which decreased steeply by 3.6 ± 3.2 points.

Conclusions
This is among the first longitudinal studies investigating the use of a passive back-support exoskeleton in the workplace. From these preliminary data, it appears that performance and perceived difficulty with the exoskeleton remained relatively stable after 4 weeks of use, disproving a "learning effect" emerging from previous works. Perceived usability of the tool generally decreased after the intervention period, especially regarding the perceived interference with working tasks, raising concerns around the long-term applicability of this device in the working population.

PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "LIVER AND TRANSPLANT SCIENCES, RARE DISEASES AND AT HIGH"

Implementing Bedside Handover in an Italian University Hospital: A longitudinal pre-post study

Ph.D. Student: Dr. Chiara DAICAMPI
TUTOR: Prof. Salvatore PIANO – CO-TUTOR: Prof. Mario DEGAN
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Liver and Transplant Sciences, Rare Diseases and At High"

Background

Bedside handover (BSH) is internationally recognised as a high-impact nursing practice that improves patient safety, engagement, and continuity of care. In Italy, however, BSH remains rarely implemented, with most handovers conducted away from the patient's bedside. This project aims to introduce and evaluate the effectiveness of BSH in four clinical units of the University Hospital of Padova, with a second phase planned to expand its adoption to additional units.

Preparatory activities included a systematic review on the organisational outcomes of BSH (Daicampi et al., Journal of Nursing Management, 2025), which provided a strong evidence base for the intervention. In addition, the HAND-Q questionnaire was developed and validated as a tool to measure handover quality and nurse satisfaction (validation study on 514 nurses; 4-factor structure; Cronbach's $\alpha \geq 0.88$).

Material and Methods

This longitudinal pre–post study involves four hospital units (stroke unit, neurosurgery, and two neurology wards), with subsequent expansion planned in the second phase.

- **Pre-implementation phase (2024):**
  A total of 244 shift handovers were observed, revealing that only 19.6% were conducted at the bedside. The average handover time was 1'08'' per patient, and critical clinical information (e.g., care priorities) was recorded in less than 30% of cases. A baseline HAND-Q survey (n = 74 nurses) highlighted a lack of standardisation, limited training (87.8% of nurses had never received formal handover training), and moderate satisfaction levels.
- **Intervention phase (February-April 2025):**
  A structured training programme on BSH was delivered to all participating nursing staff.
- **Post-implementation phase (May–October 2025):**
  BSH is currently being implemented and monitored through repeated structured observations, a follow-up administration of HAND-Q at 6 months, and the evaluation of organisational indicators such as handover duration, overtime hours, and staff workload.

Preliminary Results

Following the training sessions, preliminary data show a significant increase in bedside handovers, rising from <20% to approximately 65% of shift changes. Staff feedback suggests improved clarity, communication, and patient involvement. Baseline findings confirmed the need for cultural and organisational change, while early post-intervention analyses indicate that HAND-Q is sensitive in detecting improvements in handover quality.

Conclusions

This project represents one of the first structured attempts in Italy to implement BSH on a larger scale. By integrating evidence from the literature, validated assessment tools, and targeted training, the study will generate robust data on the impact of BSH on communication quality, nurse satisfaction, and organisational performance (e.g., reduction of overtime hours). The findings will support the broader implementation of patient-centred handover models in Italian healthcare settings.

RECTUS FEMORIS ULTRASOUND IDENTIFIES SARCOPENIA AND PREDICTS POOR OUTCOMES IN PATIENTS WITH AN ACUTE DECOMPENSATION OF CIRRHOSIS

Ph.D. Student: Dr. Roberta GAGLIARDI
TUTOR: Prof. Salvatore Silvio PIANO
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Liver and Transplant Sciences, Rare Diseases and At High"

Background: Sarcopenia is common and associated with poor outcomes in decompensated cirrhosis. While CT scan, with measurement of skeletal muscle index (SMI) at L3, is the gold standard for assessing sarcopenia in patients with cirrhosis, it is costly, exposes patients to radiation, and requires specialized software. This study aimed to evaluate the accuracy of bedside ultrasound measurement of the rectus femoris cross-sectional area (RF-CSA) in assessing sarcopenia and its prognostic value in patients with cirrhosis.
Material and Methods: A prospective two-phase study was conducted. Phase-1 analyzed correlations between RF-CSA and SMI, as well as other sarcopenia predictors, in 77 patients. In phase-2, RF-CSA was measured at the bedside in 203 patients with acute decompensation of cirrhosis, followed-up until death, liver transplant, or 90 days. Inter-operator reliability was assessed in 38 patients using the intraclass correlation coefficient (ICC).
Results: RF-CSA strongly correlated with SMI ($r=0.748$, $p<0.001$) outperforming muscle thickness and anthropometric parameters. RF-CSA was an independent predictor of sarcopenia (HR=0.27, $p<0.001$) and demonstrated high discrimination ability for sarcopenia (AUROC=0.90 in males and 0.92 in females). Lower RF-CSA was independently associated with an increased risk of developing sepsis, acute kidney injury, shock and overt hepatic encephalopathy. RF-CSA was an independent predictor of 90-day mortality (sHR=0.57, $p=0.006$). Intra- and inter-operator reliability were high (ICC=0.980 and 0.947, respectively, $p<0.001$ for both).
Conclusions: RF-CSA assessment by thigh ultrasound is an accurate, reliable, and easy point-of-care tool for assessing sarcopenia in patients with decompensated cirrhosis. It is also associated with the risk of complications and mortality.

BREAKING THE ICE OF COLD PROCESS: NORMOTHERMIC PRESERVATION FOR ISCHEMIA-FREE DCD LIVER GRAFTS

Ph.D. Student: Dr. Eugenia ROSSO
TUTOR: Prof. Umberto CILLO – CO-TUTOR: Sara MONTAGNESE
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Liver and Transplant Sciences, Rare Diseases and At High"

Background
In the context of persistent organ shortage, donation after circulatory death (DCD) offers a valuable opportunity to expand the liver donor pool. The sequential use of normothermic regional perfusion (NRP) followed by ex situ machine perfusion has been shown to reduce graft failure rates. However, DCD liver transplantation remains marginal in certain countries, such as Italy, where a mandatory 20-minute no-touch period after cardiac arrest poses significant challenges.
Materials and Methods
Between October 2017 and July 2025, a total of 83 DCD liver transplants were performed at our center under the 20-minute no-touch protocol. All grafts underwent in situ assessment via NRP prior to retrieval, followed by dynamic preservation using machine perfusion. The majority were preserved with hypothermic machine perfusion (D-HOPE).
In January 2025, we initiated a novel ischemia-free preservation protocol using normothermic machine perfusion (NMP), in which 7 DCD grafts were transferred directly from in situ NRP to ex situ NMP, entirely avoiding cold ischemia. Donor, recipient, and postoperative clinical data were prospectively collected and analyzed using both univariate and multivariate statistical methods.
Results
The overall 5-year survival rate for all DCD grafts, as analyzed via Kaplan-Meier, was 87%, showing no significant difference compared to donation after brain death (DBD) grafts despite the prolonged warm ischemia time inherent to the 20-minute no-touch period. Univariate analysis identified several prognostic factors associated with poorer outcomes: Presence of anti-HBc antibodies ($p = 0.04$), Elevated donor potassium levels ($p = 0.03$), Increased total warm ischemia time (tWIT) ($p = 0.03$). The 7 cases of ischemia-free DCD liver transplantation are currently under follow-up. All recipients are in good clinical condition with no signs of graft dysfunction. The biliary tree is being monitored prospectively using MR cholangiography.
Conclusions
Our findings demonstrate excellent patient survival outcomes using DCD grafts, comparable to those of DBD grafts, even under extended no-touch time. The combination of NRP and D-HOPE is a viable strategy for optimizing DCD liver transplantation. Additionally, our preliminary experience with ischemia-free graft preservation using NMP is promising, suggesting reduced ischemia-reperfusion injury and improved recipient outcomes. NMP may also provide a valuable platform for pharmacological graft treatment and real-time organ assessment.

PhD COURSE "CLINICAL AND EXPERIMENTAL SCIENCES"

COORDINATOR: PROF. ROBERTA RAMONDA

Curriculum "RHEUMATOLOGICAL AND LABORATORY SCIENCES"

Predictors of response to bDMARDs and tsDMARDs in Psoriatic Arthritis: the role of musculoskeletal ultrasound

Ph.D. Student: Dr. Giacomo COZZI
TUTOR: Prof. Roberta RAMONDA – CO-TUTOR: Prof. Maria Grazia LORENZIN
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Rheumatological and Laboratory Sciences"

Background

The heterogeneity of clinical manifestations in psoriatic arthritis (PsA) complicates the validation of predictors for therapy response. Ultrasound (US) has become an essential tool for enhancing clinical evaluation in PsA, with growing interest in its potential to identify early predictors of treatment efficacy and improve personalized care. This study aimed to evaluate the role of US in predicting drug retention in PsA patients initiating or switching to biologic or targeted synthetic disease-modifying antirheumatic drugs (bDMARDs/tsDMARDs). Specifically, we focused on the most clinically involved joint, enthesis, or tendon (MIJET) and the two most involved sites (2MIJET), comparing their performance to traditional clinimetric indices in predicting treatment outcomes.

Material and Methods

We conducted a prospective observational study involving PsA patients with active arthritis, enthesitis, or tendonitis across two Italian rheumatology centers. Clinical and US assessments were performed at baseline (t0), and at 1 (t1), 3 (t3), and 6 (t6) months post-treatment initiation. After 6 months of follow-up, clinicians assessed treatment efficacy and patients were divided into two groups based on drug retention, clinical Responder (cResponder) and non-cResponder. Clinimetric indices such as cDAPSA, MDA, LEI, and patient-reported outcomes (PROs) were recorded. The main endpoints were changes in US scores (MIJET, 2MIJET) over time. Between-group comparisons of normally distributed continuous variables were conducted using Student's t-test to evaluate baseline characteristics and mean changes across time points (t1, t3, t6 vs. t0).

Results

Seventy-one consecutive patients were enrolled (34 males, 47.88%), with a mean age of 54.9 (±11.19) years; 55 were cResponders, 11 were non-cResponders. Three patients were excluded from the study due to therapy discontinuation caused by adverse events, and two patients were lost to follow-up. Baseline characteristics, including disease duration, BMI, and clinimetric scores, were comparable between groups, except for a higher LEI score in non-cResponders. US scores demonstrated significant reductions in MIJET and 2MIJET at t3 and t6 among cResponders compared to non-cResponders (MIJET: Δt3-0: -2,61 vs 0,25; p=0,0044; Δt6-0: -2,348 vs 0,33; p=0,0013; 2MIJET: Δt3-0: -5,78 vs -3,75; p=0,0424; Δt6-0: -4,07 vs 0,12; p=0,0024) (***Figure 1***). Changes in clinimetric indices, such as cDAPSA, were delayed, becoming significant only at t6.
The correlation between clinimetric and ultrasound indices was evaluated to determine whether patients in clinical remission—defined as achieving MDA or cDAPSA $\leq$ 4—exhibited significant differences in ultrasound score changes compared to those not in remission. Among composite disease activity indices, MDA showed a significant correlation with changes in both MIJET and 2MIJET (p=0,0048 p=0,0413); whereas cDAPSA was significantly associated only with changes in 2MIJET (p=0,0144) (***Figure 2***). Regarding PROs, morning stiffness correlated with MIJET(p=0,0367) but not with 2MIJET, while VAS pain and patient global assessment showed no correlation with ultrasound scores.

Conclusions

This study highlights the potential of MIJET and 2MIJET as early ultrasound predictors of drug retention in PsA, complementing clinical evaluation with targeted imaging. These indices identified subclinical improvements in inflammation earlier than traditional clinimetric measures, underscoring the value of ultrasound in disease monitoring. The discrepancy between clinical and ultrasound responses, as well as the absence of significant associations between ultrasound scores and PROs, emphasizes the complexity of assessing disease activity. The presence of fibromyalgia or chronic nociplastic pain may have influenced perceived treatment efficacy. MIJET and 2MIJET's ability to differentiate inflammatory from non-inflammatory pain offers an advantage in evaluating disease activity. Further validation in larger cohorts and long-term studies is necessary to establish these indices as standard tools for predicting therapeutic success in PsA.

Unsupervised machine learning identifies distinct systemic lupus erythematosus patient endotypes with differential response to belimumab

Ph.D. Student: Roberto DEPASCALE
TUTOR: Luca IACCARINO– CO-TUTOR: Margherita ZEN
Ph.D. Course in Clinical and Experimental Sciences
Curriculum: "Rheumatological and Laboratory Sciences"

Objective:
To determine systemic lupus erythematosus (SLE) endotypes according to B cell immunophenotyping and serological profile and assess endotypes' response to belimumab.

Patients and methods:
We analysed data from 796 patients with SLE from the phase III trial BLISS-SC. Unsupervised machine learning employing factor analysis of mixed data (FAMD) and subsequent clustering determined endotypes based on B cell immunophenotyping and serological profiles. Cox regression was used to assess belimumab efficacy on inducing lupus low disease activity state (LLDAS) and Definitions of Remission in SLE (DORIS) remission within clusters.

Results:
Compared to each other, cluster 1 (n=191) displayed higher proportions of $CD19^{+}CD24b^{+}CD27^{+}$ regulatory B cells [mean ± standard deviation (SD): 35.9%±12.6%], $CD19^{+}CD20^{+}CD27^{+}$ bulk memory B cells (32.2%±9.9%), $CD19^{+}CD20^{+}CD69^{+}$ activated B cells (0.2%±0.3%), $CD19^{+}CD20^{-}CD138^{+}$ long-lived plasma cells (0.7%±1.0%), and $CD19^{+}CD38b^{+}CD27b^{+}$ SLE-associated plasma cells (6.6%±7.0%). Cluster 2 (n=366) displayed higher proportions of $CD19^{+}CD24b^{bright}CD38b^{bright}CD27^{-}$ transitional B cells (6.3%±9.0%) and $CD19^{+}CD20^{+}CD27^{-}$ naïve B cells (85.4%±7.2%), and lower proportions of $CD19^{+}CD20^{-}CD138^{+}$ peripheral long-lived plasma cells (0.2%±0.3%) and $CD19^{+}CD38b^{+}CD27b^{+}$ SLE-associated plasma cells (1.6%±2.0%). Cluster 3 (n=239) displayed a higher proportion of $CD19^{+}CD20^{+}CD138^{+}$ short-lived plasma cells (0.1%±0.1%) and higher serological activity. Use of belimumab was superior to placebo in inducing sustained LLDAS (HR: 2.22; 95% CI: 1.18-4.17; p=0.014) and DORIS remission (HR: 3.45; 95% CI: 1.2-9.94; p=0.022) in cluster 2.

Conclusion:
Three distinct SLE endotypes were identified based on B cell immunophenotyping and serological profiles, showing differential benefit from belimumab therapy.

PhD COURSE "DEVELOPMENTAL MEDICINE AND HEALTH PLANNING SCIENCES"

COORDINATOR: PROF. GIANNI BISOGNO

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

Longitudinal integrated multidimensional assessment of newborns at risk for neurological sequelae: implementation of a multidisciplinary neonatal brain research program and identification and validation of innovative diagnostic and prognostic biomarkers

Ph.D Student: Dr. Claudio ANCONA
TUTOR: Prof. Stefano SARTORI
CO-TUTOR: Dr Maria Elena CAVICCHIOLO, Dr Nicoletta MAININI, Dr Enrico VALERIO
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background: Neonatal brain injuries and their long-term neurological sequelae require multidisciplinary, integrated care throughout both the acute phase and follow-up. In addition to standardized clinical assessment and neuroimaging the integration of innovative approaches—such as metabolomics and advanced quantitative EEG (qEEG) analysis—holds promise for the identification of novel early biomarkers. They may enhance our understanding of neuronal injury mechanisms, thereby improving prognostic accuracy and neurodevelopmental interventions.
Material and Methods: This project aims to establish a multidisciplinary neonatal brain research group within the Department of Women's and Children's Health (SDB) at Padua University Hospital in Italy. The group will target 4 main neonatal neurological conditions to improve their characterization and identify innovative biomarkers with early neurodevelopmental prognostic capability: 1) Hypoxic-ischemic encephalopathy (HIE), using multi-omic technology to validate previous findings and identify metabolic acute phase fingerprints predictive of long-term outcomes; 2) neonatal cerebrovascular accidents, implementing systematic data collection in the Italian Registry of Pediatric Thrombosis (RITI) to better characterize acute manifestations and long-term outcomes; 3) pre- and perinatal exposure to toxic substances, analyzing quantitative EEG indices to characterize their alterations and assess the impact of these substances on brain activity; 4) Neurological complications and long-term neurodevelopmental outcomes in infants with Congenital Heart Disease (CHD) undergoing cardiac surgery.
Results: In the first two years of the project, a multidisciplinary neonatal brain clinical and research group was established within the SDB Department. A dedicated outpatient clinic was implemented for the neurologic and neurodevelopmental follow-up of term newborns with neurological diseases, alongside structured teaching activities focused on neonatal neurology. The first study linking acute-phase urinary metabolomic profiles and neurodevelopmental outcomes at 7 years of age in neonates with HIE was published, supporting the prognostic relevance of metabolomics. In the context of neonatal cerebrovascular accidents, a systematic review including Padua cases of neonatal subpial hemorrhage and a review on the Padua experience on neonatal cerebral venous thrombosis and deep medullary vein thrombosis were published. The collaboration with the Departments of Information Engineering and Neuroscience enabled the application of innovative EEG biomarkers—Spectral Exponent and Higuchi Fractal Dimension, HFD—in neonates. A benchmark of normative values of these indexes' values have been defined, and an article on normative HFD values in neonatal and paediatric age is currently under major revision. They will be then applied to newborns with different neurological conditions. A qEEG study in infants with CHD demonstrated significant spectral power differences compared to healthy controls.
Conclusions: The initial findings of this multidisciplinary research project highlight the potential of integrating innovative early multimodal prognostic biomarkers—such as metabolomic profiling and advanced qEEG indices—into the diagnostic framework for newborns with brain injury and/or at neurodevelopmental risk. They also emphasize the critical role of multidisciplinary follow-up, including dedicated outpatient services and structured clinical registries.

Investigation of potential non-invasive urinary biomarkers for the subclinical detection of renal transplant rejection in pediatric patients

Ph.D. Student: Dr. Benedetta ANTONIELLO
TUTOR: Prof. Giorgio PERILONGO – CO-TUTOR: Dr. Susanna NEGRISOLO
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background
Kidney transplantation is the treatment of choice for pediatric end-stage renal disease. However, the graft's half-life is approximately 15-20 years, and rejection remains the leading cause of graft loss. Currently, renal biopsy is the gold standard for diagnosing rejection and long-term graft monitoring; although, it is an invasive and costly procedure. To improve the follow-up of pediatric patients, recent research has focused on predictive biomarkers of renal damage detectable in biological fluids, such as urine. Among the emerging biomarkers in kidney transplantation, urinary CXCL10, CXCL13, and soluble CD163 have garnered particular interest due to their potential non-invasive rejection monitoring: CXCL10 has been validated as a sensitive urinary indicator of both T cell–mediated and antibody-mediated rejection in adults, CXCL13 may reflect B cell activation and humoral alloimmune responses, and soluble CD163, released by activated macrophages, has been associated with glomerular inflammation and allograft injury. Therefore, we investigated the urinary presence of these biomarkers in pediatric kidney transplant recipients during the first two years post-transplant, aiming to define a signature capable of enabling the early prediction of both cellular and humoral subclinical rejection.

Material and Methods
Urine samples were collected from 76 pediatric patients who underwent transplantation between 2016 and 2024, for a total of 117 samples. At the time of transplantation, the patients had a mean age of 13 years and included 20 females and 56 males. All patients underwent at least one protocol biopsy at six months, one year, or two years after transplantation and a urine sample was collected at the corresponding time point. After histological evaluation, the cohort was divided into two groups: 72 urine samples corresponded to time points with no subclinical rejection (Banff 1), and 45 samples corresponded to a diagnosis of subclinical rejection (Banff 2, 3, or 4). Urinary levels of the cytokines CXCL10, CXCL13, and the soluble protein CD163 were measured using ELISA assay. Furthermore, plasmatic creatinine values were considered in order to normalise the data. Statistical analysis between the two groups were performed using the Mann-Whitney test with GraphPad Prism software.

Results
The urinary concentration of CXCL10 in pediatric patients was statistically different between the group with subclinical rejection and the group without rejection (p-value = 0.0004). Moreover, the difference between the two groups remained statistically significant even after plasma creatinine levels' normalization with a p-value of 0.0005. Similarly, the difference in sCD163 concentration between the two groups was statistically significant, with a p-value of 0.002. After normalization to urinary creatinine levels, the difference between the groups was amplified and remained statistically significant, with a p-value < 0.001. Conversely, the urinary concentration of CXCL13 does not appear to differ between the two patient groups analyzed.

Conclusions
Based on the results of this study, CXCL10 and sCD163 could serve as effective early biomarkers for subclinical renal rejection in paediatric transplant patients. Measurement of their urinary levels could reduce the need for protocol biopsies, offering a faster and less invasive method for transplant monitoring. However, it is necessary to enlarge the cohort further to confirm its reliability and clinical applicability, and also to test other potential biomarkers such as miRNAs.

Can Pediatric Intermediate Care Optimize PICU Utilization? Patient Trajectories and Outcomes from a Single-Center Exploratory Study

Ph.D. Student: Dr. Francesca BENEDETTI
TUTOR: Prof. Silvia CARRARO
CO-TUTORS: Prof. Silvia BRESSAN, Dott. Tiziana ZANGARDI, Dott. Caterina AGOSTO
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background
Paediatric Intermediate Care Units (PIMCUs) are increasingly being established in paediatric healthcare to support children whose medical needs lie in between those of the general wards and the Paediatric Intensive Care Unit (PICU).
The increasing technological complexity of certain paediatric patients, combined with the rising PICU overcrowding and high associated costs, has emphasized the need to explore the role of PIMCUs in optimizing resource allocation.
This study aimed to describe the characteristics, outcomes, and resource implications of children admitted to a newly implemented PIMCU who needed escalation to PICU.

Material and Methods
A retrospective observational study was conducted at the Department of Women's and Children's Health, a tertiary paediatric hospital affiliated with the University of Padova. All paediatric patients (0–17 years) admitted to the PIMCU between November 1st, 2023, and June 30th, 2025, were included. Data collected included demographics, clinical characteristics, PEWS scores, outcomes, and resource utilization. Sub-analyses were performed on a group of children named "Low-Risk NIV Group" that, after being escalated to PICU for respiratory failure, showed: NIV duration ≤ 5 days; no sedation or only dexmedetomidine; use of simple interfaces (nasal cannulas, nasal mask or oro-nasal mask); no need for intubation. A cost simulation estimated potential savings from managing this group in the PIMCU rather than in the PICU.

Results
During the study period, 365 patients were admitted to the PIMCU (51% males, median age 3 years). Of these, 26 (7.1%) required PICU transfer; predominantly for respiratory deterioration (20, 77%), with 17 (85%) receiving NIV. All children who were treated with NIV met criteria for the Low-Risk NIV Group (47% males, median age 2 years). Five children had a chronic complex condition. Statistical analysis showed that lower PEWS scores at admission and absence of a complex chronic condition were predictors of being part of the Low-Risk NIV Group.
Cost simulation suggested that managing the Low-Risk NIV Group within the PIMCU could have saved over €100,000 annually, based on the differential between daily PICU and PIMCU costs.

Conclusions
This study provides preliminary evidence that selected pediatric patients (with low PEWS at admission and no associated complex chronic conditions) requiring NIV may be safely managed in a well-equipped PIMCU, potentially reducing the need for PICU admissions and associated costs. These findings support the implementation of NIV in PIMCUs, provided that appropriate protocols, monitoring capacity, and proximity to PICUs are ensured. Further prospective studies and pilot implementation projects are warranted to establish standardized criteria for NIV administration outside the PICU and to evaluate long-term outcomes.

OPTIMIZATION OF A DRUG SENSITIVITY PROFILING PLATFORM TO DELIVER PRECISION MEDICINE IN PEDIATRIC ONCOLOGY

Ph.D. Student: Dr. Martina CANTON
TUTOR: Prof. Giampietro VIOLA – CO-TUTOR: Dr. Elena MARIOTTO
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background

Pediatric cancers, though rare, remain the leading cause of disease-related death in children in Europe and the U.S., with over 2000 new cases annually in Italy alone. Despite significant progresses in overall survival, outcomes for high-risk, relapsed, or progressive tumors remain poor, with cure rates below 20% after recurrence. While large-scale genomic studies have identified many potential therapeutic targets, translating these findings into effective treatments is still challenging due to limited predictive power of sole genomic data and lack of reliable biomarkers. In the last years, *ex vivo* drug testing, also known as Drug Sensitivity Profiling (DSP), has emerged as a powerful translational tool able to identify patient-specific therapeutic vulnerabilities in a clinically useful timeframe, able to shorten the turnaround time in comparison to more complex personalized tools, such as patient-derived xenografts and organoids. DSP has shown promising results in predicting treatment response and reducing toxicity, thereby improving progression-free survival compared to non-guided therapies. This project aims to establish a DSP platform at the University Hospital of Padova to support clinical decisions for the treatment of high-risk and relapsed pediatric cancer patients. This will align our institution with international standards, positioning DSP as a crucial theranostic tool in pediatric oncology.

Material and Methods

Cancer-specific drug libraries have been defined following the international consensus on standard of care for Acute Lymphoblastic Leukemia (ALL) and Brain and Solid Tumors (BT/ST), as well as the latest advances in clinical trials. ALL and BT/ST samples processing and culturing conditions have been established to achieve optimal screening conditions. Moreover, each step of drug screening workflow has been optimized using a semi-automatic liquid handler, allowing the standardization of the entire process, including cell seeding, drug dispensing and readout analysis, to facilitate the identification of clinically relevant hits.

Results

ALL- and BT/ST-specific drug libraries have been designed, including 76 unique drugs, spanning from standard chemotherapeutics to newly developed drugs, e.g. multi-kinase inhibitors and epigenetic modulators. Two primary criteria have been applied for drug prioritization: i) the current drug developmental stage and ii) the internal drug accessibility, finally including 28 FDA-approved drugs for pediatric use. Standard Operating Procedures (SOP) have been defined for both ALL and BT/ST, from sample collection to drug screening endpoint. ALL biobanked samples (n=23), and ST (n=5) and BT (n=3) patient-derived cells have been successfully tested, leading to the identification of the most promising drug candidates. Inter-laboratory cross-validation has confirmed our preliminary results strengthening the robustness of our DSP platform.

Conclusions

In conclusion, this project provides a proof of concept for functional pharmacotyping for pediatric patients enrolled at the Pediatric Oncology and Hematology Unit at the University Hospital of Padova, to identify alternative therapeutic options for hard-to-treat cancers. Future steps will include testing potentially synergic combinations with chemo or other targeted agents, as well as the integration of molecular profiling data to enhance the predictive accuracy of DSP to guide alternative therapeutic strategies for refractory/relapsed tumors.

AI-Powered Surveillance of Bronchiolitis in the Nirsevimab Era: From Free-Text EHRs to Machine Learning, Deep Learning, and Large Language Models

Ph.D. Student: Dr. Marianna COSTA
TUTOR: Prof. Silvia BRESSAN
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

I am presenting the results of a study that has been recently submitted to the "Journal of Biomedical Informatics" (review is ongoing). During my current research stay abroad, I am contributing to a project at Children's Hospital Los Angeles (CHLA) focused on developing and internally validating a predictive model—enhanced by natural language processing (NLP)—to identify children at risk of suicide-related revisits to the pediatric emergency department.

Considerations The software used in Pediatric Emergency Departments (PEDs) typically collects clinical information in free-text format. While this approach enables physicians to capture detailed and nuanced clinical narratives, it limits the secondary use of data for scientific and research purposes due to its unstructured nature. Automating the extraction and classification of clinical data—coupled with the availability of structured data—can significantly enhance epidemiological surveillance, support clinical decision-making, and facilitate medical research, thereby contributing to improved quality of care. Traditionally, Machine Learning Techniques (MLTs) have been applied to classify such unstructured information. However, advanced language models—such as the Generative Pretrained Transformer (GPT)—offer an innovative alternative by enabling the interpretation of free-text input through direct textual prompts, without the need for prior training or customized model development.

Background In the era of widespread immunoprophylaxis against the primary viral cause of bronchiolitis, ongoing epidemiological surveillance remains essential. We conducted a single-center retrospective observational study evaluating and comparing the performance of traditional MLTs and pre-trained Large Language Models (LLM) such as GPT-4o in the automated extraction and classification of clinical data from the Free Text-diagnosis field of PED EHRs at the University Hospital of Padua.

Material and Methods 28 557 EHRs of infants under 1 year with complete discharge diagnoses fields were retrieved between the years 2007-2018 and manually classified by an expert pediatrician to create the gold standard diagnosis set for training the algorithm. After data pre-processing, classical ML models (Random Forest, Decision Tree, Gradient Boosting Machine, Linear Discriminant Analysis, Support Vector Machine), a Deep Learning (DL) tool, and a pre-trained LLM (ChatGPT4o) were evaluated using balanced accuracy, sensitivity, and F1 scores. The official administrative ICD-09 encoding classification accuracy was compared to the gold standard.

Results Overall, 1903 of 28,557 records (6.7%) were classified as bronchiolitis by the gold standard approach. The DL model and ChatGPT4o outperformed traditional ML models, achieving higher sensitivities (0.97, 95%CI 0.96-1·00, and 0.98, 95%CI 0.98-0.99, respectively), F1 scores (0.96, 95%CI 0.95-0.99, and 0.99, 95%CI 0.98-0.99, respectively), and balanced accuracy (0.98, 95%CI 0.98-1.00, and 0.99, 95%CI 0.99-0.99, respectively). Traditional ML models showed sensitivities between 0.77 and 0.98, F1 scores between 0.86 and 0.96, and balanced accuracies between 0.88 and 0.96. ICD-09 codes showed sensitivity of 85.9% (95%CI 84.3-87.5), and specificity of 98.5% (95%CI 98.5-98.6).

Conclusions DL and ChatGPT4o outperformed tested traditional ML-based tools in identifying bronchiolitis diagnoses and in ICD-09 diagnosis coding. AI-based tools hold significant potential for improving epidemiologic surveillance of bronchiolitis from PED EHRs.

Overcoming treatment resistance mechanisms in ALK-driven cancers

Ph.D. Student: Dr. Matteo MARZI
TUTOR: Prof. Lara MUSSOLIN
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background

Pediatric Anaplastic Large Cell Lymphoma (ALCL) accounts for approximately 10-15% of all childhood non-Hodgkin lymphomas with about 95% of cases being Anaplastic Lymphoma Kinase (ALK)-positive. Unfortunately, 20-30% of patients experience relapse. Pediatric ALCL patients are now being evaluated for new Tyrosine Kinase Inhibitor combinations like Brigatinib with standard therapies. This project aims to identify genetic mutations driving differential treatment responses and resistance and to create suitable ALCL cell models for functional studies. The clinical relevance of cell-free DNA (cfDNA) in the early detection of resistance patients will be exploited through liquid biopsy approach.

Material and Methods

Whole-exome sequencing (WES) was performed on genomic DNA extracted from tumor biopsies and matched blood samples of 38 patients (11 relapsed, 26 non-relapsed). The samples were obtained from frozen tissue and processed using the DNeasy Blood and Tissue Kits for DNA Isolation. Additionally, cfDNA was isolated from patient plasma using the QIAmp DNA mini kit. Targeted Next-Generation Sequencing (NGS) was then carried out on the cfDNA using the the Accel-Amplicon™ 56G Oncology Panel v2. To uncover genes driving resistance, CRISPR/Cas9 loss-of-function screens using the GeCKO v2 pooled sgRNA library was conducted in an ALCL cell line. These cells were exposed to chemotherapeutics and ALK inhibitors, and the depletion of sgRNAs following treatment was analysed via deep sequencing and the MAGeCK algorithm to identify resistance-associated genes.

Results

Whole-exome sequencing (WES) revealed significant heterogeneity among ALCL patients, along with generally low tumor mutational burden (TMB). However, 4 patients showed notably high TMB and microsatellites instability (MSI; interestingly all this 4 patietns were characterized by an early relapse to validate that and to expand our cohort, we performed fragment analysis on a total 17 patients, 9 matched from WES cohort and 8 new cases. WES and Fragment Analysis resulted concordant for 89%. The global cohort of 46 patients pointed out that among relapse cases, 60% of them were affected by MSI. Quantification of cfDNA indicated that the medium concentration (0,06ug/uL) at diagnosis of the relapsed patients was three times higher compared to the not-relpased cases(0,02ug/uL), with a significant p Value of 0,0029. Moreover, the preliminary results from the genome-wide CRISPR knockout screen showed the involvement in the Brigatinib resistance of ALK pathway kinases and other tumor suppressor genes such as STK11.

Conclusions

We found that major features such as MSI and the amount of cfDNA can be useful to better predict patients who will relapse. On the other hand, kinases genes such as STK11 can be a target for treating patients who will aquire resistance to Brigatinib. These preliminary results will be confirmed with other bioreplicates in the next future. Moreover, Functionally, candidate genes will be evaluated in vitro through engineered ALCL cell lines using CRISPR editing.

A protein engineering strategy for innovative anti-CD72 CAR-T therapy in pediatric AML

Ph.D. Student: Dr. Silvia MERLINI
TUTOR: Prof. Martina PIGAZZI – CO-TUTOR: Prof. Alessandra BIFFI
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background: The success of chimeric antigen receptor (CAR)-T therapy in acute myeloid leukemia (AML) is primarily limited by the challenge of identifying optimal target antigens (Ag). However, optimal Ag selection is not sufficient for effective CAR-T therapy. Therapeutic success also depends on engineering an extracellular CAR Ag-binding domain with high specificity and affinity for the target. In our laboratory, we identified CD72 as a promising target Ag for pediatric acute leukemia (Buldini, 2023). We manufactured and tested three different nanobody-based anti-CD72 CAR-T cells (Wiita, 2021). Despite a strong *in vitro* cytotoxicity against AML cell lines, their *in vivo* efficacy was limited, highlighting the need for novel, high-affinity CD72-specific binders. Here, we describe the protein engineering approach used to generate anti-CD72 binders for inclusion in CAR constructs. Our goal is to develop a successful and risk-free anti-CD72 CAR-T therapy for AML, addressing the medical need for new leukemia treatments while minimizing severe hematologic toxicity.

Materials and Methods: For binder discovery, we employed a fully *in vitro* protein engineering strategy based on yeast surface display. In this method, variants of two fully human single-domain scaffold proteins were displayed on the surface of yeast cells via fusion to the yeast agglutinin mating protein (Aga2p). The naïve library comprising 10^9 protein variants was screened using recombinant human CD72 (Acrobiosystems) as the target Ag. Binder selection involved iterative rounds of magnetic-activated cell sorting (MACS), error-prone PCR (ep-PCR) for random mutagenesis, and fluorescence-activated cell sorting (FACS) of the library. After library enrichment for CD72-binders, single clones were sequenced and expressed in *E. coli.* The resulting proteins were characterized to assess key parameters fundamental for successful CAR expression on T cells, such as thermal stability using nano-differential scanning fluorimetry (nanoDSF) and aggregation using size-exclusion chromatography (SEC). Binding affinity was tested against naturally folded CD72 on cell lines.

Results: Building on our previous work that highlighted CD72 as a promising CAR-T cell target in acute leukemia (Buldini, 2023), we are now developing specific binders to enable CAR-T cell targeting of CD72. The initial binder selection campaign began with a naïve library of 10^9 distinct proteins. Through iterative selection steps including two rounds of MACS, one round of ep-PCR, and four rounds of FACS, we enriched the library for CD72-binding clones. Plasmids encoding the selected variants were transformed into *E. coli* for sequencing. Sequence alignment and filtering were performed based on sequence-derived features, such as the absence of arginine, proline, and hydrophobic residues at key positions. This careful analysis led us to the identification of eight promising candidates. These proteins were expressed in soluble form and characterized for their biophysical properties. While they exhibited binding affinities in the mid-nanomolar range (40 nM < K_D < 200 nM), their thermal stability was suboptimal (35 °C < T_m < 50 °C). From this group, we selected three binders: clones A and B, which showed the highest affinity (A = 44 nM, B = 64 nM), and clone F, which displayed superior thermal stability (T_m = 50 °C). These best candidates were used as templates for further diversification via ep-PCR, generating second-generation libraries with approximately 10^7 variants per parent clone. Subsequent rounds of screening and selection yielded six optimized CD72 binders derived from the F-based library. These variants demonstrated enhanced affinity for endogenously folded CD72 on cell lines, improved thermal stability with no detectable aggregation, supporting efficient CAR expression in primary T cells.

Conclusions: This engineering platform enables the generation of novel CD72 binders with great affinity and stability, suitable for inclusion in CAR constructs. The newly developed CD72-specific binders are currently being incorporated into second-generation CARs for lentiviral transduction of human T cells. *In vitro* and *in vivo* studies are underway to evaluate the therapeutic potential of anti-CD72 CAR-T cells as a novel immunotherapy for pediatric AML.

Development of a lung organoid model for studying pulmonary diseases and safety assessment of RNA-based drugs

Ph.D. Student: Dr. Raquel MOLL DIAZ
TUTOR: Prof. Michela POZZOBON – CO-TUTOR: Dr. Paola BISACCIA
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background and Aim
The impact of RNA-based vaccines in the containment of the COVID-19 pandemic has provided direct evidence of how RNA-based drugs for specific targets can be rapidly and effectively developed. However, despite its beneficial application, their safety and immuno-toxicological profile must be assessed before its translation into the clinic. Lungs are highly involved in oxidative and inflammatory processes, where the presence of reactive oxygen species (ROS) could lead to oxidative stress and persistent inflammation, contributing to pulmonary diseases such as bronchopulmonary dysplasia, COVID-19 and chronic obstructive pulmonary disease. The importance to develop a drug safety platform to test emerging RNA-based drugs have become imperative. Therefore, a 3D model of lung organoid has been used for ***two specific aims***: the first one, the assessment of the safety and immunological profile of RNA-based drug candidates, as well as the use of potency assays. The second one, the use of the organoid platform for disease modelling to study the immunomodulatory activity of Mesenchymal Stromal Cell derived – Extracellular Vesicles (MSC-EVs) in oxidative-injured organoids to mitigate inflammation and cell damage.

Materials and Methods
A lung organoid model (LO) derived from adult stem cells was generated and used to test SARS-CoV2 Spike mRNA vaccine safety. The immunological compartment was added to the model using primary T lymphocytes. Moreover, an oxidative damage LO model was developed in parallel with hydrogen peroxide (H_2O_2) as a complementation to the study. EVs were administered and used as proof of principle to test the functionality of the model. Read-outs like immunofluorescence, gene expression analysis, immune system activation and viability assays were performed, along with assessments of cell death and proliferation.

Results
The characterization of LOs confirmed the presence of a mixed population of airway- and alveolar-like organoids by MUC5AC, β-TUB IV, SFTPC and RAGE gene and protein expression. mRNA vaccine preserved barrier integrity on LOs due to the presence of tight junctions (ZO-1). When the vaccine was administered the metabolic activity of cells was not compromised. An inflammatory response was observed by the upregulation of genes associated with pro-inflammatory pathways, such as *Tnf-α* and *Inf-y*. Additionally, the vaccine stimulated cell proliferation detected by an increase of Ki67+ cells. In parallel, the oxidative stress on LOs was proven by a reduced metabolic activity, the expression of an oxidative marker and the high proliferation of cells. Antioxidant proteins were markedly present after damage but significantly reduced after EVs treatment. qPCR analysis indicated a modulation of *Tnf-α*, and *Tgf-β* gene expression in damaged organoids, and a recovery after EVs, confirming their anti-inflammatory and anti-oxidative potential.

Conclusions
This *ex vivo* LO model could be useful for both disease development studies and as a drug safety platform to test new therapeutic strategies for several pediatric and adult pulmonary diseases linked to oxidative stress and inflammation.

A MULTIMODAL SINGLE-CELL STRATEGY TO ERASE JUVENILE MYELOMONOCYTIC LEUKEMIA

Ph.D. Student: Dr. Alberto PELOSO
TUTOR: Prof. Barbara BULDINI – CO-TUTOR: Dr. Silvia BRESOLIN, PhD
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background

Juvenile Myelomonocytic Leukemia (JMML) is a myeloproliferative neoplasm of early childhood, for which hematopoietic stem cell transplantation (HSCT) remains the only curative treatment. The disease is highly heterogeneous, involving mutations in *PTPN11*, *NRAS*, *KRAS*, *CBL* and *NF1*, which alone do not fully explain its different clinical outcomes. Transcriptomic and DNA methylation profiling have identified molecular subtypes with prognostic relevance, particularly the AML-like subgroup and cases with high DNA methylation levels. Despite these insights, the interplay between genetic, transcriptomic, and epigenetic layers remains poorly understood. Single-cell multi-omics provides a powerful tool to dissect this complexity by resolving tumor and immune cell populations within the bone marrow microenvironment. In particular, combining single-cell RNA sequencing (scRNA-seq) with single-cell chromatin accessibility profiling (scATAC-seq) enables the identification of regulatory programs driving disease progression and may ultimately reveal biomarkers and therapeutic targets.

Material and Methods

To investigate the cellular complexity of JMML, we performed combined single-cell transcriptomic and surface markers analysis of the bone marrow samples of six patients at diagnosis, using the BD Rhapsody platform. We set up a customized Seurat-based bioinformatic pipeline that allowed us to profile single cells by identifying their transcriptomic signatures and characterizing their maturation stage. Moreover, we assessed the transcriptomic and the chromatin accessibility profiles at single-nuclei level of JMML propagating cells obtained from PDX mouse models, using the BD Rhapsody scATAC-seq multiomic profiling and a Signac-based bioinformatic workflow.

Results

Single-cell transcriptomic data from 37,500 high-quality cells were projected onto a healthy bone marrow reference to map JMML cell identities across hematopoietic lineages. JMML cells were broadly distributed, ranging from stem and progenitor compartments to myeloid and lymphoid branches, with a marked enrichment in monocytic cells. A distinct population mapping to healthy erythroblasts exhibited elevated fetal hemoglobin gene expression and a fetal-specific gene signature. Cluster-specific markers revealed enrichment in early differentiation pathways, suggesting an immature, potentially disease-propagating phenotype. Integrated scRNA-seq and scATAC-seq analysis of JMML PDX samples revealed distinct clusters with unique transcriptional and epigenetic profiles, supporting the presence of multiple stem-like populations driving JMML progression.

Conclusions

In conclusion, this study presents the first comprehensive single-cell analysis of JMML bone marrow, integrating transcriptomic and chromatin accessibility data to reveal multilayered regulatory networks underlying disease pathogenesis.

EXPLORING THE CONTRIBUTION OF BONE MARROW ADIPOCYTES AS METABOLIC AND FUNCTIONAL MODULATORS IN PEDIATRIC ACUTE MYELOID LEUKEMIA

Ph.D. Student: Dr. Sara PERPINELLO
TUTOR: Prof. Martina PIGAZZI – CO-TUTOR: Dr. Claudia TREGNAGO
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background: Acute Myeloid Leukemia (AML) is a haematological disorder of the bone marrow (BM) and accounts for approximately 20% of pediatric leukemias. Emerging evidence highlights the pivotal role of resident BM cells in modulating AML pathogenesis and progression. Among these, mesenchymal stromal cells (MSCs) and BM adipocytes (BMAs) have emerged as major contributors to leukemic onset, influencing disease progression and therapy response.
Material and Methods: Primary MSCs (>10) were derived from the BM of AML patients at diagnosis and maintained *in vitro* in a non-differentiating (ND) medium (n=3) or underwent BMAs differentiation. Adipogenesis was assessed via morphological analysis (lipid/nuclei staining) and gene expression (qPCR). Gene expression profiling (Affymetrix U133 Plus 2.0 Array) was performed on ND AML-MSCs (n=21) or healthy BM-derived MSCs (h-MSCs, n=6) followed by Gene Set Enrichment Analysis (GSEA) to identify differentially expressed genes. AML blasts, transduced with luciferase (n=6), were cultured with BMAs, ND-MSCs, or palmitate as a single free fatty acid. We assessed AML proliferation (by luciferase activity, Ki-67, ATP production), total AML free fatty acids (FFAs) (BODIPY-FL, flow cytometry) and palmitate (BODIPY-C16, flow cytometry) uptake from BMAs, AML survival (by AnnexinV-7AAD staining) and stemness (by CFU assay) under various metabolic and cytotoxic conditions.
Results: Transcriptomic analysis revealed 608 differentially expressed genes between AML-MSCs (n=21) and h-MSCs (n=6), with 331 upregulated and 277 downregulated, indicating a distinct expression profile. GSEA uncovered a significant enrichment of gene sets related to fat cell differentiation (Gene Ontology (GO), NES=1.90), white adipocyte differentiation (Reactome, NES=2.06) and lipid storage (GO, NES=2.00) in AML-MSCs compared to h-MSCs. *In vitro*, both AML- and h-MSCs underwent efficient adipogenic differentiation in 2D culture (n=3), confirmed by lipid staining and qPCR analysis for *ADIPOQ*, *CEBPA*, and *FABP4* gene expression. However, when seeded in a 3D hydroxyapatite/collagen type I scaffold (70/30 wt%) and cultured in adipogenic medium, AML-MSCs exhibited enhanced BMAs differentiation (n=4), suggesting reprogramming toward a pro-adipogenic phenotype when cultured in a more physiological BM-like niche.
To investigate the functional role of BMAs, AML blasts were co-cultured for 7 days with BMAs or ND-MSCs. BMAs significantly promoted AML proliferation compared to ND-MSCs in both 2D (n=4, p=0.006) and 3D (n=3, p=0.048) culture conditions. Moreover, AML cells displayed increased uptake of total FFAs (n=6, p=0.007) or palmitate (n=4, p=0.007) released from BMAs compared to blasts co-cultured with ND-MSCs. This lipid uptake was partially inhibited by CD36 blockade, a FFAs transporter, using the SMS121 compound (p=0.0243), indicating that AML blasts internalized FFAs in a CD36-dependent manner. Additionally, under nutrient-deprived conditions (absence of glutamine, glucose or amino acids), AML blasts (n=3) significantly metabolized palmitate at 40h (p=0.034) and 48h (p=0.046). Furthermore, AML exposure to palmitate enhanced colony formation in semisolid media (n=2, p=0.002) even under cytotoxic (by 1µM cytarabine treatment) and mitochondrial stress (by 2µM oligomycin treatment), without significantly affecting ATP production, proliferation and overall blasts viability.
Conclusions: These findings warrant further investigation to confirm whether BMAs support metabolic reprogramming within the BM niche by preferentially sustaining leukemic stem cells rather than overall AML survival. The use of a 3D BM niche model represents a powerful and predictive platform to elucidate adipocyte–leukemia interactions and identify novel therapeutic strategies aimed at overcoming microenvironment-induced AML persistence.

Long-term assessment of myocardial and local arterial stiffness using shear wave imaging in pediatric patients treated with high doses cardiotoxic anthracyclines

Ph.D. Student: Dr. Alice POZZA
TUTORS: Prof. Luc MERTENS, Prof. Giovanni DI SALVO
CO-TUTOR: Dr. Olivier VILLEMAIN
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background: Anthracycline (AC) induced cardiotoxicity in childhood cancer survivors (CCS) is a significant concern and remains the most common non-oncological cause of premature death. Shear wave imaging is a novel, non-invasive method based on ultrafast ultrasound imaging (UUI) able to estimate myocardial stiffness throughout the cardiac cycle. Such methodology is well suited to the oncology population as the effects of long-term AC exposure are known to cause myocardial changes that increase stiffness.

Objectives: This prospective single center study at The Hospital for Sick Children aimed to assess the long-term effect of AC exposure on cardiac and local arterial stiffness estimated by induced acoustic radiation force shear waves (SW-ARF).

Material and Methods: We prospectively recruited 20 CCS (mean age at study 15±2.26 ys, 10 F) treated with AC ≥250 mg/m2 (mean AC cumulative dose 396.35±102.61mg/m2), having completed treatment since ≥5 years and 20 age-matched healthy volunteers (HV) (mean age at study 11.3±3.63 ys, 10 F). HVs and CCS underwent conventional echocardiography and UUI at the same day (mean age at study since cancer completion 10±4.01 ys). Pulse wave velocity (PWV) was measured in the left common carotid artery by direct pulse wave imaging using UUI at rest. Both PWV at the systolic foot (PWV-sf) and dicrotic notch (PWV-dn) were measured.

Results: In this preliminary analysis, we report descriptive data for the CCS cohort only, as post-processing of the HV group is still ongoing. Shear wave imaging was successfully performed in all 20 CCS participants. Myocardial stiffness assessed via SW-ARF speeds demonstrated a mean diastolic velocity of 2.31 ± 0.35 m/s and a peak systolic velocity of 3.27 ± 0.72 m/s. Local arterial stiffness assessed in the left common carotid artery showed a mean PWV-sf of 5.72 ± 0.70 m/s and PWV-dn of 8.05 ± 0.81 m/s. All measurements were feasible and reproducible in the paediatric oncology population.

Conclusions: This study demonstrates the feasibility of using shear wave imaging to non-invasively assess both myocardial and local arterial stiffness in CCS treated with high-dose AC. The observed values suggest detectable myocardial and vascular stiffness in this cohort several years post-treatment. While comparative analysis with healthy controls is ongoing, these preliminary findings support the potential of UUI techniques to serve as sensitive markers of long-term cardiovascular alterations in this high-risk population. Further statistical evaluation will clarify the degree of stiffness alteration relative to non-exposed peers.

Keywords: myocardial stiffness; ultrafast ultrasound imaging; anthracycline; children.

Possible mechanisms of relapse in the crosstalk between ALK+ ALCL and macrophages

Ph.D. Student: Rebekka Johanna Sabine SALZMANN
TUTOR: Prof. Lara MUSSOLIN
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background: Anaplastic lymphoma kinase-positive (ALK+) anaplastic large cell lymphoma (ALCL) is an aggressive peripheral T-cell lymphoma representing 10%-15% of pediatric lymphoid neoplasm. About 30% of pediatric ALK+ALCL are unresponsive or experience a relapse. We recently demonstrated that miR-146a-5p and YRNA4 are enriched in plasma small extracellular vesicles of patients with poor prognosis at diagnosis, and that that small-RNAs play an important role in macrophage polarization. Tumor-associated macrophages (TAMs) can either promote or inhibit tumor growth. Particularly alternatively activated TAMs can contribute to tumor progression. The aim is to investigate the effects of the crosstalk between macrophages and ALK+ ALCL cells. We focus on the underlying mechanisms of how tumor cells induce TAM-polarization and how TAMs in response influence ALK+ ALCL aggressiveness.
Material and Methods: We polarised THP-1 macrophages using IL4+IL13 M(IL4+IL13) and semi-quantitatively analyzed 40 inflammatory cytokines and chemokines. To study the crosstalk between ALK+ ALCL and macrophages we co-cultured ALK+ALCL cell lines with THP-1 monocytes or M(IL4+IL13). The co-culture was established using trans-well plates with a pore size that allows the exchange of the secretome only. The effect of the crosstalk between monocytes or M(IL4+IL13) and ALK+ALCL cell lines was characterized by using functional assays to test tumor cells aggressiveness and by gene expression profiling analysis. Moreover, we determined the levels of inflammatory cytokine and chemokines in the plasma of 27 patients at day of diagnosis.
Results: M(IL4+IL13) secrete higher levels of inflammatory cytokines and chemokines such as CXCL9, IL6, IL8 and TNFα than THP 1. After co-culture, ALK+ ALCL cell lines, Karpas299 and SupM2, induced polarization of THP-1 and M(IL4+IL13) towards CD163+CD14+ macrophages and of inflammatory cytokines such as IL6, CCL2 and TNFα. Co-culture with M(IL4+IL13) in turn increased Karpas299 and SupM2 migratory abilities and invasiveness, while decreasing clonogenic abilities. Moreover, the S-phase of the cell-cycle was shortened and the proliferative abilities when cultured in a matrix decreased. Gene expression profiling analysis showed a deregulation of genes in both Karpas299 and SupM2 co-cultured with M(IL4+IL13) that are enriched in IL1 regulation of the extracellular matrix, IL2 signaling, TNFα signaling via NF-κB, S100A8/9 complex and IL10 signaling. Both Karpas299 and SupM2 after co-culture with M(IL4+IL13) showed a deregulation in several genes such as TM4SF1, IL8, TNFα, EGR1, S100A8/9/12 and ESRP1. Moreover, at day of diagnosis patients that experienced relapsed within one year had significantly elevated plasma levels of IL6, IL8, IL15, CXCL9 and TNFα compared to patients under complete remission.
Conclusions: The polarization profile of THP-1 and M(IL4+IL13) upon co-culture with Karpas299 and SupM2 into CD163+CD14+ macrophages suggest a phenotype with both pro- and anti-inflammatory functions. Tumor cells in turn exhibit a more aggressive phenotype, reflected in increased migration, invasion, altered proliferation, and transcriptional changes involving key inflammatory regulators. The transcription factor EGR1 may mediate inflammatory and stress-related responses while TM4SF1 - a gene linked to cancer stem cell properties and metastasis – might play a role in relapse and resistance. The balance between the pro- and anti-inflammatory modulation of TAMs and the subsequent modulation of the tumor microenvironment could be a contributor to resistance or early relapse in ALK+ ALCL. To further validate our findings, experiments utilizing primary macrophages are currently underway. These ongoing studies aim to confirm the results observed in cell lines and provide more clinically relevant insights into disease mechanisms.

Gene Therapy in Primary Coenzyme Q10 Deficiency

Ph.D. Student: Dr. Giulia TOFFANIN
TUTOR: Prof. Eva TREVISSON – CO-TUTOR: Dr. Cristina CERQUA
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Oncohematology, Medical Genetics, Rare Diseases and Predictive Medicine"

Background

Coenzyme Q (CoQ) is an essential lipid that is formed by a quinone group, derived from 4-hydroxybenzoate (4-HB), and a polyisoprenyl tail. It is essential for the electron transport from mitochondrial complexes I and II to complex III, driving ATP synthesis, and is one of the main cellular antioxidants. Primary CoQ deficiency is caused by mutations in the genes required for CoQ biosynthesis, named *COQ* genes. Clinical conditions associated with primary CoQ deficiency are heterogenous and include multisystem disorders, encephalopathies and steroid-resistant nephrotic syndrome (SRNS), with predominantly pediatric onset. CoQ deficiency is considered one of the few treatable mitochondrial disorders since patients often respond well to oral CoQ supplementation. However, the response differs among patients, probably due to its extreme hydrophobicity, and new treatment options are needed. I investigated the supplementation with 4-HB analogues and gene therapy in a mouse model of CoQ deficiency, the *Pdss2*$^{kd/kd}$ mice, that present a mutation in *Pdss2*, one of the CoQ proteins, and manifest the SRNS. CoQ ring analogues, such as 2,4 dihydroxybenzoic acid (2,4-diHB) and vanillic acid (VA), have a good bioavailability and can enhance endogenous CoQ biosynthesis. Instead, gene therapy protocol consists in replacing the missing enzyme, Pdss2, by injecting an adeno-associated vector (AAV), restoring the genetic defect of *Pdss2*$^{kd/kd}$ mice.

Material and Methods

For the first experimental approach, WT and *Pdss2*$^{kd/kd}$ mice were supplemented with 2,4-diHB (1g/kg/day) or VA (400 mg/kg/day) at postnatal day 30 (p30) For the second experimental approach, *Pdss2*$^{kd/kd}$ mice were injected with an AAV9 containing *mPdss2* (AAV9-*Pdss2*), at a dose of 10^{12} vg/mouse, by tail vein at P30. A cohort of these mice was used to analyse their survival and urine proteinuria. Another cohort was sacrificed at 5 months, at an end-stage of disease, for tissue collection to perform kidney histology, biochemical analyses and measure the AAV9 biodistribution.

Results

Pdss2$^{kd/kd}$ mice supplemented with 2,4-diHB showed a significantly increased survival and decreased proteinuria, compared with untreated mice. However, 2,4-diHB supplementation did not rescue CoQ_9 levels and CoQ-dependent complexes activity in kidneys of 5 months old mice. Kidney electron microscopy results showed the typical signs of collapsing glomerulopathy with foot processes effacement in *Pdss2*$^{kd/kd}$ mice, while WT and *Pdss2*$^{kd/kd}$ mice treated with VA or 2,4-diHB had normocellular glomeruli with a conserved structure and lack of foot processes effacement. Regarding the gene therapy approach, the analysis of the AAV9-*Pdss2* biodistribution revealed a very low kidney transduction efficiency (~1 vg/nucleus), also confirmed by the quantification of Pdss2 protein levels through Western Blot. Moreover, the proteinuria levels were similar in uninjected and injected *Pdss2*$^{kd/kd}$ mice.

Conclusions

2,4-diHB supplementation, but not VA, significantly increases survival and decreases proteinuria of *Pdss2*$^{kd/kd}$ mice. However, 2,4diHB and VA supplementation does not rescue CoQ9 content and activity of MRC complexes I+III and II+III in kidney. AAV9-Pdss2 is not efficient in transducing the kidney.

PhD COURSE "DEVELOPMENTAL MEDICINE AND HEALTH PLANNING SCIENCES"

COORDINATOR: PROF. GIANNI BISOGNO

Curriculum "HEALTH PLANNING SCIENCES"

Bacterial and Fungal Difficult to Treat Infection in Children

Ph.D. Student: Dr. Lorenzo CHIUSAROLI
TUTOR: Prof. Vincenzo BALDO - CO-TUTORS: Dott. Daniele DONÀ, Prof. Carlo GIAQUINTO
Ph.D. Course in Developmental Medicine and Health Planning Sciences
Curriculum: "Health Planning Sciences"

Background. Difficult-to-treat bacterial and fungal infections are a growing concern in paediatrics, particularly in onco-hematological patients who are immunocompromised due to chemotherapy, neutropenia, and frequent hospital exposure. These patients face a high risk of severe infections, prolonged hospital stays, and poor outcomes. The rise of multidrug-resistant (MDR) bacteria, such as ESBL-producing *Enterobacterales*, carbapenem-resistant organisms, and MRSA, limits effective antibiotic options and complicates treatment. Similarly, invasive fungal diseases (IFDs) caused by *Candida* and *Aspergillus* species remain life-threatening, especially with emerging resistance like *Candida auris* or azole-resistant *Aspergillus*. Despite increasing awareness, data on these infections in children are limited and inconsistent. This PhD projects aims to examine current practices and challenges in managing difficult-to-treat bacterial and fungal infections in children, with a focus on onco-hematological patients and those affected by MDR pathogens.

Material and Methods. This research program aims to evaluate the impact of fungal and bacterial infections in hospitalized children and in primary care settings by describing their epidemiological features and clinical outcomes. Specifically, it will analyze hospitalization rates, length of hospital stay, and key clinical outcomes such as morbidity and mortality associated with fungal and multidrug-resistant (MDR) bacterial infections. To address these important aspects, the project proposes the implementation of an integrated assessment model for pediatric fungal infections within each hospital ward. This would involve establishing a specific clinical pathway, supported by retrospective data collection on the epidemiology of fungal infections in both hospitalized and primary care pediatric populations. Additionally, the study will examine the overall burden of fungal and MDR bacterial infections across the Pediatric Department, with the goal of establishing a prospective registry for infectious diseases in hospitalized children. As a starting point, we have conducted a review of current literature and expert opinions on this topic.

Results. During the first two years of my PhD, I conducted a review of current literature on multidrug-resistant (MDR) bacterial infections (**Chiusaroli**, 2025, Expert Opinion on Pharmacotherapy, [DOI: 10.1080/14656566.2025.2519690]; **Chiusaroli**, 2022, Antibiotics, [DOI: 10.3390/antibiotics11081088]) and fungal infections (systematic review in progress) to establish a comprehensive starting point. Building on this, we examined the overall epidemiology of fungal infections in the paediatric population (**Chiusaroli**, 2025, Pathogens, [DOI: 10.3390/pathogens14010093]) and the epidemiology of bacterial infections (Cocchio S, … **Chiusaroli**, 2025, Vaccines, [DOI: 10.3390/vaccines13030230]). We then focused on the prevention of fungal infections in onco-hematological patients within our department, specifically analyzing antifungal prophylaxis: in a retrospective study, we compared posaconazole and liposomal amphotericin B prophylaxis, and proposed a targeted antifungal strategy (**Chiusaroli**, 2025, Journal of Antimicrobial Chemotherapy, [DOI: 10.1093/jac/dkae479]). In parallel, we participated in a multicenter retrospective study of bacterial infections in onco-hematological patients, in collaboration with a national network (Zama D, … **Chiusaroli**, 2025, British Journal of Haematology, accepted for publication). From these foundations, we launched two prospective studies: one on fungal infections as part of the international registry FUNGISCOPE and one on MDR bacterial infections through the Italian Society of Paediatric Infectious Diseases (SITIP), specifically focusing on Ceftazidime–Avibactam treatment in Gram-negative infections (ESCAPE study, ongoing)

Conclusions: The initial findings from this broad research program highlight critical data on the epidemiology, management, and prevention of bacterial and fungal infections in children—especially those with onco-hematological conditions. These results support the development of tailored **preventive strategies and clinical models** that can be applied in daily paediatric infectious disease practice.

PhD COURSE "MOLECULAR MEDICINE"

COORDINATOR: PROF. ARIANNA LOREGIAN

Curriculum "BIOMEDICINE"

IDENTIFICATION OF DETERMINANTS OF BREAST CANCER METASTATIC DORMANCY

Ph.D. Student: Dr. Farouk ABOUDOU
TUTOR: Prof. Marco MONTAGNER – CO-TUTOR: Prof. Sirio DUPONT
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background

Late relapses of cancer prolong the course of the disease and increase the burden on patients. Dormancy is a widely investigated hypothesis explaining this clinical phenomenon. While cells depend on intrinsic machinery for decision making, evidences pointing at the microenvironment as dormancy regulator are accumulating: biochemical cues and mechanical components of ECM were shown to affect tumour cell growth across models and setups. We have studied breast cancer dormancy in the context of lung metastasis. In vivo, a transcriptomic experiment showed the upregulation of autophagy-lysosomal pathway in our model of disseminated dormant cancer cells DDCCs in the lungs. In vitro, we showed that DDCCs cultivated on lung organotypic system have increased lysosomal gene expression and accumulation of putative lysosomes. Culture on biomimetic substrates of low stiffness recapitulated the activation of lysosomal program. This outlines the relevance of ECM mechanical properties in DDCC biology. We set out to dissect the contribution of matrix stiffness in the activation of autophagy lysosomal pathway and the consequence of putative lysosomes accumulation on DDCC quiescence.

Material and Methods

D2.0R and D2A1 are murine models of breast cancer dissemination to the lungs. D2.0R remain quiescent over weeks while D2A1 are more aggressive after tail vein injection. D2 cells were cultivated on substrates of different stiffness. We investigated the genetic determinants for the lysosomal pathway activation by performing loss and/or gain of function experiments on key transcription factors. We took advantage of LC3-GFP-mcherry autophagy reporter to analyse the autophagy flux. We performed the DQ-BSA assay to test for lysosome hydrolytic activity and proteomic experiments on whole protein extract, and immunoprecipitated lysosomal content to characterize lysosome products in the two cell lines. We performed growth assays in conditions of up/down regulation of lysosomes for correlation analysis.

Results

Measurements on lysosome markers i.e. Lamp1 and Lysotracker increased in D2 cells on soft culture substrates. We observed decreased mRNA levels of many lysosomal genes under siRNA Tfeb and Tfe3, master regulators of autophagy and lysosome biogenesis. Immunofluorescence results were not compatible with Tfeb/3 activity suggesting the contribution of other mechano-regulated transcription factor(s). In CHiPseq database by "Hammal et al. 2022", we identified Yap as a potential regulator of lysosomal genes. DQ-BSA experiment on soft substrates showed higher specific signals in quiescent cells as compared to aggressive cells; the analysis of lysosomal content revealed the absence of number of endogenous hydrolases in aggressive cells. At last, the inhibition of lysosomes acidification with bafilomycin showed proliferation arrest and death in both cell lines.

Conclusions

Our data suggest that DDTC cells rely on lysosomal pathway activation for survival. Such response was observed on soft substrates downstream Yap/Taz inhibition. We observed impairments in lysosomes functionality in aggressive cells suggesting the presence of alternative pro survival and/or pro proliferation signals outweighing death signals. The nature of these alternative signals would further be investigated.

NEUROATTENUATED ONCOLYTIC HSV-1 ARMED WITH A PHOTOSENSITIZER PROTEIN FOR TARGETED GLIOBLASTOMA THERAPY

Ph.D. Student: Maria Vittoria FORNAINI
TUTOR: Prof.ssa Arianna CALISTRI – CO-TUTOR: Prof. Fabio MAMMANO
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background

Glioblastoma (GBM) is one of the deadliest human brain tumors. It is classified as a grade IV glioma by the World Health Organization (WHO); it accounts for approximately 57% of all gliomas and has one of the worst 5-year overall survival rates of any human cancer. Current treatments, such as chemotherapy and radiotherapy associated to chirurgical resection, are unsuccessful due to tumor resistance and relapses, which are common and severe. Alternative and promising therapeutic treatments that have been validated and extensively studied in preclinical and clinical trials are virotherapy based on the use of oncolytic viruses (OVs) and photodynamic therapy (PDT). Oncolytic viruses (OVs) are either genetically engineered or naturally occurring replication competent viruses that can selectively replicate in and destroy cancer cells. OV kill tumor cells by direct lysis and by stimulating a specific anti-tumoral immune response. On the other hand, photodynamic therapy (PDT) has received considerable attention as a therapeutic treatment for cancer and other diseases. PDT is a minimally invasive anti-tumor therapy that eradicates tumor cells through a photosensitiser-mediated cytotoxic effect upon light irradiation.

Material and Methods

In order to combine OV and PDT for the treatment of GMB, we modified the FDA and EMA approved oHSV Talimogene Laherparepvec (T-VEC) used for cancer treatment in patients with melanoma, by introducing the target sequences for a miRNA highly expressed in neuronal cells (miR-124) fused to the HSV-1 UL29 transcript, which encodes for a protein (ICP8) essential for viral lytic replication. Furthermore, the T-VEC genome was enriched with sequences encoding the reporter protein mCherry, the cellular membrane targeted killer red (mem-KR) or the mitochondria targeted KR (mt-KR) within the UL55-UL56 intergenic-region. Upon irradiation, KR should stimulate the production of reactive oxygen species (ROS) and add to the direct killing activity of the virus towards cancer cells. The replication/killing capacity of the recombinant viruses was tested in different murine and human GBM cell lines as well as patients-derived cells, both in 2D and in 3D version. Furthermore, the combined effect of PDT and OV was also assayed by adopting the same experimental models upon irradiation. Finally, the ability of primary human and murine monocytes to deliver oHSV1 to GBM cells was tested, with the aim of developing a novel approach for the systemic delivery of the oncolytic agent.

Results

Our results indicate that, while the developed oHSV-1 are attenuated in primary neurons and human brain organoids, they efficiently replicate and kill the different tested GBM cells, including the patients-derived ones. When we analyzed the effect of KR in patients-derived cells, first we found that the protein expression per se does not impair cellular growth. Next, we demonstrated that photoactivation of oHSV-1 infected cells (588 nm laser, 1 hour with 500 msec pulse) increases cell mortality when the virus expresses mem-KR. Finally, we were able to show that monocytes efficiently deliver oHSV-1 to GBM cells, thus representing a useful tool for the systemic administration of the oncolytic agent.

Conclusions

Our data pave the route to the testing of oHSV-1 expressing mem-KR in vivo in the mouse model.

PRE-EXISTING AUTOANTIBODIES AGAINST TYPE I INTERFERONS PREDICT SEVERE DISEASE IN PATIENTS WITH WEST NILE VIRUS INFECTION

Ph.D. Student: Dr. Camilla LUCCA
TUTOR: Prof. Luisa BARZON – CO-TUTOR: Prof. Marta TREVISAN
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background
West Nile virus (WNV) is an arthropod-borne neurotropic *Orthoflavivirus* primarily maintained in an enzootic cycle between mosquitoes and birds. Mosquitoes can incidentally transmit the virus to humans, which are dead-end hosts. The majority of WNV human infections are asymptomatic or associated with mild symptoms, but less than 1% of cases develop a severe neuroinvasive disease (WNND). So far, risk factors associated with severe WNV disease are still to be completely outlined. A recent study revealed that pre-existing neutralizing autoantibodies (auto-Abs) against type I interferons (IFNs) were present in about 40% of patients with WNND and in about 15% of patients with mild symptoms. The aim of this study was to analyze the prevalence of anti-IFN-α IgG auto-Abs in serum/plasma samples from a large cohort of WNV infected individuals and to investigate the association between such auto-Abs and demographic parameters, clinical presentation, virological data and serum cytokine/chemokine profile.
Material and Methods
A total of 654 subjects were included in the study, of which 34% with a laboratory-confirmed WNV lineage 1 (WNV-1) infection and 43% with WNV lineage 2 (WNV-2) infection. The study population was composed by asymptomatic blood donors (asympt., 12%), outpatients with West Nile fever (WNF, 25%), hospitalized cases of West Nile fever (hWNF, 21%) and patients with WNND (42%). Anti-IFN-α auto-Abs were detected in serum/plasma samples collected within two weeks from symptom onset or index blood donation, by using both an in-house developed ELISA assay and a commercial ELISA kit (Thermo Fisher Scientific). A panel of 34 cytokines and chemokines in serum/plasma samples were tested by using a ProcartaPlex assay based on Luminex technology.
Results
In the total cohort, the prevalence of anti-IFN-α auto-Abs significantly increased with disease severity (asympt., 1.28%; WNF, 6.06%; hWNF, 17.3%; WNND, 32.7%) ($p<0.0001$) and the antibody rate was significantly higher in males (25.3%), than in females (7.66%) ($p<0.0001$); while no significant difference was observed in patients infected with WNV-1 (20.7%) and WNV-2 (23.2%). In WNND patients, the prevalence of anti-IFN-α auto-Abs was significantly higher in patients infected with WNV-2 (47.5%), than in those with WNV-1 infection (28.7%) ($p=0.0051$). Moreover, we demonstrated that WNV-1 infection and pre-existing auto-Abs are two independent co-factors associated with disease severity. Cytokine and chemokine profiling in serum/plasma samples revealed that the levels of IL-4, IP-10 and TNFβ were significantly higher in patients with WNND than in patients with mild symptoms or in asymptomatic blood donors. Also, these cytokines were significantly higher in patients with WNND and auto-Abs, if compared with patients with WNND but without auto-Abs.
Conclusions
The presence of anti-IFN-α auto-Abs correlates with disease severity in WNV infected patients. The prevalence of such auto-Abs was significantly higher in males than in females and in patients infected with WNV-2 than in patients with WNV-1 infection. Finally, we observed that the levels of IL-4, IP-10 and TNF-β were significantly increased in patients with WNND and auto-Abs.

Development of a non-infectious assay for rapid screening of antiviral entry/fusion inhibitors

Ph.D. Student: Dr. Lorenzo LUPI
TUTOR: Prof. Alfredo GARZINO DEMO – CO-TUTOR: Prof. Arianna CALISTRI
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background: Most human infectious diseases originate from animals. While human-wildlife contact was once limited, globalization has dramatically increased these interactions, making worldwide novel pathogen spread via travel and commerce much more likely. This urgent situation highlights the need for high-throughput assays to screen antiviral compounds, especially those targeting the often-overlooked viral entry step. To meet this need, we developed a new fusion assay. It uses two cell line, one expressing hACE2 and the other SARS-CoV-2 Spike protein. When these cells fuse, a GFP signal becomes visible, and this fusion can be blocked by antivirals and neutralizing antibodies.
Material and Methods: hACE2 cell line were transduced with lentiviral vector encoding for GFP11, while 293T cells were subsequently transduced with lentiviral vectors encoding for GFP1-10 and SARS-CoV-2 Spike protein. After been transduced, cells were single cell sorted accordingly to the expression of the desired transgene to generate the final cell lines. ACE2 GFP11 were mixed with the same number of Spike GFP1-10 and the fusion was monitored in real time by fluorescent microscopy and plate reader or analyzed by flow cytometry.
Results: To verify the complementation of the two GFP subunits, 293T cells were transfected with a plasmid encoding for a single GFP subunits, or both plasmids. Only cell transfected with both plasmids emitted a fluorescence signal, indicating the complementation of the two domains. The expression of the GFP subunits and receptors from the generated cell lines was confirmed by flow cytometry. Mixing them generate cluster of cells one hours post mixing (hpm) and an observable GFP signal at 2 hpm. Fused cell, analyzed by flow cytometry, were characterized by the presence of bigger and more internal complex cells compared to the original cell lines. To test if the fluorescence can be measured with a plate reader, a different number of cells were mixed in a 96-well plate. 18 hours post mixing the plate was measured with a plate reader. The measured fluorescence signal increased accordingly to the number of cells only in well containing both cell lines. Three different neutralization antibodies were incubated with Spike GFP1-10 cells and next mixed with the same number of ACE2 GFP11 cells into a 96-well plate. The assay was able to discriminate the different neutralization potencies of the antibodies. To test if the developed assay could be adopted for a high throughput screening of a 2300 compound library, Z factor was determined using 100 µg/mL of Covi-17-LS antibody and resulted 0.66, an ideal value for a biological assay. Furthermore approximatively 800 compounds of the library were tested. Additionally, the developed assay was adopted to validate the entry inhibitor abilities of different compounds. To improve the versatility of the developed assay, Dengue 2 genome were retro-transcribed and the sequence encoding for the signal peptide, prM and Env Gene was cloned into lentiviral plasmid to generate lentiviral vectors. 293T cells expressing GFP11 were transduced with the generated lentiviral vector and single cell sorted to generate cell lines expressing Dengue 2 Env gene.
Conclusions: We developed a novel fusion assay, based on split GFP system, to evaluate the entry potency of antibodies and compounds. The assay is composed by two cell lines, one expressing hACE2 and GFP11 and the other SARS-CoV-2 Spike and GFP1-10. After mixing cells they rapidly fuse each other and start emitting a GFP signal. The assay was optimized to 96-well plate allowing for a high throughput screening 800 compounds. Finally, thanks to its plasticity, we are adapting the assay to Dengue 2.

IDENTIFICATION OF BREAST CANCER ANTIGENS

Ph.D. Student: Dr. Daniele MAFFEI
TUTOR: Prof. Stefano PICCOLO – CO-TUTOR: Prof. Paolo CONTESSOTTO
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background

Breast cancer (BC) is the most common and deadliest cancer in women worldwide, accounting for 670,000 deaths in 2022. BC is clinically subcategorized into 4 molecular subtypes having peculiar features, different therapeutic strategies and distinct prognosis, eventually. Luminal A and Luminal B are the most prevalent subtypes and are associated with a more favourable clinical outcome, whereas triple negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (HER2) positive subtypes are correlated with a more aggressive tumor behaviour and poorer prognosis.

Targeted therapies, antibody-drug conjugates and immunotherapies have now reached the clinical practice, complementing first-line treatments such as surgery, chemotherapy, radiotherapy. The efficacy of immunotherapies and the correlation between the number of tumor infiltrating lymphocytes (TILs) and response to these treatments have prompted scientists to look for the development of other immune-based therapies. Accordingly, this project is focused on the identification of breast cancer antigens to be leveraged for the development of innovative therapies for BC patients.

Material and Methods

Identification of breast cancer candidate antigens is based on the comparison between BulkRNAseq data obtained from epithelial cells sorted from the mammary gland of wild-type FVB/NJ female mice and primary breast cancer cells obtained from MMTV-PyMT in FVB/NJ female mice, a model of TNBC. Genes that resulted overexpressed in cancer were then filtered using ENCODE, a public dataset, for selecting only those with human homologues and not expressed in any other healthy human tissue. Candidate antigens have been then overexpressed in target cells (primary murine breast cancer cells and primary murine mammary epithelial cells) which, upon immunogenic cell death or senescence protocols, have been injected in wild-type syngeneic mice in a vaccination strategy. This setting aims at proving their ability to induce an antitumor immunity when mice are subcutaneously challenged with cancer cells.

Results

The 22 candidate antigens have been tested in mixes of 3 genes each by overexpressing them in primary murine breast cancer cells, resulting in the identification of 2 mixes able to induce an antitumor immunity manifested by the reduction of both volume and weight of the subcutaneous masses, with respect to control. Genes were then tested individually in the same settings and 3 of them proved to be immunogenic enough to cause a reduction in tumor growth. Of note, the reduction in tumor burden detected when testing mixes of 3 genes was higher than the effect obtained with individual overexpression.

Conclusions

Our approach has led to the identification of 22 candidate breast cancer antigens, 6 of which proved to be efficacious in a vaccination setting when tested *in vivo*. Moreover, when tested individually, 3 genes showed enough immunogenicity to reduce tumor growth. To assess whether the immune response is exclusively dependent on the overexpressed antigens rather than other tumor cell–specific factors, we are testing whether healthy epithelial cells, upon overexpression of single genes and application of an immunogenic cell death protocol, could work as vaccinating entity.

IDENTIFICATION OF NON-CANONICAL NUCLEIC ACID STRUCTURES AND THEIR INTERACTING PROTEINS IN VIRUS-INFECTED CELLS AS NOVEL ANTIVIRAL TARGETS

PH.D. STUDENT: Dr. Filippo MATTELLONE
TUTOR: Prof. Sara RICHTER – CO-TUTOR: Prof. Ilaria FRASSON
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background

Flaviviruses are a genus of positive-sense single-stranded RNA (ssRNA) viruses that include several clinically relevant human pathogens, such as West Nile virus (WNV). In recent years, the ability of arboviruses to cause large-scale outbreaks has made them a major public health concern. Despite their significant burden, effective antiviral therapies against flaviviruses remain limited, highlighting the need for a better understanding of their biology and pathogenesis.

Guanine quadruplexes (G4s) are non-canonical nucleic acid structures formed by guanine-rich sequences that fold into stacked planar tetrads. These structures are present in both human and pathogens genomes where they play key roles in the regulation of gene expression and genome replication. In the last decade, genome-wide mapping strategies for G4s has revealed G4 enrichment in human gene promoters and their correlation with increased gene transcription in several cell lines.

Growing evidence suggests that viruses can modulate host chromatin organization and DNA secondary structures, possibly including G4s, to exploit the cellular machinery for their own replication. Therefore, chromatin rearrangements and G4 dynamics may play a critical role in the host-virus interaction network.

Material and Methods, and Results

To investigate virus-induced changes at the level of the host chromatin, CUT&Tag – an innovative chromatin immunoprecipitation technique coupled with sequencing – was successfully established in a human liver cancer cell line (Huh-7) infected with WNV. The analysis focused on two histone modifications, H3K4me3 and H3K27me3 – markers of open and close chromatin, respectively – as well as G4s, during the critical time frame between viral entry and the onset of viral replication.

WNV RNA levels in Huh-7 cells were quantified by RT-qPCR at hourly intervals up to 12 hours post-infection (h.p.i.) to define the temporal dynamics of infection. Based on this, 1 and 2 h.p.i. were selected to capture early host responses to viral entry, while 6 h.p.i. marked the moment of initiation of active viral replication.

The CUT&Tag approach revealed dynamic chromatin remodeling upon infection, allowing the identification of G4-enriched genomic regions involved in the host-virus interaction network. Specifically, the formation of new G4s and the disappearance of pre-existing G4s at gene promoters highlighted the dynamism of the host G4-landscape.

Given the key role of promoters in regulating gene expression, its was investigated how G4 changes influence transcription by integrating CUT&Tag results with transcriptomic data obtained through RNA-seq performed on samples collected at the same time points. The most responsive genes to G4 alterations were primarily involved in stress responses, RNA metabolism, and translation-related pathways.

Conclusions

These findings underscore the importance of G4 dynamics in the cellular response to WNV infection and suggest that G4s may represent promising targets for the development of novel antiviral strategies.

CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS: CHARACTERIZATION OF VECTOR-VIRUS INTERACTIONS

Ph.D. Student: Dr. Michele PACCAGNELLA
TUTOR: Prof. Cristiano SALATA – CO-TUTOR: Prof. Ignazio CASTAGLIUOLO
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background
Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a tick-borne pathogen that presents a serious threat to human health, causing a severe hemorrhagic fever with high mortality rate. Ticks, notably those of the genus *Hyalomma*, serve as the principal vector for CCHFV, harboring the virus persistently and acting as its natural reservoir. However, due to biosafety concerns, limited knowledge is available regarding the virus-vector interaction. Host cycling likely contributes to the virus's evolutionary flexibility, however cross-species transmission imposes selective constraints, influencing viral adaptation. This study explores the evolutionary progression of Hazara virus (HAZV), utilized as a model for CCHFV, across different host cell lines, aiming to elucidate how pressure within host environment drives viral adaptation and impacts infectivity.
Material and Methods
HAZV was grown on human SW13 cells and used to infect *Hyalomma*-derived tick cells (HAE/CTVM8) for 30, 60 days. This first passage was used to perform four additional passages on fresh HAE/CTVM8 cells, each lasting 30 days. We evaluated both phenotypic and genotypic changes by infecting new HAE/CTVM8 cells for 5 days and SW13 for 3 days. Phenotypic changes were assessed by evaluating viral infection in ticks and SW13 cells while genotypic changes by NGS sequencing.
Results
Through analysis of HAZV propagation in HAE/CTVM8 cell line, we determined the emergence of mutations within all three viral genome segments. Notably, the passage of HAZV within this cell line appears to lead to greater stabilization of few mutations, two within the gene encoding the RdRp (Q490Q, G2112R) and four in the glycoprotein precursor (GPC) (A381T, A802T, E849K, K1415K). HAZV-adapted to tick cells demonstrated a host-dependent effect on viral infection efficiency, with higher infectivity in HAE/CTVM8 compared to mammalian cells. Conversely HAZV from SW13, employed as a control, exhibited the opposite trend.
Conclusions
Our results showed that mutations appear during the first 30 days and increase slowly until 60 days up to 30-40%. With the following passages in tick cells, mutations on the RdRp increased from 15-30% to 80-95% while on the GPC from 10-20 to 50-75% suggesting a host drive adaptation. Viral replication kinetics indicate that tick-adapted HAZV replicate better in tick cells, suggesting a potential role of the selected mutations in viral adaptation to the invertebrate host. Although genetic changes in passaged HAZV were minimal they seem to lead to an increased relative fitness and replicative ability of the virus in the homologous HAE/CTVM8 cell line. Further experiments will be performed after isolation of mutated viruses.

"This research was supported by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT)"

HUMAN CYTOMEGALOVIRUS INFECTION INDUCES A SENESCENCE-LIKE PHENOTYPE IN NEURAL STEM CELLS

Ph.D. Student: Dr. Elisa POLI
TUTOR: Prof. Arianna LOREGIAN – CO-TUTOR: Prof. Marta TREVISAN
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background – Human cytomegalovirus (HCMV) represents the most frequent cause of congenital malformations in newborns. HCMV congenital infection (cCMV) involves primarily the central nervous system (CNS), specifically targeting the neural stem cell (NSC) population of the developing brain and impairing neurogenesis. Since virus-induced senescence (VIS) has recently emerged as a pathogenetic mechanism in HCMV-infected renal epithelial cells, this study was aimed at investigating whether a similar process occurs in NSCs, potentially contributing to the onset of brain disorders associated with cCMV infection.

Material and Methods – NSCs were generated from human embryonic stem cells (hESCs) and characterized for the expression of stage-specific markers, *i.e.* SOX2, NESTIN, and PAX6, through immunofluorescence assays and quantitative Real Time PCR (qPCR). NSCs were infected with the HCMV TR strain at a multiplicity of infection (MOI) of 1 and analyzed at 3 and 6 days post-infection (dpi). For the evaluation of the senescence phenotype, several assays were performed, including SA-β-gal activity staining, 5-ethynyl-2'-deoxyuridine (EdU) incorporation assay, analysis of senescence markers expression (*i.e*., p53, $p16^{INK4A}$, p21, and LaminB1) by Western blot, analysis of senescence-associated genes (*i.e., Ki67*, *IL-6*, *IL-8*, *LaminB1*, and *TNF-α*) by qPCR, and analysis of cytokines released in cell supernatants through ELISA assays.

Results – NSCs infected with HCMV TR strain showed a significant induction of the SA-β-gal expression as early as 3 dpi, which further increased at 6 dpi, compared to mock-infected cells. Accordingly, EdU incorporation was significantly reduced at 6 dpi in infected NSCs when compared to mock-infected cells, supporting the hypothesis that HCMV infection drives a senescence phenotype in these cells. Furthermore, the cell cycle regulators p53, $p16^{INK4A}$ and p21 were significantly upregulated in HCMV-infected NSCs. Notably, p53 and $p16^{INK4A}$ protein levels were increased throughout the course of infection, whereas p21 increased only at the earliest time point in infected cells. Conversely, LaminB1, a protein of the nuclear membrane, was significantly downregulated at 6 dpi. Additionally, HCMV-induced senescence was further analyzed by qPCR. While the expression levels of *LaminB1* and *IL-6* remained unchanged between HCMV-infected and mock-infected cells, a significant decrease was observed in *Ki67* expression at 6 dpi, indicating a reduction in cell proliferation. Conversely, *TNF-α* and *IL-8* expression were significantly upregulated in infected NSCs. These results were supported by the analysis of IL-8 release in cell supernatants which was significantly increased, compared to mock-infected conditions, with a peak observed at 3 dpi. Similarly, a significant release of IL-6 from infected cells was observed at the earliest stage of infection.

Conclusions – These findings corroborate the hypothesis that HCMV infection drives a senescence-like program in NSCs, characterized by a decrease in cell proliferation, altered expression of senescence hallmarks, and a pro-inflammatory transcriptional profile.

Bacterial EVs: Big Impact on Microbial Communication and Host Interaction

Ph.D. Student: Dr. Mahmoud SHALATA
TUTOR: Prof. Paola BRUN – CO-TUTOR: Dr. Giulia BERNABE`
Ph.D. Course in Molecular Medicine
Curriculum: "Biomedicine"

Background
Bacterial Extracellular Vesicles (bEVs) are proteoliposomal nanoparticles released by both Gram-negative and Gram-positive bacteria. Ranging typically from 25 to 250 nm in diameter, they are comprised of and contain proteins, lipids, nucleic acids, and other biomolecules, such as quorum sensing (QS) molecules, derived from their parent bacterium. At first, they were thought to be merely cellular debris from bacterial cells, but they are now understood to play important roles in bacterial behaviour and interactions with other microbes and host organisms. bEVs act as a way for bacteria to transport and deliver materials, shielding these materials from being broken down, supporting optimal conditions for enzymes to work, and enabling the movement of substances over longer distances. In this study, we focused on bEVs released by *Pseudomonas aeruginosa,* which is a clinically significant opportunistic human pathogen, frequently implicated in chronic infections in vulnerable individuals, such as patients with cystic fibrosis and individuals with surgical site infections.

Materials and Methods
bEVs in this study were isolated from cultures grown overnight by ultracentrifugation, followed by an enrichment step in which size exclusion chromatography (SEC) was employed. Morphological and size characterisation of isolated EVs was then performed using techniques such as transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). Downstream processing of the bEVs was utilized to characterise the composition of cargo. This includes western blot (WB) for the identification of protein markers, enzyme-linked immunosorbent assay (ELISA) for the evaluation of bEVs' inflammatory and host interaction, and QS-specific biosensors for the assessment of bEVs as a communication system.

Results
Our findings start by determining the size and concentration of *P. aeruginosa* bEVs by TEM and NTA, respectively, followed by a confirmation of the presence of bEVs-based outer membrane marker called OprF by WB. Downstream processing confirmed the variability of the number of bEVs and the presence of a QS-molecule called Pseudomonas quinolone signal (PQS), and the variability of PQS production as well as the concentration of bEVs by the manipulation of the surrounding environment, such as the use of antibiotics and the addition of QS molecules. Finally, our results confirmed that the manipulation of the immune system by the isolated bEVs, through the detection of inflammatory and anti-inflammatory cytokines release.

Conclusion
P. aeruginosa EVs are complex biological nanoparticles with diverse and significant functions in microbial communities and the host interface. They serve as crucial mediators for cell-to-cell communication, especially for hydrophobic signalling molecules like PQS, by enabling their widespread distribution and stability. Furthermore, the increased level of *P. aeruginosa* EVs after antibiotic treatment suggests that bEVs can act as decoys, contributing more to the antimicrobial resistance dilemma. Finally, the ability of these vesicles to modulate the immune system, separate from the parent cell, provides strong evidence that they are not synthesised in an arbitrary fashion but rather in a complex and highly organised manner.

PhD COURSE "MOLECULAR MEDICINE"

COORDINATOR: PROF. ARIANNA LOREGIAN

Curriculum "REGENERATIVE MEDICINE"

SpaceTrooper: an R package for preprocessing and quality control of imaging-based spatial omics data

Ph.D. Student: Dr. Benedetta BANZI
TUTOR: Prof. Silvio BICCIATO – CO-TUTOR: Prof. Mattia FORCATO
Ph.D. Course in Molecular Medicine
Curriculum: "Regenerative Medicine"

Background: While single-cell RNA sequencing (scRNA-seq) enables high-resolution transcriptomic profiling, it requires tissue dissociation, thereby losing spatial context. In contrast, spatial omics preserves the native tissue architecture, providing critical insight into gene expression within the spatial microenvironment, a key factor in regulating key physiological and pathological processes. However, imaging-based spatial omics introduce unique analytical challenges that scRNA-seq-adapted pipelines do not fully address. In particular, quality control has been largely overlooked, and there is no standardized approach nor consensus on how to integrate expression- and spatial-derived metrics to reliably detect technical artifacts and low-quality cells. To overcome these limitations, we developed SpaceTrooper, a fully R-based package that offers an integrated framework with standardized data loading methods, robust, quantitative tools for quality control, and tailored visualization for imaging-based spatial omics data across the three major commercial platforms.
Material and Methods: SpaceTrooper implements two complementary quality control strategies. The first uses distribution-aware statistical testing on continuous metadata features to flag outlier cells, enabling the detection of numerically aberrant cells often associated to technical artifacts. The second computes a composite quality score using a multiple linear regression model designed to account for dataset- and platform-specific variability. This score, scaled with a sigmoid transformation for interpretability, integrates spatial and molecular metrics to capture subtle artifacts such as boundary effects and non-specific antibody binding, without requiring prior knowledge. The pipeline was validated on publicly available datasets spanning three commercial platforms, two omics assays, two species and four tissue types.
Results: Application of SpaceTrooper to the DBKERO CosMx Breast Cancer dataset revealed aberrant cell boundaries flagged as cell area outliers, which were independently confirmed as segmentation errors by the FastReseg pipeline. In the same dataset, the composite quality score reliably identified low-quality cells that impacted clustering results while preserving genuine biological heterogeneity. The workflow was further validated on the Xenium human lung cancer and MERFISH mouse liver datasets, where it effectively detected poorly sampled cells without introducing bias toward specific tissue structures or cell types. Finally, SpaceTrooper was applied to a CosMx human tonsil protein dataset, demonstrating that the quality score formula could be adapted to account for technology-specific artifacts, thereby improving detection of low-quality cells while maintaining its original capabilities.
Conclusions: SpaceTrooper is a fully R-based framework that provides standardized data loading, specialized quality metrics, robust quantitative tools for quality control, and computationally efficient visualization of imaging-based spatial omics. It effectively detects a wide range of artifacts, including segmentation errors, platform-specific issues, and low-quality cells that could otherwise bias downstream analysis, while preserving the dataset's biological diversity and structural integrity. Fully integrated into the Bioconductor ecosystem, SpaceTrooper offers a streamlined, all-in-one solution for analyzing imaging-based spatial omics data. The package is freely available at: https://github.com/bicciatolab/SpaceTrooper

PhD COURSE "ONCOLOGY AND IMMUNOLOGY"

COORDINATOR: PROF. ANTONIO ROSATO

Detection of Molecular Diagnostic Biomarkers in Early Hepatocellular Carcinoma (HCC) Using Liquid Biopsies

Ph.D. Student: Dr. Marah AMER
TUTOR: Prof. Stefano INDRACCOLO – CO-TUTOR: Prof. Diego BOSCARINO
Ph.D. Course in Oncology and Immunology

Background: Hepatocellular carcinoma (HCC) is often diagnosed at advanced stages due to the lack of effective early screening tools, resulting in poor survival outcomes. Common screening methods like AFP testing and imaging have limited sensitivity, while tissue biopsies are invasive and may not reflect tumor heterogeneity. Liquid biopsy, particularly circulating cell-free DNA (cfDNA), has emerged as a promising non-invasive alternative, timely molecular information. Recent studies highlight that specific somatic mutations and copy number variations in cfDNA, especially within key driver genes such as *TP53*, *CTNNB1*, and *TERT*, serve as potential early biomarkers for HCC. Targeted next-generation sequencing (NGS) of cfDNA allows for high-resolution detection of somatic mutations, enabling associated-biomarker investigation, earlier diagnosis and personalized treatment strategies in HCC.

Material and Methods: 36 genes linked to early-stage hepatocellular carcinoma (HCC) were selected from literature and COSMIC, cBioPortal, and KEGG databases. ctDNA was extracted from 9 plasma samples (8 HCC, 1 HBV). Three reference standards with 0.1%, 0.5% VAF, and wild-type, covering 10 variants across 6 overlapping genes, were included. Quality was checked using TapeStation, Qubit, and qPCR across fragment sizes (75/150, 150/300, 75/300 bp). Libraries were prepared using a UMI-based cfDNA protocol (10 ng input), pooled to 600 ng, enriched by hybrid capture, and sequenced on NovaSeq 6000. Sequencing data were processed with fgbio tool for deduplication and error correction, and variants were annotated via VarSome Clinic.

Results All extracted cfDNA samples had an average concentration of 0.2 ng/µL, with a peak fragment size around 200 bp. Sequencing yielded raw coverage between 11,000X and 30,000X, and deduplicated depth between 800X and 3,000X, highest depths were observed in the reference standards. qPCR fragment analysis, indicated high DNA integrity and minimal degradation across all samples. Coverage uniformity exceeded 98%, duplication rates were above 77%, and GC content was over 45%, consistent with expectations for UMI-based cfDNA libraries. All variants in the 0.5% VAF standard reference were detected in IGV, with allele frequencies between 0.2% and 0.6%. In contrast, only three variants from the 0.1% VAF standard reference were identified at 0.1 allele frequency. Variant annotation is currently ongoing and will be reported in detail in September.

Conclusions
This study demonstrated that the targeted cfDNA sequencing is effective in detecting low-frequency variants associated with early-stage HCC, supporting by high DNA quality and sequencing depth. Future work will expand the sample cohort and integrate additional molecular analyses, such as miRNA profiling and CNV detection to enhance understanding of HCC biology and improve early, non-invasive diagnosis strategies.

Early HBV and HDV kinetics in patients undergoing liver transplantation

Ph.D. Student: Dr. Sara BATTISTELLA
TUTOR: Prof. Francesco Paolo RUSSO – CO-TUTOR: Prof. Sabela LENS
Ph.D. Course in Oncology and Immunology

Background: Hepatitis B virus (HBV) graft infection occurs after liver transplantation (LT) despite viral source removal and prophylactic treatment. We aimed to elucidate mechanisms and timing of HBV/hepatitis delta virus (HDV) reinfection by analysing early viral kinetics in serum and tissue.

Material and Methods: Serial serum and liver samples were prospectively collected peri- and post-transplant in 12 HBV (5 HDV coinfected) LT recipients with and without hepatocellular carcinoma (HCC). Kinetics of different viral markers were analysed.

Results: HBV-DNA, HDV-RNA, HBsAg and HDAg were detected in all liver explants, while cccDNA was only detectable in HBV monoinfected patients. Residual serum HBV-DNA was detectable before LT in 5 patients but became undetectable a few hours after the anhepatic phase. HBsAg levels were low (<3logIU/mL) in HBV monoinfected patients and cleared fast (within 5 days) in those receiving hepatitis B immune globulin (HBIG) compared to those who did not (within 6 months). In HDV coinfected patients (all receiving HBIG), HBsAg clearance was fast (within 12 hours) if baseline HBsAg <3logIU/mL and slow (within 6 months) if baseline HBsAg >3logIU/ml. HDV-RNA kinetics paralleled that of HBsAg. Post-transplant biopsies, obtained at reperfusion, 3 and 12 months after LT, tested negative for all viral markers (including cccDNA), except for one patient with detectable intrahepatic HDV-RNA and HDAg at 3 months post-LT with undetectable HBV and HDV serum markers.

Conclusions: Despite varying kinetics of HBsAg clearance following LT, we did not detect intrahepatic HBV markers at different time points following transplantation. In contrast, HDV could be sporadically detected in the liver graft, highlighting the need to evaluate new prophylactic strategies in HDV coinfected patients awaiting LT.

DISTINCT DENDRITIC CELL SUBSETS DRIVE SEQUENTIAL T CELL PRIMING PHASES

Ph.D. Student: Enrico DOTTA
TUTOR: Prof. Giulia PASQUAL – CO-TUTOR: Prof. Antonio ROSATO
Ph.D. Course in Oncology and Immunology

Background
The initiation of the adaptive immune response relies on interactions between dendritic cells (DCs) and T cells during the priming phase. DC–T cell interactions must be precisely coordinated to ensures the initial engagement of rare antigen-specific T cell clones and the sustained stimulation of activated T cell. However, despite their pivotal role in shaping adaptive immunity, the dynamics of DC–T cell interactions remain poorly characterized, largely due to technical limitations and the lack of suitable tools for visualizing and studying this process *in vivo*.
In this project, we overcame this limitation investigating DC–T cell interactions *in vivo* using an enzymatic labelling system, enabling the characterization of the interacting dendritic cell populations involved in a vaccine-induced immune response.
Materials and Methods
This study was conducted entirely in mouse models. DC–T cell interactions were analyzed using a transgenic mouse strain enabling enzymatic labeling of cells upon physical contact *in vivo* (uLIPSTIC mouse model). To elicit a strong immune response, mice were immunized in the hind footpad with ovalbumin (OVA) adsorbed in alum, following adoptive transfer of naïve OVA-specific T cells. We investigated the dynamics of DC–T cell interactions by harvesting draining lymph nodes at multiple time points post-immunization and applying a combination of techniques, including flow cytometry, microscopy, and single-cell transcriptomics.
Results
Our results reveal a temporally coordinated dynamic of DC–T cell interactions following immunization. We identified a distinct peak in interaction frequency at 72 hours post-immunization. Importantly, this increase was not primarily due to the accumulation of dendritic cells or naïve T cells within the lymph node. Instead, the peak of interactions was driven by the re-engagement with DCs of proliferating T cells that had been activated during the initial priming phase. These findings revealed the existence of two distinct phases during the T cell priming: an early phase mediated by naïve T cells and a later phase involving activated, replicating T cells.
Further analyses suggest that these phases involve phenotypically and functionally distinct DC subsets. In the early, antigen-dependent phase, T cells primarily interact with CD301b^{+} conventional dendritic cells, which are enriched in chemokine and cytokine production, supporting T cell recruitment and initial activation. In contrast, the later phase is characterized by antigen-independent interactions with a CD301b^{-} cDC2 subset, distinguished by elevated expression of costimulatory molecules such as CD40 and CD70, features that promote sustained T cell activation and differentiation.
Conclusions
Our findings uncover a biphasic model of T cell priming, in which distinct dendritic cell subsets mediate sequential interactions. In the late priming phase, antigen-independent wave of DC–T cell engagement suggests a critical role for non-cognate signals in sustaining T cell activation and shaping immune responses. To confirm the functional relevance of these interactions and the involvement of specific ligand–receptor axes, we are currently employing knockout models and blocking assays to investigate changes in T cell activation during both the early and late phases of priming.

MICRO-RNA AS A DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN ORAL SQUAMOUS CELL CARCINOMA

Ph.D. Student: Dr. Piergiorgio GAUDIOSO
TUTOR: Prof. Piero NICOLAI – CO-TUTOR: Prof. Marco FERRARI
Ph.D. Course in Oncology and Immunology

Background
Oral squamous cell carcinoma (OSCC) is one of the most frequent head and neck cancers (HNC) worldwide. Despite improvements in therapeutic strategies and the introduction of multimodal treatment, the 5-year survival rate remains less than 50%. In this respect, the research interest is oriented toward improving early primary and recurrence diagnosis, and identification of factors predicting when intensification of treatment is needed.
Liquid biopsy is a laboratory test analyzing biomarkers, such as microRNAs (miRNAs), in body fluids (blood, saliva, breast milk, urine). This non-invasive approach is receiving increasing global interest as a precise diagnostic tool, providing new opportunities for detecting and monitoring cancer.
In recent years, salivary miRNAs have been described as a potential diagnostic and prognostic tool in OSCC. In 2021, Romani et al. carried out global profiling of salivary miRNAs through a microarray approach, while signature validation was performed by quantitative real-time PCR (RT-qPCR). A stringent statistical approach for microarray and RT-qPCR data normalization was applied. Salivary miR-106b-5p, miR-423-5p, and miR-193b-3p resulted in accurate OSCC detection, and their combination provided a satisfactory diagnostic performance (ROC – AUC = 0.98). Moreover, high expression of miR-423-5p was an independent predictor of poor DFS, and a significant decrease in miR-423-5p expression was observed in matched post-operative saliva samples.
This prospective study aims to investigate the potential role of liquid biopsy as a further instrument to manage OSCC, with a particular focus on the salivary miRNAs panel proposed by Romani et al.
Material and Methods
We conducted a prospective study enrolling patients with primary OSCC, confirmed by transoral biopsy and treated with curative intent. Exclusion criteria included a history of synchronous or prior HNC, previous radiotherapy to the head and neck region, treatment with non-curative intent, or inadequate salivary samples. Patient recruitment was carried out across three tertiary referral centers. Salivary samples were collected postoperatively, prior to any adjuvant radiotherapy, and subsequently at 3, 6, and 12 months following the completion of treatment. Comprehensive clinical, pathological, radiological, and follow-up data were recorded. Salivary miRNA profiling was performed to investigate its potential as a prognostic and surveillance biomarker.
Results
The study started on July 1st, 2024. To date, forty-seven patients have been enrolled. Four patients were excluded due to inadequate preoperative salivary samples. Among the included patients, twelve have experienced disease recurrence. Salivary miRNA profiling is currently ongoing.
Among the preoperative salivary samples, five were selected for exploratory analysis using shallow whole genome sequencing (sWGS). All five samples passed the integrity assessment, and tumor-derived genomic material was successfully isolated, accounting for 3.8% of the total genomic content. These findings demonstrate the feasibility of the procedure on salivary samples.
Conclusions
These preliminary findings support the feasibility of salivary liquid biopsy for genomic analysis in OSCC. Ongoing miRNA profiling may further elucidate its role in prognosis and disease surveillance.

MULTIPLEX IMMUNOHISTOCHEMISTRY STUDY OF TUMOR MICROENVIRONMENT IN EARLY AND LATE-ONSET ORAL CAVITY CANCER

Ph.D. Student: Dr. Chiara GOTTARDI
TUTOR: Prof. Valentina GUARNERI – CO-TUTOR: Dr. Maria Grazia GHI
Ph.D. Course in Oncology and Immunology

Background Early-onset oral cavity squamous cell carcinoma (OCSCC) seems to present a peculiar clinicopathological profile and risk factors compared to late-onset OCSCC. No major differences have been found in the genetic profile of young and old patients. Other factors such as the immune microenvironment have been suggested to play a role in the pathogenesis and prognosis. We investigated possible differences in tumor microenvironment (TME) composition and checkpoint expression between early and late-onset OCSCC, and their impact on patients' outcomes.

Material and Methods We retrospectively collected clinicopathological data of 464 OCSCC patients surgically treated between 2011 and 2021 at the Istituto Oncologico Veneto/AOPD in Padova (Italy) and at Cliniques Universitaires Saint Luc in Brussels (Belgium). Patients aged 50 years or less were categorized as early-onset ("young"), while patients older than 50 years were grouped as late-onset ("old"). For TME analysis, the expression of T-cells markers (CD3, CD8 and FoxP3) and immune-checkpoints (PD-L1 and IDO) were evaluated by multiplex Immunohistochemistry (mIHC) in 91 patients. The Mann Whitney test and Chi-square test were used for descriptive statistics. Spatial analysis (nearest neighbor distances G-function) was performed to assess the tumor infiltration of T cells around tumor cells, and the probability of cell-cell interaction. Outcomes of interest were locoregional failure free survival (LRFFS) and overall survival (OS). LRFFS was defined as the time from curative surgery to locoregional relapse or death for any cause; OS was defined as time from cancer diagnosis to death for any cause. The Kaplan-Meyer method was used for survival analysis.

Results Among 91 patients with available mIHC data, 29 were considered as young and 62 as old. Sixty-three percent of patients showed strong exposure to traditional risk factors (RF) such as tobacco smoke and alcohol abuse. Median CD3+ T-cell density was 1659,267 cells/mm2 for young and 996,687 cells/mm2 for old patients (p=0,055). No differences were found for CD3+/CD8+ T-cell median densities in the two groups (p=0,227). CD3+/FoxP3+ density was lower in young patients, although the difference was not statistically significant (p=0,067). PD-L1 expression was significantly higher in young patients (p=0,023), whereas no significant difference was observed for IDO (p=0,120). When stratified by RF, young patients exposed to traditional RF had significantly higher PD-L1 expression than old ones (p=0,008) while young non-smokers and non-drinkers showed a tendency towards higher IDO expression (p=0,052). According to spatial analysis reconstructions, young patients exhibited higher rates (69% vs 36%) of tumors with more intense CD3+ T-cell infiltrate, indicating a higher probability of interactions between CD3+ T-cells and tumor cells (p=0,0037). No statistically significant differences in LRFFS and OS were observed between young and old patients in relation to T-cells densities or PD-L1 and IDO expression.

Conclusions Early-onset OCSCC appears to have higher T-cell infiltrates compared to late-onset tumors. Higher PD-L1 expression and a trend towards lower CD3+/FpxP3+ T-cell densities were also observed in young patients. Furthermore, in this group of patients, traditional RF seem to modulate the TME composition. Nevertheless, these differences didn't show any significant impact on patients' outcomes. Since these data didn't show robust differences between the TME of young and old OCSCC patients, we decided to shift the focus of the project on the study of TME composition in breast cancer, as part of the "Metastasis as mechanodisease" project, which aims to develop a Mechanoscore for metastatic breast tumors.

IMPROVING ASSESSMENT OF CANCER-RELATED COGNITIVE IMPAIRMENT

Ph.D. Student: Dr. Giulia OLIVA
TUTOR: Prof. Pierfranco CONTE– CO-TUTOR: Prof. Stefano INDRACCOLO
Ph.D. Course in Oncology and Immunology

Background Cancer-related cognitive impairment (CRCI) involves cognitive decline linked to cancer or its treatments, significantly impacting quality of life. Although research is expanding, there is no consensus on the best neuropsychological tests to detect CRCI, with patients often reporting cognitive symptoms not captured by formal tests. This underscores the need for a holistic approach and brief, reliable tools to identify at-risk patients and support targeted interventions. Moreover, statistical methods to determine cognitive change over time need to be improved to support accurate long-term monitoring. To address these gaps, three main projects are ongoing: I) Study 1 aims to develop a new CRCI neuropsychological battery, collect normative data, and establish cut-off scores; II) Study 2 focuses on assessing cognitive and psycho-physical symptoms in breast cancer patients via online questionnaires and computerized tasks, developing a brief online self-report questionnaire, and comparing online with traditional in-person assessments to support hybrid monitoring models; III) Study 3 aims at improving statistical methods to assess change over time, particularly focusing on Crawford approach (Crawford & Grathwaite, 2006).
Material and Methods Study 1: Healthy female participants are completing the CRCI battery in person or via videoconference. Its psychometric properties are being evaluated in subgroups, including construct validity, inter-rater agreement, test-retest reliability after one month, and correlation between administration modes. Study 2: Breast cancer patients will complete computerized cognitive tasks and online questionnaires, including a new online tool assessing psychological and physical symptoms. Participants will also undergo standard in-person neuropsychological and clinical evaluations. The study will use network analysis to explore complex interactions among symptoms and identify key interconnected factors. Study 3: A simulation study is being developed to extend the Crawford & Grathwaite (2006) method, which estimates whether a change in test scores is meaningful, in order to apply the method at untested timepoints using assumptions about practice effects and reliability.
Results Study 1: the new neuropsychological test battery was developed based on international oncology guidelines and key tests identified in our prior umbrella review. It assesses cognitive domains commonly impaired in breast cancer patients (learning, memory, processing speed, attention, executive functions, lexical access) and can be administered both in-person and remotely, taking about one hour. The normative data collection is ongoing with 17 participants enrolled (Age: M=41.72 years, SD=17.28; Education: M=15.55 years, SD=3.26). Adequate psychometric properties are expected (e.g., $\alpha > 0.7$, test-retest > 0.8, ICC > 0.6; correlation between formats > 0.85; convergent validity > 0.6). Study 2: an online questionnaire was developed, brief (5-10 minutes), and suitable for self-administration. This includes 4 subscales, assessing: subjective cognitive symptoms, general psychological symptoms (anxiety, depression, distress), illness-related psychological symptoms (body image, illness uncertainty, coping), psycho-physical symptoms (sleep disturbances, fatigue, pain, physical symptoms). While this study is under evaluation from the Ethical Committee, a preliminary analysis of content validity is being conducted by administration to a panel of 10 experts to assess items relevance and their clarity. Two grant applications were also submitted for related projects involving a mobile app and an AI chatbot for monitoring quality of life and treatment adherence in oncology patients.
Conclusions These integrated projects aim to provide valid, scalable and time effective tools to support hybrid, patient-centred CRCI assessment. Ongoing data collection and future analyses will guide refinement and clinical translation of these tools.

THE PATHOLOGICAL ROLE OF NON-CODING RNA IN T-CELL LARGE GRANULAR LYMPHOCYTE LEUKEMIA

Ph.D. Student: Dr. Elisa RAMPAZZO
TUTOR: Prof. Renato ZAMBELLO – CO-TUTOR: Dr. Antonella TERAMO
Ph.D. Course in Oncology and Immunology

Background
T-cell Large Granular Lymphocyte Leukemia (T-LGLL) is a rare and chronic disorder marked by clonal LGLs expansions and significant biological and clinical heterogeneity. STAT3-activating mutations, common in symptomatic cases, support leukemic survival and repress miR-146b, contributing to the development of neutropenia. The involvement of other micro RNAs and emerging data on circular RNAs (circRNAs) suggest that somatic mutations may affect non-coding RNA networks. While circRNAs play roles in many diseases, their function in T-LGLL is still unknown. This study aims to profile circRNAs expression across disease subgroups and assess the impact of miR-146b restoration, toward RNA-based therapeutic strategies and improved understanding of the disease's molecular landscape.

Material and Methods
CircRNAs were identified and quantified using CirComPara2, with selected results validated by RT-qPCR and Sanger sequencing in an independent cohort. Additionally, LGLs from STAT3 WT patients were cultured with IL-6 (20 ng/mL), and those from STAT3-mutated patients with the STAT3 inhibitor STATTIC (3.5μM) for 24 hours. For miR-146b restoration, CD57+ LGLs from six CD8+ STAT3-mutated patients and CD8+ cytotoxic T lymphocytes (CTLs) from six healthy donors (HD) were purified and electroporated with either a miR-146b mimic or scramble control. After 24 hours, viability was assessed by Annexin V/PI staining, and RNA was extracted to confirm transfection efficiency, followed by RNA-sequencing.

Results
For circRNAs characterization, a total of 5,948 circRNAs (p-adj<0.05) were identified and quantified, with 358 showing differential expression in neutropenic STAT3-mutated patients compared to STAT3 WT cases, among these, the overexpression of circSETBP1, circBNC2, circZBTB46, and circPVT1 were validated in an independent cohort. In addition, IL-6 stimulation of STAT3 WT LGLs enhanced STAT3 phosphorylation (p<0.001) and upregulated circZBTB46 and circPVT1 (p<0.01), mimicking expression levels in STAT3-mutated cells. Conversely, STATTIC treatment in STAT3-mutated samples restored normal circRNAs levels (p<0.05), supporting a link between STAT3 activation and circRNAs dysregulation.
MiR-146b overexpression was effectively induced in both HD and T-LGLL samples (p<0.01), with ~60% cell viability at 24h post-transfection. In HD, the transfection caused no major transcriptomic changes, while T-LGLL cells showed marked alterations between scramble and miR-146b conditions (2,404 DEGs). The miR-146b restoration normalized known targets, notably ELAVL1, linked to T-LGLL-related neutropenia. Oncogenic and inflammatory genes, including IL32 and IL2RB, were downregulated to HD-like levels. Notably, CCL5, promoting LGL proliferation via IL-6, was significantly reduced (p<0.01). Additional analyses disclosed that several signaling pathways, including TNFα signaling via NF-κB, IL-6/JAK/STAT3 signaling, inflammatory responses and viral carcinogenesis were enriched among the genes downregulated upon miR-146b restoration.

Conclusions
Altogether, the identification of a STAT3-dependent circRNAs signature offers new insights into T-LGLL pathogenesis and progression. Additionally, miR-146b restoration results support it as a promising strategy for T-LGLL, capable of reversing disease-specific alterations without affecting normal CTLs. Ongoing studies aim to develop a nanolipid-based delivery system engineered to selectively target leukemic LGLs via CD57 binding.

Exploiting hyaluronan as a natural and effective immunological adjuvant for protein/peptide-based vaccines against HER2-expressing breast cancer

Ph.D. Student: Dr. Valentina ROSSI
TUTOR: Prof. Antonio ROSATO PhD, MD – CO-TUTOR: Dr. Debora CARPANESE
Ph.D. Course in Oncology and Immunology

Background
HER2-expressing breast cancers account for approximately 20% of all breast cancer cases and are typically associated with aggressive clinical behaviour and poor prognosis. In this context, anti-HER2 vaccines may present a promising alternative or complementary therapeutic strategy, with the potential to target early disease stages, prevent metastasis, and improve long-term patient outcomes overcoming hight cost, toxicity risk and emergence of resistance associated to current clinical therapies. However, cancer vaccines face the dual challenge of breaking immune tolerance to self-antigens and inducing durable memory, with protein/peptide platforms, though safe and easily produced, still requiring strong adjuvants. In this context, hyaluronan (HA), a biocompatible glycosaminoglycan, has recently gained attention for its immunomodulatory properties: indeed, HA fragments of approximately 200 kDa have demonstrated intrinsic adjuvant capabilities when covalently conjugated to protein antigens eliciting robust, durable immune responses.
Material and Methods
Two main mouse models were used: Balb/c mice, administered with syngeneic tumoral rHER2/neu-positive cells according to preventive or therapeutic setting, and Balb/NeuT mice that spontaneously develop rHER2/neu-expressing carcinomas, used as pre-cancerous model. In preventive and pre-cancerous settings, both models received either rHER2/HA bioconjugates or rHER2 protein formulated with aluminium hydroxide, and were, for effectiveness assessment, respectively challenged on day 30 or monitored in spontaneous tumour development. In therapeutic settings, mice were injected with tumour cells 12 days before the first dose. *In vitro* assays comprised IgG subclasses and cytokine profile characterization. Functional responses were evaluated via ^{51}Cr-release in complement/antibody-dependent cytotoxicity, as well as inhibition of cell growth quantification and mediation of antibody-dependent cellular phagocytosis. Additionally, *in vivo* depletion of B and T cells was conducted to define the role of different immune subsets in tumour control. According to structural HER2 analysis, multiple cross-species immunogenic HER2-conserved peptides were identified, then conjugated to HA for immunogenicity testing.
Results
HA-conjugated vaccines elicited strong and durable immune responses across all experimental settings, exhibiting mainly a Th1-polarized response. Antigen-specific antibodies remained functional up to one-year post-vaccination, showing remarkable results in inducing antigen-specific anti-tumoral effector functions. In the preventive model, HA-based protein vaccines conferred 100% survival, while in the therapeutic setting, partial tumour regression was observed in 2 out of 12 mice. Notably, it effectively broke tolerance to the rHER2/neu antigen and significantly delayed tumour onset in precancerous model. Furthermore, i*n vivo* depletion studies confirmed the involvement of both B and T lymphocytes in the antitumor response. Preliminary data from HER2-peptide-based vaccines identified three immunogenic candidates, currently investigated as in combinatorial formulations.
Conclusion
HA-based vaccines represent a promising strategy for inducing robust, long-lasting immune responses using low antigen doses, particularly in contexts where conventional vaccines may be less effective as low responding patients, or for relapse prevention.

ESTABLISHMENT AND CHARACTERIZATION OF PATIENT DERIVED XENOGRAFTS MOUSE MODEL OF MALIGNANT PLEURAL MESOTHELIOMA FOR THE EVALUATION OF NOVEL TARGETED THERAPIES

Ph.D. Student: Dr. Sara SANTI
TUTOR: Prof. Antonio ROSATO – CO-TUTOR: Dr. Anna DALLA PIETÀ, PhD
Ph.D. Course in Oncology and Immunology

Background
Malignant Pleural Mesothelioma (MPM) is a rare, asbestos-related tumor characterized by limited therapeutic options and poor prognosis. As current treatments fail to significantly extend patient survival, the development of novel targeted approaches supported by robust preclinical models is urgently required. The aim of this study is the investigation of two targeted therapeutic strategies: a Hyaluronic Acid-Paclitaxel (HA-PTX) bioconjugate targeting CD44 receptor, and an Antibody-Drug Conjugate (ADC) directed against the molecule TROP-2. Meanwhile, Patient-Derived Xenograft (PDX) models are being established to accurately reproduce the molecular and histopathological features of the original tumors.
Material and Methods
The expression of the two target receptors CD44 and TROP-2, HA-PTX binding, uptake, and apoptosis induction were evaluated in MPM cell lines by flow cytometry. Cytotoxicity was measured via ATP-release assays, and confocal microscopy confirmed HA-PTX internalization and microtubule disruption. *In vivo* efficacy of targeted therapies was assessed in both human and syngeneic MPM models, after treatment with HA-PTX or the anti-TROP-2 ADC injected via locoregional or intravenous route. Tumor progression was monitored by bioluminescence imaging, alongside assessment of treatment impact on overall survival. Surgical tumor tissues from MPM patients were subcutaneously implanted into NSG mice and expanded through serial passaging (P0-P1-P2-P3), with histological validation performed using H&E staining, immunohistochemistry, and multiplex immunofluorescence at each stage. GeoMx spatial transcriptomics analysis will be performed in order to compare PDXs with matched primary tumors.
Results
MPM cell lines showed high CD44 expression (~100%) and variable TROP-2 expression ranging from 10% to 80%. *In vitro* cytotoxicity assay demonstrated that HA-PTX was 10 to 70 times more potent than the control in three out of four MPM cell lines tested, inducing both microtubule disruption and apoptosis. Lastly, the preliminary *in vivo* studies showed that the treatment with HA-PTX and the anti-TROP-2 ADC significantly inhibited tumor growth and improved survival in MPM-bearing mice.
With regards to the PDX model establishment, to date, 33 patient-derived tumor samples have been implanted. One PDX has reached P3 over a two-year period and has been successfully stabilized. Histological analysis confirmed the maintenance of the original biphasic mesothelioma histotype and the expression of key mesothelioma markers (calretinin, cytokeratin 5/6, podoplanin, and WT-1). Transcriptomic analysis is currently underway. Seven out of the 33 samples are currently growing at P0, while the remaining samples either failed to engraft or, in four cases, gave rise to lymphoma-derived PDXs.
Conclusions
Overall, *in vitro* and *in vivo* results highlight the therapeutic efficacy of HA-PTX and the anti-TROP-2 ADC, supporting their potential use as effective targeted treatments for patients with MPM. Moreover, these preliminary findings demonstrate the feasibility of establishing MPM PDX models, despite low engraftment rates and extended stabilization times. The successful development of the first stabilized PDX underscores the value of these models as a preclinical tool for drug evaluation and for further *in vivo* investigation of HA-PXT and the anti-TROP-2 ADC.

Dissection of Heme Oxygenase-1's Role in Ferroptosis and Immune Suppression Induced by Bone Marrow Derived Macrophages

PhD Student: Dr. Olga SLUKINOVA
Tutor: Prof. Susanna MANDRUZZATO - Co-tutor: Dr. Sara ZUMERLE
Ph.D. Course in Oncology and Immunology

Background Ferroptosis is a recently described cell death modality mediated by iron overaccumulation and lipid peroxidation (LPO). Current research links ferroptosis to both classical and alternative activation in macrophages depending on the model and the context. Our group has shown that bone marrow-derived macrophages in the glioblastoma microenvironment are strongly immune suppressive and highly express heme oxygenase-1 (HO-1) - a key enzyme in heme catabolism. Inhibition of HO-1 by zinc protoporphyrin IX (ZnPPIX) reprograms tumor macrophages, reducing their pro-tumoral phenotype including immune suppressive activity and marker expression. Given HO-1's central role in regulating cellular iron levels, we investigated its role in the ferroptosis process. Additionally, we explored macrophages' susceptibility to ferroptosis, and how modulating this process might influence immune suppression.

Materials and Methods *In vitro* differentiated M2-like macrophages from healthy donors were used to induce ferroptosis and in the presence of HO-1 modulators. The IC50 of the ferroptosis inducer RLS3 was assessed under normoxic and 2% O2 hypoxic conditions using an MTS assay; LPO was evaluated via flow cytometry using the BODIPY-C11 fluorescent probe in treatments with DFO (ferroptosis inhibitor), RSL3 and ZnPPIX in normoxia and for RSL3 and ZnPPIX in hypoxia for comparison. Molecular mechanisms were studied by detection of selected proteins (HO-1, Bach1) by Western blotting; additionally, free Fe2+ intracellular levels were measured using the FerroOrange fluorescent probe; the functional activity of macrophages was assessed by the proliferation assay in co-culture with CellTrace-stained activated PBMC; RNASeq analysis of macrophages treated with ZnPPIX versus untreated was performed, followed by investigation of ferroptosis-related gene signatures.

Results *In vitro* differentiated macrophages are more susceptible to ferroptosis induced by RSL3 under hypoxia than normoxia ($p<0.05$), and exhibited high LPO levels, peaking after 1 hour at comparable levels in both conditions. The iron chelator DFO abrogates RSL3–induced LPO in normoxia ($p<0.01$), while HO-1 inhibition with ZnPPIX significantly reduced LPO in both normoxia and hypoxia ($p<0.001$), whether immediately before ferroptosis induction or after 24 hours rest ($p<0.01$). HO-1 level tends to increase after 4 hours of RSL3 treatment. Interestingly, ZnPPIX significantly increases levels of HO-1 after 2, 4 and 24 hours of treatment ($p<0.05$) and reduces Bach1 after 2 hours. Macrophages undergoing ferroptosis do not exhibit altered immune suppressive activity compared to untreated cells. However, blocking iron in macrophages resulted in a slight rescue of T cell proliferation in the co-culture experiment. RNAseq analysis revealed upregulation of ferroptosis negative regulator genes following ZnPPIX treatment

Conclusions Macrophages' sensitivity to ferroptosis is likely associated with a substantial labile iron pool, partly regulated by HO-1. Free iron promotes production of free radicals, potentially leading to oxidative damage. Reducing ferroptosis via iron chelation slightly reduces macrophage immune suppressive activity, suggesting that the modulation of ferroptosis may influence macrophage phenotype. Conversely, ferroptosis induction did not increase macrophage immune suppression, possibly due to their already robust immunosuppressive phenotype. Our data indicate that HO-1 plays a central role in ferroptosis, as its inhibition with ZnPPIX decreases RSL3-induced LPO and downregulates genes involved in ferroptosis suppression. However, other proteins, such as Bach1, may also contribute. Hemin treatment increased ferrous iron levels over time, while the effect of ZnPPIX (i.e. iron decrease) was minimal after 3 hours. Future experiments are required to determine whether this iron increase directly induces ferroptosis. These results suggest that iron metabolism and cytoprotective pathways are interconnected and influence macrophage activation states.

CK2 Kinase Regulates Epigenetic Plasticity and Histone Modifications in Diffuse Large B-Cell Lymphoma (DLBCL)

Ph.D. Student: Amir YAMI
TUTOR: Prof. Francesco PIAZZA - CO-TUTOR: Dr. Sabrina MANNI
Ph.D. Course in Oncology and Immunology

Background
Diffuse Large B-Cell Lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma, characterized by profound epigenetic deregulation driving tumor heterogeneity and therapy resistance. Among key regulators, Protein Kinase 2 (CK2) is a constitutively active serine/threonine kinase implicated in transcriptional control, survival signaling, and chromatin dynamics. However, its role in shaping the epigenetic landscape of DLBCL remains poorly understood.

Material and Methods
Germinal center B-cell–like (GCB) DLBCL cell lines (OCI-LY1 and OCI-LY19) were treated with selective CK2 inhibitors (CX-4945, SGC-CK2-1). Western blotting on total protein extracts and histone extractions was performed to evaluate global methylation and acetylation patterns and their impact on chromatin accessibility. Cell viability and toxicity assays were conducted using both single-agent treatments and combinational therapies, combining CK2 inhibitors with tazemetostat (EZH2 inhibitor), and venetoclax (BCL-2 inhibitor), to assess potential synergistic effects.

Results
CK2 inhibition caused profound alterations in histone modification dynamics in a cell-type–specific manner. In OCI-LY1 and OCI-LY19 cells, CK2 blockade promoted an increase in repressive methylation marks (H3K27me3, H3k9me) and shifted chromatin toward a condensed state. Acetylation patterns (H3K27ac, H3K9ac) were also analyzed by western blot and revealed significant alterations following CK2 inhibition, indicating that CK2 also regulates histone acetylation balance and chromatin accessibility. Toxicity assays revealed variable sensitivity to CK2 inhibition among GCB-DLBCL models and demonstrated enhanced cytotoxicity when CK2 inhibition was combined with epigenetic inhibitors or venetoclax (BCL-2 inhibitor), indicating strong synergistic effects. Importantly, synergy with venetoclax was observed in both GCB and ABC (RIVA) DLBCL subtypes.

Conclusion
These findings identify the probability of CK2 kinase as a critical regulator of epigenetic plasticity in DLBCL by modulating the balance between histone methylation and acetylation and altering chromatin states. By reprogramming transcriptional networks and enhancing apoptotic priming, CK2 inhibition sensitizes DLBCL cells to BCL-2 blockade**,** thereby producing potent synergy with venetoclax. These results support a therapeutic rationale for dual targeting of CK2-driven epigenetic regulation and BCL-2 dependency in both GCB and ABC DLBCL subtypes.

Keywords
DLBCL, CK2, histone methylation, histone acetylation, chromatin regulation, epigenetic therapy, tazemetostat, venetoclax

PhD COURSE "PHARMACOLOGICAL SCIENCES"

COORDINATOR: PROF. MICHELE MORARI

The novel NOP receptor ligand Sunobinop: an in vitro pharmacological characterization

Ph.D. Student: Dr. Riccardo CAMILOTTO
TUTOR: Prof. Girolamo CALÒ– CO-TUTOR: Dr. Davide MALFACINI
Ph.D. Course in Pharmacological Sciences

Background
Nociceptin/orphanin FQ (N/OFQ) modulates several biological functions via selective activation of the NOP receptor. Recently N/OFQ has been reported to promote sleep in rodents and non-human primates. This finding has been confirmed in humans with the use of sunobinop, a NOP receptor partial agonist, that demonstrated positive effect on sleep/wake function in subjects with insomnia.

Material and Methods
In the present study the pharmacological features of sunobinop have been investigated in a panel of in vitro assays.
The calcium mobilization assay was carried out on CHO cells overexpressing chimeric G proteins and the NOP, mu, delta, or kappa receptors. NOP-Gβ1 protein interaction was measured with a bioluminescence resonance energy transfer assay, employing HEK293 cells stably expressing the fusoproteins NOP-luciferase and Gβ1-green fluorescent protein. cAMP levels were measured in HEK NOP cells transfected with GloSensor. The effects of sunobinop were systematically compared to those of N/OFQ and of the peptide partial agonist UFP-113.

Results
N/OFQ displayed high values of potency and maximal effects across all datasets. UFP-113 displayed high potency and different degrees of efficacy (alpha 0.83, 0.59, 0.62, in the calcium mobilization, NOP-G protein interaction, and cAMP inhibition assay, respectively). sunobinop mimicked the effects of UFP-113 in the different assays acting as partial NOP agonist but showing lower potency. sunobinop and UFP-113 were highly selective over classical opioid receptors.

Conclusions
Sunobinop behaved as a highly selective moderate potency NOP receptor partial agonist. Similar to UFP-113, sunobinop displayed variable efficacy in the different assays depending on the efficiency of the stimulus / response coupling.

GUT MICROBIAL DIVERSITY IN PARKINSON'S DISEASE: SEPARATING MEDICATION EFFECTS FROM DISEASE IMPACT

Ph.D. Student: Dr. Gloria CARROSSA
TUTOR: Prof. Maria Cecilia GIRON – CO-TUTOR: Prof. Angelo ANTONINI
Ph.D. Course in Pharmacological Sciences

Background The gut microbiota is increasingly recognized as a potential contributor to Parkinson's disease (PD) pathogenesis. While antibiotics are well-known to impact gut microbial composition, numerous other drug classes, often underappreciated, can significantly alter the microbial composition and function[1]. Among these, medications commonly used in PD patients, such as levodopa, which undergoes microbiota-mediated intestinal metabolism, represent relevant examples of such interactions[2]. Therefore, comprehensive pharmacological profiling is essential for accurate interpretation of microbial alterations in this population. This study aimed to evaluate the distribution of pharmacological treatments in PD patients and healthy controls (HC), with a particular focus on drugs known to affect the gut microbiota, and to investigate microbial alpha diversity in both groups.

Material and Methods Ninety-seven subjects were analyzed (53 PD patients and 44 HC). Detailed clinical data and pharmacological therapy information, focusing on drugs with potential gut microbiota impact, were collected for each participant. Fecal samples were collected in specialized tubes for preservation and transport, following standardized procedures. Microbial DNA was extracted, and gut microbiota composition was assessed via 16S rRNA gene sequencing, targeting the V3-V4 regions. Alpha diversity was calculated using the Shannon index. Statistical analyses included Fisher's exact test for categorical variables and Welch's t-test for continuous variables.

Results Polypharmacy (≥5 medications) was significantly more prevalent among PD patients (73.6%) compared to controls (4.5%) ($p < 0.001$). Pharmacological therapies encompassing several drug classes with potential gut microbiota impact were analyzed. Among these, statins and proton pump inhibitors (PPIs), commonly used medications in the general population, were observed at notable frequencies. In HC group, 12 and 2 subjects were taking statins and PPIs respectively, whereas 10 subjects in the PD group were taking each medication. No statistically significant differences in the frequency of use between groups were observed (statins: $p=0.325$; PPIs: $p=0.060$, Fisher's exact test), allowing these medications to be excluded as potential confounding factors in microbiota analyses. Alpha diversity was significantly reduced in PD patients compared to HC (mean Shannon index: PD 3.79 ± 0.60; HC 4.06 ± 0.48; Welch's t-test $p = 0.019$), suggesting disease-associated alterations in gut microbial communities.

Conclusions Our results suggest a reduction in gut bacterial diversity in PD patients compared to HC. The absence of significant differences in the use of high-impact intestinal drugs between groups permits exclusion of these therapies as confounding factors in our analysis. Nevertheless, polypharmacy and high exposure to medications with potential effects on gut microbiota represent critical considerations in Parkinson's patients. Therefore, preliminary pharmacological characterization is essential for accurate interpretation of microbiota data and for identifying potential therapeutic confounders.

References

[1] Maier L, et al. Nature. 2018;555(7698):623-628.
[2] Maini Rekdal V et al. Science 2019, 364, eaau6323

MICROGLIA-MEDIATED NEUROINFLAMMATION IMPAIRS OLIGODENDROCYTE MATURATION IN AN IN VITRO MODEL OF ALZHEIMER'S DISEASE

Ph.D. Student: Dr. Chiara CHEMELLO
TUTOR: Prof. Morena ZUSSO - CO-TUTUR: Prof. Maria Cecilia GIRON
Ph.D. Course in Pharmacological Sciences

Background
Although Alzheimer's disease (AD) is traditionally characterized by neurofibrillary tangles and amyloid β (Aβ) plaques, increasing evidence implicates white matter abnormalities, particularly disrupted myelin sheath organization, in its pathogenesis. These alterations are associated with impaired maturation of oligodendrocytes (OLs), the glial cells responsible for myelination in the CNS. However, the effects of AD on OL function and myelin formation remain poorly understood. This study investigated OL maturation and myelin formation in the context of AD, with a particular focus on the role of microglia. Aβ-activated microglia release inflammatory molecules that can damage neurons and glial cells, including OLs, but the role of OL-microglia interactions in AD pathology has not been clearly defined.

Materials and Methods
Aβ42 oligomers (Aβ42Os), including low molecular weight (LMW, trimers to dodecamers) and high molecular weight (HMW, larger than dodecamers but smaller than 22-mers) species, were prepared and separated using 50 kDa Amicon ultrafiltration membranes. Their oligomeric state was verified by capillary electrophoresis before cell treatment. Primary microglia and oligodendrocyte precursor cells (OPCs) were isolated from PN1 rat cortices. OPC maturation was assessed by real-time PCR and immunofluorescence. Differentiation was quantified by measuring percentage of MBP+/Olig2+ and CNPase+/Olig2+ cells. OL morphology was analyzed by measuring the surface area of MBP+ and CNPase+ cells, and the cellular complexity was assessed using Sholl analysis.

Results
OPC treated for 48 h with non-cytotoxic concentrations of LMW or HMW Aβ42Os showed no significant changes in mRNA levels of Olig2, MBP, CNPase, and PLP compared to control cells. Immunofluorescence analysis confirmed that neither LMW nor HMW Aβ42Os affected the surface area occupied by CNPase+ and MBP+ cells, OPC morphological complexity, and the number of Olig2+ cells. To investigate the role of microglia-derived inflammatory mediators in OPC maturation, microglia were treated with LMW or HMW Aβ42Os for 24 h, and the resulting conditioned media were applied to OPCs. Although only HMW Aβ42Os significantly activated microglia, conditioned media from microglia exposed to LMW or HMW Aβ42O significantly reduced the expression of OPC maturation markers.

Conclusions
OPC maturation and morphology are not affected by direct exposure to Aβ42Os. However, inflammatory factors released by Aβ42O-activated microglia appear to impair OPC maturation, suggesting a role for microglia-mediated neuroinflammation in white matter pathology associated with AD.

Comprehensive public health protection for an aging population

Ph.D. Student: Dr. Giulia GROTTO
TUTOR: Prof. Alessandra BUJA – CO-TUTOR: Prof. Tatiana BALDOVIN
Ph.D. Course in Pharmacological Sciences

Background

The proportion of older people in the population is increasing, particularly in developed countries, and is expected to continue growing in the coming years. To promote healthy ageing, it is necessary to adopt preventive measures of proven effectiveness and encourage health-promoting behaviors.
The aim of our project was to develop tools to disseminate evidence-based health promotion and disease prevention strategies to the widest number of individuals from a public health perspective. With this goal, the following research outcomes were achieved:

1- A website dedicated to older people that brings together the main scientific evidence in the field of health promotion and disease prevention in older age.
2- A report collecting assessments of the effectiveness and certainty of evidence for digital health promotion interventions targeting older adults in three specific areas.

Material and Methods

1- The website was developed by collecting the main scientific evidence and recommendations provided by national and international guidelines, systematic reviews and meta-analyses. For some areas of health promotion where scientific literature was lacking (such as the effects on quality of life resulting from the urban environment, human-animal interactions, and spirituality), specific literature reviews were conducted.
2- To assess the effectiveness of digital health interventions, systematic reviews were conducted in the following areas: a) promotion of recommended vaccinations, b) improvement of sleep quality, c) promotion of physical activity. The GRADE framework was used to evaluate the certainty of evidence.

Results

1- The website collects recommendations on the main areas of prevention and health promotion for older adults. The information has been translated into accessible and practical messages designed to motivate and support the adoption of healthy behaviors. The approach has focused not only on disease prevention but also on the active promotion of well-being, embracing the concept of *salutogenesis*.
2- Half of the digital health interventions aimed at promoting vaccinations were effective or partially effective in increasing vaccination coverage; the certainty of evidence was rated as very low.
Regarding sleep quality improvement, two studies using cognitive behavioral therapy for insomnia and two studies offering educational interventions were found to be effective. The certainty of evidence was rated as very low and low, respectively.
Half of the digital health interventions were effective in increasing physical activity, particularly those that used motivational messages/reminders and provided tailored exercise programs. The certainty of evidence was rated as very low and low, respectively.

Conclusions

Health promotion tools were developed to provide older adults with scientifically based information and advice aimed at motivating healthy behaviors and improving quality of life in old age, while reducing the burden of disease. Assessing the certainty of evidence on the effectiveness of digital health promotion interventions can support the translation of research findings into preventive practice.

This research was developed within the project funded by Next Generation EU - “Age-It - Ageing well in an ageing society” project (PE0000015), National Recovery and Resilience Plan (NRRP) - PE8 - Mission 4, C2, Intervention 1.3”.

Project GENAME – GENetic Alcohol Metabolism Evaluation. Genetic and Epigenetic Characterization of alcoholic dependence: development and validation of an integrated SNP-based model

Ph.D. Student: Dr. Beatrice MARCANTE
TUTOR: Prof. Pamela TOZZO – CO-TUTOR: Prof. Luciana CAENAZZO
Ph.D. Course in Pharmacological Sciences

Background
The use and abuse of large quantities of alcohol is a huge public health problem worldwide. This problem is significant because it is associated with premature mortality and a high socio-economic impact. Alcohol use disorder (AUD) is a condition in which both genetic and epigenetic factors play a role in individual clinical response and subjective susceptibility. Polymorphisms in genes such as ADH1B, ALDH2, CYP2E1, and CYP2D6 are involved in the different metabolism of ethanol, as are genes involved in the neurobiological response to the substance itself, such as OPRM1 and GABRA2. Genes influence individual phenotypic variability by modulating therapeutic efficacy and the risk of developing a full-blown addiction. At the same time, several studies on DNA methylation have shown that analyses associated with methylation levels can lead to an assessment of the biological age of individuals, allowing us to investigate how it varies in subjects with a history of heavy alcohol consumption.
Material and Methods
The GENAME (GENetic Alcohol Metabolism Evaluation) project involves the study, development and validation of two different panels: the first panel, "SNPs Panel", uses a multiplex of six functional polymorphisms to evaluate and analyse the neurobiological response and different individual metabolisation of ethanol. Meanwhile, the "METs Panel" assesses and analyzes DNA methylation levels in five key genes associated with aging, which can provide an estimate of individual biological age. The study in question will be a case-control study with a total of 100 patients enrolled in each group. The study is based on molecular analyses using PCR and capillary electrophoresis.
Results
During the year, activities initially focused on a systematic review of the literature and on defining the project from a methodological and theoretical point of view. A total of 155 articles on methods for predicting biological age over the last 15 years were analysed, providing a specific overview of the evolution and progress of these techniques and enabling a comparison of the statistical methods used. At the same time, the laboratory workflow was developed, defining the criteria for the inclusion and exclusion of patients, in order to outline and produce all the necessary documentation for submission to the relevant ethics committee. Using specialised programmes, sample analyses were carried out to define the number of patients and controls needed to obtain a statistical power of at least 80% and a certain level of significance.
Conclusion
In conclusion, all these studies were necessary in order to establish an adequate theoretical and methodological basis for the launch of the experimental phase of the GENAME (GENetic Alcohol Metabolism Evaluation) translational project, which represents a starting point with potential from a clinical, pharmacological and forensic point of view. We move from personalised medicine to the assessment of subjective and individual susceptibility to developing AUD, and finally to the estimation of biological age itself. All of the activities listed above have therefore provided a solid foundation for the structure of the protocol itself.

Pharmacological modulation of LDL receptor by anti-PCSK9 siRNA: an *in vitro* study

Ph.D. Student: Giorgia MARODIN
TUTOR: Prof. Nicola FERRI – CO-TUTOR: Dr. Maria Giovanna LUPO
Ph.D. Course in Pharmacological Sciences

Background
PCSK9 is a protein that inhibits the recycling of the LDL receptor (LDLR), leading to an accumulation of circulating LDL-cholesterol. The first-line treatment for LDL-cholesterol reduction is represented by statins, which, by inhibiting HMG-CoA reductase, reduce cholesterol synthesis. Therapies targeting PCSK9 itself include anti-PCSK9 siRNAs, inclisiran, and monoclonal antibodies such as alirocumab and evolocumab. These therapies all aim to reduce PCSK9, but they act through very different mechanisms.

Aim
The aim of this study is to evaluate the pharmacological modulation of the LDLR following single or combined treatments of anti-PCSK9 siRNA, evolocumab and statins in *in vitro* cultured cells.

Material and methods
Human-derived hepatocarcinoma cell lines (HepG2 and Huh7) were treated with anti-PCSK9 siRNA (siGENOME-SMARTpool, Dharmacon), Simvastatin (Merck) and Evolocumab. Western Blotting, ELISA assay and qPCR have been used to evaluate intracellular, secreted proteins and transcripts levels, respectively. LDL-DyLight^{TM}550 uptake were determined by cytofluorimetry.

Results
anti-PCSK9 siRNA reduced intracellular PCSK9 from 4 to 8h (-30 and -35%, respectively). The reduction on secreted PCSK9 was observed from 8h (-37%) onwards. Longer time-course experiments revealed that siRNA reduced PCSK9 protein expression after 24 and 48h (-46 and -67%, respectively), while the extracellular levels showed a stronger effect after 48 and 72h (-60%). No effect was observed on apoB secretion. Surprisingly, the reduction in PCSK9 levels did not significantly increase LDLR expression.
Evolocumab itself did not show any modulation of LDLR, while incubation with human recombinant PCSK9 (hrecPCSK9) for 24h induced the receptor's complete degradation, rescued by the co-incubation with evolocumab. In line with LDLR expression, hrecPCSK9 strongly reduced the LDL-DyLight^{TM}550 uptake in Huh7, while the combination with evolocumab increased the uptake.
anti-PCSK9 siRNA was also combined with increasing concentration of simvastatin. This combination, in Huh7, increased LDLR expression in a dose-dependent manner compared to single treatments.
Further analysis with inclisiran in HepG2 cells, under the same experimental conditions, are now ongoing. After 48h, Inclisiran efficiently reduced PCSK9 protein expression in a dose-dependent manner (-45% at the highest concentration of 1μM). This silencing was followed by an increased LDLR protein expression (+50%). Also, gene expression analysis revealed a significant reduction of PCSK9 (-60%).

Conclusions
anti-PCSK9 siRNA reduced PCSK9 expression from 4 until 72h after treatment. Despite this, there is no significant modulation on LDLR. Instead, in the presence of exogenous hrecPCSK9, LDLR undergoes degradation, effect reversed by evolocumab. The combination of siRNA and simvastatin leads to a higher expression of LDLR rather than treatments alone in Huh7. Inclisiran efficiently reduced PCSK9 both in protein and gene expression, but further analyses are ongoing.

DEVELOPMENT OF CAR-T CELLS TARGETING SPECIFIC TUMOR-ASSOCIATED ANTIGENS FOR SOLID AND HEMATOLOGICAL TUMOR TREATMENTS

Ph.D. Student: Maria RASOOL
TUTOR: Prof. Monica MONTOPOLI - CO-TUTORS: Dr. Sara CAPOLLA, Dr. Michele DAL BO
Ph.D. Course in Pharmacological Sciences

Multiple myeloma (MM) remains incurable due to its high relapse, refractory, and heterogeneous nature, although several novel targets and therapies are being explored. In this context, CD138 (syndecan-1) is a potential therapeutic target, as it is highly and specifically expressed on the surface of MM cells. In recent decades, cell therapy based on chimeric antigen receptor (CAR) T cells has emerged as a groundbreaking clinical approach to treating cancers, particularly hematological tumors. However, toxicities, antigen escape, poor trafficking, restricted tumor invasion, and the tumor microenvironment are some of these issues that continue to represent major challenges to obtaining a successful treatment. In this context, the efficacy of CAR-T cell therapy that targets CD138 in MM is currently being evaluated.

Our lab has submitted a patent for a novel CD138-targeting antibody (H9) as a potential treatment for MM. With the aim of developing a customized anti-CD138 CART, we have utilized the sequence of our novel antibody to generate a single-chain variable fragment sequence (scFv)- H9HL. The developed scFv has been validated for its binding ability, specificity, and competitiveness to bind the target site in CD138-expressing tumor cell lines-RPMI-8226 by flow cytometry, immunofluorescence, and by using the CD138 recombinant protein in ELISA. In-vitro experiment of CD138 silencing in RPMI-8226 cells has also confirmed the specific binding of the developed scFv to CD138 by flow cytometry. Then, the validated scFv was used to further develop a vector for producing plasmid DNA for the production of our customized anti-CD138 CAR-T cells. At the same time, to set up and optimize the protocol of in vitro characterization of CAR-T cells, we purchased a commercial anti-CD138 CAR-T cell (Promab Biotechnologies, USA). In detail, by using the commercial anti-CD138 CAR-T, an in vitro protocol has been established to investigate the killing efficacy of CAR-T (cocultured with CD138-expressing tumor cell line, RPMI-8226, and negative control; E: T=5:1 and E: T=10:1) at the 24-hour and 48-hour time points. At both the 24-hour and 48-hour time points, the apoptosis of CD138-expressing target cells; RPMI-8226 was evaluated, as well as the activation and exhaustion of T cells (CAR-T) by considering the expression of the markers (CD25, CD95, PD-1, LAG-3, and TIM-3) by flow cytometry and the release of cytokine (IFN-γ and TNF-α) by ELISA. It was confirmed that the commercial anti-CD138 CAR-T cells were effective against the targeted MM cell line, as apoptosis increased throughout the incubation period, with a significant number of cells dying after 48 hours ($p \leq 0.001$). However, there were no significant differences found in the exhaustion and activation markers. In the next phase, to set up an optimized protocol for the production of anti-CD138 CAR-T cells using transfection with the Sleeping Beauty transposon system (SB100), we firstly set up the protocol, using a vector already available in our laboratory, for the production of anti-CD19 CAR-T cells with the aim to obtain the expression of the anti-CD19 CAR on the cell surface and its stabilization during the time in primary T cells. Results of the preliminary experiments showed that the expression (30-40%) of the anti-CD19 CAR in the primary T cells remained stable for 5-6 days. As a reliable alternative to human primary T lymphocytes, Jurkat cells were used in parallel experiments using a comparable setting to that used in primary T cells. The highest CAR expression (30-40%) in the Jurkat cell line model was found on days 2 and 4, which gradually decreased till day 7. However, further experiments are required to maintain and enhance the CAR expression. After the optimization of the CAR-T cell production protocol, our customized anti-CD138 CAR-T cells using a T cell line will be produced, evaluated, and their efficacy will be checked on the target cells, along with their in vitro characterization.

Mucosal Vaccination and Monoclonal Antibody Strategies for Glypican-1 Targeting in Pancreatic Ductal Adenocarcinoma

Ph.D. Student: Amin SAFA
TUTORS: Dr. Michele DAL BO, Prof. Sara DE MARTIN
Ph.D. Course in Pharmacological Sciences

Background
Pancreatic ductal adenocarcinoma (PDAC) has dismal outcomes and limited therapeutic options. Glypican-1 (GPC1), a cell-surface heparan sulfate proteoglycan overexpressed in PDAC, is a promising immuno-oncology target. This report evaluates a dual strategy oral mucosal vaccination and a chimeric anti-GPC1 IgM monoclonal antibody to potentiate systemic and mucosal antitumor immunity
Material and Methods
C57BL/6N mice received an optimized oral immunization with *Escherichia coli* Nissle 1917 expressing human GPC1-Flagellin (EcN-GPC1-FL) in three cycles over 30 days (days 1–3, 14–16, 28–30), with EcN-WT and PBS as controls. Serum and spleen cytokines (IL-10, IFN-γ, IL-2) were quantified by ELISA on day 32; GPC1 expression in re-isolated EcN from feces was assessed by Western blot; safety was monitored by body weight/clinical observation. In a collaborative arm, GPC1-Flagellin protein was encapsulated in PLGA/PVA nanoparticles and administered orally or subcutaneously; cytokine outputs from spleen and mesenteric lymph node (MLN) lymphocytes were measured under non-stimulated and re-stimulated conditions. A chimeric anti-GPC1 IgM was designed/expressed and characterized by ELISA (affinity/specificity), flow cytometry (BxPC-3, Panc02-GPC1), and immunofluorescence. Functional assays included MTT viability, complement-dependent cytotoxicity (CDC; LDH release, 5–20% human serum), antibody-dependent cellular cytotoxicity (ADCC) using a reporter bioassay and primary NK cell/PBMC co-cultures, and IFN-γ quantification in supernatants. Statistical analyses used ANOVA/t-tests with $p<0.05$.
Results
Oral vaccination with EcN-GPC1-FL successfully induced a measurable immune response, mainly increasing IL-10 without signs of toxicity or weight loss, and confirmed stable GPC1 expression in vivo. The nanoparticle-based vaccination strategy showed a trend toward stronger systemic and mucosal immunity with elevated cytokine secretion, although differences were not statistically significant. The chimeric anti-GPC1 IgM antibody exhibited high specificity and strong binding to GPC1-positive tumor cells. Functional assays revealed that the antibody alone did not directly inhibit tumor cell growth but mediated potent complement-dependent cytotoxicity, while antibody-dependent cellular cytotoxicity remained limited. Co-cultures with NK cells and PBMCs showed enhanced IFN-γ release at higher antibody concentrations, suggesting partial immune activation. Overall, both approaches demonstrated feasibility, safety, and biological activity, supporting GPC1 as a promising immunotherapy target in pancreatic cancer.
Conclusions
Targeting GPC1 via an optimized oral EcN-based vaccine and a chimeric anti-GPC1 IgM is feasible and biologically active. Oral vaccination achieved a favorable regulatory profile with verified antigen expression and safety, while the antibody demonstrated strong specificity/affinity and robust CDC but minimal ADCC. The combined findings support GPC1 as a viable dual-platform target and provide a preclinical foundation for translational studies integrating mucosal vaccination with complement-engaging antibody therapy for PDAC.

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

COORDINATOR: PROF. DARIO GREGORI

Curriculum "BIOSTATISTICS AND CLINICAL EPIDEMIOLOGY"

Characterization and Academic Progress of the First Five Cohorts of Students at the School of Medicine, National University of Mar del Plata, Argentina, by Gender and Parenthood Status (2017–2023). A Multidimensional Analysis

Ph.D. Student: Adrián Eduardo ALASINO
Tutor: Prof. Dario GREGORI - Co-Tutor: Prof. Giulia LORENZONI, Ph.D. Jorge UNGARO
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

General Objective: To describe and analyze the relationship between academic progress and gender/parenthood status among the first five cohorts of students at the School of Medicine, National University of Mar del Plata, Argentina, during the period 2017–2023.
Specific Objective: To identify variations in academic performance across career axes and learning units (LU) according to gender and parenthood status among students from the first five cohorts during the 2017–2023 period.
Method: Data were obtained from final evaluations of the first five cohorts of students (2017–2021). The dataset includes 51,237 records of final exam transcripts with actual student attendance. It covers final exam results in LUs from the Basic Training Cycle (BTC) (02 to 19), Clinical Training Cycle (CTC) (20 to 33), Transversal Units (34 to 39), Flexible Units (e.g., History of Argentine and Latin American Health, Ecology, Problematic Consumption, Social Medicine), and Mandatory Final Practice. Student ID numbers assigned by the system were used as record identifiers. Year of enrollment and age at enrollment were included, along with gender (F; M) and parenthood status (Yes; No). A composite variable with four categories was created: Female with children; Female without children; Male with children; Male without children. Based on the collected records, a database was constructed with entries for 4,015 students from cohorts entering between 2017 and 2021 who sat for at least one final exam. Sociodemographic variables included gender, parenthood status, gender-parenthood combination, cohort, and grades obtained in each LU. For students evaluated multiple times in the same LU, the final grade recorded was the most recent, regardless of whether the exam was passed. New variables were defined corresponding to career axes outlined in the curriculum. Each axis includes various LUs, and the values for these new variables were calculated as the average of final exam results in the respective LUs. The career axes are: Life Cycle (LC), Doctor-Patient Relationship (DPR), Health-Disease-Care Process (HDCP), Construction of Medical Knowledge (CMK), and Primary Health Care and Community Orientation (PHC). Two multivariate analyses were conducted using Principal Component Analysis (PCA), followed by Hierarchical Classification and Class Partitioning using the SPAD.N statistical package. A .SBA format database was used. In the first analysis, active variables included grades in each career axis, and supplementary variables were gender, parenthood status, gender-parenthood combination, and cohort. Two principal components were retained for hierarchical classification. A three-class partition was chosen based on the classification tree. Outputs included: a) projection of active variable vectors on the first factorial plane, b) projection of supplementary variable vectors, c) gravity centers of each class and supplementary variable categories, d) description of each class. The graphical representation and class description table clearly show the relationship between academic performance in each career axis and the gender-parenthood categories. In the second analysis, active variables were grades in LUs, and supplementary variables were the same as above. Two principal components were retained, and a four-class partition was performed. Outputs included: a) projection of active variable vectors on the first factorial plane, b) dendrogram and projection of vectors, gravity centers of each class, and projection of supplementary variable categories, c) description of each class. Again, the graphical representation and class description table clearly show the relationship between academic performance in LUs and gender-parenthood categories.
Results: In the first analysis:

- Class 1: 2,531 students with below-average performance in four of five axes ($p<0.001$). Associated modalities: "has children" ($p<0.001$) and "female with children" ($p<0.001$).

- Class 2: 565 students with below-average performance in four of five axes ($p<0.001$). Associated modalities: “no children” ($p<0.001$), male gender ($p<0.001$), and “male without children” ($p<0.001$).
- Class 3: 919 students with above-average performance in all five axes ($p<0.001$). Associated modalities: “no children” ($p<0.001$), “female without children” ($p<0.001$), and “female” ($p<0.001$).

In the second analysis:

- Class 1: 129 students with below-average performance in 23 LUs and above-average in 3. Associated modalities: “has children” ($p=0.001$) and “female with children” ($p=0.003$).
- Class 2: 3,294 students with below-average performance in 26 LUs. Associated modalities: “has children” ($p=0.001$) and “female with children” ($p=0.002$).
- Class 3: 489 students with above-average performance in 27 LUs. Associated modalities: “no children” ($p<0.001$), “female without children” ($p<0.001$), and “female” ($p=0.004$).
- Class 4: 103 students with above-average performance in 39 LUs. Associated modality: “no children” ($p<0.001$).
- **Conclusions:** Students without children generally show above-average academic performance. In particular, childless female students tend to achieve the highest performance. The “female with children” category stands out for having the lowest average performance.

Modeling Net Cancer Survival: A Simulation-Based Relative Survival Framework

Ph.D. Student: Dr. Aqsa ALI
TUTOR: Prof. Ileana BALDI – CO-TUTOR: Prof. Giulia LORENZONI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background
The present work provides a detailed, replicable framework for relative survival analysis utilizing simulated SEER-like data, designed to emulate standard population-based cancer registry frameworks.

Material and Methods
The survival outcomes were assessed using an assortment of statistical methodologies, starting with the generation of data for 10,000 simulated patients who were stratified by sex, age, tumor stage, and additional clinical variables. The Ederer II method was used to estimate the core relative survival model, which produced net survival estimates for stages I–IV over 5- and 10-year time periods.

Results
This study shows that 5-year relative survival rates varied from 0.803 (Stage I) to 0.299 (Stage IV), in line with the predicted gradients in cancer resistance. There were statistically significant changes across stages according to log-rank tests and Kaplan-Meier estimators. Using a Cox proportional hazards model and additional help from additive excess hazard modelling and flexible parametric spline-based regression, the stage effects were quantified (HR for Stage IV vs. I: 3.61). The flexible model validated stage as the principal prognostic determinant while accommodating non-linear baseline hazards. The temporal and cause-specific trends were further examined using conditional relative survival, piecewise Cox, and competing risks approaches. Based on age groups (<55 vs. ≥55), survival studies showed that younger patients had lower background mortality.

Conclusions
This simulation-based methodology offers a robust framework for survival analysis in cancer epidemiology, highlighting scientific transparency and reproducibility. The findings emphasize the importance of tumor stage in prognosis, as well as the significance of relative survival techniques in registry-based research.

Plasma Levels of Vitamin D in Residents of General Pueyrredon District, Argentina. Comparison with International Recommendations

Ph.D. Student: Gabriel Alejandro ANGELINI
TUTOR: Prof. Dolores CATELAN – CO-TUTOR: Prof. Giulia LORENZONI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Introduction: Vitamin D (VIT D) is a prohormone with crucial functions in numerous physiological processes. Beyond bone metabolism, its involvement has been described in the immune system, mitochondrial regulation, apoptosis, and DNA repair. VIT D deficiency is associated with multiple chronic diseases and has a high global prevalence, especially among elderly populations, those with limited sun exposure, or individuals with comorbidities.
General Objective: To generate the knowledge base supporting the development of a nutritional supplement that meets the potential demand for Vitamin D, based on its co-administration with other cofactors.
Specific Objectives:
- Identify plasma levels of VIT D in individuals residing in the General Pueyrredon District.
- Review current recommendations for Vitamin D supplementation and its relationship with cofactor administration.
Materials and Methods A quantitative analysis was conducted using a database of 152,450 biochemical determinations of VIT D in individuals over 18 years of age, performed over a 36-month period from December 1, 2020, to November 30, 2023, by three clinical laboratories in Mar del Plata, Argentina: Abraham, Grupo CEDEAC, and Fares Taie.
Records were identified using internal codes assigned by each laboratory. The following fields were defined: date of entry, age in years, sex, VIT D determination result (ng/mL), method used (Electrochemiluminescence, Brand: Roche, Platform: Cobas e800 / Chemiluminescence, Brand: Siemens, Equipment: Advia Centaur XPT), and originating laboratory. The first method was used by Fares Taie Laboratory, and the second by the other two.
The database was explored using Infostat software through stratified analysis with the Kruskal-Wallis H test. Results were compared by month, age group, and year. Six months (December to May) showed higher averages and medians in VIT D determinations than the remaining months. Based on this, a new variable was defined, grouping months into two semesters: a) December to May and b) June to November. Initially, nine age intervals were constructed: two six-year intervals (18–23 and 24–29), six ten-year intervals (30–39; 40–49; 50–59; 60–69; 70–79; 80–89), and one for individuals over 90 years. Age intervals with similar VIT D levels were regrouped based on the results.
The literature review included 101 international publications selected for their scientific relevance: fifteen from 2009–2018, six from 2019, twelve from 2020, seventeen from 2021, twenty from 2022, ten from 2023, thirteen from 2024, and eight from 2025.
Results Serum VIT D values across age and sex groups by semester show statistically significant differences ($p < 0.0001$) in all age groups under 90 years. In these groups, determinations from December to May (summer-autumn in the southern hemisphere) were higher than those from June to November. In all groups, the P50 is below 30 ng/mL, and P75 values are lower than those recommended internationally.
Conclusions

- High prevalence of VIT D insufficiency (plasma level < 30 ng/mL) in the general population. In all strata, the 75th percentile is below the value cited in current international literature for addressing most health issues.
- Seasonal variations with higher levels between December and May, coinciding with increased sun exposure in the southern hemisphere, though still insufficient compared to international recommendations.
- Extensive literature highlights the importance and necessity of co-administering VIT D with its cofactors. Nutrient synergy enhances VIT D's effectiveness and minimizes toxicity risk, which primarily arises from inadequate co-administration. This approach aims to support mitochondrial health—an essential condition for human health and the prevention of diseases linked to mitochondrial dysfunction.

Use of GIS Systems in Patients with Hydatidosis

Ph.D. Student: Natalia Soledad ARTOLA VINCIGUERRA
TUTOR: Prof. Dolores CATELAN – CO-TUTOR: Dr. Giorgia STOPPA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background
Hydatidosis is a parasitic zoonosis caused by *Echinococcus granulosus* and is a notifiable disease under Argentina's National Surveillance System (SNVS). Although infection often occurs during childhood, its long asymptomatic period means diagnosis typically occurs in middle adulthood. Understanding the epidemiology of hydatidosis, in particular its spatial distribution, is key to guiding targeted public health interventions. This study aims to describe the demographic and geographic characteristics of hydatidosis cases in Argentina from 2013 to 2024.

Material and Methods
Data on hydatidosis cases reported to the SNVS between 2013 and 2024 were obtained and disaggregated by department using official INDEC geo-statistical codes. Records missing information on department of residence, notification date, or age were excluded, as were duplicates. Expected cases were calculated under internal indirect standardization using national age-specific confirmed case rates and 2022 Census data. Standardized Incidence Ratios (**SIRs) were obtained at department level as the ratio between observed and expected number of cases**. Choropleth maps of SIR at department level were created.

Results
The final sample comprised 8,253 notified cases, of which 4,509 were confirmed. Median age was 46 years among notified cases and 43 years among confirmed cases. Females accounted for 53.22% and 54.6% of cases in each group, respectively. Cases were reported in all provinces, but not in all departments.
Hydatidosis distribution across Argentina was heterogeneous. Confirmed cases predominated among adults, consistent with the disease's long latency. SIR estimates revealed marked spatial variability, with higher values in departments historically linked to sheep farming and livestock activities. Several departments outside known endemic zones also showed elevated SIRs, suggesting possible shifts in the epidemiological profile, potentially linked to urbanization and domestic dog exposure.

Conclusions
Preliminary SIR-based spatial assessment indicates a heterogeneous distribution of hydatidosis in Argentina, still strongly associated with rural, livestock-linked areas. However, the emergence of higher incidence ratios in non-traditional regions points to evolving epidemiological dynamics. A comprehensive disease mapping will be conducted to visualize and explore spatial patterns accounting for overdispersion This analysis will be crucial for identifying emerging hotspots, and informing more precise public health strategies.

RECOGNITION OF DIETARY ACTIVITY VIA COMMERCIAL SMARTWATCH ORIENTATION SENSOR ANALYSIS

Ph.D. Student: Dr. Mohammad Junayed BHUYAN
TUTOR: Prof. Dario GREGORI; CO-TUTOR: Dr. Luca VEDOVELLI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background
Wearable devices, particularly smartwatches equipped with motion sensors, have emerged as promising tools for monitoring and improving individual health and lifestyle behaviors. This study focuses on applying wristwatch sensor data for detecting eating episodes, aiming to provide a non-intrusive, continuous monitoring of dietary behavior.

Material and Methods
Twenty healthy participants wore smartwatches during four meals under semi-naturalistic settings. Each meal was recorded on video and synchronized with sensor data. Two independent reviewers manually annotated eating behaviors using the video recordings. Raw sensor data were preprocessed into a structured dataset and analyzed using multiple machine learning models to classify eating vs. non-eating behavior.

Results
A total of over 130,000 sensor observations were collected and analyzed. Among the models tested, Random Forest and Decision Tree demonstrated the highest performance. Using a comprehensive feature set, Random Forest achieved a test balanced accuracy of 0.837 and an ROC-AUC of 0.958, while Decision Tree followed closely with a test balanced accuracy of 0.826 and an ROC-AUC of 0.903. Features such as the mean and maximum (within each window) of acceleration axes significantly enhanced model performance for distinguishing between eating and non-eating episodes. K-Nearest Neighbors and Gradient Boosting Machine also demonstrated moderate performance, achieving test balanced accuracies of 0.808 and 0.757, respectively. Naive Bayes and Rule Fit models showed limited capability, with balanced accuracies near random performance.

Conclusions
This study demonstrates the feasibility of using smartwatch motion sensors to accurately detect eating episodes. Machine learning, particularly ensemble tree-based models, shows strong potential for integrating dietary behavior monitoring into everyday wearable technology. Future work should address improvements in specificity and generalizability to real-world scenarios.

Funding Sources
DigitAl lifelong pRevEntion (DARE) PNC0000002

Keywords:
Wearable devices; dietary monitoring; kinetic data; food recognition

Validation of Low-Cost and Personal $PM_{2.5}$ Sensors for Exposure Assessment – A Comparison Across Different Commuting Modes

Ph.D. Student: Denise FEURER
TUTOR: Prof. Dolores CATELAN
CO-TUTORS: Prof. Francesco PIROTTI, Prof. Francesco SERA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background: Air pollution is a major global health threat, causing over 4 million premature deaths annually. Accurate exposure assessments are important for epidemiological research into the health effects of air pollution exposure and to inform policy guidelines. This study evaluates the validity of using low-cost air pollution sensors and personal monitors for exposure assessments during commutes and assesses the $PM_{2.5}$ exposure of commuters across different transportation modes.

Material and Methods: Our study was conducted in metropolitan Florence, Italy, measuring $PM_{2.5}$ exposure during commutes over 16 weeks in January - February 2019 and 2020. Each week, two participants wore personal monitors to record their georeferenced exposure during work commuting for each minute. Concurrently, 15 low-cost sensors and two reference monitors measured $PM_{2.5}$ levels. Validity checks (Pearson correlation, Lin's concordance correlation coefficient, and the correlation between differences and the mean) were used to compare the performance of low-cost sensors against personal monitors (minute level) and reference monitors (daily level). In addition, we linked personal $PM_{2.5}$ measurements to the nearest low-cost sensor with readings within 60 minutes to analyze differences across commuting modes and measurement devices.

Results: Low-cost sensors showed good performance against reference monitors with Pearson's correlation ranging from 0.82-0.97, a LIN correlation from 0.46-0.92 and a correlation between mean and difference 0.01-0.87. Compared to personal sensors, the validity of low-cost sensors was more moderate, due to larger spatial and temporal variability which the low-cost sensors were struggling to capture. The overall validity was defined by a Pearson's correlation of 0.6, a LIN correlation of 0.57 and a correlation between mean and difference of 0.3. These results varied largely across transportation mode with the best performance for bus commuting (0.75, 0.63 and 0.63) and the lowest performance for bicycle commuting (0.3, 0.19, and 0.12).
A total of 4327 observations during commuting were recorded. Personal devices and low-cost sensors measured approximately the same average exposure for all commutes (18.04 μg/m3, and 18.37 μg/m3, respectively). There were large differences across transportation modes, with bicycles measuring almost double the personal exposure compared to LCS (22.17 μg/m3, and 11.52 μg/m3), while cars had simultaneously the lowest personal exposure (14.81 μg/m3) and reversed relationship to the low-cost sensor measurement (21.12 μg/m3). The large heterogeneity of the measurement differences between devices across transportation modes (ranging from -12.12 to +10.66 μg/m3) suggests that personal devices are better at capturing the spatial and temporal variability of different transport microenvironments.

Conclusions: Low-cost sensors show good validity for more detailed ambient air pollution measurements with a large potential for application in large-scale air quality assessments and epidemiological studies. Personal monitors can capture the pollution in transport microenvironments which ambient monitors are not able to. Integrating LCS and personal sensors with existing air pollution sensor networks and public health institutions could have substantial impacts on more accurate and fine-scaled exposure assessments.

The impact of paired matched randomization on prognostic scores on operating characteristics of clinical trials

Ph.D. Student: Dr. Ajsi KANAPARI
TUTOR: Prof. Dario GREGORI – CO-TUTOR: Prof. Giulia LORENZONI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background. The choice of the randomization method is a core step in the design of Randomized Controlled Trials (RCT), that aims to minimize imbalances and predictability in data, particularly in the case of prognostic factors and biomarkers that may cause confounding on the treatment effect. The increasing availability of real-world data can be an opportunity to mitigate this effect by informing clinical trials by including historical information during their analysis stage. On the matter, the European Medicine Agency has approved the usage of prognostic scores obtained by machine learning model in the analysis of the primary outcome via a covariate adjustment.

Material and Methods. The aim of this study is to include this information during the design stage of clinical trials via a paired matched stratified prognostic score randomization, that combines the advantages of stratification, handling multiple continuous or binary prognostic covariates, while minimizing information loss and reducing residual variance. The method was evaluated via a simulation study on a two-arm setting of 300 subject per arm, by fitting several machine learning models. Eligible subjects are matched based on the prognostic score within different calipers up to a target sample size. Equal randomization is then performed within pairs. Analysis is performed using both paired and unpaired analysis. Benchmark power is obtained by equal randomization without accounting for pairing. The methods are then applied on data from two twin trials on knee osteoarthritis.

Results. This gain is evident with power that increases from 0.69 in equal up to 0.87-0.97 in paired scenarios, considering calipers of 0.01, 0.05 and 0.1. Power gain comes with some drawbacks regarding type 1 error that tends to increase particularly with larger caliper and when unadjusted paired t-test is performed. XGBoost prognostic score provided the most conservative control of the type I error particularly when the analysis involves linear mixed effect model with pairs as grouping factor. Linear covariate adjustment provided similar gains in terms of power to the paired matched randomization. Application to the trial data show that even if prediction model does not necessarily explain a high proportion of data variability, the usage of prognostic scores reduced bias and standard errors.

Conclusions. Paired matching randomization offers the opportunity to increase power and precision when appropriate caliper settings and prognostic models are used, although care is needed to avoid inflated type I error rates.

Robustness and Reproducibility of Multiverse Analysis in Cancer Staging: Systematic Assessment of Prognostic Factor Stability Across Breast, Lung, Kidney, and Prostate Cancers Using SEER Data

Ph.D. Student: Dr. Jiregna Olani KEDIDA
TUTOR: Prof. Dario GREGORI
CO-TUTOR: Prof. Corrado LANERA, Dr. Honoria OCAGLI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background:
Accurate cancer staging is essential for managing and guiding prognosis and treatment decisions. However, the reliability of identified prognostic factors usually depends on specific analytic choices, such as model selections, selections of potential variables, and data preprocessing methods. This study aimed to systematically assess the robustness and reproducibility of prognostic factors in cancer staging models for breast, lung, kidney, and prostate cancers based on Surveillance, Epidemiology, and End Results (SEER) database. The focus of this study is to evaluate the stability of these factors while restricting the Researcher degree of freedom across a broad range of analytic pathways using multiverse analysis, an approach that systematically examines how different analytic decisions affect study results. This approach provides a transparent tool for assessing the robustness of research findings, enhancing to identify an appropriate and consistent results across multiple combinations of analytic pathways.

Material and Methods
A complete multiverse analyses framework will be implemented in R software (package Multiverse), applying modelling strategies, including Cox proportional hazards, different parametric survival models (Weibull, exponential), and logistic regression based on SEER datasets to each of cancer types. The analytical pathways varied by inclusion of factors (such as age, tumor grade, receptor status, tumor size, lymph node involvement, comorbidities), variable transformation (continuous, categorical, spline), and outcome definition (overall survival, vital status, other endpoints will be included). All possible combination of analytical settings while researcher degrees of freedom is restricted will be systematically coded and implemented to list effects of methodological choices and variability and stability of estimates checked for the robustness and reproducibility of the multiverse approach.

Results
Results of breast cancer data (with small data of breast cancer patients from SEER dataset, n = 100, 000 patients) revealed substantial heterogeneity in estimated hazards ratios, odds ratios, and regression coefficients across the analytic multiverse. Some prognostic factors, such as tumor grade and lymph node involvement, exhibited robust associations with outcomes, while others were sensitive to specific model selections.

Conclusions
Multiverse analysis provides a rigorous, transparent approach to quantifying uncertainty in cancer staging. The approach expected to reveal when all dataset and all possible combination of analytical choices made.

Keywords: prognostic factors, SEER database, Researcher degree of freedom, analytic pathways, multiverse analysis

Connecting and Analyzing Electronic Health Records of Pediatric Emergency Departments in Italy: towards new frontiers from prevention to delivery of best care and health service organization strategies (ConnAEctH PED)

Ph.D. Student: Dr. Mohd Rashid KHAN
TUTOR: Prof. Danila AZZOLINA
CO-TUTORS: Prof. Dario GREGORI, Dr. Luca VEDOVELLI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background

Pediatric emergency departments (PEDs) in Italy receive hundreds of thousands of visits annually, offering a valuable yet underexploited source of electronic health records (EHR). These patient level records, particularly free-text patient diagnoses, hold significant potential for enhancing epidemiological surveillance, clinical decision-making, and health services research. This study aims to explore potential of advanced machine learning (ML) techniques to extract clinically meaningful insights from these unstructured data sources. We investigated the performance of classical machine learning (ML) models, Deep Learning (DL), and a pre-trained large language model (LLM) in classifying bronchiolitis diagnosis from the free-text-diagnosis field of the emergency department EHR. Additionally, we compare the diagnostic accuracy of the actual official administrative ICD-9 encoding with model-based bronchiolitis diagnosis.

Material and Methods

28,557 records of infants < 1 year with complete discharge diagnoses fields were retrieved between the years 2007-2018 and manually classified by an expert pediatrician to create the gold standard diagnosis set for training the algorithm. After data pre-processing, classical ML models (Random Forest, Decision Tree, Gradient Boosting Machine, Linear Discriminant Analysis, Support Vector Machine), a Deep Learning (DL) tool, and a pre-trained LLM (GPT-4o) were evaluated using balanced accuracy, sensitivity, and F1 scores. The official administrative ICD-9 encoding classification accuracy was compared to the gold standard.

Results

Overall, 1,903 of 28,557 records (6.7%) were classified as bronchiolitis by the gold standard approach. Both the DL model and GPT-4o outperformed traditional machine learning approaches, demonstrating superior performance across sensitivity, F1 score, and balanced accuracy. Traditional models showed moderate to high performance, though not on par with the deep learning approaches. In comparison, ICD-9 codes exhibited relatively high specificity but lower sensitivity.

Conclusions

DL and GPT-4o performed better than tested traditional ML-based tools in identifying bronchiolitis diagnoses and in ICD-9 diagnosis coding. AI-based tools hold significant potential for improving epidemiologic surveillance and clinical decision-making of bronchiolitis from emergency department EHRs.

An Integrated Processual Framework with Data Science Tools for Addressing Public Health Challenges: A Scoping Review on AI Interventions in HIV/AIDS Prevention

Ph.D. Student: Mauro Nicolás LIZAMA
TUTOR: Prof. Cristina CANOVA – CO-TUTOR: Isabella ROSATO
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background: Public health systems face increasing challenges in managing large volumes of data from diverse sources. Despite advances in digitalization and data science in the last years, integrating this information into operational workflows remains complex. Artificial intelligence (AI) related tools offer promising solutions to address public health problems, particularly in tackling persistent global health issues aligned with the Sustainable Development Goals (SDGs) of the United Nations. Our scoping review focused on SDG 3.3 (targeted at ending the HIV/AIDS epidemic), with the objective of mapping current AI-based interventions employed in HIV prevention. This review constitutes a foundational phase within a broader research project aimed at developing an Integrated Processual Framework to optimize data-driven decision-making in the public health field.

Material and Methods: We conducted a scoping review to outline how AI technologies are applied in HIV prevention strategies. A search strategy was designed to retrieve literature published between 2012 and June 2024 from PubMed, Scopus, and EMBASE. The screening process was structured in two phases: an initial title and abstract screening conducted using ASReview (active learning prioritization), followed by a manual full-text review. Studies were categorized into five intervention areas, according to our predefined PICO questions, and classified under two main AI approaches: Risk Identification (PICO-1) and Prevention Strategies (PICO 2-5: HIV prophylaxis, educational programs, harm reduction strategies, and testing).

Results: From 9,511 records identified across three major databases, 6,497 articles remained after duplicate removal. Title and abstract screening using ASReview reduced the selection to 301 articles for full-text review. Within the "Prevention Strategies (PICO 2-5)" category, 35 studies were identified: 10 focused on Patient-Facing interventions (including 6 chatbots), while 25 examined Non-Patient-Facing approaches applying AI at the population or system level (9 of them were social media-related studies). Regarding "Risk Identification (PICO-1)" interventions encompassed diverse AI and Machine Learning techniques aimed at estimating HIV infection risk and supporting targeted prevention strategies.

Conclusions: The findings of our scoping review provide a comprehensive overview of AI-based interventions in HIV prevention, highlighting their diverse applications across multiple domains and identifying distinct patterns in Patient-Facing and Non-Patient-Facing approaches. Notably, an important proportion of Non-Patient-Facing studies leveraged social media data to analyze behavioral patterns and guide prevention strategies, which highlights the increasing relevance of digital ecosystems in public health. These insights will guide the design of future studies contributing to the development of an Integrated Processual Framework aimed at enhancing data-driven decision-making in public health practice.

Cross-cultural adaptation of a food frequency instrument in Mar del Plata, Argentina

Ph.D. Student: Dr. Manuel Emiliano MARISCAL
TUTOR: Prof. Ileana BALDI – CO-TUTOR: Dr. Honoria OCAGLI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background

The escalating global prevalence of overweight and obesity constitutes a paramount public health crisis, with international bodies forecasting a continued upward trend. (1, 2) This epidemic is intrinsically linked to adverse health outcomes, as a substantial body of literature has established a clear correlation between malnutrition and increased morbidity and mortality from non-communicable chronic diseases (NCDs). (3) The primary methodological approach to assess population-level dietary intake has been the Food Frequency Questionnaire (FFQ), a tool that ascertains consumption habits by presenting respondents with a list of foods and typical portion sizes. (4) A prominent example is the EPIC-Norfolk FFQ; however, despite its extensive validation in diverse European contexts, a review of the literature revealed no evidence of its cross-cultural adaptation and validation for Latin American populations

Material and Methods

The FFQ is country-specific and was developed for all of the international EPIC centres. It was based on the FFQ used in the Nurses' Health Study and was designed to measure a participant's usual food intake during the previous year. A cross-cultural adaptation of the instrument was carried out to measure and understand the phenomenon in different cultures, equivalent to the original (5) (6). An initial version was prepared by two professional translators. The Spanish version was back-translated by a bilingual native English speaker. Discrepancies between the versions were addressed by a committee of three nutrition experts. The pilot test will be conducted through fieldwork during the second half of the year 2025, at new locations in the city of Mar del Plata. A sample of 100 people will be selected to complete the questionnaire, allowing for statical analysis. Upon completion of the critical appraisal of all the information collected from the previous phase, the resulting local version will be available for use in the validation process of the instrument's psychometric properties, including construct validity and composite reliability obtained through confirmatory factor analysis, in comparison with the original. REDCap (Research Electronic Data Capture) is a secure electronic data capture tool, web-based, hosted at the Department of Cardiac-Thoracic-Vascular Sciences and Public Health.

Results

Following the initial back-translation, an expert committee reviewed all identified discrepancies. These issues primarily involved food items not common to the local diet, commercially unavailable brands, and a lack of equivalents for certain beverages. Appropriate substitutions were made by identifying locally sourced products with nutritional equivalence. Subsequently, the finalized instrument was prepared for pilot testing.

Conclusions

This work will provide a cross-culturally validated nutritional assessment instrument for Hispanophone populations, establishing a pivotal resource to address the dual burden of malnutrition in the region. The instrument will empower researchers to investigate both the underlying nutritional drivers of prevalent non-communicable diseases and the persistent challenges of malnutrition.

Reference:

1. World Health Organization. Obesity and Overwight [Internet]. 2024 [cited 2024 Jul 23]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
2. Lobstein T, Powis J, Jackson-Leach R. World Obesity Atlas 2024 [Internet]. 2024. Available from: https://data.worldobesity.org/publications/?cat=22
3. Dettoni R, Bahamondes C, Yevenes C, Cespedes C, Espinosa J. The effect of obesity on chronic diseases in USA: a flexible copula approach. Sci Rep. 2023 Dec 1;13(1).
4. Horton R. Offline: Health's intercultural turn. Vol. 401, The Lancet. Elsevier B.V.; 2023. p.12
5. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the Process of Cross-Cultural Adaptation of Self-Report Measures. Spine (Phila Pa 1976). 2000;25(24):3186–91.
6. Arribas A. Adaptación transcultural de instrumentos. Guía para el proceso de validación de instrumentos tipo encuestas. Trabajo Original. Vol. 16- 2006.

Assessing the Impact of Italy's Asbestos Ban: Future Mortality Scenarios for Pleural Mesothelioma (2020-2034)

Ph.D. Student: Dr. Allegra SARTORE
TUTOR: Prof. Dolores CATELAN – CO-TUTOR: Prof. Annibale BIGGERI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background: Italy, a major European past producer, banned asbestos in 1992. Currently, a high incidence of asbestos-related diseases (ARDs) is still registered due to their long latency and the presence of residual asbestos-containing materials. Notwithstanding, few predictions of the future burden of ARDs appear in the literature, especially at the regional level. The present work aims to make projections for future years (2020-2034) of age-stratified mortality rates at national and subnational levels, separately for males and females.

Material and Methods: Data on deaths caused by malignant pleural tumors (ICD-9:163) and MPM (ICD-10:C45.0) were extracted from the Italian National Institute of Statistics death certificate archives for the period 1980-2019, both at national and regional levels. They were adjusted for misclassification of MPM in the ICD-9 classification, where a specific code for MPM did not exist. For the first time, official estimates derived from multiple cause of death registries, available since 1994, were used for this adjustment. They were aggregated in eighteen 5-year age classes (0-4, …, 85+), eight 5-year calendar periods (1980-1984, …, 2015-2019), and a total of fifteen 10-year birth cohorts were followed (1905-1914, …, 1975-1984). A Bayesian APC model was then specified to project future MPM age-specific mortality rates for the years 2020-2034. All forecasts were made both at the national level and for each administrative region, stratified by sex assigned at birth. Projected future mortality rates and counts were computed conditional on ISTAT population projections.

Results: Between 1980 and 2019, 33 889 people died due to MPM in Italy, and a total of 19 092 more deaths are expected to occur between 2020 and 2034. The M/F ratio increased from around 1.62 to 2.7 over the observation period, and is predicted to remain constant. The overall peak is expected for 2020-2024, with 6740 deaths (4946 among males and 1794 among females). Age groups up to 74 years old have already reached the peak of mortality rates. Similar trends are observed in the most impacted regions, in particular Lombardy and Piedmont. Standardized death rates (according to the WHO World Standard Population), which encompass all age classes, place the age-standardized peak in the period 2010-2014 for males and 2000-2004 for females, approximately 10-20 years earlier than the absolute peak. The smaller counts observed in the less-impacted regions and among females are reflected in the projections, which have wider credibility intervals.

Conclusions: These results are consistent with the existing literature in predicting the timing of MPM peak, even though with lower absolute numbers, and provide new insights into age-specific trends. In addition, our projected age-standardized mortality rates show that the peak has already passed. These forecasts are also useful in estimating the time lag occurring between the introduction of asbestos bans and the decline in ARDs. Specifically, an in-depth research of the literature was conducted in order to compare the national timing to that of other asbestos-banned countries, as substantial heterogeneity exists. These findings provide valuable evidence to demonstrate the efficacy of asbestos bans on M mortality and are a useful tool for national health planning.

APPLICATION OF FEDERATED LEARNING: A COMPARATIVE STUDY ON COST PROFILING OF PATIENTS AFTER ACUTE MYOCARDIAL INFARCTION USING FEDERATED LEARNING

Ph.D. Student: Dr. Shinto Pulickal THOMAS
Tutor: Prof. Dario GREGORI – Co-Tutors: Prof. Corrado LANERA, Dr. Luca VEDOVELLI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Biostatistics and Clinical Epidemiology"

Background
Classical machine learning in medical data analysis faces significant challenges due to regulatory constraints such as the General Data Protection Regulation (GDPR), vulnerability to data breaches, and the logistical hurdles of centralized data pooling. The decentralization of healthcare data across institutions complicates collaborative analytics, as data migration is often prohibited or impractical. Federated Learning (FL) has emerged as a promising paradigm to address these issues by enabling model training across decentralized data silos. By aggregating only local model updates and never sharing raw patient data, FL upholds data privacy and regulatory compliance, making it particularly suitable for multi-center medical studies.

Material and Methods
This study evaluates the efficiency and practicality of FL using the COSTAMI dataset, which is distributed across ten medical centers to simulate the heterogeneous and isolated nature of real-world clinical environments. Data from each institution are statically and externally partitioned, and split ensuring rigorous parity across all experimental paradigms. All the federated, centralized, and individual resampled data frame were modelled using mixed methods and neural network architecture, consisting of a layer with ReLU units, implemented using TensorFlow and Keras API for consistency and reproducibility. The Flower framework orchestrates federated training, securely coordinating communication between Docker-containerized clients and a central server. The performance metrics were collected in loss functions and the mean absolute error, with the weights of best round used for testing.

Results
Non-IID medical data with high heterogeneity across centers varies significantly in cost and length of stay. The predictors used were in alignment with the COSTAMI study but pertained to baseline measurements. While comparing the centralized and individual center cost for strategy, centralized mixed model outperformed their local counterpart. the same fashion observed in federated scenario. However, while privacy is preserved in FL, centralized model consistently performed better. The difference between the MAE, RMSE and R^2 is not negligible among centralized counterparts and FL.

Conclusions
This work evaluated a fair and shallow comparison between federated, centralized, and individual learning in sensitive, multi-institutional medical settings. The findings demonstrate that, when architectural and data parity are strictly enforced, federated learning can achieve accuracy which are comparable to their centralized approaches—while adhering to the privacy, regulatory, and logistical realities of decentralized healthcare data. The study also suggests heterogeneity and sample size has a negative effect on the effectiveness and efficiency of FL. The presented methodology and results offer a foundation for future research and practical deployment of federated analytics in medicine.

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

COORDINATOR: PROF. DARIO GREGORI

Curriculum "CARDIOVASCULAR SCIENCES"

Autoantibodies against G Protein Coupled Receptors in cardiovascular diseases: pathophysiological and prognostic Role

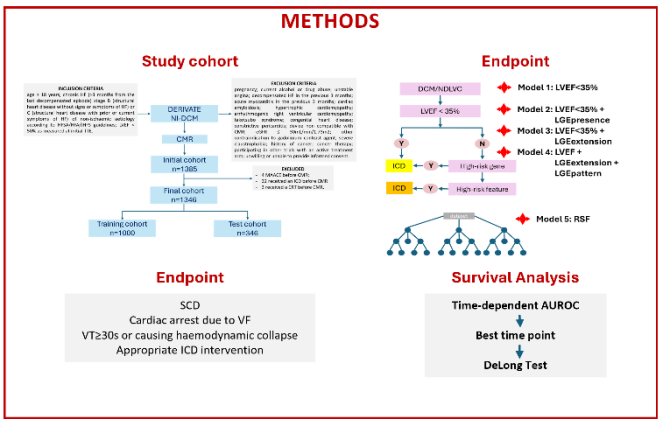
Ph.D. Student: Dr. Giovanni Riccardo Maria CIVIERI
TUTOR: Prof. Francesco TONA – CO-TUTOR: Prof. Ahmed TAWAKOL
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

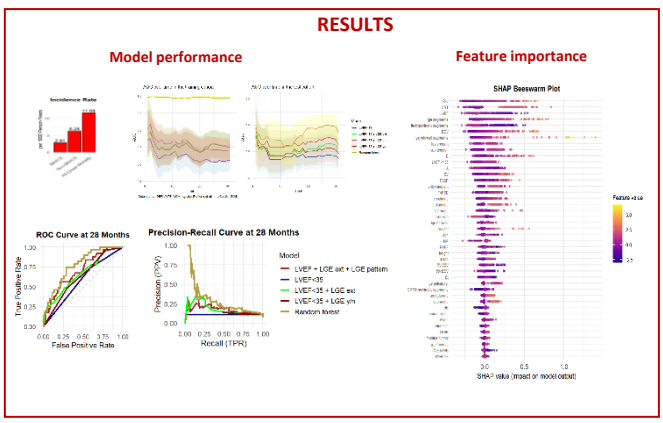
Background
angiotensin II receptor type 1 (AT1R) and endothelin-1 receptor type A (ETAR) are G-protein-coupled receptors (GPCRs) expressed on the surface of various cells and are activated by angiotensin II (AngII) and endothelin 1 (ET1), respectively. Certain autoantibodies are specific to these receptors and can regulate their function; thus, they are known as functional autoantibodies. The function of these antibodies is similar to that of natural ligands and involves vasoconstriction, inflammation, and fibrosis. The role of autoantibodies against AT1R and ETAR (AT1R-AAs and ETAR-AAs, respectively) is well described in the pathogenesis of many medical conditions, but their implications in cardiovascular diseases remain unclear. The aim of our research projects is to evaluate the role of AT1R-AAs and ETAR-AAs in different cardiovascular conditions, ranging from acute coronary syndromes to carotid atherosclerosis and from arrhythmias to cardiogenic shock.
Material and Methods
In the clinical part of our research project, we collect blood samples from patients in different settings. In the FAST (Functional Autoantibodies in STemi) study, we recruit patients with ST-elevation myocardial infarction (STEMI) from the Cardiology Intensive Care Unit of Padua University Hospital and correlate levels of AAs to 1) the occurrence of microvascular obstruction measured by cardiac magnetic resonance imaging, 2) the occurrence of left ventricular remodelling, 3) arrhythmic burden during hospital admission, and 4) long-term prognosis. In the FAAT (Functional Autoantibodies in ATherosclerosis) study, we collect carotid plaques excised in the Vascular Surgery operating theatre (Padua University Hospital) and correlate plaque characteristics with the levels of both AAs. In the FAST-NIS (Non ISchemic) substudy, we recruit patients presenting to the Cardiology Intensive Care Unit (Padua University Hospital) with cardiogenic shock of nonischemic origin and correlate the levels of AAs with multiple features of disease severity. In all setting, levels of AAs are measured via ELISA. In the preclinical part of our research project, we purify AAs derived from STEMI patients and assess their effects on cardiomyocytes and endothelial cells in vitro.
Results
In the FAST study, we recruited 302 STEMI patients and showed that the presence of AT1R-AAs and ETAR-AAs significantly increase the risk of developing microvascular obstruction after STEMI, which translated into a higher incidence of left ventricular remodeling and adverse cardiac events at follow-up. Additionally, we have shown how the two AAs display an additive effect and how they increase the risk of life-threatening arrhythmias. Moreover, both AT1R-AAs and ETAR-AAs were significantly elevated in patients with spontaneous coronary artery dissection. In the FAAT study, we recruited 62 patients, but the results are not yet available. Similarly, in FAST-NIS, we recruited eight subjects, but the results are not yet available.
In the preclinical study, we found that AT1R-AAs and ETAR-AAs induced cytoskeletal disorganization and increased oxidative stress, providing a pathophysiological explanation for the in vivo findings.
Conclusions
Our research project provides robust evidence that AT1R-AAs and ETAR-AAs contribute to microvascular obstruction and adverse prognosis after STEMI. Both AAs induce cytoskeletal disorganization, inflammatory activation, mitochondrial oxidative stress, and loss of viability, which recapitulate the clinical and histological hallmarks of microvascular obstruction. In vitro, these effects were attenuated by pharmacological blockade, highlighting a potential therapeutic avenue for microvascular obstruction. Our other ongoing projects may elucidate the role of AT1R-AAs and ETAR-AAs in atherosclerotic plaque stability, arrhythmias and cardiogenic shock.

Explainable Machine Learning for Prediction of Major Arrhythmic Events in Dilated Cardiomyopathy

Ph.D. Student: Dr. Mattia CORIANÒ
TUTOR: Prof. Francesco TONA – CO-TUTOR: Prof. Martina PERAZZOLO MARRA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

Background: Sudden cardiac death represents a significant cause of mortality in patients with dilated cardiomyopathy (DCM). Existing risk stratification guidelines primarily rely on left ventricular ejection fraction (LV-EF), which demonstrates limited predictive accuracy for arrhythmic events. This study evaluates the performance of artificial intelligence (AI) models, specifically Random Forest (RF), for predicting major adverse arrhythmic cardiac events (MAACE) in patients with DCM and compares them to guideline-based Cox regression models.
Material and Methods: The study analysed data from the DERIVATE-NICM international registry, a multicenter prospective cohort of patients with non-ischemic DCM. The dataset was randomly split into training (75%) and test (25%) cohorts. Preprocessing involved removing low-variance variables, handling missing data, and standardizing inputs. Prediction models included four Cox regression models based on LV-EF and late gadolinium enhancement (LGE) variables and an RF model incorporating clinical, echocardiographic, and CMR data. Model performance was assessed using area under the receiver operating characteristic curve (AUC) at multiple time points, with validation in the test cohort.
Results: The final cohort consisted of 1346 patients. After a median follow-up of 32 months (32-18), 84 patients died. Overall, MAACE occurred in 74 (5.5%) patients, with an incidence rate of 2.81 events per 1000person/years. The LVEF≤35% model presented the lowest performance, with a mean AUC ranging between 0.54 and 0.57 from 8 to 32 months. The addition of the LGE variables progressively improved performance: the LVEF ≤35% + LGE presence model achieving a 28-month AUC of 0.63 (0.73–0.52), while the LVEF ≤35% + LGE extension and LVEF ≤35% + LGE extension + LGE pattern models reached AUCs of 0.66 (0.77–0.55) and 0.70 (0.81–0.59), respectively. The RF showed the highest performance, with a 0.78(0.87-0.69) and 0.80(0.87-0.71) at 20 and 28 months respectively.
Conclusions: This study underscores the superiority of AI models, particularly RF, in predicting MAACE in DCM compared to guideline-based regression approaches. The findings highlight the limitations of LV-EF as a standalone predictor and support the integration of AI models for improved risk stratification. Future studies should validate these models in external cohorts and explore their integration with advanced deep learning frameworks to enhance predictive capabilities further.





In vivo bio-distribution and bio-compatibility assessment of lipoic-acid nanoparticles as new drug vectors to target therapy in cardiovascular diseases

Ph.D. Student: Dr. Ayesha JABEEN
TUTOR: Prof. Chiara CASTELLANI– CO-TUTOR: Dr. Marny FEDRIGO
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

Background
Nanoparticle-based delivery systems show significant promise for targeted cardiac therapies and represent an ideal platform due to their ability to efficiently deliver molecular cargo into specific tissues. Our group recently reported a novel in vitro and in vivo biocompatible and biodegradable polymeric nanoparticle, featuring a core made from polymerized lipoic acid derivatives (PLA) that degrade selectively in the presence of thiols. This new generation of nanoparticles has demonstrated high versatility in loading various molecules, including miRNA encapsulation.
AIM: In this study, we want to evaluate several formulations of lipoic acid-based nanoparticle systems (NPS) for the treatment of cardiovascular diseases. Our goal was to investigate their biodistribution and safety profiles and to determine the most effective route of administration to maximize therapeutic efficacy.
Material and Methods
To evaluate the biodistribution of PLA-based nanoparticles (PLA-NPs), 10 mg/kg of three rhodamine-labeled PLA-NP derivatives were injected into adult rats. Both intravenous (tail vein) and intraperitoneal routes of administration were tested. At 3 hours, 6 hours, and 24 hours post-injection, the rats were sacrificed, and organs were collected. Accumulation and tissue-specific localization of nanoparticles were assessed by confocal microscopy at multiple time points. In one group, NPs were conjugated with rhodamine and loaded with FAM-labeled mRNA. The mean fluorescence intensity (MFI) of nanoparticles and cargo was measured across whole organs using Leica Application Suite (LAS-AF) 3.1.1. software.
Results
The distribution of nanoparticles across major organs was quantitatively assessed by measuring the red channel mean fluorescence intensity (MFI) per μm^2.
In rats that received NPs intraperitoneal injection (IP), analysis of MFI values at 6hours after NPs injection showed substantial accumulation of nanoparticles in the heart, which exhibited higher fluorescence intensity (1.56×10^{-4} / $\mu m^2 \pm 5.70\times10^{-5}/\mu m^2$) compared to the kidney, liver, and spleen (all around $9\times10^{-5}/\mu m^2$). These results suggest efficient localization of nanoparticles within cardiac tissue in animal who received nanoparticles by intraperitoneal injection.
Interestingly, the group of animals that received tail vein injections showed the highest normalized MFI signal (2.57×10^{-4} / $\mu m^2 \pm 1.35\times10^{-4}$ / μm^2). This significantly elevated fluorescence in the heart, compared to animals that received intraperitoneal (IP) injections, suggests that intravenous administration results in either preferential targeting of, or retention within, the heart. This effect is observed at both 3 and 6 hours post nanoparticle (NP) injection. Statistical analysis using the Kruskal-Wallis test followed by Bonferroni correction revealed that, at 6 hours post intraperitoneal (IP) injection, the fluorescence signal in the heart was significantly higher than in the kidney (p = 0.01078) and spleen (p = 0.002). However, the difference between the heart and liver did not reach statistical significance after correction (p = 0.08208), suggesting that, following IP injection, the liver and heart may trap nanoparticles (NPs) to a similar extent.
Conclusions
In conclusion, our biodistribution studies provide compelling evidence that PLA-based nanoparticles exhibit a strong propensity to accumulate in cardiac tissue. These results not only validate the potential of PLA-NPs as promising vehicles for targeted cardiovascular drug delivery, but also highlight their strategic relevance in the development of next-generation cardiac therapies.

PROGNOSTIC ROLE OF LATE GADOLINIUM ENHANCEMENT IN ARRHYTHMOGENIC CARDIOMYOPATHY

Ph.D. Student: Dr. Marika MARTINI
TUTOR: Prof. Barbara BAUCE – CO-TUTOR: Prof. Martina PERAZZOLO MARRA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

Background: The increasing use of Cardiac Magnetic Resonance (CMR) in Arrhythmogenic Cardiomyopathy (ACM) has revealed that a considerable number of patients show left ventricular (LV) involvement through the detection of late gadolinium enhancement (LGE). While recent studies are investigating myocardial scarring across cardiomyopathies, data on the prevalence and prognostic role of LV-LGE in ACM—especially when quantified—remain limited. This study aimed to assess the prevalence of LV-LGE in a well-defined ACM cohort, explore related clinical, genetic, and imaging features and evaluate its prognostic relevance. We also aimed to identify a LGE burden threshold associated with major adverse cardiovascular events (MACE).

Material and Methods: A total of 212 patients with ACM who had undergone CMR were included in the study. Clinical, genetic, imaging characteristics and outcomes were compared based on LV-LGE presence. Univariate regression analysis was performed to assess the association between LGE and MACE. LGE burden was quantified using the 17-segment model, as percentage of total myocardial mass and as absolute volume (ml); Receiver-operating characteristic (ROC) analysis was performed to identify a segment-based threshold associated with MACE, defined as a composite of life-threatening ventricular arrhythmias, sudden cardiac death, cardiovascular death, heart failure, and hot-phase episodes.

Results: In our cohort, 105 patients (49.5%) exhibited evidence of LV-LGE. No significant differences were observed between groups in terms of sex ($p = 1.00$) or proband status ($p = 0.34$). Clinically, patients with LV-LGE more frequently presented with chest pain ($p < 0.001$). Pathogenic or likely pathogenic mutations in Desmoplakin (DSP) and Filamin C (FLNC) were more prevalent in the LV-LGE group ($p < 0.001$ and $p = 0.01$, respectively), whereas Plakophilin-2 (PKP2) mutations were significantly more common in the LV-LGE–negative group ($p < 0.001$). Electrocardiographic findings associated with LV-LGE included low QRS voltage in peripheral leads ($p < 0.001$) and T-wave inversion in lateral precordial leads ($p = 0.04$). At CMR, the presence of LV-LGE was associated with larger left ventricular end-diastolic volume (93 ± 20 vs. 82 ± 12 mL/m², $p < 0.001$) and lower mean LV ejection fraction (54% vs. 61%, $p < 0.001$). Over a median follow-up of 5.2 years (IQR 2.0–10.1), patients with LV-LGE experienced a significantly higher incidence of MACE ($p < 0.001$). Univariate regression analysis confirmed the association between LV-LGE and MACE (OR 2.7, 95% CI 1.5–5.0, $p < 0.001$). Among the three quantification methods, LGE volume in ml was the only parameter significantly associated with MACE ($p = 0.0425$). A threshold of 18.6 ml was identified as predictive of adverse events, with moderate discriminative performance (AUC 0.62).

Conclusions: LV-LGE is common in ACM patients and defines a distinct subgroup with specific genetic and phenotypic characteristics. Its presence is associated with worse outcomes. Among the available quantification methods, LGE volume was the only metric significantly associated with MACE, suggesting a potential role as an additional imaging marker within a multiparametric risk stratification approach, albeit with limited discriminative performance.

Exercise testing-induced arrhythmias and mitral valve prolapse

Ph.D. Student: Dr. Nicolò MARTINI
TUTOR: Prof. Federico MIGLIORE – CO-TUTOR: Prof. Martina PERAZZOLO MARRA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

Background
Severe mitral regurgitation (MR) in patients with mitral valve prolapse (MPV) is considered a high-risk feature. However, there is limited data on exercise stress testing (ExT) and risks in those with mild MR. We aimed to investigate patterns of ventricular arrhythmias during ExT in MVP patients with no or mild MR and to explore the relationship between these arrhythmias and the cardiac magnetic resonance (CMR) arrhythmogenic features

Material and Methods
We retrospectively identified patients with MVP and no or mild MR from CMR registries of four referral centers. All patients underwent ExT without beta-blockers or other anti-arrhythmic medications in the six months before or after CMR. Premature ventricular beats (PVBs) were classified by morphology and complexity. Patients with arrhythmias were those experiencing non-sustained ventricular tachycardia (NSVT).

Results
The study included 63 patients (52% female; median age 47 years, 54% physically active). PVBs were significantly more common during exercise (71%) compared to rest (38%, $p<0.001$) and significantly decreased during recovery (52%, $p= 0.012$). NSVT were more frequent during exercise (29% vs 3.2% in recovery, $p<0.001$). Mitral annulus disjunction (MAD) and curling were more severe in arrhythmic patients (MAD $p<0.001$, curling $p<0.001$). NSVT were more frequent in patients with LGE (46% vs 7%, $p<0.001$). In univariate logistic regression analysis, MAD, curling, and LGE emerged as independent predictors of arrhythmias ($p<0.001$; $p<0.001$; $p=0.003$, respectively).

Conclusions
VAs in MVP may be triggered by exercise. Exercise induced PVBs are associated with MAD and curling, more complex arrhythmias relate to the presence of LGE. These findings suggest a mechanical substrate for VAs in MVP, worsened by the adrenergic activation. Larger studies are needed to confirm our results.

Transcatheter Treatment of Mitral Valve regurgitation with Percutaneous Edge-To-Edge repair

Ph.D. Student: Dr. Giulia MASIERO
TUTOR: Prof. Giuseppe TARANTINI – CO-TUTOR: Dr. Chiara FRACCARO
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

Background: Mitral regurgitation (MR) is the second most common valvular disease in Western countries, and while transcatheter therapies have shown promising results in high-risk patients, real-world and long-term data are limited. The GISE registry of transcatheter treatment of mitral valve regurgitation with the MitraClip G4 (GIOTTO4) aims to confirm the safety of the device and enhance its effectiveness in a selected 'all-comers' ("more-comers") population with symptomatic severe MR undergoing or having undergone Transcatheter Edge-to-Edge Repair (TEER) with MitraClip G4 in contemporary Italian practice.
Material and Methods: This is a single arm, observational, multicentre, prospective and retrospective national registry under the auspices of Società Italiana di Cardiologia Invasiva (SICI-GISE) and conducted in a post-approval setting with an already approved indication (equivalent of phase IV study). Patients undergoing or having undergone a TEER procedure with the MitraClip G4 in selected hospitals within the GISE network, who meet all inclusion criteria and none of the exclusion criteria, will be enrolled starting from October 2023. All patients who have undergone the procedure since 13 October 2022 (the date of the first investigator meeting) may be enrolled retrospectively. The primary study outcome consists of two co-primary outcomes as percentage of participants with a residual MR grade ≤1+ the 30-day and 1-year. Two separate subgroups will be identified according to regurgitation etiology as the presence of functional (FMR) or degenerative (DMR) mitral valve disease. Several pre-specified clinical and echocardiographic secondary outcomes for safety and effectiveness will be evaluated in-hospital, at 30 days and at 1, 2, 3, 4 and 5 years. The sample size calculation is built on a historical event rate of the primary outcome at 30-day and 1-year. The sample size estimate is 534 for the FMR group and 389 for the DMR group (adjusted for a precision of 10% and a dropout rate of 10%). The enrolment phase is still ongoing. The echocardiographic examination performed at baseline, 30-day and 1 -year follow-up will be evaluated by a single, experienced and independent CoreLab. An independent adjudication committee of clinicians will adjudicate the main clinical outcomes. A Data Safety Monitoring Board will be established as well to provide external oversight to ensure safety of all trial participants.
(Preliminary) Results: As of July 2025, 537 patients (74% prospectively enrolled; 58% of the planned sample size) had been enrolled from 16 active centres, 44% in the FMR group and the remaining in the DMR group. Overall, 52% were male. Currently, data completeness in the electronic database is 80%. A mean of 1.5 ± 0.6 devices per patient have been implanted with a mean procedural time of 93 ± 43 minutes. The procedural complication rate was 3%, including acute structural failure of the implanted clip(s), vascular/structural complications, and procedure-related bleeding. At discharge, the rate of residual MR ≤1+ was 72% (historical event rates: 73% in the FMR group and 87% in the DMR group from the COAPT and Global EXPAND studies, respectively). No major adverse events were observed during the in-hospital period, including cardiovascular and non-cardiovascular death, myocardial infarction, cerebrovascular events, acute kidney injury requiring renal replacement therapy, mechanical ventilation, new-onset atrial fibrillation, infection, new ICD or CRT implantation, left ventricular assist device implantation, heart transplantation (HT), or HT listing.
Conclusions: These are the initial, encouraging safety and efficacy primary results from the GIOTTO4 registry in contemporary national real-world TEER practice for severe symptomatic MR. The enrolment phase is expected to conclude in 2026. Several pre-specified sub-group analyses are also planned (i.e. age (above vs. below median), gender (male vs female), baseline NYHA class, baseline medical therapy, MR etiology and baseline severity, baseline LVEF (above vs. below median; <30% vs. >=30%), pre-procedural right ventricular failure/pulmonary hypertension, presence of CRT, number of implanted devices, post-procedural acute technical results).

Subcutaneous Implantable Defibrillator Therapy in Patients with Brugada Syndrome: Data from a Large Multicenter Registry

Ph.D. Student: Dr. Raimondo PITTORRU
TUTOR: Prof. Federico MIGLIORE – CO-TUTOR: Prof. Martina PERAZZOLO MARRA
Supervisor of Research Fellowship and Research Collaborator activities abroad: Prof. Ivo Roca-LUQUE
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

Background
The implantable cardioverter-defibrillator (ICD) is recognized as the most effective life-saving therapy in patients with Brugada syndrome (BrS). However, transvenous ICD is associated with a notable rate of complications over time. The subcutaneous implantable cardioverter-defibrillator (S-ICD) has emerged as a promising alternative to the transvenous ICD. Nevertheless, long-term data from large cohorts of BrS patients with S-ICDs are lacking.

Objectives
This multicenter study aimed to assess the long-term outcomes of S-ICD therapy in patients with BrS.

Material and Methods
The study included 450 consecutive BrS patients (mean age 43 ± 12; 86% male) who underwent S-ICD implantation between 2014 and 2024.

Results
During a median follow-up of 52 months (25th-75th percentile: 29-72), appropriate shocks were delivered in 3% of patients (1.2%; 95% CI: 0.2-2.2, at 12 months), with a first-shock success rate of 90% (100% with 2 shocks). Inappropriate shocks occurred in 7% of patients (1.4%; 95% CI: 0.3-2.5, at 12 months). Shock zone programmed at 250 beats/min (HR: 0.40; 95% CI: 0.18-0.89; P = 0.025) and more than 1 suitable vector on screening (HR: 0.39; 95% CI: 0.17–0.87; P = 0.023) were independent protective factors against inappropriate shock. Device-related complications were reported in 4% of patients (2.5%; 95% CI: 1.0-3.9 at 12 months). The need for antibradycardia pacing was reported in 3 patients (0.7%). No device explantation because of the need for antitachycardia pacing was noted.

Conclusions
Our findings support the S-ICD as a viable alternative to the transvenous ICD for preventing sudden cardiac death in BrS patients without pacing indication.

Proof of concept of an *in vitro* model of selective endothelial decellularization in porcine hearts

Ph.D. Student: Dr. Nicola PRADEGAN
TUTOR: Prof. Gino GEROSA – CO-TUTOR: Prof. Emanuele COZZI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

Background: the shortage of suitable donors for heart transplantation compels the scientific community to seek effective therapeutic alternatives. Regenerative medicine is increasingly focusing on the development of bioengineered organs that are both functional and immuno-compatible. One promising approach in regenerative medicine involves organ decellularization followed by recellularization with recipient-derived cells; however, a standardized whole-organ protocol has yet to be established. Conversely, since the endothelium represents the primary barrier involved in immune-mediated rejection in xenotransplantation, we aimed to evaluate the preliminary efficiency of a protocol for selective decellularization of the entire porcine heart vascular endothelium.
Material and Methods: this project included an *in vitro* experimentation on whole hearts from wild-type adult pigs (n=4), using different chemical protocols: in fact, the aortic root was cannulated with appropriately sized cannulas and perfused with different detergent solutions (sodium dodecyl-sulfate -SDS- or trypsin), followed by washing solutions (distillate, normal saline or experimental cardioplegic solution) in a custom machine perfusion. Exposure time, flow, and temperature parameters were based on current literature and preliminary experiments. After perfusion, tissue samples were collected from the left anterior descending artery (LAD), the posterior descending artery (PDA), and the right (RV) and left ventricles (LV). These samples were then subjected to histological and immunofluorescence analyses. The primary objective was to qualitatively and semi-quantitatively assess the degree of endothelial decellularization. Secondary objectives included: evaluation of the integrity of the internal elastic lamina, preservation of myocardial tissue, and endothelial decellularization of the microvasculature.
Results: the four whole bio-engineered porcine hearts received an initial pre-wash with phosphate buffered saline (n=1), hypertonic solution (n=2) or experimental cardioplegic solution (n=1). Three hearts received SDS (0.05% at 21°C, 0.1% at 21°C, and 0.1% at 4°C), one heart received trypsin (at 37°C) as initial decellularizing agent. Subsequent washing was performed with distillate, normal saline, or experimental cardioplegic solution in 1, 3 and 1 case, respectively (in the first case distillate and normal saline were used together). Median (IQR) detergent exposure time (min), flow rate (mL/min) and aortic root pressure (mmHg) were 7 (6-8), 85 (79-92) and 65 (50-94), respectively. Compared to normal untreated counterparts, the 4 hearts showed signs of endothelial decellularization, with the SDS 0.1% at 4°C-protocol being the most efficient in lowering the number of endothelial cells within the coronary arteries. The different protocols did not significantly disrupt the internal elastic lamina, and myocardial cells/nuclei were not damaged or removed both around the large arterial vessels examined, or within the RV and LV. Microvascular selective dis-endothelialization was not demonstrated.
Conclusions: this project serves as a proof of concept that selective endothelial decellularization of a whole porcine heart can be successfully performed on the coronary vasculature using various protocols. Among these, the most effective is the one that closely mimics the para-physiological conditions of ex situ hypothermic perfusion of the heart. However, further studies are required to confirm the reproducibility and safety of this protocol, as well as its validation in an *in vivo* model, including chimeric repopulation of the endothelium with human cells.

The optimal time of reperfusion in high- and intermediate high-risk Pulmonary Embolism Patients

Ph.D. Student: Dr. Marco ZUIN
TUTOR: Prof. Alessandro ZORZI – CO-TUTOR: Prof. Claudio BILATO
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Cardiovascular Sciences"

BackgroundUnlike acute myocardial infarction or stroke, a definitive therapeutic time window for reperfusion in patients with high or intermediate-high-risk pulmonary embolism (PE) experiencing clinical deterioration has not been clearly established. This applies to both systemic fibrinolysis and more recently developed catheter-based interventions such as catheter-directed thrombolysis (CDTs) and mechanical thrombectomy. The aim of this study is to define the optimal time window for reperfusion therapy in high- and intermediate-high-risk PE patients undergoing systemic thrombolysis or CDT, while also considering the dynamic evolution of thrombus composition.
Material and Methods: A prospective, multicenter Italian registry, namely Optimal Time for Reperfusion in Acute Pulmonary Embolism (OPTIRAPE), has been created. Eight centers with recognized expertise in the management of acute PE are participating. The registry includes patients with confirmed high- or intermediate-high-risk PE treated with either systemic thrombolysis or CDTs. For each patient, comprehensive data are collected, including baseline clinical characteristics, PE risk factors, imaging and laboratory findings, and precise timing of reperfusion relative to hospital admission. The primary endpoint is in-hospital mortality in relation to the timing of reperfusion using systemic thrombolysis or CDT. Secondary endpoints include the incidence of cardiovascular complications and major bleeding events, also analyzed according to the timing and type of reperfusion strategy employed (*Figure 1*).
Results: As of August 1st, a total of 386 patients (mean age: 61.2 ± 10.4 years) have been enrolled across participating centers. Of these, 128 patients (33.1%) have been treated using CDT. Patient recruitment is ongoing and is expected to conclude on July 1st, 2026. The target sample size of at least 500 patients is anticipated to provide sufficient power for robust statistical analyses.
Conclusions: The OPTIRAPE registry is expected to generate valuable and novel insights into the impact of reperfusion timing in high- and intermediate-high-risk PE. It will also provide comparative data on different reperfusion modalities, helping to inform clinicians about the dynamic nature of thrombus formation and the potential benefits of early intervention. Ultimately, this study aims to identify optimal therapeutic windows for reperfusion in patients at the highest risk, a population with particularly elevated mortality rates.

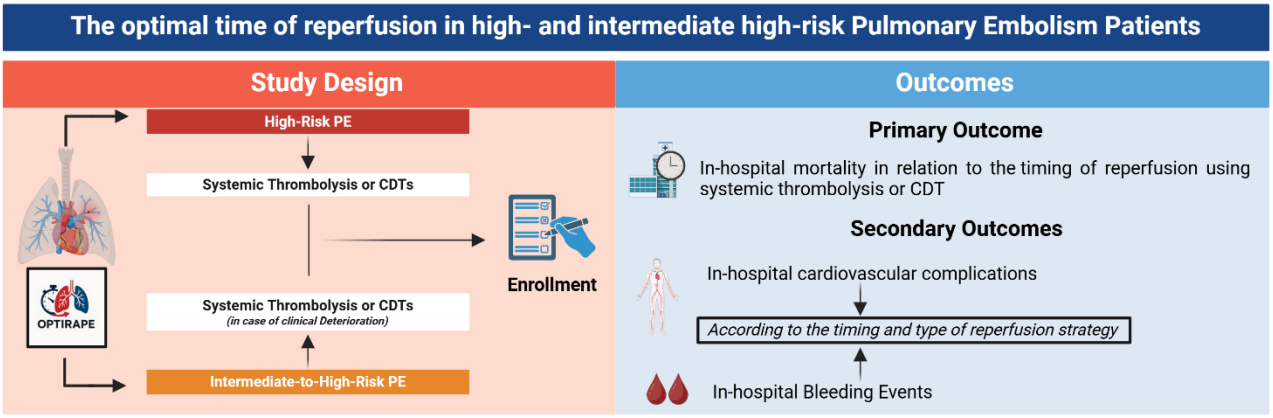


Figure 1. Study Design and Outcomes of the OPTIRAPE Registry.

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

COORDINATOR: PROF. DARIO GREGORI

Curriculum "CLINICAL AND TRANSLATIONAL NEUROSCIENCES"

New therapeutic approaches in neuropsychiatric disorders

Ph.D. Student: José Miguel BOLAÑO
TUTOR: Prof. Chiara BRIANI
CO-TUTOR: Prof. Alessandro SALVALAGGIO, Prof Mara Cristina ROMERO, Dr. Honoria OCAGLI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Clinical and Translational Neurosciences"

Background
Major Depressive Disorder (MDD) is a prevalent mental health condition with substantial impact on disability and quality of life worldwide. Despite the availability of pharmacological and psychotherapeutic treatments, response rates remain limited—only 40–50% of patients achieve remission—and many experiences adverse effects or develop treatment resistance. In this context, the use of psychedelics such as psilocybin has gained renewed attention. Preliminary studies report favorable outcomes in depressive symptoms, with faster onset, longer-lasting effects, and fewer side effects compared to conventional therapies. However, clinical implementation is hindered by the absence of standardized protocols for psilocybin formulation, dosage, and therapeutic monitoring.
Given this gap, the present study aimed to develop a reproducible protocol for cultivating psilocybin-producing mushrooms and obtaining pharmacological preparations that ensure accurate dosing and controlled patient response in the treatment of resistant depression.
Material and Methods
Mushroom production was carried out in three stages:
1- Spore Collection: Spores were obtained by placing the cap of a Psilocybe cubensis mushroom on a sterile glass surface in dry conditions.
2- Mycelium Cultivation: The resulting mycelium was used in two systems: submerged liquid culture and tray-based solid culture.
3- Fruiting and Harvesting: In the tray method, once the substrate was fully colonized, fruiting was induced under high humidity (80%), indirect lighting, and ample ventilation.
Harvested mushrooms underwent drying, followed by organic extraction of the active compound. Identification of alkaloids, including psilocin, was performed using the Dragendorff reagent for qualitative analysis.
Results
Initial cultivation trials with imported strains were successful. In this phase, a native strain, tentatively identified as Psilocybium Panaeolus, was cultivated under stable laboratory conditions. Its psilocybin content was confirmed using Dragendorff-based chemical screening.
Beyond laboratory work, dissemination efforts have included international and national academic outreach. The research was presented at the 1st World Summit of Internal Medicine and the 17th Paraguayan Congress of Internal Medicine (Asunción, Paraguay, May 2025), and in two academic activities at the Higher School of Medicine, National University of Mar del Plata (Argentina). These events addressed the translational pathway from laboratory research to clinical application in refractory depression.
Conclusions
A native psilocybin-producing strain was successfully cultivated and preliminarily characterized. Further steps include obtaining regulatory approval from Argentina's National Administration of Drugs, Food and Medical Technology (ANMAT), currently pending. Upcoming activities include completion of the literature review and preparation for publication, as well as continued academic dissemination.

Cognitive resilience in Alzheimer's: from genetics to modifiable predictors

Ph.D. Student: Luis Ignacio BRUSCO
TUTOR: Prof. Laura ASTOLFI – CO-TUTORS: Prof. Gino MARIONI, Prof. Daniel CARDINALI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Clinical and Translational Neurosciences"

Cognitive resilience is the ability to withstand brain injury. Addressing modifiable risk factors, such as blood pressure, cholesterol, head trauma, obesity and sleep quality could prolong healthy life and reduce the time lived with the disease or even prevent it.Alzheimer's disease (AD) is a complex neurodegenerative condition influenced by genetic and environmental factors. The APOE ε4 genotype is a well-established genetic risk factor for AD, but modifiable risk factors also play an important role in disease pathogenesis. This cross-sectional study investigated the relationship between modifiable risk factors and the APOE genotype in a cohort of patients with AD.

Methods
Patients with cognitive complaints attending the Alzheimer's Center at the Hospital de Clínicas of the University of Buenos Aires were recruited and underwent cognitive assessment and APOE genotyping, as well as blood markers related to risk factors (LDL,glycemia). Data were collected on modifiable risk factors, including hypertension, diabetes, obesity, smoking and lack of physical activity, and sleep quality.

Results
A high prevalence of hypertension, sleep disorders, and the ApoE4 genotype were associated with cognitive complaints. Furthermore, the presence of multiple modifiable risk factors was found to be associated with greater disease severity.

Conclusion
Our findings suggest that modifiable risk factors and the APOE genotype interact to influence AD risk. Identifying and modifying these risk factors could be an effective strategy to prevent or delay disease progression.

Keywords: Alzheimer's disease, APOE, modifiable risk factors, cross-sectional study.

TOWARDS CLINICAL AND MOLECULAR OUTCOME MEASURES IDENTIFICATION IN LIMB GIRDLE MUSCULAR DYSTROPHY R1 (LGMDR1)

Ph.D. Student: Giuliana CAPECE
TUTOR: Prof. Luca BELLO – CO-TUTOR: Prof. Elena PEGORARO
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Clinical and Translational Neurosciences"

Background
LGMDR1 is a neuromuscular disorder due to biallelic pathogenic variants in *CAPN3* gene, encoding for the muscle-specific proteolytic enzyme calpain-3. Main clinical features are proximal muscular weakness, scapular winging, hyperlordosis and joint contractures. Although muscle biopsy may display a protein deficit by Western Blot (WB), the current gold standard for LGMDR1 diagnosis involves the identification of biallelic pathogenetic *CAPN3* variants. A specific treatment is still lacking, but many pharmacological trials are ongoing. We aimed to characterize clinical and molecular features of LGMDR1 patients and to refine genotype-protein-phenotype correlations in our historical cohort. Then, we aimed to describe disease progression in a subgroup of patients over 9 years, using validated functional tests to identify sensible outcome measures.
Material and Methods
We collected demographical and molecular data of 102 patients carrying biallelic *CAPN3* variants selected from the Neuromuscular Biobank Database of the University Hospital of Padova. We also recorded functional status at last examination for 75/102 (mean age 34.57 ± 14.53 years; median age 34 years). Then, we collected relevant disease milestones and longitudinal data for 30 patients over 9 years including Manual Muscle Testing (MMT), North Star Ambulatory Assessment (NSAA), timed function tests (TFTs) and Performance Upper Limb (PUL). Patients were subdivided by the protein amount at WB, the number of null variants and the presence of variants in the protein autocatalytic domain (exon 11).
Results
In the retrospective cohort, we found significant inverse correlations between number of null *CAPN3* variants and protein amount and between protein amount and clinical severity at last neurological examination ($p \leq 0.05$).
Patients carrying two null variants showed loss of ambulation (LoA) at a median age of 34 years (95% IC, confidence interval: 21 years to NA, p 0.027) versus 53 years in all patients (95% IC, confidence interval: 48 years to NA) and earlier LoA was significantly associated with complete calpain-3 deficiency in skeletal muscle ($p \leq 0.05$). MMT revealed a phenotypic spectrum ranging from asymptomatic/mild symptomatic to severe forms, with an intermediate mainly scapulo-brachial ("Erb") phenotype especially in patients carrying missense variants in exon 11.
Patients longitudinally evaluated were characterized by age, number of null alleles, pathogenic variants in exon 11, protein amount at WB and sex. NSAA, TFTs and PUL score decrease over time ($p<0.05$), except for PUL distal level score according to longer preserved function in distal upper limbs in LGMR1. PUL score shows a linear decline of ~0.5 point/year (p 0.002), in all patients. Calpain-3 deficiency and pathogenic variants in exon 11 are negative functional prognostic factors.
Conclusions
Number of *CAPN3* null variants and calpain-3 amount at WB should be considered in patients' stratification in clinical trials. Functional outcome measures used in this study appear reliable surrogate markers of disease progression. In particular, TFTs and NSAA are reliable outcome measures in young patients, while PUL might be used in older patients. However, further studies in a larger multi-center cohort are needed to ensure the reliability of these outcome measures.

INSULIN RESISTANCE AND APO E4 IN ALZHEIMER DISEASE. CROSS SECTIONAL STUDY

Ph Student: Cynthia DUNOVITS
TUTOR: Prof. Elena PEGORARO - CO-TUTOR: Prof. Stefano MOZZETTA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Clinical and Translational Neurosciences"

Background
Insulin plays a fundamental role in the central nervous system. Beyond its metabolic role, central nervous system insulin signaling modulates neurotrophic effects and tau protein phosphorylation, processes implicated in Alzheimer's disease (AD). Cerebral insulin resistance, characterized by diminished insulin action in the brain, can arise from impaired insulin transport across the blood-brain barrier. Cerebrospinal fluid (CSF) insulin levels, reflecting peripheral levels, suggest pancreatic origin. Furthermore, the ApoE4 genotype, a major risk factor for late-onset AD, may disrupt this signaling pathway. This way, AD pathogenesis may involve neuronal insulin signaling deficits.

Material and Methods
Patients with cognitive complaints attending the Alzheimer's Center at the Hospital de Clínicas of the University of Buenos Aires were recruited and underwent cognitive assessment and APOE genotyping, as well as blood markers related to risk factors (LDL,glycemia, insulinemia, HOMA, PCR). Data were collected on modifiable risk factors, including hypertension, diabetes, obesity, smoking and lack of physical activity, and sleep quality.

Results
High prevalence of risk indicators associated with metabolic syndrome profiles: hypertension, increased LDL, altered blood glucose and insulin levels in patients with cognitive complaints.

Conclusion
Mitochondrial dysfunction, inflammation, and advanced glycation end product (AGE) accumulation appear to be common pathways linking AD and insulin resistance. The ApoE4 genotype may predispose the brain to insulin resistance. While antidiabetic agents show promise, current evidence for their efficacy in AD treatment remains limited.

Neuroscience and Skin: Mindfulness Intervention for the Management of Psoriasis

Ph.D. Student: María Alejandra FALÚ
TUTOR: Prof. Gianni SORARU – CO-TUTORS: Prof. Mauro ALAIBAC, Prof. Hugo CABRERA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Clinical and Translational Neurosciences"

Background
Neuroscience studies the nervous system and its interactions with cognition, behavior, and emotions. The skin, sharing embryonic origin (ectoderm) with the nervous system, is a sensory and emotional interface. Chronic dermatoses such as psoriasis demonstrate the brain-skin connection, with emotional stress exacerbating symptoms. Mindfulness, a form of attention-based cognitive training, has shown promise in reducing stress and improving resilience. This study explores the impact of mindfulness as an adjuvant therapy in patients with psoriasis who are unresponsive to conventional treatments.

Material and Methods
A descriptive, analytical study involving 22 adult patients with psoriasis, recruited from the Dermatology Service of Hospital San Bernardo, Salta, Argentina. After 4 months of standard treatment without significant improvement, patients will undergo an 8-week group-based mindfulness intervention (1 hour/week, in-person), from **August 8 to November 7, 2025**, while continuing their usual treatment. Patient data (clinical severity, quality of life, systemic involvement) is recorded pre- and post-intervention using a custom-designed score in clinical records. Monthly follow-ups are conducted.

Results
Preliminary: Patients represent various severities of psoriasis (mild, moderate, severe, with systemic manifestations). Variables include age, sex, phototype, comorbidities, treatment type, and quality-of-life metrics (work, sleep, self-esteem, interpersonal impact). Final results will compare pre- and post-intervention data, focusing on cutaneous and extra-cutaneous symptoms, perceived stress, and quality of life.

Conclusions
This research aims to evaluate mindfulness as a complementary strategy in managing psoriasis, improving outcomes for patients resistant to standard therapies. Integrating neuroscientific and dermatologic approaches may lead to more holistic, effective care models for chronic inflammatory skin diseases.

Development and Validation of an *In Vitro* Sound Stimulation Delivery System for Inner Ear Tissues

Ph.D. Student: Filippo HELLIES
TUTOR: Prof. Laura ASTOLFI – CO-TUTOR: Prof. Gino MARIONI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Clinical and Translational Neurosciences"

Background
This study introduces an innovative *in vitro* sound stimulation system for Noise-Induced Hearing Loss (NIHL) research. Our bioengineering platform accurately simulates the inner ear's acoustic environment to investigate NIHL cellular mechanisms and support regenerative therapies. The research focused on two primary objectives: first, to examine morphological and cytoskeletal changes in OC-k3 inner ear cells under repeated, low-intensity sound stimulation at key resonance frequencies; second, to quantify cell viability and noise-induced cell death following prolonged, high-intensity stimulation. This *in vitro* approach provides a highly controllable, ethical alternative to in vivo models, aligning with 3R principles for scientific research.

Material and Methods
The research involved two experiments using a solid-borne vibration mechanism. First, we investigated morphological changes in cultured OC-k3 cells by applying repeated low-intensity stimulation (30, 40, 50 Hz) for 30 minutes daily over three days. Viability was assessed with an MTS/PMS assay; morphological changes were analyzed via DAPI-Phalloidin staining and fluorescence microscopy. The second experiment evaluated viability under prolonged, high-intensity conditions. A thermally regulated setup with an accelerometer delivered a single stimulation (27 Hz) to high-density cells for 1-4 hours. Viability was then assessed using flow cytometry with Propidium Iodide staining.

Results
Low-intensity stimulation induced significant cytoskeletal reorganization in OC-k3 cells. Fluorescence microscopy revealed new cellular protrusions (lamellipodia, filopodia, stress fibers), indicating a dynamic migratory response not seen in controls. While MTS assays showed a slight vitality decrease, overall viability remained high, suggesting the changes were related to morphological transformation rather than widespread cell death. Morphological complexity analysis confirmed stimulated cells were significantly different from controls, with lower frequencies (particularly 30 Hz) inducing the greatest complexity. In contrast, prolonged high-intensity stimulation led to a statistically significant, time-dependent increase in cell mortality, demonstrating the system's capacity to effectively simulate dose-dependent acoustic trauma.

Conclusions
Our *in vitro* system effectively investigates cellular responses to acoustic stimuli. Low-intensity sound induced morphological changes that strongly suggest a partial Epithelial-Mesenchymal Transition (EMT), a process critical for hair cell regeneration. The frequency-dependent response, with 30 Hz causing the most significant change, offers key insights into OC-k3 mechanosensitivity. The system's dual capability—inducing non-lethal transformations and modeling dose-dependent acoustic trauma—is a key achievement. This validates our platform as a robust, ethical, and controllable alternative to in vivo models for studying NIHL and cellular regeneration. Future research will explore molecular pathways and screen otoprotective agents, advancing novel hearing restoration strategies.

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

COORDINATOR: PROF. DARIO GREGORI

Curriculum "ENDOCRINE AND METABOLIC SCIENCES"

Nutritional approach to metabolic diseases

Ph.D. Student: Dr. Giorgia GUGELMO
TUTOR: Prof. Gian Paolo FADINI – CO-TUTOR: Prof. Angelo AVOGARO
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Endocrine and Metabolic Sciences"

Background: Inherited Metabolic Diseases (IMDs) are a heterogeneous group of rare genetic disorders involving dysfunctions in fundamental metabolic pathways. These conditions often result in the accumulation or deficiency of intermediate metabolites and are typically chronic, multisystemic, and clinically complex. Nutritional management plays a pivotal role in most IMDs, especially in disorders affecting protein, carbohydrate, lipid metabolism, and lysosomal storage. Despite this, nutritional and functional evaluations are often fragmented in clinical practice.
Material and Methods: This project includes both retrospective and prospective observational components in adults followed at the Division of Metabolic Diseases of the University Hospital of Padova. Nutritional and metabolic assessments included dietary intake, body composition (BIA), indirect calorimetry, handgrip strength, physical performance (e.g., 6MWT, CPET), and biochemical markers. A completed sub-study involved 15 patients with Fabry Disease (FD), a lysosomal storage X-linked disorder, exploring gastrointestinal manifestations and a low-FODMAP diet intervention. Muscle metabolism and functional capacity were assessed through CPET and indirect calorimetry in 35 FD patients. Continuous glucose monitoring (CGM) was used in 11 adults with IMDs at risk for hypoglycemia to investigate glycemic metrics, and both diet and psychological and cognitive domains were evaluated. Data on hepatic complications were also collected from 10 patients with hepatic Glycogen Storage Disease (GSDs) and 10 with urea cycle disorders (UCDs).
Results: In FD cohort, the intervention with a low-FODMAP diet led to significant improvements in GI complaints; nutritional assessment revealed that severe phenotype males had lower BMI and fat-free mass index, and treated males showed a significantly reduced phase angle compared to untreated ones ($4.8° \pm 1.0$ vs. $7.6° \pm 0.9$; $p = 0.04$), suggesting compromised cellular integrity. The phase angle was strongly correlated with aerobic capacity (VO_2peak, $r = 0.879$, $p = 0.01$), and more than 70% of classic males displayed reduced muscle strength and physical performance, pointing to alterations in both nutritional status and physical fitness. In the IMDs at risk for hypoglycemia, two different devices were compared, and all patients were treated with personalized dietary regimens (e.g., frequent cornstarch-based meals). Preliminary findings suggest acceptable accuracy; no differences in glycemic variability patterns were observed across devices. However, nighttime hypoglycemia was associated with increased concern and altered behavioral strategies, while reduced hypoglycemia awareness—linked to higher TAR—may reflect compensatory dietary or metabolic adaptations. In the hepatic GSD and UCD patients, nutritional treatment adherence and hepatic complication data were collected, highlighting the complex interplay between dietary management, liver involvement, and overall metabolic control.
Conclusions: This project underscores that nutritional therapy remains a cornerstone in the management of many IMDs and should be personalized based on phenotype, metabolic demands, and physical performance. In particular, for conditions such as FD—where nutritional aspects have traditionally been overlooked—the integration of nutritional, metabolic, and functional assessments can uncover hidden vulnerabilities, including sarcopenia, fatigue, and metabolic inefficiency, all of which have a substantial impact on quality of life. Furthermore, CGM-based monitoring offers novel insights into the real-time interplay between glucose dynamics, cognitive-emotional functioning and dietary treatments in IMDs prone to hypoglycemia. Future challenges in the field of nutrition and IMDs may also involve translational approaches, with preclinical models such as *Drosophila melanogaster* offering promising opportunities to explore innovative and personalized dietetic therapies.

Androgens and cognitive impairment in elderly men with Metabolic Syndrome

Ph.D. Student: Demetrio Mateo MARTINEZ
TUTOR: Prof. Angelo AVOGARO
CO-TUTORS: Prof. Gian Paolo FADINI, Prof. Rodrigo O. MARAÑÓN
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Endocrine and Metabolic Sciences"

Background. Cognitive empairment is a common disorder in old age and is expected to increase in that age group over the next 30 years. Disorders such as obesity and diabetes are risk factors for dementia, and generally do not occur alone but in conjunction with others, like hypertension and dyslipidemia-defining metabolic syndrome (MS). As part of the aging process, the level of male sex hormones decreases; however, little is known about the role of these steroids in the development of cognitive decline in the elderly with metabolic syndrome. Therefore, the objective of the present study is to evaluate the influence of androgenic and MetSyn on cognitive decline in the elderly, through a systematic review and using an experimental animal model of MS.

Material and Methods. Systematic review: Database searches were performed in COVIDENCE for articles assessing cognitive performances of older subjects with hypoandrogenemia and metabolic syndrome. Briefly, the process began with the formulation of a focused research question based on the PICO framework considering the research topic. Then, a comprehensive literature searches were performed across multiple databases (Medline, EMBASE, Cochrane Library, Scielo, etc), and identified references were imported into Covidence for deduplication. The protocol will be presented in PROSPERO. Title and abstract screening were conducted by multiple reviewers, followed by full-text assessments to determine study eligibility based on predefined inclusion and exclusion criteria. Data extraction is doing structured using a customized Covidence template, focusing on key study characteristics and outcomes. The quality of included studies will assesses using appropriate tools. Data synthesis will involve qualitative analysis and, if appropiatre, meta-analysis of findings. The review adhered to PRISMA guidelines for reporting.
Experimental study: Intact and castrated animals were induced to metabolic syndrome through 10% fructose solution. We will assess cognitive decline through Novel Object Recognition test. We also will evaluate hemodynamic, lab, and histopathological parameters.

Results. Systematic review: more than half steps were done. Currently we are finishing the analysis process of the articles. Experimental study: We have preliminary hemodynamic results. We will complete the results in the coming months.

Conclusions. This study aims to elucidate the complex interplay between androgen levels and the development of cognitive decline in elderly individuals with metabolic syndrome. By integrating findings from a systematic review and an experimental animal model, we are poised to enhance our understanding of how hypoandrogenemia may contribute to cognitive impairments associated with metabolic syndrome. Preliminary results from the experimental study indicate promising directions for understanding the hemodynamic changes linked to cognitive performance. Ultimately, the outcomes of this research could inform future interventions aimed at mitigating dementia risk in older adults through hormonal and metabolic health strategies. Continued analysis of the systematic review will further clarify the clinical implications of our findings.

RISK OF PERIPHERAL ARTERIAL DISEASE AND AMPUTATION IN PEOPLE WITH DIABETES

Ph.D. Student: Dr. Mario German PAGNO
TUTOR: Giovanni SARTORE - CO-TUTOR: Prof. Annunziata LAPOLLA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Endocrine and Metabolic Sciences"

Background: Diabetes mellitus causes several microvascular and macrovascular complications, resulting in a greater risk of morbidity and mortality. In Argentina 12 % of the population knows that is diabetic.Diabetic foot ulcer is a common complication . The risk of foot ulceration is estimated to be around 25% with a point prevalence of 2% in diabetic population.
Identifying risk factors for mortality is important in the management of DFS. Although morbidity and mortality-related risk factors have been investigated for diabetic foot ulcer, there are a limited number of studies examining mortality risk factors in diabetic foot infection.
The high mortality of DF raised the importance of preventing foot ulcers.

Material and Methods: This study aimed to evaluate mortality during hospitalization of patients admitted for diabetic foot and prognostic factors, during the period 2019-2025. A cross-sectional observational study was carried out in a teaching hospital in Corrientes Argentina.
204 clinical histories were evaluated, compiling the data in a database: including mortality, days of hospitalization, location of the infection, major and minor amputations, germ obtained, Doppler of both lower limbs, glycosylated HBg, degree of arteriopathy and PEDIS score.

Results 204 type 2 diabetic patients with PAD, 13 Years of mean duration of diabetes, were admitted to the study during this period; 30 patients died during hospitalization, which representes an in-hospital mortality of 14.7%.
Poor prognostic factors included Extensive lesions ,mean PEDIS score 9, peripheral artery disease, predominantly severe , elevated inflammatory markers (PCR ESR). Gram negative bacteria (Klebsiella pneumoniae and E. coli) Tabaquism poor metabolic control. (HbgA1C 8,40)
Antibiotic resistance in lesions were associated with higher mortality. 64 patients need mayor amputation and 32 minor amputation. Ulcer recurrence or its complications required readmissions with new major amputations in those patients with previous minor amputations (27 patients) during the course of 1 year (patients with heart failure, kidney failure and sepsis at the point of splitting of skin and soft tissue).

Conclusions
Advanced age, severe peripheral artery disease, chronic kidney disease, previous minor amputations, and previous cardiovascular disease, in addition to poor diabetes control, were associated with higher morbidity and mortality. Diabetic foot is a common complication of diabetes associated with significant morbidity and mortality, readmissions and new amputations. In our center, sepsis affecting the skin and soft tissue was the most frequent cause of death. This requires greater prevention and monitoring of diabetic patients, trying to avoid the first diabetic foot lesion.

Body Fat Distribution and Its Relationship to Estrogen Levels in Perimenopausal Women with Metabolic Syndrome

Ph.D. Student: Dr. Roxana del Valle TOLEDO
TUTOR: Prof. Angelo AVOGARO
CO-TUTORS: Prof. Gian Paolo FADINI, Prof. Rodrigo MARAÑÓN
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Endocrine and Metabolic Sciences"

Background. Estrogens play a crucial role in regulating body fat composition and distribution. In premenopausal women, higher estrogen levels are associated with increased subcutaneous fat, while after menopause, lower estrogen levels contribute to an increase in visceral fat, elevating the risk of developing metabolic syndrome, vascular dysfunction, and cardiovascular events. However, the influence of estrogens on body fat distribution during perimenopause remains poorly understood. This study aims to evaluate the impact of estrogen on body fat and its role in the development of metabolic syndrome in perimenopausal women, supplemented by an experimental animal model.

Material and Methods. For the *systematic review*, comprehensive searches were conducted in Covidence for articles assessing body fat distribution in perimenopausal women with metabolic syndrome. The process began with the formulation of a focused research question using the PICO framework. Literature searches were performed across multiple databases (Medline, Cochrane, Embase, etc.), and references were imported into Covidence for deduplication. The protocol will be presented in PROSPERO. Title and abstract screenings were conducted by multiple reviewers, followed by full-text assessments to determine study eligibility based on predefined inclusion and exclusion criteria. Data extraction was structured using a customized Covidence template that emphasized key study characteristics and outcomes. The quality of the included studies will be assessed using appropriate evaluation tools, and data synthesis will involve qualitative analysis and, where applicable, meta-analysis of findings, adhering to PRISMA guidelines for reporting. In the *experimental study*, intact and ovariectomized perimenopausal female rats were induced to develop metabolic syndrome through administration of a 10% fructose solution. Body fat distribution will be assessed using computerized tomography (CT) scans, alongside evaluations of hemodynamic, laboratory, and histopathological parameters.

Results. Systematic review: more than half of the process has been completed, and we are currently finalizing the analysis of the articles. Experimental study: preliminary hemodynamic results have been obtained, and further results are expected in the coming months.

Conclusions. This study aims to provide critical insights into the role of estrogens in body fat distribution and the development of metabolic syndrome during the perimenopausal period. By integrating findings from both a systematic review and an experimental model, we seek to elucidate the implications of hormonal changes on metabolic health in perimenopausal women. The ongoing analysis will enhance our understanding of these dynamics, potentially informing future therapeutic strategies targeting hormonal and metabolic interventions in this population.

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

COORDINATOR: PROF. DARIO GREGORI

Curriculum "NURSING AND HEALTH SCIENCES"

Palliative care: Decision-making process, treatments, and communication in palliative care of patients facing life-threatening illnesses

Ph.D. Student: Dr. Sofía BUNGE
TUTOR: Prof. Paola DI GIULIO – CO-TUTOR: Dr. Matteo MARTINATO
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Nursing and Health Sciences"

Study: Patient's Decisional Control Preferences in the Palliative Care Setting. Olavarria, Argentina.

Background

Decision-making is a daily practice for all human beings. In clinical practice, patients and healthcare professionals make decisions with varying degrees of certainty and uncertainty, whether related to diagnostic or therapeutic processes or healthcare.

Today, medicine faces a growing challenge with the rise of chronic and advanced diseases, which often require a prolonged and complex approach to care. Palliative care not only focuses on the management of physical symptoms, but also addresses emotional, social and spiritual aspects, providing holistic support for both patients and their families. Decisions about care can be particularly difficult and must be aligned with the patient's values and wishes. This requires effective communication between patients, families and health professionals. However, how patients want to be involved in making decisions about their care, and treatments remains an issue that needs to be further explored.

Even today, a paternalistic view persists within the healthcare field, in which it is assumed that many patients prefer to adopt passive roles, delegating decisions to healthcare professionals. This view, however, is based more on anecdotal observations than on solid evidence. In Argentina, multicentre studies have been carried out in a public hospital in the Autonomous City of Buenos Aires (CABA), which contradict this. It is in this context that the present study was carried out in Olavarría, a city in the interior of the province of Buenos Aires. The aim of this study is to know preferences of palliative care patients regarding decision-making related to their medical care, the medical information they wish to receive and the proportion of patients who wish to receive information or not.

Material and Methods

A prospective, transversal observational quantitative study, conducted using self-administered surveys (Control Preference Scale—Modified, Yennurajalingam et al., 2013) in a population of patients with advanced cancer in palliative care follow up. The study was conducted by Palliative Care at the Hospital Especializado en Oncología L. Fortabat, in a city in the province of Buenos Aires, Argentina, between June 2024 to March 2025. The interviews collected preferences in decision making and communication.

Preliminary results

100 patients were included. 58 were females**.** 69 preferred a shared role in decision making related to their medical care, 19 a passive role, and 12 an active role. When analysing how decisions were actually made, 68 patients experienced a shared decision-making process, 19 experienced a passive process, and 13 experienced an active process. Additionally, 93% of patients expressed a desire to be fully informed about their diagnosis and 96% of their prognosis.

Conclusions

These preliminary results, which demonstrate a clear patient preference for shared decision-making, highlight the critical need to understand and evaluate the needs and preferences of the patients in order to provide tailored care and improve the competencies of the healthcare professionals involved.

Research protocol approved by the Hospital´s Ethic Committee.

MEDharmonize: Medical Data Harmonization Framework for Molecular Data based on Large Language Model and Electronic Data Capture System integration

Ph.D. Student: Dr. Cinzia Anna Maria PAPAPPICCO
TUTOR: Prof. Dario GREGORI – CO-TUTOR: Prof. Giulia LORENZONI
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Nursing and Health Sciences"

Background
The harmonization of clinical molecular data represents a major challenge for personalized medicine and multicenter oncological research. The coexistence of heterogeneous terminologies, non-interoperable local coding systems, and unstructured molecular reports hinders data standardization and slows their integration into electronic data capture (EDC) systems such as REDCap. This issue is particularly relevant within the context of the international initiative led by the International Association for the Study of Lung Cancer (IASLC), which aims to establish global registries to support clinical and translational research in lung cancer, mesothelioma, and thymic malignancies. These infrastructures require scalable solutions for the automated semantic harmonization of molecular data. Large Language Models (LLMs) offer an emerging opportunity to automate the semantic extraction and structuring of such reports, yet operational frameworks integrated with EDC systems and validated in clinical settings are currently lacking.
Material and Methods
We developed MEDharmonize, a framework integrating LLMs, REDCap, and the R environment to enable automatic extraction, classification, and semantic harmonization of oncological molecular data from unstructured NGS reports in PDF format. The analyzed reports included single-nucleotide variants, insertions/deletions, gene fusions, and clinical annotation. Text content was automatically extracted and converted into a structured natural language prompt, including task instructions, contextual information, and expected output format. Prompts were submitted to a GPT-family model via the OpenAI API, integrated within the REDCap workflow using R packages to ensure seamless interoperability and scalability. The model returned a structured output containing clinically relevant entities, which were semantically harmonized and automatically imported into REDCap via API token. All clinical data were pseudonymized locally before transmission, ensuring data security and regulatory compliance. In parallel, each report was manually annotated by a domain expert, serving as the Gold Standard. LLM outputs were compared with the GS and evaluated for accuracy, consistency, and reproducibility using the following metrics: percent agreement, Cohen's kappa, precision, recall, and F1-score. A human-in-the-loop validation process was implemented across three stages: prompt review, LLM response assessment, and resolution of discrepancies with the GS.
Results
A preliminary evaluation was conducted on four molecular reports from patients with confirmed lung cancer, yielding 10 comparable molecular entities. MEDharmonize achieved: mean F1-score: 0.93 (95% CI: 0.90–0.96), Cohen's kappa: 0.88, percent agreement: 91.2%. Accuracy in gene identification reached 98%. The average time per report decreased from 12:40 to 5:10 minutes, with a mean correction rate of 6.3% on the LLM-generated outputs.
Conclusions
MEDharmonize provides a robust and scalable solution for the automated harmonization of molecular oncology data. It significantly improves the efficiency of processing unstructured molecular reports while preserving high standards of accuracy and quality control. Aligned with the goals of the IASLC project, MEDharmonize enables semantic standardization and interoperability of real-world molecular data across global registries. Future developments will expand the dataset, extend the molecular domains, and evaluate the semantic robustness of the framework in longitudinal and multicenter settings.

BedTRACK - Advancing AI-Driven Bedside Monitoring for Fall and Pressure Ulcer Risk Assessment

Ph.D. Student: Dr. Konstantina-Thaleia PILALI
TUTOR: Prof. Paola BERCHIALLA– CO-TUTOR: Dr. Giorgia STOPPA
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Nursing and Health Sciences"

Background
Falls from bed and pressure ulcers are serious complications in hospitalized and long-term care patients, leading to higher morbidity, prolonged stays, and increased healthcare costs. Traditional risk assessments rely heavily on subjective clinical judgment, underlining the need for objective, data-driven monitoring. The BedTRACK project aims to develop a real-time predictive system using machine learning (ML) algorithms to identify patients at risk of falls and pressure injuries by integrating multi-source sensor data.

Materials and Methods
This experimental study is being conducted at the Unit of Biostatistics and Clinical Epidemiology, University of Padova. BedTRACK integrates data from a tactile matrix sensor mattress, two Garmin Fenix smartwatches for continuous movement tracking, video monitoring, and individual clinical and anthropometric data. Data were collected from 60 healthy volunteers and systematically labeled using a custom framework that synchronizes sensor signals and video-recorded movements at the frame level.
The predictive model under development uses a hybrid deep learning approach, combining Recurrent Neural Networks (RNNs) for temporal analysis with Convolutional Neural Networks (CNNs) for spatial feature extraction. Attention mechanisms and transfer learning are being tested to enhance generalizability and performance across diverse patient profiles. The neural network integrates tactile, wearable, and video features to assess movement and infer fall and pressure injury risk in real time.

Results
Early tests revealed two root causes behind initial misalignment: (i) differing frame rates between video capture and processing, and (ii) an unclear label taxonomy. These issues led models to learn noise rather than actual movement patterns.
This prompted a complete overhaul of the alignment and labeling pipeline. Frame rates were synchronized across all stages, time-stamping logic was re-engineered, and the label schema was redesigned with precise clinical definitions. Rigorous frame-level quality checks and manual cross-validation were implemented.
The new pipeline has produced a tightly aligned, high-resolution dataset now undergoing final verification. While full model training is pending, the codebase and network architectures have been updated in line with the new data structure, allowing training to begin once validation is complete.

Conclusions
BedTRACK offers an innovative AI-based approach for bedside monitoring, combining wearables, sensor mattresses, and deep learning. Real-time sensor integration promises more accurate, personalized risk assessments, supporting timely interventions and improved patient safety. Ongoing work will focus on optimizing model accuracy, validating in clinical settings, and evaluating broader applicability across patient groups.

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

COORDINATOR: PROF. DARIO GREGORI

Curriculum "THORACIC AND PULMONARY SCIENCES"

Resident and inflammatory eosinophils among different asthma age of onset and severity

Ph.D. Student: Dr. Umberto SEMENZATO
TUTOR: Prof. Graziella TURATO – CO-TUTOR: Prof. Erica BAZZAN
Ph.D. Course in Translational Specialistic Medicine "G.B. Morgagni"
Curriculum: "Thoracic and Pulmonary Sciences"

Background
Asthma is a heterogeneous chronic inflammatory disorder encompassing various clinical phenotypes and biological endotypes. Eosinophils play a pivotal role in asthma pathogenesis, yet emerging evidence suggests functional diversity within eosinophil subpopulations. Inflammatory eosinophils (iEOS) and resident eosinophils (rEOS) may contribute differently to airway inflammation and homeostasis, but their role across distinct asthma phenotypes remains underexplored. In this research we aim to investigate iEOS and rEOS levels in asthmatic patients compared to healthy controls and in different asthma phenotypes based on the age of disease onset (< 12 years: early onset asthma - EOA or > 40 years late onset asthma - LOA) or degree of disease severity.

Material and Methods
We enrolled 47 asthmatic patients, categorized as EOA, (n=17) and LOA, (n=30), along with 16 healthy controls (HC). Based on disease severity, patients were further stratified into mild-to-moderate EOA (n=14), mild-to-moderate LOA (n=14), and severe asthma (n=19; predominantly LOA, 16/19). All subjects underwent clinical and functional assessments (ACT, spirometry, DLCO, FeNO; oscillometry in selected cases). Peripheral blood eosinophil subtypes were analyzed by flow cytometry: inflammatory eosinophils (iEOS: Siglec-8^{+}CD16^{-}CD$62L^{low}$) and resident eosinophils (rEOS: Siglec-8^{+}CD16^{-}CD$62L^{high}$). Surface expression of IL-5 receptor alpha (IL-5Rα) and chemokine receptor CCR3 was also evaluated.

Results
iEOS levels were significantly higher in both EOA and LOA patients compared to HC (EOA: 4.95% vs. 3.07%, p=0.04; LOA: 5.72% vs. 3.07%, p=0.038), with no difference between EOA and LOA. CCR3 expression was significantly elevated in EOA compared to both LOA and HC (p=0.04 for both). IL-5Rα surface expression was markedly increased in severe asthma compared to mild-moderate LOA (p=0.010) and EOA (p=0.025), and higher in EOA than LOA (p=0.029).
iEOS levels positively correlated with absolute peripheral blood eosinophil count (r = 0.41, p = 0.007), supporting their role as a circulating subset of T2-associated inflammation. iEOS showed a weak inverse correlation with FEV_1/FVC (r = –0.30, p = 0.047) and MEF50 (r = –0.33, p = 0.029), and a stronger negative correlation with small airway resistance measured by oscillometry (Rtot z-score: r = –0.58, p = 0.005; R5 z-score: r = –0.52, p = 0.011). No significant correlation was found between iEOS and FeNO, total IgE, ACT score, GINA severity steps, or annual exacerbation rate.

Conclusions
Asthmatic patients exhibit significantly elevated iEOS compared to healthy controls, independent of age at disease onset. This enrichment appears to occur irrespective of clinical phenotype, yet distinct surface marker expression patterns (e.g., CCR3 and IL-5Rα) suggest possible differences in eosinophil priming based on age of onset. While iEOS and rEOS distributions do not significantly differ between EOA and LOA, iEOS levels correlate with peripheral eosinophil counts and markers of functional impairment, particularly small airway dysfunction. In contrast, no relationship was observed with conventional T2 biomarkers or clinical control measures. These findings support further investigation of iEOS as potential biomarkers for endotyping and functional assessment in asthma.

AUTHORS' INDEX

GAGLIARDI Roberta	Salvatore Silvio PIANO	-	25
GAUDIOSO Piergiorgio	Piero NICOLAI	Marco FERRARI	62
GOTTARDI Chiara	Valentina GUARNERI	Maria Grazia GHI	63
GRAZIANI Andrea	Alberto FERLIN	Giuseppe GRANDE	14
GROTTO Giulia	Alessandra BUJA	Tatiana BALDOVIN	74
GUGELMO Giorgia	Gian Paolo FADINI	Angelo AVOGARO	112
HELLIES Filippo	Laura ASTOLFI	Gino MARIONI	110
JABEEN Ayesha	Chiara CASTELLANI	Marny FEDRIGO	97
KANAPARI Ajsi	Dario GREGORI	Giulia LORENZONI	87
KEDIDA Jiregna Olani	Dario GREGORI	Corrado LANERA, Honoria OCAGLI	88
KHAN Mohd Rashid	Danila AZZOLINA	Dario GREGORI, Luca VEDOVELLI	89
KHATOON Narjis	Giulio CEOLOTTO	Brasilina CAROCCIA	10
LIZAMA Mauro Nicolas	Cristina CANOVA	Isabella ROSATO	90
LUCCA Camilla	Luisa BARZON	Marta TREVISAN	49
LUPI Lorenzo	Alfredo GARZINO DEMO	Arianna CALISTRI	50
MAFFEI Daniele	Stefano PICCOLO	Paolo CONTESSOTTO	51
MARCANTE Beatrice	Pamela TOZZO	Luciana CAENAZZO	75
MARINO Luca	Claudio LETIZIA	Luigi PETRAMALA	11
MARISCAL Manuel Emiliano	Ileana BALDI	Honoria OCAGLI	91
MARODIN Giorgia	Nicola FERRI	Maria Giovanna LUPO	76
MARTÍNEZ Demetrio Mateo	Angelo AVOGARO	Gian Paolo FADINI, Rodrigo O. MARAÑÓN	113
MARTINI Marika	Barbara BAUCE	Martina PERAZZOLO MARRA	98
MARTINI Nicolò	Federico MIGLIORE	Martina PERAZZOLO MARRA	99
MARZI Matteo	Lara MUSSOLIN	-	36
MASIERO Giulia	Giuseppe TARANTINI	Chiara FRACCARO	100
MATTELLONE Filippo	Sara RICHTER	Ilaria FRASSON	52
MERLINI Silvia	Martina PIGAZZI	Alessandra BIFFI	37
MOLL DIAZ Raquel	Michela POZZOBON	Paola BISACCIA	38
MONDIN Alessandro	Filippo CECCATO	Mattia BARBOT	15
OLIVA Giulia	Pierfranco CONTE	Stefano INDRACCOLO	64
PACCAGNELLA Michele	Cristiano SALATA	Ignazio CASTAGLIUOLO	53
PAGNO Mario German	Giovanni SARTORE	Annunziata LAPOLLA	114
PAPAPPICCO Cinzia Anna Maria	Dario GREGORI	Giulia LORENZONI	118
PELOSO Alberto	Barbara BULDINI	Silvia BRESOLIN	39
PERPINELLO Sara	Martina PIGAZZI	Claudia TREGNAGO	40
PILALI Konstantina-Thaleia	Paola BERCHIALLA	Giorgia STOPPA	119
PITTORRU Raimondo	Federico MIGLIORE	Martina PERAZZOLO MARRA	101
POLI Elisa	Arianna LOREGIAN	Marta TREVISAN	54
POZZA Alice	Luc MERTENS, Giovanni DI SALVO	Olivier VILLEMAIN	41
PRADEGAN Nicola	Gino GEROSA	Emanuele COZZI	102
RAMPAZZO Elisa	Renato ZAMBELLO	Antonella TERAMO	65
RASOOL Maria	Monica MONTOPOLI	Sara CAPOLLA, Michele DAL BO	77
RAVELLI Adele	Maria DEVITA	Giuseppe SERGI	17
ROSSI Federico Bernardo	Teresa Maria SECCIA	Michel PAQUES	12
ROSSI Valentina	Antonio ROSATO	Debora CARPANESE	66

ROSSO Eugenia	Umberto CILLO	Sara MONTAGNESE	26
SAFA Amin	Michele DAL BO, Sara DE MARTIN	-	78
SALZMANN Rebekka Johanna Sabine	Lara MUSSOLIN	-	42
SANTI Sara	Antonio ROSATO	Anna DALLA PIETÀ	67
SARTORE Allegra	Dolores CATELAN	Annibale BIGGERI	92
SEMENZATO Umberto	Graziella TURATO	Erica BAZZAN	121
SHALATA Mahmoud Elsayed Mosaad	Paola BRUN	Giulia BERNABE`	55
SLUKINOVA Olga	Susanna MANDRUZZATO	Sara ZUMERLE	68
THOMAS Shinto Pulickal	Dario GREGORI	Corrado LANERA, Luca VEDOVELLI	93
TOFFANIN Giulia	Eva TREVISSON	Cristina CERQUA	43
TOLEDO Roxana del Valle	Angelo AVOGARO	Gian Paolo FADINI, Prof. Rodrigo MARAÑÓN	115
YAMI Amir	Francesco PIAZZA	Sabrina MANNI	69
ZUIN Marco	Alessandro ZORZI	Claudio BILATO	103