CMT 2025

CONGRESO DE MEDICINA TRASLACIONAL

CIENCIA CON IMPACTO CLÍNICO

Book of Abstracts

Agencia Nacional de Investigación y Desarrollo
Ministerio de Ciencia, Tecnología, Conocimiento e Innovación
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iMPACT

Universidad de los Andes

Universidad de la Frontera

23-26 September

Universidad de los Andes
Santiago de Chile

The Translational Medicine Congress: Science with Clinical Impact CMT2025 is a unique gathering of leading national and international experts to discuss the major challenges and advances that are shaping the medicine of the future.

This event, organized by the IMPACT Center, the University of the Andes, and the University of La Frontera, is designed to be a meeting point for dialogue between science, technology, and clinical practice, promoting interdisciplinary collaboration and the effective transfer of knowledge from the laboratory to the patient's bedside.

This congress also provides the framework for two key events: the **Third UANDES-UFRO Translational Medicine Symposium**, consolidating national scientific collaboration on clinical innovation, and the **First Meeting of the Biomedical Student Network,** a space designed to empower young talent, foster interdisciplinary networks, and project the future of medicine from our classrooms and laboratories.

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El Congreso de Medicina Traslacional: Ciencia con Impacto Clínico CMT2025 se constituye como un encuentro único de destacados expertos nacionales e internacionales en torno a los grandes desafíos y avances que están dando forma a la medicina del futuro.

Este evento, organizado por el Centro IMPACT, Universidad de los Andes y Universidad de La Frontera, se proyecta como un punto de convergencia para el diálogo entre ciencia, tecnología y práctica clínica, promoviendo la colaboración interdisciplinaria y el traspaso efectivo del conocimiento desde el laboratorio a la cama del paciente.

Este congreso es también el marco para dos eventos fundamentales: el **III Simposio de Medicina Traslacional UANDES – UFRO,** consolidando la colaboración científica nacional en torno a la innovación clínica, y el **I Encuentro de la Red de Estudiantes en Biomedicina,** un espacio diseñado para potenciar el talento joven, fomentar redes interdisciplinarias y proyectar el futuro de la medicina desde nuestras aulas y laboratorios.

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Abstracts of Day 1

Oral Presentations I

Tuesday, September 23 (15:50-17:15)
Chair: Jimena Cuenca

UMBILICAL CORD MESENCHYMAL STROMAL CELLS-DERIVED SMALL EXTRACELLULAR VESICLES: ADVANCING KNEE OSTEOARTHRITIS THERAPEUTICS

Aliosha I. Figueroa-Valdés[1], Nicolás Georges[2], Yeimi Herrera-Luna[3], Patricia Luz-Crawford[1 3], Maroun Khoury[1 2 4 5 6], Francisca Alcayaga-Miranda[1 2 4 5 6].

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Introduction: Knee osteoarthritis (OA) imposes a significant global burden, profoundly affecting life quality. Our previous research employing small extracellular vesicles (sEVs) derived from umbilical cord mesenchymal stromal cells (UC-MSCs) demonstrated promising in vivo OA mitigation. Acknowledging the need for a regulatory- compliant sEV-based therapy, we have conducted an extensive characterization and in vitro studies, delving deeper in the functional capacities of UC-MSC-derived sEVs, seeking to establish therapy-release criteria and potency testing.

Methods: sEVs were isolated via differential centrifugation and characterized according to MISEV 2018 guidelines using nanoparticle tracking analysis, flow cytometry, western blot, and transmission electron microscopy. Profiling of sEV-miRNAs was performed utilizing an HTG/EdgeSeq array, followed by bioinformatic analyses to identify OA-related targets. To assess macrophage polarization, specific markers including anti-inflammatory markers CD206 and CD163, as well as pro-inflammatory markers CD86 and HLA-DR, were evaluated via flow cytometry. An ELISA analysis to determine the production of IL1-β, TNFα, IL-6, IL-10 and VEGF in sEVs-treated macrophages was established to improve their phenotype classification and as evaluation of experiment reproducibility. Furthermore, the chondroprotective effects of sEVs were evaluated on chondrocytes exposed to an apoptotic agent.

Results and Discussion: sEVs displayed an average size of approximately 150 nm and exhibited markers CD63, CD81, CD9, Flotillin-1, Syntenin-1, CD90, and CD44, with a limited presence of HLA-A/B/C MHC-class I antigens, lacking Calnexin, TOMM20, and HLA-DR/DP/DQ MHC-class II antigens. The characteristic cup-shaped morphology was observed in the sEVs. Analysis of sEV-proteomics and miRNA profiles demonstrated consistency among samples from three UC-MSC donors, potentially targeting or related to genes associated with immune system regulation, angiogenesis, and extracellular matrix modulation. Functionally, sEVs induced a notable shift in macrophage polarization towards an anti-inflammatory (M2) phenotype. Additionally, chondrocytes treated with sEVs exhibited enhanced chondroprotection when exposed to an apoptosis inducer compared to untreated cells. Therapy using umbilical cord mesenchymal stromal cell- derived small extracellular vesicles demonstrates potential for knee osteoarthritis. sEVs consistently induced anti-inflammatory macrophage polarization and enhanced chondroprotection, reinforcing a promising off- the-shell OA treatment.

Acknowledgements/Funding: ANID Centro Basal IMPACT #FB210024, Cells for Cells S.A. & Consorcio Regenero S.A.

A-TO-I(G) RNA EDITING IN MITOCHONDRIAL PROTEIN TRANSCRIPTS ASSOCIATED WITH DRUG RESPONSE IN BREAST CANCER CELL LINES

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Introduction: Breast cancer is the most prevalent malignancy among women worldwide and remains a major clinical challenge, particularly due to treatment resistance observed across disease stages. Post-transcriptional RNA modifications, such as adenosine-to-inosine (A-to-I, interpreted as A-to-G) editing, have emerged as potential molecular markers linked to therapeutic response. However, their impact on mitochondrial function and drug resistance remains poorly understood.

Materials and Methods: We analyzed 22 breast cancer cell lines classified as drug-sensitive or resistant to anthracyclines, PARP inhibitors, and alkylating agents, based on pharmacogenomic data from GDSC and DepMap. RNA-seq data were used to identify A-to-I(G) editing sites in mitochondrial protein-coding transcripts. Differential editing between resistant and sensitive groups was assessed, and functional impact was predicted in silico using Ensembl Variant Effect Predictor and Gene Ontology (GO) enrichment analyses.

Results:
We identified differentially edited sites between sensitive and resistant cell lines across all drug classes. Approximately 13–14% of predicted editing events resulted in missense changes, potentially altering protein structure and function. GO–Molecular Function enrichment revealed overrepresentation of oxidoreductase activities, cofactor binding, and active transmembrane transporter activity, suggesting that mitochondrial redox metabolism and transport pathways are modulated by RNA editing in resistant phenotypes.

Discussion: Our preliminary findings suggest that mitochondrial RNA editing may play a functional role in modulating therapeutic response in breast cancer, possibly by altering redox homeostasis and bioenergetics. These insights highlight RNA editing as a potential biomarker for drug resistance and a novel target for therapeutic intervention.

Acknowledgements: This work was supported by FONDECYT Regular Grant 1221436.

CONTROLLED HIF-1A STABILIZATION BY DEFEROXAMINE IMPROVES REDOX BALANCE AND MITOCHONDRIAL STABILITY IN PRECONDITIONED UC-MSCS

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Preconditioning of mesenchymal stem/stromal cells (MSCs) has been explored as a strategy to enhance their survival and therapeutic potential under hostile microenvironments. Hypoxia is a well-established preconditioning method; however, its effects are variable and can lead to increased oxidative and inflammatory stress. Deferoxamine (DFO), an iron chelator and hypoxia mimetic, stabilizes HIF-1α without limiting oxygen availability. Here, we compared the effects of hypoxic and DFO preconditioning on UC-MSCs, focusing on redox status, inflammatory responses, and mitochondrial function.

UC-MSCs were preconditioned for 24 h with either hypoxia or DFO . We evaluated viability, HIF-1α stabilization (qPCR, Western blot, confocal microscopy), cytokine transcription and secretion (qPCR, ELISA), ROS accumulation (DCFH-DA, MitoSOX), antioxidant enzyme expression (qPCR, Western blot), and mitochondrial dynamics/function (OPA1, DRP1, PARKIN, TOMM20, TMRE, ATP levels).

All preconditioning regimens stabilized HIF-1α and induced VEGF expression, but only DFO 150 µM preserved viability and reduced ROS. This condition increased SOD2, GPX, and catalase expression while limiting mitochondrial ROS accumulation. Both hypoxia and DFO activated NF-κB (p65 phosphorylation), yet cytokine profiles diverged: hypoxia increased IL-6 and TNF secretion while reducing IL-10 and TGF-β, whereas DFO 150 µM promoted a balanced profile with concurrent upregulation of IL-10 and TGF-β. At the mitochondrial level, all groups increased OPA1, but only hypoxia activated DRP1 and reduced ΔΨm. Conversely, DFO 150 µM improved ΔΨm stability under CCCP challenge. ATP decreased across all groups, suggesting a glycolytic shift.

Moderate DFO preconditioning promotes antioxidant defenses, preserves mitochondrial stability, and balances inflammatory responses, in contrast to the pro-oxidant and pro- inflammatory bias of hypoxia. These findings highlight DFO as a controlled hypoxia- mimetic strategy to optimize MSC-based therapies in oxidative and inflammatory diseases.

ANID-667 FONDECYT Postdoctorado N ◦ 3230447

MITOCHONDRIAL GENOME ALTERATIONS AS EARLY DRIVERS OF LUNG ADENOCARCINOMA EVOLUTION

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Introduction: Lung adenocarcinoma evolves through precursor lesions including atypical adenomatous hyperplasia (AAH), adenocarcinoma in situ (AIS), and minimally invasive adenocarcinoma (MIA). However, early detection remains clinically challenging. Given their clonality and abundance, mitochondrial DNA (mtDNA) mutations represent potential biomarkers of early tumorigenesis.

Materials and Methods: We analyzed 109 lesions from 37 NSCLC patients, encompassing AAH, AIS, MIA, and ACA. Whole mitochondrial genomes were sequenced from FFPE samples using an amplicon-based Illumina NextSeq approach. Variant detection was performed with a custom pipeline (BWA, SAMtools, GATK Mutect2).

Results: AAH lesions exhibited a diverse spectrum of somatic mtDNA alterations, withenrichment in tRNA, rRNA, and D-loop regions. Recurrent nonsynonymous variants in oxidative phosphorylation (OXPHOS) genes, such as MT-ND5 and MT-ATP8, suggested early disruption of respiratory chain integrity. RNA-seq revealed significant upregulation of mitochondrial transcripts in AAH and AIS, indicative of compensatory bioenergetic adaptation. In MIA, mutational burden peaked, accompanied by heterogeneous expression profiles. By the ACA stage, OXPHOS gene expression was markedly downregulated, reflecting a metabolic reprogramming toward glycolysis.

Discussion: These findings establish mitochondrial genomic instability and transcriptional reprogramming as early and stage-dependent events in lung adenocarcinoma evolution. The recurrence and clonal conservation of specific mtDNA mutations, together with dynamic expression changes.

Acknowledgements: We extend our special thanks to patients and clinical teams, supported by collaborative funding from Johns Hopkins University. We also gratefully acknowledge ANID for providing scholarship funding (21222011, 242230375, 752230204).

MELANOMA CELLS EXTRUDE DYSFUNCTIONAL MITOCHONDRIA TO THE TUMOR MICROENVIRONMENT AND CIRCULATION IN ASSOCIATION WITH TUMOR PROGRESSION

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Introduction: Mitochondrial quality control (MQC) safeguards cell survival by eliminating damaged organelles. While canonical mitophagy is well established, whether melanoma cells extrude dysfunctional mitochondria (MT) as an alternative MQC route remains unknown.

Materials and Methods: We employed murine melanocytes (Melan-a) and melanoma cell lines (B16-F1, B16-F10), complemented with human melanoma lines (SK-MEL-28, MEL-1). Extracellular MT were isolated by differential centrifugation and characterized by electron microscopy, nanoparticle tracking analysis, flow cytometry, mitochondrial reporters (mtGFP, mtKeima), and ΔΨm assessment. In vivo, MT release was evaluated in syngeneic melanoma mouse models and patient plasma. Transcriptomic analysis of TCGA-SKCM data was used to assess prognostic significance.

Results: Melanoma cells released significantly more extracellular MT than melanocytes, preferentially as free organelles lacking cristae and membrane potential. Under oxidative stress, melanoma cells exhibited impaired canonical mitophagy but increased MT extrusion, consistent with activation of BNIP3/NIX-mediated secretory mitophagy. In vivo, tumor-derived MT were detectable in the tumor microenvironment and plasma, correlating with tumor burden. Importantly, melanoma patient plasma contained elevated $TOMM20^{+}$ MT, and high expression of fission- and mitophagy-related genes (DRP1, BNIP3L) correlated with poor prognosis.

Discussion: Our findings identify a tumor-intrinsic mechanism whereby melanoma cells actively extrude dysfunctional MT as an alternative MQC strategy. This process intensifies with malignancy, bypasses lysosomal degradation, and may contribute to tumor progression. Circulating MT emerges as promising non-invasive biomarkers and potential modulators of immune responses.

Acknowledgements: We thank the TCGA Research Network, patients and donors for sample contributions, and colleagues for technical support. This work was funded by ANID (IMPACT FB210024; FONDECYT REGULAR #1230875, #1250955; FONDECYT INICIACIÓN #11221017; Doctoral Fellowship #21220451) and institutional research funds (FAI-UAndes; UNAB DI-06-24/REG).

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Abstracts of Day 2

Oral Presentations II

Wednesday, September 24 (17:20-17:50)
Chair: Lara Monteiro

DEEP LEARNING FRAMEWORK FOR PREDICTING HOMOLOGOUS RECOMBINATION DEFICIENCY FROM HISTOPATHOLOGICAL IMAGES IN CANCER

Andrés Lagos[1], Miguel Toro[2], Andrés Ávila[3], Carmen Ili[4 5 6], Priscilla Brebi[4 5 6]Tamara Viscarra[5], **Kurt Buchegger [1 5 6]*.**

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Introduction: Ovarian and breast cancers remain major clinical challenges due to late diagnosis and limited therapeutic options. Homologous recombination deficiency (HRD) has emerged as a key biomarker for predicting response to platinum-based therapies and PARP inhibitors. This study presents a comprehensive artificial intelligence and deep learning framework for HRD classification using digitized histopathological whole slide images, aiming to integrate cellular and morphological features for improved predictive capacity.

Methodology: Two complementary segmentation strategies were applied: tumor-focused approach using UNet++ with a ResNet34 encoder to isolate malignant regions, and a global approach capturing entire tissue architecture. Multi-scale patch extraction enabled integration of cellular and morphological information. Classification leveraged convolutional neural networks and attention-based multiple instance learning, employing slide-level labels. Models were trained and validated on TCGA-OV and TCGA-BRCA cohorts, with performance evaluated using ROC-AUC, PR-AUC, and additional metrics.

Results: Training loss decreased consistently across both cohorts. TCGA-OV validation curves exhibited oscillations and gradual increases, suggesting overfitting driven by class imbalance, while TCGA-BRCA showed stable training due to a larger sample size. ROC analysis indicated limited discrimination for TCGA-OV (AUC ≈ 0.59) with high fold variability, whereas TCGA-BRCA achieved higher and more consistent performance (AUC 0.71–0.73). Precision-Recall (PR) curves highlighted class imbalance effects: TCGA-OV reached PR-AUC values of 0.69–0.74, while TCGA-BRCA showed lower but more stable PR-AUC (0.43–0.51). These results emphasize the impact of cohort size and segmentation strategy.

Conclusions: Model performance is strongly influenced by cohort size and segmentation. Larger cohorts, as in TCGA-BRCA, enable stable training and improved ROC metrics, while small, heterogeneous datasets like TCGA-OV pose challenges reflected in higher variability and moderate PR performance. Optimizing segmentation pipelines and addressing class imbalance are critical to enhance predictive accuracy and clinical applicability.

Acknowledgements: FONDECYT N° 11250683 and 11250644, and Millennium Institute on Immunology and Immunotherapy (IMII) N° ICN2021_045

PERIODONTITIS-DERIVED EXTRACELLULAR VESICLES AS MEDIATORS LINKING PERIODONTITIS AND TYPE 2 DIABETES MELLITUS

Ramiro Gonzalez [1] , Carolina Rojas [2 3] , Jorge. Concha-Cerda [3] , Maria José. Bendek [2 3] , Joaquín. Valenzuela [4] , Katina Schinnerling [6] , Alejandra Chaparro [2 3], **María Luisa Mizgier [2 3].**

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Introduction: Periodontitis is associated with the onset, progression, and poor prognosis of type 2 diabetes (T2D), but the underlying biological mechanisms remain unclear. We aimed to evaluate the effects of host-derived extracellular vesicles from gingival crevicular fluid (Perio-EVs) and bacterial outer membrane vesicles (OMVs) from periodontal pathogens on glucose metabolism and inflammatory responses in insulin-target organs of mice.

Materials and Methods: Adult male C57BL/6 mice received daily intravenous injections for four weeks of 10^7 Perio-EVs, 10^7 OMVs isolated from Fusobacterium nucleatum (Fn), or PBS (control). Body weight, glucose tolerance (ipGTT), and insulin sensitivity (ipITT) were measured at baseline, week 2, and week 4. At endpoint, liver and skeletal muscle expressions of IL-1β, IL-6, and TNF-α was quantified, and plasma concentrations of IL-6, IL-1β, IL-4, IL-10, TNF-α, MIP-1α, MCP-1, RANTES, ICAM-1, CRP, and insulin were assessed. In parallel, pancreatic islets isolated from mice were incubated for 24 h with PBS, Perio-EVs, OMVs-Fn, or OMVs isolated from Porphyromonas gingivalis (OMVs-Pg), followed by glucose-stimulated insulin secretion assays.

Results: At baseline, glucose tolerance and insulin sensitivity were comparable across groups. By weeks 2 and 4, systemic administration of Fn-OMVs significantly impaired glucose tolerance and insulin sensitivity ($p<0.05$ vs. control). Fn-OMVs also increased hepatic TNF-α expression ($p=0.0168$). No significant systemic changes were observed with Perio-EVs. However, in vitro exposure of pancreatic islets to Perio-EVs, OMVs-Fn, and OMVs-Pg markedly impaired insulin secretion compared to controls.

Discussion: Both host- and bacteria-derived EVs promote inflammation in insulin-target tissues and disrupt glucose metabolism, while directly impairing β-cell function. These findings identify periodontal EVs as novel mediators potentially linking periodontitis to T2D.

Acknowledgements: Fondecyt 11230418 and 1211471.

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Abstracts of Day 3

Oral Presentations III

Thursday, September 25 (11:55-12:40)
Chair: Pamela Leal

LOW-VOLUME ISOLATION AND CHARACTERIZATION OF SEMINAL PLASMA EXTRACELLULAR VESICLES FOR BIOMARKER DISCOVERY IN MALE INFERTILITY

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Introduction: Conventional semen analysis provides limited diagnostic value in male fertility assessment. Extracellular vesicles (EVs), including exosomes and microvesicles, have emerged as promising biomarkers due to their roles in sperm maturation, fertilization, and immune modulation. Their protein cargo, mainly from prostasomes and epididymosomes, is essential for motility and oxidative stress protection, and its alteration has been linked to infertility. Although size-exclusion chromatography (SEC) has been previously applied to isolate seminal plasma EVs, most protocols require ≥0.5–1 mL, limiting their use when sample availability is restricted. Here, we adapted SEC to isolate EVs from low-volume seminal plasma, providing a practical platform for biomarker discovery in male infertility.

Materials and Methods: Seminal plasma, obtained as a pooled sample from three healthy male donors (100 µL each), was processed for EV isolation using size-exclusion chromatography (SEC, IZON 35-nm columns). Characterization was performed using nanoflow cytometry (nanoFCM) to determine vesicle concentration, size distribution, and surface expression of the canonical tetraspanins CD9, CD63, and CD81. Morphological validation was conducted by negative-staining transmission electron microscopy (TEM).

Results: NanoFCM revealed EVs mainly 50–100 nm (peak ~55–60 nm) at 1.23 × 10^11mparticles/mL; particles >120 nm were scarce, supporting a homogeneous exosomal population. Tetraspanin profiling showed CD9+/CD63+ double-positive EVs (9.7%), CD63+ single-positive (31.9%), CD9+ single-positive (1.6%), and a predominant CD9−/CD63− population (56.8%). TEM confirmed intact vesicles with characteristic spherical morphology.

Discussion and Conclusions: We demonstrate the feasibility of isolating and characterizing seminal plasma EVs from only 300 µL of starting material, highlighting a methodological adaptation that broadens the use of SEC in contexts where sample availability is limited. This optimized approach ensures reproducible EV isolation and offers a framework to explore their potential as biomarkers in male infertility.

Funding: This work was supported by Fondo Nacional de Investigación Científica y Tecnológica (ANID/FONDECYT). Grants 1230410 (R.S). and 1201734 (P.L).

IMPACT OF PROLONGED GENTAMICIN THERAPY ON RENAL AND HEPATIC FUNCTION IN SEPSIS SURVIVORS

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Introduction: Post-sepsis syndrome (PSS) represents a significant clinical challenge, as over 50% of survivors develop sequelae affecting multiple systems, particularly the kidney and liver. Prolonged gentamicin therapy, essential in the management of severe infections caused by gram-negative bacteria, may exacerbate persistent organ dysfunction. Clinical studies report sustained renal and hepatic alterations in post-sepsis patients, though underlying mechanisms remain poorly understood.

Objective: To establish an experimental model to evaluate systemic and local effects of prolonged gentamicin administration in sepsis survivors induced with outer membrane vesicles (OMVs) from S.Typhi.

Methodology: Male Sprague-Dawley rats (4 weeks old) were used. Sepsis was induced via S. Typhi OMVs. Survivors were identified at day 6 post-infection and treated with intraperitoneal gentamicin (120 mg/kg/day) for 8 days. At day 14, blood samples were collected for biochemical analysis (creatinine, urea, BUN, eGFR, and liver enzymes). Kidneys and livers were fixed, paraffin-embedded, and histologically assessed using tissue damage scores and immune infiltration.

Results: Gentamicin-treated PSS animals showed elevated creatinine, urea, and BUN, with a significant reduction in eGFR, reflecting renal impairment similar to human PSS. Hepatic enzyme alterations and structural damage in both organs, accompanied by inflammatory infiltration, were also observed.

Discussion: These findings indicate that prolonged gentamicin therapy in PSS, commonly used for severe infections, worsens renal and hepatic dysfunction, reproducing clinical observations in humans. The model emphasizes the importance of long-term clinical follow-up to timely control functional alterations and provides an experimental platform to evaluate and develop therapeutic strategies to mitigate post-sepsis organ injury.

Acknowledgements: Laboratory of Genetics and Bacterial Pathogenesis, Andrés Bello University.

SUBGLOTTIC PRESSURE AND IMMERSION DEPTH: KEY DETERMINANTS OF VOICE OUTCOMES IN WATER RESISTANCE THERAPY

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Introduction: Water Resistance Therapy (WRT) is a type of semi-occluded vocal tract exercise (SOVTE) used for voice training and rehabilitation in people with dysphonia (voice disorders). Although its clinical application has demonstrated benefits for improving voice quality, vocal economy, and the sensation of vocal ease, gaps remain in our understanding of the physiological mechanisms underlying its therapeutic effects.

Purpose: This study aimed to evaluate the effects of two physical variables (1) immersion depth and (2) subglottic pressure (Psub) on (a) oscillatory characteristics of oral pressure (Poral) (frequency, amplitude, jitter, shimmer), (b) glottal adduction measured via electroglottographic contact quotient (CQ), and (c) the subjective perception of phonatory ease during water resistance therapy (WRT).

Methods: Forty participants were recruited: twenty with normal voices and twenty diagnosed with functional dysphonia (confirmed laryngoscopically). The assessment included subjective ratings of phonatory ease and objective measurements of aerodynamic and electroglottographic variables. Participants produced sustained vowel-like phonation into a tube submerged at five depths (2, 4, 6, 8, and 10 cm). Subsequently, they phonated at three targeted Psub levels (10, 15, and 20 cm H2O) while maintaining a constant immersion depth. Phonatory ease was self-rated after each condition. Objective measures of Poral and CQ were recorded simultaneously.

Results: Both immersion depth and Psub significantly affected CQ and perceived phonatory ease in all participants. Poral oscillation parameters (frequency, amplitude, jitter, shimmer) were also significantly influenced by both variables. Significant correlations were found among the measured variables.

Conclusion: The results confirm that modulating immersion depth and Psub during WRT induces significant changes in vocal physiology. Higher loading conditions (deeper immersion or increased Psub) produced a progressive increase in CQ, greater instability of Poral oscillations, and a significant reduction in perceived phonatory ease. Furthermore, both variables influence bubble characteristics and Poral dynamics, which may underlie the massage-like sensation reported in WRT. Precise control of these parameters is therefore essential for optimizing and individualizing voice therapy outcomes.

Acknowledgements: The authors would like to acknowledge the Universidad de los Andes (Chile) for providing the facilities and infrastructure where the entire experimental phase of this study was conducted. We also extend our gratitude to the Universidad San Sebastián for the kind loan of essential equipment. This research was supported by the Doctoral Program in Medical Sciences at the Pontificia Universidad Católica de Chile. Furthermore, the lead author was funded by a National Doctoral Scholarship from ANID.

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Abstracts of Day 3

Oral Presentations IV

Thursday, September 25 (14:25-15:10)
Chair: Francesca Velarde

DRUG DELIVERY OF POLYPHENOLS FROM SS-CYCLODEXTRIN-FUNCTIONALIZED POROUS SILICON PARTICLES: A STEP TOWARD OSTEOARTHRITIS THERAPY

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Introduction: Osteoarthritis (OA) is a degenerative joint disease characterized by cartilage loss, synovial membrane alterations, and chronic inflammation. Current pharmacological and surgical treatments offer limited long-term efficacy, underscoring the need for novel therapeutic approaches. Polyphenols such as pinocembrin (PIN) and caffeic acid phenethyl ester (CAPE) exhibit antioxidant and anti-inflammatory properties that may protect cartilage and modulate OA progression. Porous silicon (nPSi) nanoparticles, with their high surface area and tunable porosity, combined with β-cyclodextrin (βCD) functionalization, provide an efficient platform for sustained drug delivery.

Materials and Methods: nPSi particles were synthesized by electrochemical etching, followed by in situ βCD polymerization using citric acid. CAPE and PIN were loaded into nPSi-βCD via adsorption. Physicochemical characterization included UV-Vis spectroscopy, dynamic light scattering (DLS), zeta potential, and ATR-FTIR. In vitro release studies were conducted in PBS (pH 7.4, 37 °C) for 8 h, with quantification by UV-Vis at 324 nm (CAPE) and 322 nm (PIN). Release kinetics were analyzed using first-order, Higuchi, and Weibull models.

Results: UV-Vis spectra showed a characteristic absorption peak at ~280 nm for all formulations, indicating preservation of the nPSi optical properties after functionalization and loading. DLS analysis revealed an increase in hydrodynamic diameter from 228.6 nm (nPSi) to 348.0 nm (nPSi-βCD), and further to 563.8 nm (CAPE) and 384.6 nm (PIN) upon loading. Zeta potential shifted from –42.9 mV (nPSi) to –30.8 mV (nPSi-βCD) and –24.8 mV / –22.3 mV after CAPE and PIN loading, respectively. ATR-FTIR confirmed polyphenol incorporation via additional CH bending modes. In vitro release profiles demonstrated slower, more sustained release from βCD-functionalized particles compared to non-functionalized controls. Weibull modeling yielded the best fit ($R^2 > 0.94$), with $\beta \leq 0.75$, indicating Fickian diffusion in fractal or highly disordered matrices.

Conclusion: βCD-functionalization of nPSi particles effectively modulates surface properties and enables sustained polyphenol release, providing a promising delivery strategy for future evaluation in an IL-1β-stimulated chondrocyte OA model.

Funding: Fondecyt N° 1230553 and Dirección de Investigación, Universidad de La Frontera, Temuco, Chile, Grant number PP24-0003.

ALPHA 7 NICOTINIC RECEPTOR STIMULATION PREVENTS SEPSIS-INDUCED COAGULOPATHY IN A PRE-CLINICAL MODEL OF ENDOTOXEMIA

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Introduction: Sepsis remains a global health burden due to its high incidence and mortality. A major complication is Sepsis-Induced Coagulopathy (SIC), affecting up to 80% of septic patients and being strongly associated with worse outcomes. SIC is diagnosed using a scoring system based on organ (Truncated SOFA: PaO2/FiO2 ratio, creatinine, bilirubin) and coagulation dysfunction markers (INR, platelet count); a score ≥4 indicates SIC. Cholinergic stimulation via the α7 nicotinic acetylcholine receptor (α7nAChR) improves survival in sepsis models due to anti-inflammatory effects. However, its role in coagulation remains poorly explored. This study evaluated the effect of α7nAChR stimulation in a preclinical model of SIC.

Methods: Male Sprague-Dawley rats (230–260 g) were pre-treated with GTS-21 (5 mg/kg, i.v.) or vehicle 10 min before LPS infusion (30 mg/kg, i.v., over 1 h). Three hours later, blood was collected to assess SOFA components and coagulation markers. A rat-adapted SIC score, previously developed in our laboratory, was applied. Saline-treated animals served as healthy controls.

Results: All control animals had SIC scores <4. In the LPS group, 80% met SIC criteria (score ≥4), while none of the GTS-21–treated animals reached this threshold (n=5 per group). GTS-21 alone did not induce SIC.

Conclusion: α7nAChR stimulation prevented SIC during endotoxemia, suggesting a protective role beyond inflammation and supporting its translational relevance as a therapeutic strategy in sepsis.

Acknowledgements: FONDECYT 1241072 and National Doctoral Fellowship (ANID), Beca INI 2024 (Universidad Andrés Bello), and IMII.

CROSS-SPECIES IMPACT OF STREPTOCOCCUS MUTANS BACTERIAL EXTRACELLULAR VESICLES ON STREPTOCOCCUS SANGUINIS EARLY ADHESION

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Introduction: *Streptococcus mutans* is a cariogenic bacterium that produces bacterial extracellular vesicles (bEVs) that mediate cellular communication. The growth effect of bEVs in the antagonistic bacteria *Streptococcus sanguinis* has previously been investigated; nevertheless, their impact on early adhesion remains unknown. Thus, this research aimed to quantify single-cell adhesion of bEV-exposed S. *sanguinis* using atomic force microscopy-based single-cell force spectroscopy (AFM-SCFS).

Material and Methods: S. *mutans* UA159 was grown as biofilms on collagen substrates and in planktonic state at 37 °C for 24 hours. Subsequently, bEVs were obtained using filtration and ultracentrifugation. S. *sanguinis* were incubated with bEVs at 37 °C and 5% CO2 and immobilized onto 0.1% w/v poly-L-lysine functionalized AFM cantilevers for SCFS. For each treatment, 400 force curves were generated to determine maximal adhesion force, single unbinding rupture lengths, rupture forces, and contour lengths.

Results: S. *sanguinis* treated with biofilm-derived S. mutans bEVs generated a reduction in adhesion to collagen substrates, while treatment with planktonic bEVs increased the adhesion, as reflected by the resulting AFM-SCFS data at 5-second contact times.

Discussion: S. *mutans* EVs from the biofilm state have more impact in the reduction of S. *sanguinis* adhesion compared to S. *mutans* bEVs from the planktonic state. This demonstrates the differential effect of S. mutans bEVs depending on planktonic vs biofilm origin.

Acknowledgements: ANID Fondecyt Regular #1220804, Scholarship #21220799 and Imaging Facility at the Faculty of Dentistry UofT, Canada.

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Abstract of Day 3

Poster session

Thursday, September 25

DEFORMABLE LIPID NANOCARRIERS FOR PEPTIDE DELIVERY: EDGE ACTIVATOR-BASED APPROACH FOR GLP-1 RA

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Introduction: Bile salts have been widely reported as effective edge activators (EA) in nanocarriers due to their ability to enhance membrane fluidity. Their incorporation into nanostructured lipid carriers (NLCs) can increase deformability and improve drug permeation and bioavailability. This study focused on the development of deformable NLCs loaded with a GLP-1 receptor agonist (GLP-1 RA), incorporating sodium deoxycholate (SDC) and sodium deoxyglycocholate (SDGC) as functional excipients.

Material and Methods: NLCs were prepared by a low-energy hot emulsification technique using a blend of solid and liquid lipids and adding SDC or SDGC. Hydrodynamic diameter (HD) and polydispersity index (PdI) were measured by dynamic light scattering; Zeta potential (ZP) by laser Doppler electrophoresis. Deformability was assessed by extrusion through polycarbonate membranes. Transmission Electron Microscopy (TEM) was performed to visualize the NLC morphology. Stability was monitored over four weeks at 4 °C. Encapsulation efficiency (%EE) and drug loading (%DL) were determined by fluorimetry. One-way ANOVA ($p < 0.05$) was used for statistical analysis.

Results: Incorporation of EA significantly reduced the HD from 180.4 nm (control) to 137.2 nm (SDC) and 119.3 nm (SDGC). ZP shifted from -7.20 mV to -28.50 mV, indicating enhanced colloidal stability. Both modified NLCs showed increased deformability compared to control. EE reached 82.4% with a DL of 0.256%. TEM images revealed spherical structures, supporting the role of EA in modulating membrane elasticity.

Discussion: Our findings demonstrate that incorporating SDC and especially SDGC improves NLC performance by optimizing HD, ZP, and structural flexibility. These features are advantageous for enhancing buccal permeability and the bioavailability of peptide drugs like GLP-1 RA.

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ACTIVATION OF THE A3 NICOTINIC RECEPTOR PREVENTS ENDOTHELIAL CELL ADHESION AND THE ACQUISITION OF A PROCOAGULANT PHENOTYPE UNDER ENDOTOXEMIC CONDITIONS.

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Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection, commonly triggered by Gram-negative bacteria. The endotoxin (LPS) in their outer membrane can induce endotoxemia, a core condition in sepsis pathophysiology. A main complication is the exacerbated activation of the coagulation system, which promotes a procoagulant state known as **sepsis-induced coagulopathy (SIC),** affecting up to 60% of patients. This condition is caused by **endothelium activation** and increased endothelial adhesion of platelets and neutrophils, a crucial early event and a reliable **marker for coagulopathy.** This adhesion is mediated by enhanced expression of adhesion molecules like CAMs and selectins. With no effective therapies currently available, new strategies are urgently needed to modulate this procoagulant response.

Recently, stimulating the cholinergic system has been proposed to influence this response. The alpha 3 nicotinic receptor **(α3nAChR),** expressed in endothelial cells, has been linked to regulating this activation. Although previous studies associated α3nAChR antagonism with more pronounced procoagulant processes, its anti-adhesive role and contribution to coagulopathy during sepsis remain unclear.

This study aimed to determine the effect of endothelial α3nAChR stimulation on the procoagulant phenotype induced by endotoxin. Human endothelial cells (EA.hy926) were pretreated with an agonist, an antagonist, or both, then exposed to LPS. Platelet and neutrophil adhesion, alongside VCAM-1 and P-selectin expression, were assessed via fluorescence microscopy. Our results showed that α3nAChR stimulation significantly reduced cell adhesion to the endothelium and adhesion molecule expression, while inhibition increased them. These findings suggest that α3nAChR activation provides a protective effect against endothelial adhesion and the procoagulant phenotype, highlighting its potential as a therapeutic target to improve clinical outcomes in sepsis.

POST-ISCHEMIC ADMINISTRATION OF AMINOGUANIDINE ATTENUATES RENAL INJURY, SUPPORTING ITS POTENTIAL AS A TIMELY PHARMACOLOGICAL INTERVENTION FOR THE MANAGEMENT OF ACUTE KIDNEY INJURY.

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Background: Renal ischemia-reperfusion (IR) is a major cause of acute kidney injury (AKI), yet current therapeutic options remain limited. We previously demonstrated that aminoguanidine (AG), an inhibitor of inducible nitric oxide synthase (iNOS), has renoprotective effects when administered before inducing renal ischemia in a mouse model. However, this preventive approach has limited clinical applicability, since renal insults are often sudden and unanticipated. Therefore, assessing whether AG remains effective when given after the ischemic event is essential to determine its translational potential. In this study, we evaluated the therapeutic effect of AG administered after an experimental renal IR protocol in C57BL/6J mice.

Methods. Ischemia was induced for 30 minutes, followed by 48 hours of reperfusion. Mice received vehicle or AG (50 mg/kg, intraperitoneally) one hour post-ischemia. Clinical outcomes were evaluated with serum creatinine (SCr), blood urea nitrogen (BUN), and histopathology. In addition, markers of oxidative stress, tubular injury, endothelial activation, pericyte, cell proliferation, mesenchymal transition, ferroptosis, and inflammation were assessed.

Results. From a clinical perspective, the main findings of post-ischemic AG treatment were (i) improved renal function (SCr and BUN), (ii) reduced histological kidney damage, (iii) decreased cell proliferation, and (iv) diminished medullary lipid peroxidation. Additionally, AG restored the expression of genes associated with endothelial integrity disrupted by IR injury.

Conclusions. Our findings demonstrate that post-ischemic administration of a single AG dose attenuates renal injury, supporting its potential as a timely pharmacological intervention for AKI, and providing a therapeutically relevant approach that better reflects clinical realities.

Acknowledgements: The assistance of the animal care and veterinarian of Nataly Quezada from the Services of the Universidad de los Andes is gratefully acknowledged. The technical support of the histology, Dr. Wilfredo González, from the Histology Unit of the Universidad de los Andes, is gratefully acknowledged.

EVALUATE THE MECHANISM OF ACTION OF SMALL EXTRACELLULAR VESICLES (SEVS) ON MACROPHAGE POLARIZATION IN THE CONTEXT OF OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA) is a degenerative joint disease involving sustained inflammatory in the synovial environment, linked to macrophage polarization toward a pro-inflammatory (M1) phenotype. Despite its high prevalence, there is currently no cure, posing a global health burden. Recent studies show that small extracellular vesicles (sEVs) from umbilical cord mesenchymal stromal cells (UC-MSCs) can shift non-activated (M0) macrophages toward an anti-inflammatory (M2) phenotype. This research aims to induce the repolarization of macrophages toward an M2 phenotype using sEVs, starting from M1 macrophages previously polarized from M0 using IL-1β and TNF-α (both typically found at high level in OA's environment).

Methods: Flow cytometry will be used to determine the expression of pro-inflammatory (HLA-DR and CD86) and anti-inflammatory (CD163 and CD206) markers. An ELISA will allow quantify cytokines associated with macrophage polarization (IL-10, VEGF, IL-6, TNF-α, and IL-1β). Transcripts levels of genes of interest will be evaluated by RT-qPCR.

Results: Ten batches of UC-MSCs sEVs were obtained via ultracentrifugation and were characterized by nanoparticle tracking analysis (size and concentration), and by flow cytometry for sEVs markers (CD63, CD81, and CD9). An experiment was conducted where macrophages were polarized using a range of IL-1β concentrations to assess the induction of a pro-inflammatory phenotype. Pro-inflammatory markers increased slightly, while CD206 increased considerably. ELISA analysis is still required to confirm the polarization outcome. Further steps include standardizing IL-1β concentrations and incorporating TNF-α to enable consistent polarization towards a pro-inflammatory phenotype and to assess its repolarization into M2 using sEVs.

Discussion: This preliminary work will help us advance toward establishing the mechanism of action of sEVs by developing protocols for macrophage repolarization in a setup that mimics OA's inflammatory state *in vitro.*

Acknowledgements: ANID Centro Basal IMPACT #FB210024

THERAGNOSTIC POTENTIAL OF CYTIDINE DEAMINASE PROTEIN IN PREDICTING CHEMORESISTANCE IN GALLBLADDER CANCER

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Introduction: Gallbladder cancer (GBC) is an aggressive tumour usually diagnosed at advanced stages with poor prognosis. The high recurrence rates and limited clinical responses to current regimens reflect the highly drug-resistant phenotype of GBC cells due to intrinsic or acquired mechanisms. The identification of theragnostic markers that provide targets for diagnosis and therapy at the same time can greatly improve clinical outcomes. Cytidine deaminase (CDA) is a key enzyme in the nucleotide salvage pathway and participates in adaptive stress responses to chemotherapy. Highly expressed CDA catalyzes and inactivates cytidine analogues, contributing to increased gemcitabine resistance. Tetrahydrouridine (THU) is a well characterized and potent inhibitor of CDA that has been used in combination with cytidine analogs, such as gemcitabine, both preclinically and clinically for some decades without documentation of toxic side effects.

Material and Method: Expression of CDA was analyzed by Western blot in parental and gemcitabine-resistant cells, NOZ, TGBC-1 NOZ- GemR, and TGBC1-GemR, both in cell lysate and in cell supernatant. Drug sensitivity assay was performed by treating cells with stepwise 3-fold serial dilutions of gemcitabine and incubating with or without THU. Cell viability was assessed by incubating the cells with MTS–PMS colorimetric solution. The half-maximal inhibitory concentration (IC50) was calculated from the dose-response curve.

Results: We observed an overexpression of CDA protein in gemcitabine-resistant cells, both in cell lysates and in cell supernatants compared to their parental counterpart. Combination therapy with gemcitabine and THU improves gemcitabine sensitivity in parental and gemcitabine-resistant cells.

Discussion and Conclusions: These preliminary results indicate that CDA has potential as a non-invasive biomarker of chemoresistance in GBC as well as a promise new therapeutic target. Our results suggested that a combination regimen of THU and gemcitabine could be used to address the pharmacologic limitations of gemcitabine in GBC.

Acknowledgements: Supported by ANID/FONDECYT. Grants 1201734 and 11180987.

TRANSCRIPTOMIC PROFILING REVEALS SOX8 OVEREXPRESSION AS A CANDIDATE BIOMARKER ASSOCIATED WITH CARBOPLATIN RESISTANCE IN OVARIAN CANCER.

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Introduction: Ovarian cancer is one of the most lethal gynecological cancers, due to late diagnosis and the lack of effective methods for early detection. In advanced stages, carboplatin (CBDCA) has been shown to be as effective as cisplatin, and both are first-line therapies. However, despite the favorable initial response, 5-year survival remains at only 20–30%, mainly due to the development of resistance to chemotherapeutic agents. SOX transcription factors are involved in tumor processes such as apoptosis, epithelial- mesenchymal transition (EMT), metastasis, angiogenesis, and therapeutic resistance. SOX8 has been linked to cisplatin resistance through the suppression of senescence and induction of glycolytic metabolism, as well as being associated with aggressive tumor phenotypes. However, its involvement in platinum resistance is still poorly understood.

Material and methods: Differential gene expression analysis was performed using RNA-seq data from the TCGA, as well as from carboplatin-sensitive and carboplatin-resistant A2780 cell lines. Bioinformatic analysis included the use of DESeq2 and correlation models to evaluate the association between expression profiles and resistance phenotypes.

Results: In cell lines, SOX8 showed significant overexpression in resistant cells (log2FC =7.81; padj < 0.001), supporting its involvement in acquired resistance. In contrast, in TCGA tumor samples, SOX8 was found to be repressed compared to normal tissue, suggesting a possible loss of its suppressor function in early stages of carcinogenesis. Likewise, moderate overexpression of PAX2 was observed in resistant cells (log2FC = 1.26), which could suggest possible functional cooperation with SOX8 in resistance.

Discussion: These findings suggest that SOX8 may have a dual role: suppressed during initial progression and reactivated in platinum resistance. This positions it as a potential predictive biomarker and therapeutic target in ovarian cancer. Future studies will seek to validate its expression and interaction with PAX2 in resistant cell models.

Acknowledgements: We thank the Laboratorio de Biología Integrativa, Centro de Excelencia en Medicina Traslacional (CEMT-BIOREN), for their support and for providing the cell line used in this study. This work was supported by collaborative funding from Universidad de La Frontera and by the Fondecyt Initiation Project No11250683.

CYTOTOXIC ACTIVITY OF METABOLITES ISOLATED FROM THE ANTARCTIC FUNGUS OIDIODENDRON SP. AGAINST CASTRATION-RESISTANT PROSTATE CANCER CELL LINES 22RV-1 AND DU-145.

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Prostate cancer is one of the most prevalent neoplasms worldwide, with 400,000 deaths per year globally. Its high mortality rate is mainly associated with late diagnosis and, therefore, detection at an advanced stage of the disease. Disease progression is androgen receptor (AR) dependent, and thus first-line treatment typically consists of androgen deprivation therapy (ADT), which generally yields a good initial response. However, most of patients develop resistance to treatment within 2 to 3 years, entering the castration-resistant phase of the disease (CRPC), in which life expectancy decreases to 12–24 months due to the limited availability of effective drugs. Therefore, there is a need to search for new agents capable of controlling CRPC. In this context, Antarctic fungi represent a novel source for the discovery of new bioactive metabolites against CRPC.

The Antarctic fungus *Oidiodendron* sp. was inoculated in approximately 80 flasks containing YM medium and incubated at 15 °C for 28 days. Extractions were performed with EtOAc to the culture medium. The total extract was fractionated by affinity chromatography on silica gel. Fractions were evaluated using MTS assays in the 22Rv-1 and DU-145 cell lines. The active fractions were purified by size-exclusion chromatography and high-performance liquid chromatography (HPLC). The putative molecular structures of the purified compounds were assessed by mass spectrometry.

Approximately 40 liters of culture medium yielded 4,303 mg of crude extract, from which 20 fractions were obtained. MTS assays showed that the most active fractions were F15–F17, which were identified as Benzonidazole, Diethyl Phthalate, and a carboxyl-aldehyde-type molecule, respectively. However, all three compounds need to be further analyzed by NMR and X-ray diffraction to determine their exact structures. And the pure compounds should be further studied against CRPC.

Acknowledgements: We acknowledge the funding provided by the ANID Doctoral Fellowship, INACH Project DG-08-23, and Fondequip EQM-220161.

LACTIC ACID BACTERIA-DERIVED CELL-FREE SUPERNATANT AS NOVEL ADJUVANTS TO ENHANCE CISPLATIN EFFICACY IN GASTRIC CANCER

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Introduction: Gastric cancer is the fifth most incident malignancy and fourth leading cause of cancer-related mortality worldwide, with most patients diagnosed at advanced stages, where chemoresistance limits cisplatin (CDDP) efficacy. Lactic acid bacteria (LAB) have demonstrated anticancer potential via apoptosis induction, proliferation inhibition, and enhancement of chemotherapeutic responses. Cell-free supernatants (CFS) provide a model to study postbiotic activity.

Material and methods: LAB-A and LAB-B were evaluated for synergy with cisplatin (CDDP) in AGS-WT, AGS-RCDDP, SNU-601, and SNU-638. Cells were seeded, incubated 24 h, and treated with CDDP alone or combined with CFS. After 72 h, viability was assessed by MTT, and synergy analyzed using SynergyFinder 3.0 (ZIP, Bliss, Loewe, HSA models). IC50 shifts and Resistance Index (IR = IC50 resistant / IC50 sensitive) were calculated. Ki67 proliferation assays were performed in sensitive and resistant lines after 48 h of CFS treatment and analyzed with Muse® cell analyzer.

Results: CFS showed synergistic or additive effects with CDDP across sensitive and resistant lines. Combined treatment reduced CDDP IC50 and significantly decreased IR in resistant cells (AGS-RCDDP: 2.74 → 0.87, $p < 0.05$; SNU-638: 6.82 → 1.62, $p < 0.001$). Ki67 analysis suggested potential proliferation inhibition after 48 h of CFS treatment.

Discussion: Observed synergism suggests LAB-derived metabolites may act as chemosensitizers, partially restoring cisplatin sensitivity. While mechanisms remain unclear, modulation of apoptosis, oxidative stress, and drug transport—previously reported for LAB metabolites in cancer and other systems—may underlie these effects. The reduction in resistance indices underscores the potential of LAB-derived metabolites to overcome cisplatin refractoriness. Future studies should focus on biochemical characterization of active compounds and mechanistic validation in more complex models.

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TRANSLATIONAL MEDICINE IN CHILE: STRATEGIC PUBLIC-PRIVATE PARTNERSHIPS FOR ADVANCING DIAGNOSTIC INNOVATIONS

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Introduction: Translational medicine in Chile faces the challenge of accelerating the transition from biomedical research to concrete clinical applications that impact population health. In this context, partnerships between the private sector and academia are essential for the development of robust clinical studies and the validation of new diagnostic technologies.

Materials and Methods: Grupo BIOS established collaborative models with national universities, research centers, and biotechnology companies. These partnerships enabled the generation of clinical evidence and the advancement of companion diagnostics, a key tool for personalized medicine that links specific biomarkers to targeted therapy selection.

Results: These collaborative projects have enabled the integration of complementary capabilities—technological infrastructure, clinical expertise, and research networks—enhancing the generation of high-impact scientific and clinical results.

Discussion: This model highlights that health innovation in Chile requires a coordinated ecosystem in which academia, industry, and the public sector cooperate to accelerate knowledge transfer and improve patient outcomes. Strategic alliances effectively accelerate translational research and position Chile as a relevant player in innovative diagnostics tailored to local needs.

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F5 AS A PROGNOSTIC BIOMARKER IN GASTRIC CANCER

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Introduction: Gastric cancer (GC) is a major cause of morbidity and mortality worldwide, characterized by late diagnosis and tumor heterogeneity. Identifying prognostic and theranostic biomarkers is key for earlier detection and effective treatment. This study aimed to identify such markers through gene ontology analysis.

Methods: Data from The Human Protein Atlas (THPA), including TCGA transcriptomic and protein profiles, were analyzed. From 354 GC patients, 171 genes associated with unfavorable prognosis were selected. Functional enrichment was conducted with Metascape and Cytoscape-ClueGO to identify pathways and protein complexes (MCODE). Gene deregulation was validated with TCGA RNA-seq (484 samples) and GTEx (359 controls) using DESeq2 (log2FC ≥1; adjusted P <0.05). Expression in normal and tumor tissues was evaluated using GeneCards and GEPIA2.

Results: Among enriched pathways, F5 emerged as a relevant marker, along with vWF, FN1, THBS1, and PCDH7. F5, a coagulation factor related to invasion, metastasis, and tumor grade, is involved in coagulation, extracellular matrix remodeling, angiogenesis, vasculature development, endothelial migration, adhesion, vasculogenesis, and VEGFA-VEGFR2, Wnt, and TGF-β signaling. RNA-seq confirmed F5 overexpression in gastric adenocarcinoma (fold change 4.4) across all stages. Its expression is low in normal stomach tissue but high in circulatory and immune tissues, and linked to D-dimer, supporting its biomarker potential.

Discussion: F5 deregulation in GC and its involvement in tumor-promoting pathways suggest prognostic value. Further validation, including immunohistochemistry, is required to confirm clinical relevance.

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HYPOXIC DERIVED SMALL EXTRACELLULAR VESICLES: A STRATEGY TO IMPROVE CARDIOMYOCYTE SURVIVAL FROM HYPOXIA/REPERFUSION INJURY.

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Introduction: Cardiovascular diseases (CVD) remain the leading cause of mortality globally, with ischemia-reperfusion (I/R) injury exacerbating the myocardial damage, posing a significant public health challenge. Small extracellular vesicles (sEV), released by cardiomyocytes under stress conditions such as hypoxia, have emerged as potential sources of novel biomarkers and therapeutic targets. Our group has previously identified that sEV carrying hERG1 (a regulator of cardiac electrical activity) and HSP47 (a mediator of fibrotic remodeling) can serve as biomarkers of CVD. In this study, we investigated whether hypoxia-derived sEV from human cardiomyocytes (AC-16) exerts a protective effect against hypoxia/reoxygenation (H/R)-induced cell death.

Method: Cardiomyocytes were preconditioned overnight with sEV or conditioned medium derived from hypoxic cells (AC-16), exposed to 4 h hypoxia/4 h reoxygenation stress, and the cell mortality was assayed. To assess the contribution of specific membrane vesicular proteins, sEV were incubated with anti-hERG1 or anti-HSP47 antibodies before preconditioning.

Results: Our results showed that sEV preconditioning prevented the cardiomyocyte mortality induced by H/R. Blocking hERG1 and HSP47 on sEV membranes with specific antibodies partially reduced this protective effect, suggesting their involvement, though other factors likely contribute.

Conclusion: Hypoxia-induced sEV confer cytoprotective signals that enhance cardiomyocyte resilience to I/R injury. These findings support the role of sEV as both therapeutic candidates and biomarkers in ischemic heart disease.

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SEGATELLA COPRI OUTER-MEMBRANE VESICLES DRIVE A PRO-INFLAMMATORY PROFILE OF HUMAN DENDRITIC CELLS.

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Introduction: Outer-membrane vesicles (OMVs) mediate host–microbiota signaling. The gut commensal *Segatella copri* (Sc) is enriched in inflammatory diseases such as rheumatoid arthritis, yet the impact of its OMVs on human immunity is unknown. Because dendritic cells (DCs) orchestrate T-cell programming, we investigated whether *Sc*-OMVs are internalized by human DCs and modulate their phenotype and function.

Materials and Methods: OMVs from *S. copri* DSM18205 were characterized by nanoparticle tracking analysis and TEM. Human monocyte-derived DCs (moDCs) were generated with GM-CSF/IL-4 for 4 days. Uptake of CFSE-labeled OMVs was analyzed by flow cytometry and confocal microscopy with endocytosis inhibitors (cytochalasin D, dynasore). DC activation was assessed by flow cytometry in moDCs *in vitro* (24 h) and *ex vivo* in whole blood DCs (6 h). The capacity of *Sc*-OMV-exposed moDCs to prime autologous T-cell proliferation and cytokine production was evaluated in co-cultures.

Results: *Sc*-OMVs are spherical vesicles of 80–90 nm. Approximately 75% of moDCs internalized OMVs within 30 min and uptake decreased with cytochalasin D but not dynasore, indicating actin dependence. In moDCs, 10 µg/mL *Sc*-OMVs increased maturation/antigen-presentation markers (CD83, HLA-DR, CD86). *Ex vivo, Sc*-OMVs upregulated HLA-DR, CD86, CD83 and CCR7 and induced IL-12p40, TNF-α and IL-6. Nevertheless, *Sc*-OMV-conditioned DCs did not enhance Th1/Th17 responses in co-culture.

Discussion: *Sc*-OMVs rapidly enter human DCs and drive maturation and a pro-inflammatory cytokine profile. Yet this innate activation does not translate into stronger Th1/Th17 responses, implying a "semi-mature/tolerogenic or unlicensed" DC state. It remains to be investigated whether an anergic or tolerogenic state is induced in T lymphocytes. Further research is required to elucidate whether *Sc*-OMVs can play pathogenic roles in inflammatory and systemic diseases, such as rheumatoid arthritis.

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VALIDATION OF AN AUTOMATED ALGORITHM FOR IMMUNOHISTOCHEMICAL ANALYSIS IN BREAST CANCER USING QUPATH SOFTWARE

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Introduction: Breast cancer stands as one of the primary causes of mortality among women, and its classification into molecular subtypes (such as Luminal A, Luminal B, HER2-positive, and triple-negative) is based on the immunohistochemical (IHC) of ER, PR, Ki67, and HER2. The process can be improved in terms of efficiency and objectivity of the observer using digital tools like QuPath, which enable automated quantification, thereby improving diagnostic precision. This study aimed to compare the effectiveness of visual assessment versus automated quantification using QuPath software for diagnostic biomarkers in breast cancer through digital images.

Materials and methods: A retrospective cohort (2023-2024) study assessed by examining 400 digitized images of invasive breast carcinoma. Cases were selected through random sampling. Slides were digitized using Easy-Scan (MOTIC) and analyzed with the open-source QuPath. The variables included the expression levels of the biomarkers ER, PR, Ki67, and HER2, focusing on intensity and positive proportion. Pathologists conducted visual evaluations, while QuPath facilitated automated quantification. The analytical statistics was the intraclass correlation coefficient (ICC) and Pearson (R).

Results: The ICC calculation in the statistical analysis revealed high reliability between both methods of ER, PR, Ki67, and HER2 quantification. Additionally, ICC values exceeding 0.85 and R values greater than 0.80 for all biomarkers.

Discussion: Digital quantification is an efficient tool for evaluating IHC biomarkers in breast cancer. The results are comparable to those obtained through visual evaluation, offering advantages in terms of standardization, speed, and clinical applicability. These findings support the implementation of digital pathology as an innovative contribution to cancer diagnosis.

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CONSENSUS MOLECULAR CLASSIFICATION ALGORITHM FOR COLORECTAL CANCER BASED ON A PANEL OF PREDICTIVE BIOMARKERS

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Introduction: Colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide. Classification by molecular subtypes (CMS1–4) enables improved therapeutic guidance based on tumor biology. However, many countries, including Chile, lack accessible clinical diagnostic tools to implement this classification. The objective of this study was to apply a multimarker panel combining protein and gene analysis to support CMS-based molecular classification and enhance treatment stratification in CRC patients.

Methodology: A total of 100 CRC samples were analyzed. The panel included: MSI; BRAF V600E by PCR (CMS1); nuclear β-catenin and SALL2 loss (CMS2); KRAS mutation by RT-qPCR (CMS3); and ZEB1+SALL2 positivity (CMS4). Algorithm outputs were benchmarked against the gold standard IHC-CMS classifier (Li et al., 2021), assessing sensitivity, specificity, concordance (kappa index), and area under the curve (AUC). A stratified dataset (75% training, 25% testing) with 20-fold StratifiedKFold validation and automated hyperparameter tuning was employed to test linear, tree-based, neural, probabilistic, and instance-based algorithms for CMS subtype classification.

Results: The CMS algorithm achieved 85% concordance with the gold standard and an overall diagnostic accuracy of 89%. Subtype-specific AUCs were high: CMS1 = 0.88, CMS2 = 0.85, CMS3 = 0.90, and CMS4 = 0.99. Sensitivity and specificity ranged from 78–93% and 80–96%, respectively. Relative importance analysis of the Linear SVM model showed KRAS contributing 31% to patient classification, while the SALL2/β-catenin/CDX2 axis contributed 6%, highlighting their differential impact on CMS subtype discrimination in the Chilean clinical setting.

Discussion: The multimarker panel demonstrated utility in identifying CMS molecular subtypes, providing a valuable tool to support personalized therapeutic decision-making in colorectal cancer.

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A VERSATILE PLATFORM TO ISOLATE MONOCLONAL ANTIBODIES (MABS) AGAINST BIOMARKERS OVEREXPRESSED IN THE MOST LETHAL CANCERS: TOWARDS THE NATIONAL SUSTAINABLE PRODUCTION OF PROTEINS WITH BIOPHARMACEUTICAL AND BIOTECHNOLOGICAL PURPOSE

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Abstract. Cancer is one of the most causes of decease worldwide. Actually, in term of treatment, immunotherapy though the use of mAbs, has been considered as the cornerstone of modern cancer therapy. Despite the benefits of the use of mAbs in the treatment of cancer, it is still an inaccessible treatment in underdeveloped countries, and even in developed ones, due to the high cost of treatment. For this reason, strategies that lead to the more accessible and autonomous production of mAbs for therapeutic and diagnostic use are essential. A plausible alternative that allows the faster production of mAbs is through phage display technology, which involve 1) generation of robust immune gene libraries; 2) recombinant production of a panel of cancer antigens; 3) the process of library screening and determination of variable regions sequence of mAbs by an immobilized antigens (previously produced in step 2; 4) recombinant production of mAbs in desired format (scFv, FAB, diabodies, bivalents, IgG, etc.) and finally, 5) characterization and validation of this molecules. Since there is already a large and growing panel of molecular targets for treatment of various types of cancer, mainly those overexpressed in the most lethal cancer such as PD-1, CTLA-4, CLDN-18, Nectin-4, CD19 and CD20, in addition of construction of an immune library GALLBLA1, in this proposal it is contemplated the sequencing of a GALLBLA1 library and through bioinformatics tools the isolating of scFv sequences against a panel of antigens present in most lethal cancer worldwide, that it may have biotechnological purposes.

Through NGS sequencing, the GALLBLA1 library showed to contain about forty thousand monoclonal antibodies which were isolated as least two scFv sequences against both PD-1 as well as CD19.

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CONSTRUCTION OF AN EXPRESSION PLASMID FOR RECOMBINANT NECTIN-4 AS AN ANTIGEN FOR FUTURE MONOCLONAL ANTIBODY PRODUCTION.

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Introduction: Nectin-4 is a cell adhesion molecule overexpressed in several malignancies, including breast, lung, and ovarian cancers, making it a promising biomarker and target for monoclonal antibody development. This work aimed to construct an expression plasmid containing the extracellular domain of NECTIN-4 to enable future recombinant protein production.

Materials and Methods: The structure of NECTIN-4 was analyzed using bioinformatic tools(ColabFold, PyMOL), and the extracellular region was selected for cloning. Primers with SacI and KpnI restriction sites were designed for PCR amplification. The ~1,031 bp amplicon was purified, digested, and ligated into the pCMV-GFP expression vector. Recombinant plasmids were transformed into E. coli competent cells and screened by colony PCR and sequencing.

Results: Amplification of the extracellular NECTIN-4 fragment was successful, and multiple recombinant clones were obtained. Restriction analysis and sequencing confirmed correct insertion and orientation within the expression vector.

Discussion: The construction of this plasmid provides the basis for expressing recombinant NECTIN-4 in mammalian systems. This resource is essential for subsequent protein purification, structural characterization, and the generation of monoclonal antibodies against this clinically relevant biomarker.

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CHARACTERISATION OF POLYMERIC NANOPARTICLES FOR NOSE-TO-BRAIN DELIVERY OF THERAPEUTIC NUCLEIC ACIDS

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Introduction: Recently, several nucleic acids (NAs) have been reported to show therapeutic potential in animal models of central nervous system (CNS) diseases. However, conventional administration routes do not efficiently enable transit across the blood–brain barrier. This study aimed to develop polymeric nanoparticles (NPs) loaded with NAs for nose-to-brain delivery.

Materials and Methods: NPs were synthesised using biodegradable PEG and PLA polymers (mPEG-PLA) through a double-emulsion method and loaded with model NAs. We characterised NA-loaded NPs (NP- mPEG-PLA-NA) in terms of hydrodynamic diameter (HD), polydispersity index (PdI), zeta potential (ZP), and stability. HD, PdI, and ZP were measured by Dynamic Light Scattering (DLS). NP concentration was quantified by Nanoparticle Tracking Analysis (NTA), and morphology visualised by Scanning Transmission Electron Microscopy (STEM). Cytotoxicity was evaluated in HT-22 hippocampal neuronal cells using MTT assay.

Results: The NPs exhibited a mean HD of 165 ± 10 nm, a PdI of 0.21–0.32, and a ZP of –30 to –25 mV, indicating good colloidal stability. NP-mPEG-PLA-NA remained stable for at least four weeks at 4 °C with only minimal variations. Cytotoxicity assays in HT-22 cells showed that viability remained above 97 % after 24 h of exposure to 100 µg/mL.

Discussion: These results indicate that the NPs have a narrow size distribution and exhibit strong electrostatic repulsion, reducing their tendency to aggregate. Importantly, they exerted no significant effect on HT-22 cell viability at the tested concentrations, supporting biocompatibility. Their physicochemical properties render them promising candidates for nose-to-brain delivery with potential applications in CNS disorder models. Further in vivo studies will be required to confirm biodistribution and therapeutic efficacy.

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AS A TRANSLATIONAL TOOL FOR RELIABLE HBA1C QUANTIFICATION IN TYPE 2 DIABETES MELLITUS IN CHILE

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Introduction: Type 2 diabetes mellitus (T2DM) is one of the major public health challenges in Chile, with a prevalence of nearly 12% among adults and a high impact on chronic complications. Glycated hemoglobin (HbA1c) is the reference biomarker for diagnosis and monitoring, as it reflects the average glycemic level of the previous 2–3 months and correlates with the occurrence of micro- and macrovascular events.

Materials and Methods: The accuracy of HbA1c measurement depends on the analytical technology employed. High-performance liquid chromatography (HPLC) has emerged as the gold standard, offering high sensitivity and specificity, along with the capacity to detect hemoglobin variants and analytical interferences that may be overlooked by alternative methods.

Results: HPLC has demonstrated advantages in heterogeneous populations, such as that of Chile, where hemoglobin variants may compromise clinical interpretation. Its systematic use ensures robust and reproducible data, enabling accurate early diagnosis, reliable monitoring, and high-quality population-based studies in T2DM.

Discussion: The adoption of HPLC as the preferred method for HbA1c quantification represents a translational strategy that bridges biomedical research and clinical practice. By ensuring reliable results, HPLC supports better therapeutic decision-making, contributes to the optimization of management strategies, and strengthens the integration of scientific evidence into public health policies in Chile. Importantly, its systematic introduction across all levels of the healthcare system is essential to guarantee equity, improve early detection, and standardize diabetes management nationwide.

Acknowledgments: We acknowledge the contributions of Grupo BIOS and its collaborators to the promotion of innovative diagnostic technologies in Chile.

PATIENT-DERIVED SCFV LIBRARY CONSTRUCTION AND SELECTION AGAINST CLAUDIN-18.2 FOR GASTRIC CANCER VIA PHAGE DISPLAY

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Cancer is a leading cause worldwide and the primary cause of death in Chile; gastric cancer remains highly prevalent. We aimed to generate single-chain variable fragment (scFv) antibodies against Claudin-18.2 (CLDN18.2), an actionable biomarker overexpressed in gastric tumors, using phage display. Peripheral blood from patients was used to amplify immunoglobulin VH and VL regions, assemble scFv, and clone into a phagemid to build a display library. Library quality and diversity were assessed by electrophoresis and next-generation sequencing; recombinant phage production was verified by SDS-PAGE/Western blot. Three biopanning rounds were carried out on recombinant CLDN18.2, and specificity was evaluated by dot-blotting versus bovine serum albumin (BSA). The library comprised >48,000 unique candidates; an ~38 kDa scFv band confirmed expression. After panning, pools were enriched for CLDN18.2 binders, and three scFv clones showed clear binding to CLDN18.2 without reactivity to BSA. These findings indicate that a patient-derived scFv library can deliver CLDN18.2-specific binders, providing leads for targeted diagnostics and therapeutics in gastric cancer; future work will prioritize affinity maturation, epitope mapping, and cell-based validation.

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EFFECT OF OVERWEIGHT IN MALE REPRODUCTION: IMPACT ON SPERM PARAMETERS AND CELLULAR FUNCTION

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Introduction: Overweight is a condition characterized by an increase in adipose tissue, which is becoming increasingly prevalent worldwide. It has been reported to cause an imbalance in cellular redox status and alter male reproductive function, potentially contributing to some idiopathic causes of infertility. This study examined sperm parameters and functionality in men of normal and overweight body mass index (BMI).

Materials and Methods: Preliminary results were obtained from a total of 12 healthy male volunteers aged between 21 and 35 years with no history of genital tract disease. The donors had previously agreed to participate and signed an informed consent form approved by the Scientific Ethics Committee at the Universidad de La Frontera. The participants were divided into two groups: normal weight (n = 4) and overweight (n = 8). Native semen samples were analyzed by flow cytometry to evaluate viability, mitochondrial membrane potential, reactive oxygen and nitrosative species production, thiol oxidation and lipid peroxidation. Sperm parameters were also evaluated according to World Health Organization guidelines**.**

Results: Compared to the normal weight group, the overweight group showed an increase in reactive oxygen species (ROS) levels in both live ($p = 0.0144$) and dead ($p = 0.0263$) cells. An increase in thiol group oxidation was also observed in overweight males compared to normal-weight males ($p = 0.0052$). There were no significant differences in sperm parameters or functional test results between the two study groups.

Discussion: Our results indicated an increase in ROS and alterations in the redox balance, suggesting that being overweight negatively affects cell integrity, even before reaching the obese category. Oxidative stress plays an important role in the etiology of male infertility, as excessive levels of ROS have detrimental effects on human sperm function. At the molecular level, excessive ROS production in sperm cells is induced by an increase in intracellular calcium overload, which has a detrimental effect on the sperm membrane. These findings emphasize the importance of early detection and treatment of oxidative changes in overweight men to prevent the progressive deterioration of sperm quality.

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TGF-β1 AND NRF2 SIGNALING IN AN IN VIVO MODEL OF DEMYELINATION

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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS), characterized by demyelination, inflammation, and neurodegeneration. The latter has been strongly associated with pathological microglial activation, which compromises the viability of oligodendrocytes, cells that are responsible for the formation and maintenance of the myelin sheath at the CNS. In this context, both the cytokine TGF-β1 and the nuclear factor Nrf2 pathways have been extensively implicated in neuroprotective and pathological processes associated with MS. TGF-β1 would play a dual role by promoting remyelination in certain contexts but also contributing to glial dysfunction in others. Nrf2 regulates the expression of antioxidant genes, being actively involved in the modulation of neuroinflammation. In the present study, a cuprizone (CPZ)-induced demyelination model was used to evaluate the effect of the pharmacological inhibition of TGF-β1 receptor using galunisertib (GAL, 10 mg/kg). This treatment significantly reduced the expression levels of Nrf2, catalase, and TGF-β1 during the demyelination phase. Complementarily, we studied the activation of a microglia cell line (BV-2 cells) under similar inflammatory/demyelinating stimulation by lipopolysaccharide and lysophosphatidylcholine (LPC, a demyelinating agent). By RT-qPCR analysis, we identified pro- and anti-inflammatory profiles, namely the expression of IL-6, IL-1, IL-23, IL-17, and CCL2 and TGF-β1, IL-10, respectively.

Conclusion: A potential cross-talk between TGF-β and NRF2 pathways occurs in microglia; however, further studies are needed to clarify its link to the pro-inflammatory cytokines observed in our in vitro model.

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NEUROANGIOGENIC PROTEINS AND BLOOD–BRAIN BARRIER DYSFUNCTION IN SCHIZOPHRENIA: CLINICAL AND EXPERIMENTAL EVIDENCE

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Schizophrenia is a chronic and disabling psychiatric disorder, characterized by a highly heterogeneous clinical presentation. It negatively impacts the quality of life of patients. The origin of the pathology lies in neurodevelopment, and recent findings have reported an associated neurovascular dysfunction, including alterations and implications involving the blood–brain barrier (BBB), suggesting a vascular and multisystemic component to the disease. This study aims to identify and analyze biomarkers in a Chilean population of patients diagnosed with schizophrenia, focusing on proteins related to the Neurovascular Unit. Serum samples from patients were analyzed using ELISA to detect the presence of neuroangiogenic proteins, such as BDNF, VEGF, and NTN-1. Results showed that Serum Netrin-1 levels are significantly altered in patients with schizophrenia. In addition, representative samples were submitted for metabolic analyses to identify altered metabolic pathways associated with the disorder. In parallel, the relevance of these findings is being assessed in vitro using BBB models such as the human cerebral microvascular endothelial cell line HCMEC/D3, and in vivo using the chicken embryo model. Patient serum and antipsychotic drugs will be tested to determine endothelial responses through changes in angiogenesis and vascular permeability upon exposure to these stimuli, with particular attention to the WNT/β-Catenin signaling pathway, which plays a key role in regulating blood–brain barrier properties.

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INTRANASAL PANX1 BLOCKERS DIFFERENTIALLY MODIFY GLIAL INFLAMMATORY PHENOTYPES IN A MURINE MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an irreversible, chronic and inflammatory demyelinating disease of the central nervous system (CNS) characterized by the loss of myelin (i.e. demyelination). The demyelinated regions (or lesions) are focal brain areas that undergo an inflammatory gliosis leading ultimately to neuronal death. Pannexin 1 (Panx1) channels are ubiquitously expressed in CNS, facilitating ATP release from intracellular to extracellular space in neurons and glial cells. Panx1-channel activity has been largely involved in neuroinflammatory and neurodegenerative diseases, including MS. However, the mechanisms underlying the role of these channels have not been fully described. Here, we investigate the effect of intranasal Probenecid and 10Panx, Panx1-channel blockers, on astrocytes, microglia and oligodendrocytes in a preclinical model of MS, based on the stereotaxic injection of lysolecithin (LPC, a demyelinating agent) into CNS white matter tracts. As expected, at the peak of demyelination both astrocytes and microglia showed an inflammatory phenotype associated with myelin loss. However, when animals were treated with Panx1 blockers, both myelin loss and the astrocytic gliosis response were reduced significantly, while microglia were not susceptible to this treatment. Together, these results suggest a novel association between the Panx1-channel activity and the inflammatory response of astrocytes during demyelination in a pathological context such as MS.

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EXTRACTION AND STRUCTURAL PROFILING OF BUFADIENOLIDES FROM KALANCHOE DAIGREMONTIANA AS CANDIDATES FOR FUNCTIONAL ANTICANCER ANALYSIS IN GALLBLADDER CANCER

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Introduction: Traditional medicine has utilized plants for the treatment of diseases for centuries, taking advantage of their biological diversity[1] . In this context, the *Kalanchoe* genus has been studied for its anticancer potential, particularly due to the presence of polyphenols and bufadienolides—compounds that have been shown to inhibit key processes in cancer progression, such as cell adhesion, migration, and proliferation[2-7]. Since gallbladder cancer is a highly prevalent disease in Andean regions, especially in Chile[8-9], it is essential to explore new anticancer therapies that may improve its management.

Material and Methods: Leaves collected from greenhouse-propagated *Kalanchoe* daigremontiana plants were subjected to maceration using different solvents, according to results an optimization of the chloroform-based protocol was carried, by the establishment of an initial delipidation step with hexane to reduce lipid interference. The resulting extracts were analyzed by MALDI-TOF mass spectrometry, confirming a significant reduction in lipid content and revealing characteristic metabolite profiles. Subsequent chromatographic fractionation was performed[10] using size-exclusion chromatography on Sephadex LH-20, and fractions were monitored by thin-layer chromatography (TLC) under UV light. Combined fractions were analyzed by nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C), enabling preliminary structural characterization and comparison with reference data for bufadienolides.

Results and Discussion: These complementary approaches allowed the establishment of a workflow for the generation, purification, and structural profiling of bufadienolide-containing fractions from K. *daigremontiana*. The ^{1}H and ^{13}C NMR spectra of some fractions revealed characteristic signals of the α-pyrone system typical of bufadienolides, thus confirming their presence. These results provide a chemical foundation for subsequent studies aimed at evaluating the antitumoral effects of these natural products on GBC cell lines, with a particular focus on the modulation of spleen tyrosine kinase (SYK) activity and cell adhesion processes. Collectively, this work contributes to the bioprospecting of *Kalanchoe* species as a source of novel anticancer compounds.

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References:

1. Zhan, X., Wu, H., Wu, H., Wang, R., Luo, C., Gao, B., Chen, Z., & Li, Q. (2020). Metabolites from Bufo gargarizans (Cantor, 1842): A review of traditional uses, pharmacological activity, toxicity and quality control. Journal of Ethnopharmacology, 246, 112178. https://doi.org/10.1016/j.jep.2020.112178
2. Nascimento, L.B.d.S., Casanova, L.M. & Costa, S.S. (2023) Bioactive Compounds from Kalanchoe Genus Potentially Useful for the Development of New Drugs. Life, 13, 646. https://doi.org/10.3390/life13030646
3. Huang, H. C., Chang, W. T., Lee, M. S., Chen, H. Y., Chen, Y. H., Lin, C. C., & Lin, M. K. (2021). Three bufadienolides induce cell death in the human lung cancer cell line CL1-5 mainly through autophagy. Bioorganic & Medicinal Chemistry Letters, 31, 127715. https://doi.org/10.1016/j.bmcl.2020.127715
4. Hsieh, Y. J., Huang, H. S., Leu, Y. L., Peng, K. C., Chang, C. J., & Chang, M. Y. (2016). Anticancer activity of Kalanchoe tubiflora extract against human lung cancer cells in vitro and in vivo. Environmental Toxicology, 31, 1663-1673. https://doi.org/10.1002/tox.22211
5. Arias-González, I., García-Carrancá, A. M., Cornejo-Garrido, J., & Ordaz-Pichardo, C. (2018). Cytotoxic effect of Kalanchoe flammea and induction of intrinsic mitochondrial apoptotic signaling in prostate cancer cells. Journal of Ethnopharmacology, 222, 133-147. https://doi.org/10.1016/j.jep.2018.04.004
6. Hernández-Caballero, M. E., Sierra-Ramírez, J. A., Villalobos-Valencia, R., & Seseña- Méndez, E. (2022). Potential of Kalanchoe pinnata as a cancer treatment adjuvant and an epigenetic regulator. Molecules, 27, 6425. https://doi.org/10.3390/molecules27196425
7. Stefanowicz-Hajduk, J., Hering, A., Gucwa, M., Sztormowska-Achranowicz, K., Kowalczyk, M., Soluch, A., & Ochocka, J. R. (2022). An in vitro anticancer, antioxidant, and phytochemical study on water extract of Kalanchoe daigremontiana Raym.-Hamet and H. Perrier. Molecules, 27, 2280. https://doi.org/10.3390/molecules27072280
8. Leal, P., Garćia, P., Sandoval, A., Letelier, P., Brebi, P., Ili, C., Álvarez, H., Tapia, O., & Roa, J. C. (2013). Immunohistochemical expression of phospho-mTOR is associated with poor prognosis in patients with gallbladder adenocarcinoma. Archives of Pathology and Laboratory Medicine, 137(4), 552–557. https://doi.org/10.5858/arpa.2012-0032-OA
9. Roa, I., Ibacache, G., Muñoz, S., & De Aretxabala, X. (2014). Gallbladder cancer in Chile: Pathologic characteristics of survival and prognostic factors: Analysis of 1,366 cases. American Journal of Clinical Pathology, 141(5), 675–682. https://doi.org/10.1309/AJCPQT3ELN2BBCKA
10. Gomes, K. S., Coelho, J. A., Gomes, R. N., Bosquetti, L. M., Lange, C. N., Batista, B. L., Cerchiaro, G., & Lago, J. H. G. (2024). Dehydrodieugenol isolated from Ocotea cymbarum induces cell death in human breast cancer cell lines by dysregulation of intracellular copper concentration. Chemico-Biological Interactions, 396, 111039. https://doi.org/10.1016/j.cbi.2024.111039

IMPLEMENTING GLP TO ENHANCE INTEGRITY AND QUALITY IN BIOMEDICAL RESEARCH

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Introduction: Ensuring data integrity and quality is a growing challenge in biomedical research, especially in complex translational and preclinical settings. Good Laboratory Practice (GLP) offers a structured framework to minimize errors, standardize processes, and build trust with regulatory agencies, industry partners, and the scientific community.

Material and Methods: The TRACE Unit was created at the IMPACT Center to lead the implementation of GLP principles, with its framework and procedures in alignment with FDA's 21 CFR Part 58 and ISP's Technical Standard 139. Key steps included defining organizational roles, conducting gap analyses, implementing biosafety systems, upgrading infrastructure and equipment, drafting SOPs, forming a Quality Assurance Unit (QAU), developing monitoring tools such as the Document Quality Index (DQI), adopting REDCap for secure data management, and applying continuous improvement strategies.

Results: The progressive implementation of GLP at the IMPACT Center enabled the identification and resolution of previously undetected non-conformities, and increased quality, traceability and operative efficiency. The TRACE DQI showed sustained improvement in document quality, while the TRACE Weighted Severity Index (WSI) indicated an increased awareness and compliance with biosafety and GLP practices. A structured training plan for staff established a baseline for standardizing GLP knowledge.

Discussion: Our experience demonstrates that GLP can be effectively implemented even in resource-limited settings, offering biomedical centers a competitive advantage. By integrating GLP, research groups can strengthen collaborations with industry, meet regulatory expectations, and streamline the path from bench to clinic. The TRACE Unit now positions IMPACT as a technical reference for GLP implementation, offering third-party support services in preclinical research under rigorous regulatory standards and transferring expertise to other biomedical centers.

Acknowledgments: We thank the Universidad de los Andes, the Regenero Consortium, and the IMPACT center and IMPACT staff. The TRACE Unit welcomes collaborations and service inquiries from institutions aiming to enhance research quality and regulatory compliance.

ENHANCING NUCLEIC ACID ANALYSIS THROUGH TISSUELYSER II: IMPLEMENTATION IN CHILEAN REFERENCE LABORATORIES TO SUPPORT HIGH-THROUGHPUT MOLECULAR DIAGNOSTICS

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Introduction: Precise DNA and RNA extraction is critical for downstream molecular applications, including clinical diagnostics, infectious disease surveillance, and biomedical research. Efficient sample homogenization ensures nucleic acid integrity and reproducibility, which are particularly relevant in settings with increasing testing demands.

Materials and Methods: Grupo Bios introduced the TissueLyser II (QIAGEN) in Chilean laboratories, including the national reference center at the Instituto de Salud Pública (ISP) and Servicio Medico Legal (SML). The TissueLyser II enables high-throughput disruption and homogenization of challenging biological samples (bone, shell, soil, skin, hair, etc), providing consistent and scalable workflows for nucleic acid extraction.

Results: Implementation of the TissueLyser II has improved laboratory throughput and reproducibility, supporting accurate downstream DNA and RNA analyses. Laboratories reported enhanced reliability in nucleic acid yield and purity, which directly benefits subsequent applications such as qPCR, NGS, and molecular surveillance programs. The increased efficiency has been particularly valuable in handling higher sample volumes.

Discussion: The adoption of robust homogenization technologies such as the TissueLyser II represents a key step toward strengthening molecular diagnostics in Chile. By ensuring high-quality nucleic acid preparation, laboratories are better equipped to deliver reliable results that support timely clinical decision-making and public health strategies. Broader implementation of these tools can contribute to advancing precision medicine and addressing the growing demand for molecular testing.

Acknowledgments: We thank the Instituto de Salud Pública, SML and partnering laboratories for their collaboration in the implementation of TissueLyser II.

LINC02258 AS A POTENTIAL REGULATOR OF CARBOPLATIN RESISTANCE IN OVARIAN CANCER CELL LINES

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Introduction: Ovarian cancer is the most lethal gynecological malignancy, largely due to late diagnosis and the development of drug-resistance to platinum-based therapies. Recent transcriptomic analyses have highlighted the role of long non-coding RNAs (lncRNAs) in drug resistance. LINC02258 has been identified as highly expressed in carboplatin-resistant ovarian cancer cells, although its biological function remains poorly understood.

Methods: Total RNA was extracted from ovarian cancer cell lines (A2780 parental/resistant, UCI-101, SKOV-3, OVCAR-3) using TRIzol™. Following DNase I treatment, cDNA synthesis was performed with M-MLV reverse transcriptase. LINC02258 expression was quantified by SYBR Green-based qPCR, normalized to β-actin, and analyzed using the 2-ΔΔCt method. Carboplatin sensitivity was determined by MTT-based viability assays, and IC_{50} values were calculated from dose-response curves.

Results: qPCR analysis revealed a significant overexpression of LINC02258 in A2780 resistant cells compared with their parental counterpart ($p < 0.05$). Elevated LINC02258 levels were also observed in SKOV-3 and OVCAR-3, both known for reduced carboplatin sensitivity. Viability assays demonstrated higher IC_{50} values in A2780 resistant (≈48 µM) and SKOV-3 (≈53 µM) relative to A2780 parental (≈39 µM). A positive trend between LINC02258 expression and carboplatin IC_{50} was observed.

Discussion: These findings suggest that LINC02258 may contribute to carboplatin resistance in ovarian cancer models. Its elevated expression in resistant lines aligns with decreased drug sensitivity, supporting its potential role as a regulator of chemoresistance. Functional validation through knockdown/overexpression studies is warranted to confirm its therapeutic and biomarker value.

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METAGENOMIC MINING OF BIOSYNTHETIC GENE CLUSTERS (BGCS) WITH ANTIMICROBIAL POTENTIAL FROM DECEPTION ISLAND, ANTARCTICA

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Deception Island is an active Antarctic volcano with geothermal activity and fumaroles that produce unique environmental conditions which have probably shaped the functional and metabolic activity of the microbial communities inhabiting this island. This makes Deception island a potentially invaluable source of microorganisms capable of producing novel secondary metabolites with specialized antimicrobial functions. The aim of this study was to determine the genetic potential to produce secondary metabolites with antimicrobial activity in Deception Island soil metagenomes. For this, metagenomic DNA was extracted from five soil samples and subsequently sequenced on Illumina Novaseq6000 platform. Metagenomes were assembled using MEGAHIT v1.2.9, and a genome reconstruction strategy (binning) was performed using Metabat2 v2.12.1, Maxbin2 v2.2.4, CONCOCT v1.0.0, and DAS_Tools v1.1.6. Taxonomic profiling of the bins was carried out using GTDB-tk tool(KBASE platform) using Refseq database. Finally, the determination of the biosynthetic potential of the bins was performed with anti-SMASH v8. A total of 47 bins with contamination <10% and completeness >90% were obtained, among them, a 95.8% corresponded to the Bacteria domain and 4.2% to the Archaea domain, however 91,5% of the bins were not classified either for the bacteria or archaea domain. Anti-SMASH indicated that a 66.3% of the identified BGCs within the bins are associated with antimicrobial potential. Among them, the most abundant were RiPPs (16.0%), NRPSs (6.8%), terpenes (27.3%), terpene precursors (19.6%) and acyl-amino acids (30.5%). This study provides crucial information on previously unregistered microorganisms that possess a significant genetic potential for antimicrobial activity. The discovery of these novel biosynthetic pathways is highly relevant for bioprospecting efforts and may provide new leads in the urgent fight against the growing global threat of antibiotic resistance.

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BIOSENTINEL: BIOINFORMATIC PLATFORM FOR AUTOMATED ANALYSIS OF MICROBIAL COMMUNITIES BASED ON NANOPORE AND ILLUMINA SEQUENCING DATA

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The bioinformatic analysis of microbiome data still a challenge for most microbiologist. Furthermore, the recent arrival of third-generation sequencing platforms such as Nanopore has brought new difficulties in terms of data processing due to the intrinsic characteristics of Nanopore reads. In this context, comprehensive and user-friendly bioinformatic pipelines that covers from the raw data analysis to graphical interpretation are scarce. The aim of this work was to develop Biosentinel, a user-friendly and automated bioinformatic web platform for Nanopore and Illumina 16S rRNA data analysis. For this, we packaged bioinformatic protocols for the management of microbiome data from raw data (fastq) to microbiome profiling and statistical analysis under an architecture built in python, R, html and php languages. Biosentinel includes packages and tools such as Kraken2, Emu2, "phyloseq", "dplyr", "ggplot2", "metagenomeSeq", "microbiomeMarker" and "qiime2". The integrated platform is composed of a user friendly and easy-to-use front-end that allows the obtention of alpha and beta diversity plots, LEFSe barplot, cladograms, relative abundance barplots, correlation plots, among others. Performance tests indicated that Biosentinel takes 15 minutes to process a dataset of ~1 million of Illumina reads, while for a dataset of ~1 million of Nanopore reads, the platform took 1.5 hours. All tests were performed on a computer equipped with a conventional i7 intel processor and 32Gb of RAM memory. Results indicate that Biosentinel allows the automated and user-friendly analysis of 16S rRNA sequencing data from Nanopore and Illumina platforms through an interactive dashboard avoiding the necessity of having bioinformatic scripting skills, covering an important gap mainly for Nanopore sequencing data.

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CHEMOKINE EXPRESSION PROFILE AND MORPHOLOGICAL CHARACTERIZATION IN A THREE-DIMENSIONAL MODEL OF CISPLATIN- RESISTANT GASTRIC CANCER.

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Introduction: Gastric cancer (GC) is the third deadliest and fifth most common cancer globally. In Chile, it is the second most common cancer, with a high mortality rate in the Araucanía region. The main obstacles to curing GC are late diagnosis and the development of cisplatin-resistant cells. The mechanism of this resistance has not been fully elucidated; however, it has been associated with the tumor microenvironment and the secretion of inflammatory mediators such as cytokines and chemokines, which promote tumor progression and the acquisition of a resistant phenotype. Three-dimensional (3D) culture models, better reproduce the tumor microenvironment than conventional 2D cultures and are valuable tools for studying chemoresistance. The objective of this study was to identify the chemokine expression profile and morphologically characterize spheroids in cisplatin-sensitive and cisplatin-resistant gastric cancer cell lines.

Materials and Methods: Spheroids were generated from the gastric cancer cell lines AGS- WT, AGS-RCDDP, SNU-601, and SNU-638. Morphological parameters and cell viability were evaluated. Thirty-eight human chemokines were evaluated using an antibody microarray from the cell culture supernatants of all lines. The chemokine microarray images were analyzed using ImageJ.

Results: Spheroids generated showed similar circularity, roundness, and solidity between resistant and sensitive counterparts. Viability remained high across all groups on day 4. Chemokine analysis revealed that CXCL8 and CXCL12 remained unchanged in AGS- RCDDP, whereas CXCL9 decreased compared to AGS-WT. In SNU-638, resistance was associated with an increase in CXCL8 and CXCL12, coupled with the loss of CCL3, compared to SNU-601.

Discussion: In this 3D model, AGS-RCDDP showed decreased CXCL9, suggesting immune evasion, while SNU-638 showed increased CXCL8 and CXCL12 together with CCL3 loss, indicating a pro-inflammatory and remodeling microenvironment. These findings suggest that distinct chemokine profiles may contribute to cisplatin resistance mechanisms.

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DNA DELIVERY NANOSYSTEM BY IONIC INTERACTION WITH METFORMIN

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Introduction: Gene therapy requires efficient delivery systems to protect nucleic acids from enzymatic degradation, improve cellular uptake, and minimize immune responses [1]. Non-viral nanosystems, such as lipid or polymeric complexes, are safer but often less efficient than viral vectors [2,3]. Guanidine-containing molecules, due to their high pKa and stable positive charge, can promote DNA condensation [5]. Metformin, a strong biguanide base (pKa 12.4), is positively charged at physiological pH and has shown potential to form stable DNA nanostructures [4,5]. This study evaluates the DNA-condensing ability of metformin at different N/P ratios.

Materials and Methods: Metformin/DNA complexes were prepared in nuclease-free water at N/P ratios (amine groups/phosphate groups) from 2 to 30. Hydrodynamic diameter (DH), zeta potential (ZP), and polydispersity index (PDI) were measured by dynamic light scattering (DLS).

Results: Complex size and ZP varied with N/P ratio: N/P2 – 432 nm, -43.8 mV; N/P5 – 399 nm, -39.3 mV; N/P10 – 366 nm, -32.0 mV; N/P20 – 353 nm, -23.2 mV; N/P30 – 461 nm, -50.0 mV. Higher N/P ratios up to 20 reduced particle size and increased ZP toward neutrality, indicating DNA condensation. At N/P30, size increased and ZP became more negative, suggesting a structural rearrangement of the complexes. PDI values were ~0.5 up to N/P20.

Discussion: Metformin condenses DNA into nanoparticles with moderate size and stability, although excessive cationic excess (N/P30) may disrupt optimal packing. These findings support further evaluation of metformin as a guanidine-based non-viral transfection agent, including studies on stability, association efficiency, and DNA release profiles.

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References:

1. Jafari M, Soltani M, Naahidi S, N. Karunaratne D, Chen P. Nonviral Approach for Targeted Nucleic Acid Delivery. Curr Med Chem. 1 de enero de 2012;19(2):197-208.
2. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nat Rev Genet. agosto de 2014;15(8):541-55.
3. Dizaj SM, Jafari S, Khosroushahi AY. A sight on the current nanoparticle-based gene delivery vectors. Nanoscale Res Lett. diciembre de 2014;9(1):252.
4. Huang WX, Desai M, Tang Q, Yang R, Vivilecchia RV, Joshi Y. Elimination of metformin–croscarmellose sodium interaction by competition. Int J Pharm. marzo de 2006;311(1-2):33-9.
5. Qian H, Wang D, He B, Liu Q, Xu Y, Wu D, et al. Assembling defined DNA nanostructures with anticancer drugs: a metformin/DNA complex nanoplatform with a synergistic antitumor effect for KRAS- mutated lung cancer therapy. NPG Asia Mater. Diciembre de 2022;14(1):81.

ACETATE PRODUCED BY COMMENSAL *ESCHERICHIA COLI* ENHANCES THE VIRULENCE OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN A *GALLERIA MELLONELLA* INFECTION MODEL.

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Introduction: The gastrointestinal microbiota plays a crucial role in intestinal homeostasis, with significant compositional changes occurring during diarrheal episodes. Shiga toxin-producing *Escherichia coli* (STEC) is a relevant pathogen responsible for severe diseases through the production of Shiga toxin. Acetate, a short-chain fatty acid produced by commensal *E. coli,* has been linked to modulating STEC virulence. In our laboratory, metagenomic analysis of STEC-positive diarrheal samples from Chilean children revealed elevated acetate levels, positively correlated with an increased abundance of non-pathogenic *E. coli.* These findings suggest that *E. coli* may modulate the intestinal metabolic environment, potentially enhancing STEC virulence.

Materials and Methods: Commensal *E. coli* strains were isolated from stool samples of children under five years old with diarrhea positive for STEC, using MacConkey agar and biochemical profiling. The supernatants of the isolated *E. coli* strains were quantified for acetate production using high-performance liquid chromatography (HPLC), and the strain exhibiting the highest acetate levels was selected for subsequent analysis. To evaluate the impact on STEC virulence, the *Galleria mellonella* infection model was used, and survival analysis was performed using the Kaplan-Meier method.

Results: 52 commensal *E. coli* strains were isolated from STEC-positive diarrheal samples and screened for acetate production under anaerobic conditions using HPLC. Acetate levels varied between 10.81 mM and 26.97 mM, and the highest-producing strain (AM-2) was selected for further analysis. In a *Galleria mellonella* infection model, larvae infected with STEC resuspended in the sterile supernatant of AM-2 showed significantly increased mortality compared to those infected with STEC alone ($p = 0.0155$). The supernatant itself was non-lethal, suggesting that metabolites derived from commensal *E. coli,* particularly acetate, may enhance STEC virulence *in vivo.*

Conclusion: These results suggest that the supernatant from *E. coli* strains isolated from STEC-positive diarrheal samples, which contains acetate, can enhance STEC virulence *in vivo.* As a future direction, we aim to evaluate the effect of bacterial consortia derived from DEC-associated microbiota, incorporating the selected commensal *E. coli* strain, on STEC virulence using the *Galleria mellonella* infection model. This approach will contribute to a better understanding of the polymicrobial and metabolic interactions that influence STEC pathogenesis.

Keywords: STEC, *Escherichia coli,* acetate, virulence, fecal gut microbiota, *Galleria mellonella.*

CARDIORESPIRATORY BRAINSTEM CENTERS SHOW ALTERATIONS IN MYELIN AND GLIAL CELLS IN ANIMAL MODEL OF ALZHEIMER'S DISEASE.

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Alzheimer'disease (AD) is a neurodegenerative pathology associated with gradual memory loss, being the leading cause of dementia in older adults. It is characterized by the presence of amyloid-beta (Aβ) protein deposits and the formation of neurofibrillary tangles of hyperphosphorylated TAU protein (NFTs). These protein aggregates trigger glial-mediated neuroinflammation in the damaged areas. Although a major hallmark of AD is memory and cognitive impairment, there is also evidence showing cardiorespiratory dysfunction in both clinical and pre-clinical AD studies. In this regard, experimental research on AD has been largely focused on cortical and hippocampal regions, but much less is known about brainstem regions involved in cardiorespiratory control. Here, using an APP/PS1 transgenic mice and wild type controls we analyzed the immunofluorescence-based expression of Aβ, myelin and glial markers such as Olig2 (oligodendroglia), GFAP (astrocytes) and Iba1 (microglia) in major cardiorespiratory centers at the brainstem, namely: nucleus of the solitary tract (NTS), rostral ventrolateral medulla (RVLM) and retrotrapezoid nucleus (RTN). Our results revealed a significant loss of myelin (i.e. demyelination) across all the examined nuclei with differential effects on astrocytes and microglia population, as revealed by marker´s expression and Sholl analysis. Aβ deposits were observed in RVLM and RTN, but not in NTS, suggesting different Aβ production/clearance balance between regions.

Keywords: Alzheimer's disease, APP/PS1, myelin, glial cells, cardiorespiratory nuclei, brainstem.

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THE BLOOD CONCENTRATION OF EXTRACELLULAR VESICLES CONTAINING THE HSP70 IS ASSOCIATED WITH REDUCED ISCHEMIA/REPERFUSION-INDUCED KIDNEY INJURY IN MICE.

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Introduction: Acute kidney injury (AKI) consists of a loss of renal function in approximately 20% of hospitalized patients and is associated with the development of chronic kidney disease (CKD). One of the most important causes of AKI is renal ischemia and reperfusion (I/R), which is linked to oxidative stress processes and the activation of apoptosis. Small Extracellular vesicles (sEV) play a key role in cell-to-cell communication. It has been reported that sEV may exert a protective effect on the heart and kidney. The protein HSP70 can be released and anchored to the membrane of these EVs (sEV-HSP70) under conditions of cellular stress and inflammation, suggesting an anti-apoptotic role of Hsp70. We hypothesize that sEV-HSP70 prevents renal I/R injury.

Methods: C57BL/6 mice were exposed to renal ischemia (30 minutes) and reperfusion (48 hours), and renal clinical outcomes were evaluated by SCr, BUN, and histology. In addition, kidney damage biomarkers (TIMP-2, IGFBP-7, and NGAL) were measured. Besides, mice were treated with (geranylgeranyl acetone, GGA), an HSP70 inducer, and the sEV-HSP70 levels were quantified by ELISA.

Results: Increased SCr, BUN, kidney damage biomarkers, and histology alterations were observed in C57BL/6 mice subjected to I/R compared to the sham group. The GGA administration prevented this phenomenon. Besides, the levels of sEV-HSP70 were modified by the I/R group, and GGA recovered its plasma level.

Discussion: The sEV-HSP70 blood levels are associated with renal I/R-induced injury, and it is a potential biomarker and therapy strategy to reduce the acute kidney injury.

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EVALUATION OF THE MAPK SIGNALING PATHWAY IN GASTRIC CANCER CELL LINES AND ITS ROLE IN PLATINUM-BASED DRUG RESISTANCE FOR THE IDENTIFICATION OF A POTENTIAL PROTEIN BIOMARKER.

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Introduction: Gastric cancer is one of the leading causes of cancer mortality, with 70–90% of patients developing cisplatin resistance, resulting in metastasis and therapeutic failure. Inflammatory mediators have been implicated in this process, particularly the chemokine CCL5 and its receptor CCR5, which activate multiple signaling cascades. Among them, the MAPK pathway stands out as a potential contributor to chemoresistance. Understanding the role of the CCL5/CCR5–MAPK axis may provide valuable insights for the identification of novel therapeutic biomarkers in response to platinum-based treatments.

Material and Methods: The MAPK pathway was evaluated by Western blot, while the transcriptional expression of the chemokine CCL5 was analyzed by qPCR in resistant and parental gastric cancer cell lines AGS and MKN-28.

Results: Significant differences in CCL5 expressions were observed between AGS WT and R-CDDP cells, whereas no changes were detected in MKN-28 WT and R-CDDP cells. Regarding MAPK activation, phosphorylation remained active in both AGS WT and R-CDDP cells.

Discussion: In AGS WT and R-CDDP cells, CCL5 appears to be involved in the cisplatin-resistant phenotype, whereas in MKN-28 cells resistance may be mediated by other chemokines such as CCL11 or CCL21. MAPK signaling in AGS cells did not appear to be associated with cisplatin resistance, suggesting a potential role of STAT3 instead. In contrast, the increased MAPK phosphorylation observed in MKN-28 R-CDDP cells suggests its possible involvement in resistance to cisplatin. These findings highlight CCL5 and MAPK signaling as potential biomarkers for differential mechanisms of cisplatin resistance in gastric cancer, which could contribute to the development of targeted therapeutic strategies.

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MITOCHONDRIAL DELIVERY FROM MELANOMA IMPAIRS NK CELL ACTIVITY

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Introduction: Mitochondrial transfer between different cell types within the tumor microenvironment has emerged as an immunosuppressive mechanism. Based on the evidence that cancer cells transfer dysfunctional mitochondria to lymphocytes, we hypothesize that also exists mitochondrial transfer from melanoma cells to natural killer cells (NK), a phenomenon that diminishes their antitumor capacity.

Materials and Methods: Mitochondrial transfer from SK-MEL-28 melanoma cells to peripheral blood–derived NK cells was assessed *in vitro* at different co-culture intervals. SK-MEL-28 mitochondria were stained with MitoTracker Green, allowing their detection in NK cells by flow cytometry. NK cells were classified as mitochondria-positive or -negative and compared for expression of granzyme B, perforin, CD107a, NKp30 and NKp46, as well as cytotoxicity. Both mitochondrial transfer and effector-molecule levels were quantified by flow cytometry; NK cytotoxicity against K562 cells was determined using a calcein release assay.

Results: Co-culture of SK-MEL-28 melanoma cells with NK cells resulted in the detection of tumor-derived mitochondria in a subset of NK cells within the first 24 hours, indicating that mitochondrial transfer occurs rapidly after cell–cell interaction. NK mitochondria-positive showed reduced cytotoxic activity against K562 leukemic cells, suggesting a functional impairment. This was accompanied by lower levels of degranulation, and decreased levels of granzyme B, perforin, NKp30 and NKp46, pointing to a diminished degranulation capacity and reduced activation level compared to NK cells without mitochondrial acquisition from neoplastic cells.

Discussion: These results suggest melanoma-to-NK mitochondrial transfer promotes immune evasion by impairing cytotoxicity and effector/degranulation functions, weakening antitumor responses and fostering tumor progression.

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SR-B1 IS EXPRESSED IN KIDNEY TUBULAR EPITHELIAL CELLS, AND ITS DEFICIENCY EXACERBATES KIDNEY DAMAGE.

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Introduction. Acute kidney injury (AKI) is a prevalent clinical condition associated with high morbidity and mortality. Scavenger receptor class B type 1 (SR-B1) is a multiligand membrane receptor that plays a crucial role in lipid metabolism, uptake of lipid-soluble vitamins, and anti-inflammatory properties. Here, we investigate the role of SR-B1 in kidney function.

Method. The SR-B1 wild-type (WT) and knockout (KO) mice were used to test the role of SR-B1 in a unilateral nephrectomy (UNI) model of renal mass reduction. The renal function was assessed after 24 hours of surgery.

Results. Notably, our results showed for the first time that SR-B1 is expressed in the kidney epithelial tubular cells in mice. In addition, SR-B1 KO mice display basal renal inflammation, oxidative stress, and ferroptosis, suggesting a pivotal role for SR-B1 in renal homeostasis. SR-B1 KO mice undergoing unilateral nephrectomy exhibit exacerbated kidney damage characterized by increased oxidative stress, inflammation, progeroid phenotype, fibrosis, and ferroptosis. These findings suggest that SR-B1 plays a protective role in the kidney by mitigating these detrimental processes.

Conclusion. While our study does not discern whether the role of SR-B1 in renal homeostasis is attributed solely to renal SR-B1 or involves the contributions of this receptor expressed in other organs, it unequivocally highlights the significance of SR-B1 in maintaining renal homeostasis.

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DBC-1 INTERACTION WITH ECE-1C MODULATES ITS STABILITY IN COLORECTAL CANCER CELLS

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The Endothelin Converting Enzyme-1c (ECE-1) isoform is a protein that activates the Endothelin-1 peptide in diverse conditions, but it promotes malignant progression in cancers. Interestingly, the cytoplasmatic domain of ECE-1c is phosphorylated by protein kinase CK2, enhancing its stability and promoting a malignant phenotype in colorectal, glioblastoma and lung cancer cells. However, the mechanism associated with the regulation of stability of ECE- 1c and promotion of malignity remains unknown. Our laboratory studied ECE-1c binding proteins by immunoprecipitation, pull-down, stability assays, and a proteomic approach. One protein that seems to interact with stable ECE-1c is DBC-1 (CCAR2), which is related to regulation of stability of certain target proteins, as well as activation of the canonical Wnt pathway. Thus, plasmid coding for DBC-1 and specific siRNAs against it, were used in colorectal cancer cells for studying ECE-1c protein levels upon overexpression and downregulation of DBC-1, respectively. We observed that DBC-1 interacts with ECE-1c, and its ectopic expression increases ECE-1c protein levels. These findings suggest that ECE-1c stability is regulated by binding to DBC-1 protein. Whether the DBC-1/ECE-1c interaction promotes a malignant phenotype in colorectal cancer cells is uncovered yet.

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EXPANDING ACCESS TO DIAGNOSTICS FOR PRIMARY IMMUNODEFICIENCIES IN CHILE: A TRANSLATIONAL PERSPECTIVE

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Introduction: Primary immunodeficiencies (PIDs) remain underdiagnosed in Chile, delaying timely treatment. Functional complement assays, particularly CH50, are internationally recognized as first-line tests to detect classical pathway deficiencies and guide specialist referral [IDF; Figueroa et al., *Clin Immunol*, 2019].

Materials and Methods: We propose integrating CH50 testing with targeted clinician education to raise awareness of PID clinical features, promote appropriate test requests, and improve referral pathways. This combination aims to link laboratory availability with knowledge dissemination to strengthen the continuum from suspicion to diagnosis and management.

Results: International and regional evidence demonstrates that complement testing enhances detection of PIDs and guides timely referral [Jonsson et al., *Front Immunol*, 2018]. Expanding availability of CH50 in diagnostic labs, together with physician training, could increase test utilization, reduce diagnostic delays, and ensure timely access to therapies covered by Law 20.850 (GES).

Discussion: This integrated strategy exemplifies translational medicine by combining diagnostic innovation with education. Expanding CH50 access while increasing clinician knowledge addresses gaps in both test availability and clinical utilization. The approach is expected to reduce inequities, improve early detection, and optimize outcomes for patients with immunological disorders in Chile. Collaboration between academia, clinical networks, and private laboratories is essential to sustain diagnostic capacity.

Acknowledgments: We thank clinicians and academic partners supporting PID recognition, diagnosis, and management.

REDUCTION OF CENTRAL AND PERIPHERAL NERVOUS SYSTEM IMPAIRMENT IN A PRECLINICAL MODEL OF MULTIPLE SCLEROSIS BY IN VIVO VAGAL NERVE STIMULATION

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Multiple Sclerosis (MS) is a neurodegenerative disease characterized by the loss of myelin, a specialized oligodendrocyte (OL)-derived membrane. At early stages of the demyelinating process, damaged regions (i.e. demyelinated lesions) are characterized by the presence of OLs precursor cells (OPCs), reactive astrocytes and proinflammatory microglia. These proinflammatory events aggravate the symptoms and promote the disease progression. Available treatments for MS reduce the progression of the disease, however, none of them induce the recovery of the symptoms. A promising treatment for reducing inflammatory (peripherical and central) conditions is the stimulation of vagus nerve (VNS), which is able to reduce inflammatory cytokines and gliosis (i.e. reactive microglia and astrocytes). To study the physiological effects of in vivo VNS in both the pathological progression-like MS, we induced multiple focal demyelinated lesions by microinjecting lysolecithin (LPC) in C57BL/6 mice (PN70-90). One week after the LPC injection (i.e. maximum demyelination reached) mice were treated with *in vivo* VNS. Afterwards, pathophysiological parameters of MS progression and brain lesioned tissue were evaluated. Immunostaining of myelin, glial and neuronal markers, as well as cytokines expression examination of demyelinated lesions showed increased remyelinated area, reduction in the population of astrocytes and a change from proinflammatory- to an antiinflammatory-like microglial phenotype in the VNS treated animals. Complementarily, locomotor and autonomic dysfunction were rescue in the same animals detected. Our results suggest that VNS revert the MS progression signs, improving remyelination by modulation of proinflammatory environment of lesions.

TRANSCRIPTIONAL PROFILING OF IMMUNE-RELATED GENES AND ITS ASSOCIATION WITH CLINICAL PROGNOSIS IN HIGH-GRADE SEROUS OVARIAN CANCER

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Introduction: High-grade serous ovarian cancer (HGSOC) shows a high mortality rate, explained by the frequent development of drug-resistance and tumor recurrence. A study has described that the overexpression of genes associated with the inflammasome, is related to poor survival in patients, suggesting their contribution to drug-resistant phenotype.

Materials and Methods: HGSOC data were obtained from a cohort derived from The Cancer Genome Atlas (TCGA), and enriched from others NCBI bioprojects. ReadCounts of healthy ovarian tissue were extracted from Genotype-Tissue Expression (GTEx). The analysis was performed with DESeq2 v.1.36.0 in R (4.2.1), considering overexpression in genes with log2 FC >1 and STAT = 1.96. Gene ontology analysis was performed with ClusterProfiler v.3.14.3 in R (3.6.3), and survival analysis with Kaplan-Meier using Survminer v.0.5.0 and Survival v.3.8-3 in R (4.5.0), where $P<0.05$ values were considered statistically significant.

Results: Overexpression was observed in inflammasome and/or PANoptosome-associated genes, including NLRP3, PYCARD, AIM2, CASP1, NLRP12, RIPK3, in HGSOC. These findings were confirmed with NCBI data, which also showed overexpression of NAIP and CASP8. Gene ontology analysis revealed immunological processes associated with adaptive immune response, T cell activation, and response to bacteria infection. Survival analysis showed that NLRP12 and FADD are associated with reduced overall survival.

Discussion and Conclusions: Gene Ontology analysis revealed an immunologically active microenvironment in HGSOC, suggesting mechanisms of immune evasion. Among the overexpressed genes, only FADD and NLRP12 were associated to poor survival. FADD, although pro-apoptotic, paradoxically supports cancer progression and drug resistance. NLRP12 was associated to immune infiltration and tumor–immune interactions, highlighting its theranostic value for prognosis and therapeutic strategy development in HGSOC.

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PREDICTING THREE, SIX AND TWELVE-MONTH MORTALITY IN NON-ONCOLOGICAL PALLIATIVE CARE USING MACHINE LEARNING: EXPERIENCE FROM PRIMARY CARE IN COPIAPÓ

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Introduction: Universal palliative care aims to improve quality of life for people with advanced or terminal illnesses through a multidisciplinary approach. A key challenge is the timely identification of patients with limited life expectancy, particularly in non-oncologic populations where validated prognosis tools are scarce. We aimed to develop and evaluate machine-learning prognosis models to forsee three, six and up to twelve-month mortality in a local cohort enrolled in a Universal Palliative Care program.

Methods: We conducted a retrospective observational study of 166 patients admitted to the Universal Palliative Care program in Copiapó, Chile, between 2022 and 2025. The study was authorized by the Teaching Coordination Committee of the Municipality of Copiapó. Five algorithms—Extra Trees, logistic regression, random forest, XGBoost, and LightGBM—were trained to predict three, six and up to twelve-month mortality using 17 clinical variables available at admission. Performance was estimated with stratified nested cross-validation (five outer folds and three inner folds for hyperparameter tuning). The performance of all models was evaluated and compared using the area under the receiver operating characteristic curve (AUC) as the primary metric.

Results: Logistic regression was the best performing algorithm for the 3-month mortality scenario with an average AUC of 0.704 (95% CI: 0.648–0.759). XGBoost was best for the 6 and 12-month scenario, with an average AUC of 0.72 (95% CI, 0.661–0.778) and 0.700 (95% CI: 0.537–0.863), respectively.

Conclusions: Machine-learning models can estimate three, six and up to twelve-month mortality risk in palliative-care patients without additional testing. Despite moderate performance, their reliance on routinely collected variables suggests practical utility as decision-support tools in primary care and resource-limited settings.

INCORPORATION OF LYOPHILIZED L-PRF ENHANCES THE BIOACTIVITY OF 3D-BIOPRINTED SCAFFOLDS FOR BONE REGENERATION

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Introduction: Three-dimensional (3D) bioprinting enables the fabrication of cell-laden scaffolds for biomedical applications. Leukocyte- and platelet-rich fibrin (L-PRF) is a platelet concentrate that releases bioactive molecules to promote tissue regeneration. However, its application in 3D-bioprinted scaffolds for bone regeneration has been scarcely reported.

Materials and Methods: Composite hydrogels composed of alginate, gelatin, and nanohydroxyapatite supplemented with 10% lyophilized L-PRF were bioprinted with 1×10^6 dental pulp stem cells (DPSCs)/mL using a CellInk printer. Conditioned media collected at 1, 7, 14, and 21 days from the bioprinted scaffolds were analyzed for PDGF-BB, EGF, VEGF, FGF-2, and BMP-2 using Luminex. Scaffold-derived conditioned medium was used to stimulate DPSCs, and Ki67 expression was evaluated by immunostaining. Finally, bioprinted scaffolds were cultured in osteogenic medium, and the expression of RUNX2, ALP, COL1A, BMP-2, TGF-β, VEGF, OSX, OCN, OPN, OPG, and RANKL was determined by RT-PCR. Kruskal–Wallis and Student's t-tests statistical analyses were applied.

Results: DPSCs exhibited high viability within the bioprinted scaffolds. Growth factors were released up to 14 days from bioprinted scaffolds, and conditioned media recovered from the scaffolds promoted DPSC proliferation. Osteogenic differentiation was confirmed at 14 days by the upregulation of RUNX2, ALP, and COL1A, while high expression of OPN, OPG, and OCN was observed at 21 days of osteogenic medium incubation.

Discussion: L-PRF sustained the release of functional growth factors from the bioprinted scaffolds. Osteogenic differentiation of DPSCs was also evident, highlighting its potential as a bioactive additive for bone tissue engineering. Further in vitro and in vivo studies are warranted.

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CONSTRUCTION OF A RECOMBINANT VECTOR INCORPORATING THE HUMAN NON-SOLID TUMORBIOMARKER CD19: TOWARDS FUTURE BIOTECHNOLOGICAL APPLICATIONS

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Non-Hodgkin's lymphoma and leukemia are the most common malignant hematological neoplasms worldwide, while in Chile, leukemia is the cancer with the highest incidence and mortality in children and adolescents. CD19 is a protein that acts as a BCR co-receptor and plays a fundamental role in signal transduction. This protein has become a valuable biomarker due to its strong expression in the aforementioned pathologies, serving as a target for diagnosis and targeted immunotherapy. The objective is to design and validate a recombinant genetic construct that enables the future expression of CD19. In silico assays were performed using SnapGene software for the construction of the recombinant plasmid and primer design. Cloning was carried out by restriction and ligation, chemically competent cells were transformed, and vector insertion was verified by colony PCR. From the in silico analysis, the extracellular region of the protein was obtained, incorporating the His-tag and the TEV sequence; digestion with the restriction enzymes NheI and XhoI followed by ligation generated the recombinant vector pCMV-CD19. After transformation, colonies were selected and 8 clones tested positive by colony PCR. The use of affinity tags allows detection and facilitates protein purification, while the TEV sequence enables tag cleavage by TEV protease. The pCMV is a constitutive promoter frequently used for recombinant protein expression in mammalian cells, providing robust and sustained expression.

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STUDY ON THE BIOCOMPATIBILITY OF A CYANOBACTERIA-BASED PERFUSION SOLUTION FOR APPLICATION IN INTRAVASCULAR PHOTOSYNTHETIC THERAPY

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Introduction: Hypoxia remains a major limitation in the ex vivo preservation of organs intended for transplantation. As a potential solution, we propose a photosynthetic perfusion solution (PSOP) incorporating the cyanobacterium Synechocystis sp. PCC 6803, capable of generating oxygen in situ via photosynthesis.

Objective: To evaluate the hemocompatibility and cytotoxicity of PSOP using in vitro and in vivo experimental models.

Methods: Human blood was used to perform coagulation and hemolysis assays to assess hemocompatibility. Cytotoxicity was evaluated through MTT assays in HUVEC endothelial cells, alongside zebrafish larval toxicity assays following 24-hour PSOP exposure.

Results: PSOP did not induce significant coagulation or reduce cell viability when compared to control conditions. Hemolysis remained below 2% at the optimal concentration (109 cells/mL), yet increased substantially at higher concentrations. In zebrafish larvae, no significant differences in viability or morphological alterations were observed post-exposure.

Conclusions: Under the tested conditions, PSOP demonstrated good biocompatibility and safety in early-stage in vitro and in vivo models. However, the increase in hemolysis at higher concentrations highlights the importance of further optimizing physicochemical parameters such as osmolality and viscosity. Future research should focus on evaluating immune response, systemic distribution, and long-term safety in murine models to support potential clinical applications.

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GLOBAL MIRNA PROFILING IN VISCERAL ADIPOSE TISSUE SUGGEST HAD-D36 INFECTION AFFECT LONG-TERM METABOLIC PROCESSES RELATED TO GLYCEMIC CONTROL AND BONE HEALTH

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Objective: To evaluate the impact of prior HAdV-D36 infection on global miRNA expression in visceral adipose tissue and its association with metabolic pathways related to glycemic control and bone health.

Background: Adenovirus 36 (HAdV-D36) infection is related to adipogenic differentiation of mesenchymal stem cells (MSC) and high risk of obesity. Paradoxically, HAdV-D36 also showed to improve glycemic control in animal models and humans. There is no information about epigenetic mechanisms involved in maintenance of HAdV-D36 related pro-adipogenic or antidiabetic effects. We evaluated the effect of previous HAdV-D36 infection on global expression of microRNAs (miRNAs) in visceral adipose tissue (VAT) of humans.

Methods: Subjects were grouped in seropositive (n=8) and seronegative (n=8) according to serology. VAT was obtained from individuals submitted to surgical intervention and total RNA was isolated. miRNA libraries were prepared using the TruSeq small RNA Library Prep and sequenced using the MiSeq platform (Illumina Inc., USA). Sequence quality analysis and differential expression analysis of miRNAs were performed using bioinformatic tools in the Galaxy platform (FastQC; MirDeep2; DESeq2). Prediction of target genes and metabolic pathways regulated by differentially expressed miRNAs were evaluated in the mirNet 2.0 platform using experimentally validated interactions from Tarbase 9.0.

Results: 14 miRNAs (up-regulated: hsa-miR-5683, hsa-miR-888-5p, hsa-miR-3117-3p, hsa-miR-1283, hsa-miR-1283.1, hsa-miR-518f-3p, hsa-miR-518a-5p.1, hsa-miR-517c-3p, hsa-miR-527, hsa-miR-518a-5p; down-regulated: hsa-miR-196b-3p, hsa-miR-4454, hsa–miR–211–5p, hsa-miR-196b-5p) were differentially expressed in VAT from HAdv-D36 seropositive subjects. Enrichment analysis of predicted genes in KEGG demonstrated HAdV-D36 participates in biological processes related to insulin signaling, metabolism of eNOS and adipocytokine signaling pathway. Further gene ontology analysis using Reactome also identified signaling by bone morphogenic proteins (BMP) as an important process regulated by dysregulated miRNAs.

Conclusions: The study of metabolic pathways regulated by differentially expressed miRNAs in VAT from HAdv-D36-seropositive obese individuals suggests the involvement of posttranscriptional epigenetic mechanisms in the regulation of long term HAdV-D36-induced cardiometabolic effects and particularly glycemic control though insulin signaling pathway. Regulation of BMPs could have a role in MSC differentiation affecting regulation of bone health as a new potential role of HAdV-D36.

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Ethical approval and informed consent The study protocol was approved by de ethics committee of the Universidad de la Frontera (Protocol # 050/14). All individuals were informed about the study protocol and signed the informed consent form.

CHARACTERIZATION AND EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM UMBILICAL CORD MESENCHYMAL STROMAL CELLS (EVS-UC-MSCS) LOADED WITH MIR-365A-5P IN MEMORY CD4+ T CELLS

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Introduction: Autoimmune/inflammatory diseases are characterized by an imbalance between pro-inflammatory (Th1/Th17) and regulatory (Treg) CD4+ T cells, posing a persistent challenge for the regulation of these diseases. MSCs exert an immunomodulatory effect mainly through secretion of EVs, although with variable clinical outcomes. Metabolic reprogramming of UC-MSCs towards glycolysis and their microRNA content could enhance this effect. We propose that miR-365a-5p could contribute to the immunosuppressive action of EVs-UC- MSC on memory CD4+ T cells.

Materials and Methods: EVs were isolated by ultracentrifugation from basal UC-MSCs and UC-MSCs reprogrammed towards glycolysis. They were electroporated with miR-39 (uptake control) or with miR-365a-5p. They were characterized by NTA, flow cytometry, and TEM. miRNA cargo and uptake were quantified by qPCR. Human memory CD4+ T cells were treated with EVs, EVsglyco, or EVs loaded with miR-365a-5p, and the Th1, Th17, and Treg phenotype were analyzed by FACS. IL-10 secretion was evaluated by ELISA.

Results: Electroporation preserved EV size, morphology, and CD63 expression. qPCR confirmed the presence of miR-39 in EVs electroporated with this miRNA and its uptake in memory CD4+ T cells. EVs electroporated with miR-365a-5p showed higher levels of this miRNA compared with non-electroporated EVs. In memory CD4+ T cells treated with EVs enriched with miR- 365a-5p, a trend of immunomodulation was observed, with lower Th1/Th17 frequency and a higher proportion of Treg and IL-10.

Discussion: Our data indicates that electroporation maintains EV identity and allows efficient transfer of miRNAs. The trend toward immunomodulating CD4+ T cells by EVs enriched with miR-365a-5p preliminarily supports the hypothesis that miR-365a-5p may contribute to the immunosuppressive activity of EVs-UC-MSC. Future studies will delve into the role of this miRNA to establish its therapeutic relevance in EV-based acellular strategies.

PHARMACOLOGIC DEMETHYLATION RESTORES MTUS1/ATIP1 EXPRESSION IN HUMAN BREAST, GASTRIC, AND COLORECTAL CANCER CELLS

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Introduction: Breast, gastric, and colorectal cancers account for a substantial proportion of the global cancer burden. Besides genetic alterations, epigenetic changes, particularly promoter DNA methylation, can silence tumor-suppressor genes. MTUS1/ATIP1 acts as a tumor suppressor, and promoter hypermethylation has been reported in other malignancies, such as lung cancer and gliomas. Objective: To determine whether pharmacologic demethylation restores MTUS1/ATIP1 expression in human breast, gastric, and colorectal cancer cells.

Materials and Methods: In silico prediction of CpG islands in the MTUS1 promoter was performed with MethPrimer. Cells were then exposed to 5-aza-2′-deoxycytidine (30 or 50 µM) for 5 days. Cell viability was evaluated by the trypan blue exclusion assay. For expression analysis, RNA was extracted, cDNA synthesized, and MTUS1/ATIP1 mRNA quantified by qPCR using the $2^{-\Delta\Delta Ct}$ method. Human lines used: breast (MDA-MB-231), gastric (AGS), colorectal (HT-29), and non-tumor gastric epithelial cells (GES).

Results: MethPrimer identified a CpG island within the MTUS1 promoter. After 5-aza-2′-deoxycytidine exposure, cell viability remained ≥95% at all concentrations. MTUS1/ATIP1 expression increased versus control in all cancer cells with a dose-response (50 µM > 30 µM); fold changes were 1.45- and 2.59-fold in breast cancer cells, 0.76- and 2.00-fold in gastric cancer cells, and 0.52- and 1.03-fold in colorectal cancer cells at 30 and 50 µM, respectively. Basal expression in non-tumor cells exceeded that of the three cancer cell types.

Discussion: The presence of a promoter CpG island together with re-expression after demethylation, achieved without compromising viability, supports promoter methylation as a contributor to MTUS1/ATIP1 downregulation. Pharmacologic demethylation restores MTUS1/ATIP1 across breast, gastric, and colorectal cancer cells, with the greatest recovery in breast cancer cells, endorsing MTUS1/ATIP1 as a potential epigenetic biomarker and re-expression target.

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MSC-MEDIATED MITOCHONDRIAL TRANSFER RESTORES REDOX BALANCE AND BOOST CYTOTOXICITY IN NK CELLS AGAINST LEUKEMIC CELL LINE.

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Natural killer (NK) cells are immune effectors capable of killing cancer cells by recognizing surface ligands without prior antigen presentation. NK-based therapies, including allogeneic NK and CAR-NK cells, are promising options for treating hematological malignancies, as clinical trials have shown their safety. These therapies do not induce graft-versus-host disease and carry lower risk of severe side effects like cytokine storms. While NK-based therapies show promise for hematological malignancies, their efficacy is hampered by elevated reactive oxygen species (ROS) leading to exhaustion. Mitochondrial metabolism critically regulates NK cytotoxicity, suggesting a potential target.

Mitochondrial transfer from mesenchymal stem cells (MSCs) has been shown to regulate ROS levels in fibroblast lines with mitochondrial disorders. In this study, we assessed whether NK cells could acquire mitochondria from MSCs and if this transfer modulates the redox state of peripheral blood NK under an oxidative environment and whether mitochondrial transfer could enhance the cytotoxic activity of stressed NK cells against cancer cell targets.

The NK cells were isolated from peripheral blood and then underwent mitochondrial transfer. Subsequently, they were challenged with menadione, an oxidative stress-inducing agent. Then, we evaluated the cytotoxicity against K562, a leukemic cell line, using a calcein-realese assay.

We successfully demonstrated mitochondrial transfer from MSCs to NK cells by Flow cytometry. Importantly, this transfer reduced ~34% of the ROS levels in NK cells exposed to oxidative stress and enhanced their cytotoxic activity against K562 cells under these conditions.

Mitochondrial augmentation in NK cells through MSC-mediated transfer emerges as a novel strategy to restore redox balance and enhance cytotoxicity under oxidative stress, paving the way for innovative cell therapies against solid tumors. This approach holds strong potential to overcome tumor microenvironment constraints and improve the effectiveness of NK-based immunotherapies.

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DIVERSITY AND PREVALENCE OF THIRD-GENERATION CEPHALOSPORIN-RESISTANT GRAM-NEGATIVE BACILLI IN HEALTHY DOGS FROM THE ARAUCANÍA REGION: A PRELIMINARY APPROACH OF ONE HEALTH SURVEILLANCE

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Antimicrobial resistance mediated by extended-spectrum β-lactamase (ESBL)-producing bacteria represents a global threat, with third-generation cephalosporin-resistant (C3GR) Gram-negative bacteria reported in humans and animals, where they act as reservoirs and potential vectors. Within the One Health framework, this study assessed the diversity and prevalence of C3GR bacteria in healthy dogs and the risk factors associated with colonization in the Araucanía Region.

A cross-sectional study from March–July 2025 was conducted in dogs from Lautaro, Temuco, Padre Las Casas, and Nueva Imperial. Owners answered a questionnaire on household characteristics, pet habits, and environment. Rectal swab samples were enriched and cultured on MacConkey agar supplemented with cefotaxime (2 µg/mL), and bacterial identification was performed using MALDI-TOF. Statistical associations were evaluated using χ^2 tests and odds ratios (OR, 95% CI), with significance set at $p<0.05$.

Among 495 dogs, C3GR colonization prevalence was 19.5%, with higher rates in Nueva Imperial (27.5%) and Temuco (22.3%). Identified species included *E. coli, Pseudomonas aeruginosa, P. taetrolens, P. brassicacearum, P. fragii, P. koreensis, Citrobacter braaki, C. freundii, Klebsiella pneumoniae, K. aerogenes, Enterobacter hormaechei, Hafnia alvei, Salmonella* spp., and *E. ludwigii.*

Risk factors for colonization were owners employed in healthcare (OR=2.65; 95% CI: 1.83–4.4), pets of unknown origin (OR=2.04; 95% CI: 1.30–3.22), and age >2 years (OR=2.46; 95% CI: 1.44–4.22). Protective factors included living indoors (OR=0.27; 95% CI: 0.17–0.44), close interaction with owners (OR=0.51; 95% CI: 0.32–0.76), and neutering (OR=0.56; 95% CI: 0.33–1.95).

C3GR strain diversity in dogs encompassed *Enterobacteriaceae* and *Pseudomonas* spp., indicating their role as reservoirs and possible sources of resistance dissemination. Some of these bacteria have also been detected in community-acquired human infections. The human–animal interface, particularly when owners work in healthcare, and environmental exposure appear to be key factors for canine carriage of C3GR bacteria.

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EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM GLYCOLYTIC MESENCHYMAL STEM/STROMAL CELLS ON THE PHENOTYPIC AND METABOLIC REPROGRAMMING OF SYNOVIAL MACROPHAGES IN PATIENTS WITH OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA) is a chronic inflammatory disease for which no cure exists; only palliative treatments are available. OA is characterized by an exacerbated inflammatory response primarily driven by the activity of synovial macrophages. Mesenchymal stem/stromal cells (MSCs) are well known for their therapeutic potential; however, alternative strategies are essential to enhance their immunomodulatory properties. We show that glycolytic MSC (MSCglyco) and their small extracellular vesicles (EVs) holds promise for developing new therapies for OA.

Methods: MSCs were isolated from the umbilical cords of healthy donors, with informed consent obtained for the use of these samples. MSCs were treated with oligomycin for 24 hours to induce glycolytic metabolism. EVs were isolated from the MSCglyco (MSCglyco-EVs) using ultracentrifugation, quantified by nanoparticle tracking analysis (NTA), and characterized by transmission electron microscopy (TEM) and flow cytometry. Macrophages were isolated from the synovial membranes of OA patients (OAM), for which informed consent was also obtained. EVs from MSCs or MSCglyco were added to the culture media of the OAM at various doses. After 24 hours, OAM were recovered to evaluate EV internalization using qPCR, surface marker expression through flow cytometry, cytokine secretion via ELISA, and glycolytic flux using SCENITH, a flow cytometry assay.

Results: The metabolic reprogramming of MSCs does not alter the phenotype or size of the released EVs, nor their capacity of internalization. OAMs treated with MSCglyco-EVs internalize these EVs, leading to an increased expression of CD206, which indicates an anti-inflammatory phenotype, while decreasing HLA-DR and CD86 associated with a proinflammatory phenotype. Additionally, OAMs treated with MSCglyco-EVs exhibit a reduction in the secretion of inflammatory mediators.

Discussion: EVs from metabolically reprogrammed MSC have improved therapeutic properties. We demonstrate that MSCglyco-EVs dose-dependently reduce inflammatory profiles in OAM and increase anti-inflammatory profiles, providing compelling evidence for their enhanced therapeutic effects. This offers a promising approach for the development of novel acellular therapies for OA, focusing on immunomodulation using EVs from metabolically reprogrammed MSCs.

ENHANCING NK CELL ANTI-SOLID TUMOR FUNCTION VIA MITOCHONDRIAL TRANSFER FROM MESENCHYMAL STEM CELLS

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Natural Killer (NK) cells are key effectors of the innate immune system with the ability to eliminate tumor cells without prior sensitization. In solid tumors, the tumor microenvironment (TME) impairs NK cell function by inducing mitochondrial dysfunction, oxidative stress, and decreased cytotoxicity, limiting the efficacy of NK-based immunotherapies. In this context, mitochondrial transfer from mesenchymal stem cells (MSCs) has emerged as a promising strategy to restore immune cell metabolism and bioenergetics.

This study evaluates the effect of mitochondrial transfer from umbilical cord-derived MSCs on primary NK cell survival, proliferation, and cytotoxicity. NK cells were isolated from PBMCs and mitocepted using a centrifugation-based protocol. Survival and proliferation were analyzed by flow cytometry, while cytotoxicity was assessed against breast and lung solid tumor cell lines (MDA-MB-231, A549 and MCF-7) using Calcein-AM release assays. In addition, NK cells were co-cultured with tumor cells for six hours to evaluate activation and inhibition receptors (NKp30, NKp46, KIR3DL1, and NKG2a) and effector machinery, including IFN-γ, CD107a, perforin, and granzyme B.

Our results demonstrate that mitochondrial transfer does not impair NK cell survival and may support cell maintenance. No significant differences in proliferation were observed in total NK cell, or in the CD56Dim and CD56Bright subsets. Notably, mitocepted NK cells exhibited enhanced cytotoxic activity against cancer cell lines of breast and lung. Preliminary data suggest modulation of receptor expression and effector molecule release, indicating improved immune function.

These findings highlight mitochondrial transfer as a selective enhancer of NK cell antitumor activity, presenting a potential strategy to augment NK-based adoptive immunotherapies in metabolically restrictive TMEs.

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ENGINEERING A BIOMIMETIC LUNG HYDROGEL: A POTENTIAL 3D PLATFORM TO ASSESS EVS DERIVED FROM BREAST CANCER METASTASIS-STAGE 1

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Introduction: Progression of breast cancer often leads to lung metastasis and causes mortality. This process occurs through the formation of tumoral extracellular vesicles (EVs) and pre-metastatic niche (PMN). Tumor-derived EVs can interact with the lung extracellular matrix, modulating the stiffness of PMN. In this study, we developed a biomimetic hydrogel from murine lung tissue (mLECM-gel) as a 3D culture platform to investigate the role of tumoral EVs on the stiffness of PMN.

Materials and Methods: Murine lung tissue was decellularized (dECM) using detergents and DNase I, visualized by fluorescent and histological analysis, and lyophilized to ECM-rich powder. This powder was re-solubilized with pepsin and thermopolymerized (37°C) to create mLECM-gel hydrogel. Native lung and gel stiffness are currently being characterized by Young's modulus and dynamic oscillation. EVs were isolated from breast cancer cells (MDA-MB-231), quantified by NTA and analyzed by gene ontology.

Results: Gene ontology analysis revealed significant structural and remodeling elements of the ECM in MDA-MB-231 secreted EVs (FN1, FLNA). The dECM successfully showed a significant ($p<0.05$) reduction of DNA concentration in murine lung tissue (34.4 ng/µL), compared to a native lung tissue control (642.5 ng/µL). Moreover, collagen staining and fluorescent analyses also indicate cell removal. These results were consistent across both mice (C57BL/6) and rats (Sprague Dawley). As reference, native lung stiffness achieved values <3.5 kPa.

Discussion: This study introduces a potential biomimetic hydrogel derived from murine lung tissue that closely replicates the native lung ECM, offering a physiologically relevant model. Although further mechanical and biological characterization is needed, these promising results are crucial for advances in cancer metastasis research and to investigate how MDA-MB-231 EVs influence lung PMN formation and stiffness modulation.

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THERMOSENSITIVE SUPRAMOLECULAR SCAFFOLDS BASED ON INJECTABLE MICROGELS OF PNIPAM AND METHACRYLATED CHONDROITIN SULFATE FOR TISSUE ENGINEERING

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Introduction: Granular hydrogels (GH), composed of microgels, are injectable biomaterials that self-assemble into supramolecular scaffolds (SS). Poly(N-isopropylacrylamide) (pNIPAM) is widely studied for its thermosensitivity, forming SS via hydrophobic interactions above ~32 °C. However, this transition causes syneresis, releasing water and compromising cell viability. To overcome this issue, methacrylated chondroitin sulfate (CSMA) was incorporated as a crosslinker to modulate syneresis and enhance bioactivity.

Materials and Methods: pNIPAM microgels with 5–15wt% CSMA were synthesized by precipitation radical polymerization; pNIPAM having N', N'-methylenbisacrylamide (BIS) was used as control. The injectability of GH (10% w/v in PBS) was tested by compression. A rheometer was used to determine the gelation temperature (Tgel), the evolution of G′ and G″, SS stability, and shear-thinning behavior. UC-MSC viability was evaluated using PrestoBlue® and Live/Dead® assays.

Results: Injection forces of GH ranged from 12.2 to 13.8 N, and combined with the shear thinning flow, demonstrate its suitability for delivery. At 25 °C, G″ > G′ indicates the absence of coalescence, while at 37 °C, G′ > G″ confirms the development of an SS. CSMA reduced Tgel compared to BIS. Stable SS were obtained with 5–10% CSMA, with 10% pNIPAM-CSMA showing the highest UC-MSC viability.

Discussion: Injection force values and shear-thinning behavior align with manual delivery requirements. The thermal shift in G′/G″ reflected scaffold formation, while frequency sweeps supported structural stability under physiological conditions. CSMA incorporation lowered Tgel and reinforced G′, improving network integrity and hydration. These effects explain the enhanced cell viability in 10% CSMA scaffolds, whereas 15% CSMA likely created unfavorable microenvironments, such as local pH alterations, reducing compatibility.

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EFFECT OF SMALL EXTRACELLULAR VESICLES FROM PERIODONTITIS PATIENTS ON THE INFLAMMATORY PROFILE OF MENSTRUAL-DERIVED MESENCHYMAL STEM CELLS.

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Introduction: Extracellular vesicles (EVs) are nanosized particles released by human and bacterial cells. Among them, outer membrane vesicles (OMVs), derived from Gram-negative bacteria, carry immunogenic molecules and virulence factors capable of reaching distant tissues and modulating immune responses. Our previous proteomic analysis of periodontal EVs (PerioEVs), isolated from gingival crevicular fluid of pregnant women with periodontitis, revealed an inflammatory protein cargo profile and major bacterial virulence factors. Here, we evaluate the effects of PerioEVs, P. gingivalis OMVs (PgOMVs), and F. nucleatum OMVs (FnOMVs), key periodontal pathogens, on the inflammatory profile of menstrual mesenchymal stem cells (MenSCs), which play a central role in endometrial receptivity. Proinflammatory cytokines such as IL-6, IL-1β, TNF-α, and MCP-1 were analyzed to assess potential immune activation.

Materials and Methods: PerioEVs, PgOMVs, and FnOMVs were isolated and used to stimulate MenSCs for 24 h (n=2). EVs' uptake by MenSCs was assessed using confocal immunofluorescence microscopy. mRNA expression of IL-6, IL-1β, TNF-α, and MCP-1 were quantified by RT-qPCR. Experiments were performed using two independent MenSC lines and conducted in biological duplicate per condition.

Results: MenSCs successfully internalized PerioEVs and bacterial OMVs, as confirmed by immunofluorescence and confocal microscopy. IL-6 expression was significantly upregulated following stimulation with PerioEVs ($p < 0.05$) and PgOMVs ($p < 0.01$).

Discussion: PerioEVs and PgOMVs elicited a proinflammatory response in MenSCs. These findings support the hypothesis that periodontal-derived vesicles may function as long-range inflammatory messengers, potentially contributing to the link between oral inflammation and reproductive dysfunction. Further analyses are warranted in a higher sample size.

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ENVIRONMENTAL DETECTION OF VIABLE *HELICOBACTER PYLORI* IN SURFACE WATERS OF SOUTHERN CHILE: A POTENTIAL PUBLIC HEALTH RISK

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Helicobacter pylori is a World Health Organization (WHO) priority pathogen associated with chronic gastritis, peptic ulcer disease, and gastric cancer. Although person-to-person transmission is the main route, water has been proposed as a relevant environmental reservoir. However, the inherent limitations of culturing this microorganism hinder its reliable detection in such environments. In Chile, where gastric cancer remains one of the leading causes of cancer-related mortality, investigating the environmental persistence of *H. pylori* is critical to understanding its epidemiology and guiding preventive strategies. This study assessed the presence of viable *H. pylori* in surface waters from the lacustrine zone of the Araucanía Region. Thirteen samples were collected from rivers, lakes, and canals, with physicochemical parameters recorded and bacterial cells concentrated through filtration and enrichment. Molecular detection was performed by qPCR targeting the 16S and *UreC* genes, complemented with PMA-qPCR to discriminate between viable and non-viable cells. Results confirmed the presence of *H. pylori* DNA. In the urban area of Villarrica, all samples contained viable cells, indicating a latent health risk in a highly populated area. In surrounding rural areas, viable cells were detected in 3 of 4 sites. In Huincacara only non-viable cells were observed. In Pucón and Curarrehue, detection was restricted to non-viable states, with very low genetic loads and no evidence of cell viability. Overall, bacterial viability was primarily associated with temperatures above 10 °C and intermediate levels of organic matter. These findings confirm the environmental persistence of *H. pylori* in the Villarrica Lake basin and support the hypothesis of waterborne transmission as a contributing factor in the regional epidemiology of *H. pylori* infection. This study underscores the importance of integrated water quality surveillance and provides translational evidence linking environmental microbiology with clinical impact.

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IMPLICATIONS OF SIALYLTRANSFERASES EXPRESSION ON PROGNOSIS ACROSS CONSENSUS MOLECULAR SUBTYPES AND STAGES OF COLORECTAL CANCER

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Introduction: Colorectal cancer (CRC) is among the top causes of cancer-related deaths globally. Changes in sialylation driven by sialyltransferases (SiaTs), have been linked to tumor growth and unfavorable prognosis. In humans, twenty SiaTs are grouped into four distinct families based on their regioselectivity and the specificity of their acceptors. Despite this classification, there has been limited research on their connection to clinical and consensus molecular subtypes, as well as the mutation frequency within CRC. This study aimed to identify variations in the expression levels of SiaTs in CRC through analyses of databases and their molecular and clinical relevance.

Materials and Methods: Gene expression data sourced from TCGA were evaluated using UALCAN, Cbioportal and Kaplan–Meier survival analysis tools (KMPlotter). The expression patterns of 20 identified SiaTs were examined in relation to CRC CMS categories, mutation characteristics, stages, and survival plots. Statistical assessments involved log-rank tests to identify survival differences and ANOVA for comparing expressions.

Results: Out of the 20 SiaTs examined, ST3GAL1, ST3GAL6, and ST8SIA4 showed notable correlations with overall survival rates. Elevated expression was linked to reduced survival, especially within the CMS1 and CMS4 subgroups. Mutation levels were observed a maximum of 11% in the case of ST3GAL1. The prognostic impact was particularly evident in more advanced clinical stages and CMS1 and CMS4.

Discussion: The study underscores the potential of specific SiaTs as biomarkers for classifying CRC and predicting prognosis. The connection with adverse outcomes in immune-related CMS subtypes suggests their role in interactions between tumors and the immune system. These findings advocate for further functional validation and investigation of SiaTs as potential therapeutic targets.

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ROS/NF-KB–MEDIATED REGULATION OF LOX-1 BY PALMITATE CONNECTS LIPID METABOLISM WITH COLORECTAL CANCER PROGRESSION

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Colorectal cancer (CRC) progression is strongly influenced by modifiable risk factors such as obesity, which is associated with elevated circulating saturated fatty acids, including palmitate. The oxidized low-density lipoprotein receptor (LOX-1) is implicated in tumor progression and is regulated by palmitate in several cell types; however, its role in CRC remains poorly understood.

To investigate this, HCT116 and HT-29 cells were treated with palmitate and LOX-1 expression was quantified by RT-PCR. To explore the underlying mechanisms, we evaluated NF-κB activation, intracellular ROS production, and NADPH oxidase expression. Co- treatments with palmitate and oxLDL (LOX-1 ligand) were performed to evaluate epithelial- mesenchymal transition (EMT) markers. CRC patient tumor tissue samples were used to generate organoids.

Palmitate induced dose-dependent LOX-1 overexpression in HCT116 and HT-29 cells. This effect was associated with increased ROS and NOX expression, leading to NF-κB activation. Co-treatment with palmitate and oxLDL enhanced EMT marker expression, reflecting increased cancer progression. We established CRC organoids to validate our findings in a more physiologically relevant model in the future.

These findings suggest that palmitate, abundant in obesity, upregulates LOX-1 in CRC through a ROS/NF-κB–dependent mechanism, promoting cancer progression processes. The study establishes a mechanistic link between lipid metabolism and cancer cell signaling, highlighting LOX-1 as a potential translational target for therapeutic intervention in CRC patients.

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SIALYLTRANSFERASES EXPRESSION IS ASSOCIATED WITH IMMUNE INFILTRATION AND PREDICTS RESPONSE TO THERAPY IN COLORECTAL CANCER

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Introduction: The immune microenvironment of tumors plays a crucial role in colorectal cancer (CRC) outcomes and response to immunotherapy. Sialyltransferases (SiaTs) expression is associated to cancer cell surface hypersialylation in CRC, promoting immune evasion and poor prognosis. However, the link between SiaTs expression, immune microenvironment, and therapy response remains unclear. This research aims to explore the relationship between the expression of SiaTs, the tumor microenvironment, and the therapeutic response in colorectal cancer by combining gene expression data with bioinformatics tools.

Material and Methods: To explore the relationship between SiaTs and immune infiltration, TIMER2.0 and GEPIA2 were applied. ROCplot assessed links between SiaT expression and clinical response to immunotherapy or chemotherapy in CRC.

Results: The analysis of immune infiltration in COAD tumors demonstrated a strong association between SiaTs expression and specific immune subsets ($p < 1e-15$). ST6GAL1 was notably present in regulatory T cells and M2 macrophages, with moderate levels in resting NK cells. ST3GAL6 showed minimal expression in Tregs and NK cells but was significantly higher in M2 macrophages. High levels of SiaTs expression were linked with decreased infiltration of effector T cells and increased infiltration of immunosuppressive cells. Additionally, analysis with ROCplot indicated that high expression of these SiaTs was related to a poor response to several treatments.

Discussion: These results underscore the role of SiaTs in modulating the CRC immune landscape and their potential as predictive markers of immunotherapy response. Expression of ST6GAL1, ST3GAL6, and ST3GAL1 is associated with an immunosuppressive microenvironment characterized by higher M2 macrophage infiltration.

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MENSTRUAL FLOW AS A SOURCE OF ENDOMETRIAL EPITHELIAL ORGANOIDS IN ENDOMETRIOSIS RESEARCH

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Introduction: Endometriosis is a prevalent gynecological disorder associated with chronic pelvic pain, infertility, and impaired quality of life. Progress in understanding its pathophysiology has been limited by the scarcity of models that reproduce the endometrial microenvironment. Patient-derived organoids (PDOs) have recently emerged as powerful tools for modeling epithelial biology, enabling long-term culture and functional fidelity. Menstrual flow represents a minimally invasive, accessible source of endometrial cells with great potential for organoid establishment. While most studies focus on stromal cells, the epithelial compartment remains insufficiently explored.

Material and Methods: Two women of reproductive age (18–45 years), one healthy control and one laparoscopically confirmed endometriosis, were recruited (Ethics ID: CEC2023041, Universidad de Los Andes). Menstrual flow samples were digested with collagenase/dispase and cultured following Hewitt SC, *et al*. Organoids were characterized by immunofluorescence and confocal microscopy using EPCAM and phalloidin.

Results: Epithelial organoids were successfully established from two menstrual flows, one with endometriosis and one control. Organoids exhibited classical morphology with three-dimensional organization, central lumen, basal-out and apical-in polarization, and preserved epithelial features, confirming their structural and functional fidelity relative to native endometrium.

Discussion: This study provides proof-of-concept for generating endometrial epithelial organoids from menstrual flow in both conditions. This approach offers a novel method for comparative analyses of epithelial biology in endometriosis, supporting biomarker discovery and therapeutic testing. Menstrual flow as a cell source expands access to patient-derived material while offering an ethical, non-invasive, and scalable platform to advance research in this disease.

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EFFECT OF RESISTANCE TRAINING ON THE REVERSAL OF SARCOPENIA INDUCED BY CHRONIC CHOLESTATIC LIVER DISEASE

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Introduction: Sarcopenia, defined as the loss of muscle strength, mass, and physical function, is a major health concern, particularly when linked to chronic cholestatic liver disease (CCLD), as it predicts post-transplant mortality. No drugs are approved for this condition, although nutritional support and resistance training improve age-related sarcopenia. The impact of resistance training on CCLD-induced sarcopenia remains unclear. This study evaluated whether resistance training can reverse CCLD-induced sarcopenia in mice.

Materials and Methods: Male C57BL/6J mice (16 weeks) were fed standard chow or hepatotoxin-supplemented diets for 11 weeks. From week 3, animals were assigned to sedentary or trained groups. Muscle strength and physical performance were tested at weeks 0, 3, 8, and 11. At week 11, mice were euthanized to assess muscle mass, maximal force (by electrostimulation), and fiber diameter. Data were analyzed using two-way ANOVA with Tukey's post-hoc test (n=5–9).

Results: CCLD impaired strength and physical performance from week 3. By week 11, sedentary mice displayed significant reductions in maximal force, muscle mass, and fiber diameter. Resistance training reversed these effects, improving strength, function, maximal force, and preserving muscle mass and fiber morphology.

Discussion: Resistance training reversed CCLD-induced sarcopenia in mice. These findings support exercise as a potential therapeutic strategy to prevent muscle decline and improve outcomes in chronic liver disease.

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