



# **CEL-GEN 2026**

## **NOVEMBER 03 TO 05**

### **PRODIGY SANTOS DUMONT-RJ**

#### **CONGRESS OF THE BRAZILIAN ASSOCIATION OF CELL AND GENE THERAPY**

## **Book of Abstracts**

Brazilian Society of Cell and Gene Therapy (ABTCEL-GEN)  
November 3-5, 2026 — Prodigy Santos Dumont, Rio de Janeiro — RJ, Brazil

203 abstracts · 24 oral presentations · 179 posters

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# Oral Presentations

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## Day 1 · Viral Delivery

### Abstract 80 | Oral Presentation

#### **NEUROGENIN 2 OVEREXPRESSION IN CORTICOSPINAL NEURONS MODULATES EARLY NEURONAL AND MICROGLIAL RESPONSES AFTER SPINAL CORD INJURY**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Maria Carolina Silva

**Institution:** Universidade Federal de Minas Gerais — Belo Horizonte - Minas Gerais

**Introduction:** Traumatic spinal cord injury (SCI) disrupts descending pathways, including the corticospinal tract, and triggers neurodegenerative processes. Beyond the local spinal damage, axonal injury may induce retrograde changes in the cell bodies of corticospinal neurons located in the motor cortex. Because these neurons have limited regenerative capacity, strategies that promote neuroprotection, axon regeneration and modulate the cortical cellular environment are therapeutically relevant. Neurogenin 2 (NGN2) is a transcription factor involved in neuronal development and axonal growth, predominantly expressed during embryogenesis, and may therefore represent a molecular target for gene therapy. **Objective:** To test whether NGN2 overexpression in corticospinal neurons modulates early neuronal and glial responses in the motor cortex after SCI. **Methods:** Rats received stereotaxic injections of recombinant adeno-associated viral (rAAV) vectors expressing NGN2 (rAAV.NGN2) or a control vector (rAAV.CTRL) into the sensorimotor cortex. After viral transduction, animals underwent a dorsal SCI at T8. One week later, the animals were euthanized, and the transduced cortical region was processed for NeuN, Iba1, and GFAP immunofluorescence followed by confocal microscopy. NeuN-positive area, Iba1 and GFAP immunoreactivity, and microglial morphological parameters were quantified. **Results:** The rAAV.NGN2 group showed a larger NeuN-positive area than the rAAV.CTRL group, together with an increased minimum neuronal soma diameter, suggesting preservation of cortical neuronal morphology after injury. No differences were detected in neuronal circularity or maximum soma diameter. NGN2 overexpression also increased the Iba1-positive area, microglial skeletonization index, and total branch length, indicating microglial morphological remodeling. In contrast, no differences in GFAP immunoreactivity were observed between groups. **Conclusion:** NGN2 overexpression in corticospinal neurons induced changes consistent with neuronal preservation and microglial remodeling in the motor cortex during the early phase after SCI. Long-term studies are required to determine whether these effects translate into sustained neuroprotection and support the use of NGN2-based gene therapy for neural repair.

**Keywords:** spinal cord injury, neurogenin 2, gene therapy, neuroprotection, microglia.

## **Abstract 153 | Oral Presentation**

### **AAV-mediated overexpression of cannabinoid CB1 receptors preserves functional responses in a mouse model of retinitis pigmentosa: Perspectives for a nationally developed gene therapy in a rare disease**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** RAFAEL DE FREITAS AZEVEDO REPOSSI; ANNA CATHARINA MILAGRES MACHADO; CAIO MOTA MACHADO; GABRIELA SILVA ALMEIDA; HILDA PETRS SILVA; LUCIANNE FRAGEL MADEIRA; MARIANA SANTANA DIAS; VICTOR GUEDES DE ARAÚJO

**Institution:** Universidade Federal do Rio de Janeiro — Rio de Janeiro-RJ

Retinitis pigmentosa (RP) is the most prevalent form of inherited retinal degeneration. RP arises from mutations in genes essential for photoreceptor homeostasis, leading to progressive photoreceptor death and blindness (1). Current gene therapy approaches (e.g., Luxturna) target only RPE65 mutations, leaving ~99% of patients without therapeutic options (2). Results from our group show that pharmacological upregulation of cannabinoid signaling in a retinitis pigmentosa model provides transient protection to photoreceptors (3). Thus, our goal was to evaluate whether AAV-mediated cannabinoid CB1 receptor (CB1R) overexpression can slow photoreceptor degeneration due to the neuroprotective actions of cannabinoids. To test this, we designed plasmids for overexpression of WT-CB1 and DN22-CB1 (a mutant receptor lacking mitochondrial targeting). To validate expression and functionality of recombinant CB1R, HEK293 cells were transfected with WT-CB1 or DN22-CB1 using calcium phosphate. Transfection induced robust CB1 expression, confirmed by immunofluorescence (24 h post-transfection) and Western blotting (72 h). Confocal super-resolution imaging of HEK293 cells immunolabeled for CB1 and TOMM20 (an outer mitochondrial membrane protein) revealed that DN22-CB1 transfected cells lacked mitochondrial targeting, whereas WT-CB1 transfected cells showed CB1/TOMM20 co-localization. To ensure receptor functionality, we assayed the ratio of ERK1/2 phosphorylation. Agonist application (WIN55212-2) induced an increase in p-ERK in transfected cells, which was blocked by treatment with a selective CB1 receptor antagonist (SR141716A). With the plasmids validated, we proceeded with in house AAV-vector production in HEK293T cells, obtaining highly concentrated titers of  $1.44 \times 10^{12}$  vg/mL (WT-CB1) and  $1.22 \times 10^{12}$  vg/mL (DN22-CB1). HEK293 cells transduced with these AAVs produced robust receptor expression, as confirmed by immunofluorescence. AAV-vectors were then subretinally injected into rd10 mice and functional responses were analyzed. DN22-CB1 treated animals displayed preservation of electroretinographic response, with a 3.3-fold increase in b-wave amplitude ( $76.34 \pm 16.61$   $\mu$ V, N=8) compared to controls ( $22.83 \pm 5.43$   $\mu$ V, N=8). No significant difference was observed in WT-CB1 treated animals ( $37.22 \pm 10.78$   $\mu$ V, N=5). Visual acuity was determined using the optomotor test; both DN22-CB1 ( $0.31 \pm 0.06$  c/d, N=8) and WT-CB1 ( $0.29 \pm 0.03$  c/d, N=10) treated animals had preserved visual acuity compared to controls ( $0.19 \pm 0.02$  c/d, N=6). We have designed and produced AAVs for targeted CB1 expression in an animal model of RP. Our results show improved functional response in treated animals. Additionally, overexpression of CB1 in the mitochondria seems to limit the protective effect of CB1 overexpression as WT-CB1 failed to preserve electroretinographic responses, despite improving visual acuity. Next, we are evaluating cellular and morphological responses in the retina of treated animals.

**Keywords:** Retinitis Pigmentosa, Gene Therapy, Cannabinoids, Rare diseases

## Day 1 · IPSCs

### Abstract 37 | Oral Presentation

#### **Bioengineering a human lung-on-a-chip platform toward immune-informed preclinical modeling of pulmonary advanced therapies**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** ARTUR CHRISTIAN GARCIA DA SILVA

**Institution:** Universidade Federal de Goiás — Goiânia - GO

Pulmonary advanced therapy products, including cell-, gene-, and extracellular vesicle-based approaches, have gained increasing relevance for the treatment of respiratory diseases. However, their preclinical development is still limited by the lack of human-relevant models capable of reproducing the structural, cellular, and dynamic features of the pulmonary barrier. Conventional two-dimensional cultures fail to capture tissue-level responses, while animal models often poorly predict human airway physiology and immune-mediated reactions. In this context, lung-on-a-chip systems emerge as promising bioengineered platforms to model the epithelial-endothelial pulmonary interface under controlled dynamic conditions, supporting more predictive preclinical assessment of advanced therapeutic strategies. This study aimed to develop and characterize a human cell-based lung-on-a-chip model as a platform for future preclinical assessment of Advanced Therapy Medicinal Products. The model was constructed using human bronchial epithelial cells (BEAS-2B) and human endothelial cells (EA.hy926) cultured in transwell inserts for seven days under static and dynamic conditions. Tissue viability was assessed by Live/Dead assay, while structural organization and phenotype maintenance were evaluated by conventional and confocal fluorescence microscopy. Immunofluorescence staining was performed to detect MUC1 as a bronchial epithelial marker and CD31 as an endothelial marker. After seven days of culture, the bioengineered tissue remained viable under both static and dynamic conditions. Fluorescence microscopy confirmed the establishment of two organized cellular compartments within the transwell system, consistent with an epithelial-endothelial pulmonary interface. Both cell types maintained the expression of their respective phenotypic markers, with MUC1 detected in the bronchial epithelial compartment and CD31 in the endothelial compartment. Confocal imaging further supported the compartmentalized architecture of the model, indicating preservation of the bronchial and endothelial layers after dynamic culture. In parallel, HL-60 cells were successfully differentiated into neutrophil-like cells, as indicated by increased CD11b and CD18 expression and morphological changes compatible with granulocytic differentiation. The next step includes the addition of these leukocytes into the microfluidic channels to allow their recirculation and simulate inflammatory aspects of pulmonary diseases. The developed lung-on-a-chip remained viable and preserved epithelial and endothelial phenotypic markers under dynamic culture, supporting its relevance as a bioengineered model of the pulmonary interface. This platform may provide a human-relevant preclinical tool to model inflammatory aspects of pulmonary diseases and support future assessment of advanced therapy products for respiratory applications.

**Keywords:** lung-on-a-chip; pulmonary interface; preclinical model; advanced therapy products; respiratory inflammation; bioengineering.

## **Abstract 66 | Oral Presentation**

### **Development and Validation of a CAPN1 Knockout SH-SY5Y Cell Model via CRISPR-Cas9 for SPG76 Studies**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Melaine Maiara Da Silva Lopes

**Institution:** Universidade Federal do Rio Grande do Sul (UFRGS) — Porto Alegre (RS)

**Introduction:** Hereditary spastic paraplegias (HSP) comprise a genetically heterogeneous group of inherited motor neuron disorders characterized by the progressive degeneration of the corticospinal tract, leading to lower limb spasticity and locomotion impairment. To date, more than 90 genetic subtypes have been described, reflecting the molecular complexity of these conditions. Among these subtypes, HSP type 76 (SPG76) is caused by loss-of-function variants in the CAPN1 gene, which encodes the calcium-dependent cysteine protease calpain-1. This condition follows an autosomal recessive inheritance pattern and can present as either a pure or a complex phenotype, associated with cerebellar ataxia, dysarthria, peripheral neuropathy, and cognitive impairment. In Brazil, SPG76 is one of the most prevalent autosomal recessive HSPs. Currently, no disease-modifying therapy is available, and treatment remains symptomatic. Furthermore, the scarcity of in vitro cellular models limits the understanding of its pathophysiology and the initial screening of therapies based on adeno-associated virus (AAV) vectors. **Objective:** To develop and validate a CAPN1 knockout (KO) cellular model using the SH-SY5Y lineage via CRISPR-Cas9, aiming for its future application in efficacy and safety assays of the AAV9-CAPN1 vector. **Methods:** Wild-type human neuroblastoma SH-SY5Y cells were cultured in T25 flasks ( $1 \times 10^6$  cells/flask) in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Following expansion,  $2.5 \times 10^5$  cells underwent electroporation with a CRISPR-Cas9 ribonucleoprotein (RNP) complex targeting non-homologous end joining (NHEJ) repair. The edited population was expanded in 6-well plates until confluence, followed by genomic DNA extraction, PCR amplification, and purification for Sanger sequencing. The cleavage profile and efficiency were determined via digital deconvolution using the ICE (Inference of CRISPR Edits) tool. Subsequently, single-cell sorting was performed in 96-well plates to obtain purified clones. **Results:** Sequencing of the edited bulk population revealed an initial insertion/deletion (indel) rate of 73%. Following the cloning process and re-analysis using the ICE software, 5 homozygous clones were identified, showing an indel efficiency and Knockout Score (KO-Score) of up to 97%, with a high mathematical model fit ( $R^2 = 0.97$ ), confirming a frameshift mutation profile. **Conclusions:** The established protocol demonstrated high efficiency in generating CAPN1 knockout SH-SY5Y clones. As a subsequent step for model characterization for SPG76, Western blot analyses will be conducted to confirm the absence of the calpain-1 protein, validating the cell line for future therapeutic assays.

**Keywords:** AAV9-CAPN1; Hereditary Spastic Paraplegia Type 76; Gene Therapy; SH-SY5Y; Gene Editing;

## Day 1 · Bioengineering and Tissue Engineering

### Abstract 19 | Oral Presentation

#### EXPERIMENTAL EVOLUTION OF ZIKA VIRUS FOR ADAPTATION IN DIFFERENTIATED CELLS OF GLIOBLASTOMA TO ENHANCE ONCOLYTIC ACTIVITY

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Matheus Villanueva Andrade

**Institution:** Universidade Federal do Rio de Janeiro (UFRJ) — Rio de Janeiro

Glioblastoma (GBM) is the most common and aggressive malignant tumor of the central nervous system, characterized by high cellular heterogeneity, and resistance to current therapeutic approaches. Despite medical advances, the average patient survival remains below 15 months, highlighting the urgent need for new therapeutic strategies (1). Oncolytic viruses have emerged as promising candidates for their direct cytolytic activity and they may convert immunologically "cold" tumors into inflamed tumors that become susceptible to immune-mediated activity (2). In this context, Zika virus (ZIKV) has recently emerged as a promising oncolytic agent due to its natural tropism for GBM stem cells, but not for differentiated GBM cells (3). The present study aims to experimentally adapt ZIKV seeking to enhance the oncolytic activity of ZIKV in differentiated GBM cells while preserving GBM selectivity. Viral adaptation was performed through ten serial passages in the differentiated human glioblastoma cell line U-87 MG. The phenotypes of parental and adapted viruses were compared by LDH viability assays, plaque assays, and plaque morphology analysis after infection in U-87 MG. Other cell lines such differentiated GBM cell lines such as T98G, human brain microvascular endothelial cells (HBMEC) and the glioblastoma stem cell line TG1 were included to evaluate oncospecificity. To evaluate whether the adapted phenotype was maintained in vivo, Swiss mice bearing intracranial GBM were injected intracranially with either parental and adapted ZIKV. The infectious viral particles and viral RNA in brain tissues were quantified. Compared with the parental virus, the adapted ZIKV produced significantly larger plaques and exhibited enhanced cytotoxicity specifically in differentiated U-87 MG and T98G cells, while maintaining lower cytotoxicity in HBMEC and TG1 cells. These findings indicate that experimental evolution can generate ZIKV variants with enhanced tumor cell-specific fitness without compromising their selective tropism. In vivo, the adapted virus showed lower production of infectious viral particles in brain tissue than the parental strain. We propose that experimental evolution of ZIKV represents a promising platform not only for enhanced oncolytic virotherapy but also for future combination approaches aimed at immune reprogramming and cell-based immunotherapies in glioblastoma.

**Keywords:** Glioblastoma, Zika, Oncolytic Virus, Oncolytic Virotherapy

## Abstract 24 | Oral Presentation

### Air-Jet-Generated Alginate Microgels Enable Controlled and Functional Release of AAV Vectors for Gene Therapy Applications

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** LARISSA EMLYM ALVES PINTO; HILDA PETRS SILVA; ROBERTA SESSA STILHANO YAMAGUCHI

**Institution:** Faculdade de Ciências Médicas da Santa Casa de São Paulo — São Paulo - SP

Adeno-associated virus (AAV) is the most in vivo vector used in the gene therapy field due to their high transduction efficiency, robust transgene expression, and broad applicability. However, vector readministration remains a major translational limitation, as immune responses against viral components may compromise the efficacy of subsequent administrations. In this context, biomaterial-based delivery systems, such as alginate hydrogels, represent a promising strategy to protect viral vectors and modulate their release kinetics. This study aimed to standardize alginate microgels using the Air-Jet system for the encapsulation and controlled release of viral vectors. A total of 33 formulations were evaluated using low-molecular-weight alginate at different concentrations and oxidation degrees of 2% and 3%. Microgel degradation was monitored for 30 days in water, PBS, and DMEM by spectrophotometry at 250 nm, while morphological features were assessed by bright-field and fluorescence microscopy. Circularity and area were quantified using ImageJ. For AAV-GFP encapsulation, 2 uL of AAV-GFP suspension ( $1 \times 10^9$  TU/uL) was diluted in 70 uL of alginate solution prior to microgel production. After fabrication, the AAV-GFP-loaded microgels were added to HEK293T cells previously seeded in 24-well plates at a density of  $3 \times 10^4$  cells/well. Overall, no significant changes in microgel area were observed during the first four days across all formulations. After this period, microgels showed increased degradation in saline solution and greater stability in DMEM ( $p < 0.05$ ), findings that were corroborated by spectrophotometric analysis. The 1% (w/v), 3%-oxidized formulation showed the most pronounced size reduction over time, reaching complete degradation after 18 days in PBS. In DMEM, the 1.5% (w/v) formulations with 2% and 3% oxidation displayed the greatest reduction in size ( $p < 0.05$ ). Based on their degradation and stability profiles, the 1.5% (w/v) formulations with 2% and 3% oxidation were selected for viral vector release assays. AAV-GFP-loaded microgels were incubated with HEK-293T cells over time. After 24 hours of contact, functional vector release was observed, as evidenced by GFP expression. Transduction was assessed by fluorescence microscopy and flow cytometry, revealing an initial transduction rate of approximately 20%, followed by a linear reduction and stabilization at around 6% by day 7. Both formulations exhibited similar behavior, although the 1.5% (w/v), 3%-oxidized microgel demonstrated a higher release rate and transduction efficiency. These findings demonstrate the feasibility of producing alginate microgels using the Air-Jet system and support the successful standardization of a formulation capable of preserving viral vector functionality for at least seven days after release. Therefore, alginate microgels represent a promising biomaterial-based platform for the controlled delivery of viral vectors in gene therapy applications.

**Keywords:** Alginate microgels; AAV; Air-Jet; controlled release; viral vectors; gene therapy

## Day 2 · Novel tools in gene editing

### Abstract 107 | Oral Presentation

#### Sustained reactivation of fetal hemoglobin by adenine base editors in human hematopoietic stem cells for sickle cell disease

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** DANIELLE GONÇALVES DE CARVALHO; ARIANE BARROS DINIZ; BIANCA BONETTO MORENO GARCIA; CARLA MARTINS FRAGA DE MELO; DAVI COE TORRES; FLÁVIA PEIXOTO ALBUQUERQUE; GRACIELLE APARECIDA LIMA; KARINA GRIESI DE OLIVEIRA; KARINA TOZATTO MAIO; LIDIANE DE SOUZA TORRES; LUCAS ASSIS PEREIRA; MARIANA TEREZA DE LIRA BENICIO; MICHELLIN PEREIRA DE ALBUQUERQUE; PAULO ALFONSO SCHUOFF; PRISCILA KEIKO MATSUMOTO MARTIN; Ricardo Weinlich

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Sickle cell disease (SCD) is an inherited blood disorder caused by a point mutation in the beta-globin gene (HBB) that leads to the production of sickle hemoglobin S (HbS). Under deoxygenated conditions, HbS polymerizes, resulting in red blood cells sickling, hemolysis, vaso-occlusion, and chronic inflammation. Fetal hemoglobin (HbF) inhibits HbS polymerization, and its sustained expression is associated with improved clinical outcomes. Gene-editing strategies that reactivate HbF therefore represent a promising curative approach, offering an alternative to allogeneic hematopoietic stem cell transplantation, thus avoiding donor dependence and graft-versus-host disease. Here, we developed an ex vivo adenine base-editing (ABE) strategy to induce HbF in human CD34+ hematopoietic stem and progenitor cells (HSPCs) by recapitulating non-deletional hereditary persistence of fetal hemoglobin (HPFH). Candidate guide RNAs were screened in HSPCs and based on editing efficiency and HbF induction, two guides (HBG-123 and HBG-175) were selected for further evaluation. These guides were subsequently validated using ABE8e and ABE8e-NG, with ABEmax-NG evaluated for the HBG-175 strategy only. Electroporation of HSPCs resulted in high on-target A-to-G editing efficiencies. Edited HSPCs retained normal erythroid differentiation capacity, with viability and maturation comparable to mock controls, and exhibited robust and sustained HbF reactivation throughout a 21-day differentiation protocol. Both F-cell frequency and intracellular HbF levels increased progressively over time, peaking at day 21. Colony-forming assays demonstrated preserved clonogenic capacity, with comparable total colony numbers and lineage distributions between edited and mock cells, as well as maintenance of on-target edits in clonogenic progenitors. Importantly, experiments using CD34+ HSPC derived from patients with SCD showed similarly high on-target editing levels, preserved erythroid differentiation capacity, and robust HbF reactivation and reduced HbS levels. Notably, edited healthy HSPCs exhibited robust long-term repopulating capacity in vivo following primary and secondary transplantation into NBSGW mice. All three editing strategies resulted in 100% engraftment of primary recipients in the bone marrow, spleen, and peripheral blood, as well as 100% engraftment of secondary recipients in the bone marrow, with multilineage contribution. These results demonstrate preservation of long-term, self-renewing hematopoietic stem cell function following base editing. Collectively, these data establish three comparable adenine base-editing strategies that recapitulate non-deletional HPFH, enabling durable HbF reactivation in human HSPCs while preserving erythroid differentiation potential, clonogenic capacity, and long-term multilineage engraftment, both in healthy and SCD patients, further supporting the translational potential of these strategies as curative therapies for sickle cell disease.

**Keywords:** Sickle Cell Disease, HSCs, Gene Therapy, Base Editor, hemoglobinopathy

## Abstract 28 | Oral Presentation

### Gene Therapy with Adeno-Associated Viral Vector Serotype 9 in Cellular Models for Glutaric Acidemia Type I Developed via CRISPR/Cas9 Gene Editing

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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Glutaric Acidemia type 1 (GA-I) is an inborn error of metabolism caused by Glutaryl-CoA dehydrogenase deficiency, which is involved in the metabolism of L-lysine, L-hydroxylysine and L-tryptophan, resulting in the accumulation of glutaryl-CoA, glutaric acid and the 3-hydroxyglutaric acid, especially in the brain. The incidence of GA-I is estimated at 1:100,000 live births. GA-I is characterized by acute encephalopathic crises triggered by catabolic states (such as fever or infections), which culminate in acute striatal injury, associated with high morbidity and mortality, representing the primary consequence of the disease. In patients, different cell types are affected by the disease, such as neurons, astrocytes, and hepatocytes, each playing key roles in the chronology of symptoms. Thus, investigating these cell types may address historical gaps in the pathophysiology of GA-I and serve as a foundation for exploring new treatment alternatives. Therefore, we developed GA-I cellular models using CRISPR-Cas9 system editing via ribonucleoprotein complexes in the commercial cell lines SH-SY5Y, U-87 MG, and HEPG2, aiming for the knockout of the GCDH gene. The editing resulted in a varied number of clones; for each cell line, a knockout clone was selected to test for the presence of the GCDH enzyme via Western Blot (WB), analyse glutaric acid (GA) levels in the extracellular medium by GC/MS, and investigate oxidative stress markers such as SOD, GPx, GR, LDH, Lactate, GSH, DCF, and TBARS. All knockout clones, C2 for SH-SY5Y, C10 for U-87 MG, and E3 for HEPG2, showed an absence of the GCDH enzyme via WB, as well as elevated GA levels in the extracellular medium compared to Wild Type (WT) cells. Furthermore, the U-87 MG cell line demonstrated significant oxidative damage across all analysed markers. Based on these data, we tested the application of Adeno-Associated Viral Vector Serotype 9 (AAV9) with a specific capsid mutation to deliver the functional GCDH gene, aiming to restore the investigated markers to levels close to those of control cells (WT). The application of AAV9-hGCDH was able to restore the presence of the enzyme (via WB) in U-87 MG cells after 96 hours of exposure to the vector at a dose of  $1 \times 10^5$  vg/cell. Therefore, the construction of cellular models, the analysis and establishment of markers, and AAV-mediated gene therapy enabled an advanced functional genomics study and the evaluation of the AAV9 vector's in vitro delivery capacity, paving the way to establish a preclinical gene therapy protocol for Glutaric Acidemia type I in Brazil. Accordingly, this study consolidates the importance of gene-edited cellular models for rare genetic diseases, the validation of diagnostic techniques for marker detection, the reproduction of severe metabolic aspects presents in inborn errors of metabolism, and the search for new treatment alternatives, such as gene therapy.

**Keywords:** Glutaric Acidemia type I; Lysine; CRISPR/Cas9; Biochemical findings; AAV9; Gene Therapy.

## Day 2 · GMP Manufacturing

### Abstract 88 | Oral Presentation

#### Lentiviral Vector Platform based on Packaging Cell Line Engineering and GMP-Oriented Process Development

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Carlos Melo

**Institution:** Advanced Therapies Unit, Instituto Distrital de Ciencia Biotecnología e Innovación en Salud - IDCBIS — Bogotá D.C.

**Background:** Lentiviral vectors (LVs) are essential gene delivery tools for advanced cell and gene therapies, including CAR-T cell manufacturing. Their clinical implementation requires robust manufacturing platforms capable of consistently producing high-quality vectors under Good Manufacturing Practice (GMP) conditions. However, improving vector performance while establishing scalable, GMP-compliant manufacturing processes remains a challenge. **Methods:** To address these challenges, we developed an integrated LV manufacturing platform combining packaging cell line engineering with GMP-oriented process development. A HEK293-based packaging cell line was genetically engineered to stably express ProAssembly Factor (PAF), a proprietary protein engineered to enhance lentiviral particle assembly and increase vector titer. In parallel, an adherent upstream production process based on calcium phosphate-mediated transient transfection was optimized and scaled from 40 mL research-scale process to a 400 mL GMP manufacturing process using multilayer cell culture vessels. Downstream processing included clarification and tangential flow filtration (TFF)-based concentration. A comprehensive quality control panel was established, including residual host-cell DNA and protein quantification, sterility testing, adventitious agent detection, replication-competent lentivirus (RCL) testing, and vector genome titration by qPCR. **Results:** Stable PAF expression consistently increased functional LV titers across multiple vector constructs, although the magnitude of improvement was transgene-dependent. The optimized manufacturing platform achieved a tenfold increase in vector yield, from approximately  $2 \times 10^8$  to  $2 \times 10^9$  transducing units per production run following scale-up. The downstream workflow enabled reproducible vector recovery and implementation of GMP-compatible quality control assays. Vectors generated using this platform have been successfully employed in validation activities supporting CAR-T cell manufacturing. **Conclusions:** We describe an integrated manufacturing platform that combines host cell engineering with GMP-oriented process development to enhance lentiviral vector production. This strategy increases vector productivity while establishing a scalable manufacturing and quality control framework that supports the clinical translation of advanced cell and gene therapies. Furthermore, the development of a GMP-compatible lentiviral vector manufacturing platform represents an important step toward strengthening regional biomanufacturing capacity and technological independence for advanced cell and gene therapies in Latin America.

**Keywords:** Lentiviral Vector, Good Manufacturing Practice (GMP), Transduction, Titration, Upstream, Downstream, ProAssembly Factor (PAF)

## Abstract 76 | Oral Presentation

### Establishing GMP-Compliant CAR-T Cell Manufacturing at INCA: Infrastructure Adaptation and Manufacturing Readiness for an Upcoming Pediatric Clinical Trial in Brazil

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Daniela Tupy de Godoy

**Institution:** Laboratório de Imunoterapia Celular e Gênica, Instituto Nacional de Câncer, Rio de Janeiro, Brazil. — Rio de Janeiro-RJ

**Background:** Local manufacturing is essential to expand access to chimeric antigen receptor T-cell (CAR-T) therapy within the Brazilian Unified Health System. Through a technology transfer from the Children's Hospital of Philadelphia (CHOP), the Brazilian National Cancer Institute (INCA) is implementing the local manufacture of autologous CD19-directed CAR-T cells for a planned clinical trial in pediatric patients with relapsed or refractory B-cell acute lymphoblastic leukemia. We describe an integrated clinical-readiness strategy encompassing manufacturing training, product quality assessment, and adaptation of INCA's infrastructure to Brazilian regulatory requirements for Advanced Therapy Medicinal Products (ATMP). **Methods:** Two manufacturing training runs were conducted using leukapheresis-derived peripheral blood mononuclear cells and the closed, semi-automated CliniMACS Prodigy platform. T cells were enriched, activated, lentivirally transduced with an anti-CD19 CAR, expanded, harvested, and cryopreserved. Final products were evaluated against predefined acceptance criteria for cell identity, viability, transduction efficiency, residual B cells, sterility, endotoxin, mycoplasma, vector copy number (VCN), and replication-competent lentivirus (RCL). In parallel, a dedicated manufacturing area was designed, incorporating Grade B and C environments, a bubble airlock, dedicated HVAC with HEPA filtration and pressure control, segregated flows, and a quality management system. **Results:** Both training runs met all evaluated acceptance criteria. Viability was 91% and 95%, CD3+ cells represented 88% and 89%, and CAR expression was 37% and 27% in rounds 1 and 2, respectively. Residual CD19+ and CD20+ cells ranged from 0.00% to 0.01%. Endotoxin was <0.250 EU/mL in both runs; sterility showed no growth at day 14; and mycoplasma was not detected. VCN was 2.6 and 2.0 copies per transduced cell, and RCL was not detected. The first run yielded  $1.8 \times 10^9$  total cells after six days of expansion. In addition, the infrastructure adaptation project was approved by the Brazilian Health Regulatory Agency (Anvisa), supporting the institutional pathway toward compliant manufacturing of investigational ATMP. **Conclusion:** Reproducible manufacturing performance across two training runs, combined with product quality assessment, regulatory planning, and infrastructure adaptation, demonstrates INCA's progress toward integrated clinical readiness for local CAR-T-cell production. These activities support the planned initiation of the clinical study in the first half of 2027 and represent an important step toward expanding access to advanced cell therapies in Brazil.

**Keywords:** Advanced Therapy Medicinal Products, chimeric antigen receptor T-cell (CAR-T), Good Manufacturing Practices (GMP)

## Day 2 · New Cell Therapies: CAR-M, CAR-TREG

### Abstract 14 | Oral Presentation

#### A Lentiviral EDV Platform for simultaneous CAR Engineering and CRISPR Gene Editing in Primary T and NK Cells

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Lays Adrienne Mendonça Trajano Silva; Davi Coe Torres; Ingrid Ferreira de Souza; Jamile Ramos da Silva; Julia Carnaz Benincasa; Larissa Cardoso Zanetti; Lucila Nassif Kerbauy; Nelson Hamerschlag; RICARDO WEINLICH; Thiago Giove Mitsugi

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Efficient and safe delivery of CRISPR-Cas9 components remains a major challenge for next-generation cell therapies. Although viral and non-viral delivery methods have enabled genome editing, limitations related to manufacturing complexity, cellular fitness, and multi-step engineering workflows remain. Enveloped Delivery Vehicles (EDVs) have emerged as a promising platform for transient CRISPR-Cas9 delivery, enabling efficient genome engineering while preserving cell viability and functionality. Here, we optimized a lentiviral-based EDV platform for simultaneous CAR engineering and CRISPR-mediated gene editing in primary T and NK cells. EDVs were generated using different formulations containing varying ratios of Gag-Pol- and Gag-Cas9-encoding plasmids together with envelope glycoproteins (VSV-G or BaEV), a BCMA-IL15 CAR-encoding plasmid, and an sgRNA targeting B2M. Multiple formulations were systematically evaluated to optimize both CAR transduction and gene-editing efficiency. In primary human T cells, the optimized formulation achieved approximately 25% CAR expression together with ~27% B2M knockout, demonstrating the feasibility of simultaneous CAR engineering and genome editing using a single EDV platform, without any impact on expansion rate. Further optimization to improve both CAR transduction and gene-editing efficiency is ongoing. In cord blood-derived NK (CB-NK) cells, optimized EDVs achieved 44.3% CAR expression and 58.2% B2M knockout (n=2) while maintaining expansion comparable to non-transduced controls. Collectively, these findings demonstrate that EDVs provide a versatile one-step platform for simultaneous CAR engineering and CRISPR-mediated genome editing across both adaptive and innate immune cells. By combining efficient CAR engineering with transient CRISPR-Cas9 delivery while preserving cellular expansion, this strategy has the potential to simplify manufacturing, reduce process-associated cellular stress, expand the versatility of genome engineering, decrease manufacturing costs, and facilitate the development of next-generation gene-edited CAR-T and CAR-NK therapies.

**Keywords:** Cell Engineering; Enveloped Delivery Vehicles; Cell Therapy

## Abstract 79 | Oral Presentation

### Cell therapy targeting fungal pathogens: optimization of Mannan-Specific Chimeric Antigen Receptor (M-CAR)

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Julia Garcia Guimarães 1

**Institution:** 1 São Paulo State University (UNESP), Araraquara, São Paulo, Brazil; 2 University of São Paulo, Ribeirão Preto, São Paulo, Brazil — Araraquara-SP

Invasive fungal infections (IFIs) are a global health threat associated with high morbidity and mortality. *Candida albicans* is a major IFI, and non-*albicans* *Candida* species rise as public health concern worldwide. Additionally, *Rhizopus oryzae* is the most common cause of mucormycosis, a severe and often life-threatening IFI. Host immune response against IFIs depends on effector T cells, and most of the patients affected by candidiasis shown T-cell activity compromised. To address this, chimeric antigen receptor (CAR) technology presents a promising therapeutic strategy to engineer T cells, redirecting these modified cells to target fungal species. Our group developed a novel CAR, designated M-CAR, capable of inducing T-cell activation upon recognizing different *Candida* species and *R. oryzae*. The antigen-recognition domain of M-CAR consists of a single-chain variable fragment (scFv) specific to  $\alpha$ -1,6 mannose backbone found in the fungal cell wall. M-CAR is composed of an N-terminal FLAG-tag, a CD8 hinge/transmembrane region, and a CD3 $\zeta$  signaling domain. The original M-CAR construct contains CD137 molecule as costimulatory domain. The current work, both second- and third-generation M-CARs variants were generated to identify costimulatory domains capable of enhancing target-specific T-cell activation. Second-generation M-CAR variants were generated by incorporating either CD28 or iCOS as costimulatory domains. Moreover, two third-generation M-CAR variants composed of iCOS plus CD137 or iCOS plus CD28, both linked with CD3 $\zeta$  were developed. The M-CAR constructs were generated using Gibson Assembly. Lentiviral particles were produced via HEK 293T cell transfection and used to transduce both Jurkat cell line and primary human T cells (PBMCs). M-CAR+ cells were subsequently co-cultured with soluble mannan (*S. cerevisiae*), various *Candida* species/forms, or *R. oryzae* spores. All M-CAR variants were efficiently expressed on the surface of Jurkat cells and primary human T cell. Co-culture of modified Jurkat cells with mannan, heat-killed *C. albicans* and *C. tropicalis* hyphae, *C. parapsilosis* pseudohyphae, *C. glabrata* yeast, and *R. oryzae* spores up-regulated CD69 and increased IL-2 production. While M-CAR variants incorporating iCOS, CD28, iCOS-CD28, or iCOS-CD137 costimulatory domains induced the Jurkat cell activation, the M-CAR containing the CD137 domain alone induced the strongest T cell activation. Primary T cells were successfully modified with second-generation constructs, and preliminary data demonstrate high levels of IFN- $\gamma$  production in response to *C. albicans*. These data pave the way to evaluate the direct fungicidal activity of human T cells expressing M-CAR variants against the fungal species. Funding: FAPESP 2024/00060-8, FAPESP 2018/18538-0, CAPES - Finance Code 001

**Keywords:** Cell Therapy, *Candida*, Chimeric Antigen Receptor (CAR)

## Day 2 · RNA/DNA based therapeutic strategies

### Abstract 42 | Oral Presentation

#### **Isoform-resolved lncRNA remodeling as a secondary readout of SCN2A dosage and CRISPRa-mediated activation in human iPSC-derived cortical excitatory neurons**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Adne Vitória Rocha de Lima

**Institution:** Instituto Gonçalo Moniz - Fiocruz, Bahia — Salvador, Bahia

SCN2A haploinsufficiency is a leading monogenic cause of autism spectrum disorder and developmental epileptic encephalopathy, and restoring endogenous gene dosage is an emerging therapeutic strategy. CRISPR activation (CRISPRa) can up-regulate a haploinsufficient allele from its native promoter (Matharu et al., Science 2019), including at SCN2A (Tamura et al., Nature 2025; and our unpublished thesis data). Beyond the coding response, long non-coding RNAs (lncRNAs) act on chromatin state and on transcriptional and post-transcriptional processing during neuronal differentiation, and lncRNAs at neurodevelopmental loci such as CASC15 (6p22.3) shape neural differentiation in an isoform-specific manner (Mondal et al., 2018; Russell et al., 2015). Whether an endogenous-dosage CRISPRa therapy leaves a discrete, interpretable RNA-regulatory footprint remains unexplored. Building on our SCN2A haploinsufficiency model (group preprint), we asked whether lncRNA isoforms provide secondary regulatory readouts of the haploinsufficient state and of CRISPRa-mediated SCN2A activation. We reanalyzed transcript-level RNA-seq (GENCODE v49; Salmon-Terminus-swish) from donor-derived hiPSC-NGN2 cortical excitatory neurons, comparing SCN2A haploinsufficient versus control-donor neurons and, within the haploinsufficient background, CRISPRa-treated versus untreated cultures at 2 and 4 weeks. We quantified differential lncRNA transcripts, named versus unannotated entries, multi-isoform loci, and coexpression between differentially expressed lncRNAs and a curated SCN2A/excitability gene panel (Spearman; absolute  $\rho > 0.8$ ,  $p < 0.01$ ; candidate associations, not direct regulation). Haploinsufficient neurons showed broad lncRNA remodeling (803 and 818 differential lncRNA transcripts at 2 and 4 weeks; a substantial fraction unannotated). By contrast, direct CRISPRa-versus-untreated comparisons produced far fewer transcripts (14 and 16), mostly up-regulated and non-overlapping between the two time points. After deduplication, coexpression yielded 307 unique lncRNA-coding associations across 120 lncRNA genes and seven panel genes; the strongest candidate hubs were the potassium-channel genes KCNC1 and KCNJ6, whereas SCN2A itself had a single lncRNA partner. At the locus level, CASC15 was the only directly CRISPRa-responsive lncRNA also present in the coexpression network; through a distinct isoform, it linked the treatment-responsive signature to a KCNJ6-associated module. These exploratory data support a two-layer model: SCN2A haploinsufficient neurons carry a broad lncRNA dysregulation architecture, whereas endogenous CRISPRa activation is associated with a small, time-dependent lncRNA response, specific at the isoform level, that touches that architecture at a single candidate entry point (CASC15). Isoform-resolved lncRNA signals nominate candidate excitability-associated modules as secondary readouts of SCN2A dosage, now being prioritized for isoform-specific validation.

**Keywords:** lncRNA isoform; SCN2A; ASD; Crispra

## Abstract 34 | Oral Presentation

### From cells to patients: gene therapy with p14ARF and interferon- $\beta$ reveals antitumor and immunomodulatory mechanisms in canine neoplasms

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** JEAN CARLOS DOS SANTOS DA LUZ; Bethânia Almeida Gouveia; Bianca Naomi Niitsuma; BRYAN ERIC STRAUSS; Fernanda Antunes; Gissele Rolemberg Oliveira Silva; Nathalia Leal Dovaizem

**Institution:** Instituto do Câncer dos Estado de São Paulo - Universidade de São Paulo — São Paulo - SP

**Introduction:** Previous studies by the group demonstrated, in murine, human, and canine cell lines treated with species-specific adenoviral vectors encoding p14ARF and interferon- $\beta$  (IFN $\beta$ ), induction of immunogenic cell death, DAMP release, and control of tumor growth in vivo in mice (1-4). Based on these results, this project extended the investigation to more complex in vitro models and to veterinary clinical application. **Objective:** To evaluate the antitumor and immunomodulatory effects of the AdRGD-PG-cp14ARF and AdRGD-PG-cIFN $\beta$  vectors in 2D and 3D models of canine cell lines and in patients with spontaneous oral melanoma. **Methods:** The D17 (osteosarcoma) and TLM-1 (oral melanoma) cell lines were transduced with the vectors alone or in combination (MOI 25). Cell cycle was assessed by flow cytometry (72h), 3D spheroid volumetry (D17, days 0-10), and relative gene expression (MDM2, p21, IFN $\beta$ , MHC-I, CXCL10) by RT-qPCR. In the clinical study, three dogs with spontaneous oral melanoma (wild-type TP53 confirmed by sequencing) received intratumoral applications of the vectors ( $5 \times 10^9$ - $1 \times 10^{10}$  TU, two doses, 7-day interval), with sample collection pre- and post-treatment for immunohistochemistry and RT-qPCR. **Results:** In vitro, IFN $\beta$ , alone or combined with ARF, induced G1-phase cell cycle arrest with a reduction in the S-phase fraction in both cell lines, without a significant increase in sub-G1, indicating a predominantly cytostatic mechanism at 72h post-transduction. ARF alone had a more modest effect on the cell cycle. RT-qPCR revealed modulation of the MDM2/p21 axis by the ARF vector, and upregulation of MHC-I and CXCL10 in the IFN $\beta$  and ARF+IFN $\beta$  groups, suggesting activation of antigen presentation pathways and lymphocyte recruitment. In D17 3D spheroids, ARF+IFN $\beta$  produced the greatest volumetric reduction (~57% relative to control), with progressive structural disintegration. In the clinical study, all three patients showed increased tumor-infiltrating lymphocytes: patients 1 and 3 with elevated CD45; patients 1 and 2 with increased CD3; patient 3 with a marked increase in granzyme B and MHC II. Tumor RT-qPCR revealed activation of p21 and TLR3 (via the p53/p14ARF pathway) in patient 2, and of IRF1 and CD47 (via the IFN pathway) in patient 3. Neutralizing antibodies were induced post-treatment, with no hematological adverse events. **Conclusion:** Cell cycle modulation, activation of immunostimulatory genes in vitro, and lymphocytic infiltration in treated patients converge to demonstrate the translational potential of this gene therapy, positioning canine oral melanoma as a relevant clinical model for comparative oncology (5-7).

**Keywords:** Immunotherapy Melanoma Viral Vectors Comparative Oncology Dogs Canine

## Day 2 · Non-viral delivery

### Abstract 170 | Oral Presentation

#### Optimization and Preclinical Validation of an mRNA-LNP Vaccine Candidate Against Chikungunya Virus

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Flávia Alves França

**Institution:** Universidade Federal de Minas Gerais — Belo Horizonte-MG

**INTRODUCTION:** Chikungunya virus (CHIKV) is an arbovirus with major health and economic impact due to its potential for chronic disease<sup>1</sup>. As there is no commercialized vaccine for CHIKV in Brazil, its development is a national priority. Nucleic acid vaccines attracted attention for safety and rapid production<sup>2</sup>. Among these, mRNA vaccines promote faster immune responses than conventional platforms<sup>3</sup>. However, due to the instability and its delivery challenges, lipid nanoparticles (LNPs) emerged as a strategy to protect mRNA and enhance delivery<sup>4</sup>. Here, we present an mRNA vaccine against CHIKV using LNPs. **METHODOLOGY:** Three LNPs encapsulating luc-mRNA were synthesized by microfluidic and characterized for size, polydispersity index (PDI), and zeta potential by DLS. In vitro transfection was performed in JAWSII cells to determine a dose-response curve and assess viability. In vivo transfection was assessed in C57BL/6 mice by i.m injection and biodistribution was monitored at various timepoints by IVIS. The best LNP was used to encapsulate mRNA encoding CHIKV proteins (LNP-CHIKV). Antigen production was evaluated in HEK293T cells. For in vivo studies, mice received a prime-boost vaccination with mRNA doses of 1, 5 and 10 µg per animal, followed by CHIKV challenge 15 days post-boost. Outcomes including weight, acute and chronic pain, clinical score, paw edema, viral load and humoral response were measured. **RESULTS:** All LNPs exhibited near-neutral zeta potential, a size suitable for intramuscular administration and a PDI indicating monodispersity. LNP-Vac was selected for in vivo experiments due to higher transfection efficiency. LNP-CHIKV induced antigen expression in HEK293T cells. In vivo, a dose-dependent response occurred across the tested mRNA doses, with higher doses improving immunogenicity. Mice receiving 10 µg showed pain, clinical scores, body weight, paw edema and ankle viral load comparable to mock controls at 7 dpi, with reduced viremia at 3 dpi. Neutralizing antibodies were detected, indicating a robust humoral response. Ninety days post-challenge, only animals vaccinated with 10 µg of mRNA showed a non-significant difference compared to the mock group in the adhesive removal test, indicating that this dose of the vaccine also provides protection against the chronic phase of the disease. **CONCLUSION:** An optimized LNP was selected for vaccine delivery and showed protection and immunogenicity against acute and chronic CHIKV. These findings highlights the potential and relevance of LNP-CHIKV in accelerating vaccine development for emerging viruses.

**Keywords:** LNP; Vaccine; mRNA delivery

## Abstract 57 | Oral Presentation

### Scaling up the non-viral production of CD19 CAR-T cells using a minicircle-based Sleeping Beauty transposon system.

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Arthur da Silva Neumann; DANIELA TUPY DE GODOY MAZZA; EDUARDO MANNARINO CORREIA; EMMANUEL ARTHUR ALBUQUERQUE ARAGAO; Leonardo Ribeiro Batista Silva; LÍVIA PIMENTEL DE SANT'ANA DOURADO; LUIZA DE MACEDO ABDO; MARTÍN BONAMINO; SABRINA ALVES DOS REIS

**Institution:** Instituto Nacional do Câncer — Rio de Janeiro

**INTRODUCTION:** Chimeric Antigen Receptor T-cell (CAR-T) therapy targeting CD19 has demonstrated remarkable clinical efficacy in patients with B-cell malignancies. However, conventional CAR-T manufacturing relies on viral vectors, making the production process complex, costly, and challenging to scale. To address these limitations, our group developed a non-viral platform based on the Sleeping Beauty (SB) transposon system using minicircle (MC) DNA vectors to generate anti-CD19 CAR-T cells. MCs are simplified DNA vectors produced in engineered bacteria expressing an inducible recombinase, resulting in DNA molecules that contain only the gene of interest and lack bacterial backbone sequences, thereby improving the safety and efficiency of gene delivery. **OBJECTIVE:** To scale up the production of anti-CD19 CAR-T cells using the Sleeping Beauty transposon system delivered by minicircle DNA vectors. **METHODS:** T lymphocytes isolated from healthy donors were activated with anti-CD3 beads, nucleofected with the PT4-19BBz minicircle vector, and expanded in G-Rex culture devices. Later, the CAR-T cells were infused into animal models at different dose levels to evaluate their antitumor efficacy. **RESULTS:** Starting from an initial population of  $5 \times 10^7$  T lymphocytes, we generated approximately  $1.0 \times 10^8$  CAR-positive cells after 12 days of culture in both experiments. MC-derived CAR-T cells showed high viability (94.7% in Fig. 1 and 96.8% in Fig. 2) and consistent CAR expression (21.3% in Fig. 1 and 22.5% in Fig. 2). Furthermore, in vivo study demonstrated that MC-derived CAR-T cells exhibited an efficient dose-dependent antitumor activity. **CONCLUSION:** These findings demonstrate that the Sleeping Beauty minicircle-based platform represents a safe, efficient, and cost-effective approach for CAR-T cell manufacturing, with strong potential for scalable production and point-of-care clinical applications.

**Keywords:** CAR-T; leukemia; immunotherapy; minicircle

## Day 3 · New strategies on CAR-T

### Abstract 142 | Oral Presentation

#### Phage display selection of scFvs specific for the HER2 S310F mutation from rationally designed libraries based on pertuzumab

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

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**Introduction:** HER2-positive gastric cancer is treated with anti-HER2 antibodies such as trastuzumab and pertuzumab; however, mutations in the extracellular domain of the receptor may reduce therapeutic efficacy. The S310F mutation is the most frequent activating alteration in this region and the second most common hotspot in gastric cancer, occurring in approximately 7% of patients. The substitution of serine by phenylalanine alters the epitope conformation, sterically preventing pertuzumab binding while preserving trastuzumab recognition. The mutation also promotes HER2 heterodimerization with EGFR, sustaining oncogenic signaling despite HER2 blockade. Given the reduced efficacy of pertuzumab and the absence of cellular therapies for gastric cancer, a CAR-T directed against mutant HER2 represents an innovative strategy with high tumor specificity and minimal cross-reactivity with wild-type HER2. **Objective:** To select, by phage display, single-chain variable fragments (scFvs) specifically recognizing the mutant ERBB2 S310F peptide from libraries rationally designed based on pertuzumab. **Methods:** Based on structural mapping of the pertuzumab-HER2 interface, four residues within the pertuzumab VH domain (CDR1 and CDR2) contacting Ser310 were randomized, generating a theoretical diversity of  $19^4$  (~130,000 variants). Two libraries were constructed, differing only in the terminal CDR3 residue: one retaining tyrosine (TYR) and the other containing alanine (ALA). Biopanning was performed against a biotinylated mutant peptide immobilized on streptavidin-coated plates with increasing washing stringency (5, 10, and 15 washes in rounds 1–3). In the third round, competitive negative selection with soluble wild-type peptide eliminated wild-type binders, recovering only phages bound to the immobilized mutant peptide. **Results:** Input/output analysis demonstrated progressive enrichment of phage populations throughout selection, indicating depletion of nonspecific clones and accumulation of high-affinity mutant binders. The ALA library achieved ~100,000-fold enrichment and the TYR library ~300,000-fold enrichment from the first to the third round. A further reduction after competitive negative selection confirmed efficient discrimination between mutant and wild-type epitopes, consistent with the steric hindrance previously described for pertuzumab-derived antibodies against the S310F mutation. **Conclusion:** Mutation-specific scFv selection by phage display from rationally designed pertuzumab-based libraries proved feasible and efficient. The enriched sequences will be used to construct a chimeric antigen receptor (CAR) expected to specifically recognize mutant HER2 (S310F) while sparing wild-type HER2, establishing an innovative and potentially scalable platform for targeting oncogenic point mutations in solid tumors.

**Keywords:** HER2-positive gastric cancer; HER2 S310F mutation; Pertuzumab; Phage display; CAR-T cell therapy.

## Abstract 6 | Oral Presentation

### Engineering CAR-T Cells to Enhance Tumor Infiltration in Solid Tumors

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** ELOAH RABELLO SUAREZ; Fernando Luiz Affonso Fonseca; Najla Santos Pacheco de Campos

**Institution:** Universidade Federal do ABC/ Universidade Federal da São Paulo/ Centro Universitário FMABC — Santo André-SP

**Introduction.** Despite the clinical effectiveness of chimeric antigen receptor T cells (CAR T cells) in the treatment of some hematological tumors, their efficiency against solid tumors remains limited by critical barriers, including inefficient migration and infiltration within the tumor site<sup>1</sup>. Clear cell renal carcinoma (ccRCC) and other hypoxic solid tumors overexpress carbonic anhydrase IX (CAIX), making this enzyme a strategic target for the development of cellular therapies, particularly given its restricted physiological expression<sup>2</sup>. In this project, we developed and validated strategies to enhance the migration of anti-CAIX CAR-T cells co-expressing cell migration modulators (CMMs) for treating ccRCC, using a polycistronic lentiviral vector. **Methods.** Lentiviruses were produced by transient transfection, concentrated, titrated, and used to transduce CD4:CD8 T cells at a 2:1 ratio. T cells were purified from the peripheral blood mononuclear cell fraction of healthy donors. The resulting CAR-T cells products were immunophenotyped and assessed for proliferative potential and transduction efficiency. Anti-CAIX CAR-T cells co-expressing CMMs were evaluated for migratory capacity using transwell plates coated with endothelial cells and their cytotoxicity against luciferase-positive ccRCC cells was determined by measuring luciferase activity. These CAR T cells were also tested in orthotopic NSG-SGM3 mouse models implanted with human ccRCC cells. The number, activation status, and exhaustion profile of tumor-infiltrated T cells were subsequently assessed. **Results and Discussion.** The CAR-T cells showed high proliferation and transduction rates. CMMs co-expression was validated both in vitro and in vivo. One specific variant, protected by patent secrecy (CMM A), significantly increased CAR-T cell infiltration into the tumor microenvironment (TME) and enhanced antitumor efficacy in orthotopic models. This produced statistically significant reductions of 60% in emitted bioluminescence and 85% in tumor weight after 21 days of treatment. The data suggest attenuation of terminal exhaustion among tumor-infiltrated T cells, with a relevant fraction maintaining a central memory phenotype, consistent with greater persistence potential. In contrast, the effects observed with variant CMM B were less pronounced and did not persist over prolonged periods. Safety profiles were supported by appropriate levels of serum markers of hepatic and renal function. **Conclusions.** Co-expression of T cell migration modulators represents a promising strategy to overcome the physical and biological barriers imposed by solid tumors. This approach may be incorporated into CAR vectors targeting different targets expressed by solid tumors, providing a relevant foundation for the development of more effective next-generation cellular therapies against aggressive solid malignancies. CEP/UNIFESP CAAE: 84533524.0.0000.5505/ CEUA/UFABC: 4272191124. Supported by FAPESP: 2023/03631-3.

**Keywords:** Chimeric antigen receptor T cells; T cell migration; solid tumors; renal cell cancer

## Day 3 · In vivo gene therapy

### Abstract 17 | Oral Presentation

#### **Construction and evaluation of an HSV-1-based oncolytic virus expressing the pro-apoptotic protein sfTRAIL, for the treatment of head and neck tumors.**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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Head and neck squamous cell carcinoma (HNSCC) comprises a group of malignant tumors arising from the squamous epithelial cells lining the mucosa of the upper aerodigestive tract. Globally, more than 900,000 new cases are diagnosed each year, making HNSCC one of the most prevalent malignancies worldwide. Despite advances in the molecular and clinical understanding of these tumors, the five-year overall survival rate remains between 35% and 70%, underscoring the need for novel therapeutic strategies. This study aimed to generate oncolytic viruses derived from herpes simplex virus type 1 (HSV-1) expressing the soluble form of the tumor necrosis factor-related apoptosis-inducing ligand (sfTRAIL). Viral genetic engineering was performed through homologous recombination in bacterial systems. The sfTRAIL gene was inserted in place of the US3 gene to promote viral attenuation, using a wild-type HSV-1 genome (strain 17+) maintained in *Escherichia coli* SW102. Additionally, sfTRAIL was inserted into the UL55-UL56 intergenic region of an attenuated genome harboring the same gene deletions as T-VEC using the bacterial strain GS1783. Recombinant clones were validated by restriction enzyme digestion, PCR, and Sanger sequencing. Viral transfection and amplification were carried out in Vero cells, and viral titers were determined by plaque assay. Plaque diameters were measured using Feret's diameter analysis in images obtained by light microscopy and processed with ImageJ software. sfTRAIL expression was confirmed by Western blotting, while cell viability was assessed in HNSCC cell lines (FaDu, UD-SCC-2, VU-SCC-120, and VU-SCC-147) using the MTS assay at different multiplicities of infection (MOIs) (0.01, 0.1, and 1.0). Recombinant viruses expressing sfTRAIL produced significantly larger plaques compared with their parental viruses (HSV-1 TRAIL BR: 320.75% increase relative to HSV-1 WT; HSV-1 TRAIL IT: 137.27% relative to HSV-1 AC). Additionally, genomes attenuated by deletion of the US3 gene generated plaques with larger diameters. Overall, all oncolytic viruses reduced cell viability in the tested cell lines, with increased sensitivity observed in UD-SCC-2, VU-SCC-120, and VU-SCC-147 cells infected with sfTRAIL-expressing viruses. These findings suggest that sfTRAIL expression may enhance the oncolytic activity of HSV-1, highlighting the potential of this strategy as a promising therapeutic approach for the treatment of HNSCC.

**Keywords:** Cell death; head and neck squamous cell carcinoma; HSV-1; TRAIL protein; apoptosis; oncolytic virus.

## Abstract 146 | Oral Presentation

### Therapeutic DNA Vaccination Targeting HPV16 E5 and E7 Oncoproteins: Evaluation of Different Immunization Regimens in a Preclinical Tumor Model

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Ingrid Andrêssa de Moura; Anna Jéssica Duarte Silva; ANTONIO CARLOS DE FREITAS; Jacinto da Costa Silva Neto; Julliano Matheus de Lima Maux; Karina Mayumi Tani Bezerra de Melo; Larissa Silva de Macedo; Ligia Rosa Sales Leal; Lucas Matheus Barreto Santana; Maria da Conceição Viana Invenção; Samara Souza de Pinho; Tiago Henrique dos Santos Souza

**Institution:** Federal University of Pernambuco (UFPE) — Recife-PE

Persistent infection with high-risk human papillomavirus (HPV), particularly HPV16, is a major cause of cervical cancer and the incidence of orogenital malignancies. Therapeutic DNA vaccines targeting viral oncoproteins represent a promising strategy to induce antitumor immune responses. However, the impact of antigen combinations in bicistronic vectors and the immunization sequence on therapeutic efficacy remains poorly understood. Thus, this study aimed to evaluate the preclinical antitumor efficacy of therapeutic DNA vaccines targeting HPV16 E5/E7 oncoproteins in C57BL/6 mice with C3 tumors and using different immunization schedules. The regimens included doses of DNA vaccines encoding E5 (E5CP), E7 (E7CP), or an E5CP-IRES-E7CP bicistronic construct (E5E7), using various prime-boost combinations such as E5/E7, E7/E5, E5/E5E7, E7/E5E7, and E5E7/E5E7, with PBS and pVAX1 as controls. During the experiment, tumor growth was monitored using a caliper and the mice were observed for signs of discomfort and weight loss. At the experimental endpoint, tumors and spleens were collected for histological and immunological analyses. Splenocytes were frozen and subsequently analyzed by flow cytometry following ex vivo stimulation with IL-2 and peptides derived from E5 and E7. The supernatant was also evaluated for the presence of nitric oxide using the Griess reaction. Compared to the PBS group, tumor volumes presented reductions of 57.0% (E5/E7), 60.9% (E5/E5E7), 53.7% (E7/E5E7), and 69.1% (E5E7/E5E7) whereas the E7/E5 regimen did not significantly reduce tumor volume. Differences in tumors were also identified among the treatment groups concerning fibrosis, inflammatory infiltrate, angiogenesis, fibroblasts, and edema. Distinct changes in the CD4+, CD8+, CD80+, and CD206+ cell populations were observed across the different regimens, as well as a strong negative correlation ( $r = -0.86$ ) between nitric oxide concentration and tumor reduction. In this regard, immunotherapy with DNA vaccines targeting HPV16 E5 and E7 was able to inhibit, to a certain level, the tumor growth of C3 cells in this preclinical study. The strongest antitumor effect was seen after repeated doses of the E5E7 bicistronic vaccine and in regimens with E5 priming, suggesting that giving the E5 antigen first may lead to stronger responses. Furthermore, this research illustrates the dual nature of nitric oxide and underscores the importance of antigen selection and immunization sequencing, as well as the use of strategies targeting the E5 protein in therapeutic HPV vaccination. Although the vaccination regimens did not achieve complete tumor regression, their potential use in combination with conventional treatments could represent a viable alternative. Finally, the future incorporation of novel delivery systems into these constructs could diversify administration routes and enhance the immune response through activation and uptake by immune system cells.

**Keywords:** Immunotherapy; human papillomavirus; DNA vaccines; preclinical models; bicistronic vaccine

## Day 3 · Extracellular Vesicles

### Abstract 59 | Oral Presentation

#### Investigation of the neuroprotective role of extracellular vesicles from physical exercise and associated with FNDC5/irisin in preclinical models of Alzheimer's disease

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Tayná Rody

**Institution:** Universidade Federal do Rio de Janeiro — Rio de Janeiro, RJ

Alzheimer's disease is a neurodegenerative disorder characterized by neurodegeneration, neuroinflammation, and memory loss. Due to the lack of disease-modifying treatments, the search for new therapeutic strategies is necessary. Physical exercise has been investigated as a non-pharmacological therapy to prevent and improve AD, as preclinical studies demonstrate neuroprotective effects related to exercise in animal models, including improvements in learning and memory. Physical exercise upregulates irisin, a myokine derived from FNDC5, previously shown to mediate beneficial actions of exercise on memory in AD mouse models. Exercise also causes an overall increase in plasma extracellular vesicles (EVs). EVs are membranous particles that play an important role in communication between the periphery and brain, carrying biomolecules from donor to recipient cells, notably modulating levels of different myokines during exercise. Interestingly, EVs have been reported to modulate some physiological roles of FNDC5/irisin in peripheral tissues. However, the involvement of EV-associated FNDC5/irisin in the brain has not yet been investigated. Herein, we aim to investigate extracellular vesicles (EVs) as a potential therapeutic approach. We hypothesize that exercise modulates irisin production in EVs, which might elicit beneficial effects on cognition. Our results show that FNDC5/irisin is increased in plasma EVs of exercised mice and in mice that overexpress FNDC5. We report that intravenous treatment with EVs obtained from exercised wild-type mice, but not from exercised FNDC5 KO mice, elevates cortical BDNF expression and rescues memory in APP/PS1 mice, a transgenic AD model. In addition, EVs from mice overexpressing FNDC5/irisin also rescue memory in APP/PS1 mice. These results indicate that EVs may be necessary for FNDC5/irisin-related crosstalk between periphery and brain, providing insight into the role of EVs as carriers of FNDC5/irisin during exercise and highlighting the therapeutic potential of EVs carrying irisin to restore cognition in AD.

**Keywords:** Extracellular vesicles, FNDC5/irisin, exercise.

## Abstract 104 | Oral Presentation

### 3D Bioprinted Wound Dressings Containing 5-Azacytidine-Preconditioned Human Umbilical Cord Mesenchymal Stromal Cells for the Treatment of Severe Cutaneous Wounds in a Murine Model of Sickle Cell Disease

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Waldir César Ferreira de Oliveira; Adriana Oliveira; Carolina Caliári Oliveira; Clarissa Azevedo; JOSILENE ALVES SANTOS; Juliana Magro Ribeiro; KELEN CRISTINA RIBEIRO MALMEGRIM DE FARIAS; Maria Carolina Oliveira

**Institution:** Universidade de São Paulo — Ribeirão Preto

Chronic cutaneous ulcers are a major clinical complication in patients with sickle cell disease (SCD), a condition characterized by systemic inflammation, endothelial dysfunction, and impaired wound healing. Conventional therapies often fail to achieve complete wound resolution, resulting in reduced quality of life and increased healthcare costs. Mesenchymal stromal cells (MSCs) have emerged as a promising regenerative therapy due to their secretion of trophic factors. This study aimed to evaluate the therapeutic efficacy of 3D-bioprinted wound dressings containing 5-azacytidine (5-AZA)-preconditioned human umbilical cord-derived mesenchymal stromal cells (hUCMSCs) in a murine model of SCD. We investigated whether combining cellular preconditioning with delivery through a bioprinted wound dressing could enhance regenerative outcomes by accelerating wound closure and improving re-epithelialization and tissue remodeling. MSCs were preconditioned with 5-AZA and incorporated into a 3D-bioprinted wound dressing. Therapeutic efficacy was evaluated using the Townes murine model of SCD bearing splinted excisional wounds, which closely recapitulates the impaired healing observed in human chronic wounds. In vitro, conditioned medium from preconditioned MSCs exhibited enhanced immunomodulatory activity, as demonstrated by increased secretion of IL-4, IL-10, and LIF, together with a markedly enhanced angiogenic profile, including 67-fold higher FGF, 47-fold higher PDGF, and 3-fold higher VEGF levels compared with non-preconditioned MSCs. In addition, 5-AZA preconditioning enhanced the secretion of regenerative factors involved in angiogenesis (VEGF, PDGF-BB, FGF, and PlGF), immunomodulation (TGF- $\beta$ 1), and extracellular matrix remodeling (uPA, Activin, Endothelin, MMP-8, and MMP-9). Both hUCMSC- and hUCMSC-AZA-loaded wound dressings accelerated wound healing by promoting earlier and more complete re-epithelialization and restoring skin architecture, including dermal appendages, by day 14. Animals treated with hUCMSC-AZA-loaded wound dressings displayed a more pronounced angiogenic profile and achieved faster wound closure than the comparison groups. Complete restoration of normal histological architecture was observed in animals treated with MSC-containing wound dressings. These findings demonstrate that 5-AZA preconditioning enhances the therapeutic efficacy of hUCMSCs, and that its combination with 3D-bioprinted wound dressings represents a promising translational strategy for the treatment of chronic cutaneous ulcers associated with sickle cell disease.

**Keywords:** Sickle Cell Disease; Mesenchymal Stromal Cells; 5-Azacytidine; Cell Preconditioning; 3D Bioprinting; Chronic Cutaneous Wounds; Regenerative Medicine; Tissue Remodeling.

## Day 3 · Clinical Applications of Gene Therapy in Brazil

### Abstract 187 | Oral Presentation

#### Will It Reach Patients? An AI Model Predicts Clinical Translation of Gene Therapies from Modifiable Preclinical Design Choices

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Maria Mascarenhas; Cynthia Silva Bartolomeo; Camila Cohen Kaleka; Diego Costa Astur; Luana de Mambro; Moises Cohen; Pedro Debieux; Roberta Sessa Stilhano

**Institution:** Faculdade de Ciências Médicas da Santa Casa de São Paulo — São Paulo-SP

**Background:** Most preclinical gene therapy (GT) studies never reach patients, and the factors that decide clinical translation are poorly defined. We combined statistical inference with machine learning (AI) to identify which preclinical characteristics associate with translation and to predict it. **Methods:** We assembled a disease-matched case-control corpus of preclinical (animal) GT studies. Cases translated - the same therapeutic program reached a registered human interventional trial; disease-matched controls did not. Translation was verified against ClinicalTrials.gov, EudraCT and CTIS in a three-round, blinded, adversarial audit under a strict same-program criterion (identical target, vector class and development lineage). From full texts we extracted ~100 standardized variables, each assessed solely from information reported in the preclinical study (no post-hoc trial data), spanning vector engineering, delivery, efficacy, preclinical rigor, product/manufacturing representativeness and disease context. Association used disease-stratified conditional logistic regression on pre-specified modifiable factors. Prediction used gradient-boosted trees with disease-grouped (out-of-distribution) cross-validation, tested only on unseen diseases; discrimination is reported with bootstrap 95% CIs and calibration. **Results:** The curated set comprised 468 studies (254 cases, 214 controls). In disease-stratified conditional regression, four modifiable factors independently predicted translation: a clinically representative product (OR 40.2, 95% CI 11.0-146.4), large-animal confirmation (4.5, 1.8-11.0), a large standardized effect (1.8 per SD, 1.2-2.5) and independent replication (1.7 per unit, 1.2-2.3); all  $p \leq 0.002$ . Other factors (positive primary outcome, adequate controls, clinical co-authorship, translational funding) were significant univariately but attenuated after conditioning on disease. The AI model predicted translation on unseen diseases with AUC 0.89 (95% CI 0.86-0.92; calibration slope 1.12) using modifiable design factors alone, essentially equal to a model using all preclinical factors (AUC 0.90) and well above resource-only baselines (~0.70). In a leakage-controlled analysis excluding preclinical papers published after the corresponding trial began, AUC remained 0.92. **Conclusions:** A disease-generalizable AI model predicts clinical translation of gene therapies, and the discriminative signal is carried by modifiable preclinical design choices - above all testing a clinically representative product - rather than by fixed disease context. Because these factors are actionable, the model can guide preclinical study design and help prioritize scarce translational resources, a need that is acute in settings such as Brazil.

**Keywords:** gene therapy; clinical translation; preclinical research; machine learning; predictive modeling; case-control study

## **Abstract 198 | Oral Presentation**

### **Safety and Preliminary Efficacy of a Brazil-Developed CMV pp65-Specific T-Cell Therapy for Clinically Significant CMV Infection After Allogeneic Hematopoietic Stem Cell Transplantation: Interim Results of a Phase I Study**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Anita Campos Mendonça Silva; Afonso Celso Vigorito; Luciana Cavaleiro Marti; Bruno Garcia Pires; Celso Arrais-Rodrigues; Clarisse M Machado; Fernanda Ferraz Assir; Iago Colturato; Mariana Nassif Kerbauy; Nelson Hamerschlag; Vinícius Campos de Molla

**Institution:** Hospital Israelita Albert Einstein — São Paulo - SP

Background: Cytomegalovirus (CMV) infection remains a major complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT), particularly in patients with resistant/refractory infection or toxicity limiting conventional antiviral therapy. Adoptive transfer of CMV-specific T cells (CMV-VSTs) may restore CMV-specific cellular immunity. We report interim safety and preliminary efficacy results from an ongoing Phase I study of CMV phosphoprotein 65 (pp65) -specific T-cell product. Methods: This multicenter, single-arm Phase I study evaluates the safety and preliminary efficacy of CMV pp65-specific T cells in adult allo-HSCT recipients with clinically significant CMV infection. CMV-VSTs are generated from platelet-donor leukocytes following stimulation and expansion with overlapping peptide pools derived from CMV pp65, followed by selection of IFN- $\gamma$ -secreting cells. For each patient, a cell product is selected based on HLA class I compatibility and infused (1-3 doses) at a dose of up to  $2 \times 10^4$  cells/kg. The primary endpoint is safety and tolerability, including infusion-related adverse events (AEs), serious AEs (SAEs), and acute/chronic graft-versus-host disease (GVHD). Efficacy is assessed at Day 30 based on CMV viral load reduction or improvement of clinical response and classified as complete response (CR; undetectable), partial response (PR;  $\geq 50\%$  reduction), or no response (NR; no reduction). Results: Six patients received CMV-VSTs and comprised the safety population. No infusion-related AEs were observed. Five patients with CMV infection without end-organ disease comprised the virologic efficacy cohort; median follow-up was 101 days (99-377). In these patients, 23 AEs were reported, 22 of which occurred after infusion; 19/23 (82.6%) were Grade 1-2. Three SAEs occurred, all considered unlikely to be related to the investigational product, and two non-serious AEs were considered possibly related to treatment. One patient developed mild chronic GVHD (oral lichen planus-like lesions), which was resolved and was considered unrelated to treatment. The sixth patient, treated for CMV retinitis, died two months after infusion from thrombotic microangiopathy/thrombotic thrombocytopenic purpura; unlikely to be related to the investigational product. On Day 30, two of five patients achieved CR, two achieved PR, and one had NR, for a protocol-defined virologic response rate of 80% (4/5). The patient with NR at D+30 achieved a CR 30 days after the second infusion. Conclusions: These interim findings suggest that CMV pp65-specific T-cell therapy is well tolerated, with no infusion-related AEs or treatment-related SAEs. The single GVHD event was mild and unrelated to treatment, and the fatal event was unlikely related to the investigational product. Four of five patients (80%) in the virologic efficacy cohort achieved a protocol-defined response by Day 30. Continued enrollment and follow-up will further characterize its safety and efficacy.

**Keywords:** Cytomegalovirus (CMV); Virus-Specific T Cells (VSTs); Adoptive T-Cell Therapy; Allogeneic Hematopoietic Stem Cell Transplantation (allo-HSCT); Resistant/Refractory CMV Infection.

# **Poster Session Day 1**

November 3, 2026 · 6:45 PM – 8:15 PM

# Poster Session Day 1

November 3, 2026 · 6:45 PM – 8:15 PM

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### Abstract 3 | Poster | Poster Session Day 1

## Beyond Motor Neurons: Astrocytic Reactivity and Glutamate Transporter Dysfunction in Amyotrophic Lateral Sclerosis

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Robertta Silva Martins

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**Introduction:** Amyotrophic Lateral Sclerosis (ALS) is characterized by progressive motor neuron loss associated with glutamatergic excitotoxicity and dysfunction of EAAT1/EAAT2 transporters. Neuroinflammation further modulates astrocytes toward neurotoxic (A1) or neuroprotective (A2) phenotypes through cytokines such as TNF- $\alpha$ , IL-1 $\alpha$ , and IL-1 $\beta$ . **Objective:** To investigate EAAT1/EAAT2 dysfunction and astrocyte reactivity in iPSC-derived astrocytes from ALS patients. **Methods:** Three control astrocyte cell lines (CTRL) derived from neurotypical individuals (XY, 62; XY, 37; XY, 31 years) and two cell lines from ALS patients were used: ELAf SOD1 (F21C) (XT, 49 years), ELAf VAPB (P56S) (XY, 58 years), and ELAs VAPB (SNP rs2234487). The cells were characterized by immunocytochemistry (GFAP, vimentin, EAAT1/EAAT2) and western blot, and [ $^3$ H]-D-Aspartate uptake was evaluated with blockers (DL-TBOA, DHK - 10, 25, 50 and 100  $\mu$ M) and after treatment with TNF $\alpha$  (1, 2, 5, 5 and 10 ng/ml), IL-1 $\alpha$  and IL-1 $\beta$  (1, 5, 10 and 50 ng/ml). Postal Code: 1.763.786. **Results:** All cell lines showed positive staining for GFAP and vimentin. Uptake was lower in astrocytes from ALS patients compared to CTRLs ( $F(5,151)=12.31; P<0.0001$ ), with lower uptake in SOD1 versus familial VAPB ( $P=0.0190$ ). The blocker DL-TBOA reduced uptake in CTRLs (XY,62:  $F(4,88)=19.75; P<0.0001$ ; XY,37:  $F(4,106)=4.29; P=0.0029$ ; XY,35:  $F(4,54)=8.03; P<0.0001$ ), while in ELA cell lines the effect occurred from 25  $\mu$ M in SOD1 ( $F(4,38)=4.00; P=0.0083$ ) and 10  $\mu$ M in familial ( $F(4,85)=11.52; P<0.0001$ ) and sporadic ( $F(4,91)=34.33; P<0.0001$ ) VAPB. The DHK blocker reduced uptake in CTRL (XY,62:  $F(4,83)=5.31; P=0.0007$ ; XY,37:  $F(4,95)=5.06; P=0.0010$ ) and showed a partial effect in the XY,35 cell line ( $F(4,64)=2.29; P=0.0687$ ), while in SOD1 ( $F(4,67)=2.858; P=0.0300$ ) and familial VAPB ( $F(4,71)=2.201; P=0.0399$ ) it occurred only at 100  $\mu$ M. In the sporadic VAPB cell line, DHK did not exert significant effects ( $F(4,43)=0.6540; P=0.6272$ ). ELAf SOD1 astrocytes showed a reduction in EAAT1 ( $F(2,7)=18.26; P=0.0017$ ) and an increase in the total density of EAAT2 ( $t=3.37, df=6; P=0.0149$ ). TNF $\alpha$  reduced uptake in all CTRL cell lines (XY,62:  $F(4,25)=5.23; P=0.0033$ ; XY,37:  $F(4,88)=10.66; P<0.0001$ ; XY,35:  $F(4,35)=9.49; P<0.0001$ ), and in SOD1 ( $F(4,65)=5.263; P=0.0010$ ), but in VAPB the effect was from 2.5 ng/mL ( $F(4,49)=3.82; P=0.0087$ ). IL-1 $\alpha$  showed a tendency to reduce SOD1 uptake (Baseline:  $100\pm6.99$ ; 1 ng/ml:  $90.87\pm7.19$ ; 5 ng/ml:  $87.57\pm6.09$ ; 10 ng/ml:  $85.86\pm10.41$ ; 50 ng/ml:  $98.41\pm6.10$ ), and there was a reduction in CTRL ( $F(4,21)=165.3; P<0.0001$ ). IL-1 $\beta$  increased uptake by 5 ng/mL in CTRL ( $F(4,21)=5.27; P=0.0042$ ), but there was a reduction in SOD1 ( $F(4,22)=11.11; P<0.0001$ ). **Conclusion:** ALS astrocytes exhibit impaired glutamate uptake and altered cytokine responsiveness. EAAT2 emerges as a potential therapeutic target, and inflammatory signaling plays a key role in modulating astrocyte neuroprotective versus neurotoxic responses. **Funding:** FAPERJ, CNPQ, Capes

**Keywords:** ALS, astrocytes, EAATS, glutamatergic excitotoxicity and Neuroinflammation

## **Abstract 4 | Poster | Poster Session Day 1**

### **MARTINLAB IN SCHOOLS: PROMOTING SCIENCE COMMUNICATION THROUGH EDUCATIONAL BOARD GAME CARTELAS**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Manoela Ribeiro Bastos; Andreia Maranhão

**Institution:** Instituto Nacional de Câncer - INCA — Rio de Janeiro - RJ

**INTRODUCTION AND OBJECTIVE** The “MartinLab in Schools” is an outreach activity aimed at promoting science communication in the field of advanced therapies such as immunotherapy with CAR-T cells. The project utilizes the educational board game CARTelas as a tool to facilitate the understanding of key concepts including cancer, immune system, and CAR-T cell therapies. The objective is to promote the dissemination of knowledge with an emphasis on CAR-T cell immunotherapies among high school students through educational and interactive activities. **MATERIAL AND METHOD** The educational activity was conducted with high school students from Colégio Estadual Souza Aguiar (CESA), and CIEP 092 Federico Fellini (CIEP 092). Participants used the CARTelas game kit, which included printed 3D and non 3D boards, instruction manual, tokens, scientific questions, and action cards. The game is structured in three phases per round: scientific questions, maintenance of cell dynamics, and player actions for controlling or eliminating cancer cells. Victory is achieved by eliminating or containing tumor cells; uncontrolled proliferation results in defeat. **RESULTS AND CONCLUSION** We performed three educational visits, two in CESA, and one in CIEP 092. High school students played the game and at the end all groups engaged well in the activity. Most of the groups eliminated the tumor and won the game, some of them lost it as tumor cells expanded through the board. Most of the students didn’t know CAR-T cells, as well as immunotherapy. In contrast, most knew someone who had cancer in their social circle. The initiative successfully facilitated the understanding of key scientific concepts related to the immune system and CAR-T cell therapy in an interactive manner. The initiative proved effective in disseminating scientific knowledge, with future plans to refine questions for different levels and enhance tactile learning with more 3D-printed components.

**Keywords:** CAR-T, immunotherapy, science communication

## **Abstract 5 | Poster | Poster Session Day 1**

### **Functional Application of the iCasp9 Suicide Gene System as a Safety Mechanism for CAR-T Cells**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Bernardo Monteiro de Vasconcelos; EDUARDO MANNARINO CORREIA; JOÃO VITOR STEIMBACH; MARTÍN BONAMINO

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According to the World Health Organization, cancer is one of the leading causes of death worldwide, with more than 1 million cases registered in 2022 in Latin America alone. Recent advances in technology and immunology have introduced new tools for fighting cancer, such as biological "living drugs" like modified T-cells expressing a Chimeric Antigen Receptor (CAR-T cells). Although demonstrating promising results, CAR-T cells have introduced new challenges for patients, like Adverse Events (AEs) related to Cytokine Release Syndrome (CRS). To address the need for a safety switch to eliminate these modified cells in the event of severe AEs, the iCasp9 suicide gene system was proposed as a rapid and effective mechanism for this immunotherapy. The goals of this work are to implement, validate and replicate the iCasp9 system by producing a Jurkat iCasp9-CAR population using non-viral vectors and demonstrating that, upon contact with the dimerization drug AP20187, the modified Jurkat population undergoes apoptosis via the caspase-9 pathway. This demonstrates that the system specifically affects the modified population with no adverse effects on the wild-type population. For this project, Jurkat wild-type (WT) cell lines were first cultivated in RPMI medium supplemented with 10% Fetal Bovine Serum (FBS). Following cultivation, the cells were electroporated with the iCasp9-CAR plasmid using a transposon system. Lonza 2B nucleofector was utilized at the high viability setting X-001. After a recovery period in culture, the cells were labeled with anti-CAR antibodies. The cells were subsequently sorted to isolate the iCasp9-CAR positive population, aiming for a 100% modified population. Currently, we have successfully modified the Jurkat WT cell line into an 100% iCasp9-CAR positive population and have initiated treatment with AP20187, analyzing the effects of the drug on both the WT Jurkat and the modified populations. At present, the literature indicates that the iCasp9 suicide gene is highly efficient and does not induce significant toxicity in unmodified cells. This suggests that the system represents a promising alternative for enhancing the safety of CAR-T cell treatments in the Brazilian national health system.

**Keywords:** CAR-T, Safety mechanism, Cellular therapy.

## **Abstract 7 | Poster | Poster Session Day 1**

### **Dual CAR-Based Cell Therapy with Anti-CAIX CAR T Cells or CAR Microglia Secreting Anti-PD-L1 Antibodies for the Preclinical Treatment of Glioblastoma**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** ELOAH RABELLO SUAREZ

**Institution:** Federal University of ABC/ Federal University of São Paulo/ University of São Paulo — Santo André-SP/ São Paulo-SP

**Introduction:** Glioblastoma (GBM) is a brain tumor characterized by hypoxia and about 60% overexpression of carbonic anhydrase IX (CAIX) and programmed cell death ligand 1 (PD-L1), favoring T cell exhaustion(1,2). The use of immune cells expressing chimeric antigen receptors (CARs) against a tumor-associated antigen that also blocks immune checkpoints by antibody co-secretion emerges as a promising strategy for advanced solid tumors. **Objectives:** We aim to evaluate the efficacy of CAIX-targeted CAR-T cells (CAR-T) and CAR microglia secreting anti-PD-L1 antibodies against human GBM cells in vitro and in mice. **Methodology:** GBM cell lines (U251 and A172) were evaluated regarding CAIX/PD-L1 expression. Primary T cells and a microglia lineage were transduced with lentiviruses to express anti-CAIX CAR, capable of anti-PD-L1 co-expression. Cytotoxicity, cytokines secretion, and immunophenotyping assays were performed. The efficacy of CAR-T was validated in NSG-SGM3 and NSG-SGM3-hIL15 mice, followed by analysis of tumor-infiltrating lymphocytes (TIL) and residual expression of tumorigenesis-related markers by flow cytometry. In vivo assays for CAR microglia are underway. **Results:** In vitro, CAR-T and CAR-microglia targeting only CAIX showed greater cytotoxicity and cytokine exchange; however, CAR-microglia also showed increased cytokine release in cells when targeting CAIX/PD-L1. However, the dual-target strategy for CAR-T (CAIX/PD-L1) was superior in controlling tumor progression in NSG-SGM3 animals, and there was an increase in TIL in this group, accompanied by a reduction in CAIX, PD-L1, and Ki67 in the tumor. In NSG-SGM3-hIL15 animals, cells targeting only CAIX have shown greater efficacy, with increased TILs and decreased CAIX, PD-L1, and Ki67 in tumors. **Conclusion:** We propose CAR-microglia as a new platform to be explored for GBM treatment. Local PD-L1 blockade was crucial for the in vivo antitumor efficacy of CAR T in the NSG-SGM3 model, promoting greater lymphoid recruitment and tumor regression. hIL-15 animals demonstrate that the efficacy of co-stimulatory domains depends on cytokine support from the microenvironment, with hIL-15 signaling being a relevant factor for the success of conventional anti-CAIX CAR T therapy against GBM, although it can impair the therapeutic efficacy of checkpoint blockade in vivo.

**Keywords:** Chimeric antigen receptor T cells; immune checkpoint blockade; T cell exhaustion; glioblastoma; microglia

## Abstract 9 | Poster | Poster Session Day 1

### MULTIGENE TESTING IN HEREDITARY BREAST AND OVARIAN CANCER IN A BRAZILIAN PUBLIC HEALTH REGISTRY

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Marjorie Pereira Gualter; Felipe Cavalcanti Carneiro Silva; Francisca Rafaela Ferreira de Souza; Helber Alves Negreiros; Igor Gabriel Barbosa de Sousa; João Marcelo de Castro e Sousa; Kamila Bezerra Fernandes Diocesano; Luisa Vitória de Sá Carneiro; Maria dos milagres Carvalho Santos; Vladimir Costa Silva

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Hereditary breast and ovarian cancer (HBOC) represents approximately 5-10% of all breast cancer cases and BRCA1/2 are the major cancer predisposition genes. Multigene panel have become the preferred approach for evaluating individuals with suspected HBOC, since it increases the likelihood of identifying pathogenic mutations (up to 15% more), provide more precise cancer risk and personalized prevention strategies. Brazil, as a continental country, does not have precise data on HBOC cases of triple-negative and luminal B breast cancer. Thus, the study aimed to identify pathogenic mutations in a multigene panel through next-generation sequencing in HBOC-suspected patients. For this, 36 young women with breast cancer treated by the public health system were selected for molecular diagnosis. Sixteen hereditary genes were sequenced using genomic DNA isolated from lymphocytes through next-generation sequencing (Miseq, Illumina). Most patients had triple-negative (TN) tumors (27 cases) and luminal B (6 cases). Of the 27 TN cases, five patients presented a known pathogenic mutation in BRCA1, two had a BRCA2 missense variant of unknown significance (VUS), and one had a BRCA2 truncating VUS; and of the 6 Luminal B cases, one had a pathogenic mutation in BRCA1 and two unrelated patients had the same in-frame VUS. Interesting, one triple negative patient presented two pathogenic mutation (frameshift and truncating) in the BRCA1 and PALB2 genes. The frequency of pathogenic mutation was 17%, including 18.5% (5/27) for TN and 16.7% (1/6) for luminal B. The mean age at breast cancer diagnosis for patients with pathogenic mutations was 41.4 years, with a tendency for earlier diagnosis in triple-negative molecular subtypes. Genetic testing impacted clinical management, allowing for individualized handling strategies. Patients with mutations showed favorable clinical progression during the follow-up period, reinforcing the importance of early identification of women carrying mutations in breast cancer predisposing genes.

**Keywords:** Hereditary cancer, sequencing, pathogenic mutations, screening

## **Abstract 10 | Poster | Poster Session Day 1**

### **Development, Physicochemical Characterization and Preliminary Ocular Safety of Cationic Liposomes Carrying CRISPR/Cas9 and IDUA Plasmids for Topical Gene Editing in Mucopolysaccharidosis Type I**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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Mucopolysaccharidosis type I (MPS I) is a rare inherited lysosomal disorder caused by mutations in the  $\alpha$ -L-iduronidase (IDUA) gene, leading to a deficiency of the IDUA enzyme and progressive accumulation of glycosaminoglycans (GAGs) in multiple tissues. Ocular involvement, including corneal clouding, retinopathy, and optic nerve damage, represents a major cause of morbidity, while current treatments, such as intravenous enzyme replacement therapy, show limited efficacy in immune-privileged tissues such as the eye. In this context, advanced therapies based on non-viral vectors have emerged as promising strategies for gene delivery and gene editing. This study aimed to develop, characterize, and evaluate the preliminary safety profile of cationic liposomes carrying CRISPR/Cas9 and IDUA plasmids for topical ocular gene editing in MPS I. Liposomes composed of DOTAP, DOPE, and DSPE-PEG2000 were prepared by lipid bilayer hydration followed by microfluidization (800 psi, 5 cycles, LV1, Microfluidics, Newton, MA, USA). Lipid composition was optimized using a  $2^3$  full factorial design based on hydrodynamic diameter and zeta potential. Plasmids were complexed at a +4/-1 charge ratio. Physicochemical characterization included particle size, polydispersity index (PDI), zeta potential, agarose gel electrophoresis to evaluate plasmid complexation, and stability in simulated tear fluid. Preliminary safety was assessed using the Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) assay and histological evaluation (H&E staining) of fresh porcine corneas after exposure to the formulations. The optimized formulation achieved a composite desirability of 0.90, with a mean particle size of  $93 \pm 6$  nm, PDI of  $0.31 \pm 0.05$ , and zeta potential of  $+61.7 \pm 1.1$  mV. Following plasmid complexation, particle size increased to  $287 \pm 38$  nm, PDI to  $0.52 \pm 0.03$ , while zeta potential decreased to  $+29.6 \pm 2.5$  mV, consistent with electrostatic interaction between the cationic liposomes and negatively charged plasmid DNA. Agarose gel electrophoresis confirmed efficient plasmid complexation, as only free plasmids migrated through the gel, and no decomplexation was observed in simulated tear fluid, demonstrating formulation stability. In the HET-CAM assay, both unloaded and plasmid-loaded liposomes showed no signs of hemorrhage, coagulation, or vasoconstriction, indicating a non-irritating profile. Likewise, histological analysis of porcine corneas revealed no epithelial damage or tissue deterioration after exposure to the complexes, in contrast to the positive control. Conclusions: The cationic liposomes demonstrated suitable physicochemical characteristics for plasmid delivery, effective stabilization of CRISPR/Cas9 and IDUA plasmids through electrostatic complexation, stability in simulated tear fluid, and a favorable preliminary ocular safety profile. These findings support the potential of this non-viral nanocarrier as a platform for topical ocular gene delivery and MPS I gene editing.

**Keywords:** cationic liposomes, gene editing, mucopolysaccharidosis type I

## **Abstract 11 | Poster | Poster Session Day 1**

### **THE IMPORTANCE OF IMMUNOREGULATION FOR THE SUCCESS OF AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH TYPE 1 DIABETES**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

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Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by the destruction of  $\beta$  cells, leading to insulin deficiency that requires replacement therapy, which does not prevent complications. Autologous hematopoietic stem cell transplantation (AHSCT) promotes immune renewal and restores endogenous insulin production. The procedure involves the mobilization of hematopoietic stem cells, collected by leukapheresis. The patient undergoes an immunoablative regimen, followed by infusion of the collected cells. However, after a median period of 3.5 years, patients experience disease recurrence. The lack of mechanistic studies limits the application of AHSCT. This study aims to characterize the profile of regulatory T cells (Tregs) and IL-10, both essential for immunoregulation, in patients with T1D submitted to AHSCT, correlating them with clinical outcomes. This retrospective study (CAAE: 74991923.2.3001.5440) used peripheral blood mononuclear cell (PBMC) and serum samples from 17 patients with T1D, with a mean age of  $16.8 \pm 3.7$  years, submitted to AHSCT between 2004 and 2012. Patients were divided according to how long they remained insulin-independent: short independence ( $<3.5$  years) or long independence ( $>3.5$  years). Samples from the pre-transplant period and up to 2100 days after transplantation were used. PBMC samples were used for phenotyping by flow cytometry and serum samples for cytokine analysis by ELISA. There was an increase ( $p<0.01$ ) in Tregs 60 days after transplantation compared with the pre-transplant period. At baseline, patients in the long-independence group already had a higher frequency of Tregs than those in the short-independence group. In addition, patients in the long-independence group showed detectable IL-10 levels at all analysed time points, whereas most patients in the short-independence group had no detectable levels of this cytokine. Treg frequency showed a strong positive correlation with IL-10 levels ( $r=0.72$ ;  $p<0.05$ ). IL-10 levels showed a moderate positive correlation with the duration of insulin independence ( $r=0.6$ ;  $p=0.08$ ). The dynamics of Treg reconstitution are of interest because of their early recovery in the periphery, a compensatory phenomenon in response to immunoablation. Patients who responded better to AHSCT had higher baseline Treg levels, indicating that these cells may influence clinical outcome after transplantation. Moreover, the positive correlation between Tregs and IL-10 suggests that this cell population may be a source of this cytokine and that its expression could help reduce pancreatic injury, determining longer periods of insulin independence. Finally, patients with a long response had higher IL-10 levels, which may be an indicator of the success of the procedure.

**Keywords:** Type 1 Diabetes Mellitus, Hematopoietic Stem Cell Transplantation, Regulatory T Cells

**Abstract 12 | Poster | Poster Session Day 1**  
**PRODUCTION OF AMPLICON-TYPE VIRAL VECTORS FOR GENE THERAPY: A NEW PURIFICATION SYSTEM BASED ON SITE-SPECIFIC CRE/LOX RECOMBINATION.**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Ana Rubia Alcantara Peloso

**Institution:** Instituto Nacional de Câncer — Rio de Janeiro, RJ

Amplicon vectors, derived from human herpes simplex virus type 1 (HSV-1), are extremely versatile viral vectors that are simple to produce and are used in several areas of molecular biology, such as gene transfer, monoclonal antibody synthesis, the study of neuromotor diseases and vaccine design. Nowadays, however, these vectors have features that limit their use in gene therapy protocols. Among these undesirable features are (i) contamination by helper viral particles and (ii) the presence of bacterial DNA in their genome (derived from the amplicon plasmid), which are responsible for immune response activation and for transgene silencing. The main objective of the present project is therefore to build a new amplicon vector production system based on site-specific Cre/lox recombination and to evaluate the production of amplicon vectors generated by this system. The proposed system can be regarded as a "floxed" amplicon module (packaging sequences, viral origin of replication, transgene and reporter gene), devoid of the bacterial sequences commonly present in amplicon plasmids, and cloned into the locus of one of the glycoproteins of the recombinant HSV-1 genome. Amplicons were produced in a cell line transcomplementing essential viral genes, transiently transfected with Cre recombinase, using two strategies for introducing the plasmid DNA: lipofection and electroporation. In the production carried out by lipofection, an amplicon vector titre of  $1.37 \times 10^4$  TU/mL and helper viral particle contamination of  $2.27 \times 10^5$  PFU/mL were obtained. In the production carried out by electroporation, an amplicon titre of  $3.4 \times 10^4$  TU/mL and a helper particle titre of  $2.5 \times 10^5$  PFU/mL were observed. Taken together, the results show that both approaches allowed the generation of amplicon vectors, with better performance for electroporation in terms of yield. However, the detection of helper particles under both conditions shows that the system used still has limitations regarding the purity of the viral preparations, despite its capacity to produce amplicon vectors.

**Keywords:** Amplicon vector, HSV-1, Gene Therapy

## Abstract 13 | Poster | Poster Session Day 1

### GeneTips: using social media for gene therapy science communication

**Category:** Manufacturing, quality control, clinical studies and science communication

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**Background:** by mid 2026, more than 50 gene therapies (GTs) had received global regulatory approval. Despite this expanding clinical landscape, public and healthcare student knowledge of GT remains limited. At the same time, social media has become a major source of health information, where misinformation spreads rapidly. To address these gaps, we created GeneTips, a platform for accurate and engaging GT science communication (SciComm). This case study examines the development and early outputs from the project. **Methods:** GeneTips began in 2023 as an initiative by students in a GT laboratory under faculty supervision. In 2025, the project became the outreach component of a CNPq funded GT research project developed in the laboratory. The project involves undergraduate and graduate student contributors and publishes recurring content across Instagram and LinkedIn, its most established platforms, as well as Substack, TikTok, YouTube, Spotify, and a website. On June 28, 2026, we collected metrics from the native Instagram and LinkedIn insights dashboards. For the content sample, we purposively selected the ten most recent posts on GT or related genetics topics, excluding event announcements, opportunities, awareness days, posts focused on team members, and one outlier. We also qualitatively inspected comments and profile interactions to explore audience trends. **Results:** the Instagram account had 1,678 followers and 316 posts. Over 90 days (March 30 to June 27, 2026), it reached 156,128 views and 56,198 accounts, gaining 539 new followers (56.8% aged 18-34; 70.7% women; 96.4% from Brazil). The sample of ten posts had a mean of 2,956 views, 104 likes, and 16 shares; nine were developed collaboratively with other SciComm projects. One post outside the sample achieved viral reach: 78,598 views, 2,300 likes, 1,200 shares, and 382 new followers. On LinkedIn, posts about professional opportunities such as courses and events consistently received more impressions than other topics. Qualitative inspection of interactions on both platforms suggested a diverse audience including healthcare undergraduates, postgraduate students, researchers, and other academic stakeholders. On Instagram, families of patients with rare diseases also appear to be part of the audience. **Discussion:** metrics indicate that GeneTips's multiplatform presence attracts different audience profiles and content preferences. The viral Instagram post may reflect the combined effects of timely publication, a compelling narrative, and a high-impact discovery, although achieving this balance is challenging. Because engagement metrics capture visibility but not learning or long-term impact, outcome-based evaluation is needed. **Conclusion:** GeneTips is a proof of concept for social media GT SciComm. It suggests that a collaborative multiplatform project can generate visibility while maintaining scientific accuracy. These observations can guide the expansion of GeneTips and inform similar initiatives.

**Keywords:** Scientific outreach, gene therapy, social media

## **Abstract 15 | Poster | Poster Session Day 1**

### **DEVELOPING NANOBODY DERIVED CARS TARGETING B CELL LEUKEMIA MARKERS: CD19, CD20 AND CD22.**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Joao Duarte de Paula Coutinho Marques; Andrea queiroz maranhao; Carla Celedonio; MARTÍN BONAMINO; Maurício Tilburg

**Institution:** INCA — Rio de Janeiro - RJ

T cells genetically modified to express transgenic chimeric antigen receptors (CAR-T cells) to treat hematological malignancies, such as B-cell acute lymphoblastic leukemia (B-ALL) and multiple myeloma (MM), have shown great efficacy. However, in around 10% of the cases treating B-ALL with CAR-T cells targeting CD19, patients relapse with tumor cells not expressing the targeted antigen. With this perspective, this project aims to develop and test bi-specific CAR-T cells targeting two out of three antigens present in B cell membrane: CD19, CD20 and CD22; expecting to reduce the relapse rate and increasing the treatment efficacy. Bispecific CARs will be built using nanobody (VHH) domains selected by phage display from camelid libraries immunized with whole Raji cells. The Lama glama immune response is being measured by ELISA against Raji membrane extract, cytosolic extract and against the whole cell in a cell-based-ELISA. Selected VHHs will be humanized and cloned into the Sleeping Beauty vector pT4-19BBz. Modified T cells will be tested in vitro against Nalm-6 and Raji lines expressing different antigen combinations (GFP-labeled), and in vivo in NSG mice using luciferase-tagged tumor cells to assess cytotoxic activity and tumor control. CD19, CD20 and CD22 were detected on the membrane extract by Dot Blot; the immune response measured against the membrane extract by ELISA reached titers of 103, but measuring it against the whole cell, the serum, reached titers of 104. Having reached these levels, the immune library will be prepared. The immunization with the whole cell (Raji) generated a higher immune response against cytosolic proteins, later immunizations were performed using only the membrane extract, for a more intense immune response on the desired targets. This work aims to develop brand-new sequences of bispecific nanobody-based CARs targeting multiple B-cell leukemia antigens. A sufficient immune response was detected by ELISA against whole Raji cells, enough to build an immune library against the three targets: CD19, CD20 and CD22. This approach has the potential to improve the robustness and persistence of CAR-T cell therapy, offering a promising strategy to prevent tumor relapse due to antigen loss and to expand the therapeutic options for hematological cancers.

**Keywords:** CAR-T, Nanobodies, bi-specific

## **Abstract 16 | Poster | Poster Session Day 1**

### **Wharton's Jelly-Derived Mesenchymal Stem Cells Improve Functional Recovery and Reduce Neuroinflammation Following Experimental Intracerebral Hemorrhage**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Maria Carolina Peixoto Rodrigues; JULIANA FERREIRA VASQUES; Marcelo Felipe Santiago; Poliana Pires de Oliveira; Robertta Silva Martins; ROSALIA MENDEZ OTERO; Tayná Rodrigues de Almeida da Silva

**Institution:** Universidade Federal do Rio de Janeiro — Rio de Janeiro

Intracerebral hemorrhage (ICH) is a severe form of stroke associated with high mortality and long-term disability, with limited therapeutic options available. Mesenchymal stem cells (MSCs) derived from Wharton's jelly have emerged as a promising cell-based therapy due to their immunomodulatory and neuroprotective properties. This study investigated the therapeutic potential of Wharton's jelly-derived MSCs in an experimental model of ICH. Adult male and female Wistar rats were subjected to ICH induction through stereotaxic intrastriatal injection of collagenase. Twenty-four hours after injury, animals received either an intraperitoneal administration of MSCs or vehicle. Motor function was assessed using the rotarod test. Neurodegeneration was evaluated by Fluoro-Jade C staining, while neuroinflammatory responses were investigated through immunohistochemical analysis of glial markers, including glial fibrillary acidic protein (GFAP) for astrocytes and ionized calcium-binding adaptor molecule 1 (Iba-1) for microglia. Microglial morphology was also analyzed to characterize activation states. MSC-treated animals exhibited significant improvements in motor performance compared with vehicle-treated controls, regardless of sex. Histological analyses revealed a marked reduction in neuronal degeneration in both the striatum and ipsilateral cortex of MSC-treated animals. Furthermore, MSC therapy significantly decreased GFAP and Iba-1 immunoreactivity in both brain regions, indicating attenuation of astroglial and microglial activation. Morphological assessment of microglia demonstrated a shift from a predominantly amoeboid phenotype in control animals to a more ramified phenotype in MSC-treated animals, suggesting a less reactive and potentially neuroprotective inflammatory profile. These effects were observed at both short- and long-term evaluation time points. Collectively, these findings demonstrate that intraperitoneal administration of Wharton's jelly-derived MSCs promotes functional recovery, reduces neuronal degeneration, and modulates neuroinflammatory responses following experimental ICH. The observed changes in glial activation and microglial morphology support the hypothesis that MSC-mediated immunomodulation contributes to neuroprotection and improved neurological outcomes. These results highlight the therapeutic potential of Wharton's jelly-derived MSCs as a promising strategy for the treatment of hemorrhagic stroke.

**Keywords:** Cell therapy; mesenchymal stem cells; ICH; Neuroprotection

## **Abstract 18 | Poster | Poster Session Day 1**

### **Bioengineering of CD19-CAR Monocytes Incorporating Dectin-1 or FcγRI as Costimulatory Domains for Targeting B-Cell Malignancies**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Victor Gabriel Santos Silva; Gustavo Matheus Amaro; Isabele Held Lemos; Júlia Garcia Guimarães; Milena Barrocali de Araújo Melo; Nathália Ferreira Fregonezi; Pedro Augusto Macedo Teixeira; Thiago Aparecido da Silva

**Institution:** São Paulo State University (UNESP), School of Pharmaceutical Sciences — Araraquara, SP, Brazil.

CD19-targeted chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of relapsed or refractory B-cell malignancies. However, important limitations, including cytokine release syndrome, neurotoxicity, and the current requirement for autologous cell manufacturing, have driven the development of alternative immune cells platforms, such as genetically engineered NK cells, monocytes and macrophages. Therefore, the current work established a platform using THP-1 monocytic cell line expressing CD19-CAR variants containing Dectin-1, FcγRI, or CD137/CD3ζ-derived costimulatory domains for targeting B-cell malignancies. CD19-positive Nalm-6 and Daudi cell lines were engineered to stably express luciferase, enabling the assessment of antitumor activity by bioluminescence. Lentiviral particles carrying the coding sequence of different CD19-CAR constructs were produced, and an MOI of 5 was used to modify THP-1 cells. CD19-8H-Dectin-1-CAR-, CD19-8H/TM-FcγRI-CAR-, and CD19-8H/TM-137ζ-CAR-engineered THP-1 cells achieved transduction efficiencies of 75.6%, 87.6%, and 64%, respectively. The enrichment of CAR-THP-1 cells was performed by cell sorting using EGFP as reporter, and 99% EGFP-positive cells was obtained. CAR expression on the cell surface was evaluated by flow cytometry using anti-F(ab')<sub>2</sub> antibody or Protein L-PE staining, and all construction exhibited robust expression. Untransduced (UTD) or CD19-CAR THP-1 cells were co-cultured with Nalm-6-Luc or Daudi-Luc cells (E:T ratios of 1:1, 3:1, and 5:1) for 6 and 24 h. Functionally, CD19-8H/TM-137ζ-CAR THP-1 cells induced a significant reduction of the bioluminescence of both Nalm-6-Luc and Daudi-Luc cells only after 6 hours. Interestingly, CD19-8H-Dectin-1-CAR and CD19-8H/TM-FcγRI-CAR THP-1 cells, which incorporate costimulatory domains associated with myeloid/macrophage activation, significantly reduced Nalm-6-Luc bioluminescence at both 6 and 24 hours, whereas Daudi-Luc bioluminescence was reduced only after 6 hours. These findings demonstrate antitumor activity against in vitro models of B-cell acute lymphoblastic leukemia and Burkitt lymphoma, indicating that Dectin-1- and FcγRI-derived costimulatory domains improved the antitumor response. Thus, THP-1 cells expressing CD19-CAR variants associated with costimulatory domains sourced from myeloid/macrophage are an important preclinical platform for the development of CAR macrophages targeting hematological malignancies.

**Keywords:** Chimeric Antigen Receptor; Monocytes; Dectin-1; FcγRI; B-cell malignancies.

## **Abstract 20 | Poster | Poster Session Day 1**

### **A gene therapy strategy for liposarcoma using p14ARF to inhibit MDM2 and IFN- $\beta$ for immune activation**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** SORAIA BARBOSA DE OLIVEIRA; BRYAN ERIC STRAUSS; Fernanda Antunes

**Institution:** Instituto do Cancer do Estado de São Paulo — São Paulo

**Introduction:** Dedifferentiated and well-differentiated liposarcomas (DDLPS/WDLPS) are characterized by MDM2 amplification, resulting in suppression of the p53 pathway and resistance to conventional therapies. Given the limited efficacy of current treatments, novel therapeutic strategies are needed. We investigated a gene therapy approach combining p14ARF and interferon-beta (IFN- $\beta$ ) delivered by non-replicative RGD-modified adenoviral vectors. While p14ARF restores p53 activity through MDM2 inhibition, IFN- $\beta$  promotes antitumor immune responses and immunogenic cell death. **Objective:** To evaluate the antitumor activity of combined p14ARF and IFN- $\beta$  gene transfer in liposarcoma cell lines with distinct TP53 status and compare its effects with pharmacologic intervention. **Methods:** Human liposarcoma cell lines 93T449 (MDM2-amplified, TP53 wild-type) and SW872 (TP53-mutant) were treated with adenoviral vectors encoding p14ARF and/or IFN- $\beta$ . Vector functionality was validated by luciferase assays, immunofluorescence, and RT-qPCR. Pharmacological controls included Nutlin-3a and recombinant IFN- $\beta$ . Treatment responses were evaluated by cell viability, membrane permeability, SubG1 DNA fragmentation, and expression of p53- and interferon-related genes. **Results:** In 93T449 cells, Nutlin-3a activated canonical p53 targets, including p21, MDM2, and BAX, whereas recombinant IFN- $\beta$  induced TLR3, TRAIL, and IRF9 expression. However, the strongest cytotoxic effect was observed following combined p14ARF and IFN- $\beta$  gene transfer, resulting in the accumulation of sub-G1 cells (65%) accompanied by extensive membrane damage. In SW872 cells, Nutlin-3a showed minimal activity, consistent with the TP53-mutant status. Although recombinant IFN- $\beta$  induced interferon-responsive genes, it produced limited cytotoxicity. In contrast, IFN- $\beta$  gene transfer conferred significant antitumor activity independently of p53 status. Combined p14ARF and IFN- $\beta$  treatment further enhanced cell death, where membrane permeability and DNA fragmentation were seen in approximately 77% and 30% of cells, respectively, suggesting activation of non-apoptotic cell death mechanisms. These findings are consistent with previous studies from our group demonstrating that p14ARF and IFN- $\beta$  gene transfer induce immunogenic cell death and immune activation in multiple tumor models. **Conclusions:** Combined p14ARF and IFN- $\beta$  gene therapy demonstrated significant cytotoxicity in MDM2-amplified liposarcoma models. As compared to Nutlin-3a and recombinant IFN- $\beta$  treatment, the gene therapy has shown a greater anti-tumor activity in cell lines that differ in their p53 status, indicating the possible involvement of p53-dependent and p53-independent mechanisms. These results support further investigation of this strategy in preclinical in vivo and patient-derived models as a potential therapeutic approach for liposarcoma.

**Keywords:** Gene therapy; Liposarcoma, p14ARF, IFN- $\beta$  and MDM2 amplification.

## **Abstract 21 | Poster | Poster Session Day 1**

### **Enhancing cytotoxic cancer therapies through tumor metabolic reprogramming using novel therapeutic RNA strategies**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Fernanda Antunes

**Institution:** Centro Translacional de Investigação em Oncologia (CTO) - Instituto do Câncer do Estado de São Paulo (ICESP) — São Paulo - SP

Cancer progression is associated with genetic alterations, immune evasion, resistance to cell death, and metabolic reprogramming. A hallmark of this process is the “Warburg effect,” in which tumor cells preferentially rely on glycolysis rather than oxidative phosphorylation (OXPHOS), enabling adaptation to hostile microenvironments and resistance to therapy. This metabolic plasticity makes tumor metabolism an attractive therapeutic target. Although fasting and fasting-mimicking diets can enhance the efficacy of antitumor therapies, patient adherence remains a major clinical limitation. This project proposes the use of therapeutic RNAs to reproduce the metabolic effects of nutrient deprivation and enhance the efficacy of anticancer therapies. The strategy focuses on modulating glycolysis, OXPHOS, and mitochondrial components using RNA interference and/or miRNA/mRNA delivery via lentiviral vectors or nucleic acid nanoparticles (NANPs) carrying therapeutic RNAs. Quantitative shotgun proteomics based on mass spectrometry will be employed to identify metabolism-related proteins that are differentially expressed following the different treatment combinations. Our previous findings demonstrated that nutrient deprivation using Earle’s Balanced Salt Solution (EBSS, hereafter referred to as STV) enhanced cisplatin- and sorafenib-induced cytotoxicity in melanoma models (CHL-1 and SK-Mel-28), with significant mitochondrial involvement 1,2. Here, we show that sorafenib + STV, 5-fluorouracil + STV, and oxaliplatin + STV potentiated cell death, as determined by flow cytometric analysis of the sub-G1 population following propidium iodide staining. These treatments also induced early mitochondrial depolarization, assessed by MitoTracker Deep Red FM staining followed by flow cytometry, in melanoma (UACC62 and SK-Mel-147), hepatocellular carcinoma (HuH7 and HepG2), and colorectal cancer (HCT116 and HT29) cell lines. Alternative nutrient deprivation protocols using high- or low-glucose DMEM supplemented with 2% FBS resulted in significantly lower sensitization than STV. Subsequently, STV combined with cytotoxic agents will be subjected to quantitative proteomic analyses to identify metabolism-related proteins, supporting the development of adaptable RNA-based adjuvant therapies targeting cancer metabolism and ultimately improving the efficacy of current anticancer treatments.

**Keywords:** Metabolism, proteomics, therapeutic RNA, cancer, cell death

## **Abstract 22 | Poster | Poster Session Day 1**

### **Addressing LNP Storage Instability Through Optimization of Sucrose Concentration and Storage Temperature**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Júlia Alves Tanjoni; BIANCA BONETTO MORENO GARCIA; RICARDO WEINLICH

**Institution:** Hospital Israelita Albert Einstein, São Paulo (SP), Brazil. — São Paulo-São Paulo

**Introduction:** Lipid nanoparticles (LNPs) have emerged as efficient delivery systems for vaccines and mRNA therapies. However, their instability during storage remains a major challenge for their widespread application. Although freezing is commonly used for preservation, temperature fluctuations and ice crystal formation may compromise nanoparticle integrity and reduce biological activity, highlighting the need for storage conditions capable of preserving both physicochemical properties and functionality. **Objective:** To investigate the effects of sucrose concentration and storage conditions on the physicochemical and functional stability of LNPs. **Methods:** LNPs containing EGFP mRNA were prepared at an N/P ratio of 3 and diluted (1:1 v/v) in a cryoprotective medium composed of Tris buffer (20 mM) and increasing sucrose concentrations (0%, 5%, 8%, and 10%). Samples were stored at different temperatures. Physicochemical characterization was performed by Dynamic Light Scattering (DLS) to determine mean particle size, polydispersity index (PDI), and zeta potential over 28 days. Functionality was evaluated in HEK-293T cells by flow cytometry through transfection efficiency and mean fluorescence intensity (MFI). **Results:** LNPs maintained adequate physicochemical characteristics during refrigerated storage, although differences were observed depending on time and sucrose concentration. Under freezing conditions, formulations containing lower sucrose concentrations showed a marked increase in particle size, suggesting aggregation. In contrast, higher sucrose concentrations demonstrated a partial protective effect on LNP physical stability. Despite this, functionality was significantly impaired after freezing, with a progressive reduction in transfection efficiency throughout storage. Nevertheless, cell viability remained high under all evaluated conditions. **Conclusion:** The results demonstrate that preservation of physicochemical characteristics does not necessarily predict the functional performance of LNPs after storage. Although the addition of 10% sucrose partially reduced aggregation, it was insufficient to preserve mRNA bioactivity. Overall, refrigeration proved to be the most effective storage strategy within the evaluated period, highlighting the need to improve cryoprotection approaches for LNP-based applications.

**Keywords:** Lipid Nanoparticles; mRNA delivery; Cryoprotection; Sucrose

## **Abstract 23 | Poster | Poster Session Day 1**

### **Establishment of Recombinant Viral Vector (NVVR) Core Facility to Support Gene Therapy and Biomedical Research**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Ana Laura Vieira Alves

**Institution:** Cancer Institute of Sao Paulo (ICESP) — São Paulo

Background: Lentiviral vectors are essential tools for stable genetic cell modification and are widely used in functional studies, target validation, development of cellular models, and investigation of disease mechanisms. However, their production and use requires specialized infrastructure, standardized protocols, quality control, and compliance with biosafety regulations. In this context, dedicated core facilities represent an effective strategy to expand access to these technologies while improving experimental reproducibility. Aim: To present the organization, services, and initial performance indicators of the Núcleo de Vetores Virais Recombinantes (NVVR), a core facility that provides technical and scientific support for plasmid preparation, lentiviral and adenoviral vector production, viral titration, and cell modification for research purposes. Methods: The facility was established following standardized technical and regulatory workflows, including an initial consultation with investigators, evaluation of vector-related information, and definition of the requested services and associated costs. Available services include plasmid preparation at different scales, production of non concentrated and ultracentrifuge-concentrated lentiviral vectors, adenoviral vector production, viral titration, and modification of cell lines with viral vectors. All services are performed according to predefined technical criteria, with delivery of a technical data sheet describing the generated product and clear definition of the responsibilities of both the NVVR and the user. The facility operates under research-grade standards and does not perform Good Laboratory Practice (GLP)-compliant activities. Results and Conclusions: Since its implementation, the NVVR has supported 13 research laboratories from FMUSP, USP, UNIFESP, UNESP, as well as biotechnology companies (Celluris and Nicell). More than 50 viral vector production processes, including non concentrated and concentrated lentiviral vectors and adenoviral vectors, have been completed. In addition, approximately 20 different cell lines have been modified using viral vectors with different genes of interest or reporter genes such as luciferase, GFP, tdTomato, and mCherry. The establishment of the facility enabled the standardization of critical steps in viral vector production, centralized biosafety procedures, optimized use of specialized infrastructure, and expanded access to viral vector technologies for research groups without dedicated expertise. Overall, the NVVR strengthens institutional infrastructure for biomedical research by reducing technical and operational barriers and supporting functional studies, cellular models, and translational research based on gene transfer technologies.

**Keywords:** Lentiviral vectors; Core facility; Cell modification

## **Abstract 25 | Poster | Poster Session Day 1**

### **Study of the mechanisms associated with the therapeutic efficacy of mesenchymal stem cell-derived extracellular vesicles against experimental cutaneous leishmaniasis**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Camila Nascimento Fernandez; Douglas Barroso de Almeida; Herbert Leonel de Matos Guedes

**Institution:** Universidade Federal do Rio de Janeiro — Rio de Janeiro-RJ

**Introduction:** Leishmaniasis is a global public health problem caused by pathogens of the genus *Leishmania*. One of the etiological agents involved in the clinical form with the highest incidence in Brazil is the species *Leishmania amazonensis*, responsible for clinical manifestations of the cutaneous form. Previous studies have shown that adipose tissue-derived mesenchymal stem cell (ADSC) therapy and, later, derived extracellular vesicles (ADSC-EVs) have a therapeutic potential for disease improvement, being effective in reducing the progression of lesions in infected C57BL/6 mice. **Objectives:** To better understand the mechanisms involved in these effects, we sought to investigate possible pathways of action of EVs-ADSC in in vitro models, using peritoneal macrophages infected with *L. amazonensis*, and in vivo, using C57BL/6 mice deficient in pro- and anti-inflammatory cytokines. **Methodology:** To perform the in vitro experiments, peritoneal macrophages of C57BL/6 mice were plated on 24-well plates, infected with *L. amazonensis* promastigotes in the proportion of 5 or 10 parasites per macrophage and subsequently treated with ADSC-EVs in quantities of  $1 \times 10^7$ ,  $1 \times 10^8$  or  $1 \times 10^9$  per well. After incubation, the cells were stained and the number of intracellular amastigotes was manually quantified. In addition, nitric oxide (NO) and reactive oxygen species (ROS) levels were evaluated by spectroscopy. In the in vivo assays, C57BL/6 wild-type (WT) mice deficient for IFN- $\gamma$  (IFN- $\gamma$  KO), IL-10 (IL-10 KO), IL-22 (IL-22 KO) and IL-23 (IL-23 KO) were infected intradermally with  $2 \times 10^6$  promastigotes of *L. amazonensis* and treated with three doses of  $1 \times 10^9$  EVs-ADSC by the same route, with a weekly interval between applications. Animals treated with PBS were used as controls. The evolution of the lesions was monitored for approximately six weeks with the aid of a caliper and, at the end, the parasitic loads of the paw, lymph node and spleen were quantified. **Results:** In vitro, a significant reduction in the parasite load was observed in macrophages infected and treated with EVs-ADSC, in a dose-dependent manner. However, this effect did not seem to be associated with increased NO production, but possibly ROS. In vivo, the treated WT, IL-22 KO and IL-23 KO groups showed control of lesion progression, remaining stable for a few weeks, while the PBS groups continued to evolve. In the IFN- $\gamma$  KO group, no differences were observed after treatment. On the other hand, IL-10 KO mice treated with EVs-ADSC showed worsening of the lesion. Despite this, no significant differences were observed in the parasite loads of the organs analyzed. **Conclusion:** Thus, our data suggest that EVs-ADSC acts mainly in the healing of cutaneous leishmaniasis wounds through an immunomodulatory mechanism of action instead of leishmanicidal, through the regulation of cytokines, such as IL-10.

**Keywords:** extracellular vesicles; mesenchymal stem cell; cutaneous leishmaniasis; immunomodulation

## **Abstract 26 | Poster | Poster Session Day 1**

### **From Phenotype to Target: Gene2Edit as a cell-type-agnostic functional genomics platform for target discovery**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Heloísa Berti Gabriel

**Institution:** EditBio Biotechnology LTDA — Ribeirão Preto, São Paulo, Brazil

Despite major advances in biotechnology, the discovery of therapeutically relevant targets remains a major bottleneck in innovative drug development. Disease-associated phenotypes are controlled by complex genetic networks that cannot be fully addressed through candidate-gene approaches alone. Functional genomics, particularly genome-wide CRISPR screening, provides a powerful strategy to systematically identify genes that regulate disease-relevant phenotypes. However, scalable and adaptable screening platforms remain scarce in emerging biotechnology ecosystems, including Brazil. To address this gap, we developed Gene2Edit, a modular, cell-type-agnostic functional genomics platform for phenotype-driven target discovery in mammalian cells. As a proof of concept, we applied Gene2Edit to investigate macrophage polarization and reprogramming in cancer, where immune cell plasticity shapes the tumor microenvironment (TME) and influences therapeutic responses. Tumor-associated macrophages (TAMs) can adopt either pro- or antitumoral functions depending on TME-derived signals, making them attractive targets for immunotherapy. We established an in vitro macrophage polarization and re-polarization model using Cas9-expressing THP-1 cells differentiated into macrophage-like cells and exposed to defined cytokine combinations that mimic key TME conditions. The model enabled phenotypic characterization by flow cytometry and reproduced relevant TAM-like immune states. THP-1-derived macrophages displayed context-dependent modulation of markers associated with immunosuppression and antigen presentation, including TREM2, PD-L1, and HLA-DR, supporting the suitability of this system for functional CRISPR screening. Ongoing R&D efforts include optimization of pilot CRISPR screens, expansion of the platform to primary human monocytes, and validation of candidate targets in physiologically relevant models. By integrating functional genomics, immune phenotyping, and phenotype-driven target discovery, Gene2Edit provides a scalable platform to identify novel therapeutic targets and accelerate early-stage drug discovery. In solid tumors, macrophage reprogramming has the potential to reshape the TME, reduce immunosuppression, enhance antigen presentation, and improve responses to cancer immunotherapy. Developed in Brazil, Gene2Edit strengthens regional biotechnology capabilities while generating globally relevant assets for next-generation therapeutic development. This work is supported by FAPESP (2025/22834-8).

**Keywords:** Macrophages, Gene2Edit, CRISPR-screening, Target Discovery

## **Abstract 27 | Poster | Poster Session Day 1**

### **A CRISPR-Generated HEK293 Cellular Model of Mucopolysaccharidosis type IVA (MPS IVA)**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Náthali Paiva; Andrew Oliveira Silva; Esteban Alberto Gonzalez; GUILHERME BALDO

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Mucopolysaccharidosis type IVA (MPS IVA) is a rare genetic syndrome, inherited in an autosomal recessive manner, caused by mutations in the GALNS gene, which encodes the enzyme N-acetylgalactosamine-6-sulfatase (GalN6S). Deficiency of this enzyme results in the accumulation of glycosaminoglycans (GAG), primarily affecting bone and cartilage tissues, leading to improper development and maturation of these structures and causing postural, mobility, and growth issues. In this context, the present study aimed to establish and characterize a new human cell model, with gene editing of the GALNS gene using CRISPR/Cas9 technology, capable of mimicking the cellular profile of patients with MPS IVA, enabling in vitro morpho-functional and therapeutic studies. The HEK293 cells were nucleofected with the Cas9/gRNA complex, and clonal subpopulations were isolated by Single-Cell Sorting. Gene editing was evaluated by sequencing the amplicon of exon 1 of the GALNS gene, and GalN6S activity was assessed using an enzymatic assay with a fluorogenic substrate. Subpopulations with residual, intermediate, normal enzymatic activity were selected for proliferation analysis (Cumulative Population Doubling, CPD, over 22 days), morphometry (FSC × SSC by flow cytometry), and lysosomal content (LysoTracker Red DND-99). After nucleofection, 70 clonal subpopulations were isolated, from which three clones were selected: one with 74% indel and 96% reduction in GalN6S activity (GALNS-KO), another with 26% indel and 48% enzymatic activity (GALNS-knockdown), and one without editing, with activity close to normal (GALNS-WT). Subsequently, cell proliferation was analyzed using the Cumulative Population Doubling (CPD) assay over 22 days, revealing a reduced proliferative capacity in GALNS-KO and, consequently, an increased population doubling time (1.47 days) compared to the wild-type population (1.02 days). Morphometric characteristics of size and intracellular complexity were also measured via Flow Cytometry, showing increases in both parameters in the knockout clone and a similar, albeit smaller, profile in the GALNS-knockdown compared to the wild-type control. Finally, intracellular acidic environment analysis was performed using LysoTracker™ Red DND-99 staining followed by Flow Cytometry, which showed an increased density of acidic compartments in GALNS-KO and GALNS-knockdown relative to the WT control, a phenomenon consistent with the pathophysiology of MPS IVA. These results show that the edited GALNS-KO clone reproduces key features of MPS IVA, including enzymatic deficiency, reduced proliferation, morphometric alterations, and lysosomal overload, confirming the establishment of a cellular model deficient in GalN6S activity, suitable for more in-depth studies of this disease.

**Keywords:** Gene Therapy, Mucopolysaccharidosis Type IVA (MPS IVA), GALNS, Inborn Errors of Metabolism, CRISPR/Cas9, In Vitro Cell Model, RNP Complex, Gene Knockout.

## **Abstract 29 | Poster | Poster Session Day 1**

### **DEVELOPMENT OF A BIOENGINEERING PLATFORM USING HUMAN MESENCHYMAL CELLS: ANALYSIS OF EXOSOMES AND CELLULAR MORPHOLOGY IN THREE-DIMENSIONAL CULTURE STRATEGIES FOR ADVANCED THERAPIES**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Thais Alessandra dos Santos; Alessandra Alves Thole; Ana Lúcia Rosa Nascimento; Cherley Borba V. de Andrade; Erika Afonso Costa Cortez Marques; Gabriela Santos Oliveira; Genilza Pereira de Oliveira; Isabela de Souza Marques; Simone Nunes de Carvalho; Vitor Silva de Carvalho

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**Introduction:** Mesenchymal stem cells (MSCs) and their small extracellular vesicles, including exosomes, have been investigated in tissue engineering and regenerative medicine due to their immunomodulatory and reparative potential. However, the application of three-dimensional systems and bioprinting requires careful evaluation of cellular morphology and the integrity of the extracellular vesicles produced. **Objective:** Evaluate the morphology of human MSCs subjected to spheroid aggregation and 3D bioprinting, as well as small extracellular vesicles isolated from MSCs, to establish a bioengineering platform for regenerative medicine and advanced therapy products based on MSCs. **Methodology:** MSCs derived from human adipose tissue, approved by the Research Ethics Committee (CAAE: 84423224.3.0000.5259), were obtained by mechanical and enzymatic dissociation and expanded in supplemented medium. After flow cytometry characterization, cells were maintained under conventional two-dimensional culture or aggregated into spheroids using microwell molds. Then, free cells or spheroids were incorporated into the bioink and bioprinted in a gyroid pattern using a BioEnder Termo bioprinter (BioEdTech), followed by crosslinking and culture for up to 5 days. Cellular and spheroid morphology were monitored by phase-contrast optical microscopy. Exosomes were isolated from the secretome of conventional cultures by ultracentrifugation, processed by negative staining, and analyzed using a JEM 1400 FLASH transmission electron microscope (Jeol). **Results:** MSCs maintained under conventional culture conditions exhibited typical fibroblast-like morphology and a phenotypic profile compatible with MSCs, expressing CD73, CD90, and CD105 while being negative for CD45. After spheroid aggregation and bioprinting, constructs containing free cells and spheroids maintained morphological stability throughout the 5-day culture. Bioprinted free cells remained viable and predominantly rounded within the constructs, whereas spheroids preserved their compaction without significant structural loss or expressive cellular migration toward the external limits of the bioprinted matrix. Exosomes exhibited preserved spherical morphology, intact membranes, and relatively homogeneous size distribution, compatible with characteristics described for exosomes and other small extracellular vesicles, validating our isolation protocol. Cell aggregation and bioprinting preserved the structural organization of the three-dimensional models. The maintenance of spheroid compaction and the absence of expressive cellular migration may indicate physical stability of the bioprinted system and adequate cell-matrix interaction. **Conclusions:** The morphological preservation of exosomes isolated using our protocol reinforces the feasibility of using these cultures as a platform for obtaining secretome and exosomes for regenerative medicine and the development of regenerative wound dressings and skin-mimetic constructs.

**Keywords:** 3D bioprinting, rheology, bioink, mesenchymal stem cells, tissue engineering

## Abstract 30 | Poster | Poster Session Day 1

### STANDARDIZATION OF HUMAN ADIPOSE TISSUE COLLECTION AND PROCESSING FOR OBTAINING MESENCHYMAL STROMAL CELLS: A METHODOLOGICAL PROPOSAL FOR APPLICATIONS IN REGENERATIVE MEDICINE

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Thais Alessandra dos Santos; Simone Nunes de Carvalho; Alessandra Alves Thole; Gabriela Santos Oliveira; Genilza Pereira de Oliveira; Marcos Bettini Pitombo; Maria Antonia Oakim Mourão; Maria Lídia de Abreu Silva; Michel Luciano H. T. Vaena; Paula Sampaio de Azevedo; Samuel de Navarro Abreu

**Institution:** Universidade do Estado do Rio de Janeiro (UERJ) — Rio de Janeiro

**Introduction:** Mesenchymal stem cells (MSCs) have been widely explored in regenerative medicine owing to their self-renewal capacity, multipotent differentiation potential, immunomodulatory properties, and secretion of bioactive molecules. Among the available tissue sources, adipose tissue is considered particularly attractive because of its abundance, minimally invasive harvesting, and high cellular yield. Nevertheless, the absence of standardized isolation protocols continues to limit experimental reproducibility and interstudy comparability. **Objective:** To establish a standardized protocol for the isolation of MSCs from human adipose tissue obtained from different surgical sources. **Methodology:** Human adipose tissue samples were collected from surgical procedures performed at Pedro Ernesto University Hospital (HUPE/UERJ), in collaboration with the Departments of General Surgery and Plastic Surgery, following approval by the Research Ethics Committee (CAAE: 84423224.3.0000.5259). Samples obtained from hernioplasty, lipoma excision, and reduction mammoplasty were transported in DMEM/F-12 supplemented with antibiotics. Following tissue weighing, samples underwent mechanical and enzymatic dissociation using 0.2% type II collagenase (1 mL/g tissue), followed by incubation at 37 °C under orbital agitation for 45 min. After enzyme neutralization, the cell suspension was filtered, centrifuged, and the resulting pellet was resuspended for in vitro expansion. **Results:** Sixteen human adipose tissue samples were obtained from hernioplasty (n=5), lipoma excision (n=8), and reduction mammoplasty (n=3). Donors were 29–54 years old, with body mass index ranging from 23.6 to 30.0 kg/m<sup>2</sup>, and tissue samples weighing between 0.546 and 12.907 g. Cell yield varied according to the surgical source. Hernioplasty samples yielded approximately  $9.1 \times 10^5$  nucleated cells/g of tissue, whereas lipoma and reduction mammoplasty samples yielded  $1.5 \times 10^6$  and  $1.6 \times 10^6$  nucleated cells/g, respectively. Immunophenotypic characterization demonstrated CD73 and CD90 expression ranging from 78% to 100%, together with the characteristic fibroblast-like morphology expected for MSCs. **Conclusions:** The observed variability highlights the influence of the surgical source and initial tissue processing on cell yield. Nevertheless, the proposed protocol enabled efficient isolation and in vitro expansion while preserving the morphological characteristics and phenotypic profile of MSCs, indicating that its standardization may contribute to greater experimental reproducibility and support future translational applications. **Acknowledgements.** Funding: Capes, CNPq, and Faperj.

**Keywords:** mesenchymal stem cells; MSCs, adipose tissue; methodology; regenerative medicine.

## Abstract 31 | Poster | Poster Session Day 1

### PRODUCTION AND EVALUATION OF A NOVEL YEAST-DERIVED RECOMBINANT IDURONATE-2-SULFATASE (I2S)

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Natalia Cavallari Novakowski

**Institution:** Hospital de Clínicas de Porto Alegre - Universidade Federal do Rio Grande do Sul - Pontifícia Universidad Javeriana — Porto Alegre - Rio Grande do Sul

Mucopolysaccharidosis type II, or Hunter syndrome, is an X-linked disorder caused by pathogenic variants in the IDS gene, leading to deficient iduronate-2-sulfatase activity and progressive accumulation of dermatan sulfate and heparan sulfate. Although enzyme replacement therapy with idursulfase is available, its high cost, chronic intravenous administration, limited tissue distribution, and poor ability to cross the blood-brain barrier, support the development of alternative recombinant enzymes. This study evaluated a novel recombinant iduronate-2-sulfatase produced in the yeast *Komagataella phaffii*, named prIDS, as a potential alternative enzyme replacement therapy for Hunter syndrome. prIDS was tested in IDS-deficient HEK293 cells created using CRISPR-Cas technology and in IDS-knockout mice after intravenous administration, using idursulfase as a reference enzyme. IDS activity, glycosaminoglycan accumulation, lysosomal alterations, cellular uptake, serum activity, and tissue biodistribution were analyzed. In vitro, IDS-deficient cells showed markedly reduced IDS activity ( $p < 0.0001$ ), confirming the cellular model. Treatment with prIDS significantly increased intracellular IDS activity compared with untreated MPS II cells ( $p < 0.001$ ), although idursulfase induced higher activity than prIDS at the same concentration ( $p < 0.001$ ). prIDS reduced glycosaminoglycan accumulation in IDS-deficient cells ( $p < 0.01$ ), while idursulfase produced a stronger reduction ( $p < 0.0001$ ). LysoTracker analysis showed increased fluorescence in untreated MPS II cells compared with wild-type cells ( $p < 0.01$ ), indicating lysosomal alterations. Both prIDS and idursulfase reduced LysoTracker fluorescence compared with untreated MPS II cells, suggesting restoration of lysosomal homeostasis ( $p < 0.01$  and  $p < 0.004$ , respectively). Cellular uptake assays showed that mannose reduced prIDS internalization by approximately 60%, while mannose-6-phosphate (M6P) almost completely blocked enzyme uptake, suggesting internalization predominantly by M6P receptor. In vivo, prIDS showed detectable IDS activity in several organs of IDS-knockout mice, including liver, spleen, kidney, lung, heart, and brain. Compared with idursulfase, prIDS presented a distinct biodistribution profile. Idursulfase showed higher activity in heart and liver, whereas prIDS was detected in tissues relevant to Hunter syndrome pathophysiology, including brain, kidney, spleen, and lung. Yeast-derived prIDS was functionally active, internalized by IDS-deficient cells mainly through the mannose-6-phosphate receptor pathway, reduced glycosaminoglycan accumulation, improved lysosomal alterations, and reached relevant tissues in vivo. These findings support prIDS as a promising alternative recombinant enzyme for Hunter syndrome and justify further preclinical studies to evaluate its long-term therapeutic efficacy and safety.

**Keywords:** idursulfase. Hunter. Mucopolysaccharidosis type II. lysosomal. biodistribution. receptor. mice.

## Abstract 32 | Poster | Poster Session Day 1

### Development of a qPCR Method for Quality and Safety Assurance of Non-Viral CAR-T Cell Products

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** SABRINA ALVES DOS REIS; ANA LETICIA DE SOUZA GUTIERREZ; ARTHUR DA SILVA NEUMANN; DANIELA TUPY DE GODOY MAZZA; EMMANUEL ARTHUR ALBUQUERQUE ARAGAO; LÍVIA PIMENTEL DE SANT'ANA DOURADO; LUIZA DE MACEDO ABDO; MARTÍN BONAMINO

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CAR-T cell immunotherapy has transformed cancer treatment, yet conventional manufacturing remains limited by the high cost and complexity of viral vectors. To address these limitations, our group has optimized a non-viral approach utilizing the Sleeping Beauty (SB) transposon system via plasmid electroporation, offering a safer and more economically viable alternative. For clinical translation, regulatory agencies mandate stringent quality control, particularly the quantification of Vector Copy Number (VCN) within the host genome to ensure product safety. Quantitative PCR (qPCR) stands out as a robust and precise methodology for tracking and quantifying these integrated sequences. To meet this demand, we established and developed a qPCR assay designed for vector copy number determination, optimizing the quality control assessment of non-viral anti-CD19 CAR-T cells generated through the Sleeping Beauty system. To achieve this, CAR-T cells were produced by electroporating T cells with the PT4-19BBz transposon and SB100X transposase plasmids. Following genomic DNA extraction, qPCR amplification was performed targeting the FMC63 scFv transgene. Specific primers and probes were designed to evaluate amplification efficiency, linearity, and correlation coefficient ( $R^2$ ) in a multiplex reaction. To prevent overestimation caused by non-integrated plasmids, enzymatic digestion with DpnI and EcoRI was evaluated. Final data were analyzed to determine the accuracy and reproducibility of the method. As result of these evaluations, the developed qPCR assay successfully amplified the FMC63 scFv target sequence from the genomic DNA of SB-engineered CAR-T cells. Standard curves exhibited strong linearity, with correlation coefficients  $R^2 > 0.98$  and efficiency of both curves (FMC63 CAR gene and RNase P reference gene) between 90% e 110%, confirming optimal quantification performance. While amplification efficiency remained consistent across replicates, initial assessments indicated that residual episomal plasmid DNA could inflate VCN values. The integration of DpnI and EcoRI enzymatic digestion step successfully eliminated this non-integrated plasmid background, refining the assay's precision for integrated copies. Consequently, this developed assay allows for accurate and reproducible VCN determination. In conclusion, this work highlights that we successfully developed a qPCR-based framework for the detection and quantification of integrated transgene copies in non-viral CAR-T cell products. The preliminary evaluation demonstrated robust analytical performance and linearity. Incorporating enzymatic digestion ensures precise vector copy number assessment, directly aligning the platform with strict regulatory standards. This methodology represents an indispensable, cost-effective tool for the routine quality assurance and safety profiling of CAR-T cells manufactured via the Sleeping Beauty system.

**Keywords:** CAR-T cells; Vector copy number; Quality control

## **Abstract 33 | Poster | Poster Session Day 1**

### **Evaluation of the Administration Sequence and Dosage of Adenoviral Vectors Encoding p14ARF and IFN- $\beta$ in Tumor Cell Lines.**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Giovanna Lagonegro Amanatidis; BRYAN ERIC STRAUSS; Fernanda Antunes

**Institution:** Instituto do Câncer do Estado de São Paulo (ICESP) — São Paulo - SP

Cancer is characterized by the uncontrolled proliferation of cells, which generally exhibit metastatic potential, leading to high mortality<sup>1</sup>. Despite the availability of numerous therapeutic approaches, the development of new treatment strategies remains necessary<sup>2</sup>. Our laboratory has developed an immunotherapy based on the gene transfer of p14ARF and interferon- $\beta$  (IFN- $\beta$ ) using improved adenoviral vectors. Previous studies have demonstrated that this combination induces tumor cell death while also activating an antitumor immune response, with p14ARF gene transfer sensitizing cells to the effects of IFN- $\beta$ <sup>3</sup>. However, these studies have consistently employed two vectors administered simultaneously at a 1:1 ratio, without investigating whether the therapeutic response could be enhanced by alternative vector ratios or sequential gene delivery. We hypothesize that, due to the secretion of IFN- $\beta$ , this vector may be administered at a lower dose than the p14ARF-encoding vector. Furthermore, delivering p14ARF first may sensitize cells to subsequent treatment with the IFN- $\beta$ -encoding vector. The aim of this project is to optimize the use of these vectors by modifying their ratio and delivery sequence to maximize tumor cell death. The cell lines used were HCT116 (human colorectal cancer) and UACC62 (human cutaneous melanoma). The techniques employed included viral transduction, luciferase reporter assay, cell death analysis (Sub-G1 and propidium iodide (PI) exclusion), and RT-qPCR assays. This study began with the optimization of the multiplicity of infection (MOI), revealing a dose-dependent response in which an MOI of 50 using the GFP-encoding vector resulted in the transduction of 77% of HCT116 cells. When the luciferase-encoding vector was used (MOI 50 for HCT116 and 200 for UACC62), reporter activity reached  $6.7 \times 10^4$  RLU and  $2 \times 10^4$  RLU for HCT116 and UACC62, respectively. At the selected MOI for each cell line, transduction-associated toxicity did not exceed 22% for HCT116 and 7% for UACC62. Treatment with the combination of p14ARF and IFN- $\beta$  (1:1 ratio, simultaneous delivery) resulted in increased induction of cell death (14% and 12% for HCT116 and UACC62, respectively). The results obtained suggest that the HCT116 cell line is not compatible with the objectives of this study, as vector-associated toxicity outweighs the potential therapeutic effects of the treatments. In contrast, the UACC62 cell line showed lower toxicity induced by the vector, allowing for a more accurate evaluation of the therapeutic effects of p14ARF and IFN- $\beta$ . Additional cell lines will be evaluated to obtain more robust results.

**Keywords:** Gene therapy, p14ARF, IFN- $\beta$ , Colorectal cancer, Melanoma

## **Abstract 35 | Poster | Poster Session Day 1**

### **Bioengineering the retinal neurovascular unit using human corneal stromal stem cell-derived RGC-like cells: insights into in vitro modeling of diabetic retinopathy**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Artur Christian Garcia da Silva

**Institution:** Universidade Federal de Goiás — Goiânia - GO

Diabetic retinopathy (DR) is a neurovascular disease in which retinal neuronal dysfunction and endothelial activation occur early and contribute to disease progression. Human-relevant in vitro platforms integrating both compartments are needed to investigate pathogenic mechanisms and support preclinical screening of regenerative strategies. This study aimed to develop a bioengineered co-culture model of the retinal neurovascular unit by combining retinal ganglion cell-like (RGC-like), obtained from human corneal stromal stem cell (hCSC), with human umbilical vein endothelial cells (HUVECs) under diabetic-like inflammatory conditions. Neuronal commitment of hCSC was assessed by immunofluorescence for  $\beta$ 3-tubulin, BRN3A, NeuN, and Pax6 after differentiation. HUVECs were exposed to diabetic medium containing 33 mM glucose, IL-6, and TNF- $\alpha$  and evaluated by MTT, LDH, tube formation, migration, ICAM-1 expression, cytokine secretion, and active caspase-3. Differentiated hCSC-derived progenitors were also exposed to diabetic conditions and evaluated for cytokine secretion and active caspase-3 expression. For the bioengineered model assembly, hCSCs were differentiated in the apical compartment of transwell inserts, and HUVECs were seeded in the basolateral compartment after 14 days. The co-culture was maintained for seven days in harmonized medium and characterized by Live/Dead assay and immunofluorescence. hCSCs acquired neuronal-like morphology and showed increased  $\beta$ 3-tubulin, BRN3A, and NeuN expression, with reduced Pax6 after 14 days, supporting differentiation toward an RGC-like phenotype. HUVECs exposed to diabetic medium remained viable and did not show increased active caspase-3, but developed endothelial dysfunction characterized by impaired tube formation, increased migration, ICAM-1 overexpression, and increased IL-6 and IL-8 secretion. In contrast, hCSC-derived neuronal progenitors displayed increased IL-6 and IL-8 secretion and higher active caspase-3 expression, suggesting early neuroinflammatory and apoptotic responses. During model standardization, neuronal progenitors remained predominantly viable in transwell inserts between 21 and 28 days, defining a suitable window for DR induction. The final co-culture remained viable with preserved apical and basolateral compartments, showing  $\beta$ 3-tubulin+ and BRN3A+ neuronal cells and CD31+ endothelial cells. Preliminary results also demonstrated that the co-culture model also increased the production of IL-6 and IL-8 after being exposed to diabetogenic conditions. These findings demonstrate the feasibility of a hCSC-derived bioengineered neurovascular retinal model that reproduces key early features of DR, including endothelial inflammatory dysfunction and neuronal susceptibility to diabetic injury. This platform may support mechanistic studies of neurovascular damage and preclinical screening of Advanced Therapy Medicinal Products for retinal diseases.

**Keywords:** Diabetic retinopathy; neurovascular unit; tissue engineering; corneal stromal stem cells; retinal ganglion cell-like cells; in vitro model.

## Abstract 36 | Poster | Poster Session Day 1

### Unlocking preserved donor corneas as a source of functional stromal stem cells for ocular cell therapy

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Artur Chistian Garcia da Silva

**Institution:** Universidade Federal de Goiás — Goiânia - GO

Stem cell therapies based on human corneal stromal stem cells (hCSCs) represent a promising approach for ocular diseases therapy. However, the operational reliance on fresh versus stored corneal tissue dictates downstream functional consistency. This study evaluated the feasibility, phenotype, and functional kinetics of hCSCs extracted via cellular migration from human donor explants, establishing a direct comparative baseline between fresh and preserved tissues. Explant-derived hCSCs were successfully isolated through cellular out-migration, resulting in high cell yields suitable for downstream expansion and characterization. Phenotypic profiling via flow cytometry confirmed a robust mesenchymal signature, with high marker expression for CD90 (98.7%–99.9%) and CD105 (99.1%–99.9%), alongside variable CD73 (50.6%–87.5%), neural/ocular markers (Nestin, Pax6,  $\beta$ III-Tubulin), and minimal hematopoietic background (CD45 <0.5%). Functional proliferation tracking via CFSE over 120 hours demonstrated a statistically significant reduction in population doubling time for preserved corneas ( $14.89 \pm 0.96$  h) compared to fresh corneas  $17.24 \pm 1.47$ h;  $p = 0.0101$ ). Clonogenic capacity differed significantly ( $p = 0.0077$ ), with preserved tissues showing higher macroscopically stained colony counts ( $14.39\% \pm 33.03\%$ ) versus fresh explants ( $9.07\% \pm 14.85\%$ ). Propidium iodide cell-cycle analysis revealed stable replication kinetics with no significant phase distribution variation  $p > 0.05$ . Paracrine profile mapping via ELISA on 30-fold concentrated culture supernatants tracked neuroprotective mediators. In fresh corneas, baseline outputs reached  $73.02 \pm 27.03$   $\mu$ g/mL for Serpin F1,  $13.35 \pm 3.75$  ng/mL for BDNF,  $16.44 \pm 0.28$  ng/mL for CNTF, and  $280.00 \pm 56.57$  pg/mL for  $\beta$ -NGF. Overall, cellular out-migration enabled the isolation of viable hCSCs from both fresh and preserved human corneal explants. Preserved tissues retained cell-cycle stability and showed favorable proliferative and clonogenic features. These findings support the use of preserved donor corneal tissues as a feasible source of hCSCs for ocular cell engineering and preclinical regenerative medicine applications.

**Keywords:** Cell proliferation; Clonogenicity; Flow cytometry; Secretome.

## Abstract 38 | Poster | Poster Session Day 1

### Age-Dependent Progression of the Behavioral Phenotype in a Murine Model of Calpain-1 Deficiency (SPG76): Identifying Potential Therapeutic Windows.

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** ISABELA CAVALCANTE SCHAMES; Graciela Luana Ruidiaz Correa; GUILHERME BALDO; Jonas Alex Morales Saute; Melaine Maiara da Silva Lopes; Nevton Teixeira da Rosa Junior; Willian Carlos Côrrea Padilha

**Institution:** Universidade Federal do Rio Grande do Sul — Porto Alegre- RS

Hereditary spastic paraplegias (HSPs) comprise a group of 93 heterogeneous rare disorders characterized by progressive degeneration of the corticospinal tract, muscle weakness, lower-limb spasticity, and consequent impairment of locomotion. Spastic paraplegia type 76 (SPG76) is caused by biallelic variants in the CAPN1 gene, resulting in loss of function of the protease calpain-1. There are limited reports describing the phenotype of Capn1 knockout (Capn1<sup>-/-</sup>) mice, and such investigations are essential to advance the understanding of disease pathophysiology and to support the development of disease-modifying therapies, including gene therapy. Our aim was to characterize the natural history of SPG76 in Capn1<sup>-/-</sup> mice to identify therapeutic intervention windows. Wild-type (WT) and Capn1<sup>-/-</sup> mice were evaluated using behavioral tests at 2 months of age (WT: n = 16; Capn1<sup>-/-</sup>: n = 9) and 6 months of age (WT: n = 16; Capn1<sup>-/-</sup>: n = 4). In the gait test, 2-month-old Capn1<sup>-/-</sup> mice exhibited early differences in forepaw stride length (p = 0.035) and forepaw step length (p = 0.041), with significantly increased values compared with WT mice. At later ages, these parameters showed a change in trajectory, with lower mean values relative to baseline, although these differences did not reach statistical significance. Open-field analysis revealed significant differences at 2 months in total immobility time (p = 0.004), total number of immobility episodes (p = 0.005), total distance traveled (p = 0.018), number of center-zone entries (p = 0.047), and grooming time (p = 0.048). Capn1<sup>-/-</sup> mice also showed a trend toward lower average speed (p = 0.061), consistent with a less active behavioral phenotype. At 6 months, no statistically significant differences were detected between groups, possibly due to the reduced sample size. However, Capn1<sup>-/-</sup> mice showed trends toward shorter total distance traveled (p = 0.077) and lower average speed (p = 0.062). No significant differences were observed in rotarod performance between WT and Capn1<sup>-/-</sup> mice at either 2 or 6 months of age. Together, these findings indicate an early, age-dependent behavioral phenotype in Capn1<sup>-/-</sup> mice. A comprehensive temporal characterization of these behavioral phenotypes will provide a foundation for defining therapeutic windows and for evaluating phenotypic rescue in future preclinical studies.

**Keywords:** Spastic paraplegia type 76; Natural history; Murine Model; Behavioral Phenotype; Therapeutic windows.

## Abstract 39 | Poster | Poster Session Day 1

### Development of Non-viral anti-CLDN18.2 CAR-T Cells for Gastric Cancer

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** JOÃO VITOR STEIMBACH; EDUARDO MANNARINO CORREIA; MARTÍN BONAMINO; Paulo Pimentel de Assumpção

**Institution:** Instituto Nacional de Câncer — Rio de Janeiro-RJ

According to the World Health Organization, gastric cancer (GC) is the fifth most common malignancy and one of the leading causes of cancer-related deaths worldwide. In Brazil, its incidence is heterogeneous, being highly incident in the Southern and Northern regions. Although curative treatment relies on gastrectomy combined with chemotherapy and monoclonal antibody-based immunotherapy, the prognosis of advanced-stage patients remains poor. Cellular therapies (CT), including tumor-infiltrating lymphocytes (TIL) and, more recently, anti-Claudin 18.2 CAR-T cells have been investigated for advanced GC. Claudin 18.2, a tight junction protein whose extracellular epitope becomes exposed predominantly in malignant gastric tissues, represents an attractive target for cellular immunotherapy. Anti-Claudin 18.2 CAR-T cells were recently approved for the treatment of CLDN18.2-positive gastric cancer, representing the first CAR-T product approved for solid tumors. However, as reported in a phase-2 clinical trial, anti-Claudin 18.2 CAR-T cells exhibit limited persistence and expansion, reaching peak levels up to tenfold lower than those observed in B-cell acute lymphoblastic leukemia (B-ALL). We hypothesize that these suboptimal outcomes are driven by T cell exhaustion and antigen loss within the tumor microenvironment. So, this project aims to develop CT products derived from either healthy donor lymphocytes or TIL from advanced-stage patients, capable of inducing robust and sustained antitumor responses. For this, Anti-Claudin 18.2 CAR-T cells were generated from healthy donor peripheral blood using either the SB100X transposase system or CTLA4-targeted knock-in mediated by Cas9. Their antitumor activity was assessed in vitro against the AGS GC cell line. In parallel, TIL were isolated from 400 mL of ascitic fluid obtained from an advanced GC patient and expanded in vitro for 26 days in the presence of high-dose IL-2, followed by immunophenotypic characterization. Anti-Claudin 18.2 CAR-T cells were produced with FDA-compliant efficiency using both non-viral vector approaches. These cells demonstrated potent cytotoxic activity, eliminating tumor cells at effector-to-target ratios as low as 1:2. Regarding TIL expansion, 800,000 cells were initially isolated and expanded to approximately 75 million cells after 26 days. Expanded TIL displayed a highly functional effector memory phenotype (CD45RO+/CD95+) with low expression of exhaustion markers (PD1-/LAG3-). Collectively, these findings demonstrate the successful preclinical development of two scalable, non-viral CT platforms targeting GC. The use of transposon- and knock-in-based strategies may contribute to reducing manufacturing complexity and cost. Furthermore, the robust expansion and functional profile of patient-derived TIL support future strategies combining both approaches, including the generation of CAR-T cells from TIL to enhance persistence and antitumor efficacy in solid tumors.

**Keywords:** CAR-T; GASTRIC CANCER; CELL THERAPY; INHIBITORY RECEPTORS

## **Abstract 40 | Poster | Poster Session Day 1**

### **Development and Characterization of a Recombinant Zika Virus Expressing a Suicide Gene to Enhance Oncolytic Activity Against Glioblastoma**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Carolina Filgueiras Sanchez; Elibio Leopoldo Rech Filho; Enzo Oliveira Barone; Fábio Monteiro; Luiza Higa; Rayane Nunes Lima

**Institution:** Universidade Federal do Rio de Janeiro — RJ

Glioblastoma (GBM) is a highly aggressive primary brain tumor characterized by marked cellular heterogeneity, diffuse invasion, tumor microenvironment immunosuppression, and high resistance to therapies. At the core of this aggressiveness are glioblastoma stem cells (GSCs), which are responsible for tumor maintenance and disease recurrence. As a promising alternative, oncolytic virotherapy utilizes Zika virus (ZIKV) due to its natural tropism for neural progenitors and GSCs, enabling selective cellular destruction. To enhance this action, the insertion of a suicide gene (SG) into ZIKV emerges as a strategy to convert prodrugs into their active, cytotoxic metabolites restricted to infected cells within the tumor microenvironment. The expression of this gene induces localized cell death and subsequent tumor lysis, releasing antigens and danger signals capable of activating the immune system and reversing microenvironmental immunosuppression. The objective of this study was to develop and characterize a recombinant ZIKV strain expressing an SG to expand antitumor activity against GBM. The recombinant infectious clone (rZIKV-SG) was constructed via Gibson Assembly, followed by cellular transfection. Assembly efficiency was evaluated by RT-qPCR, and viral titer was determined by plaque assay. Synthetic virus assembly was confirmed with optimal molecular parameters, displaying a Ct of 14 and a quantification of  $8,8 \times 10^{10}$  genome copies/mL. During rZIKV-SG passaging, a marked cytopathic effect (CPE) was observed starting at 3 days post-infection (dpi), confirming the genomic stability and replicative competence of the recombinant virus. In conclusion, preliminary data confirm the successful construction of rZIKV-SG. This step overcomes the primary methodological bottleneck of the project, generating a viable, replication-competent virus capable of inducing a robust cytopathic effect. The optimal synthetic assembly parameters (Ct of 14 and  $8,8 \times 10^{10}$  RNA copies) enable the subsequent steps of the research, which include confirming construct stability via Sanger sequencing, characterizing replicative kinetics and lysis assays, as well as evaluating cell viability across different cell lines and mechanisms of death (apoptosis/necrosis) to determine oncolytic selectivity in glioblastoma.

**Keywords:** Glioblastoma; Zika virus; Oncolytic virotherapy; Suicide gene; Recombinant virus; Gene therapy.

## **Abstract 41 | Poster | Poster Session Day 1**

### **Yeast *S. cerevisiae* mitochondria transplantation enhances retinal oxidative phosphorylation and protects rats from glaucomatous neurodegeneration**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** CAIO MOTA MACHADO; CLAUDIO AKIO MASUDA; HILDA PETRS SILVA; MARIANA SANTANA DIAS; RAFAEL FREITAS AZEVEDO; RAFAEL LINDEN; THAÍS COSTA PORTO MARINHO; VICTOR GUEDES DE ARAUJO

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Mitochondrial transplantation consists in the systemic or local delivery of functional mitochondria to a tissue or cell under stress (Brestoff et al., 2025). This approach has been shown to be effective in animal models of ischemic insult, neurodegeneration and cancer (Li et al., 2025). Also, it has been successfully tested in pediatric cardiac patients with major ischemic heart lesions (Emani & McCully, 2018; Guariento et al., 2021). Despite the recent success, the transplant is expensive, laborious, and dependent on a donor to obtain mitochondria. We propose yeast *Saccharomyces cerevisiae* mitochondria as a potential substitute. Yeast mitochondria are cheap to obtain, scalable, and can be stored frozen for future use. We showed that Yeast mitochondria are internalized by human embryonic kidney cells (HEK293) and rat retina cells. The uptake leads to an increase in phosphorylative oxidation in both cases, without alterations in retinal mitochondria ROS production. Yeast mitochondria transplantation didn't induce major inflammatory response or vascular alterations observed by eye funduscopy. Furthermore, mitochondria transplantation protects retinal ganglion cells from cell death induced by high intraocular pressure in rats and preserves retinal function, as observed by pattern electroretinography. This data demonstrates that yeast mitochondria transplantation protects retinal ganglion cells from glaucomatous neurodegeneration and could be a low-cost potential substitute for human-derived mitochondria in transplantation.

**Keywords:** Mitochondria, transplant, glaucoma, yeast

## **Abstract 43 | Poster | Poster Session Day 1**

### **MORPHOMETRIC AND CELL VIABILITY ASSESSMENT OF ALGinate SPHERES ASSOCIATED WITH BIOLOGICAL MATRICES**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Gabriel Brito Ribeiro

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Cell encapsulation in three-dimensional matrices may contribute to cell retention, protection, and maintenance of viability, while also promoting the sustained release of molecules of therapeutic interest. The physicochemical and structural properties of the matrices used for encapsulation may influence both sphere morphology and the survival of encapsulated cells. This study evaluated the morphometry and cell viability of alginate spheres associated with chondroitin sulfate (CS), porcine placental hydrogel (PPH), and bovine corneal hydrogel (BCH), aiming to identify suitable formulations for further cell encapsulation studies. Alginate spheres at 2.5%, combined with supplementary matrices at different concentrations, were produced by ionic crosslinking and controlled extrusion using a 26G needle at a flow rate of 0.04 mL/min. Dimensional characterization was performed using ImageJ software by calculating the equivalent circular diameter from the area of the spheres. Cell viability was assessed by High-Content Analysis using the Live/Dead assay. Morphometric analysis revealed dimensional differences among the formulations. Compared with the control group ( $0.238 \pm 0.010$  mm), the addition of 1% BCH increased the mean diameter to  $0.323 \pm 0.049$  mm and resulted in greater measurement dispersion. Increasing the BCH concentration to 10% reduced the mean diameter to  $0.249 \pm 0.021$  mm, bringing it closer to that of the control group. The 10% PPH and 1% CS formulations showed mean diameters of  $0.285 \pm 0.010$  mm and  $0.287 \pm 0.012$  mm, respectively. All evaluated conditions maintained cell viability above 86%, with the highest value observed in the 10% BCH group (94.8%). The incorporation of supplementary matrices into alginate influenced the dimensional characteristics of the spheres. Among the evaluated conditions, the formulation containing 10% BCH showed a diameter close to that of the control group and the highest percentage of viable cells, supporting its evaluation in future studies on cell functionality and therapeutic factor release.

**Keywords:** alginate; cell encapsulation; cell viability; biomaterials; biological matrices.

## Abstract 44 | Poster | Poster Session Day 1

### Effect of Molecular Architecture and pH Responsiveness on the Interfacial Behavior of Chitosan Derivatives

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Gabriel Jose De Carli; Dayane dos Santos Alvares; MARCIO JOSÉ TIERA

**Institution:** IBILCE - UNESP — São José do Rio Preto - SP

In non-viral gene therapy, endosomal escape is a major barrier to polymer-based delivery systems. Chitosan is often presented as promising material due to its biocompatibility, low toxicity, and biodegradability, whereas some limitations are low solubility at neutral pH and poor endosomal escape. Chemical architecture and pH sensitivity are believed to be key factors governing endosomal escape; therefore, in this work, we aimed to investigate how chitosan derivatives featuring different pH sensitivities and molecular architectures interact with lipid monolayers using a Langmuir monolayer model. Chitosan derivatives were synthesized from Sigma-Aldrich low-molecular-weight chitosan by grafting diethylaminoethyl (DEAE), resulting in derivatives with 12, 26, and 34% DEAE (D1, D2, and D3, respectively). Each derivative underwent degradation to shorten its chain length (OD1, OD2, and OD3), and OD3 was grafted with lauric acid, resulting in derivatives with 5% (PD5) and 10% (PD10) substitution degrees. Chitosan derivatives were characterized by  $^1\text{H}$  NMR and SEC-MALS to determine substitution degrees and molecular masses, respectively. Potentiometric and conductometric titrations were performed to determine degree of ionization ( $\alpha$ ). Langmuir monolayer compressions were performed using a KSV NIMA equipped with a Wilhelmy plate at  $25 \pm 1$  °C. Tertiary zwitterionic (POPC:SM:Chol) and quaternary anionic (POPC:SM:Chol:POPS) lipid mixtures were compressed subphases consisting of PBS buffer, pH 7.4 (zwitterionic, ZW), PBS buffer, pH 6.3 (anionic, EE), or acetate buffer, pH 5.5 (anionic, LE) and chitosan derivative (30 mM) at a rate of 7 mm<sup>2</sup>/min. Surface pressure-area ( $\pi$ -A) isotherms and compression modulus ( $\text{Cs}^{-1}$ ) were measured. Chitosan derivatives showed a positive correlation between the presence of DEAE side chains and degree of ionization. In ZW isotherms (tertiary lipid mixture in PBS, pH 7.4), high-molecular-weight derivatives promoted greater monolayer expansion at lower surface pressures and increased monolayer rigidity; however, they were expelled back into the subphase as the monolayer was compressed. In contrast, chitosan oligomers (ZW) did not promote early monolayer expansion but remained interspersed at higher surface pressures, increasing monolayer fluidity. Hydrophobically modified chitosan showed a hybrid response, expanding the monolayer while increasing its rigidity. As pH decreased (EE and LE), all derivatives promoted greater monolayer expansion. Furthermore, for the most highly ionized and hydrophobic derivative, monolayer rigidity increased as pH decreased. These results suggest that chitosan acts as a potential biomembrane disruptor. Moreover, chitosan pH sensitivity, chain length, and hydrophobicity influence its interfacial behavior, enabling it to remain interspersed within the membrane and alter its physical properties in a context relevant to non-viral gene therapy.

**Keywords:** Chitosan, Langmuir Monolayer, Endosomal escape, POPC, Cholesterol, Sphingomyelin, POPS

## **Abstract 45 | Poster | Poster Session Day 1**

### **Integrin $\beta 4$ of Retinal Pigment Epithelial (ARPE-19) Cells Mediates the Interaction with Fibrin Scaffolds for Retinal Regeneration: An Experimental and Computational Approach**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Pedro Henrique dos Santos Dantas; Alice Tâmara de Carvalho Lopes; Ana Karulline Garcia Ungaratti; Juliana Moraes Dias; Marize Campos Valadares; Thiago de Andrade Silva Santana Lima

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The retina is the neurosensory tissue responsible for converting light energy into electrical impulses, which are transmitted to the brain through the optic nerve, enabling vision. However, diseases such as diabetic retinopathy, age-related macular degeneration, and retinitis pigmentosa can impair this structure, leading to irreversible damage and permanent vision loss. In this context, strategies aimed at promoting retinal pigment epithelium (RPE) regeneration have emerged as promising therapeutic approaches for these disorders. Among these, cell-based therapies combined with platelet-rich fibrin (PRF) biomembranes have shown potential to promote the morphofunctional restoration of retinal tissue in refractory macular holes and macular degeneration. Cell adhesion to fibrin scaffolds depends on the interaction between integrins and extracellular matrix components. ARPE-19 cells, widely used as a human RPE model, exhibit high expression of integrin  $\beta 4$  (ITGB4). However, the molecular mechanisms through which this integrin mediates cell adhesion to fibrin remain poorly understood. Therefore, this study investigated the role of ITGB4 in the interaction between ARPE-19 cells and fibrin scaffolds using computational and in vitro experimental approaches. Initially, molecular docking was performed between ITGB4 and the  $\alpha$ ,  $\beta$ , and  $\gamma$  fibrin chains. Subsequently, fibrin scaffolds were prepared from human plasma and seeded with ARPE-19 cells at a density of  $2 \times 10^5$  cells for 24 hours. At the end of the culture period, cell viability was assessed using the LIVE/DEAD™ Fixable Dead Cell Stain assay. Molecular docking results demonstrated high binding affinity of ITGB4 for the  $\alpha$  and  $\beta$  fibrin chains, with Gibbs free energies of  $-157.98$  kcal/mol and  $-167.22$  kcal/mol, respectively. In contrast, no significant interaction was observed with the  $\gamma$  chain, suggesting that cell adhesion occurs predominantly through the  $\alpha$  and  $\beta$  fibrin chains. Cell viability assays revealed high survival of ARPE-19 cells on the scaffolds, with a predominance of viable cells and a low frequency of dead cells. Collectively, these findings demonstrate that ITGB4 plays a key role in the adhesion of ARPE-19 cells to fibrin scaffolds, contributing to cell attachment and maintenance of cell viability. In addition to elucidating the molecular mechanisms underlying the interaction between retinal pigment epithelial cells and fibrin-based biomaterials, this study provides a foundation for the rational design of biomaterials capable of modulating cellular responses, enhancing tissue-biomaterial integration, and improving retinal tissue engineering and regenerative medicine strategies.

**Keywords:** Retinal pigment epithelium; ARPE-19 cells; Integrin  $\beta 4$ ; Fibrin scaffolds; Retinal regeneration.

## **Abstract 46 | Poster | Poster Session Day 1**

### **Evaluation of the immunomodulatory effect of extracellular vesicles derived from hUCMSCs on human monocytes and neutrophils**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Caio Marques Landeiro Torres

**Institution:** Federal University of Bahia (UFBA), Gonalo Moniz Institute (IGM/FIOCRUZ-BA) — Salvador, BA

**Background:** Extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) exhibit low immunogenicity and strong immunomodulatory potential. EVs are thus considered strong candidates for the treatment of inflammatory diseases. We previously investigated the therapeutic capacity of hUCMSC-EVs to modulate lesion development in experimental Cutaneous Leishmaniasis caused by *Leishmania braziliensis*, a disease characterized by the presence of inflamed ulcers in the skin. In the present work, we aim to understand the effect of hUCMSC-EVs on cells of the human immune system. Considering the central role of monocytes and neutrophils in the inflammatory response and in the balance between resolution and persistence of inflammation, our work aims to investigate how hUCMSC-EVs impact the phenotype and function of these human cells. **Methods:** Human monocytes were isolated from PBMCs of healthy donors by negative selection. Human neutrophils were isolated from whole blood following the Polymorphprep medium protocol. Cells were cultured in complete RPMI medium. Monocytes were stimulated with LPS for 6 hours, after which they were treated with hUCMSC-EVs for 24 hours. Neutrophils were pretreated with hUCMSC-EVs for 1 hour and then stimulated with recombinant TNF for 4 hours. Cytokine levels were measured by ELISA, and cell viability and surface marker expression were measured by flow cytometry. **Results:** Our preliminary results showed a significant increase in cytokine production (TNF and IL-10) in monocytes stimulated with hUCMSC-EVs alone, conversely, the surface markers evaluated (CD80, CD86, and CD163) showed no significant difference. Among neutrophils, initial assays showed that the group pre-treated with hUCMSC-EVs exhibited a reduction in IL-8 secretion and higher viability. Furthermore, uptake assays performed on monocytes suggest that EVs are taken up more rapidly in the presence of LPS. **Conclusions:** hUCMSC-EVs demonstrated immunomodulatory capacity in human monocytes and neutrophils. Further experiments will be conducted to describe how monocytes respond to simulation with hUCMSC-EVs in terms of cytokine production and expression of surface markers.

**Keywords:** Extracellular Vesicles, Mesenchymal Stem Cells, Monocytes, Neutrophils, Inflammation

## Abstract 47 | Poster | Poster Session Day 1

### Engineered MSC Secretome Expressing sTβRII Suppresses Fibro-Adipogenic Progenitor Proliferation and Restores Myogenic Differentiation

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Luana De Mambro

**Institution:** Universidade Federal de São Paulo, Faculdade de Ciências Médicas da Santa Casa de São Paulo, Universidade da California, Davis — São Paulo

Background and Objective Skeletal muscle fibrosis is driven by the aberrant proliferation of fibro-adipogenic progenitors (FAPs)<sup>1</sup> in response to TGF-β1 signaling.<sup>2</sup> Cell-free strategies based on the secretome of engineered mesenchymal stem cells (MSCs) represent a clinically relevant alternative to cell transplantation.<sup>3</sup> We hypothesized that human MSCs (hMSCs) engineered to secrete the soluble TGFβ type II receptor (sTβRII) — a decoy receptor that sequesters TGF-β1 — would produce a bioactive secretome capable of attenuating FAP proliferation and preserving myogenic differentiation. Methods hMSCs were transiently transfected with plasmids encoding sTβRII or Sdf-1 (Fugene 4K; ~25-30% efficiency) and stimulated with LPS 100 ng/mL for 48 h. Conditioned media (CM) were collected and analyzed by ELISA for TGF-β1, sTβRII, and Sdf-1 (BCA for protein quantification). FAP proliferation was quantified by DNA-content assay (Hoechst) after 48 h of treatment with CM. Myogenic differentiation of C2C12 cells was assessed by qRT-PCR ( $2^{-\Delta\Delta Ct}$ ) for MyoD, MyoG, and MyH7 (reference gene: GAPDH). Statistical analysis: one-way ANOVA, Welch's ANOVA, Kruskal-Wallis, and independent t-test ( $\alpha = 0.05$ ). Results LPS stimulation increased total protein secretion ~11-fold relative to unstimulated controls (ANOVA,  $F(3,8) = 48.14$ ;  $p < 0.0001$ ). sTβRII was detected exclusively in the CM of engineered cells ( $1,172 \pm 332$  pg/mL; Kruskal-Wallis  $p = 0.005$ ,  $n = 4/\text{group}$ ), confirming transgene expression and secretion. The sTβRII secretome reduced TGF-β1 concentration by 64.2% relative to control CM (Welch's ANOVA  $p = 0.0014$ ). Critically, treatment with the sTβRII secretome reduced FAP proliferation by 42.7% compared to the TGF-β1-stimulated positive control ( $p = 0.0113$ , Cohen's  $d = 3.5$ ) and by 56.0% compared to the secretome of unengineered hMSCs ( $p = 0.0086$ ,  $n = 3/\text{group}$ ). In C2C12 cells, TGF-β1 strongly suppressed MyoG expression, and the sTβRII CM partially protected MyoD levels (one-way ANOVA: MyoD  $F(5,11) = 15.58$ ,  $p = 0.0001$ ; MyoG  $F(5,12) = 104.8$ ,  $p < 0.0001$ ; MyH7  $F(5,12) = 10.49$ ,  $p = 0.0005$ ). Conclusion Engineering hMSCs to secrete sTβRII generates a cell-free secretome with potent anti-fibrotic activity. The sTβRII-enriched CM suppressed FAP proliferation by more than 50%, reduced TGF-β1 in the conditioned medium, and partially protected myogenic gene expression. These findings support a secretome-based strategy as a promising cell-free therapy for skeletal muscle fibrosis.

**Keywords:** muscle fibrosis; FAP; TGF-β1; sTβRII; MSC secretome; cell-free therapy; myogenic differentiation

## **Abstract 49 | Poster | Poster Session Day 1**

### **A Fully Feeder-Free based Sendai Virus Reprogramming Strategy Accelerates Human Urine-Derived iPSC Generation With Colony Emergence Within Only Seven Days**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** CAROLINA ORRICO MELO FERREIRA DE JESUS; GABRIEL AUGUSTO PIRES DE SOUZA; MILENA BOTELHO PEREIRA SOARES

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The generation of induced pluripotent stem cells (iPSCs) has revolutionized regenerative medicine, cell therapy, and disease modeling. However, many reprogramming protocols still depend on feeder cells or require subsequent adaptation to feeder-free conditions, increasing experimental variability and limiting standardization and reproducibility. This study aimed to develop and evaluate a refined protocol for the reprogramming of human urine-derived epithelial cells obtained by a non-invasive collection method using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) in a fully feeder-free culture system from viral transduction to colony expansion. Throughout the entire procedure, cells were cultured on Geltrex™-coated plates, eliminating the need for feeder cells or transitions between different culture systems. As a methodological refinement, SB431542 and sodium butyrate (NaBut) were added during the early phase of reprogramming in combination with ReproTeSR™ medium, followed by StemFlex™ for long-term maintenance of pluripotency. Cell cultures were monitored daily by phase-contrast microscopy to evaluate colony formation, morphology, and expansion. Emerging colonies were mechanically selected using EDTA and expanded while maintaining the same fully feeder-free conditions. A major finding of this study was the appearance of colonies with typical iPSC morphology as early as day 7 after reprogramming, considerably earlier than the time reported in most conventional Sendai virus-based protocols. These colonies exhibited progressive expansion, high cellular compaction, a high nucleus-to-cytoplasm ratio, epithelial organization, and well-defined borders while preserving their proliferative capacity after the first passages. At passage 2 (P2), pluripotency was confirmed by immunofluorescence through the expression of OCT4, NANOG, and TRA-1-60. The rapid emergence of colonies together with the maintenance of pluripotency under fully feeder-free conditions suggests that the proposed strategy increased reprogramming efficiency while reducing experimental variability and eliminating the need for adaptation between feeder-dependent and feeder-free culture systems. In conclusion, this methodological refinement enabled the establishment of human urine-derived iPSCs with robust colony formation within only seven days and successful maintenance of pluripotency in a fully feeder-free Geltrex™-based culture system. This approach provides a simplified, reproducible, and potentially more translatable platform for iPSC generation, supporting future applications in regenerative medicine, disease modeling, and cell therapy. All procedures involving human cells were approved by the Institutional Research Ethics Committee (CAAE 90283925.0.0000.5544).

**Keywords:** Induced pluripotent stem cells, Feeder-free culture, Urine-derived cells

## **Abstract 50 | Poster | Poster Session Day 1**

### **Evaluation of the correlation between a gene expression signature in hematopoietic stem cell populations and engraftment levels in xenotransplantation assays.**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Eduardo Chohfi Ramos; Bueno Moreira Gustavo; Griesi-Oliveira Karina

**Institution:** Faculdade Israelita de Ciências da Saúde Albert Einstein — São Paulo - SP

Hematopoietic stem cells (HSCs) are responsible for maintaining hematopoiesis and are essential for the treatment of onco-hematological and hereditary diseases. However, therapeutic success is strongly associated with the number of long-term hematopoietic stem cells (LT-HSCs), the only HSC subset capable of indefinite self-renewal and long-term hematopoietic reconstitution. Their isolation remains challenging due to the lack of definitive and stable markers. Since the current gold-standard method for quantifying functional LT-HSCs is serial xenotransplantation into immunodeficient mice, a costly and time-consuming approach, the development of alternative, scalable, and cost-effective methods is of great interest. To address this challenge, our group previously identified a 315-gene transcriptional signature whose expression is associated with LT-HSC enrichment in heterogeneous cell populations. In the present study, we aimed to evaluate the correlation between the expression of this gene signature and engraftment levels following xenotransplantation. Transcriptomic data from engrafted cell populations were analyzed, and principal component analysis (PCA) was used to summarize the expression of the 315-gene signature through the first principal component (PC1). Additionally, biologically informed gene sublists were generated to identify a smaller set of genes capable of predicting the relative abundance of LT-HSCs in heterogeneous samples. Correlation analyses between the PC1 values derived from each gene list and relative engraftment levels showed that none of the evaluated signatures exhibited a positive and statistically significant correlation with engraftment. These findings suggest that the 315-gene signature may primarily reflect the phenotypic abundance of LT-HSCs rather than their functional competence and long-term repopulating capacity, which may explain the lack of association with engraftment outcomes. As a next step, we will perform exploratory mathematical analyses of both the global transcriptomic dataset and the 315-gene panel to identify unbiased gene subsets with improved predictive value for engraftment, alongside conducting validation experiments in a second xenotransplantation assay. We expect that this approach will identify a more representative gene signature that correlates with engraftment levels, paving the way for more accurate clinical assessment of functional LT-HSC content.

**Keywords:** RNA-seq; Xenotransplantation; Genomic Panel; LT-HSC.

## Abstract 51 | Poster | Poster Session Day 1

### Three-Dimensional Bioprinting of Undifferentiated Human iPSCs Enables Sequential Neural Differentiation Within a Single Construct

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** CAROLINA ORRICO MELO FERREIRA DE JESUS; ARTHUR JOÃO REIS LIMA RODOVALHO; BRENO CARDIM BARRETO; CARINE MACHADO AZEVEDO CARDOSO; DAHARA KEYSE CARVALHO SILVA; GABRIEL AUGUSTO PIRES DE SOUZA; GIRLAINE CAFÉ SANTOS; JAQUELINE LEITE VIEIRA; LETICIA ROCHA QUINTINO SOUZA; MILENA BOTELHO PEREIRA SOARES; ROSALIA MENDEZ OTERO

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Human induced pluripotent stem cells (hiPSCs) represent a valuable platform for regenerative medicine, disease modeling, drug screening, and cell therapy due to their self-renewal capacity and ability to differentiate into multiple specialized cell types. However, efficient neural differentiation remains challenging, particularly in conventional two-dimensional cultures that fail to reproduce the complexity of the native neural microenvironment. Three-dimensional (3D) bioprinting has emerged as a promising strategy to generate biomimetic constructs that promote cell-cell and cell-matrix interactions, improving tissue organization and supporting cellular maturation. Although most neural bioprinting approaches use previously differentiated neural progenitors or mature neural cells, the direct bioprinting of undifferentiated hiPSCs followed by neural differentiation within the construct remains poorly explored. In this study, we evaluated a strategy based on the 3D bioprinting of undifferentiated human hiPSCs encapsulated in 4% sodium alginate-Geltrex microspheres, followed by sequential neural differentiation directly within the printed construct. hiPSCs remained encapsulated throughout the differentiation protocol, initially undergoing neural induction and subsequently cultured for 14 days in N2/B27 neural maturation medium. Differentiation was evaluated by RT-qPCR, immunofluorescence, and cell viability analyses. After 14 days of neural maturation, the bioprinted constructs maintained high cell viability, preserved their three-dimensional architecture, and remained structurally stable throughout the culture period. Gene expression analysis demonstrated downregulation of the pluripotency marker OCT4, indicating progression from the undifferentiated state. Concomitantly, increased expression of the neuronal markers DCX and TUBB3 (TUJ1) was observed, demonstrating neuronal commitment. In addition, elevated expression of the astroglial markers GFAP and ALDH1L1 suggested differentiation toward the glial lineage, indicating the establishment of a mixed neural population within the constructs. Immunofluorescence analysis was performed to further characterize the expression of neuronal and glial markers following differentiation, complementing the molecular findings. These results demonstrate that 4% sodium alginate-Geltrex microspheres provide a supportive three-dimensional microenvironment capable of maintaining hiPSC viability and promoting sequential neural differentiation directly after bioprinting while preserving construct architecture. This strategy eliminates the need for prior neural differentiation before printing, simplifying construct production and expanding the potential application of hiPSC-derived 3D neural models for disease modeling, drug screening, tissue engineering, and regenerative medicine. All procedures involving human cells were approved by the Institutional Research Ethics Committee (CAAE 60531516.0.3001.5261).

**Keywords:** Human induced pluripotent stem cells (hiPSCs), Neural tissue engineering, Bioprinting

## **Abstract 52 | Poster | Poster Session Day 1**

### **DEVELOPMENT OF CRISPR RNAS TO BLOCK HIV-1 REACTIVATION FROM LATENCY IN LYMPHOID AND MYELOID CELLS EXPOSED TO LATENCY-REACTIVATING AGENTS**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** MARIANA DE OLIVEIRA THEODORO DA SILVA; Amilcar Tanuri (in memoriam); Átila Duque Rossi; Gabriel Cardoso Atella; Johari Larias Chequer; Lendel Correia da Costa

**Institution:** UNIVERSIDADE FEDERAL DO RIO DE JANEIRO (UFRJ) — RJ

Antiretroviral therapy (ART) has markedly improved the survival of people living with HIV-1 by sustaining long-term viral suppression and preventing millions of AIDS-related deaths. However, ART is unable to eliminate latent virus reservoirs, which remain a major challenge to an HIV-1 cure. CRISPR-based epigenetic repression represents a promising block-and-lock strategy to reinforce viral latency and prevent HIV-1 reactivation. Therefore, this study aimed to develop repressor CRISPR guide RNAs (gRNAs) targeting the HIV-1 transcription start site (TSS) and evaluate their ability to suppress the reactivation of latent HIV-1 in lymphoid and myeloid cells exposed to different latency-reactivating agents (LRAs). Five gRNAs targeting different sites of HIV-1 promoter region were designed and cloned into a lentiviral vector encoding the CRISPR/dCas9-KRAB transcriptional repressor system. Following confirmation of the recombinant constructs, J-Lat 10.6 cells were transduced, selected with puromycin and subsequently exposed to four LRAs with distinct mechanisms of transcription reactivation: PMA, Panobinostat, JQ1, and AZD5582. LRA-induced HIV-1 reactivation was quantified by flow cytometry to assess the repressive activity of each gRNA. All five constructs were successfully generated, with the construct targeting the TSS5 site demonstrating the most pronounced effect, achieving a 98% inhibition of HIV-1 reactivation following the first transduction event. However, the magnitude of this repression varied substantially across independent transductions, suggesting that differences in lentiviral delivery efficiency and construct expression remain important sources of variability. Taken together, our results provide proof-of-concept evidence that the CRISPR/dCas9-KRAB system targeting HIV-1 TSS can achieve highly efficient silencing of latent HIV-1 reactivation, although reproducibility of this effect depends on further optimization of lentiviral transduction and stable expression of the constructs in target cells.

**Keywords:** dCas9-KRAB; block and lock; gene editing; retrovirus

## **Abstract 53 | Poster | Poster Session Day 1**

### **Lipid nanoparticle-mediated delivery of anti-CD19 CAR plasmid DNA for non-viral engineering of lymphoid cells**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Maria Letícia Cardoso Bina; Pedro Pires Goulart Guimarães

**Institution:** Universidade Federal de Minas Gerais (UFMG) — Belo Horizonte, Minas Gerais

Chimeric antigen receptor (CAR)-based immunotherapy has revolutionized the treatment of several hematological malignancies. However, lymphoid cell engineering still relies mainly on viral vectors, which present important limitations, including complex manufacturing processes, high production costs, and regulatory challenges, making ionizable lipid nanoparticles (LNPs) a promising non-viral alternative for engineering lymphoid cells with transient CAR expression. Therefore, this study developed and evaluated a lipid nanoparticle (LNP)-based platform for the non-viral delivery of plasmid DNA encoding an anti-CD19 CAR into Jurkat cells and NK-92 cells, both an immortalized lymphoid cell lines, for cancer immunotherapy applications. LNPs encapsulating a plasmid encoding a CAR anti-CD19 were synthesized by microfluidic mixing. Their physicochemical properties, including particle size, polydispersity index (PDI), and zeta potential, were characterized by dynamic light scattering (DLS). Encapsulation efficiency (EE) was determined using the PicoGreen™ assay. Transfection efficiency was evaluated by flow cytometry in both Jurkat cells and NK-92 cells. Finally, the antitumor activity of the engineered NK-92 cells was assessed in co-culture with CD19-positive, luciferase-expressing NALM-6 cells using the OneGlo™ luminescence assay. The LNPs exhibited physicochemical characteristics consistent with a stable formulation and efficiently encapsulated the plasmid DNA. Moreover, the LNP platform successfully mediated plasmid delivery to both Jurkat and NK-92 cells. Engineered NK-92 cells demonstrated functional antitumor activity in co-culture with NALM-6 cells, resulting in reduced luminescence compared with control groups. These findings demonstrate the potential of this LNP platform as a non-viral strategy for plasmid DNA delivery into immune cells and highlight its promise for the development of next-generation CAR-based cancer immunotherapies.

**Keywords:** Lipid nanoparticles, pDNA delivery, CAR-NK cells, cell engineering

## **Abstract 54 | Poster | Poster Session Day 1**

### **Efficient In Vitro Gene Editing via CRISPR-Cas9 Delivered by Lipid Nanoparticles**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Natália Jordana Alves da Silva; Lays Cordeiro Guimarães; Pedro Guimarães

**Institution:** Universidade Federal de Minas Gerais — Belo Horizonte - MG

**INTRODUCTION:** The CRISPR-Cas9 system has emerged as a powerful and versatile tool for gene editing (Doudna, et al. 2014), being a key method in a range of applications, such as immunotherapy and genetic disease treatment (Liu, et al. 2023). However, its efficiency relies on effective intracellular delivery of both Cas9 endonuclease and single guide RNA (sgRNA). Lipid nanoparticles (LNPs) have shown great promise as delivery systems for nucleic acids, providing a tool to overcome barriers in Cas9 based gene editing (Palanki, et al. 2024). **OBJECTIVE:** To develop and characterize a LNP platform capable of efficiently delivering mRNA encoding Cas9 as well as a sgRNA. **METHODOLOGY:** LNPs were formulated by microfluidic mixing using ionizable and helper lipids, and loaded with Cas9 mRNA and sgRNA targeting GFP (sgRNA-GFP) at a 2:1 weight ratio. Particle size, polydispersity index (PDI), and zeta potential were assessed by dynamic light scattering (DLS). In vitro assays were performed using HeLa-GFP+ cells treated with increasing mRNA doses (75, 150, 300, and 600 ng/well). Untreated cells and cells transfected with Lipofectamine MessengerMAX (150 ng of mRNA) were used as controls. Gene editing efficiency was evaluated by fluorescence reduction using live cell imaging and flow cytometry. After determining the optimal treatment dosage, gene editing efficiency was further evaluated at different timepoints. **RESULTS:** The LNP formulation exhibited favorable physicochemical properties, including an average size of 150 nm, PDI of 0.09, and zeta potential of -10 mV. Both imaging and flow cytometry analyses demonstrated a significant reduction in GFP fluorescence in cells treated with 300 and 600 ng doses after 72 hours. Kinetic analysis using 300 ng/well revealed the onset of fluorescence reduction at 48 hours post-treatment. **CONCLUSION:** The developed LNP platform displays suitable nanotechnological characteristics and demonstrates strong potential for efficient intracellular delivery of CRISPR-Cas9 components. These findings support its applicability as a promising strategy for gene editing and future gene therapy approaches.

**Keywords:** Lipid nanoparticle, gene delivery, CRISPR

## Abstract 55 | Poster | Poster Session Day 1

### OBTENTION, CHARACTERIZATION, AND COMPARATIVE ANALYSIS OF POLYMERIC SPHERES ENRICHED WITH BIOMACROMOLECULES FOR OCULAR REGENERATIVE MEDICINE

**Category:** Tissue engineering, biomaterials and advanced in vitro models

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Stem cell therapies are prominent in ocular regenerative medicine, but poor localized retention and limited survival in inflamed microenvironments hinder efficacy. Hydrogel encapsulation offers a protective physical barrier. Sodium alginate features high biocompatibility, yet its lack of cell-adhesion motifs necessitates blending with bioactive macromolecules like hyaluronic acid (HA), bovine gelatine (BG), or corneal hydrogel (CH) to enhance structural and secretory microenvironments. This study compared four microcapsule types—pure Sodium Alginate (SA 2.5%), SA+0.1% HA, SA+0.1% BG, and SA+0.1% CH—for human corneal stromal cell (hSSCs) delivery. Cells were suspended at  $5 \times 10^7$  cells/mL and extruded into a 0.9% CaCl<sub>2</sub> bath. Across three repetitions, microcapsules were characterized regarding diameter, encapsulation efficiency, SEM morphology, swelling, and 7-day degradation, alongside ELISA quantification of neuroprotective factors: SERPIN F1, CNTF, BDNF, and  $\beta$ -NGF. Biomacromolecules significantly modified physical profiles. Average diameters varied: pure SA reached 712.78  $\mu\text{m}$ , whereas blending altered it in SA+HA (608.67  $\mu\text{m}$ ), SA+BG (767.22  $\mu\text{m}$ ), and SA+CH (784.44  $\mu\text{m}$ ). Thicker blends minimized cell sedimentation, yielding higher encapsulation yields for SA+HA (100.24%), SA+CH (83.31%), and SA+BG (80.43%) relative to pure SA (72.33%). Over 7 days, pure SA exhibited high swelling (24.48%), whereas enriched groups formed compact networks with suppressed swelling: SA+HA (5.63%), SA+BG (3.58%), and SA+CH (3.31%). Degradation remained low across groups (0.18%–0.23%). SEM verified superior structural preservation for enriched matrices compared to porous pure SA. Notably, ELISA showed enhanced secretion of neuroprotective factors in modified formulations. SA+HA demonstrated the highest overall paracrine performance, with SERPIN F1 and CNTF concentrations reaching  $\sim 24 \mu\text{g/mL}$  and  $\sim 9.5 \text{ ng/mL}$  (per 112 beads), respectively, alongside increased  $\beta$ -NGF secretion ( $>1.0 \text{ pg/mL}$ ); BDNF was detected at lower ranges across all groups, while SA+BG showed reduced neurotrophic expression. Enriched formulations successfully sustained functional hSSCs, showing that macromolecular additions govern hydrogel architecture and improve secretory yields. Future designs will focus on covalent crosslinking to optimize cell-matrix interactions for targeted retinal regenerative therapies.

**Keywords:** Biomaterials; Hyaluronic acid; Neuroprotection; Secretome; Hydrogels

## **Abstract 56 | Poster | Poster Session Day 1**

### **Bone Marrow-Derived Mesenchymal Stromal Cells Improve Kidney Injury in Experimental Chronic Kidney Disease and Are Associated with Enhanced Parkin - Related Mitochondrial Quality Control**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** GENILZA PEREIRA DE OLIVEIRA; ALESSANDRA ALVES THOLE; ALINE DE ALMEIDA; ERIKA AFONSO COSTA CORTEZ MARQUES; KARINA R. SILVA; LAIS CARVALHO; SIMONE NUNES DE CARVALHO; YANCA AZEVEDO

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Chronic kidney disease (CKD) secondary to renal artery stenosis is characterized by persistent ischemia, renal fibrosis, mitochondrial damage, and progressive loss of kidney function. Mitochondrial quality control mechanisms are essential for maintaining cellular homeostasis and may represent important therapeutic targets in CKD. This study investigated whether bone marrow-derived mesenchymal stromal cells (MSCs) improve renal injury and are associated with alterations in Parkin-related mitochondrial quality control in the two-kidney, one-clip (2K1C) model of chronic renovascular kidney disease. Male Wistar rats were allocated into four groups: Sham, 2K1C, 2K1C+MSC7d, and 2K1C+MSC15d. Four weeks after renal artery clipping, when fibrosis was established,  $1 \times 10^6$  MSCs were transplanted into the renal subcapsular space. Kidneys were collected 7 or 15 days later for histological, ultrastructural, and protein expression analyses. The 2K1C group developed hypertension, increased plasma creatinine and urea levels, proteinuria, reduced serum total protein levels, elevated renin expression, and marked renal fibrosis. Transmission electron microscopy revealed fragmented and structurally disorganized mitochondria, altered cristae morphology, and extensive mitochondria-endoplasmic reticulum contacts in tubular epithelial cells. Parkin expression in the 2K1C group remained comparable to Sham levels. MSC transplantation significantly reduced blood pressure, creatinine, urea, proteinuria, renin expression, and collagen deposition while restoring serum total protein levels. In parallel, MSC treatment increased Parkin expression by 175% and 200% at 7 and 15 days, respectively ( $p < 0.05$  vs. both 2K1C and Sham). Ultrastructural analyses demonstrated progressive mitochondrial recovery, characterized by preservation of mitochondrial cristae, improved organelle organization, and the presence of mitochondria-containing autophagosomes in treated kidneys. In conclusion, MSC transplantation attenuated renal dysfunction, hypertension, and fibrosis in ischemic kidneys and was associated with increased Parkin expression and mitochondrial ultrastructural remodeling. The combination of enhanced Parkin expression and the presence of mitochondria-containing autophagosomes is consistent with improved Parkin-associated mitochondrial quality control and may contribute to the renoprotective effects of MSC therapy in chronic kidney disease.

**Keywords:** Mesenchymal stromal cells, Chronic kidney disease, Parkin, Mitochondrial quality control, Renal fibrosis

## Abstract 58 | Poster | Poster Session Day 1

### Optimization of a non-viral CRISPR-dCas9 delivery system for FNDC5/irisin activation in human adipose-derived mesenchymal stem cells

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Rafaela Luiza Costa Franco; Fernanda Guarino De Felice; Matheus Melo Fabiano; Tayná Rody Souza Ferreira; Thyago Leal Calvo

**Institution:** Federal University of Rio de Janeiro (UFRJ) — Rio de Janeiro-RJ

Human adipose-derived mesenchymal stem cells (AdMSCs) have emerged as a promising approach for neurodegenerative disease therapy due to their low immunogenicity, neuroprotective properties, and abundant secretion of bioactive extracellular vesicles. Their therapeutic potential may be enhanced by increasing endogenous neuroprotective factors. Among these, FNDC5/irisin is a promising therapeutic target, as it mediates exercise benefits on the brain. Exercise improves brain health and protects against Alzheimer's disease, in part through increased FNDC5 expression and irisin release, which can cross the blood-brain barrier and rescue memory and cognition. In this context, the CRISPR-dCas9 system has been explored as a tool for regulating endogenous gene expression. CRISPR activation (CRISPRa), based on a catalytically inactive Cas9 (dCas9) fused to transcriptional activators, enables gene upregulation without DNA cleavage. However, despite successful CRISPR-based modulation in AdMSCs using viral transduction approaches, efficient non-viral delivery remains challenging due to low transfection efficiency. Non-viral strategies offer potential advantages for therapeutic applications, including reduced risk of genomic integration and improved safety profiles. Here, we aimed to establish a non-viral CRISPR-dCas9 delivery strategy in human AdMSCs for FNDC5 upregulation. CRISPRa constructs with fluorescent reporters were validated in HEK293T cells before evaluation in AdMSCs. Transfection efficiency was compared between calcium phosphate precipitation and the cationic lipid-based reagent Lipofectamine 2000 using a GFP reporter plasmid. Lipofectamine 2000 resulted in an eight-fold increase in arbitrary fluorescence units (AU) compared with calcium phosphate after 24 h of transfection. Fluorescence images were acquired using a Cytation 5 Cell Imaging System and analyzed with ImageJ. Subsequently, different cationic lipid reagents, including Lipofectamine 2000, Lipofectamine 3000, and Lipofectamine Stem, were compared. Lipofectamine Stem showed the highest efficiency, achieving a six-fold increase compared with Lipofectamine 3000 and a two-fold increase compared with Lipofectamine 2000. Under optimized conditions, two CRISPR-dCas9 activation systems, dCas9-VPR-mCherry (~15 kb) and dCas9-VP64-GFP (~8.5 kb), were evaluated. Detectable fluorescence was exclusively observed with the smaller dCas9-VP64-GFP construct using Lipofectamine Stem, whereas no expression was detected with dCas9-VPR-mCherry. The optimized CRISPR-dCas9 system and FNDC5-targeting sgRNAs are currently being co-transfected into AdMSCs, followed by evaluation of FNDC5 upregulation by RT-qPCR. In conclusion, Lipofectamine Stem combined with the dCas9-VP64 activation system represents a promising non-viral strategy for CRISPR-dCas9 delivery in human AdMSCs. This approach may enable FNDC5/irisin activation and support the development of epigenetic therapies for neurodegenerative diseases.

**Keywords:** Epigenome editing; CRISPR/dCas9; FNDC5/Irisin; Adipose-derived mesenchymal stem cells; Neurodegenerative diseases

## Abstract 60 | Poster | Poster Session Day 1

### Amphiphilic Oligochitosan Nanocarriers Bearing Tertiary Amines and Cholesterol for mRNA Delivery

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** ANA LAURA FERRAZ TEZOTO; Felipe Atílio Nunes; Gabriel José De Carli; MARCIO JOSÉ TIERA; Vera Aparecida de Oliveira Tiera

**Institution:** UNESP/IBILCE — São José do Rio Preto-SP

Polymeric polyplexes are versatile non-viral platforms for the delivery of siRNA and mRNA IVT, as cationic segments condense and protect these molecules while simultaneously promoting cellular uptake and endosomal escape. Beyond electrostatic complexation, efficient transfection requires optimized amphiphilicity. Hydrophobic moieties, especially cholesterol, reinforce polyplex stability, favoring mRNA condensation, serum resistance, and cellular internalization [1-2]. In this work, low-molecular weight chitosan was functionalized with diethylaminoethyl (DEAE) or diisopropylaminoethyl (DIPEA) groups using the corresponding 2-chloroethyl tertiary-amine reagent under controlled pH. The products were purified by dialysis, lyophilized and then oxidatively depolymerized to yield CHDDEAE and O-DIPEA-CH. Cholesterol was conjugated to CHDDEAE and O-DIPEA-CH in dimethylformamide (DMF) via EDC/NHS coupling chemistry using cholesterol succinate. The polymers were characterized by <sup>1</sup>H NMR, potentiometric titration (protonation/ionization behavior), and gel permeation chromatography (GPC). Cholesterol grafting was confirmed, affording O-DIPEA-CH<sub>4</sub> (GS<sub>Chol</sub> ≈ 3.9%), O-DIPEA-CH<sub>7</sub> (≈ 7.0%), and CHDChol with four degrees of substitution (GS ≈ 2.4%; GS ≈ 3%). GS ≈ 4% and GS ≈ 4.5%). The correlation between cholesterol grafting and the aggregation behavior of CHDDEAEChol was investigated by determining the critical aggregation concentration (CAC), which demonstrated that cholesterol hydrophobicity favors nanoparticle formation at lower polymer concentrations (0.05 g/L to 0.018 g/L). Additionally, gel electrophoresis assays demonstrated that the CHDDEAE oligomer efficiently encapsulated mRNA at N/P ratios of 3 and 5, serving as the basis for subsequent analyses with CHDChol derivatives, which achieved encapsulation at N/P ratios ranging from 3 to 15. Cholesterol-modified O-DIPEA-CH<sub>X</sub> formed nanometric siRNA polyplexes: at N/P 10, the hydrodynamic diameter (D<sub>h</sub>) decreased from ~250 nm (DIPEA-CH) to ~70–120 nm (O-DIPEA-CH<sub>4</sub>), while the zeta potential (ζ) remained slightly positive. Particle size depended on both pH and N/P (e.g., pH 7.4: ~265 nm for DIPEA-CH vs ~90 nm for the cholesterol derivative at N/P 10). The low-M<sub>w</sub> O-DIPEA (~10.2 kDa) showed weak binding (D<sub>h</sub> ~1000–1700 nm), whereas incorporation of ~7% cholesterol (O-DIPEA-CH<sub>7</sub>) reduced D<sub>h</sub> to ~300 nm. Overall, these results demonstrate precise control over oligomer charge density and hydrophobicity and highlight the role of cholesterol in promoting stable nanometric polyplexes in the ~100–300 nm range. Overall, these results demonstrate precise control over oligomer charge density and hydrophobicity, highlighting the role of cholesterol in promoting the formation of stable nanoscale polyplexes for efficient complexation, supporting their potential to deliver mRNA and siRNA.

**Keywords:** Chitosan, siRNA, mRNA, Nanoparticles, Cholesterol

## Abstract 61 | Poster | Poster Session Day 1

### Synthesis and Characterization of Amphiphilic Chitosan Oligomers for mRNA Delivery

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Laura Aiex Fernandes; Gabriel José De Carli; MARCIO JOSÉ TIERA; Vera Aparecida de Oliveira Tiera

**Institution:** UNESP-IBILCE — São José do Rio Preto - SP

Non-viral delivery of messenger RNA (mRNA) has emerged as a highly promising gene-therapy strategy due to its ability to enable transient expression of therapeutic proteins. However, its clinical performance critically depends on safe and efficient delivery systems that protect mRNA from degradation, promote cellular uptake, and facilitate endosomal escape. In this context, chitosan, a biocompatible and mucoadhesive polymer, was modified with ionizable tertiary amine moieties and long alkyl chains to overcome these barriers, relying on the balance between electrostatic complexation with the mRNA phosphate groups at physiological pH and hydrophobic intermolecular association of the polycation (1,2). These amphiphilic chitosan-based polymers were synthesized and evaluated as potential carriers for mRNA delivery. Low molecular weight chitosan was functionalized by nucleophilic alkylation with 2-chloroethyl diisopropylethylamine chloride, yielding diisopropylaminoethyl chitosan (CHD) with a targeted substitution of 15% in glucosamine units (3). Subsequently, oxidative depolymerization with sodium nitrite afforded the corresponding oligomer (OCHD). Finally, N-alkylated derivatives (OCHDC8X) were prepared by reductive amination with octyl aldehyde and sodium cyanoborohydride (4). After each step, the products were purified by dialysis and then lyophilized. The polymers were characterized by proton nuclear magnetic resonance ( $^1\text{H}$  NMR), gel permeation chromatography (GPC), potentiometric titration and fluorescence spectroscopy.  $^1\text{H}$  NMR confirmed incorporation of DIPEA moieties (signal at  $\sim 1.8$  ppm) and n-octyl chains ( $\delta$  1.8–1.2 ppm), enabling estimation of the DIPEA substitution degree (13.8%) and verification of controlled octyl substitution (5.3%, 10.0%, and 18.5%). GPC confirmed the reduction of molecular weight after depolymerization while preserving the monomer composition. Potentiometric titration showed that such decrease markedly increased the fraction of protonated groups at physiological pH, from  $\sim 20\%$  for CHD to  $\sim 40\%$  for OCHD, indicating improved ionization and solubility. The fluorescence spectroscopy studies showed that higher hydrophobic substitution promoted nanoparticle formation at lower polymer concentrations from 0.310 g/L for OCHD to 0.093 g/L for OCHDC818,5% indicating enhanced tendency toward self-aggregation in solution. Furthermore, the mRNA complexation was evaluated in the nanoparticles with gel electrophoresis at different N/P ratios. The OCHD polymer effectively encapsulated mRNA at pH 7 for N/P ratios above 1.6, supporting the potential of the system as a vector for nucleic acid binding. Overall, the resulting polymers possess suitable physicochemical properties for mRNA complexation and the development of stable nanoparticles and represent promising candidates for non-viral mRNA delivery systems.

**Keywords:** Chitosan, mRNA, Nanoparticles, Gene Therapy, Amphiphilic Polymers

## **Abstract 62 | Poster | Poster Session Day 1**

### **Therapeutic evaluation of PLGA-dECM biomembranes using an in vitro model of outer retinal oxidative degeneration**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** ANA KARULLINE GARCIA UNGARATTI; MARIA CLAUDIA PASSOS; MARIZE CAMPOS VALADARES; PEDRO HENRIQUE DOS SANTOS DANTAS; THIAGO DE ANDRADE SILVA SANTANA LIMA

**Institution:** UNIVERSIDADE FEDERAL DE GOIÁS — GOIÂNIA-GO

**Introduction:** Retinal degenerative diseases, such as age-related macular degeneration (AMD), are progressive pathologies characterized by severe oxidative damage to the retinal pigment epithelium (RPE). To counter this, PLGA biomembranes functionalized with decellularized extracellular matrix (dECM) offer a dual regenerative approach: providing structural support for cell anchoring while retaining native bioactive molecules to mitigate cellular stress. Crucially, screening these advanced platforms demands in vitro models that recapitulate human pathology, reducing the reliance on animal testing. **Objective:** To evaluate the cytoprotective efficacy of a previously developed poly(lactic-co-glycolic acid) (PLGA) biomembrane functionalized with decellularized corneal extracellular matrix (dECM) applied as a treatment to an in vitro ARPE-19 barrier model under H<sub>2</sub>O<sub>2</sub> induced oxidative stress. **Methodology:** An in vitro retinal barrier model was established by culturing human RPE cells (ARPE-19) in Transwell inserts. To simulate AMD-like degeneration, the established barrier was exposed to H<sub>2</sub>O<sub>2</sub>-induced oxidative stress, followed by the addition of the PLGA-dECM biomembrane as a therapeutic intervention. The cytoprotective performance was evaluated at three distinct time points through cytotoxicity analysis (LDH release) and cell survival quantification (Live/Dead via High-Content Analysis). Mechanistic stress responses were investigated over time using immunofluorescence for NRF2 expression. **Results:** The introduction of the PLGA-dECM biomembrane established a highly cytoprotective microenvironment under severe oxidative insult. Live/Dead assays demonstrated significantly higher viable cells in the treated group compared to the untreated control across all evaluated time points ( $p < 0.05$ ). This was strongly corroborated by LDH assays, indicating significantly higher cytotoxicity in untreated injured cells ( $p < 0.05$ ). Crucially, immunofluorescence revealed that the biomembrane treatment attenuated the H<sub>2</sub>O<sub>2</sub> induced stress response, showing reduced NRF2 expression and a qualitative decrease in pyknotic nuclei over time compared to untreated cells. This sustained protection confirms a robust beneficial mechanism mediated by paracrine effects from the biofunctionalized matrix. **Conclusion:** These findings suggest that the PLGA-dECM biomembrane holds potential as a supportive therapeutic strategy against AMD-like oxidative stress. By reducing cytotoxicity, helping preserve cell viability, and attenuating the NRF2 mediated stress response, the treatment demonstrated a relevant protective effect on RPE cells. Furthermore, this ethically sustainable in vitro approach provides a valuable alternative model for the preliminary screening of retinal therapies, contributing to the reduction of animal use in early-stage research.

**Keywords:** ARPE-19; Cytoprotection; Hydrogen peroxide; NRF2; Tissue engineering.

## **Abstract 63 | Poster | Poster Session Day 1**

### **Role of IFNL1 in endothelial cell reprogramming by glioblastoma cells: impacts of Zika virus infection**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Gabriel Cardoso Atella; Enzo Olivera Barone; Gabriella Pinheiro Alves de Freitas; Lendel Correia da Costa; Luiza Mendonça Higa; Mariana Theodoro; Matheus Villanueva de Andrade

**Institution:** Universidade Federal do Rio de Janeiro — Rio de Janeiro

Glioblastoma (GBM) is the most common and aggressive primary cancer of the central nervous system (CNS). Current treatment consists of surgical resection of the tumor, radiotherapy, and chemotherapy. Treatment is ineffective, and most patients experience recurrence. GBM presents a heterogeneous tumor microenvironment (TME) composed of tumor and non-tumor cells, which play an important role in tumor progression and recurrence. Endothelial cells play a central role in the TME by mediating angiogenesis, supplying the tumor with oxygen and nutrients for cell proliferation. Zika virus (ZIKV) exhibits oncolytic activity in glioblastoma cells both in vitro and in vivo. ZIKV induces the death of GBM cells and promotes antitumor immunity. A transcriptomic analysis of infected tumor cells identified interferon type 3 (IFNL1) as the most differentially expressed gene (DEG). We plan to produce IFNL1 knockout GBM cells, and IFN-unresponsive endothelial cells by knocking out the IFNL1 receptor (IFNLR1) using the CRISPR-Cas strategy to create IFNL1 and IFNLR1 gene knockout cell lines. We also plan to produce GBM cells with the IFNL1 gene super-activated with the dCRISPR-Cas9 SAM complex. We have already evaluated the IFNL1 expression by mock and ZIKV-infected WT GBM cells using RT-qPCR to validate transcriptomics data and showed that ZIKV-infected cells produce IFNL1 at MOIs of 1 and 3, while no expression was detected in mock-infected cells. Additionally, after cloning the IFNL1 and IFNLR1 sgRNAs in vectors, we transfected them in GBM and endothelial cells, and utilizing the gene reporter GFP sorted the positive populations of each cell line utilizing the cell sorting strategy based on fluorescence emission. Currently we are validating the sorted cells populations by Sanger sequencing and using the High Resolution Melting assay. By using knockout and super-activated cells, we plan to perform phenotypic assays to assess the role of the IFNL1 in the oncolytic effect induced by the ZIKV infection in GBM cells.

**Keywords:** Oncolytic virus, CRISPR-Cas9, IFNL1, ZIKV, Glioblastoma

## **Abstract 64 | Poster | Poster Session Day 1**

### **Comparative Evaluation of Minicircle and Conventional Plasmid DNA for Sleeping Beauty-Mediated CD19 CAR-T Cell Generation: Improved Manufacturing Efficiency Without Compromising Antitumor Activity**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** LÍVIA PIMENTEL DE SANT'ANA DOURADO; ARTHUR DA SILVA NEUMANN; EMMANUEL ARTHUR ALBUQUERQUE ARAGAO; Leonardo Ribeiro Batista Silva; LUIZA DE MACEDO ABDO; SABRINA ALVES DOS REIS

**Institution:** INCA — Rio de Janeiro

**Background:** Chimeric Antigen Receptor T cell (CAR-T) immunotherapy targeting CD19 has achieved remarkable clinical outcomes in patients with B-cell malignancies. However, current manufacturing protocols largely rely on viral vectors for gene transfer, making the process expensive, technically complex, and difficult to scale. To overcome these limitations, our group implemented a non-viral vector system using minicircle (MC) plasmids based on the Sleeping Beauty (SB) transposon to generate anti-CD19 CAR-T cells. MCs are simplified, non-viral DNA vectors derived from parental plasmids using engineered bacteria that express an inducible recombinase. This process generates vectors containing only the CAR transgene under a specific promoter, devoid of prokaryotic backbone sequences, thereby enhancing safety and compactness for gene delivery applications. **Objective:** To compare CD19 CAR-T cell generation using the SB transposon system delivered via either minicircle (MC) DNA or a conventional full plasmid (FP). **Methods:** Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by density-gradient centrifugation (Ficoll). T lymphocytes were enriched using anti-CD3 magnetic beads and activated with anti-CD3/CD28 beads for 48 h. A total of  $5 \times 10^6$  T cells were nucleofected (4D-Nucleofector, Lonza) with the PT4-19BBz construct in either MC or FP format and expanded in G-Rex culture plates in the presence of IL-2. CAR expression, cell viability, and expansion were monitored throughout the culture period. In vivo antitumor activity was evaluated in a xenograft mouse model. **Results:** MC-derived CAR-T cells exhibited high viability ( $83 \pm 13.5\%$ ) and robust CAR expression ( $52.5 \pm 12.5\%$ ) after 8–12 days of culture. Based on a concentration experiment (2.75–11  $\mu\text{g}$ ), 5.5  $\mu\text{g}$  of MC DNA was selected for subsequent experiments as it yielded the highest CAR-T cell expansion. After 12 days of expansion, the MC platform generated approximately four-fold more CAR-T cells than the FP platform, demonstrating superior CAR expression and proliferative capacity. In vivo, MC-derived CAR-T cells displayed antitumor efficacy comparable to FP-derived CAR-T cells, indicating that the use of MC vectors improves manufacturing efficiency without compromising therapeutic activity. **Conclusions:** The Sleeping Beauty minicircle-based platform represents a safe, efficient, and cost-effective non-viral strategy for CAR-T cell manufacturing. By enhancing CAR expression and cell expansion while maintaining equivalent in vivo antitumor efficacy, this approach provides a promising alternative to conventional plasmid-based systems and supports the development of scalable, decentralized, and point-of-care CAR-T cell manufacturing.

**Keywords:** CAR-T cells; Sleeping Beauty transposon; Minicircle DNA; Non-viral gene delivery;

## **Abstract 65 | Poster | Poster Session Day 1**

### **Liposomal CRISPR/Cas9-mediated gene editing induces IFN- $\beta$ expression in fibroblast-like synoviocytes from patients with rheumatoid arthritis and attenuates experimental inflammatory arthritis**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** MARIA LUÍSA GASPARINI; GUSTAVO FLORES CHAPACAIS; JÚLIA DE AZAMBUJA VANIEL; LANA GOTTARDI SCHUSTER; NATÁLIA HAHN LUMERTZ; RICARDO MACHADO XAVIER; ROSELENA SILVESTRI SCHUH; VINICIUS MONTEAGUDO DE BARROS; WILLIAN DA SILVA CARNIEL

**Institution:** UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL — PORTO ALEGRE/RS

**Introduction:** Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent synovial inflammation and progressive joint destruction. Although current therapies improve disease control, many patients remain refractory or experience adverse effects. Non-viral CRISPR/Cas9 delivery using liposomal vectors represents a promising strategy for localized therapeutic gene expression. **Objective:** To develop liposomal vectors carrying CRISPR/Cas9 plasmid DNA (pDNA) and a donor pDNA to induce IFN- $\beta$  expression in fibroblast-like synoviocytes (FLS) from patients with RA and in a collagen-induced arthritis (CIA) mouse model. **Methods:** Primary FLS isolated from synovial fluid of RA patients were treated with liposomal CRISPR/Cas9 complexes. Cell viability was assessed by MTT assay (n=3), liposome uptake by flow cytometry after 24 and 48 h, and IFN- $\beta$  secretion by ELISA. For in vivo evaluation, male DBA/1J mice with CIA received intra-articular administration of saline (n=4), naked liposomal vector (n=5), or IFN- $\beta$  CRISPR/Cas9 liposomal vector (n=5). Paw edema and clinical arthritis scores were monitored throughout the study. IFN- $\beta$  and TNF- $\alpha$  levels were quantified by ELISA in cell supernatants and joint samples. Statistical analyses included one-way ANOVA with Dunnett's post hoc test and Spearman correlation. **Results:** Liposomal complexes maintained FLS viability above 70% at all tested concentrations. Efficient intracellular uptake was observed, with fluorescence increasing by approximately 30% after 24 h and 40% after 48 h compared with untreated cells. Treatment significantly increased IFN- $\beta$  secretion at both 24 h ( $15.42 \pm 10.78$  vs.  $1.00 \pm 0.00$  pg/mL;  $p < 0.01$ ) and 48 h ( $77.61 \pm 28.49$  vs.  $1.00 \pm 0.00$  pg/mL;  $p < 0.01$ ). In CIA mice, intra-articular administration of the liposomal CRISPR/Cas9 vector significantly reduced paw edema ( $0.09 \pm 0.01$  vs.  $0.12 \pm 0.00$ ;  $p = 0.019$ ), showed a strong trend toward lower clinical arthritis scores ( $5.6 \pm 2.19$  vs.  $9.33 \pm 1.15$ ;  $p = 0.054$ ), and increased intra-articular IFN- $\beta$  levels ( $67.26 \pm 28.71$  vs.  $27.07 \pm 15.56$  pg/mL;  $p = 0.035$ ). IFN- $\beta$  levels negatively correlated with disease severity, whereas TNF- $\alpha$  positively correlated with both clinical score and paw edema. **Conclusion:** Liposomal CRISPR/Cas9 delivery efficiently promoted IFN- $\beta$  expression in vitro and in vivo, while reducing experimental arthritis severity. These findings support this platform as a promising and potentially safer strategy for localized gene therapy in rheumatoid arthritis.

**Keywords:** Rheumatoid arthritis; CRISPR/Cas9; Liposomes; Non-viral gene delivery.

## Abstract 67 | Poster | Poster Session Day 1

### Imidazole-Functionalized Oligomeric Chitosan Derivatives for mRNA Delivery.

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Milena Garcia Ferreira; Gabriel José de Carli; MARCIO JOSÉ TIERA; Vera Aparecida de Oliveira Tiera

**Institution:** Instituto de Biociências, Letras e Ciências Exatas - UNESP/Ibilce — São José do Rio Preto-SP

Polymeric polyplexes are attractive non-viral platforms because their chemical structure can be tuned to adjust charge density, molecular weight, hydrophobicity, biodegradability, buffering capacity, and intracellular responsiveness [1]. In cationic polymer systems, mRNA complexation must be strong enough to condense and protect the cargo, but sufficiently reversible to allow cytoplasmic release after endocytosis. High molecular weight or highly charged polycations may bind nucleic acids too strongly, increasing cytotoxicity and impairing intracellular release. Imidazole-containing groups are useful because the imidazole ring has a pKa near 6, being less protonated at physiological pH but increasingly protonated in the mildly acidic endosomal environment. This pH window can enhance buffering, counteract endosomal acidification, and contribute to endosomal membrane destabilization [2]. Imidazole-functionalized oligomeric chitosans were successfully synthesized and showed improved capacity to complex mRNA. The derivatives were characterized by potentiometric titration and <sup>1</sup>H-NMR spectroscopy, while gel permeation chromatography revealed oligomeric chains with a molecular weight of 6.3 kDa and a PDI of 1.8, confirming the production of oligomeric chitosan with a relatively narrow molecular weight distribution following depolymerization. Potentiometric titration indicated protonatable groups over a broad pH range, consistent with improved buffering capacity. NMR analysis confirmed imidazole incorporation through characteristic signals at 5.37-5.42 ppm and 6.77-9.16 ppm. Integration of the imidazole signals relative to the chitosan backbone signals allowed the determination of substitution degrees of 32.0%, 42.3%, and 50.7%. The successful chemical modification was further supported by the preservation of the characteristic chitosan backbone resonances together with the appearance of new signals assigned to the imidazole moieties. Moreover, the progressive increase in the relative intensity of the imidazole proton signals was consistent with the increasing degree of substitution among the synthesized derivatives, demonstrating that the reaction proceeded in a controlled manner. Increasing imidazole substitution reduced the N/P ratio required for complete mRNA retention, confirming that the introduced pH-responsive groups modulate polymer-mRNA interactions. Gel electrophoresis showed that mRNA complexation improved with the degree of imidazole substitution. The derivative containing 32% imidazole substitution fully retained mRNA at N/P ratios 10, whereas the 42.30% derivative achieved complete retention from N/P ratios 5 and the 50.7% derivative complexed mRNA at even lower N/P ratios. These results support imidazole-functionalized oligomeric chitosans as promising candidates for the development of polymeric mRNA nanocarriers with tunable complexation and pH-responsive behavior.

**Keywords:** Chitosan, Amphiphilic Polymer, Nanoparticles, Cytoplasmic Delivery, mRNA, Gene Therapy.

## **Abstract 68 | Poster | Poster Session Day 1**

### **Optimization of CRISPR-Based Non-Viral Strategies for Allogeneic CAR-T Cell Generation Using Targeted Knock-In and Transposon-Mediated CAR Integration**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

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**INTRODUCTION AND OBJECTIVES:** Chimeric antigen receptor (CAR)-T cell therapy has demonstrated remarkable clinical efficacy for hematological malignancies. However, current manufacturing strategies are predominantly autologous and rely on viral vectors, resulting in high production costs, prolonged manufacturing times, variability in starting material, and limited scalability. Generation of allogeneic CAR-T cells requires disruption of the T-cell receptor (TCR) to prevent graft-versus-host disease (GVHD). Non-viral approaches, including Sleeping Beauty (SB)-mediated transposition and CRISPR/Cas9-mediated knock-in (KI) into the TRAC locus, represent attractive alternatives. Although targeted KI may improve CAR regulation and functionality, its efficiency remains limited. Inhibition of the non-homologous end joining (NHEJ) pathway using M3814 may enhance homology-directed repair, while base editors (BE) offer a safer alternative to conventional CRISPR/Cas9 by avoiding double-strand DNA breaks. Building on our previous generation of functional allogeneic CAR-T cells using CRISPR combined with SB or KI, this study aimed to optimize KI efficiency using M3814 and to standardize BE for combination with SB-mediated CAR insertion. **MATERIALS AND METHODS:** PBMCs were isolated, CD3<sup>+</sup> T cells were purified, and cells were electroporated using the 4D-Nucleofector with CRISPR/Cas9 ribonucleoproteins (RNPs), SB plasmids, donor DNA, or BE mRNA. Experimental groups included Mock, RNP, RNP+SB, SB alone, KI alone, BE alone, and BE+SB. TCR knockout and CAR expression were evaluated by flow cytometry, while cell expansion and antitumor activity were also assessed. **RESULTS AND CONCLUSION:** M3814 increased the frequency of CAR<sup>+</sup> KI cells but reduced overall cell expansion, without affecting antitumor activity after drug removal 24 hours post-electroporation. BE optimization demonstrated that 3 and 5 µg of mRNA achieved comparable TCR knockout efficiencies of up to 33%. However, combining BE with SB failed to generate either TCR knockout or CAR<sup>+</sup> cells, in contrast to the RNP+SB approach. Interestingly, RNP+SB resulted in preferential CAR expression within the TCR-knockout population. To determine whether this phenomenon was specific to SB, additional transposon platforms, including PiggyBac (PB) and minicircle (MC) vectors, were evaluated. Similar enrichment of CAR expression in TCR-knockout cells was observed regardless of the transposon system, without compromising antitumor activity. Overall, M3814 improved KI efficiency at the expense of cell expansion, whereas BE efficiently mediated TCR disruption but was incompatible with transposon-mediated CAR delivery under the conditions tested. Furthermore, preferential CAR expression in TCR-knockout cells appears to be independent of the transposon platform, providing new insights for the development of non-viral allogeneic CAR-T manufacturing strategies.

**Keywords:** CRISPR/CAS9, Immunotherapy, CAR-T; Base Editor

## **Abstract 69 | Poster | Poster Session Day 1**

### **Gene Therapy for Neurodegeneration Based on miR-431 Overexpression**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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Neurodegeneration in the central nervous system (CNS) is a complex, multifactorial process observed in traumatic spinal cord injury and in chronic progressive diseases such as Alzheimer's disease. Neurodegeneration is characterized by synapse loss, axonal degeneration and neuronal death, which lead to permanent neurological deficits. There are no effective treatments capable of blocking neurodegeneration or promoting CNS regeneration; however, gene therapy strategies are emerging as promising alternatives. In this context, the use of microRNAs (miRNAs) as a gene therapy strategy offers a multifactorial approach to neurodegenerative diseases and traumatic injuries, based on the broad modulation of gene expression. miR-431 specifically has been identified as a regulator capable of preventing  $\beta$ -amyloid-induced synapse loss through silencing of the Kremen1 pathway, resulting in activation of the WNT/ $\beta$ -catenin pathway, which is essential for neurogenesis and axonal regeneration. Nevertheless, the role of miR-431 in neuroprotection and regeneration is still poorly understood. The general objective of this study is to test, in vitro, whether miR-431 overexpression in primary cortical neurons is able to promote neuroprotection, axonal growth and regeneration. Specifically, we investigate the effect on neurite outgrowth, on the complexity of neurite arborization, on the capacity for axonal regeneration after axotomy and on the neuroprotective potential against N-methyl-D-aspartate (NMDA)-induced damage, in addition to identifying the miR-431 target genes involved in the observed effects. For this purpose, recombinant adeno-associated virus (rAAV) vectors expressing miR-431 (rAAV.miR-431) and a control vector (rAAV.CTRL), expressing only the mCherry reporter gene, were produced. The rAAV.miR-431 and rAAV.CTRL vectors efficiently transduced cortical neurons in vitro, as shown by mCherry expression. Preliminary data suggest that the rAAV.miR-431 vector had no effect on the survival of neurons exposed to NMDA compared with the rAAV.CTRL vector. On the other hand, transduction with the rAAV.miR-431 vector appears to increase neurite outgrowth and axonal regeneration of cortical neurons plated in microfluidic chambers when compared with transduction with the rAAV.CTRL vector. Finally, the effect of rAAV.miR-431 on NMDA-induced synapse loss and the target genes of this miRNA are being evaluated by bioinformatics analyses. These results indicate that miR-431 may be a promising candidate for rAAV-based gene therapy strategies aimed at axonal regeneration in neurodegenerative diseases.

**Keywords:** gene therapy; axonal regeneration; miR-431; neuroprotection; regeneration; neurodegeneration

## **Abstract 71 | Poster | Poster Session Day 1**

### **Proliferative Potential Characterization of Human Limbal Epithelial Stem Cells from Cadaveric Donors: Advancing CALEC Therapy Implementation**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** MARIZE CAMPOS VALADARES

**Institution:** Tox In, School of Pharmacy, Federal University of Goiás — Goiânia

Limbal epithelial stem cell (LESC) deficiency represents a major cause of corneal damage, and Cultivated Autologous Limbal Epithelial Cells (CALEC) transplantation has emerged as a promising strategy for ocular surface restoration. The implementation of this therapeutic approach requires the establishment of robust protocols for LESCs isolation, expansion, and characterization. In this context, corneoscleral tissues derived from cadaveric donor's ineligible for transplantation constitute a valuable source for translational research and for the development of cell therapy platforms. This study aimed to characterize LESCs regarding their biomarkers profile and proliferative potential, generating evidence to support the implementation of protocols for future regenerative therapies of the ocular surface based on the CALEC concept. LESCs were isolated from ocular globes of cadaveric donor's ineligible for transplantation, provided by the Goiás Eye Bank Foundation (FUBOG). Cell isolation was performed by sequential enzymatic dissociation using Dispase II and TrypLE Express, followed by cell viability determination. Cells were cultured under standardized conditions and the biomarkers CK3, CK12, CK14, CK15, ZO-1, Ki67, TP63, and C/EBP were assessed by immunocytochemistry (passage 0), with quantitative analysis performed by high-content fluorescence microscopy (High-Content Analysis, HCA; Thermo Fisher Scientific). The proliferative potential of the cells was evaluated by a clonogenic assay at passages 0 and 1 to determine the Colony Forming Efficiency (CFE). Cell viability exceeded 41% for donors. In the clonogenic assay, donors exhibited holoclone formation at passage 0, whereas at passage 1 a predominance of meroclones and paraclones was observed. The evaluation of proliferation and differentiation biomarkers revealed, for both donors, high expression of CK14 and moderate to high expression of CK15, a profile consistent with progenitor LESCs and corroborated by the clonogenicity assays, in addition to the preservation of epithelial occlusive junctions, as evidenced by ZO-1 staining. Furthermore, donors exhibited low-to-moderate expression of TP63 and C/EBP, as well as low or absent expression of CK3 and CK12, suggesting a predominance of cells with progenitor characteristics and reduced differentiation. The Ki67 expression, indicative of proliferative capacity, was moderate in the evaluated cells. LESCs derived from cadaveric donors demonstrated viability, clonogenic capacity, with a biomarker profile consistent with progenitor cells, confirming the potential of these tissues as a source for stem cell expansion protocols. These findings represent a significant step toward the implementation of regenerative therapies for the ocular surface based on the CALEC concept.

**Keywords:** Corneal epithelium; Cell therapy; Limbus; Regenerative Medicine

## Abstract 72 | Poster | Poster Session Day 1

### BISPECIFIC CAR WITH MODULAR ASSEMBLY VIA IGG DOMAINS: A NEW PLATFORM FOR DUAL TARGETING

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Paulo Vitor Dias de Souza; Andrea Queiroz Maranhão; Dyane Rocha Aragão; Igor Cabral Studart; Isabel Garcia Sousa; Isabela Vitoria Tavares Werneck; Marcelo de Macedo Brigidio

**Institution:** universidade de Brasília - UnB — Brasília - DF

Chimeric antigen receptors (CARs) are synthetic proteins that combine antibody binding specificity with T-cell activation machinery, redirecting the immune system against tumor targets. Although effective in treating hematological malignancies, CAR T-cell therapy targeting a single antigen, such as CD19, faces therapeutic limitations due to antigen escape and subsequent relapse. Simultaneous dual targeting of two antigens, like CD19 and CD20 for leukemia, can mitigate this resistance. To achieve this, conventional bispecific constructs can be applied, but they often suffer from structural instability. In this regard, we propose an innovative bispecific architecture based on the natural heterodimerization of immunoglobulin constant domains (CH1 and Ck), establishing a stable modular platform for multispecific receptors. A bispecific CAR was developed containing two antigen-recognition domains (scFv): FMC63 scFv (anti-CD19) containing an amino terminus MYC tag and fused to the CH1 constant domain (heavy chain), which also contains the other CAR domains: hinge, transmembrane and intracellular CD3 zeta and 4-1BB signaling domains; and Rituximab scFv (anti-CD20) containing an amino terminus HA tag fused to the Ck constant domain. These chains are directed to the membrane by CD8 and IL-2 signal peptides, respectively. The constructs were cloned into the pBCAG vector (PiggyBac system) in a bicistronic way in two orientations: anti-CD19 - peptide T2A - anti-CD20 (MB19/20) and anti-CD20 - peptide T2A - anti-CD19 (MB20/19), where the processing of T2A generates the two polypeptides described. The two constructs were transfected into Expi293 cells. Expression was evaluated by flow cytometry at 48h (single anti-MYC staining) and 72h (single anti-HA staining) post-transfection. Flow cytometry revealed distinct expression kinetics between MB19/20 and MB20/19. At 48h, mock background was 0.63%. For MB19/20, transposon:transposase ratios of 1:2 and 1:1 resulted in 0.98% and 1.06% CAR+ cells, and 2:1 resulted in 12.2%. For MB20/19, percentages were 22.0% (1:2), 56.4% (2:1), and 62.2% (1:1), surpassing the conventional CAR control (40.7%). At 72h, MB19/20 showed 0.77% (1:2), 30.2% (2:1), and 35.4% (1:1). For MB20/19, the 1:2 ratio resulted in 83.1% CAR+ cells, 2:1 showed 57.4%, and 1:1 reached 93.7% positive cells. These results confirm the feasibility of surface expression for the bispecific CAR via molecular heterodimerization using CH1 and Ck domains. The variations between MB19/20 and MB20/19 suggest that the positional order of the scFvs and the adjustment of the molecular ratio between transposon and transposase determine expression efficiency, consolidating this innovative modular architecture as a baseline model for future multi-targeting developments. As subsequent steps, binding assays against CD19 and CD20 antigens and transfection assays in human PBMCs will be conducted.

**Keywords:** CAR T; bispecific; immunotherapy; lymphocyte.

## **Abstract 73 | Poster | Poster Session Day 1**

### **Rare maternal coding variants in IRF3 and TNF identified in a congenital Zika syndrome case series: investigating their potential role in inborn errors of immunity**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Renato Gontijo

**Institution:** Universidade Federal do Rio de Janeiro (UFRJ) — Rio de Janeiro (RJ)

Case-control epidemiological studies have demonstrated associations between common maternal variants in immune-related genes and susceptibility to congenital Zika syndrome (CZS). However, the functional role of rare variants in this context remains poorly understood. Here, we identified 13 rare maternal variants in a case series comprising 28 mothers of children with CZS from Campina Grande, Brazil, where the second-highest prevalence of microcephaly among newborns was reported during the 2015 ZIKV outbreak. These variants were prioritized according to their predicted functional impact, with occurrence in the homozygous state considered an additional criterion. Two variants stood out through this approach: a TNF variant detected in two mothers, one homozygous and one heterozygous, and an IRF3 variant detected in the heterozygous state in another mother. The corresponding children were then genotyped, confirming transmission of the respective variants to the affected probands. To investigate whether these variants may underlie defects in innate immunity consistent with inborn errors of immunity, we generated candidate knockout cellular models for TNF and IRF3. Guide RNA sequences targeting these genes were designed using the Broad Institute's CRISPick tool and cloned into CRISPR/Cas9 vectors. The vectors were enzymatically digested in parallel with guide RNA oligonucleotide phosphorylation. The oligonucleotides were subsequently ligated into the vectors using T4 DNA ligase, followed by heat-shock transformation into *Escherichia coli* DH10 $\beta$  cells. Recombinant clones were confirmed by colony PCR and agarose gel electrophoresis. The constructs were then transfected into HBMEC and HEK293T cell lines using Lipofectamine 3000, followed by the enrichment of construct-positive cells through fluorescence-activated cell sorting. Following knockout validation, the established cellular models will be transfected with plasmids encoding wild-type TNF or IRF3, or their corresponding mutants, and then challenged with ZIKV to evaluate the effects of these variants on cytokine responses, cell survival, and viral replication.

**Keywords:** CRISPR/CAS9, innate immunity, susceptibility, infectious disease

## **Abstract 74 | Poster | Poster Session Day 1**

### **Reprogrammed synthetic Zika Virus as both an oncolytic virotherapy and a delivery platform for glioblastoma immunotherapy**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Enzo Oliveira Barone; Carolina Filgueiras Sanchez; Elibio Leopoldo Rech Filho; Fábio Luís Lima Monteiro; Gabriel Cardoso Atella; Lendel Correia da Costa; Luiza Mendonça Higa; MARTÍN BONAMINO; Rayane Nunes Lima; Stevens Kastrup Rehen

**Institution:** Universidade Federal do Rio de Janeiro — Rio de Janeiro

Glioblastoma (GBM) is the primary malignant tumor of the central nervous system with the highest incidence and aggressiveness, characterized by marked cellular heterogeneity and resistance to currently available therapies, including immunotherapy. At present, patients diagnosed with GBM have a median survival of less than 15 months, highlighting the need to develop alternative therapeutic approaches. In this context, oncolytic virotherapies stand out; these are composed of viruses engineered to selectively infect, replicate in, and destroy tumor cells while sparing healthy cells. Among candidate viruses for an oncolytic virotherapy against GBM is Zika virus (ZIKV), a mosquito-borne flavivirus with an 11 kb positive-sense RNA genome, that exhibits intrinsic tropism for glioblastoma stem cells. This natural tropism, coupled with its ability to induce cytotoxic effects and promote local immune activation by recruiting immune cells, positions ZIKV as a tool for disruptive glioblastoma therapies. Recently, Chimeric Antigen Receptor T-cell (CAR-T) therapy, an immunotherapeutic strategy in which patient-derived T cells are genetically engineered to recognize and eliminate tumor cells expressing specific antigens, have also risen as an alternative to treat glioblastoma alongside other strategies. In this study we engineered a synthetic ZIKV that expresses CD19, a powerful and well-characterized antigen for CAR-T cells therapy, in order to turn the already oncolytic ZIKV in a delivery method for the CAR-T's target, fusing two therapeutic strategies in just one virus. Using the Brazilian ZIKV-PE strain (PE243/2015) as a chassis, the CD19 gene was inserted into the viral genome through CPER (Circular Polymerase Extension Cloning). Moreover, to increase safety, two targeted microRNA responsive elements (MREs) were incorporated into the viral genome to attenuate viral replication in healthy cerebral parenchymal cells (1). The generated viruses were functionally characterized in vitro through genomic sequencing and the analysis of replication kinetics. Using GBM stem-cells (GSCs) and patient-derived glioblastoma neurospheres, the inherent oncotropism of the ZIKV constructs was confirmed, as evidenced by their selective infection and significant oncolytic damage to tumor cells. The expression of CD19 was accessed through an immunofluorescence assay using an anti-CD19 antibody, which confirmed the expression in infected cells. Now the generated viruses will be used in in vitro and in vivo assays to determine if CD19-recognizing CAR-T cells can use the antigens expressed in infected glioblastoma cells to identify and kill the tumoral infected cells.

**Keywords:** Glioblastoma, ZIKV, CD19, CAR-T

## **Abstract 75 | Poster | Poster Session Day 1**

### **Construction of the RRV-P14ARF Replicative Retroviral Vector and Validation in Solid Tumor Models**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Mariana Barbosa de Souza

**Institution:** ICESP - Instituto do Câncer do Estado de São Paulo — São Paulo - SP

Cancer immunotherapy has emerged as a promising strategy to overcome limitations of conventional treatments, particularly in solid tumors. Among these approaches, oncolytic gene therapy aims to selectively target tumor cells while stimulating antitumor immune responses. Replicative retroviral vectors (RRVs) are an attractive platform due to their selective replication in dividing cells and stable genomic integration, enabling sustained transgene expression. However, clinical strategies using suicide genes, such as Toca511 (pAC3-yCD2), have shown limited efficacy, highlighting the need for alternative genetic payloads. We propose the development of RRVs encoding the tumor suppressor gene p14ARF as a novel oncolytic strategy. p14ARF activates the p53 pathway through inhibition of MDM2, promoting cell cycle arrest and apoptosis, and has been associated with immunogenic cell death. The workflow has been initiated with validation, amplification, and production of pAC3-yCD2 and pAC3-emd (GFP) plasmids using calcium phosphate transfection. Viral particles were generated and titrated by TaqMan qPCR, yielding titers of  $3.56 \times 10^5$  and  $3.33 \times 10^5$  IU/mL for GFP and CD constructs. Preliminary monitoring of viral replication in HT1080 cells demonstrated progressive expansion of GFP-positive cells over 72h in a time and dose-dependent manner, with GFP-positive cells increasing from 3.1% to 45.5% at the highest viral input (20  $\mu$ L). In contrast, cultures treated with azidothymidine (AZT) showed no increase in GFP fluorescence, confirming that viral spread was replication-dependent and supporting the functionality of the platform. In parallel, construction of RRV-p14ARF and RRV-NanoLuc vectors is underway. The viral backbone was processed by restriction digestion and agarose gel purification. Inserts were amplified by PCR using template plasmids. Cloning was performed using Gibson Assembly followed by electroporation into DH10B bacteria. Although positive constructs haven't yet been obtained, optimization is ongoing. Following vector construction, functional validation will be performed in melanoma cell lines (UACC62, SKMel-147, SKMel-29) in 2D and 3D systems. Viral expansion will be monitored by flow cytometry, luminescence assays, and immunofluorescence. Oncolytic activity will be assessed through metabolic assays, cell cycle analysis, and caspase activation, while modulation of immune-related genes will be evaluated by RT-qPCR. We expect RRV-p14ARF to demonstrate efficient replication in tumor cells and induce oncolytic effects via ARF/p53 activation. In addition to direct cytotoxicity, modulation of immune-related genes may support immunogenic cell death. Even with limited cytolysis, the vector may contribute to tumor microenvironment remodeling and enhance combination therapies such as CAR-T cells. Overall, this study aims to establish RRV-mediated delivery of p14ARF as a gene therapy approach for solid tumors, with translational potential in cancer immunotherapy.

**Keywords:** Replicative Retroviral Vectors (RRVs), Oncolysis, p14ARF

## **Abstract 77 | Poster | Poster Session Day 1**

### **Normative Argumentation in Bioethical Literature on Somatic Gene Therapy: An Interpretative Review**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** DIOGO FELIPE CORECHA DO NASCIMENTO; Murilo Mariano Vilaça

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As somatic gene therapies enter routine clinical practice, questions concerning equitable implementation, governance, reimbursement, and long-term responsibility have become increasingly prominent in bioethical debate. This interpretative review examined how recent bioethical literature organizes explicit normative argumentation concerning somatic gene therapy. Publications from 2021 to 2025 were identified through searches in PubMed, Google Scholar, LILACS, and PhilPapers. Studies addressing somatic gene therapy or closely related cell and gene therapy contexts were included when they developed explicit normative argumentation grounded in justificatory reasoning. Each publication was interpretatively classified according to the predominant ethical problem structuring its principal normative argument. Thirty-two publications met the inclusion criteria. Based on this analysis, 25 studies (78.1%) were classified as implementation-centered and 7 (21.9%) as intervention-centered. Implementation-centered publications primarily addressed equitable access, distributive justice, pricing and reimbursement, governance, regulation, healthcare organization, and institutional responsibility, whereas intervention-centered publications focused on risk-benefit assessment, informed consent, uncertainty, moral permissibility, and the distinction between therapy and enhancement. The review also identified numerous publications that referred to ethical implications without developing sustained normative argumentation, underscoring the analytical importance of distinguishing ethical concerns from explicit normative argumentation. These findings suggest that, within the analyzed corpus, normative bioethics increasingly evaluates the institutional conditions required for responsible implementation alongside the genetic intervention itself. As somatic gene therapies move into routine clinical practice, governance, equitable access, reimbursement, long-term follow-up, and sustainability emerge as integral components of responsible clinical translation. Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

**Keywords:** somatic gene therapy; bioethics; clinical translation; implementation; governance.

## Abstract 78 | Poster | Poster Session Day 1

### Overcoming biological barriers in non-viral CAR-T engineering: The impact of polymer to plasmid ratio on nano-polyplex performance

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Ana Beatriz Caribé dos Santos Valle; Helvécio Vinicius Rocha Antunes; Juliana Palermo Tobler

**Institution:** Fundação Oswaldo Cruz — Rio de Janeiro - RJ

CAR-T cell immunotherapy has established itself as a breakthrough pillar in oncology, yet the reliance on viral vectors for cellular engineering imposes severe challenges regarding cost, safety, and industrial scalability [1]. In this context, the development of non-viral platforms, such as nano-polyplexes designed for gene delivery, presents a highly attractive strategy for CAR-T cell generation [2]. The success of this approach, however, relies on the nanovector's ability to efficiently complex, protect, and release the genetic material within the intracellular environment [3]. Consequently, fine-tuning the polymer to DNA ratio becomes a fundamental parameter to correlate the physicochemical stability of the complex with biological transfection efficiency [4]. In this sense, this study aimed to develop, physicochemically characterize, and evaluate in vitro nano-polyplexes to express an anti-CD19 CAR. Initially, a 180:1 polymer:plasmid ratio was evaluated using PBLR7 and PBLR7-3D polymers. The hydrodynamic diameter of pure PBLR7 and DNA-complexed PBLR7 was 151.6 nm (PDI 0.45) and 272.6 nm (PDI 0.18), respectively, while PBLR7-3D ranged from 163.5 nm (empty) to 208.3 nm (with DNA), maintaining a stable PDI of 0.18 in both formulations. Nanoparticle tracking analysis showed a mean size of 85 and 92 nm and a particle concentration of approximately 2.6 and 2.35E+11 particle/mL for PBLR7 and PBLR7-3D, respectively. The size and PDI were also evaluated after polyplexes exposure to cell medium to understand the stability of the formulation during transfection and they remained stable for at least four hours, unlike the formulation kept at room temperature. Agarose gel retention assay demonstrated PBLR7-3D plasmid complexation, outperforming PBLR7 (~50%). This is probably due to the addition of 3D cap that enhances the number of amino groups which are anchor points for plasmid complexation. Additionally, the polyanion competition assay with heparin revealed a concentration-dependent disassembly of the complex, with the PBLR7-3D polyplex remaining remarkably stable up to a heparin concentration of 10 µg/mL. Despite the favorable physicochemical profile at the 180:1 ratio, transfection assays in HEK cells resulted in only 3% efficiency. To overcome this biological barrier, a 360:1 ratio was tested, which achieved 100% transfection in the same cell line by delivering polyplexes of around 300 nm with full plasmid complexation. These results highlight that tuning the polymer to plasmid ratio is a critical factor for successful non-viral transfection. Future perspectives will focus on the detailed physicochemical characterization of the new 360:1 formulation, alongside the incorporation of specific antibodies onto the polyplex surface to enable targeted and optimized transfection in peripheral blood mononuclear cells-derived primary lymphocytes, which are the target cells.

**Keywords:** Poly(beta-amino ester), gene therapy, immunotherapy, anti-CD19

## **Abstract 81 | Poster | Poster Session Day 1**

### **Extracellular Vesicles Derived from Human Corneal Stromal Stem Cells: Isolation, Characterization, and Purity Assessment for Regenerative Medicine**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Voltaire Vieira Vasconcelos

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Extracellular vesicles (EVs) are nanoparticles delimited by a lipid bilayer, secreted by virtually all cell types, and mediate intercellular communication through the transport of proteins, lipids, and nucleic acids. These characteristics, combined with their biocompatibility, low immunogenicity, and ability to deliver bioactive molecules to specific tissues, make EVs a promising therapeutic strategy for applications in regenerative medicine. The objective of this study was to standardize methods for the extraction, isolation, quantification, and characterization of EVs derived from corneal stromal stem cells for the treatment of retinal neurodegenerative diseases. Stromal cells were characterized by flow cytometry to evaluate the expression of the CD90, CD73, and CD105 markers. The conditioned medium was collected after the cells reached 70% confluence and were incubated for 48 hours in DMEM/F12 medium without fetal bovine serum (FBS). The conditioned medium was subjected to ultracentrifugation and evaluated using the bicinchoninic acid (BCA) protein assay, sulfophosphovanillin (SPV) lipid assay, transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), NanoAnalyzer (NanoFCM), and immunoblotting for the EV-specific markers TSG101, CD63, and CD81. Total protein quantification in the extracellular vesicle samples was performed using the BCA method, yielding a mean protein concentration of 0.65 µg/µL of protein. Lipid quantification was performed using the SPV method, yielding a mean lipid concentration of 0.14 µg/µL. Subsequently, the protein-to-lipid (P/L) ratio was calculated as an indicator of sample purity, yielding an average of 7.7. Based on the BCA results, immunoblotting of three supernatants was performed, showing positive immunoreactivity for TSG101. TEM revealed spherical structures, confirming the morphological integrity of the isolated EVs, with diameters predominantly ranging between 50 and 150 nanometers. NTA, NanoFCM, and DLS yielded complementary particle size distribution profiles ranging from 60 to 180 nanometers, demonstrating that the combination of these techniques is essential for the robust physical characterization of the samples. NanoFCM analysis of the conditioned media using TSG101, CD63, and CD81 antibodies conjugated with secondary antibodies confirmed the presence of EVs in the samples. Following this characterization, cellular internalization assays will be conducted with these EVs. Thus, this study established standardized and reproducible protocols for the isolation and characterization of extracellular vesicles derived from human corneal stromal stem cells.

**Keywords:** Exosomes, Ultracentrifugation, Retinal neurodegenerative diseases, Nanoparticles, Biomarkers

## **Abstract 82 | Poster | Poster Session Day 1**

### **Porcine Placental Matrix Hydrogel: A Promising Biomaterial for Wound Healing**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Maria Claudia Passos

**Institution:** Laboratory of Research and Education in In Vitro Toxicology, Faculty of Pharmacy, Federal University of Goiás, Brazil — Goiânia - GO

The extracellular matrix (ECM) plays a fundamental role in tissue repair processes, providing structural support and biochemical signaling that guide cell migration, angiogenesis, and matrix remodeling. Biomaterials derived from decellularized ECM have emerged as a promising strategy for wound treatment, as they preserve bioactive components essential for healing. In this context, porcine placenta emerges as an advantageous alternative source, due to its abundance, low cost, and richness in structural proteins and growth factors. The aim of this study was to develop and characterize a biomaterial derived from porcine placental ECM with potential application in wound healing. Porcine placentas were subjected to a decellularization process using SDS, and the resulting ECM was processed to obtain a hydrogel, which was tested at different concentrations (1.5 to 10.8 mg/mL) for its gelation capacity. The material was characterized in terms of total protein, collagen, and glycosaminoglycan (GAG) content, as well as quantification of the growth factors bFGF and VEGF. Chemical composition was assessed by FTIR, protein profile by SDS-PAGE, and matrix architecture by scanning electron microscopy (SEM). Hydrogel biocompatibility was evaluated in keratinocyte (HaCaT) and human neonatal dermal fibroblast (HDFn) cultures. The SDS-based decellularization process proved effective in obtaining porcine placental ECM, preserving its main structural and bioactive components. Biochemical analyses confirmed the maintenance of total proteins, collagen, and glycosaminoglycans (GAGs), as well as the presence of the growth factors bFGF and VEGF, molecules essential for angiogenesis and the wound healing process. FTIR and SDS-PAGE corroborated the preservation of ECM-characteristic protein components, including type I and type IV collagen and fibronectin, while SEM revealed a three-dimensional fibrillar architecture, fundamental for structural support and cell migration. The resulting hydrogel demonstrated good gelation capacity, with stability maintained for up to 7 days, as well as biocompatibility in HaCaT and HDFn cultures. The results demonstrate that ECM derived from porcine placenta preserves structural components and bioactive growth factors, in addition to exhibiting the ability to form stable, biocompatible hydrogels. A cell migration assay (scratch assay) is currently underway, and future steps include evaluating the hydrogel in a human skin radiodermatitis model, aiming to validate its therapeutic potential for the treatment of this condition.

**Keywords:** Extracellular matrix; Porcine placenta; Hydrogel; Wound healing; Biomaterial

## Abstract 84 | Poster | Poster Session Day 1

### Development of CD19-CAR variants for the modification of CD3 $\zeta$ -expressing NK-92 cells

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Isabele Held Lemos; Gustavo Matheus Amaro; Michele Procópio Machado; Milena Barrocali de Araújo Melo; Nathália Ferreira Fregonezi; Thiago Aparecido da Silva; Victor Gabriel Santos Silva

**Institution:** Universidade Estadual Paulista "Júlio de Mesquita Filho" — Araraquara - SP

**Introduction:** CD19-Chimeric Antigen Receptor (CAR)-T cell therapy has transformed the treatment of B-cell malignancies. However, severe adverse events, including cytokine release syndrome and neurotoxicity, together with manufacturing limitations, have motivated the modification of other immune cells using CAR. Natural killer (NK) cells expressing CD19-CAR are a promising strategy based on their safety profile and potential for allogeneic application. The NK-92 cell line is an important platform for CAR engineering; however, the lack of CD3 $\zeta$  expression may impair signaling transduction domain triggered by CD19-CAR. Moreover, CD3 $\zeta$  expression is essential to contribute to the cytotoxic effect mediated by NKp30 receptor. Then, the current work proposed the modification of NK-92 cells to express both CD3 $\zeta$  and CD19-CAR containing NKp30 as costimulatory domain, which will be evaluated against malignant B cells. **Methods:** Lentiviral vectors encoding CD3 $\zeta$  and three CD19-CAR variants were generated, as follows: CD19-NKp30-CAR, CD19-CD8-NKp30-CAR, and CD19-NKp46-NKp30-CAR. The constructions were validated by restriction enzyme digestion and PCR. Lentiviral particles were produced in HEK-293T cells and used to transduce NK-92 cells by spinoculation. Transgene expression was evaluated by flow cytometry, followed by fluorescence-activated cell sorting. CAR expression on the cell surface was performed using Protein L and anti-Fab antibody. The cytotoxicity activity of modified NK-92 cells was evaluated using luciferase-expressing Daudi and NALM-6 cells. **Results:** Lentiviral vectors encoding CD3 $\zeta$  or CD19-CAR variants were successfully validated. Lentiviral transduction efficiencies reached 17.4% for CD3 $\zeta$ , 18.0% for CD19-NKp30-CAR, 10.7% for CD19-CD8-NKp30-CAR, and 11.2% for CD19-NKp46-NKp30-CAR. Transduced populations were subsequently enriched by cell sorting. The approach using Protein L or anti-Fab antibody to detect CAR expression on the cell surface showed that CD19-CAR variants did not anchor in the membrane. Cytotoxic assay demonstrated that CD3 $\zeta$ -engineered NK-92 cells exhibited increased cytotoxicity against Daudi and NALM-6 target cells compared with unmodified NK-92 cells. **Conclusions:** NK-92 cells are suitable for the expression of CD3 $\zeta$  or CD19-CAR variants, retaining expression even after freezing and thawing. CD3 $\zeta$  expression enhanced NK-92 cytotoxic function, supporting its role in the NK-92 cell activation. In addition, CD19-CAR variants containing NKp30 as costimulatory domain need optimization to improve CD19-CAR expression on the cell surface. These findings provide novel insights for the development of CD19-CAR-NK-92 cells.

**Keywords:** Chimeric Antigen Receptor Therapy; Leukemia, Lymphoid; Natural Killer T-Cell

## Abstract 85 | Poster | Poster Session Day 1

### Selection of Novel Fully Human Anti-CD19 scFvs for CAR-T Cell Development

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Gilvan Pessoa Furtado; Ana Virginia Frota Guimarães; Daniel Pascoalino Pinheiro; Jucilene Pereira de Sousa; Marcos Roberto Lourenzoni; Marcus Rafael Lobo Bezerra; Ronny Petterson dos Santos Araujo

**Institution:** Fundação Oswaldo Cruz, Fiocruz Minas, Instituto René Rachou — Belo Horizonte/MG

**Introduction.** Single-chain variable fragments (scFvs) are key antigen-recognition domains in chimeric antigen receptor (CAR) T-cell therapies. CD19 is a well-established B-cell marker and one of the most clinically validated targets for the treatment of B-cell leukemias and lymphomas. Although murine-derived scFvs, such as FMC63, have demonstrated clinical efficacy, fully human binders may reduce immunogenicity and support the development of improved CAR constructs. Here, we combined phage display, next-generation sequencing (NGS), and phage ELISA to identify novel fully human scFvs targeting the extracellular domain of CD19 (CD19-ECD). **Methods.** A human scFv phage-display library was subjected to four rounds of biopanning against immobilized commercially available CD19-ECD, with progressively increased washing stringency. The naïve library (R0) and selected pools (R1-R4) were sequenced by NGS, and VH and VL repertoires were independently analyzed using ATTILA 2.0 (Automated Tool for Immunoglobulin Analysis), developed by our group, to determine clonal enrichment. In parallel, 278 individual clones from R3 were screened by phage ELISA using recombinant CD19-ECD produced in Expi293F cells. After subtraction of the nonspecific binding control signal, 17 clones with the highest positive/negative signal ratios were retested in duplicate in the presence or absence of immobilized antigen. **Results.** Phage titration showed a progressive increase in the output/input ratio in later selection rounds, consistent with enrichment of CD19-binding clones. NGS analysis demonstrated reduced repertoire diversity during biopanning. ATTILA 2.0 identified highly enriched VH and VL sequences relative to R0, with maximum fold-enrichment values of 2,153 and 15,253, respectively. Among the 17 retested clones, four displayed the highest antigen-present/antigen-absent signal ratios and were selected for Sanger sequencing. Notably, the VH sequence of one ELISA-positive clone showed high identity to the sixth most enriched VH sequence identified by ATTILA 2.0, supporting convergence between sequence-based enrichment and experimental binding data. The selected scFvs are currently being cloned into pET-28-SUMO for recombinant expression and subsequent biochemical and functional characterization. **Conclusion.** The integration of phage display, NGS-based repertoire analysis, and phage ELISA established a robust workflow for the discovery of novel fully human anti-CD19 scFvs. The selected binders represent promising antigen-recognition domains for the development of CD19-targeted CAR-T cells and are undergoing further validation.

**Keywords:** scFv; CD19; CAR-T cells; phage display; B-cell malignancies

## **Abstract 86 | Poster | Poster Session Day 1**

### **A novel CD19 second-generation fast CAR-T product demonstrates robust antitumor responses in a xenograft leukemia model: biomedical innovation to expand access to CAR-T therapy in Colombia and Latam**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Flávia Midori Ishikawa

**Institution:** Advanced Therapies Unit, Instituto Distrital de Ciencia, Biotecnología e Innovación en Salud – IDCBIS; National Institute of Health — Bogotá-DC

**Introduction:** CD19-directed chimeric antigen receptor T-cell (CAR-T) therapy has revolutionized treatment of B-cell malignancies, yet access in Latin America remains limited due to high costs and underdeveloped local biopharma infrastructure. Affordable, scalable and efficient manufacturing strategies, as well as innovative CAR designs could impact not only on product consistency, persistence, and efficacy but also promote better access opportunities in middle-income countries. To address these challenges, we developed a novel second-generation CART-19 construct (COL01) and established two distinct GMP-compliant manufacturing platforms: an automated closed system and an open, fast protocol. Using a xenograft human B-cell leukemia model, we investigated whether manufacturing strategy impacts on CAR-T expansion, persistence, antitumor activity, and survival. **Methods:** COL01 construct (CD19-specific scFv and 4-1BB costimulatory domain), was produced in a pharma-grade lentiviral vector platform at IDCBIS. CAR-T cells were generated using two approaches: (i) automated CliniMACS Prodigy protocol; (ii) semi-automated G-REX 10-day protocol; and (iii) semi-automated G-REX 3-day fast protocol. A validated academic vector served as reference (Ref). NSG mice were injected with NALM-6 and distributed into groups: untreated, non-transduced T cells, CART-Ref, CART-CliniMACS, CART-GREX, and CART-FAST. Leukemia progression, CAR-T persistence, tissue distribution, and survival were monitored for up to 8 weeks. **Results:** CART-FAST strategy achieved the highest transduction efficiency (70%), compared with CART-GREX (45%), CART-Ref (50%) and CART-CliniMACS (30%) and displayed antitumor cytotoxic activity. CART-FAST cells showed enhanced activation markers (CD28, CD69, CD95). In vivo, untreated and non-transduced groups showed rapid leukemia progression (median survival <24 days), while CAR-T-treated mice exhibited sustained leukemia control, tumor burden near 2%, and survival >45 days. CART-FAST demonstrated superior persistence in blood (>70% at 4 weeks), liver (85%), and spleen (80%), outperforming automated and reference products. **Conclusion:** We demonstrate robust in vivo expansion, persistence, and antileukemic activity of CAR-T COL01 cells. Importantly, the fast protocol yielded highly transduced, functionally active CAR-T cells with superior expansion and persistence compared with automated and semi-automated methods. Overall, open and rapid protocols may enable scalable, cost-effective production and help expand CAR-T access in Latin America.

**Keywords:** CAR-T-19, fast manufacturing protocol, xenograft leukemia model, preclinical model, NALM-6

## **Abstract 87 | Poster | Poster Session Day 1**

### **Engineered human IL-15/IL-15 $\alpha$ promotes robust CD8<sup>+</sup> T cell and NK cell-mediated antitumor effect leading to melanoma control in a humanized mouse model**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Flávia Midori Ishikawa

**Institution:** Advanced Therapies Unit, Instituto Distrital de Ciencia, Biotecnología e Innovación en Salud – IDCBS; National Institute of Health — Bogotá-DC

**Introduction:** Advances in tumor immunology have led to the development of novel cancer immunotherapies; however, clinical responses remain limited in most patients. Interleukin-15 (IL-15) is a potent immunostimulatory cytokine that promotes the activation, proliferation, and survival of cytotoxic CD8 T cells and natural killer (NK) cells through IL-15 $\alpha$ -mediated transpresentation. Although IL-15 has demonstrated promising antitumor activity in syngeneic mouse models, its mechanisms of action within a functional human immune system remain poorly understood. Here, we evaluated the antitumor effects of membrane-bound and soluble engineered human IL-15/IL-15 $\alpha$  complex using a humanized mouse model of melanoma. **Methods:** Human A375 melanoma cells were genetically engineered using third-generation lentiviral vectors to constitutively express IL-15/IL-15 $\alpha$  either membrane-bound (mbIL-15) or soluble (sIL-15); wild-type (WT) cells served as controls. Humanized NRG mice were generated by transplantation of  $4 \times 10^5$  human umbilical cord blood CD34<sup>+</sup> hematopoietic stem cells into irradiated recipients. Upon hematopoietic/immune reconstitution,  $1 \times 10^6$  tumor cells were subcutaneously implanted in mice. Tumor growth was monitored by caliper measurements, peripheral blood immune reconstitution was evaluated weekly by flow cytometry, and immune cell infiltration in tumors and lymphoid organs were analyzed at the experimental endpoint. **Results:** Humanized mice achieved stable reconstitution (20–45% CD45<sup>+</sup>) of human CD4 and CD8 T cells, B cells, NK cells, monocytes, and other myeloid populations with leukocytes detected in peripheral blood from week 6 onward. Human immune cells (>40% CD45) were also detected in bone marrow, thymus, spleen, and lymph nodes. A375 cells were successfully engineered to express mbIL-15 (43%) or sIL-15 (91%) before implantation into humanized mice. In the presence of functional human immune system, both mbIL-15 and sIL-15 induced potent tumor regression mediated predominantly by CD8<sup>+</sup> T cells and NK cells. Maximum tumor volumes were reduced to 610 mm<sup>3</sup> (mbIL-15) and 340 mm<sup>3</sup> (sIL-15), compared with 2,017 mm<sup>3</sup> in WT tumors ( $p < 0.001$ ). Tumor control was associated with increased circulating and intratumoral NK and CD8 T cells and a lower CD4/CD8 T-cell ratio than WT tumors. NK-cell infiltration reached 28% and 53% in mbIL-15 and sIL-15 tumors, respectively, compared with <1% in WT tumors. Most infiltrating NK cells displayed an activated NKp46<sup>+</sup>CD56<sup>+</sup>CD16<sup>-</sup> phenotype, whereas WT tumors were predominantly infiltrated by CD33 myeloid cells. **Conclusions:** Human IL-15/IL-15 $\alpha$  signaling promotes robust CD8 T cell- and NK cell-mediated antitumor immunity in vivo by enhancing immune cell expansion, tumor homing, and activation, resulting in marked melanoma growth control. These findings establish humanized mouse models as a clinically relevant platform for investigating human tumor immunology and support the development of IL-15-based cancer immunotherapies.

**Keywords:** Humanized mice model, HSC, IL-15, IL-15/IL-15 $\alpha$ , NK cells, CD8 T cells, in vivo, melanoma, A375

## **Abstract 89 | Poster | Poster Session Day 1**

### **Generation and functional evaluation of a novel anti-CD19 CAR-T cell design for hematologic and solid tumors: Building a translational platform for gene and cell immunotherapy in Colombia.**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** ADRIANA PARRA

**Institution:** Advanced Therapies Unit, Instituto Distrital de Ciencia Biología e Innovación en Salud-IDCBIS — Bogotá

Cancer remains one of the leading causes of mortality worldwide. Although surgery, chemotherapy, and radiotherapy form the basis of cancer treatment, their effectiveness in certain patients is still limited. In this context, immunotherapy based on chimeric antigen receptor T (CAR-T) cells has revolutionized the treatment of hematologic malignancies. However, access to these therapies remains limited in many countries, including Colombia, due to the technological and manufacturing complexity required for their development. To contribute to the establishment of national capabilities for the future implementation of this therapeutic strategy in Colombia, we developed an experimental platform for the generation and functional preclinical evaluation of anti-CD19 CAR-T cells. A CAR construct specific for human CD19 was designed, and the production of third-generation lentiviral vectors was standardized, achieving stable CAR expression of approximately 30% in T lymphocytes isolated from healthy donors. In addition, T-cell activation, transduction, and expansion protocols were optimized to promote the generation of minimally differentiated T cells, a phenotype associated with enhanced proliferative capacity and functional persistence. Expanded T cell phenotype including differentiation, activation, senescence and exhaustion markers were thoroughly assessed across several experimental conditions. We were able to optimize protocols for CAR-T production that led to optimal T cell phenotype that account for non exhausted, stem-like memory cell stages. As experimental tumor models, K562, A375, and A549 cell lines were engineered to express CD19 and used as target cells in functional assays. NALM6, were used as constitutively CD19 expression cell target. CAR-T cells exhibited potent in vitro antitumor activity, achieving cytotoxicity levels >80% against CD19-positive target cells. Furthermore, three-dimensional tumor spheroids were established to evaluate CAR-T cell activity to represent a better model of tumor microenvironment. CAR-T cells achieved were able to infiltrate and trigger cytotoxicity against A375 CD19+ (80%) and A549 CD19+ (60%) spheroids. Collectively, these findings demonstrate the feasibility in the generation and preclinical evaluation of novel anti-CD19 CAR-T cells. This data support future steps towards local implementation of CAR-T therapies, creating new opportunities to expand access to innovative treatments for hematologic malignancies, with potential future applications in solid tumors.

**Keywords:** CAR-T therapy, chimeric antigen receptor (CAR), CD19, immunotherapy, cancer, genetically modified T lymphocytes, lentiviral vectors, antitumor cytotoxicity, hematologic malignancies, solid tumors.

## **Abstract 91 | Poster | Poster Session Day 1**

### **Apoptotic Bodies from Whartons Jelly Mesenchymal Stromal Cells induce Macrophage Reprogramming and trigger potent Immune Therapeutic response in a humanized model of Graft-Versus-Host Disease (GVHD).**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Karl Michael Beltran Ricaurte; Gustavo Salguero; Monica Cruz

**Institution:** Instituto Distrital de Ciencia Biotecnología e Innovación en Salud - IDCBIS — Colombia - Bogotá

Mesenchymal stromal cells (MSC)-based immunotherapies represent promising tools for the treatment of inflammatory and autoimmune diseases. Despite increasing evidence showing MSC as powerful immune modulators, cell products currently lack substantial therapeutic efficacy, making still challenging their clinical translation. MSC seem to be greatly impacted by impaired in vivo biodistribution via increased retention in lung and spleen following intravenous injection, leading to activation of apoptosis and release of apoptotic bodies (AB). Since an interplay between MSC-derived AB and several components of the immune system has been recently proposed, here we postulate that MSC-AB released by apoptotic MSC might exert synergistic immunomodulatory effects under inflammation both in vitro and in vivo. Umbilical cord-derived MSC were obtained from Wharton's jelly (WJ) by a disaggregation process. Exposure to ionizing irradiation (25Gy) efficiently induced apoptosis in WJ-MSC, as shown by DNA fragmentation, phosphatidyl serine translocation, 3/7 caspase expression and loss of membrane permeability. MSC-AB were isolated, characterized and used for further immune assays. Upon anti-CD3/anti-CD28-activation of human lymphocytes, MSC-AB did not induce immune modulation as compared to MSC controls. However, preconditioning of CD14+ human monocyte/macrophages with MSC-AB, induced a very strong M2 phenotype and triggered a potent inhibitory effect on activated-CD3+ lymphocyte proliferation (>90%). Importantly, MSC-AB treatment induced immunomodulatory phenotypes in monocyte/lymphocytes co-cultures characterized by overexpression of immunological checkpoint molecules and secretion of anti-inflammatory cytokines. RNA-seq analysis of human macrophages upon treatment with MSC-AB demonstrated the upregulation of a broad set of genes involved in multiple immunological processes and confirmed an enhanced immunoregulatory profile of AB treated CD14+ cells, as compared to untreated controls. Finally, intravenous treatment of MSC-AB in previously established humanized graft-versus-host disease (GvHD) in NOD.SCIDnull.IL2yRnull (NSG) mice, significantly prolonged survival, reduced levels of xenogenic T cells and enriched M2-macrophages in lung and liver of AB-treated mice. Altogether, these findings suggest a pivotal role of MSC-AB in reprogramming strong immune effector cell responses by potentially turning activated macrophages into immunomodulatory phenotypes M2 during severe inflammation.

**Keywords:** Apoptotic Bodies , Mesenchymal Stromal Cells , extracellular vesicles, Macrophage Reprogramming, humanized model and Graft-Versus-Host Disease (GVHD).

## Abstract 95 | Poster | Poster Session Day 1

### Bioengineering of membrane particles from mesenchymal stromal cells and cationic liposomes as non-viral vectors for gene editing

**Category:** Vectors and gene delivery systems (viral and non-viral)

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**Institution:** Programa de Pós-Graduação em Ciências Farmacêuticas – Universidade Federal do Rio Grande do Sul (PPGCF-UFRGS) — Porto Alegre/RS

**Introduction:** Gene therapy is a promising strategy for treating various diseases, however, the use of viral vectors is still limited by immunogenicity, safety, and restricted loading capacity. In this context, non-viral lipid vectors emerge as attractive alternatives, aiming to circumvent these issues. **Objectives:** To develop and evaluate bioengineered liposomes (BL) fused with membrane particles (MP) derived from mesenchymal stromal cells (MSC) for the efficient delivery of CRISPR/Cas9 plasmids for gene editing. **Methodology:** MSC were cultured in DMEM supplemented with 20% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). To obtain the particles, MSC were placed in hypotonic medium, followed by extrusion using a needle and syringe or via microfluidization. Hydrodynamic size and zeta potential ( $\zeta$ ) analyses were performed using dynamic light scattering and electrophoretic mobility, respectively. Protein quantification was determined using the Lowry method. Cationic liposomes (CL) were obtained through lipid film formation. The fusion of CL and MP was carried out through 4 cycles of microfluidization, using 3 different liposome:MP ratios: 9:0.25, 9:0.5, and 9:1. The hydrodynamic size and  $\zeta$ -potential of the BL were evaluated, and the efficiency of complexation with plasmids (pDNA) was verified through agarose gel retention assay. To assess the internalization of BL/pDNA complexes, HEK-293 cells were cultured in DMEM, supplemented with 10% BFS and 1% P/S, until 80% confluence, and seeded into a 24-well plate for subsequent treatment with rhodamine-PE-labeled BL. Fluorescence images were acquired 4 and 24h after treatment using a fluorescence microscope. **Results:** The MP obtained by syringe and needle extrusion exhibited a more homogeneous profile compared to those obtained by microfluidization. Protein quantification indicated a higher concentration in the samples processed by syringe and needle extrusion, which was 4 times higher than in the samples processed by microfluidization. The MP produced by syringe and needle extrusion were used for bioengineering along with the CL. The bioengineering liposomes showed hydrodynamic diameters around 113.89 nm (LB 9:0.25), 103.37 nm (LB 9:0.5), and 109.32 nm (LB 9:1). The corresponding PDI values were  $0.286 \pm 0.010$ ,  $0.303 \pm 0.035$ , and  $0.322 \pm 0.012$ , respectively, while the  $\zeta$ -potential remained highly positive for all formulations ( $+57.28 \pm 8.85$ ,  $+63.07 \pm 1.17$ , and  $+56.84 \pm 9.00$  mV, respectively), indicating homogeneous nanoparticles with good colloidal stability. The BL proved to be effective in complexing with pDNA, as no migration of nucleic acids was observed when they were associated with the nanostructure. The fluorescence images indicate internalization of the BL/pDNA complexes by HEK cells at both 4 and 24h. **Conclusions:** The methodology for producing LBs proved promising based on the tests conducted, and an in vitro biological evaluation of the vector's transfection efficiency is planned.

**Keywords:** Bioengineering, liposomes, gene editing

## **Abstract 96 | Poster | Poster Session Day 1**

### **Second-Generation CAR-T Cells display potent antitumor response in Humanized Murine Models of Melanoma**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** MANUELA LLANO LEÓN

**Institution:** 1. Advanced Therapies Unit, Instituto Distrital de Ciencia, Biotecnología e Innovación en Salud – IDC BIS, Bogotá, Colombia 2. Animal Facility, National Health Institute, Bogotá, Colombia — BOGOTÁ D.C

Tumor microenvironment (TME) plays a critical role in the progression of solid tumors by promoting immunosuppressive mechanisms that limit effective antitumor responses. Although chimeric antigen receptor T-cell (CAR-T) therapy has demonstrated remarkable clinical success in hematological malignancies, its efficacy in solid tumors remains limited due to challenges associated with cellular infiltration, persistence, and functionality within the TME. This study aimed to evaluate the antitumor activity of second-generation CAR-T cells in preclinical murine models of melanoma. In a first experimental setup, human A375 melanoma cells expressing CD19 at different proportions (10%, 20%, 30%, and 100%) were implanted subcutaneously. Second-generation anti-CD19 CAR-T cells were administered intravenously either on day 3 or day 12 following tumor implantation. Tumor growth was monitored twice weekly, and CAR-T cell persistence was assessed longitudinally in peripheral blood. In a second model, immunodeficient mice were humanized via human hematopoietic progenitor cells transplantation 12 weeks prior tumor implantation. Following confirmation of human immune cell reconstitution by flow cytometry, A375-CD19+ melanoma cells expressing CD19 were inoculated, and anti-CD19 CAR-T cells were administered intravenously on day 12. In both experimental approaches, tumor infiltrate, peripheral blood, spleen, bone marrow, and thymus were assessed by multiparametric flow cytometry. In the xenograft model, all CAR-T-treated groups exhibited delayed tumor growth compared with control mice. Complete tumor regression was observed in mice bearing tumors with 100% CD19 expression. In this group, a tumor rechallenge was performed to assess the durability of the CAR-T-mediated antitumor response. In the humanized model, CAR-T-treated mice showed reduced tumor growth kinetics relative to controls, associated to marked heterogeneity of immune effector cell populations in tumor and lymphoid organs. Analyses of CAR-T cell persistence, tumor infiltration, activation, memory, and exhaustion phenotypes were performed. Collectively, CAR-T cells showed antitumor activity in human melanoma, both in xenograft and in experimental humanized immune setting. The delayed tumor growth observed in both models, along with the presence of infiltrating immune effector cells in humanized mice, suggest synergistic processes that could enhance the therapeutic potential of CAR-T therapy in solid tumors. Thus, humanized mice emerge as valuable tools to assess TME in the context of human immune system.

**Keywords:** CAR-T cells, Solid tumors, Tumor microenvironment, Humanized mouse model, Immunotherapy, Immune reconstitution

## **Abstract 97 | Poster | Poster Session Day 1**

### **COL7A1 Variant Associated with Dystrophic Epidermolysis Bullosa Replicated via CRISPR-Cas9 Gene Editing in MRC-5 Cells: Phenotypic Characterization and Investigation of Molecular Markers**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Rafaela Feijó Vieira; Carlos Robson Costa Cruz; JEFFERSON PEDROSO

**Institution:** Universidade Federal do Rio Grande do Sul (UFRGS) — Porto Alegre - RS

The COL7A1 gene encodes type VII collagen, an essential component of anchoring fibrils that ensure dermo-epidermal adhesion. Pathogenic variants (mutations) resulting in loss of function lead to severe phenotypes of dystrophic epidermolysis bullosa (DEB), characterized by skin fragility, chronic blistering, severe scarring, and an increased risk of aggressive squamous cell carcinoma. Given the lack of available treatments and the need for a deeper understanding of the condition, creating a cellular model could provide new insights into its pathological mechanisms and the development of novel therapies. To this end, we edited the MRC-5 cell line (human fibroblasts) using the CRISPR/Cas9-HDR system to reproduce a rare human COL7A1 variant found in DEB, but whose effects on the protein remain unknown. For editing, we used a ribonucleoprotein (RNP) complex—consisting of a guide RNA conjugated to Cas9-SpyFi (Aldevron®) and a DNA template containing the variant—delivered via nucleofection (4D-Nucleofector® X Unit, Lonza Bioscience). The edited population was expanded, sequenced, and subsequently plated for clonal expansion and verification of the variant's presence in all gene copies. One clone, in which the variant was confirmed by Sanger sequencing, was selected and designated C7-KI (COL7A1 knock-in). We then performed a functional wound-healing assay (creating a gap in the cell monolayer) to assess whether the variant directly affected cell migration, given the protein's essential role in anchoring fibrils. We demonstrated that the C7-KI clone closed the gap significantly faster than the non-mutated (wild-type/WT) group. By excluding the effects of differences in cell division via a cumulative population doubling (CPD) assay, we demonstrated that the ability of C7-KI cells to close the gap in the monolayer was not due to increased cell division but rather to enhanced migration, consistent with previous studies on COL7A1 knockout cells. Next, we evaluated migration-related marker genes by assessing gene expression with TaqMan probes via RT-qPCR, using the GAPDH gene as an endogenous control. We observed that the genes COL1A1, COL3A1, COL7A1 (collagen matrix), TGF- $\beta$ 1 (upregulation of COL7A1), and THBS1 (cell migration) showed significantly higher expression compared to the control group, whereas the  $\beta$ -ACTIN gene (cell motility) showed lower expression in C7-KI cells compared to control cells lacking the variant (WT); this demonstrates that the model exhibits a differential expression profile for genes known to be related to DEB pathology. Thus, the MRC-5 cellular model for DEB represents a crucial step in preclinical research on the disease—caused by rare variants of uncertain significance—as well as in the identification of morphological, functional, and molecular markers to understand its pathophysiology and evaluate new therapeutic alternatives.

**Keywords:** collagen; therapy; cell model; drug repurposing; and VUS.

## Abstract 98 | Poster | Poster Session Day 1

### De novo Design of Next-Generation Anti-FAP Recognition Domains for CAR-T Cell Therapy

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Vinicius Pinto Costa Rocha; Allan Souza dos Santos; Gabriel Azevedo Ponciano; Girlaine Café Santos; Júlia Gomes Reis; Lívia Batista Galinari; MILENA BOTELHO PEREIRA SOARES; Sabrina de Azevedo Silveira; Tiago Antônio de Oliveira Mendes

**Institution:** 1- Instituto Gonçalo Moniz, Fiocruz-Bahia; 2- Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa; 3- Departamento de Informática, Universidade Federal de Viçosa — Salvador-BA; Viçosa-MG

Chimeric antigen receptor (CAR)-T cell therapy has transformed the treatment of hematological malignancies, however, conventional single-chain variable fragments (scFvs), which remain the predominant antigen-recognition domains in CAR constructs, are associated with challenges including tonic signaling, immunogenicity, and limited structural stability<sup>1,2</sup>. Computationally designed de novo binding proteins have recently emerged as promising alternatives, offering compact architectures enhanced stability, and the possibility of tailoring antigen recognition<sup>3,4</sup>. In this study, we aimed to design novel recognition domains targeting fibroblast activation protein (FAP), which is markedly upregulated under pathological conditions, including fibrosis, atherosclerosis, arthritis, autoimmune diseases and a broad range of solid tumors. This expression profile has made FAP an attractive target for CAR-T cell therapy, with growing interest in applications targeting tumor stroma, cardiac and liver fibrosis<sup>5</sup>. Protein binders were generated using the BindCraft platform through multiple rounds employing distinct hotspot configurations on the dimeric human FAP<sup>6</sup>. Candidate structures were filtered according to structural confidence metrics (ipSAE, ipTM, and consistency after AlphaFold3 and Chai-1 refolding), structural diversity, and predicted toxicity<sup>7</sup>. Selected binders were cloned into a 2nd-generation CAR construct, followed by in vitro mRNA transcription and transient expression analysis in HEK293T cells. Seven independent design rounds generated 22 candidate binders ranging from 76 to 150 amino acids, all displaying predominantly  $\alpha$ -helical architectures. Structural filtering identified ten high-confidence candidates, of which seven structurally diverse binders were retained after removing redundant designs. Six binders were successfully cloned into the CAR expression vector together with a reference anti-FAP scFv construct. One candidate repeatedly failed during cloning, likely due to its elevated GC content. Sequence verification confirmed correct assembly of all remaining constructs except one binder containing mutations. Capped mRNAs were successfully produced with yields ranging from approximately 0.5 to 1 mg. Preliminary expression analysis demonstrated successful surface expression of the reference scFv CAR in approximately 80% of transfected HEK293T cells, establishing the experimental platform for subsequent evaluation of the de novo binders. These results demonstrate the feasibility of integrating computational protein design with CAR engineering to generate structurally diverse recognition domains. Ongoing studies will evaluate the surface expression and FAP-binding capacity of the engineered CAR binders. Their functional performance will then be investigated in T cells using co-culture assays with FAP+ fibroblasts, assessing antigen-dependent activation, proliferation, cytotoxicity, and persistence in comparison with a conventional scFv-based CAR.

**Keywords:** CAR T Cell. mRNA. Cell Therapy. Artificial intelligence

## **Abstract 99 | Poster | Poster Session Day 1**

### **Development and Characterization of a Decellularized Amniotic Membrane-Based Scaffold for the Expansion of Limbal Mesenchymal Stromal Cells**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Geovanna Carla Amaro da Silva; Caroline Nascimento Barquilha; Damiana Pedro de Oliveira; HELGA CAPUTO NUNES HOLZHAUSEN; João Pedro Arantes Aun Migueis; Mariane Aparecida Risso; Mônica de Cassia Alves

**Institution:** Universidade Estadual de Campinas, UNICAMP — Campinas-SP

Ocular surface diseases (OSD) affect distinct corneal layers, significantly impairing patients' quality of life. OSD compromises the epithelium, tear film, and conjunctiva. Common interventions include autologous limbal tissue transplantation from the healthy eye to the injured eye in unilateral limbal stem cell deficiency, or allogeneic transplants from live and deceased donors, which can be immunologically rejected by the patient. In this context, limbal mesenchymal stromal cells (limbal MSCs) are recognized for their therapeutic potential and low immunogenicity, particularly for their self-renewal capacity, genetic stability, immunomodulatory properties, and ability to remodel the corneal stroma. The human amniotic membrane (AM) has also been used in ophthalmology, especially as a three-dimensional, tissue-specific scaffold for cell growth and expansion. In clinical practice, AM is widely used as a biological dressing due to its transparent structure and lack of immunogenicity. To address this challenge, this project proposes expanding limbal MSCs within a bioactive construct derived from decellularized AM to enhance cell growth and functionality. To this end, we first standardize AM dissection, packaging, and transportation; washing and decontamination; and two methods for maintaining AM: cryopreservation or freeze-drying. AMs samples were obtained from 5 placentas from women undergoing elective cesarean sections at the UNICAMP maternity hospital in Brazil, who authorized the use of the material by consent, and tested negative for infectious diseases (Ethical Approval Code: 45209521.4.0000.5404). Second, we standardized the isolation and characterization of MSCs from 5 corneoscleral rings provided by the Brazilian Biosciences National Laboratory (LNBio) Biobank (Ethical Approval Code: 82902024.3.0000.540). We are now testing protocols to decellularize the AM, characterize it, and standardize the preservation methods before combining the scaffold with the MSCs. Transmission electron microscopy, DNA analysis, and sulfated glycosaminoglycan quantification are being used for this purpose.

**Keywords:** Ocular surface diseases, Limbal mesenchymal stromal cells, Amniotic membrane, Decellularized scaffold, Tissue engineering, Cell therapy

## **Abstract 100 | Poster | Poster Session Day 1**

### **Engineering PEG-Lipid Composition in Lipid Nanoparticles Enhances mRNA Delivery to Antigen-Presenting Cells for Cancer Immunotherapy**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** GABRIEL HENRIQUE COSTA DA SILVA

**Institution:** UFMG — Belo Horizonte

Messenger RNA (mRNA)-based vaccines have transformed the field of immunotherapy, largely due to advances in lipid nanoparticle (LNP) technology, which enables efficient nucleic acid delivery. Among the structural components of LNPs, PEG-lipids play a key role in regulating nanoparticle stability, circulation, and cellular uptake. Nevertheless, the combined influence of PEG-lipid molar ratio, polyethylene glycol molecular weight, and lipid anchor length on mRNA delivery efficiency has not been fully elucidated. In this work, we investigated the impact of PEG-lipid composition on LNP performance with the goal of improving mRNA delivery to antigen-presenting cells (APCs) for cancer vaccination. A panel of mRNA-loaded LNP formulations was generated by varying PEG-lipid content (1%, 2.5%, and 5%), PEG molecular weight (2000 and 5000 Da), and lipid tail length (C14, C16, and C18). Physicochemical characterization included particle size, polydispersity index, zeta potential, and encapsulation efficiency. Biological performance was initially assessed in dendritic cells and macrophages, and the most effective formulation was subsequently evaluated in a prophylactic B16F10-OVA melanoma model. Altering PEG-lipid composition markedly affected both nanoparticle characteristics and biological activity. Increasing PEG-lipid content produced smaller nanoparticles while increasing size heterogeneity, whereas longer lipid chains resulted in larger particles. Among all formulations, the LNP containing 1% PEG-C14-2000 (LNP F1) achieved the highest transfection efficiency in myeloid APCs. In vivo, this formulation elicited a strong humoral immune response, with anti-OVA IgG detected after the primary immunization and significantly enhanced following the booster dose. Moreover, vaccination with LNP F1 provided substantial protection against tumor development, with only a small proportion of immunized animals developing tumors and exhibiting tumor volumes more than ten-fold lower than those observed in control animals. Collectively, these findings demonstrate that fine-tuning PEG-lipid composition is an effective strategy to optimize mRNA-LNP delivery to APCs, ultimately improving the efficacy of mRNA cancer vaccines and providing valuable insights for the rational design of next-generation LNP formulations.

**Keywords:** Lipid nanoparticles, PEG-lipid, vaccine

## Abstract 101 | Poster | Poster Session Day 1

### Development and Optimization of Lipid Nanoparticles for Adenine Base Editor Delivery to Hematopoietic Stem Cells

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** RICARDO WEINLICH; ARIANE BARROS DINIZ; BIANCA BONETTO MORENO GARCIA; CARLA MARTINS FRAGA DE MELO; DANIELLE GONÇALVES DE CARVALHO; DAVI COE TORRES; GRACIELLE APARECIDA LIMA; JULIA ALVES TANJONI; LIDIANE DE SOUZA TORRES; LUCAS ASSIS PEREIRA; MICHELLIN PEREIRA DE ALBUQUERQUE; PAULO ALFONSO SCHUOFF; PRISCILA KEIKO MATSUMOTO MARTIN

**Institution:** Hospital Israelita Albert Einstein — São Paulo - SP

Background: Sickle cell anemia is one of the most prevalent monogenic diseases worldwide and is caused by a mutation in the HBB gene, leading to abnormal hemoglobin (HbS) production. Gene therapy offers curative potential by correcting the HBB gene or reactivating fetal hemoglobin expression in autologous hematopoietic stem cells (HSCs). However, current ex vivo approaches rely on specialized infrastructure, limiting scalability and accessibility. Thereby, lipid nanoparticles (LNPs) have emerged as promising non-viral platforms for nucleic acid delivery due to their biodegradability, favorable safety profile, and potential for targeted in vivo delivery, reducing costs while increasing specificity and accessibility. Objective: To develop and optimize a library of LNPs for the efficient and safe delivery of Adenine Base Editing (ABE) tools to HSCs. Methods: Forty LNP formulations were prepared by nanoprecipitation and characterized by DLS and zeta potential measurements. Functional screening was performed in HEK-293T cells and primary human CD34<sup>+</sup> cells from healthy donors using mRNA-GFP as cargo. Cells were evaluated by fluorescence microscopy, and transfection efficiency, MFI, and cell viability were assessed by flow cytometry. Selected formulations were then optimized by evaluating production methods, N/P ratios, mRNA doses, and analysis time points. The optimized LNP was subsequently tested for the delivery of mRNA-ABE and sgRNA, and editing efficiency was quantified by Sanger sequencing. Results: The LNP library exhibited an average size of  $136 \pm 36$  nm, PDI of  $0.18 \pm 0.07$ , and zeta potential of  $-8 \pm 5$  mV, confirming the robustness of the production method. In HEK-293T cells, 80% of formulations achieved high transfection efficiency ( $>70\%$ ) with preserved viability, whereas in CD34<sup>+</sup> cells, only 17% reached transfection efficiencies above 70%, highlighting the complexity of this target. Notably, Lipofectamine, despite achieving  $>80\%$  transfection efficiency in HEK-293T cells, showed minimal activity in CD34<sup>+</sup> cells ( $<0.2\%$ ), reinforcing the need for dedicated delivery platforms. Following additional optimization, including implementation of an HTS production method and optimization of N/P ratios, mRNA dose, and incubation time, LNP-L6 achieved transfection efficiencies of  $97.1 \pm 2.4\%$  while maintaining cell viability of  $96.1 \pm 1.4\%$  across three independent donors. Delivery of editing tools resulted in editing efficiencies of up to 65% without significant cytotoxicity ( $>85\%$  viability). Further optimization efforts to improve nanoparticle homogeneity and maximize editing performance are ongoing. Conclusion: Our results demonstrate that LNPs are a promising non-viral platform for efficient and safe gene-editing cargo delivery to primary HSCs. The identification of HSC-tropic formulations and the establishment of a robust HTS workflow provide a foundation for developing scalable and accessible gene therapies for sickle cell disease and other hemoglobinopathies.

**Keywords:** Lipid Nanoparticles, HSCs, Gene Therapy, Base Editor, hemoglobinopathy

## Abstract 102 | Poster | Poster Session Day 1

### CRISPR/Cas9-mediated targeting of the HBG1/HBG2 promoters induces fetal hemoglobin expression in HUDEP-2 cells

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Gabriela da Silva Arcanjo; Carolina Lanaro; Dulcinéia Albuquerque Nunes dos Santos; Fernando Ferreira Costa; Ryo Kurita; Victor Gabriel de Faria Pastre; Yukio Nakamura

**Institution:** Hematology and Hemotherapy Center, State University of Campinas — Campinas-SP

Sickle cell anemia (SCA) is one of the most common inherited disorders worldwide and is characterized by vaso-occlusion and progressive organ damage resulting from the polymerization of sickle hemoglobin (HbS). Increased fetal hemoglobin (HbF) levels reduce HbS polymerization and disease severity, making HbF induction a major therapeutic strategy 1. Naturally occurring non-deletional hereditary persistence of fetal hemoglobin (ndHPFH) variants, including the Brazilian HBG1 –195 C>G mutation, are associated with sustained HbF expression into adulthood 2. Here, we aimed to evaluate a CRISPR/Cas9 gene editing strategy targeting the HBG promoter as an approach to reactivate HbF expression in the HUDEP-2 erythroid cell line. An HDR-based CRISPR/Cas9 strategy was employed using the Alt-R™ CRISPR-Cas9 system. Ribonucleoprotein (RNP) complexes were assembled using Alt-R™ S.p. HiFi Cas9 Nuclease V3 and two sequence-specific single-guide RNAs (sgRNAs). RNP complexes were co-electroporated with a donor DNA template carrying the –195 C>G substitution into  $1 \times 10^6$  HUDEP-2 cells 3 using the Lonza 4D-Nucleofector™ system. Control cells consisted of mock-electroporated cells, while experimental groups received the donor template together with RNP complexes containing either sgRNA1 or sgRNA2. HbF-positive cells were quantified by flow cytometry 96h after electroporation, and HBG1/HBG2 and HBB mRNA expression levels were determined by qPCR. Comparative analysis demonstrated superior performance of sgRNA1, which generated 51.2% HbF-positive cells, compared with 30.9% for sgRNA2. Consistently, sgRNA1 increased HBG1/HBG2 expression by approximately 13-fold relative to control cells (1.49 vs. 0.11 arbitrary units (A.U)), whereas sgRNA2 induced a 3.9-fold increase (0.44 vs. 0.11 A.U.). Conversely, compared to controls, HBB expression decreased approximately 4-fold with sgRNA1 (0.37 vs. 1.68 A.U.) and 1.6-fold with sgRNA2 (1.00 vs. 1.68 A.U.), indicating higher HbF production with sgRNA1 with compensatory decreased levels of beta globin. Considering that editing efficiency has not yet been confirmed by targeted sequencing, the observed HbF induction likely reflects a heterogeneous population of edited cells, including precise HDR events, repair products generated by non-homologous end joining, and non-edited cells. Ongoing sequencing of the HBG1 promoter and clonal isolation will determine the frequency of the intended –195 C>G edit and establish its specific contribution to HbF reactivation. These preliminary findings demonstrate that CRISPR/Cas9-mediated editing targeting the HBG promoter region efficiently induces HbF expression in HUDEP-2 cells. Confirmation of the precise introduction of the Brazilian HPFH mutation will provide further evidence for the therapeutic potential of this strategy, supporting its future evaluation in CD34<sup>+</sup> hematopoietic stem and progenitor cells from patients with SCA.

**Keywords:** Sickle cell disease; Hereditary persistence of fetal hemoglobin; Gamma globin; Genome editing

## **Abstract 103 | Poster | Poster Session Day 1**

### **Nanoemulsions thickened with poloxamer 407 as a non-viral intra-articular platform for CRISPR/Ca9 and dexamethasone delivery in mucopolysaccharidosis type II**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Júlia de Azambuja Vaniel; Flávia Nathiely Silveira Fachel; GUILHERME BALDO; Helder Ferreira Teixeira; Maria Luísa Gasparini; ROSELENA SILVESTRI SCHUH; URSULA DA SILVEIRA MATTE; Vinicius Monteagudo de Barros; Willian da Silva Carniel

**Institution:** Universidade Federal do Rio Grande do Sul — Porto Alegre - RS

**Introduction:** Mucopolysaccharidosis type II (MPS II) is a rare X-linked lysosomal storage disorder caused by deficiency of iduronate-2-sulfatase (IDS), leading to progressive accumulation of the glycosaminoglycans heparan and dermatan sulfate. This accumulation underlies multisystemic manifestations, among which joint stiffness and cartilage damage are the most debilitating and least responsive to treatment. Current therapies fail to adequately control articular disease progression, as enzyme replacement therapy has limited access to avascular, poorly perfused joint tissue. Gene editing strategies capable of restoring local IDS expression within the joint represent a promising alternative, provided a safe and efficient non-viral delivery vector is available for intra-articular administration. **Objective:** To develop and characterize thickened poloxamer 407-based nanoemulsions (NE) as a non-viral intra-articular delivery platform for dexamethasone, CRISPR/Cas9 plasmids, and an IDS donor sequence for gene editing in MPS II. **Methodology:** Cytotoxicity was assessed by MTT assay in fibroblast-like synoviocytes (FLS). Cellular internalization kinetics were evaluated by flow cytometry and fluorescence microscopy across a 2 to 48h time course. Transfection efficiency was determined via IDS enzymatic activity, both in FLS in vitro and after intra-articular administration in a murine model, alongside biodistribution analysis to assess systemic exposure. **Results:** NE exhibited a favorable biocompatibility profile, with no significant cytotoxicity observed under any tested conditions. Cellular internalization was detected as early as 2 hours post-exposure and remained sustained up to 48 hours, indicating efficient cellular uptake of the complexes. Both in vitro transfection and in vivo intra-articular administration significantly increased IDS enzymatic activity relative to untreated controls, confirming efficient plasmid delivery and functional gene expression within the joint microenvironment. Biodistribution analysis showed no detectable systemic exposure after intra-articular administration, supporting a localized and potentially safer therapeutic profile. **Conclusions:** The poloxamer 407-based nanoemulsion platform demonstrated favorable biocompatibility, sustained cellular internalization, and functional restoration of IDS enzymatic activity in nonclinical assays, with no evidence of systemic biodistribution. These findings support the feasibility of this delivery platform as a non-viral, joint-restricted gene editing strategy for MPS II and provide a nonclinical foundation for future translational studies.

**Keywords:** Gene therapy; Nanoemulsions; Non-viral vectors; Gene editing.

## Abstract 105 | Poster | Poster Session Day 1

### Optimization of Post-Thaw Functional Recovery in NK-92 and CAR-NK-92 Cells

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Bruno Correia de Oliveira; Camilly Melo Garcia Ferreira; Heloisa Brand; Leticia Maria Ribeiro Bassi; Luis Roberto Fonseca; Matheus Henrique dos Santos; Sima Ebrahimabadi; VIRGINIA PICANCO E CASTRO

**Institution:** Blood Center of Ribeirão Preto and the Center for Cellular Therapies (CTC) of the University of São Paulo, Ribeirão Preto, Brazil. — Ribeirão Preto - SP

Cryopreservation is essential for the development of standardized, off-the-shelf NK-cell products. However, post-thaw stress can compromise cell survival, metabolic fitness, and antitumor function. This limitation is particularly relevant for NK-92- and CAR-NK-92-based products, in which consistent activity after thawing is required for reliable manufacturing, quality control, and clinical translation. Here, we investigated an *ex vivo* conditioning approach designed to improve post-thaw functional recovery of NK-92 and anti-CD19 CAR-NK-92 cells. NK-92 and anti-CD19 CAR-NK-92 cells were subjected to an *ex vivo* conditioning protocol before cryopreservation. Fresh and thawed cells were evaluated in co-culture assays with CD19-positive and CD19-negative hematologic target cells. Functional analyses included cytotoxicity, degranulation, and cytokine production. Post-thaw recovery was assessed through apoptosis and growth kinetics. Metabolic profiling was performed using extracellular flux analysis, complemented by flow cytometric assessment of mitochondrial parameters. The conditioning strategy enhanced the cytotoxic activity of fresh NK-92 cells against CD19-negative targets and of CAR-NK-92 cells against CD19-positive targets. This functional improvement was accompanied by increased degranulation and cytokine production. Following cryopreservation, conditioned NK-92 and CAR-NK-92 cells retained improved antitumor activity against their respective target cells, together with reduced apoptosis and enhanced post-thaw recovery. Metabolic profiling identified changes consistent with a distinct functional state in conditioned cells, supporting the hypothesis that *ex vivo* modulation may influence NK-cell adaptation to cryopreservation-associated stress. These findings support the development of an *ex vivo* conditioning strategy to improve functional recovery of cryopreserved NK-92 and CAR-NK-92 cells. By preserving antitumor activity after thawing, this approach may contribute to the manufacture of more consistent off-the-shelf NK-cell products. Ongoing studies are investigating the molecular basis of this effect and its applicability to additional CAR constructs and NK-cell sources.

**Keywords:** NK-92, CAR-NK-92, cryopreservation, recovery

## **Abstract 111 | Poster | Poster Session Day 1**

### **Genetic profiling of epidermolysis bullosa in a large Brazilian cohort**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** PRISCILA KEIKO MATSUMOTO MARTIN

**Institution:** Debra Brasil, Hospital Israelita Albert Einstein — São Paulo- SP

Background: Gene therapy is rapidly transforming the treatment landscape for epidermolysis bullosa (EB). However, successful implementation of mutation-specific therapies depends on accurate molecular diagnosis and a comprehensive understanding of population-specific genetic variants. Brazil has one of the world's largest admixed populations, yet its EB mutational spectrum has remained largely underexplored. Objectives: To characterize the genetic landscape of EB in a nationwide Brazilian cohort and evaluate its implications for precision medicine and the development of gene therapy strategies. Methods: Whole-exome sequencing (WES) was performed in 393 individuals from 349 unrelated families with a clinical or suspected diagnosis of EB recruited throughout Brazil. Variants were classified according to ACMG guidelines, and genotype-phenotype correlations were established to determine EB subtype distribution and identify recurrent and novel pathogenic variants. Results: A definitive molecular diagnosis was achieved in 91.6% of participants. Overall, 211 pathogenic or likely pathogenic variants were identified across 12 EB-associated genes, including 63 previously unreported variants. Recessive dystrophic EB (RDEB) represented the predominant subtype (74.1%), followed by EB simplex (18.7%), junctional EB (6.7%), and Kindler EB (0.6%). The most frequent pathogenic variants were COL7A1 c.6527\_6528insC and COL7A1 c.5047C>T, highlighting important therapeutic targets for the Brazilian population. Importantly, the genetically confirmed diagnosis differed from the initial clinical diagnosis in 45.7% of patients, emphasizing the limitations of phenotype-based classification alone and reinforcing the value of molecular diagnosis for patient management and eligibility for emerging mutation-specific therapies. Conclusions: This study represents the largest genetic characterization of EB in Brazil and one of the largest worldwide. The identification of recurrent founder variants and numerous novel pathogenic variants provides an essential framework for developing personalized therapeutic strategies, including gene editing, exon-specific therapies, and future clinical trials. Notably, the high prevalence of the COL7A1 c.5047C>T variant supports ongoing efforts to develop a non-viral base-editing therapy specifically targeting this mutation. These findings also strengthen genetic counseling, improve diagnostic accuracy, facilitate patient stratification for precision medicine, and establish a valuable resource for future international collaborations in gene therapy for EB.

**Keywords:** Epidermolysis Bullosa, Whole-Exome Sequencing, Precision Medicine

**Abstract 113 | Poster | Poster Session Day 1**  
**Optimization of CRISPR/Cas Nickase Platforms for HDR-Mediated Correction of a Prevalent Brazilian COL7A1 Pathogenic Variant in Recessive Dystrophic Epidermolysis Bullosa**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Laurent Ketlen Leão Viana

**Institution:** Hospital Israelita Albert Einstein — São Paulo- SP

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a severe genetic disorder caused by mutations in the COL7A1 gene, which encodes type VII collagen (C7), a key component of the anchoring fibrils essential for dermal-epidermal adhesion (1). The frequent Brazilian pathogenic variant c.5047C>T introduces a premature stop codon, resulting in a loss of functional C7 (2). This study aims to establish an ex vivo gene correction strategy for this mutation using the Dual CRISPR/Cas9 Nickase (Cas9n) platform in immortalized neonatal foreskin fibroblasts (BJ-hTERT c.5047C>T ). The Cas9n system employs a D10A mutant enzyme to generate single-strand breaks, thereby reducing off-target effects compared to the wild-type nuclease. To achieve double-strand breaks, two pairs of sgRNAs were designed to flank the mutation site. Following in silico off-target analysis, the selected sgRNAs were complexed into two delivery platforms: Cas9n as ribonucleoproteins (RNP) and Cas9n mRNA (IVT) via nucleofection. Transfection efficiency was optimized and confirmed (>94%) using GFP-encoding mRNA. While individual sgRNA delivery did not yield significant editing, co-transfection of both pairs proved highly effective. Next-Generation Sequencing analysis via CRISPResso2 demonstrated robust activity, with indel frequencies reaching 95% for RNP and 83% for IVT in the mutant line. To achieve precise correction, we evaluated single-stranded donor oligonucleotides (ssODN) at different concentrations combined with the chemical modulator Alt-R HDR Enhancer V2 (IDT). The two platforms exhibited distinct repair profiles: in the RNP system, V2 treatment promoted a significant 23.5-fold increase in perfect HDR ( $P=0.0157$ ), although this gain was accompanied by a three-fold increase in genomic deletions ( $P=0.0021$ ). In contrast, the IVT system was refractory to chemical enhancement and exhibited high sensitivity to donor dose; increasing the ssODN concentration from 0.5  $\mu\text{M}$  to 1.0  $\mu\text{M}$  led to a significant 'silencing' effect, reducing global editing activity ( $P=0.0003$ ). Furthermore, the ssODN1 (PAM-inactivated) design demonstrated superior fidelity in the IVT system, generating significantly lower genomic noise (imperfect HDR) compared to ssODN2 ( $P<0.01$ ). Our findings establish that while the RNP platform offers superior plasticity for chemical modulation, the IVT strategy requires precise donor dose optimization to mitigate cytotoxicity and maintain genomic integrity. Consistent with this limitation, our initial experiments yielded correction rates below 1% in BJ-hTERT c.5047C>T cells in the absence of pathway modulation. These findings highlight the inherent difficulty of precise genome editing in this cellular model and underscore the need to further investigate the biological factors limiting HDR, including the response of the IVT-based system to chemical modulation and the impact of donor DNA dosage.

**Keywords:** Homology-directed repair; Gene Editing; CRISPR/Cas9 nickase; Gene Therapy

## **Abstract 115 | Poster | Poster Session Day 1**

### **FUNCTIONAL ENGINEERING OF NATURAL KILLER (NK) CELLS TO COMBAT MYELOID NEOPLASMS**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Beatriz L Adjafre; Allana Guimarães de Carvalho; Camila Araújo Bernardino Garcia; Isabela Arruda Catarin; Larissa Sarri Binelli; Letícia Maria R. Bassi; Letícia Olops Marani; Lorena Lobo de Figueiredo-Pontes; MARA ELISAMA DA SILVA JANUÁRIO; VIRGINIA PICANCO E CASTRO

**Institution:** Universidade de São Paulo — Ribeirão Preto/SP

Myeloid neoplasms represent a heterogeneous group of malignant hematological disorders with a poor prognosis for cure. The hallmark of these neoplasms is the dysregulated clonal proliferation of hematopoietic stem cells, leading to the expansion of immature cells in the bone marrow and peripheral blood (1-3). Despite advances in understanding the pathophysiology of these diseases and the development of new drugs, clinical responses to available treatments are often limited, especially in refractory or relapsed cases, resulting in low cure rates for many patients (4). In this context, innovative approaches are being explored, particularly immunotherapy and cell therapy, with increasing interest in the use of Natural Killer (NK) cells. These cells exhibit cytotoxicity against leukemic cells without the need for prior sensitization and play a key role in immune surveillance against hematological malignancies. However, the clinical efficacy of NK cell-based therapies remains limited (5, 6). Tumor immune evasion mechanisms, such as the expression of inhibitory ligands or the activation of negative regulatory pathways, impair the antitumour activity of NK cells. Previous data from our research group indicate a predominant role of inhibitory pathways in sustaining dysregulated hematopoietic stem cells, particularly through the activation of the proteins CISH, KIR2DL1, NKG2A, and PD-1 (7). In this context, gene editing of these proteins emerges as a promising strategy to enhance NK cell immune responses by knocking out their encoding genes, generating NK cells capable of overcoming tumor resistance mechanisms. This approach aims to expand the therapeutic arsenal available for the treatment of myeloid neoplasms.

**Keywords:** NK cells; myeloid neoplasms; cell therapy

**Abstract 116 | Poster | Poster Session Day 1**  
**Targeting YAP/TAZ and MRTF signaling to inhibit proliferative mechanotransduction in osteosarcoma models**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** André Carlos Pereira de Matos; Rosângela Vieira de Andrade

**Institution:** Universidade Católica de Brasília — Brasília-DF

Osteosarcoma is the most common primary malignant bone tumor affecting children, adolescents, and young adults. It is characterized by high aggressiveness, frequent recurrence, therapeutic resistance, and a strong metastatic potential. Recent evidence has demonstrated that tumor progression is strongly influenced by mechanotransduction, a process by which mechanical stimuli from the tumor microenvironment are converted into biochemical signals that regulate cell proliferation, migration, survival, and plasticity. Among the main effectors of this process are the YAP/TAZ and MRTF/SRF signaling pathways, whose activation is promoted by the high stiffness of the extracellular matrix, a hallmark of bone tissue. Consequently, these pathways represent promising therapeutic targets for the development of more effective treatment strategies against osteosarcoma. This study aims to investigate the role of the YAP/TAZ and MRTF pathways in osteosarcoma aggressiveness using two-dimensional (2D) and three-dimensional (3D) experimental models, as well as to evaluate the therapeutic potential of pharmacological YAP/TAZ inhibition by verteporfin (VT) combined with MRTF1 gene silencing using siRNA. The human osteosarcoma cell line U2-OS was employed as the in vitro experimental model. The methodology included siRNA transfection, methylcellulose-based spheroid formation, cell viability assays, determination of the selectivity index, gene expression analysis by RT-qPCR, fluorescence microscopy, and pharmacological synergy assays. Following 24 hours of treatment, VT exhibited an  $IC_{50}$  of 6.97  $\mu$ M in 2D cultures, which decreased to 0.91  $\mu$ M when combined with MRTF1 siRNA. Chou-Talalay analysis demonstrated a strong synergistic interaction, yielding a combination index (CI) of 0.03 and a verteporfin dose reduction index (DRI) of 38.13. RT-qPCR confirmed more than 80% of MRTF knockdown after 24 hours of treatment, also revealing modulation of the YAP/TAZ and MRTF associated genes MYL9, SRF, MMP2, TAGLN, ANKRD1, AREG, ITGA5, RHOA, VCL, MKI67, YAP1, CPNE3 and BIRC5. Cytotoxicity assays performed in HFF fibroblasts revealed a selectivity index of 2.43 for osteosarcoma cells, indicating a favorable therapeutic window. Three-dimensional spheroid formation was successfully established using 0.6% methylcellulose in hanging-drop and U-bottom plate cultures, generating spheroids with an average diameter of approximately 350  $\mu$ m after 24 hours. This culture system provides a microenvironment free from the artificial focal adhesions characteristic of monolayer cultures, making it particularly suitable for investigating mechanotransduction-dependent cellular events. Overall, these findings provide strong evidence of synergism between verteporfin and MRTF1 silencing, suggesting that the simultaneous inhibition of the YAP/TAZ and MRTF pathways enhance the antitumor response and represent a promising strategy for the development of next-generation therapies for osteosarcoma.

**Keywords:** Osteosarcoma, mechanotransduction, verteporfin, gene silencing, 3D cell culture

## Abstract 117 | Poster | Poster Session Day 1

### Development of a Reliable RT-qPCR Assay for DEAF1 Gene Expression Analysis in SH-SY5Y Cells

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Simoneide Souza Titze-de-Almeida; Ana Clara Tejo Freire; Evilly Lopes Gomes; Raphael Severino Bonadio; Ricardo Titze-de-Almeida; Sabrina Simplicio de Araújo Romero Ferrari

**Institution:** Universidade de Brasília — Brasília-DF

Development of a Reliable RT-qPCR Assay for DEAF1 Gene Expression Analysis in SH-SY5Y Cells Simoneide S. Titze-de-Almeida<sup>1</sup>, Tayná C. Santos<sup>1</sup>, Sabrina S.A.R. Ferrari<sup>1</sup>, Ana Clara Tejo Freire<sup>1</sup>, Evilly Lopes Gomes<sup>1</sup>, Raphael Severino Bonadio<sup>1,2</sup>, Ricardo Titze-de-Almeida<sup>1</sup> 1 University of Brasília - Central Institute of Sciences, Research Center for Major Themes 2 Instituto DEAF1 e Autismos Sindrômicos Defining the mRNA expression of genes of interest is a key step in biotechnological projects aimed at modulating gene expression. This issue is particularly important for DEAF1 (Deformed Epidermal Autoregulatory Factor 1), as this gene regulates several important targets and also exerts autoregulatory control through DEAF1 dimerization. Furthermore, the existence of multiple DEAF1 splice isoforms poses an important challenge for the design of qPCR primers. From a pathobiological perspective, alterations in DEAF1 expression resulting from genetic variants may lead to DEAF1-Associated Neurodevelopmental Disorder (DAND), a condition marked by cognitive impairment, delayed language acquisition, autism-related features, reduced muscle tone, sleep abnormalities, and epilepsy that is often refractory to treatment. The present study established an RT-qPCR protocol capable of measuring the basal expression of wild-type DEAF1 mRNA in the neuron-like SH-SY5Y cell line. Human SH-SY5Y neuroblastoma cells were cultured in DMEM supplemented with fetal bovine serum and antibiotics. Total RNA was extracted using the RNeasy® Plus Mini Kit (QIAGEN), quantified using a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific), reverse-transcribed into cDNA using the SuperScript® First-Strand Synthesis System (Invitrogen), and analyzed by RT-qPCR using SYBR™ Green chemistry (Applied Biosystems) on a QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher Scientific). DEAF1 expression levels were evaluated using five DEAF1-specific primer pairs (Exxtend). In addition, three endogenous reference genes were tested for normalization: GAPDH, GAPB1, and ACTB (β-actin). Relative gene expression was calculated using the 2<sup>-ΔΔCt</sup> method. In our neuronal cell model, two DEAF1 primer pairs showed satisfactory amplification performance, yielding Ct values between 23 and 24. Among the reference genes tested, GAPDH demonstrated the greatest expression stability. In conclusion, we established experimental conditions for the accurate measurement of DEAF1 expression in SH-SY5Y cells, providing a reliable platform for future studies investigating DEAF1 gene regulation and gene-silencing strategies. Supported by: UnB, Instituto DEAF1 e Autismos Sindrômicos

**Keywords:** DEAF1; mRNA; gene-silencing; autism

## Abstract 118 | Poster | Poster Session Day 1

### Molecular Effects of DEAF1 Silencing on Its Regulatory Target Genes in a Neuronal Cell Model

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Ana Clara Tejo Freire; Evilly Lopes Gomes; Raphael Severino Bonadio; Ricardo Titze-de-Almeida; Sabrina Simplício de Araújo Romero Ferrari; Sarha Nery Santos; Simoneide S. Titze-de-Almeida

**Institution:** University of Brasília - Central Institute of Sciences, Research Center for Major Themes — Brasília-DF

DEAF1 (Deformed Epidermal Autoregulatory Factor 1) is a transcription factor highly expressed during central nervous system development and involved in the regulation of multiple genes. While pathogenic variants in DEAF1 have been implicated in DEAF1-associated neurodevelopmental disorders (DAND), the molecular mechanisms underlying the effects of altered DEAF1 function remain poorly understood. Characterizing the regulatory network controlled by DEAF1 is therefore essential to elucidate its biological role. Silencing DEAF1 is a viable strategy for investigating the consequences of reduced DEAF1 content on the expression of specific target genes. This study aimed to investigate changes in the expression of selected DEAF1-regulated genes, including UBE2M, B4GAT1, and RABEP2, after DEAF1 silencing in a neuronal cell model. Human SH-SY5Y neuroblastoma cells were transfected with a synthetic siRNA targeting DEAF1 at 300 pmol. Total RNA was extracted after 24h using RNeasy® Plus Mini Kit (QIAGEN), quantified with a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific) followed by reverse transcription and RT-qPCR analysis. DEAF1, UBE2M, B4GAT1, and RABEP2 expression levels were assessed using specific primers and normalized to the endogenous reference gene *Papoula*. Preliminary results demonstrated that DEAF1 silencing induced an increased RABEP2 expression relative to the control, supporting its regulatory activity within the DEAF1 regulatory network. The expression levels of UBE2M, B4GAT1, showed no significant changes following DEAF1 silencing. Further analyses will evaluate the effects of DEAF1 silencing on additional target genes, cellular phenotypes, and the expression and localization of DEAF1 and RABEP2 proteins in cellular, animal, and brain organoid models. These studies will contribute to a better understanding of DEAF1 function and its molecular regulatory networks, while providing a foundation for the future development of targeted gene-silencing tools and therapeutic strategies for DAND. Supported by: UnB, Instituto DEAF1 e Autismos Sindrômicos

**Keywords:** DEAF1; siRNA; Gene silencer

## **Abstract 120 | Poster | Poster Session Day 1**

### **GENERATION OF ANTI-GD2 CAR-T CELLS BY SLEEPING BEAUTY TRANSPOSON SYSTEM**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Layla Maria Vieira; EMMANUEL ARTHUR ALBUQUERQUE ARAGAO; MARTÍN BONAMINO; Sabrina Azevedo Machado

**Institution:** Instituto Nacional de Câncer — RIO DE JANEIRO

Immunotherapy with T cells modified with Chimeric Antigen Receptors (CARs) is one of the most promising antitumor strategies currently available. The CAR gene can be integrated into the genome using the Sleeping Beauty system, which employs two vectors: one carrying the CAR gene within the transposon, and another encoding the transposase that mediates genomic integration. Some solid tumors, such as retinoblastoma, neuroblastoma, osteosarcoma, melanoma, and glioblastoma, commonly express the ganglioside GD2, making it a good target for CAR-T cell immunotherapy. The aim of this work was to synthesize and validate the anti-GD2 CAR plasmid (clone 14G2A), generate CAR-T cells from peripheral blood mononuclear cells (PBMCs), and evaluate its antitumor effect. To this end, 20 µg of the anti-GD2 CAR plasmid and 1 µg of the SB transposase plasmid were electroporated into  $3 \times 10^7$  pre-activated PBMCs, which were then analyzed by flow cytometry for up to 8 days post-electroporation; CAR-T cells were also co-cultured with  $3 \times 10^4$  T-98G glioblastoma cells at effector-to-target (E:T) ratios of 5:1 and 1:1, and after 1, 3, and 5 days of co-culture the remaining adherent tumor cells were stained with crystal violet and absorbance was measured at 592 nm, while effector cytokine production (IL-2, TNF-α, and IFN-γ) was quantified by ELISA in co-culture supernatants collected after 24 hours. The average frequency (n = 7) of anti-GD2 CAR-T positive cells was 7.63%, 17.9%, and 16.1% on days 1, 6, and 8 of expansion, respectively, and after 72 hours of co-culture the anti-GD2 CAR-T (14G2A) group reduced absorbance by 76.3% and 74.5% at the 5:1 ratio, and by 59.1% and 74.3% at the 1:1 ratio, compared to the T-98G alone and MOCK groups (PBMCs electroporated without the CAR plasmid), respectively; regarding cytokine secretion, at the 5:1 ratio the anti-GD2 CAR-T (14G2A) group showed a statistically significant increase, with mean concentrations of  $708.01 \pm 197.30$  pg/mL of TNF-α (3.6-fold higher than control; p = 0.0312),  $930.93 \pm 76.78$  pg/mL of IL-2 (2.1-fold; p = 0.0156), and  $481.25 \pm 124.19$  pg/mL of IFN-γ (4.2-fold; p = 0.0312), while at the 1:1 ratio, although no statistically significant differences were observed, cytokine levels also trended higher than control, reaching  $291.77 \pm 76.42$  pg/mL of TNF-α (2.7-fold),  $449.72 \pm 68.20$  pg/mL of IL-2 (3.0-fold), and  $141.12 \pm 48.22$  pg/mL of IFN-γ (3.8-fold). These results demonstrate stable expression of the anti-GD2 CAR plasmid (14G2A) in PBMCs following electroporation using the Sleeping Beauty system, along with in vitro antitumor activity and effector cytokine secretion upon recognition of GD2+ tumor cells, together supporting the potential of this non-viral platform for generating functional CAR-T cells, with future steps in this project including additional functional assays, such as further cytotoxicity assays using GD2+ cell lines and in vivo validations, further reinforcing its translational relevance.

**Keywords:** CAR-T Cell; GD2; Sleeping Beauty.

## **Abstract 121 | Poster | Poster Session Day 1**

### **Advances in CAR-T Cell Engineering: Optimized expression transposases based on PiggyBac and Sleeping Beauty Cassettes as Low-Cost Non-Viral Alternatives.**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** MARIA EDUARDA DE OLIVEIRA MACHADO; ARTHUR DA SILVA NEUMANN; Eduardo Mannarino Correia; EMMANUEL ARTHUR ALBUQUERQUE ARAGAO; LUIZA DE MACEDO ABDO; MARTÍN BONAMINO

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Cancer remains one of the major public health challenges worldwide, the tumor resistance and aggressiveness of conventional treatments leads the search for development of innovative therapeutic strategies, such as chimeric antigen receptor T-cell (CAR-T) therapy, which relies on the genetic modification of T lymphocytes to recognize tumor-associated antigens through the expression of a chimeric antigen receptor. The widespread clinical application of CAR-T therapy remains limited by the high cost on individualized manufacturing. In this context, non-viral transposon-based systems have emerged on enable stable integration of the transgene into the host genome while simplifying the genetic engineering of CAR-T producibility. The present study aimed to evaluate the functionality of a novel hybrid non-viral vector developed by our research group, designed based on the hypothesis that the superior performance previously observed in PiggyBac system could be enhanced by combining structural elements from the currently available in Sleeping Beauty and PiggyBac transposon systems. The hybrid vectors, designated SBNew and PBNew, were compared with their corresponding unmodified non-viral vectors SB and PB. CAR-T cells were generated from T lymphocytes isolated from peripheral blood mononuclear cells (PBMCs) obtained from blood donation filters provided by INCA donors. Cells were electroporated with 5µg of transposon and 0.5µg of SB100X for the SB and SBNew groups, and with 2.5µg of transposon and 5µg of transposase for the PB and PBNew groups. Following genetic modification, the cells were expanded and subjected to in vitro assays to characterize the functional performance of CAR-T cells against tumor target cells. The results obtained during cell expansion demonstrated good viability throughout the culture period, with the SBNew group exhibiting higher viability than SB, whereas no significant differences were observed between PBNew and PB. By day 8, PB, PBNew, SB, and SBNew reached 10.5, 10.3, 10.7, and 11.0 million cells, with CAR expression of 53.7%, 43.4%, 84.2%, and 28.4%, respectively, confirming successful genetic modification in all groups. Functional cytotoxicity assays performed by co-culturing effector and target cells at a 1:1 ratio (15,000 cells of each population) confirmed the functionality of the newly developed vectors and enabled the evaluation of the cytotoxic activity of the genetically modified cells. Representative Mock and Nalm-6 groups exhibited marked tumor cell expansion, whereas the PB, SB, PBNew, and SBNew conditions showed reduced tumor-associated signal and no significant tumor cell expansion. Among the evaluated systems, SBNew reached a response against tumor cells earlier than SB, while no apparent differences were observed between PB and PBNew. Collectively, these findings enabled the functional performance of the proposed hybrid vector as a non-viral alternative for the genetic engineering of T lymphocytes.

**Keywords:** Immunotherapy, CAR-T, Transposon, Non-viral vector

## **Abstract 124 | Poster | Poster Session Day 1**

### **Functional characterization and immunophenotypic profiling of Tumor-Infiltrating Lymphocytes (TIL) Reactive to HPV E6/E7 oncoproteins: a foundation for TCR-T cell therapy in Cervical Cancer (CC).**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Martín Esteban Escobar Poveda

**Institution:** Instituto Distrital de Ciencia Biotecnología e Innovación (IDCBIS) — Bogotá D.C

Background: Human Papillomavirus (HPV)-associated cervical cancer (CC) represents the second leading cause of cancer mortality among women in Colombia. The limited availability of effective therapeutic options for patients with advanced disease leads to poor prognoses and high mortality rates, highlighting the urgent need for novel treatment alternatives suited to the local population. T-cell receptor (TCR)-engineered T-cell (TCR-T) therapy has emerged as a promising strategy for treating CC. However, the development of these therapies relies on identifying tumor-infiltrating lymphocyte (TIL) subpopulations reactive to HPV antigens. Therefore, this project aims to identify and functionally characterize TIL reactive to antigenic presentation of peptides derived from HPV E6/E7 oncoproteins in Colombian CC patients. Methods: TIL derived from 10 tumor samples of CC patients were isolated and expanded under stimulation with high-concentration IL-2 (6000 UI/ml). Subsequently, co-cultures were performed with autologous B-cell lines (LCL) pulsed with E6 and E7 oncoprotein peptides specific to each patient's HPV genotype. Antigen reactivity was evaluated by quantifying IFN- $\gamma$  release via ELISA and immunophenotypic characterization using spectral flow cytometry, assessing the activation marker CD137 alongside key therapeutic markers. Results: Of 100 tumor fragments evaluated, 36 (36%) demonstrated functional reactivity to HPV E6/E7 peptides: 11 TIL cultures were E6-reactive, 13 were E7-reactive, and 12 exhibited dual reactivity to both E6 and E7. Peptide stimulation induced robust IFN- $\gamma$  secretion and a significant upregulation of CD137 expression compared to unstimulated controls. Comparative immunophenotypic analysis between reactive and non-reactive fragments revealed no significant baseline differences in the expression profiles of the evaluated therapeutic markers. Conclusions: Functional activation reliably identifies HPV E6/E7-reactive TILs. The absence of a distinctive baseline immunophenotypic signature between reactive and non-reactive fragments indicates that antigen recognition is strictly governed by intrinsic TCR specificity, coupled with a favorable phenotypic state. Consequently, these findings enable the isolation of reactive subpopulations via immunoselection (magnetic beads and cell sorting) for subsequent TCR sequencing. This will facilitate TCR gene transfer into peripheral blood T cells using lentiviral vectors, followed by preclinical validation in animal models. Overall, this project represents a fundamental step toward identifying and sequencing HPV-specific TCRs, laying the groundwork for developing TCR-T cell therapy for Colombian patients with cervical cancer.

**Keywords:** cervical cancer; Human Papillomavirus; tumor-infiltrating lymphocytes; T-cell receptor; immunotherapy; TCR-T.

## **Abstract 126 | Poster | Poster Session Day 1**

### **Evaluation of RNA Platforms in Lipid Nanoparticles for Anti-CD19 CAR-T Cell Generation**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Leticia dos Santos Nogueira

**Institution:** Center for Cell-based Therapy, University of São Paulo, Ribeirão Preto Blood Center — Ribeirão Preto - SP

Our group has successfully developed an anti-CD19 CAR-T cell therapy that is currently being evaluated in a phase I/II clinical trial for B-cell malignancies. To increase treatment accessibility and reduce the cost and complexity associated with conventional ex vivo manufacturing, we are now investigating the adaptation of this clinically translated CAR construct for direct in vivo delivery using ionizable lipid nanoparticles (LNPs). Anti-CD19 CAR-T cells have shown remarkable efficacy in B-cell malignancies; however, conventional ex vivo manufacturing relies on viral vectors and remains limited by high costs, long production times, scalability challenges, and the risks of permanent genomic integration. Non-viral nucleic acid delivery represents an alternative for inducing transient CAR expression without genomic integration, reducing the risk of insertional mutagenesis. Among available technologies, LNPs are promising nucleic acid delivery platforms, providing efficient intracellular transport and protection against degradation. In this study, different RNA-based architectures encoding the same anti-CD19 CAR were evaluated as distinct expression strategies: Modified messenger RNA (mRNA), incorporating m6A and N1-methylpseudouridine modifications was designed to reduce innate immune activation and enhance translation efficiency; circular RNA (circRNA) has a structure that increases resistance to degradation and prolongs protein production; and self-amplifying RNA (saRNA) contains replicase-encoding sequences that promote intracellular transcript amplification. As a delivery system for these RNAs, LNPs were formulated and characterized: LNPs encapsulating mRNA, circRNA and saRNA showed mean particle sizes ranging from 109 to 125 nm, with low polydispersity indices, indicating homogeneous nanoparticle populations. The formulations exhibited encapsulation efficiencies of 67.8–92.9%, and zeta potentials between –15.6 and +4.0 mV, consistent with the physicochemical profile of LNP formulations. Preliminary results from Jurkat cell transfection with LNP-mRNA-GFP showed consistently high transfection efficiency (~95%) across all tested mRNA doses (25–1200 ng) over three days, with the highest mean fluorescence intensity (MFI) observed at 100 ng. This dose was selected for LNP-mRNA-CAR transfection, resulting in nearly 25% CAR-positive Jurkat cells on day 1, while LNP-circRNA-CAR achieved a mean CAR expression of nearly 8%. Based on these preliminary results, the next step will be to compare different RNA constructs using electroporation and lipid nanoparticles to evaluate their ability to induce and sustain CAR expression in T cells, supporting the future development of an in vivo CAR-T cell generation. This approach may contribute to the establishment of a safer and more scalable alternative to conventional ex vivo CAR-T cell manufacturing.

**Keywords:** Lipid nanoparticles; CAR-T; RNA

## **Abstract 128 | Poster | Poster Session Day 1**

### **Evaluation of anti-LRRC15 and anti-HER2 CAR T cells in HER2+ breast cancer**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Beatriz Faria de Oliveira; EMMANUEL ARTHUR ALBUQUERQUE ARAGAO; MARTÍN BONAMINO; Tatiana Martins Tilli

**Institution:** Instituto Nacional de Câncer (INCA) — Rio de Janeiro - RJ

HER2-positive breast cancer is an aggressive subtype that accounts for 15–20% of all breast cancer cases. Although targeted therapies have significantly improved patient outcomes, therapeutic resistance and treatment-related adverse effects remain major clinical challenges. In this context, chimeric antigen receptor T cell (CAR-T) therapy has emerged as a promising immunotherapeutic strategy. However, its application in solid tumors remains limited by tumor heterogeneity. Recently, our research group identified the overexpression of the leucine-rich repeat containing protein 15 (LRRC15) in tumor tissues from HER2-positive breast cancer compared with non-tumoral breast tissue. Based on this finding, this project aims to evaluate the antitumor efficacy of anti-LRRC15 CAR-T cells, alone and in combination with anti-HER2 CAR-T cells, for the treatment of HER2-positive breast tumors. Sleeping Beauty transposon-based vectors encoding anti-LRRC15 and anti-HER2 CARs containing a 4-1BB co-stimulatory domain were used in this study. Human T lymphocytes were isolated from leukocyte filters obtained from blood bags donated to the blood bank of the Instituto Nacional de Câncer (INCA) and genetically modified by electroporation to generate CAR-T cells. CAR expression was subsequently evaluated by flow cytometry. The expression of LRRC15 was confirmed in the HCC-1954 and MDA-MB-231 cell lines. An enriched HCC-1954 GFP+/Luc+ cell line was also established for functional assays. Anti-LRRC15 CAR constructs including two murine (mu209 and mu210) and three humanized (huM25, hu209 and hu210) scFv, as well as the anti-HER2 CAR construct (4D5), were successfully confirmed by restriction enzyme digestion. CAR expression was initially validated in HEK-293FT cells and subsequently confirmed in PBMCs. Representative results from one donor demonstrated that the hu210, mu210, and huM25 constructs exhibited the highest frequencies of CAR-positive cells after eight days of expansion, reaching 19.3%, 11.9%, and 7.12%, respectively. These findings support the advancement to functional characterization of the generated CAR-T cells and highlight the potential of this strategy for the treatment of breast cancer.

**Keywords:** CAR T cells; HER2-positive breast cancer; LRRC15

## **Abstract 136 | Poster | Poster Session Day 1**

### **Modification of chimeric antigen receptor (CAR) vector for the inclusion of genetic elements to modulate T lymphocyte exhaustion phenotype.**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Isabela Vitória Tavares Werneck

**Institution:** Universidade de Brasília — Brasília-DF

CAR-T cell immunotherapy employs genetically engineered T lymphocytes to target tumor cells. However, T-cell exhaustion remains a major challenge due to persistent antigen stimulation, upregulation of inhibitory receptors, and the immunosuppressive tumor microenvironment. Gene regulation strategies have been investigated to overcome this limitation. Among the most promising targets are the transcription factor TOX, a key regulator of the epigenetic program associated with T-cell exhaustion, and miR-29a, whose increased expression has been shown to inhibit exhaustion-related pathways while promoting the differentiation of memory-like T cells. To reprogram T lymphocytes, we propose the incorporation of mirtron-based genetic elements into the therapeutic transgene. Mirtrons are short introns processed into miRNAs or siRNAs through the splicing machinery. CAR vectors were designed to express mirtrons targeting these molecules, aiming to reduce the exhausted phenotype. The anti-CD19 CAR vector pT4 BcA MSCV'U3 19BBz (2-26) was modified to introduce mirtrons into different regions of the coding transgene. Three siRNAs targeting TOX and miR-29a were designed in the mirtron format and inserted into either the 5' untranslated region (5' UTR) or the coding sequence (CDS). Eight vectors were generated, together with a ninth psiCHECK-2-XNS-based reporter vector, for cotransfection and evaluation of siRNA and miRNA activity. Plasmids were transformed into *Escherichia coli* by heat shock, followed by clonal expansion and plasmid preparation. Expi293 and HEK293T cells were then transfected using FreeStyle™ MAX and Lipofectamine™ 3000, respectively. After 48 h, CAR expression was evaluated by flow cytometry, gene regulation by dual-luciferase assay, and total RNA was extracted to confirm mirtron splicing. Vector production was confirmed by agarose gel electrophoresis. Flow cytometry revealed cell population shifts comparable to that of the control vector lacking mirtrons, indicating CAR expression and proper membrane localization, and suggesting that mirtron insertion did not significantly interfere with CAR translation or localization. The dual-luciferase assay showed a reduced Renilla/Firefly ratio in groups transfected with specific mirtron constructs, indicating their ability to modulate reporter gene expression. These preliminary results suggest expression of the modified CAR and proper mirtron processing. Ongoing studies include RNA analysis, PBMC electroporation, and evaluation of TOX expression together with additional exhaustion markers. Once optimized, this strategy has the potential to mitigate T-cell exhaustion and preserve the effector and memory functions of CAR-T cells.

**Keywords:** CAR-T; anti-CD19; T lymphocytes; exhaustion; mirtron

# **Poster Session Day 2**

November 4, 2026 · 6:45 PM – 8:15 PM

# Poster Session Day 2

November 4, 2026 · 6:45 PM – 8:15 PM

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## **Abstract 83 | Poster | Poster Session Day 2**

### **$\alpha 6\beta 4$ Integrins of Retinal Pigment Epithelium (ARPE-19) Cells Interact with Alginate Scaffolds for Retinal Regeneration: An In Silico Study**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** THIAGO DE ANDRADE SILVA SANTANA LIMA

**Institution:** Universidade Federal de Goiás — Goiânia-GO

**Introduction:** The retinal pigment epithelium (RPE) plays an indispensable role in supporting photoreceptors and preserving visual health. Structural and functional impairment of the outer blood-retinal barrier is central to debilitating ocular diseases, such as age-related macular degeneration (AMD), which can lead to severe and irreversible vision loss. To address this clinical challenge, cell therapy combined with tissue engineering has emerged as a promising strategy for retinal regeneration. The success of cell transplantation relies on supporting biomaterials (scaffolds). Among these, sodium alginate-based scaffolds have shown significant potential in ophthalmological regenerative medicine due to their biocompatibility and tunable properties. The survival and anchoring of transplanted cells to these scaffolds depend directly on the interaction between membrane receptors and the material's surface. In ARPE-19 cells, a standard model of human RPE, the  $\alpha 6$  and  $\beta 4$  integrins are known to play a crucial role in mediating adhesion to extracellular matrix components. However, the exact molecular mechanisms by which these cells adhere to sodium alginate remain elusive. Therefore, we postulate that the  $\alpha 6\beta 4$  integrin heterodimer is essential for mediating this cell-biomaterial attachment. **Objective:** This study aimed to investigate the structural interaction between the  $\alpha 6\beta 4$  integrin complex and sodium alginate using computational approaches. **Methods:** To evaluate the interaction between the cellular receptor and the biomaterial, an in silico approach was employed. Initially, the three-dimensional structure of the human  $\alpha 6\beta 4$  integrin heterodimer was modeled using the AlphaFold 3 server. Concurrently, the 3D chemical conformer of the sodium alginate ligand was retrieved from the PubChem database. Both structures were then submitted to the CB-DOCK 3.0 server, which performed automated cavity detection and blind molecular docking simulations to identify the most favorable binding poses and calculate the lowest binding free energy. **Results:** The molecular docking simulations demonstrated that sodium alginate binds with high affinity to the  $\alpha 6\beta 4$  integrin heterodimer, yielding a highly favorable binding free energy of approximately -9.7 kcal/mol. **Conclusion:** Together, these preliminary findings suggest that the  $\alpha 6\beta 4$  heterodimer presents affinity and thermodynamic potential to interact with sodium alginate, and may be involved in the attachment of ARPE-19 cells to this material. In addition to contributing to the understanding of the physical interaction between RPE cells and the polymer, this study provides useful theoretical insights to guide the design of more efficient biomaterials, aiming to improve future tissue engineering strategies for retinal regeneration.

**Keywords:** Biomaterials; Cell therapy; Molecular docking; Regenerative medicine; Tissue engineering.

## **Abstract 92 | Poster | Poster Session Day 2**

### **Next-Generation Biomaterials: Lyophilized Human Platelet Lysate as a Bioactive Enhancer for Bone Regeneration**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Yury Katherine Herrera Gomez; Gabriel Flecher; Gustavo Salguero; Mariana Cañas

**Institution:** Instituto Distrital de Ciencia Biotecnología e Innovación en Salud – IDCBIS — Colombia - Bogotá

Human platelet (HP)-derived factors have emerged as promising tools for tissue engineering and bone regeneration applications due to their high concentration and potent bioactivity. These mediators play a pivotal role in cell proliferation, migration and differentiation, as well as in angiogenesis and tissue repair. Lysis and lyophilization of HP pools allow standardized manufacture, enabling pharmaceutical reproducibility and batch certification. Moreover, in combination with structural materials such as bone cements and biomaterials, HP represents an innovative approach to enhance graft integration in reconstructive bone surgery. Currently, bone grafts such as GENEX® or GENTAFIX® provide mechanical stability and effectively fill bone defects, although, they lack intrinsic bioactivity and do not actively promote tissue regeneration. Similarly, human lyophilized bone (HLB) is widely used as osteoconductive scaffold that supports new bone tissue formation by promoting cell adhesion, proliferation, and migration leading to graft integration with host bone. In this context, HP-derived factors may contribute to enhance HLB regenerative response. Here, we present a novel formulation combining lyophilized HP lysate (HPL) with either GENEX® (GEN) or GENTAFIX® (GFX) or HLB, to evaluate in vitro bioactivity by monitoring the release of platelet-derived growth factors and its ability to support proliferation of mesenchymal stromal cells (MSC). Batches of lyophilized HPL were produced under GMP-compliant conditions. Quality control and batch release process was successfully implemented. Combination of HPL (12% w/w) with GEN (GEN+HPL) and GFX (GFX+HPL) generated solid scaffolds that maintained expected structural specifications. Similarly, HLB-HPL combination and embedding in stable plasma clots was consistently produced. In culture, GEN+HPL, GFX+HPL and HLB-HPL exhibited sustained release and prolonged accumulation (up to 168 hours) of platelet-derived factors reaching levels comparable to HPL-supplemented culture control. Importantly, in co-culture with MSC, HPL-containing scaffolds significantly enhanced MSC proliferation, in absence of growth supplement. These results demonstrate that HPL, when combined with synthetic or biologic bone graft materials maintain active and sustained release of regenerative and immunomodulatory factors, capable of promoting cell growth. Thus, HPL might support integration of these biomaterials and improve graft bioactivity and bone regeneration processes, representing a promising approach for the development of advanced therapies in regenerative orthopedics.

**Keywords:** Biomaterials, Lyophilized Human Platelet Lysate, Bone Regeneration, Orthobiologics.

## **Abstract 93 | Poster | Poster Session Day 2**

### **Open-system manufacturing as a sustainable strategy for GMP production of anti-CD19 CAR-T therapies for Hematolymphoid Malignancies.**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Mariana Cañas Arboleda

**Institution:** Instituto Distrital de Ciencia, Biotecnología e innovación en Salud (IDCBIS) — Bogotá

**Introduction:** B-cell leukemia and non-Hodgkin lymphoma are major hematologic malignancies with high incidence, mortality, and healthcare burden [1-2]. Anti-CD19 CAR-T cell therapy is a promising strategy for achieving high remission rates and improving overall survival in B-cell malignancies [3]. However, financial toxicity and limited access limit its clinical use [4-5]. This study aimed to introduce a non-automated methodology (G-Rex®) to generate sustainable, GMP-compliant second-generation anti-CD19 CAR-T cell therapies. **Methods:** Leukapheresis from healthy volunteers served as starting material. G-Rex® methodology [6] initiated with selected CD4+/CD8+ cells or unfractionated mononuclear (MNC) cells. Activation was performed using TransAct reagent at 1:100, 1:250, 1:500, 1:750 or 1:1000 dilutions, with activation beads serving as control. In both protocols, cells were transduced at a multiplicity of infection (MOI) of 4 with a proprietary vector LV-CARCD19. In-process controls on days 3, 7, and 10 including cell number, CAR expression, and immunophenotype were assessed. Batches (n=4) produced in a CliniMACS Prodigy system served as standard reference. **Results:** anti-CD19 CAR-T cultures initiated with 79,49% T cells in selected CD4+/CD8+ vs. 55,12% CD3+ in non-selected MNC material. Both beads and TransAct induced activation. Importantly, transduction efficiency evaluated by anti-CD19 CAR expression was  $\geq 74,00\%$  in all conditions and comparable to CliniMACS Prodigy protocol (aprox. 87,27%). Furthermore, upon expansion all conditions yielded highly enriched CD3+ product with low proportion of CD3- cells, regardless of the starting material. Final anti-CD19 CAR-T product met identity (CAR expression  $\geq 19,00\%$ ) and potency (cytotoxicity  $\geq 81,00\%$ ) criteria, with no significant differences compared with the reference product (CAR expression  $\geq 31,00\%$ ; cytotoxicity  $\geq 87,00\%$ ). Memory phenotype analysis showed predominantly naive and central memory T cells, mainly within the CD4+ subset. LAG-3 and TIM-3 were the major exhaustion markers, with no differences among conditions. In contrast, Prodigy manufacturing showed a distinctive effector memory and terminally differentiated effector memory cells. **Conclusion:** anti-CD19 CAR-T cells can be successfully manufactured from unfractionated mononuclear cells, without prior cell selection. The G-Rex® system combined with optimized TransAct conditions generated an anti-CD19 CAR-T product with greater persistence and proliferative capacity as compared to the reference product, while meeting quality attributes for potential clinical use.

**Keywords:** Anti-CD19 CAR-T therapy, G-Rex® methodology, GMP manufacturing, Hematolymphoid Malignancies

## **Abstract 94 | Poster | Poster Session Day 2**

### **Real-time stability as strategy to define the shelf life of Mesenchymal Stromal Cell therapy medicinal products**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Daniel Sebastian Bejarano Velandia

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Wharton's jelly-derived mesenchymal stromal cells (MSC) have demonstrated significant therapeutic potential owing to their immunomodulatory, anti-inflammatory, and regenerative properties [1]. However, storage stability is essential to maintain critical quality attributes (CQAs) and establish a shelf-life that supports proper clinical use [2]. We have previously defined a shelf life for MSC formulations of maximum 24 hours post thaw. Here we present a real-time stability approach aimed at exploring the extension of shelf life of MSC formulations beyond 24 hours. Final batches were stored at 2-8 °C for up to 72 hours to evaluate CQAs during extended storage. Assessments were performed at 24, 48, and 72 hours using one product unit per time point, with baseline (time 0) corresponding to the analytical results obtained immediately after product formulation and filling. CQAs included physical characterization (color, appearance, consistency, and presence of suspended particles) and evaluation of identity, purity, and potency [2]. Cell identity was confirmed by the expression of CD73, CD90, and CD105, and the absence of hematopoietic (CD45 and CD34), immunological (HLA-DR) [3], and endothelial (CD31) markers. Growth kinetics, including analysis of cell morphology, plastic adherence [3], population doubling, and population doubling time were also evaluated. Purity was determined by viability, viable cell recovery, and the absence of microbiological contamination through sterility testing and endotoxin detection [2]. Potency was assessed using an in vitro functional assay based on MSC-mediated inhibition of anti-CD2/CD3/CD28 activated CD3<sup>+</sup> T-cell proliferation [4], and induction of CD274 (PD-L1) expression, a functional marker closely associated with MSC immunomodulatory mechanisms and therapeutic activity [5]. Data were assessed against established specifications for each CQA using descriptive statistics and ANOVA. As expected, we were able to reproduce previous stability analyses for final products released at 24 hours post-thaw. Importantly, we observed compliance with all CQAs after 48 hours of extended storage. Moreover, at 72 hours, only minor, non-significant variations were detected in batches, specifically in some phenotype markers and immunomodulatory potency. Overall, these results demonstrate that final MSC products maintain compliance with predefined specifications, preserving their quality attributes. No alterations were observed that could compromise product quality, safety, or functionality, supporting the stability of the formulation throughout the extended storage period. Thus, real-time stability approaches are feasible methodologies to evaluate and verify the shelf life of MSC-based cell therapy products for clinical use.

**Keywords:** mesenchymal stromal cells (MSC), Real-time stability, shelf life, critical quality attributes (CQAs).

## Abstract 106 | Poster | Poster Session Day 2

### Engineering Extracellular Vesicles Enriched with miRNA as a Platform to Investigate miRNA-Mediated Immunomodulation in Systemic Lupus Erythematosus

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Ricardo Cardoso Castro; FABIANI GAI FRANTZ; KELEN CRISTINA RIBEIRO MALMEGRIM DE FARIAS

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Extracellular vesicles (EVs) are important mediators of intercellular communication by transporting regulatory RNAs, including microRNAs (miRNAs) (1). Among these, miR-155-5p has emerged as a key regulator of immune responses and has been implicated in autoimmune diseases such as systemic lupus erythematosus (SLE) (2,3). However, the molecular network through which EV-associated miR-155-5p regulates FOXP3 signaling and regulatory T-cell (Treg) dysfunction in SLE remains poorly understood. Despite growing interest in EV-based therapeutics, efficient strategies to enrich specific miRNAs into EVs remain limited. To establish an experimental strategy to generate EVs enriched with miR-155-5p and to evaluate their suitability as a platform for investigating miRNA-mediated immunomodulation through integrated computational and experimental approaches. Predicted targets of hsa-miR-155-5p were identified using miRDB. Protein interaction networks and pathway enrichment were performed using STRING and Reactome database, focusing on regulatory pathways associated with FOXP3 signaling and Treg biology. THP-1 cells were differentiated into macrophage-like cells and transfected with a miR-155-5p mimic using Lipofectamine. Conditioned media were collected for EV isolation by size-exclusion chromatography (SEC), while cell pellets were used for RNA extraction. miR-155-5p expression was quantified in both cell lysates and isolated EVs by RT-qPCR. In silico analyses identified several predicted targets of miR-155-5p interacting with proteins involved in FOXP3 signaling and regulatory T-cell function, including RUNX1, NFATC2, IL2, CBFB, FOXP1, FOXP2, FOXP4, and RORC, supporting the relevance of this miRNA in immune regulatory pathways. The expanded interaction network further identified chromatin remodeling proteins and transcriptional regulators potentially associated with Treg stability. Experimentally, transfection with the miR-155-5p mimic resulted in a marked increase in intracellular miR-155-5p expression compared with negative control-transfected cells. Importantly, EVs isolated from transfected cells exhibited robust enrichment of miR-155-5p, demonstrating efficient loading of the miRNA into secreted EVs following SEC purification. These findings establish a reproducible strategy for generating miRNA-enriched EVs suitable for downstream functional studies investigating EV-mediated immune regulation. These preliminary data establish a platform for engineering EVs enriched with miRNA through cellular transfection and SEC-based purification. Integrating computational prediction with experimental validation identified candidate molecular networks linking miR-155-5p to FOXP3-associated signaling while demonstrating efficient incorporation of this miRNA into EVs. This strategy provides a framework for future studies investigating EV-mediated miRNA delivery and its potential application in immunomodulatory therapies targeting Treg dysfunction in autoimmune diseases.

**Keywords:** Extracellular vesicles; miR-155-5p; Regulatory T cells; Immunomodulation; Systemic lupus erythematosus

## **Abstract 108 | Poster | Poster Session Day 2**

### **Characterization and evaluation of the chondrogenic potential of ROR2+ synovial fluid-derived mesenchymal stem/stromal cells**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Diego de Carvalho Carneiro

**Institution:** Fundação Oswaldo Cruz (FIOCRUZ), Instituto Gonçalo Moniz — Salvador-BA

**Introduction:** Synovial fluid-derived mesenchymal stem/stromal cells (SF-MSCs) have emerged as a promising cell source for cartilage regeneration due to their easy accessibility and intrinsic association with the joint microenvironment. However, SF-MSCs are a heterogeneous population, and identifying subpopulations with enhanced chondrogenic potential remains a major challenge. Receptor tyrosine kinase-like orphan receptor 2 (ROR2), a key regulator of skeletal development and cartilage formation, has been identified as a marker of progenitor MSC cells with superior chondrogenic capacity. We aim to be the first to identify, characterize and evaluate the chondrogenic potential of ROR2+ SF-MSCs, which can therefore improve the selection of cells for regenerative therapies targeting osteoarthritis and cartilage injuries. **Methods:** Immunomagnetic cell sorting of ROR2+ MSCs and characterization by flow cytometry, cytokine proteome profile and RNAseq, comparing with the ROR2- SF-MSC and unsorted cell populations. 2D and 3D-pellet cultures under defined chondrogenic conditions to evaluate the chondrogenic potential of ROR2+ compared to ROR2- and unsorted SF-MSCs. Chondrogenic differentiation assessment by quantifying glycosaminoglycan (GAG) and pellet dry weight content, histological and immunohistochemical staining for collagen type II and aggrecan, and expression of the chondrogenic marker genes COL2A1, ACAN and SOX9 by RT-qPCR. **Results:** Human SF-MSCs were isolated from synovial fluid samples of 3 patients undergoing osteoarthritis treatment and expanded under standard culture conditions. Initial analysis by flow cytometry with anti-ROR2 antibodies showed that the patient samples contained about 13-16% of ROR2+ cells. Following chondrogenic induction, the ROR2+ SF-MSCs demonstrated cartilage matrix deposition and increased expression of chondrogenic markers compared to ROR2- and unsorted SF-MSCs. **Conclusions:** ROR2 is a potential marker to identify a synovial fluid-derived MSC subpopulation with enhanced chondrogenic potential, supporting its use as a biomarker for selecting cells with improved regenerative capacity. These findings may highlight the therapeutic potential of ROR2+ SF-MSCs for cartilage tissue engineering, cell therapy and regenerative medicine, providing a promising platform to improve MSC and MSC-derived extracellular vesicles therapies for osteoarthritis and regeneration of focal cartilage defects.

**Keywords:** Cell therapy, MSCs, ROR2, osteoarthritis, cartilage regeneration

**Abstract 109 | Poster | Poster Session Day 2**  
**ESTABLISHING A MASTER CELL BANK OF PRIMARY MENSTRUAL MESENCHYMAL STROMAL CELLS: FROM COLLECTION TO CRYOPRESERVATION**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Helga Caputo Nunes Holzhausen; Caroline Nascimento Barquilha; Ingrid Iara Damas; Mariane Aparecida Risso; Monica Alves; Samarah Vargas Harb

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Mesenchymal stromal cells (MSCs) are among the most investigated cell types for the development of advanced therapy medicinal products (ATMPs) due to their immunomodulatory, trophic, anti-inflammatory, and tissue repair properties. Among the various sources, menstrual blood-derived MSCs (MenSCs) have emerged as a particularly attractive alternative because of their non-invasive collection, abundant availability, rapid expansion capacity, genetic stability, and favorable safety profile. Previous studies have demonstrated that MenSCs fulfill the novel minimal criteria proposed by the International Society for Cell & Gene Therapy (ISCT), including terminology, expression of positive and negative MSC surface markers, description and type of mechanism of action, tissue of origin, extensive information about the tissue donors and culture conditions<sup>1</sup>. Furthermore, menstrual blood collection presents a lower ethical burden and reduced contamination risk when appropriate screening procedures are applied. Clinical interest in MenSCs has increased substantially over the last decade. Robust clinical and preclinical evidence, supports their application in reproductive medicine. Clinical studies have investigated MenSCs for intrauterine adhesions, thin endometrium, premature ovarian insufficiency, infertility, and endometrial regeneration, reporting encouraging safety and efficacy outcomes<sup>2,3</sup>. These findings highlight the relevance of establishing a Master Cell Bank (MCB) to support translational research and future ATMP development. For this, the establishment of a MCB at CNPEM represents a strategic opportunity to support MSC in cell therapy, tissue engineering, New Approach Methodologies (NAMs). Good Laboratory Practices (GLP) were developed covering donor recruitment and screening, self-collection, isolation, expansion, characterization, microbiological quality control, and cryopreservation. Challenges associated with self-collection were reported; however, 6 out of 10 donors provided suitable samples for successful isolation. Three isolation methodologies were evaluated, and Method 2 demonstrated the highest culture establishment success rate and was selected for further development. Cell culture performance was monitored through post-seeding adherence after 72h, cell composition, predominant morphology, microbiological contamination assessment, and confluence after one week culture during the transition from passage 0 to 1. Characterization studies included trilineage differentiation assays, demonstrated robust adipogenic, chondrogenic, and osteogenic differentiation potential consistent with MSC identity. Next steps will include the immunophenotyping by flow cytometry to quantify the positive MSC markers and assess the negative expression of negative markers, and xeno-free GLP production chain will be finished. In conclusion, MenSCs represent a feasible, accessible, and scalable MSC source for the establishment of MCB.

**Keywords:** Menstrual blood derived mesenchymal stromal cells, Master Cell Bank, Good Laboratory Practices, New approach methodologies, Advanced therapy medicinal products.

## **Abstract 110 | Poster | Poster Session Day 2**

### **Assessment of the Potential Nose-to-Brain Delivery of miR-124-Loaded-Lipid Nanoparticles as a New Approach to Treat Neurodegenerative Diseases**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** João Victor soares Vieira; Alfeu Zanotto Filho; Elenara Lemos-Senna; Katherine Athayde Teixeira de Carvalho; Mariana Côcco Funghetti; Mariele Paludetto Sanches; Natália Marcelli Stephanes

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Neurodegenerative diseases (NDDs) are characterized by the progressive loss of neurons in the central nervous system (CNS) and represent a heterogeneous group of diseases with different clinical manifestations. Genetic therapies have emerged as an innovative approach for treating NDDs by targeting their molecular mechanisms, using nucleic acids, such as microRNAs (miRs). miR-124 is the most abundant miR in the brain, playing a crucial role in neurogenesis, synaptic function, and the progression of neuroinflammation. In NDDs, miR-124 is frequently dysregulated, contributing to the neuroinflammation process and consequently, the progression of neurodegeneration [1,2]. Therefore, miR-124 supplementation appears to be a promising approach for protecting neurons from damage and hindering the progression of NDDs. In the last decade, lipid nanoparticles (LNPs) have been successfully employed to carry therapeutic RNAs, overcoming their limitations of low biological stability and poor ability to enter cells [3]. In this study, we investigated the potential of LNPs to deliver miR-124 by the nose-to-brain route. miR-124-loaded LNPs were prepared by the ethanol dilution method, using an ionizable (DODMA) and cationic (DOTAP) lipid, a helper lipid (DSPC), a pegylated lipid (DSPE-PEG2000), and cholesterol in a molar ratio of 25:25:10:1,5:38,5. The total lipid concentration was 12.5 mM, and the nitrogen to phosphate (N/P) ratio was 10:1. The LNPs were characterized according to their size, polydispersity index (PDI), zeta potential, drug loading, and encapsulation efficiency. The ability of LNPs to permeate through the olfactory nasal mucosa was evaluated using a vertical Franz cell and porcine olfactory nasal mucosa as a membrane model. Besides, cell viability and internalization studies were carried out using the human neuroblastoma cell line (SH-SY5Y). For both studies, the green-fluorescent NBD-cholesterol was incorporated into the LNP formulations. LNPs exhibited a mean size of around 120 nm, surface charge close to neutrality, and a PDI lower than 0.3. LNPs displaying miR-124 loading of around 150 µg/mL and encapsulation efficiency higher than 95% were obtained. In vitro permeation studies demonstrated the ability of NBD-cholesterol-loaded LNPs to permeate across the porcine olfactory mucosa, reaching the basolateral side of the membrane. In addition, miR-124-loaded LNPs were taken up by the SH-SY5Y cells depending on their concentration and incubation time, maintaining cellular viability over 72 hours. Overall, this study evidenced the potential of LNPs to deliver miR-124 to the CNS via the nose-to-brain route, aiming to treat NDDs.

**Keywords:** Neurodegenerative diseases; Lipid Nanoparticles; Nose-to-brain delivery; miRNA-124

## Abstract 112 | Poster | Poster Session Day 2

### 5-Azacytidine preconditioning promotes mitochondrial transfer from umbilical cord-derived mesenchymal stromal cells into CD4<sup>+</sup> T cells

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** BRUNA RAFAELA VIELLI; KELEN CRISTINA RIBEIRO MALMEGRIM DE FARIAS; PIETRA BURATTO DE SANTIS; Waldir César Ferreira de Oliveira

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Mesenchymal stromal cells (MSCs) exert potent immunomodulatory effects through the secretion of soluble mediators, direct cell-cell interactions, and the induction of regulatory T cells (Tregs). Recent evidence has identified intercellular mitochondrial transfer as an additional mechanism by which MSCs regulate immune cell metabolism and function. Preconditioning strategies, such as treatment with 5-azacytidine (5-AZA), have been shown to enhance the biological properties of MSCs, including mitochondrial metabolism and immunomodulatory activity. However, the effect of 5-AZA preconditioning on MSC-mediated mitochondrial transfer remains poorly understood. This study aimed to determine whether 5-AZA preconditioning enhances mitochondrial transfer from human umbilical cord-derived MSCs into CD4<sup>+</sup> T cells. Human umbilical cord-derived MSCs were expanded and characterized according to the International Society for Cell & Gene Therapy (ISCT) criteria, preconditioned with 5  $\mu$ M 5-AZA, and labeled with MitoTracker™ Deep Red FM before coculture with human peripheral blood lymphocytes for 30 minutes. Mitochondrial transfer was quantified by flow cytometry through the detection of MitoTracker-positive lymphocyte subsets. Preconditioned MSCs showed a trend toward increased mitochondrial transfer compared with untreated MSCs. Preferential mitochondrial transfer was observed in CD4<sup>+</sup> T cells, indicating selective interactions with immune populations involved in immunoregulation. These preliminary findings suggest that 5-AZA preconditioning enhances the capacity of MSCs to transfer mitochondria into CD4<sup>+</sup> T cells, supporting the hypothesis that metabolic reprogramming contributes to MSC-mediated immunomodulation. Ongoing studies will determine whether mitochondrial transfer promotes the induction, phenotypic stability, and suppressive function of regulatory T cells, providing mechanistic insights for optimizing MSC-based therapies for inflammatory and immune-mediated diseases.

**Keywords:** mesenchymal stromal cells; mitochondrial transfer; CD4<sup>+</sup> T cells; regulatory T lymphocytes; immunoregulation, 5-azacytidine.

## **Abstract 114 | Poster | Poster Session Day 2**

### **The First Year of Science Communication at The Brazilian National Institute of Gene Therapy (INTERGEN): Expanding Public Engagement and the Visibility of Gene Therapy Research**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Nathalia Freitas Lima

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**Background:** Gene therapy is transforming the treatment of inherited, rare, and acquired diseases, creating new opportunities for precision medicine. However, the complexity of these technologies, limited public understanding, and the rapid spread of health misinformation remain major barriers to their societal adoption. Research institutes dedicated to gene therapy have a unique responsibility to translate scientific advances into accessible, evidence-based information, strengthening scientific literacy and fostering dialogue among researchers, healthcare professionals, patients, policymakers, and the general public. The Brazilian National Institute of Gene Therapy (INTERGEN) is a nationwide collaborative network that brings together leading research groups across Brazil to advance gene therapy research, innovation, and the development of nationally driven therapeutic solutions. As part of its institutional mission, INTERGEN established a coordinated science communication program to increase awareness of gene therapy and promote evidence-based public engagement. This study describes the implementation and first-year outcomes of this initiative. **Methods:** Science communication activities were launched in May 2025 through a coordinated multiplatform strategy including Instagram, LinkedIn, a website, a newsletter, podcasts, and scientific events. Platform analytics and website traffic reports were analyzed to assess audience growth, engagement, and geographic reach during the first year of the initiative. **Results:** During its first year, INTERGEN established a consistent digital presence across multiple communication platforms. By July 2026, the Instagram account had reached 1,253 followers, reaching 39,015 accounts over the previous 90 days. LinkedIn expanded to 2,374 followers, generating 162,234 impressions over the previous year and strengthening the institute's visibility among researchers, healthcare professionals, and other stakeholders. The INTERGEN newsletter reached 254 subscribers and generated more than 2,500 views during its first year. Website analytics demonstrated international visibility, with visitors from multiple countries across the Americas, Europe, and Asia, highlighting the growing global reach of INTERGEN's science communication activities. Beyond digital platforms, the communication program supported the dissemination of major scientific initiatives through coverage of national and international events, including the ASGCT Annual Meeting (USA), the 1st Brazilian Gene Therapy Forum, the Gene and Cell Therapy 2025 - ABTCel-Gen & INTERGEN Meeting, the 1st INCT-INTERGEN Workshop, and the Science, Technology & Innovation Week for SUS. In addition, six podcast episodes were produced to broaden access to evidence-based information on gene and cell therapy. **Conclusion:** INTERGEN demonstrates that science communication can be effectively integrated into the mission of a national gene therapy research institute. Beyond disseminati

**Keywords:** Gene therapy; Science communication; Public engagement; Health communication; Translational research.

## **Abstract 119 | Poster | Poster Session Day 2**

### **Analysis of the DEAF1 Gene Silencing Profile Using Synthetic siRNA**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Ana Clara Tejo Freire; Evilly Lopes Gomes; Raphael Severino Bonadio; Ricardo Titze-de-Almeida; Sabrina Simplício de Araújo Romero Ferrari; Simoneide S. Titze-de-Almeida

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DEAF1 (Deformed Epidermal Autoregulatory Factor-1) gene encodes a transcription factor that is highly expressed during central nervous system development, functioning as both a transcriptional activator and repressor of several genes, including UBE2M, B4GAT1, RABEP2, as well as regulating its own expression. Pathogenic mutations in DEAF1 are directly associated with DEAF1-Associated Neurodevelopmental Disorders (DAND), which are characterized by intellectual disability, language delay, autism spectrum disorder, hypotonia, sleep disturbances, and treatment-resistant epilepsy. Despite the remarkable advances in therapies based on synthetic oligonucleotides, such as small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs), no therapeutic strategies targeting DEAF1 modulation are currently available. In this context, the present study aimed to evaluate the silencing efficiency of the wild-type DEAF1 gene using synthetic siRNAs as a proof of concept for the future development of gene therapies. Human SH-SY5Y neuroblastoma cells were cultured in DMEM supplemented with fetal bovine serum and antibiotics and transfected with a synthetic siRNA (Thermo Fisher Scientific) targeting the DEAF1 transcript using Lipofectamine™ as the transfection reagent. The siRNAs were tested at concentrations of 150, 300, and 450 pmol. Non-transfected cells and cells transfected with a scramble sequence were used as controls. Total RNA was extracted after 24 and 48 hours using the RNeasy® Plus Mini Kit (QIAGEN), quantified using a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific), reverse-transcribed into cDNA using the SuperScript® First-Strand Kit (Invitrogen), and analyzed by RT-qPCR using SYBR™ Green (Applied Biosystems) on a QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher Scientific). DEAF1 expression levels were determined using the DEAF1-1 primer (Exxtend) and normalized to the endogenous reference gene *Papoula*. Preliminary results demonstrated that the synthetic siRNA promoted dose-dependent silencing of DEAF1, reducing its expression to approximately 0.5-fold relative to the control, thereby validating the proposed methodology and indicating that siRNAs designed to target pathogenic DEAF1 variants may represent a promising strategy for the development of gene therapies for DAND. Supported by: UnB, Instituto DEAF1 e Autismos Sindrômicos.

**Keywords:** DEAF1; siRNA; Gene silencer.

## Abstract 122 | Poster | Poster Session Day 2

### Defining the Skeletal Muscle Phenotype of Vascular Vulnerability to Guide Engineered MSC Secretome-Based Regenerative Therapies

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

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**Institution:** Departamento de Ciências Fisiológicas, Faculdade de Ciências Médicas da Santa Casa de São Paulo — São Paulo, SP

Cell-free therapies based on engineered mesenchymal stromal cell (MSC)-derived secretomes have emerged as promising strategies to promote skeletal muscle regeneration through the combined modulation of tissue repair and extracellular matrix remodeling. However, the skeletal muscle alterations associated with cardiovascular vascular vulnerability remain poorly characterized, limiting the rational development of targeted regenerative therapies. We investigated the skeletal muscle phenotype of ApoE<sup>-/-</sup>Fbn1C1039G/+ mice, a model of vascular vulnerability, to establish a biological framework for future engineered MSC secretome-based interventions. Seventeen histomorphometric parameters were quantified in the adductor (MA), gastrocnemius (MG), and quadriceps (MQ), including vascular remodeling, regenerative markers, myofiber morphometry, and compartment-specific extracellular matrix composition. Vascular vulnerability induced a muscle-specific remodeling phenotype rather than generalized degeneration. The adductor muscle was the most susceptible, exhibiting reduced myogenin-positive area ( $0.71 \pm 0.10$  vs.  $1.16 \pm 0.26\%$ ;  $p = 0.0277$ ), decreased total myofiber cross-sectional area ( $914 \pm 53$  vs.  $1433 \pm 81 \mu\text{m}^2$ ;  $p = 0.0006$ ), and reduced slow-fiber cross-sectional area ( $568 \pm 81$  vs.  $1186 \pm 76 \mu\text{m}^2$ ;  $p = 0.0006$ ), indicating impaired regenerative capacity associated with selective muscle atrophy. In contrast, the gastrocnemius displayed increased microvessel density ( $6.38 \pm 0.96$  vs.  $4.43 \pm 0.57$  vessels/ $\text{mm}^2$ ;  $p = 0.0068$ ) without changes in CD31-positive area, suggesting localized vascular remodeling rather than endothelial expansion. Importantly, vascular vulnerability did not induce diffuse fibrosis but promoted compartment-specific extracellular matrix remodeling, characterized by a consistent reduction of type III collagen surrounding intramuscular blood vessels across all three muscles ( $p = 0.0058$ – $0.0428$ ), representing the only extracellular matrix alteration shared among muscles. These findings identify impaired myogenic signaling and perivascular extracellular matrix remodeling as actionable biological targets for next-generation engineered MSC secretome therapies and provide a preclinical rationale for developing cell-free regenerative strategies aimed at restoring skeletal muscle homeostasis under conditions of cardiovascular vascular vulnerability.

**Keywords:** Engineered MSC secretome; Cell-free therapy; Vascular vulnerability; Skeletal muscle regeneration; Extracellular matrix remodeling

## Abstract 123 | Poster | Poster Session Day 2

### Development and Evaluation of Cationic Nanoemulsions as a Strategy for Intra-articular Gene Therapy in Mucopolysaccharidosis Type II

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Willian da Silva Carniel; Flávia Nathiely Silveira Fachel; Gabriela Fusinato de Oliveira; GUILHERME BALDO; Helder Ferreira Teixeira; JÚLIA DE AZAMBUJA VANIEL; Maria Luísa Gasparini Vieira; Patrícia Weimer; ROSELENA SILVESTRI SCHUH; URSULA DA SILVEIRA MATTE; Vinicius Monteagudo de Barros

**Institution:** Programa de Pós-Graduação em Ciências Farmacêuticas – Universidade Federal do Rio Grande do Sul (PPGCF-UFRGS) — Porto Alegre/RS

**Introduction:** Mucopolysaccharidosis type II (MPSII) is a rare and hereditary disease caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS), which is responsible for the breakdown of glycosaminoglycans. Among the treatments currently available, enzyme replacement therapy does not provide effective distribution to certain organs; more importantly, it is unable to reach bones and cartilage, thus necessitating new therapeutic approaches, such as gene editing using the CRISPR-Cas9 system. **Objectives:** To develop and evaluate the performance of a cationic nanoemulsion (NE), thickened with poloxamer 407, as a co-carrier of dexamethasone and plasmids containing the CRISPR-Cas9 system and the IDS gene donor sequence, for intra-articular administration, with the aim of gene editing. **Methodology:** Evaluation of physicochemical properties by dynamic light scattering (DLS) and electrophoretic mobility (EM); evaluation of NE complexation with plasmids by agarose gel electrophoresis; stability of the complexes upon exposure to simulated synovial fluid (SSF); evaluation of viscosity and rheological profile by capillary and rotational viscometry; determination of the sol-gel phase transition temperature using a parallel-plate rheometer; a cell uptake protocol was conducted on synoviocyte-like fibroblasts (FLS) treated with NE containing NBD-PE, a fluorescent marker. Images were captured using a fluorescence microscope 2, 24, and 48 hours after treatment. Flow cytometry analyses were performed to quantify the percentage of cells that internalized the NE/pDNA complexes. **Results:** Analysis of the physicochemical characteristics revealed droplets with a diameter of  $183.10 \pm 1.60$  nm, a polydispersity index of less than 0.1, and a zeta potential of  $+25.4 \pm 0.5$  mV—desirable characteristics for the formulation. Complex formation proved effective, as no migration of the NE/plasmid complexes was observed on the agarose gel, unlike when naked plasmids were applied. The NE/plasmid complexes proved stable for up to 48 hours when incubated in FSS, as evidenced both by the presence of viable complexes on the agarose gel and by the values obtained in the physicochemical characterization following incubation in the fluid. The viscosity of the formulation thickened with poloxamer 407 was  $16.87 \pm 1.66$  cP, with a Newtonian rheological profile. The phase transition temperature was 42 °C, demonstrating that poloxamer acts as a thickener and not as a thermoresponsive agent at body temperature (37 °C). The fluorescence images obtained indicate internalization of the NE/pDNA complexes at all time points, and flow cytometry corroborates this with high percentages of fluorescence detected in the cells. **Conclusion:** The nanoemulsions exhibit favorable characteristics for application via the desired route of administration and show promising results for in vitro and in vivo testing.

**Keywords:** gene therapy, nanoemulsion, Mucopolysaccharidosis

## **Abstract 125 | Poster | Poster Session Day 2**

### **Impact of seeding and harvesting conditions on cell health and vector yield during rAAV2 production**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** MARIANA SANTANA DIAS; CAIO MOTA MACHADO; GABRIELA SILVA ALMEIDA; HILDA PETRS SILVA

**Institution:** Federal University of Rio de Janeiro — Rio de Janeiro - RJ

Adeno-associated viral vectors (rAAVs) are nowadays the most common vectors used for in vivo gene therapy and represent a powerful tool for human gene therapy. They promote high transduction efficiency in several tissues with stable gene expression and have low toxicity and immunogenicity when compared to other viral vectors. Since the first cloning of the AAV genome, the production process of its vectors has been developed and optimized over time, but is still laborious, costly, and vector titers need to be improved, especially for clinical applications. In this work, we have compared different conditions of cell seeding and harvesting regarding cell health status and vector yield aiming to optimize the production process of rAAV2 in our laboratory. Each rAAV2 production was carried in a single 225 cm<sup>2</sup> flask, in which HEK293T cells were seeded and transfected after 24h with polyethylenimine (PEI) and both principal and helper plasmids for vector production. Protocol variations included seeding density, 4E+4 vs 7E+4 cells/cm<sup>2</sup>, harvesting time after transfection, 48h or 72h, and harvesting method, directly in the cell media or in DPBS. Evaluated parameters comprised cell growth rate, oxygen consumption, transfection efficiency, vector genome titer and productivity. Analyses of cell growth and respiration showed that the lower seeding density, 4E+4 cells/cm<sup>2</sup>, led to higher routine oxygen consumption at 24h and higher cell growth rate at 72h after transfection. Both seeding conditions had similar transfection efficiency, but vector productivity (vg per cell) was higher for the lower seeding at both 48h and 72h. When comparing harvesting conditions, results indicated higher vector recovery when collection was performed at 72h after transfection and directly in the cell culture media. Overall, cells seeded at the lower density had better metabolic conditions at the initial stages of vector production, with higher vector productivity at the end, and harvesting at 72h directly in the cell culture media led to higher titers. In this sense, it was possible to establish optimized production parameters for rAAV2, from seeding to harvesting.

**Keywords:** adeno-associated; viral vectors; gene therapy; production; optimization

## Abstract 127 | Poster | Poster Session Day 2

### In vivo biodistribution and enzymatic activity of liposome-complexed IDUA mRNA in a murine model of mucopolysaccharidosis type I

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Vinicius Monteagudo de Barros; Carlos Frederico Martins Menck; GUILHERME BALDO; Natália Cavalari Novakowski; ROSELENA SILVESTRI SCHUH; Willian Carlos Corrêa Padilha; Willian da Silva Carniel

**Institution:** Universidade Federal do Rio Grande do Sul (UFRGS) — Porto Alegre (RS)

**Introduction:** Mucopolysaccharidosis type I (MPS I) is a rare progressive genetic disorder caused by mutations in the IDUA gene, resulting in a deficiency of the alpha-L-iduronidase enzyme. Without this enzyme, the accumulation of glycosaminoglycans (GAGs), specifically heparan sulfate and dermatan sulfate, occurs in tissues, leading to cellular damage and multisystemic manifestations. To overcome the limitations of current therapies, mRNA therapy emerges as a promising and safe alternative to traditional gene therapy, alongside its delivery via non-viral vectors. **Objective:** To evaluate the in vivo biodistribution of liposomes complexed with IDUA mRNA in a murine model of MPS I. **Methodology:** The experimental design consisted of 5 study groups (n=3 animals per group), including wild-type (WT) control and MPS I mice. Animals received a single intravenous administration (via the tail vein) of the liposome-mRNA complex in a tiered volume ranging from 10µL to 100µL (adjusted according to body weight), with three distinct doses evaluated: 0.25, 0.5, and 1.0 mg/kg. For biodistribution determination, peripheral blood aliquots were collected on filter paper at 6 h, 24 h, and 72 h post-injection. Animals were euthanized 7 days post-treatment, followed by the collection of target tissues and organs (such as liver, heart, spleen, kidney, and brain). IDUA enzymatic activity in all biological samples (blood and macerated tissues) was quantified using a fluorimetric assay. **Results:** Enzymatic activity measurements were calculated as a percentage of activity relative to the WT group. Peripheral blood collected on filter paper 6 hours post-administration showed that the mean IDUA enzymatic activity ( $\pm$  standard deviation) in the 1.0 mg/kg group ( $37.21 \pm 15.51$ ) was significantly higher than that of the 0.25 mg/kg group ( $2.39 \pm 4.68$ ,  $p < 0.0093$ ) and the 0.5 mg/kg group ( $1.83 \pm 1.30$ ,  $p < 0.00537$ ), though it showed no statistically significant difference compared to the untreated MPS I controls ( $20.34 \pm 11.39$ ,  $p > 0.123227$ ). For all statistical analyses, a one-way ANOVA followed by Tukey's post-hoc test was performed. For the remaining time points (24 h and 72 h on filter paper) and across the analyzed tissues, no significant IDUA enzymatic activity was detected via the fluorimetric method. **Conclusions:** The present study presents preliminary results that highlight the need for an increased sample size, as well as the evaluation of different mRNA concentrations and administration volumes. Nevertheless, these findings demonstrate the potential of this approach as a viable therapeutic strategy.

**Keywords:** Liposome, mRNA Therapy, Non-viral Vector, MPS I

## **Abstract 129 | Poster | Poster Session Day 2**

### **Evaluation of Collagen Deposition and Gene Expression Following CDKN1B Silencing in Fibroblasts Cultured in Two- and Three-Dimensional Models**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** ERIC ALMEIDA MEDEIROS; André Carlos Pereira de Matos; Isabela Karoline Ferreira Lima; Lara Fernanda Pachêco Guimarães; Max Alves Bastos Lopes; Rosângela Vieira de Andrade

**Institution:** Universidade Católica de Brasília - UCB — Taguatinga-DF

Maintenance of tissue homeostasis depends on the strict regulation of the balance between cell proliferation and entry into quiescence, a process primarily coordinated by cell cycle regulators such as cyclin-dependent kinases and their inhibitors. Disruption of this balance contributes to the development of several pathological conditions, including fibrosis, dysplasia, and degenerative diseases. Among these regulators, CDKN1B plays a central role by inducing cell cycle arrest and restricting proliferative progression. Its activity is complemented by contact inhibition, a physiological mechanism in which cell-cell and cell-extracellular matrix interactions limit proliferation when cell density becomes high. Because these interactions are poorly represented in two-dimensional cultures, three-dimensional (3D) models provide a more suitable approach for investigating this phenomenon under conditions that more closely resemble the tissue microenvironment. In fibroblasts, which are essential cells for tissue repair and extracellular matrix synthesis, CDKN1B is associated with the maintenance of quiescence and may also contribute to restraining the production of ECM components, such as type I collagen. The aim of the present study was to elucidate the involvement of CDKN1B in regulating proliferative status and the characteristics of the extracellular matrix produced by fibroblasts. Human fibroblasts were subjected to CDKN1B gene silencing using siRNA and compared with untreated control cells and cells treated with scramble siRNA. The treatments were performed in both monolayer cultures and three-dimensional spheroids generated using the hanging-drop method. Protein interaction network analysis was conducted using the STRING platform with default parameters in the Disease Pathways mode, employing CDKN1B as the query term. RT-qPCR was performed to evaluate CDKN1B knockdown efficiency and to determine the expression levels of CDKN1B, RHOA, ACTB, TP53, COL1A1, FN1, MMP2, MKI67, FGF2, CCND2, and BCL2. Extracellular matrix secreted by the different experimental groups was collected for relative quantification using the Sirius Red assay. The protein interaction network identified potential associations between CDKN1B and TP53, ACTB and RHOA. CDKN1B knockdown efficiency exceeded 80%, resulting in altered RNA expression levels of CDKN1B, COL1A1, FN1, MMP2, MKI67, FGF2, CCND2, RHOA, ACTB, TP53, and BCL2. Furthermore, total collagen deposition differed between the 2D and 3D culture systems as well as between their respective CDKN1B-silenced conditions. Spheroids exhibited higher collagen deposition than monolayer cultures. Moreover, the 3D model showed a greater increase in collagen deposition following CDKN1B silencing than the corresponding monolayer cultures. Collectively, these findings indicate that CDKN1B acts as a direct regulator of collagen synthesis and deposition in fibroblasts, while its silencing promotes increased cellular proliferation.

**Keywords:** CDKN1B, fibroblasts, quiescence, extracellular matrix, gene silencing.

## Abstract 130 | Poster | Poster Session Day 2

### Nonviral CRISPR-Cas13d-mediated EGLN1 silencing enhances angiogenic signaling for Peripheral Artery Disease

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Cynthia Bartolomeo; ROBERTA SESSA STILHANO YAMAGUCHI

**Institution:** Faculdade de Ciências Médicas da Santa Casa de São Paulo — São Paulo - SP

Peripheral artery disease (PAD) is a progressive vascular disorder characterized by arterial obstruction and impaired tissue perfusion, which may lead to ischemic pain, nonhealing ulcers, and limb loss. Therefore, strategies capable of promoting functional neovascularization are urgently needed. Prolyl hydroxylase domain protein 2 (PHD2), encoded by EGLN1, is a key negative regulator of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ). EGLN1 downregulation stabilizes HIF-1 $\alpha$  and enhances the expression of proangiogenic mediators, including vascular endothelial growth factor (VEGF), representing a promising approach for therapeutic angiogenesis. Here, we investigated CRISPR-Cas13d-mediated EGLN1 silencing and established a nonviral minicircle (MC) platform for future delivery of the system. Five gRNAs targeting EGLN1 were selected by in silico analysis based on predicted efficiency and minimal off-target potential and cloned into a Cas13d expression vector. Silencing efficiency was assessed by RT-qPCR in HEK293T cells. At 48 h after calcium phosphate-mediated transfection, gRNAs 2 and 5 reduced EGLN1 expression by 83.4% and 85.6%, respectively, compared with the control. EGLN1 downregulation was also confirmed in induced human skeletal muscle cells (iHSM) 48 h after nucleofection. Consistent with activation of the HIF-1 $\alpha$  pathway, ELISA showed 5.5- and 4.8-fold increases in VEGF secretion following treatment with gRNAs 2 and 5, respectively. To evaluate functional angiogenic activity, a stable GFP-expressing human umbilical vein endothelial cell line (HUVEC-GFP) was generated by lentiviral transduction and fluorescence-activated cell sorting. Conditioned medium from iHSM cells nucleofected with the selected gRNAs promoted endothelial sprouting in HUVEC-GFP, demonstrating that Cas13d-mediated EGLN1 silencing induces a proangiogenic secretory response. In parallel, a GFP-expressing MC was developed to validate the production and delivery platform. Transfection of HEK293T cells resulted in approximately 95% GFP-positive cells. Different arabinose concentrations were tested to optimize MC induction and purification, and 1% arabinose yielded the lowest levels of residual parental plasmid and miniplasmid. Additional treatment with I-SceI endonuclease or Plasmid-Safe DNase further reduced contaminating DNA species, with DNase showing the highest purification efficiency. These findings demonstrate that CRISPR-Cas13d efficiently silences EGLN1, increases VEGF secretion, and promotes endothelial sprouting. The successful establishment of an efficient MC platform supports the development of a compact, nonviral RNA-targeting gene therapy for therapeutic angiogenesis in PAD.

**Keywords:** Peripheral artery disease, EGLN1, CRISPR-Cas13d, minicircle

## Abstract 131 | Poster | Poster Session Day 2

### Investigating the Role of IQSEC1 in Congenital Zika Syndrome: From Epigenetic Modulation to Functional In Vitro Models

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Rafael Ferreira Lima

**Institution:** UFRJ — RJ

The Zika virus (ZIKV) is an arbovirus belonging to the Flaviviridae family, primarily transmitted to humans by *Aedes* mosquitoes. While symptomatic infection in adults is generally mild, vertical transmission during pregnancy poses severe public health concerns. Within the fetal environment, ZIKV exhibits a strong tropism for neural progenitor cells, leading to microcephaly and other severe neurological malformations that characterize Congenital Zika Syndrome (CZS). Despite recent advances in understanding ZIKV replication and neuropathogenesis in the developing central nervous system (CNS), the epigenetic mechanisms underlying host-pathogen interactions remain poorly understood. To address this gap, our group performed a genome-wide epigenetic association study (EWAS) on brain methylomes from fetuses affected by congenital ZIKV infection compared to controls. This analysis identified differentially methylated regions (DMRs) across four candidate genes: IFI44, IQSEC1, THBS2-AS1, and OR8B8. Among them, IQSEC1—a gene vital for neurodevelopment and highly expressed in the cerebral cortex during embryonic stages—was selected for further validation due to its biological plausibility. In a pregnant Swiss mouse model infected in utero via intraventricular injection, ZIKV infection significantly downregulated murine *Iqsec1* expression at developmental stages E18 and P0. Although global methylation of the *Iqsec1* promoter showed no broad alterations, site-specific analyses revealed significant hypomethylation at two CHH sites ( $\text{FDR} < 0.05$ ) and suggestive shifts at 12 additional non-CpG/CpG sites ( $\text{FDR} < 0.06$ ), providing compelling evidence of ZIKV-induced epigenetic modulation. Building upon these epigenetic and transcriptomic findings, we are currently establishing in vitro human neuronal cell models featuring CRISPR-Cas9-mediated overexpression and repression of IQSEC1. By challenging these engineered overexpression and knockdown cell lines with ZIKV at different multiplicities of infection (MOIs), we aim to evaluate whether differential IQSEC1 expression directly alters viral kinetics and replication dynamics. Ultimately, this project seeks to validate IQSEC1 as a novel target for gene therapy interventions designed to suppress ZIKV viral load in fetal neural tissues, potentially mitigating the devastating neurodevelopmental impacts of Congenital Zika Syndrome.

**Keywords:** Zika Virus, Congenital Zika Syndrome, IQSEC1, DNA methylation

**Abstract 132 | Poster | Poster Session Day 2**

**DESENVOLVIMENTO DE DELIVERY POR NANOPARTÍCULAS LIPÍDICAS PARA CRISPR/CAS9 COM POTENCIAL DE EXCISÃO DO PROVÍRUS HIV-1 LATENTE.**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Johari Iarias Chequer

**Institution:** Universidade Federal do Rio de Janeiro — Rio de Janeiro, RJ

Genome editing techniques are extensively investigated for a wide range of biotechnological and therapeutic applications. Currently, systems based on the CRISPR-Cas9 machinery stand out and have already been explored in the treatment of latent HIV-1 infection through excision of proviral DNA in chronically infected cells. In this context, the development of efficient delivery strategies for this system into lymphoid cells is essential for clinical applications of this methodology. Thus, many studies aim to identify improved CRISPR delivery strategies for lymphoid cells, seeking low toxicity and higher delivery efficiency. Therefore, the objective of this work is to develop LNPs capable of efficiently delivering the CRISPR system, using the CRISPR-Cas9RFP vector in J.Lat cells, enabling excision of the HIV-1 provirus with low cellular toxicity. To attain this goal, the CRISPR-Cas9-RFP vector was selected, and its expression efficiency was tested in HEK293T cells. Subsequently, the guide RNA sequence was ligated into the vectors, and successful ligation was confirmed by PCR and colony screening. After confirming the presence of the guide RNA, the vectors were incorporated into LNPs formulated using microfluidics. The characteristics of the lipid nanoparticles were then evaluated, followed by transfection of the LNPs into J-Lat 10.6 cells. This will allow assessment of CRISPR/Cas9 vector delivery and the potential for provirus excision in these cells. As a result, we expect to successfully assemble vectors containing the guide RNA and to develop LNPs with characteristics within acceptable parameters, exhibiting high packaging and transfection capacity, along with low cytotoxicity, particularly compared to Lipofectamine. Finally, we hope for the excision of the HIV-1 provirus mediated by CRISPR/Cas9 molecules in cells transfected with these formulations.

**Keywords:** HIV; CRISPR-CAS9; LNP

## **Abstract 133 | Poster | Poster Session Day 2**

### **MicroRNAs as Biomarkers in Neurodegenerative Diseases: Causal Prospecting in HMSS v4.0**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Max Alves Bastos Lopes; André Carlos Pereira de Matos; Eric Almeida Medeiros; Fábio Gonçalves da Silva; Isabella Ribeiro da Silva; Júlia da Mata Vieira

**Institution:** Universidade Católica de Brasília (UCB) — Brasília, DF

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate protein translation through mRNA silencing or degradation. Owing to their stability in biological fluids (such as blood and cerebrospinal fluid), they stand out as promising biomarkers and therapeutic targets for central nervous system disorders. Objective: To map and quantify causal relationships between miRNAs and five neurodegenerative diseases using bioinformatics analyses. Methodology: The HMSS v4.0 bioinformatics platform was used in “causality” mode under the “diseases” option to identify and quantify curated causal association hits. Searches were restricted to official Disease Ontology ID (DOID) codes: Alzheimer’s disease (DOID:10652), Parkinson’s disease (DOID:14330), Multiple sclerosis (DOID:2377), Huntington’s disease (DOID:12858), and Amyotrophic Lateral Sclerosis (DOID:332). Results and discussion: HMSS v4.0 mining identified a total of 61 curated hits. Alzheimer’s disease (DOID:10652): 32 curated hits were recorded, highlighting miRNAs that regulate amyloid precursor protein and BACE1 (miR-101, miR-124, miR-128, miR-33, miR-339), tau pathology (miR-132, miR-212, miR-922), and neuroinflammation (miR-146a, miR-206). Parkinson’s disease (DOID:14330): 12 curated hits were identified, related to  $\alpha$ -synuclein regulation (miR-16-1, miR-205, miR-214, miR-7), Nlrp3-mediated neuroinflammation (miR-30e), and neuroprotection (let-7d, miR-22, miR-221). Multiple sclerosis (DOID:2377): 8 curated hits were found, associated with myeloid inflammatory response and Th17 cell differentiation (miR-132, miR-155, miR-15b, miR-17, miR-223, miR-448). Huntington’s disease (DOID:12858): 7 curated hits were mapped, with miRNAs targeting the HTT gene (miR-125b, miR-146a, miR-150, miR-214) and regulation of mitochondrial morphology (miR-214/MFN2). Amyotrophic lateral sclerosis (DOID:332): 2 curated hits were identified, involving preservation of neuromuscular junctions (miR-206) and motor neuron survival (miR-125b). Conclusion: MicroRNAs play an essential role in gene regulation and emerge as promising tools in neurodegenerative diseases. Their stability and detectability in biological fluids make them relevant biomarkers for early diagnosis, monitoring disease progression, and defining prognosis. Furthermore, they represent potential therapeutic targets, opening perspectives for innovative strategies in personalized medicine. Continuous investment in research is indispensable to translate their potential into clinical advances for addressing diseases such as Alzheimer’s, Parkinson’s, Multiple sclerosis, Huntington’s disease, and Amyotrophic Lateral Sclerosis.

**Keywords:** MicroRNAs; Neurodegenerative diseases; Gene regulation; Molecular biomarkers; HMSS v4.0.

## **Abstract 134 | Poster | Poster Session Day 2**

### **Mesenchymal stem/stromal cells genetically modified to overexpress interleukin-15 increase lymphoproliferation and inhibit tumor growth in an experimental model of subcutaneous melanoma**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Diego de Carvalho Carneiro

**Institution:** Laboratory of Tissue Engineering and Immunopharmacology, Oswaldo Cruz Foundation, Gonçalo Moniz Institute (Fiocruz-BA) — Salvador-BA

**Introduction:** Mesenchymal stem/stromal cells (MSCs) are promising candidates for cell-based therapies due to their immunomodulatory properties and intrinsic tumor-homing capacity. However, their effects on tumor progression remain controversial. Genetic engineering offers an opportunity to enhance the therapeutic potential of MSCs by enabling the localized delivery of immunostimulatory molecules. Interleukin-15 (IL-15) is a potent cytokine that promotes the activation and proliferation of T lymphocytes and natural killer (NK) cells, making it an attractive candidate for cancer immunotherapy. This study evaluated the antitumor potential of MSCs genetically modified to overexpress IL-15 in a murine model of melanoma. **Methods:** Bone marrow-derived MSCs from C57BL/6 mice were transduced with second-generation lentiviral vectors encoding mouse IL-15. Genetically modified MSCs (MSC-IL-15) were characterized by morphology, immunophenotyping, trilineage differentiation, RT-qPCR, and ELISA. The biological activity of secreted IL-15 was assessed using a splenocyte lymphoproliferation assay. For in vivo evaluation, C57BL/6 mice bearing subcutaneous B16F10 melanoma received intravenous injections of MSC-IL-15, wild-type MSCs (MSC-WT), or saline on days 3 and 7 after tumor inoculation. Tumor growth was monitored until day 15, and histopathological analyses were performed. **Results:** MSC-IL-15 retained the characteristic fibroblast-like morphology, mesenchymal immunophenotype, and multilineage differentiation capacity following genetic modification. RT-qPCR and ELISA confirmed robust IL-15 overexpression and sustained cytokine secretion. Conditioned medium from MSC-IL-15 significantly enhanced ConA-induced splenocyte proliferation in a concentration-dependent manner, demonstrating the biological activity of the secreted cytokine. In vivo, MSC-IL-15 treatment significantly reduced melanoma growth compared with MSC-WT and saline-treated animals, with differences becoming significant by day 15. Histological evaluation revealed extensive necrotic areas within tumors from MSC-IL-15-treated mice, supporting enhanced antitumor activity. **Conclusion:** Engineering MSCs to overexpress IL-15 preserved their phenotypic characteristics while substantially enhancing their immunostimulatory and antitumor properties. Localized delivery of IL-15 by MSCs promoted lymphocyte proliferation and significantly inhibited melanoma progression in vivo, supporting the use of genetically modified MSCs as targeted cellular platforms for cancer immunotherapy. These findings provide proof-of-concept for the development of MSC-based cytokine delivery strategies and warrant further investigation into their mechanisms of action and translational potential.

**Keywords:** Mesenchymal stromal cells, IL-15, immunotherapy, melanoma, genetic modification, tumor inhibition

## **Abstract 135 | Poster | Poster Session Day 2**

### **Standardized Production, Quality Control, and Safety Assessment of an ITS-Conditioned Mesenchymal Stromal Cell Secretome Ophthalmic Formulation for Canine Use**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** CARLA LUJAN PEREIRA VILLARROEL; Hilana dos Santos Sena Brunel; Mauricio Antonio Silva Peixer; Patricia Furtado Malard; Rinaldo Wellerson Pereira

**Institution:** Universidade Católica de Brasília — Brasília-DF

**Introduction:** Ocular surface diseases are a major cause of ocular pain and visual impairment, requiring a highly coordinated tissue repair process to restore corneal integrity and transparency. Although current therapies effectively control inflammation, infection, and ocular surface lubrication, their capacity to promote functional tissue regeneration remains limited. In this context, the mesenchymal stromal cell (MSC)-derived secretome has emerged as a promising cell-free therapeutic strategy with high regenerative potential. However, its clinical application still depends on the development of reproducible manufacturing processes, quality control criteria, and safety data. **Objective:** To develop an ophthalmic formulation, initially intended for use in dogs, based on the insulin-transferrin-selenium (ITS)-conditioned MSC secretome, including defining production conditions, establishing quality control criteria, and assessing product safety. **Methods:** Different inflammatory (TNF/IFN and TNF/IFN + ITS) and non-inflammatory (ITS and melatonin) culture conditions were initially compared using human adipose-derived MSCs to identify the most suitable strategy for secretome production, based on cell morphology, cytokine profile (IL-6, IL-10, TNF- $\alpha$ , and IFN- $\gamma$ ), total protein content, and process standardization potential. The selected protocol was subsequently used to sequentially produce the canine adipose-derived MSC secretome, which was concentrated by 100-kDa ultrafiltration and formulated with 0.2% sodium hyaluronate. A pooled secretome from multiple donors was prepared to minimize donor-to-donor variability. Quality control included total protein content, pH, and microbiological testing. Product safety was assessed using the Ames test, the in vitro micronucleus assay, and the bovine corneal opacity and permeability (BCOP) assay. **Results:** ITS conditioning for 48 h under serum-free conditions was selected as the production strategy because it provided the best cell morphology in culture and an immunomodulatory profile comparable to that induced by inflammatory licensing for the cytokines evaluated, while offering greater process reproducibility and standardization potential. This production strategy consistently generated concentrated secretome batches that met the predefined quality criteria for the formulation. Furthermore, the final product showed no evidence of mutagenicity, genotoxicity, or ocular irritation under the evaluated experimental conditions. **Conclusion:** The proposed process enabled the development of an MSC secretome-based ophthalmic formulation with reproducible quality and a favorable initial safety profile. These findings complement the previously demonstrated regenerative activity of the formulation and support further in vivo efficacy and dose-finding studies using dogs with naturally occurring ocular surface lesions and represent an advance in the development of standardized cell-free products for regenerative medicine.

**Keywords:** mesenchymal stromal cells; secretome; cell licensing; insulin-transferrin-selenium; cell-free therapy; veterinary medicine.

## **Abstract 137 | Poster | Poster Session Day 2**

### **ANTI-HER2 CAR-T CELLS EVALUATION IN AN OVARIAN TUMOR PRECLINICAL MODEL**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Emmanuel Arthur Albuquerque Aragão

**Institution:** Instituto Nacional de Câncer (INCA) — RJ

In CAR-T cell therapy, T lymphocytes are genetically modified to express a chimeric antigen receptor (CAR), enabling tumor recognition and activation of an antitumor immune response. This approach has transformed hematological cancer treatment, offering options for patients refractory to conventional therapies. However, its application in solid tumors remains limited, requiring further research. A major challenge in anti-HER2 CAR-T therapy—targeting an antigen present in various tumors—is selecting the optimal extracellular domain for antigen recognition. This study aimed to evaluate and compare the efficacy of producing two anti-HER2 CARs (4D5 and FRP5) in peripheral blood mononuclear cells (PBMCs), as well as their antitumor capacity in vitro and in vivo. PBMCs were electroporated using a 2:1 transposase-to-transposon ratio and cultured for 12 days. The resulting CAR-T cells were analyzed by flow cytometry and tested functionally. CAR expression (~25%) and a predominant central memory phenotype (CD45RO<sup>+</sup> CCR7<sup>+</sup>) were observed, with low exhaustion, especially in CD8<sup>+</sup> FRP5<sup>+</sup> cells. CAR-4D5 showed greater antitumor activity, inducing more SK-OV-3 and U2OS cell lysis and higher IL-2, TNF- $\alpha$ , and IFN- $\gamma$  production. In vivo, NSG mice with SK-OV-3 tumors received  $8.9 \times 10^6$  cells ( $1.3 \times 10^6$  CAR<sup>+</sup>). Tumor regression occurred in the CAR-4D5 group, but necrosis led to fatalities; no response was seen with CAR-FRP5. A second experiment tested different administration routes in early-stage tumors. Intraperitoneal injection showed the best outcome, with 4/5 mice tumor-free after one year and no toxicity, while other routes caused side effects and reduced survival. Both CARs showed central memory phenotypes and low inhibitory receptor expression. CAR-4D5 was more effective, especially via intraperitoneal delivery.

**Keywords:** Immunotherapy, CAR-T, HER2, Ovarian tumor

**Abstract 138 | Poster | Poster Session Day 2**

**GENERATION OF VIRUS SPECIFIC T CELLS EXPRESSING A CD19 CAR (VST.CD19 CAR-T) FOR TREATMENT OF VIRAL INFECTIONS AND B CELL NEOPLASIA RELAPSE POST HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** MARIANA TORRES MAZZI

**Institution:** INCA — RIO DE JANEIRO-RJ

Viral infections and disease relapse post allogeneic hematopoietic stem cells transplantation (HSCT) remain a significant burden, accounting for the majority of deaths after the procedure. Antiviral drug options are limited and highly toxic. Adoptive T cell therapy to reconstitute T cell immunity has proved safe and effective in HSCT recipients, but post-transplant relapse carries a dim prognosis. Patients with relapsed /refractory B cell malignancies can be rescued by immunotherapy with T-cells genetically modified with chimeric antigen receptors (CARs) for CD19 (CD19.CARTs). However, CART therapy access is limited in developing countries, due to high costs and technology availability. Integration of virus specific T cell (VST) and CAR-T platforms would address two of the most relevant problems after allogeneic HSCT and by designing an inexpensive manufacturing protocol, we planned to reduce cost. We used a non-viral platform (Sleeping Beauty - SB transposon system) to generate VST.CD19 CAR-T cells which preserve the functionality of both receptors, TCR and CAR, and direct cytotoxic activity against tumor cells and virus-infected cells. The protocol consists of magnetic selection of the memory fraction of T cells (CD45RA negative) from peripheral blood mononuclear cells (PBMCs) from healthy donors; activation with anti-CD3/CD28 for 48 hours followed by electroporation of isomolar fractions of minicircles, the first containing the gene of interest (CAR anti-CD19) and the second containing the transposase (SB). Minicircles are minimal expression cassettes generated by removing bacterial genes from parental plasmids, thereby significantly reducing cellular toxicity. Following electroporation, cells are activated with irradiated autologous PBMCs pulsed with viral pepmixes (CMV, EBV, BKV, and ADV). On day 11, the percentage of cells expressing CAR ranged from 12-47% (n = 7 donors). Viability remained above 90%, with cells expanded 6 to 10-fold. IFN- $\gamma$  ELISPOT analysis showed preservation of viral specificity, especially for CMV and EBV. The immunophenotype profile consists mostly of effector T cells (Teff and Temra), with low expression of exhaustion markers. In vitro lysis assays against CD19+ cell lines showed cytotoxic activity at effector:target ratios up to 1:2. VST.CD19 CAR-T single cell RNA sequencing analysis is under process. The results confirm the proposed protocol allows for generation of VST.CD19 CAR-T cells using a non-viral platform with a transposon system, and minicircles as DNA vectors. This platform is GMP-compliant and compatible with the manufacture of clinical products. VST.CD19 CAR-T cells have the potential to address two of the main complications of hematopoietic stem cell transplantation (HSCT), and the cost reduction may increase patient access to new technologies within the Brazilian public health system (SUS).

**Keywords:** VST, VST.CD19 CAR-T cells, CAR-T cells, HSCT, viral reactivation

## Abstract 139 | Poster | Poster Session Day 2

### Cost-effectiveness of the Development of a Master Cell Bank of Perinatal Tissue-Derived Mesenchymal Stromal Cells under Good Laboratory Practice Conditions

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** Petrus de Paula Ribeiro; Helga Caputo Nunes Holzhausen; Mônica Alves

**Institution:** Universidade Estadual de Campinas, Faculdade de Ciências Médicas, Laboratório de Pesquisa em Doenças de Superfície Ocular. — Campinas-SP

Perinatal tissue-derived mesenchymal stromal cells (MSCs) are among the most promising cell sources for advanced therapies and regenerative medicine because of their high proliferative capacity, immunomodulatory properties, and ethically accessible procurement<sup>1,2,3</sup>. Establishing a Master Cell Bank (MCB) under Good Laboratory Practice (GLP) conditions is essential to ensure traceability, reproducibility, safety, quality, and scalability for translational research and future clinical applications. However, cost analyses of MSC banks remain scarce. This study evaluated the cost-effectiveness of establishing a perinatal tissue-derived MSC MCB by mapping all direct and indirect production costs. An Activity-Based Costing (ABC) model covered tissue collection, transport, cell isolation, ex vivo expansion, cryopreservation, quality control, and batch release, including infrastructure, specialized labor, quality management, and testing for identity, viability, sterility, stability, cell concentration, and biological potency. Assuming  $5 \times 10^6$  initial cells, four passages with a fourfold expansion per passage, and a 20% batch failure rate<sup>4</sup>, the model estimated 205 effective aliquots, corresponding to  $1 \times 10^9$  banked cells. Total batch cost was estimated at BRL 141,000.00, corresponding to BRL 687.39 per aliquot and BRL 137.48 per million cells. GLP infrastructure and quality control represented the largest cost components, accounting for 48% (BRL 67,700.00) and 20.3% (BRL 28,638.00) of total costs, respectively. Indirect costs represented 81% of total investment, whereas cellular inputs and labor accounted for 18.6% (BRL 26,178.00). Sensitivity analysis identified cell expansion efficiency as the main determinant of unit cost. At a 20% batch failure rate, cost per aliquot ranged from BRL 2,175.00 (3.0-fold expansion) to BRL 282.00 (5.0-fold expansion), while the baseline scenario (4.0-fold) resulted in BRL 688.00 per aliquot. These findings indicate that the initial investment in an MCB is offset by generating a standardized, qualified, and scalable cell stock that reduces batch variability and ensures a continuous supply of biological material for research and advanced therapy medicinal product development. Establishing perinatal MSC Master Cell Banks under GMP-compliant conditions is therefore a cost-effective strategy to support regenerative medicine, accelerate clinical translation, and strengthen the national infrastructure for advanced cell therapies.

**Keywords:** mesenchymal stromal cells; Master Cell Bank; GLP; advanced therapies; cost-effectiveness; cost analysis; quality control.

## Abstract 140 | Poster | Poster Session Day 2

### RNA interference targeting CSF1R delivered by *Pichia pastoris*: A strategy for macrophage modulation

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** KARINA MAYUMI TANI BEZERRA DE MELO; ANNA JÉSSICA DUARTE SILVA; ANTONIO CARLOS DE FREITAS

**Institution:** Universidade Federal de Pernambuco — Recife, PE

Tumor microenvironment (TME) is a heterogeneous environment that plays an important role in tumor development [1]. It consists of cellular and extracellular components that interact with one another promoting several carcinogenic processes [1]. Modulating the TME components is an interesting approach for inducing antitumor responses. Among these components, tumor associated macrophages (TAMs), specifically M2 phenotype, are promising targets [2]. This class of macrophage exhibits a pro-tumoral profile, promoting carcinogenesis. A receptor responsible for the polarization and maintenance of M2 macrophage is the colony-stimulating factor 1 receptor (CSF1R) [3]. Blocking this signaling pathway shows potential for reducing M2 macrophage and reversing the TME profile, this blockage also represents a strategy for cancers with high CSF1R expression, such as cervical cancer [4,5,6]. One way to modulate the expression of CSF1R is through RNA interference (RNAi). However, one of the challenges in establishing immunomodulatory approaches is ensuring specific delivery to the cells [7,8]. Yeast has proven to be a promising carrier due to their interaction with macrophage and dendritic cell receptors, emerging as potential vectors for delivering DNA, mRNA and RNAi [9,10]. Yeast also allows a better delivery by protecting the genetic material and enabling a higher delivery dose. Thus, this study proposes the development of a gene therapy product using *Pichia pastoris* as a delivery system for RNAi targeting the CSF1R to modulate the expression of M2 macrophage, aiming the induction of an antitumor profile in macrophages associated with HPV positive tumors. To this end, a short hairpin RNA (shRNA) sequence was selected and inserted in the pLT-hU6-miR30 expression vector, targeting the *Mus musculus* CSF1R mRNA based on their potential to bind to the mRNA and not interfere with other genes. In addition, the vector pEGFP-mCSF1R was constructed to verify the modulation of CSF1R expression through the fluorescence of GFP (Green Fluorescent Protein) gene and flow cytometry. Flow cytometry analysis revealed the potential of the pLT-hU6-miR30-shRNA3 to reduce the expression of the mCSF1R gene, at a higher ratio, such as 3:1 ratio (pLT-hU6-miR30-shRNA3:pEGFP-mCSF1R). The pLT-hU6-miR30-shRNA3, also has an autonomously replicating sequence (ARS), which is important to deliver a higher dose of genetic material to the macrophages, increasing the therapeutic response. This study also involved the insertion of the vector into *P. pastoris*, allowing targeted delivery to macrophage. Further studies should be conducted to confirm the modulation of CSF1R expression and the macrophage profile using yeast as a delivery vector, as well as the antitumor potential of the resulting product.

**Keywords:** RNA interference. Gene delivery. Yeast.

## **Abstract 141 | Poster | Poster Session Day 2**

### **AAV-mediated miR-146a expression as a gene therapy strategy for neuroprotection and axonal regeneration in human iPSC-derived neurons**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Carolina Gomes Ferreira

**Institution:** Universidade Federal de Minas Gerais — Belo Horizonte - MG

Neurodegeneration is a characteristic process of neurodegenerative disorders affecting the central nervous system (CNS), including chronic neurodegenerative diseases and traumatic lesions. It is initially characterized by axonal degeneration, leading to the loss of neurological functions. For a neuron to be able to restore its functions, regeneration of damaged axons is necessary; however, neurons of the CNS of adult mammals have a limited capacity for regeneration. Regulating gene expression through microRNAs, using adeno-associated viral (AAV) vectors as delivery tools, is a promising alternative to promote CNS repair after injury. The aim of this work is to test the effect of AAV-mediated expression of miR-146a, an important regulator of neuroinflammation, in neuroprotection and axonal regeneration using human neurons derived from induced pluripotent stem cells (iPSC). First, we established a 2D culture model of human neurons and astrocytes derived from iPSC. For this, human neural stem cells (NSC) derived from iPSC were seeded in cell culture plates or microfluidic devices and cultured up to eight weeks. We evaluated the expression of PAX6, a neural stem cell marker, class III beta-tubulin (Tuj-1) and MAP2, to identify neurons, and GFAP, to stain astrocytes, by immunofluorescence. We found that PAX6 is expressed preferentially in early time points, while Tuj-1, MAP2 and GFAP staining increased from 2 weeks onwards. Moreover, NSC plated on microfluidic devices were able to differentiate into neurons, which extended their axons through microgrooves into the axonal compartment. To express miR-146a in human neurons, an AAV vector expressing the human sequence of miR-146a under the control of the human Synapsin promoter (hSyn) was produced (AAV.miR-146a), which also expresses the mCherry reporter gene under the control of a second hSyn promoter. As a control, we produced an AAV vector expressing only mCherry (AAV.CTRL). Subsequently, we tested the transduction efficiency of both AAV vectors and observed that over a period of four weeks the vectors were able to efficiently and specifically transduce neurons, which was evidenced by the expression of mCherry only in MAP2-positive neurons. We are currently evaluating the effects of AAV.miR-146a on neurite outgrowth, axonal regeneration and degeneration and synapse loss. Finally, in silico target prediction/validation analyses have also been performed to identify miR-146a target genes. Preliminary results show 77 validated and 112 predicted targets of miR-146a and functional enrichment analyses performed on the predicted targets, show a predominance of genes associated with biological processes and cellular components related to synapses. In conclusion, this study will allow a better understanding of the role of miR-146a in neurodegeneration and regeneration of human neurons and may provide new perspectives for the development of gene therapies for neurodegenerative disorders.

**Keywords:** stem cell, microRNA, neurodegeneration, gene therapy

## **Abstract 143 | Poster | Poster Session Day 2**

### **RANKL siRNA Gene Therapy Using Ionizable Hybrid Lipid-Polymeric Nanoparticles in Bone Metastasis Co-culture and In Vivo Prostate Cancer Models**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Breno Francisco Figueiredo Maia; Andréa Teixeira-Carvalho; Carolina Fontanella de Araújo; Eloy Ramos Seixas; Kelly Alves Bicalho; Marco Túllio Rodrigues Alves; Pedro Henrique Rodrigues; Pedro Luís Araújo Antônio; Pedro Pires Goulart Guimarães; Walison Nunes da Silva

**Institution:** Instituto René Rachou - Fiocruz Minas — Belo Horizonte - Minas Gerais

Bone metastasis represents one of the main complications of advanced prostate cancer and is associated with reduced survival and the development of skeletal related events, including pain, pathological fractures, nerve compression, and malignant hypercalcemia. In this context, RANKL overexpression plays a central role in osteoclast activation and disruption of the bone microenvironment. Although antiresorptive therapies, such as bisphosphonates and denosumab, are used in clinical practice to reduce these events, such approaches have limited impact on overall patient survival. Therefore, RNA interference based strategies have emerged as promising alternatives for the modulation of molecular targets involved in disease progression. The present study aimed to develop and evaluate ionizable lipid polymeric nanoparticles (LNPs) containing RANKL targeting siRNA to promote gene silencing in in vitro and in vivo prostate cancer models. The formulations were produced by microfluidic technology using ionizable lipid polymers (7C1) and C14PEG2K (PEG) and characterized regarding hydrodynamic diameter, polydispersity index (PDI), zeta potential, and apparent pKa. Gene silencing efficiency was evaluated by RT qPCR, while cytotoxicity was assessed using the alamarBlue assay and cellular uptake was investigated by fluorescence microscopy. Additionally, the profile of cytokines, chemokines, and soluble factors was analyzed by Luminex in SaOs 2 and PC 3 Luc2 cell lines and in a co culture system. Finally, the therapeutic potential of the selected formulation was investigated in a murine prostate adenocarcinoma model. The developed formulations exhibited suitable physicochemical characteristics for intracellular siRNA delivery, with an average diameter below 110 nm, low polydispersity, and near neutral zeta potential. Among the evaluated sequences, siRNA-RANKL1@LNPs demonstrated superior gene silencing performance, promoting reduced RANKL expression in SaOs 2 cells, PC-3 Luc2 cells, and the co-culture model. Cell viability assays demonstrated the absence of significant cytotoxicity at the tested concentrations. Cellular uptake studies revealed a progressive increase in nanoparticle internalization over the evaluated time points, confirming efficient nanoparticle delivery. Luminex analyses demonstrated significant changes in the production of cytokines, chemokines, and factors associated with angiogenesis and bone remodeling in the co culture system, highlighting the influence of tumor osteoblastic cell interactions on the modulation of the tumor microenvironment. In the murine model, siRNA-RANKL1@LNPs promoted efficient RANKL silencing, with the highest efficacy observed after 48 hours of treatment. Overall, these results demonstrate the potential of the developed LNPs as siRNA delivery platform for RANKL silencing, representing a promising strategy for the treatment of prostate cancer associated bone metastasis.

**Keywords:** Prostate cancer; bone metastasis; RANKL; Ionizable lipid polymeric nanoparticles; Gene therapy; RNA interference.

## **Abstract 144 | Poster | Poster Session Day 2**

### **Targeting Pathogenic Immunity in Acquired Aplastic Anemia with an Off-the-Shelf CAR-NK-92 Platform**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Maria Cláudia M. T. Cavallini

**Institution:** Faculdade de Medicina de Ribeirão Preto - FMRP/USP — Ribeirão Preto-SP

Immune aplastic anemia (AA) is a rare and life-threatening acquired bone marrow failure syndrome characterized by the immune-mediated destruction of hematopoietic stem and progenitor cells, leading to hypocellular bone marrow and consequent pancytopenia. Autoreactive cytotoxic lymphocytes and sustained inflammatory signaling play central roles in disease pathogenesis. Although immunosuppressive therapy and allogeneic hematopoietic stem cell transplantation have significantly improved clinical outcomes, treatment-related toxicity, relapse, refractoriness, clonal evolution, and donor availability remain important challenges. Novel strategies capable of controlling disease-driving immune cells more selectively than systemic immunosuppression are therefore needed. Here, we developed an engineered CAR-NK-92 platform directed against a disease-relevant immune antigen associated with the pathogenic immune response in AA. NK-92 cells constitute a clinically evaluated platform with a favorable safety profile and offer reproducible, donor-independent manufacturing, efficient genetic engineering, scalable expansion, and rapid off-the-shelf availability. Importantly, pre-infusion irradiation restricts their proliferation and persistence in vivo, providing a controllable therapeutic window that may reduce the risk of prolonged on-target, off-tissue toxicity, an especially relevant safety feature when targeting immune cells in a nonmalignant disorder. Based on this rationale, we developed a novel CAR-NK-92 platform directed against an immune target involved in the pathogenic activation of autoreactive T cells in AA and engineered to minimize fratricide. A lentiviral vector encoding the CAR construct was successfully generated and used to establish stable CAR-NK-92 cells following transduction and selection. Functional characterization demonstrated that engineered CAR-NK-92 cells maintained robust proliferative capacity compared with parental NK-92 cells under both IL-2-supplemented and IL-2-deprived culture conditions, confirming the functional activity of the construct. Furthermore, CAR-NK-92 cells exhibited significantly enhanced cytotoxicity against target cells compared with unmodified NK-92 cells, demonstrating the functional efficacy of the engineered platform. Collectively, these findings demonstrate the feasibility of this CAR-NK-92 platform and provide encouraging proof-of-concept for further investigation of its therapeutic potential, supporting its future application as a targeted cellular immunotherapy for immune aplastic anemia.

**Keywords:** CAR; CAR-NK-92; Immune Aplastic Anemia

## **Abstract 145 | Poster | Poster Session Day 2**

### **High-resolution analytical platform for quality control of critical attributes required for CAR-T therapy safety and efficacy**

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** BELKIS XIOMARA CLAVIJO SANCHEZ

**Institution:** INSTITUTO DISTRITAL DE CIENCIA BIOTECNOLOGIA E INNOVACIÓN EN SALUD (IDCBIS) — BOGOTÁ

The manufacturing and clinical translation of Advanced Therapy Medicinal Products (ATMPs) such as chimeric antigen receptor T-cells (anti-CD19 CAR-T), demand strict analytical control. To comply with international regulations (FDA, EMA, WHO and Pharmacopoeias) [1-3], the development of analytical specifications for biotechnological products requires systematic verification of Critical Quality Attributes (CQAs) of these products [4]. In line with the ICH framework [5], we aimed to implement a comprehensive, high-resolution, integrated platform intended to release of anti-CD19 CAR-T therapies with focus on identity, purity, potency, and biosafety of the final product for clinical administration [4]. Following the identification of CQA, each analytical method was standardized through detailed procedures defining reagents, equipment, controls, acceptance criteria, and data analysis. Validation was conducted according to ICH Q2(R2), including evaluation of specificity, accuracy, precision, linearity, range, robustness, and detection or quantitation limits [5]. As a result, identity and purity parameters comprised the evaluation of cell count, viability, and advanced immunophenotypic characterization (CD3+, CD4+, CD8+, and CAR CD19 receptor expression). Regarding biological safety, we executed automated sterility analysis, endotoxin detection via the kinetic-chromogenic LAL method, molecular screening of 12 *Mycoplasma* spp., testing for Replication-Competent Lentivirus (RCL), and confirmation of genomic stability by assessing the Vector Copy Number (VCN) using PCR. Finally, potency was validated by quantifying the in vitro cytotoxic effect against the target cell line Nalm-6 (CD19+). During the manufacturing and final formulation stages, this analytical panel demonstrated high precision and operational robustness. The ten (10) evaluated batches consistently met the established specifications for each CQA, evidencing the high biological efficacy and safety of the CAR-T product. Collectively, these findings support the technical and translational research capacity of IDCBIS in developing robust analytical methodologies that support and guarantee quality. All analytical procedures proved to be fit for purpose and capable of reliably supporting the batch release of CAR-T for clinical applications in the Colombian context.

**Keywords:** Advanced Therapy Medicinal Products (ATMPs), CAR-T therapy, Critical Quality Attributes (CQA), Analytical method validation.

## Abstract 147 | Poster | Poster Session Day 2

### Using RecombinaseA (RecA) along with the CRISPR-Cas9 system to enhance homologous recombination in Mucopolysaccharidosis I focusing on cardiovascular analysis

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Willian Carlos Corrêa Padilha; Angela Maria Vicente Tavares; Angélica Salatino de Oliveira; Esteban Alberto Gonzalez; GUILHERME BALDO; Nancy Carlini Vieira Martins; Roberto Giugliani; ROSELENA SILVESTRI SCHUH; Willian da Silva Carniel

**Institution:** Universidade Federal do Rio Grande do Sul (UFRGS) — Porto Alegre-RS

**Introduction:** Mucopolysaccharidosis (MPS) I is caused by deficiency on the IDUA gene, leading to low activity of  $\alpha$ -L-iduronidase, and subsequently accumulation of glycosaminoglycans (GAGs) across tissues. It has multisystemic manifestations, such as organomegaly, cognitive decline and heart impairment. Previously, we used the CRISPR/Cas9 gene editing system as a candidate treatment for MPS I, however the rate of homologous recombination (HDR) and gene correction was low and could be improved. **Aim:** Here, we are testing whether adding RecA (an Escherichia coli factor that improves HDR) to the CRISPR-Cas9 system increases HDR in mice, focusing on heart parameters. **Methods:** We produced a liposomal vector carrying plasmids with CRISPR-Cas9 system components (the Cas9 gene, a gRNA aiming at the ROSA26 locus, the IDUA cDNA donor sequence) with or without the RecA gene in vivo applying intranasally. Two groups of MPS I (IDUA KO) mice were treated for 30 days, starting when they were 30 days old. The first one (CRISPR, n = 8) received the CRISPR system without RecA, while the other one received the system plus RecA (CRISPR-RecA). They were compared to wild type (WT; n = 7) and MPSI untreated mice (n = 7). Ecocardiographies were performed when the animals were six months old, followed by euthanasia and tissue collection (including heart and blood). Also, GAGs levels were measured by tandem mass spectrometry. All data were analyzed by the non-parametrical Kruskal-Wallis test followed by Wilcoxon's U test for pair comparisons, and expressed by mean  $\pm$  sd. **Results:** The MPS I left ventricular diastolic diameter ( $0.32 \pm 0.04$  cm) was lower than the WT ( $0.41 \pm 0.07$  cm,  $p=0.02$ ) and the CRISPR ( $0.041 \pm 0.08$  cm;  $p=0.032$ ) groups. The estimated mass of the MPS I hearts ( $0.13 \pm 0.037$  cm) was lower than the WT ( $0.19 \pm 0.043$  cm;  $p=0.026$ ) and CRISPR ( $0.19 \pm 0.046$  cm;  $p=0.037$ ) ones, although the RecA group showed a tendency to significance ( $0.19 \pm 0.049$  cm;  $p=0.062$ ). The GAGs levels on RecA hearts ( $119.85 \pm 36.44$  nmol/mg protein) were lower than the MPS I ( $189 \pm 44.19$  nmol/mg protein;  $p=0.012$ ), but have not reached the WT ( $0.075 \pm 0.09$  nmol/mg protein;  $p>0.0001$ ) levels yet. The GAG serum levels on MPSI ( $8.72 \pm 6.72$  nmol/mg protein) were higher than both the RecA ( $1.5 \pm 0.58$  nmol/mg protein;  $p=0.0043$ ) and CRISPR ( $1.43 \pm 0.62$  nmol/mg protein;  $p=0.0101$ ) groups, although none of them reached the WT ( $0.00 \pm 0.00$  nmol/mg protein;  $p=0.0055$ ) levels. **Conclusions:** These preliminary results showed that both gene editing treatments may benefit affected mice, by reducing clinical heart impairment symptoms of MPS I. To confirm and validate, further analysis are underway, like evaluation of enzymatic activity in other tissues, quantification of HDR events and functional parameters.

**Keywords:** CRISPR/Cas9, RecA, liposomal vector, MPS I

## **Abstract 148 | Poster | Poster Session Day 2**

### **Immunophenotypic characterization of tumor-infiltrating lymphocytes before and after ex vivo expansion in triple-negative breast cancer**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Abril Serna López

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Adoptive tumor-infiltrating lymphocyte (TIL) therapy requires ex vivo expansion to generate sufficient cells for clinical application(1). While this manufacturing process enables the production of therapeutic TIL products, it may also reshape the cellular composition and immunophenotypic characteristics of the original tumor immune infiltrate (2). Understanding these changes is essential for interpreting downstream functional studies and establishing robust strategies for TIL product characterization. This study compares the immunophenotypic composition of freshly isolated tumor infiltrates with their corresponding expanded TIL products from patients with triple-negative breast cancer (TNBC). Fresh tumor specimens were obtained from five TNBC patients undergoing surgical resection. Tumor fragments were used to establish TIL cultures, while matched tumor tissue underwent enzymatic and mechanical dissociation to characterize the baseline tumor immune infiltrate (day 0). Expanded TIL products were generated from tumor fragments using IL-2, OKT3, and agonistic anti-4-1BB stimulation and harvested at the pre-rapid expansion (pre-REP) stage upon completion of the initial expansion phase. Fresh tumor digests and matched pre-REP TIL products were immunophenotyped by multiparameter flow cytometry to evaluate immune cell composition, T-cell differentiation, activation, and exhaustion. Ex vivo expansion successfully generated pre-REP TIL products from all five tumor samples. Compared with the original tumor immune infiltrate, expanded TIL products showed a marked enrichment of CD8+ T cells and a significant reduction in CD4+ T cells (both  $p=0.015$ ). Within CD8+ cells, expanded TIL products exhibited a pronounced decrease in CD103 expression ( $p=0.030$ ) together with a significant increase in CD39 expression ( $p=0.016$ ), whereas PD-1 expression, NK-cell frequencies, and the distribution of CD8+ memory subsets (effector memory [TEM] and terminal effector memory [TEMRA]) remained unchanged. These findings demonstrate that ex vivo expansion selectively remodels the immunophenotypic composition of the original tumor immune infiltrate, enriching CD8+ T cells while altering markers associated with tissue residency and antigen experience (3,4). These results underscore the impact of the manufacturing process on the final cellular product and support comprehensive immunophenotypic profiling as an important component of TIL product characterization for adoptive cell therapy (5,6).

**Keywords:** Tumor-infiltrating lymphocytes (TILs), Adoptive cell therapy, Ex vivo expansion, Triple-negative breast cancer, Immunophenotyping, T-cell exhaustion

## **Abstract 149 | Poster | Poster Session Day 2**

### **Molecular characterization of mesenchymal stromal cells from the ocular limbal niche**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** João Pedro Arantes Aun Migueis

**Institution:** UNICAMP — Campinas-SP

Ocular surface diseases, especially in the most severe cases, require new lines of treatment. The use of limbal mesenchymal stromal cells (LMSC) in a cell-based therapy proposes a new therapy tool in development, as this cell lineage is essential and naturally participates in corneal epithelial wound healing. Thus, this project aimed to standardize the isolation and characterization process of LMSCs under quality control, considering future clinical applications, especially in the national context. Six samples of corneoscleral rings were obtained in partnership with the Biobank of the Brazilian Biosciences National Laboratory (LNBio/CNPEM, Campinas, SP, Brazil - CAAE: 82902024.3.0000.5404). Cells are being characterized by morphological analyses, Population Doubling Time (PDT), potential to tri-lineage differentiation, immunophenotyping (flow cytometry), and gene expression pattern (Real Time-PCR). The corneoscleral tissues were first fragmented into small pieces and washed with PBS, then the fragments were incubated overnight with decontamination solutions, followed by overnight digestion with Collagenase I in DMEM at 37°C. The remaining tissue was dissociated, filtered, and centrifuged. The pellet of cells was resuspended, counted, and cultured. The obtained LMSCs population presented fibroblastic morphology, typical of mesenchymal stromal cells, and the average PDT was 37.16 hours. Furthermore, the results indicated that >90% of LMSCs population expressed CD90 and CD73, while <5% expressed CD34, CD45, HLA-DR, CD11b, CD19, and CD14. Meanwhile, the overall mean expression of CD105 was 34%, showing considerable inter-donor variability. Also, the cells were able to differentiate into osteogenic, adipogenic, and chondrogenic cells. Besides, the preliminary results through Real Time-PCR demonstrated that LMSC presented positive expressions to PTGDS, OCT4, TSPAN7, SOX2, NANOG, AQP3, and KRT14. However, samples from different patients showed variable relative gene expression among the analyzed targets, emphasizing the heterogeneity among donors, wise the necessity to establish quality control for different cell therapy products. To conclude, the study focused on the characterization of LMSCs in hopes to amplify the knowledge and comprehension of LMSCs while making its first steps for future clinical applications in the ophthalmological field of regenerative medicine.

**Keywords:** Mesenchymal Stromal Cells; Eye treatment; Characterization.

## Abstract 150 | Poster | Poster Session Day 2

### DEVELOPMENT AND RHEOLOGICAL CHARACTERIZATION OF A BIOINK FOR 3D BIOPRINTING WITH HUMAN MESENCHYMAL CELLS FOR REGENERATIVE WOUND DRESSINGS

**Category:** Tissue engineering, biomaterials and advanced in vitro models

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**Institution:** 1Laboratório de Pesquisa em Células-Tronco, Departamento de Histologia e Embriologia, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro (UERJ), 2 Grupo de Reologia, Departamento de Engenharia Mecânica, Pontifícia Univ — Rio de Janeiro - RJ

**Introduction:** Chronic wounds represent a public health issue, particularly in diabetes mellitus, generating high healthcare costs and impacting quality of life. In this scenario, bioprinted wound dressings containing mesenchymal stem cells (MSCs) and their secretory derivatives emerge as a promising strategy for regenerative medicine. However, the translational applicability of these platforms depends on the rheological properties of the bioinks, which influence extrudability, structural stability, and architectural maintenance after printing. **Objective:** Aim to develop and rheologically characterize gelatin- and alginate-based bioink formulations intended for 3D bioprinting of constructs containing MSCs. **Methodology:** Bioinks were prepared using 5% type A gelatin and 2% sodium alginate (both Merck) in sterile PBS according to the laboratory protocol. Two conditions were tested: (A) a fluid composed of gelatin, sodium alginate, and calcium chloride (CaCl<sub>2</sub>) as a crosslinking agent, analyzed at 37 °C (post-extrusion and crosslinking temperature, corresponding to the post-bioprinting cell culture stage); and (B) a fluid composed of gelatin and sodium alginate without crosslinking, analyzed at 24 °C (hydrogel extrusion temperature prior to CaCl<sub>2</sub> crosslinking). After preparation, the hydrogels were subjected to rheological testing using an HR-30 rheometer (Discovery Series, TA Instruments) at the Department of Mechanical Engineering of PUC-Rio. **Results:** The formulations exhibited pseudoplastic behavior, evidenced by progressive viscosity reduction with increasing shear rate, a desirable characteristic for extrusion-based processes. Oscillatory tests demonstrated viscoelastic contributions compatible with post-printing structural maintenance, with increased elastic moduli under specific experimental conditions. Formulation A, containing CaCl<sub>2</sub>, exhibited greater structural organization but also significant temporal variability and lower repeatability among assays, suggesting aging-dependent phenomena and microstructural reorganization. In contrast, formulation B demonstrated more stable and reproducible rheological behavior. Additionally, bioprinted constructs maintained viability-associated metabolic status comparable to conventional 2D cultures, according to our laboratory analyses, indicating that the rheological characterization agrees with the literature and MSC bioprinting protocols under experimental validation. The formulations show rheological properties compatible with 3D bioprinting, including shear-thinning behavior and partial structural recovery after shear stress. However, CaCl<sub>2</sub> significantly influenced bioink stability, indicating that balancing crosslinking, extrudability, and repeatability is critical for system standardization. **Conclusions:** Rheological characterization proved essential for defining parameters supporting the fabrication of bioprinted constructs for tissue engineering and the development of regenerative wound dressings.

**Keywords:** 3D bioprinting, rheology, bioink, mesenchymal stem cells, tissue engineering

## Abstract 151 | Poster | Poster Session Day 2

### Development and Preclinical Characterization of Humanized Bispecific CD19/CD20 Tandem CAR-T Cells to Overcome Antigen Escape in B-Cell Malignancies

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** IGOR CABRAL STUDART; ANDREA QUERIOZ MARANHÃO; DYANE ROCHA ARAGÃO; GILVAN PESSOA FURTADO; ISABEL GARCIA SOUSA; ISABELA VITÓRIA TAVARES WERNECK; JOÃO DUARTE DE PAULA COUTINHO MARQUES; LUIZA DE MACEDO ABDO; MARCELO DE MACEDO BRÍGIDO; MARIA EDUARDA DE OLIVEIRA MACHADO; MARTÍN BONAMINO; PAULO VITOR DIAS DE SOUZA

**Institution:** Universidade Federal do Ceará / Universidade de Brasília — Fortaleza-CE / Brasília-DF

Background: CAR-T cell therapy has transformed the treatment of B-cell malignancies; however, antigen escape caused by heterogeneous or lost target antigen expression remains a major limitation. Bispecific tandem CARs (TanCARs) may overcome this challenge by recognizing either of two target antigens through an "OR" gate logic. Additionally, humanized and harmonized single-chain variable fragments (scFvs) may reduce immunogenicity and aggregation propensity, potentially minimizing tonic signaling and improving CAR-T functionality. Methods: Four second-generation CD19/CD20 TanCARs combining murine and humanized/harmonized scFvs derived from FMC63 and Rituximab were rationally designed using in silico approaches. Human peripheral blood mononuclear cells from leukopaks were electroporated with PiggyBac transposon/transposase vectors ( $7 \times 10^6$  cells per condition) to generate TanCAR-T cells, while monospecific CAR-T cells served as controls. CAR expression was evaluated by flow cytometry in viable CD3<sup>+</sup> T cells, followed by comparative analyses of activation, memory and exhaustion profiles. Cytotoxicity was assessed by live-cell imaging (Incucyte) using GFP-expressing Nalm-6 cells with CD19<sup>+</sup>/CD20<sup>-</sup> or CD19<sup>+</sup>/CD20<sup>+</sup> phenotypes. CAR-positive effector frequencies were normalized across groups before co-culture. Additional CRISPR-engineered target cell variants (CD19<sup>-</sup>/CD20<sup>+</sup> and CD19<sup>-</sup>/CD20<sup>-</sup>) are being generated to complete the antigen escape model. In parallel, recombinant murine and harmonized bispecific scFvs are being characterized regarding antigen binding and aggregation propensity. Results: Primary human T cells successfully expressed all TanCAR constructs and supported downstream functional analyses. Initial cytotoxicity assays demonstrated efficient elimination of both CD19<sup>+</sup>/CD20<sup>-</sup> and CD19<sup>+</sup>/CD20<sup>+</sup> target cells, while memory and exhaustion profiles were comparable to those observed in monospecific CAR-T cells. Ongoing studies will incorporate all antigen combinations together with biochemical and biophysical characterization of the recombinant scFvs to investigate the relationship between reduced aggregation propensity, tonic signaling and CAR-T function. Conclusions: This work establishes a preclinical platform for evaluating humanized and harmonized CD19/CD20 TanCAR-T cells designed to overcome antigen escape. The study also explores whether rational scFv engineering can improve CAR-T performance while preserving a favorable functional phenotype.

**Keywords:** CAR-T cell therapy; Tandem CAR; Antigen escape; Humanized scFv; Tonic signaling; CD19/CD20.

## **Abstract 154 | Poster | Poster Session Day 2**

### **In Vitro Evaluation of the Wound Healing Potential of Human Umbilical Cord-Derived Mesenchymal Stromal Cells Genetically Modified to Overexpress LIF**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Jaqueline Leite Vieira

**Institution:** Instituto Gonçalo Moniz, Fundação Oswaldo Cruz — Salvador

In Vitro Evaluation of the Wound Healing Potential of Human Umbilical Cord-Derived Mesenchymal Stromal Cells Genetically Modified to Overexpress LIF Jaqueline Leite Vieira 1,2, Girlaine Café Santos<sup>1,2</sup>, Josiane Dantas Viana Barbosa<sup>1</sup>, Milena Botelho Pereira Soares<sup>1,2</sup> <sup>1</sup> Institute for Technology in Health (ITS), SENAI CIMATEC, Salvador, Bahia, Brazil. <sup>2</sup> Laboratory of Tissue Engineering and Immunopharmacology (LETI), Instituto Gonçalo Moniz (IGM), Fundação Oswaldo Cruz (Fiocruz Bahia), Salvador, Bahia, Brazil. Wounds remain a major clinical challenge, affecting millions of patients worldwide and placing a substantial burden on healthcare systems, particularly when associated with conditions such as diabetes, cardiovascular diseases, and advanced age. The complex process of tissue repair is not yet fully understood, and effective therapeutic options remain limited. In this context, advanced therapy medicinal products have emerged as promising alternatives, with mesenchymal stromal cells (MSCs) receiving considerable attention due to their regenerative and immunomodulatory properties. MSC-derived growth factors can enhance wound healing through multiple mechanisms, and among them, Leukemia Inhibitory Factor (LIF) has attracted increasing interest because of its reported therapeutic potential in tissue repair. Therefore, this study evaluated the wound-healing properties of human umbilical cord-derived MSCs in their native form and genetically modified to overexpress LIF using in vitro assays. H<sub>2</sub>O<sub>2</sub>-induced oxidative damage, scratch, and collagen deposition assays using L929 and the MSCs at a 1:1 and 5:1 ratio were performed to investigate whether these cells could represent a promising therapeutic strategy for wound healing. Indirect co-culture (transwell) of L929 fibroblasts with MSCs significantly attenuated H<sub>2</sub>O<sub>2</sub>-induced oxidative damage evaluated at 48, 72, and 96 h after exposure. Both WT and LIF MSCs exhibited a protective effect, with the highest concentration of modified cells showing the greatest recovery of cell viability. Indirect co-culture also significantly increased collagen deposition by L929 fibroblasts. Enhanced collagen deposition was observed in all LIF treatments and in the high dose of WT groups, whereas the lower WT group did not differ significantly from the control, suggesting that the LIF retained pro-regenerative activity at a lower cell concentration. In an in vitro scratch assay, LIF cells promoted significantly greater wound closure than WT cells at the lower cell concentration, while no significant difference was observed between WT and modified cells at the higher concentration. These findings indicate that genetically modified mesenchymal stromal cells enhance fibroblast regenerative responses and may represent a promising strategy for improving wound healing.

**Keywords:** Wound Healing; Mesenchymal Stromal Cells; LIF, Tissue regeneration

## **Abstract 155 | Poster | Poster Session Day 2**

### **GITRL coexpression mitigates exhaustion, enhances resistance to Treg-mediated immunosuppression, and boosts CAR-T cell cytotoxicity against glioblastoma spheroids and organotypic tumor slices**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** IZADORA PETER FURTADO

**Institution:** Center for Cell-based Therapy, Hemotherapy Center of Ribeirão Preto, University of São Paulo (USP), Brazil — Ribeirão Preto

The CAR-T cell therapies have revolutionized hematological malignancies treatment. However, their efficacy against solid tumors in early-phase trials showed modest responses. This is largely due to the heterogeneous and immunosuppressive tumor microenvironment (mediated predominantly by regulatory T cells), which impairs CAR-T cell trafficking, persistence and function. Furthermore, the lack of reliable and predictive preclinical platforms to elucidate CAR-T cell behavior within tumor tissues poses a significant obstacle for clinical translation. Notably, conventional two-dimensional assays fail to recapitulate the complexity of the tumor microenvironment, lacking essential immunosuppressive cell populations as well as the physiological gradients of oxygen and nutrients. Adopting more reliable and predictive preclinical platforms, and based on previous reports, we hypothesized that the glucocorticoid-induced TNFR-related protein ligand (GITRL), through engagement of its receptor GITR, could potentiate T cell activity in CAR-T cells and overcome Treg-mediated suppression in 3D glioblastoma cocultures containing regulatory T cells, as well in organotypic tumor slices. To this purpose, we generated by lentiviral transduction human anti-GD2 CAR-T cells coexpressing GITRL (CAR.GD2/GITRL) also CAR-T cells expressing the GD2-specific CAR alone (CAR.GD2). Firstly, to assess their in vitro functional activity we tested both CAR-T cells against T98G glioblastoma cells. By incucyte analysis, CAR.GD2/GITRL showed superior lysis capacity against tumor cells in all effector:target ratios (E:T). Phenotypic analysis of CAR-T cells recovered from cocultures revealed reduced expression of exhaustion markers, such as PD-1 and LAG-3, in CAR.GD2/GITRL cells. To reliably test our CAR-T cells, we established subcutaneous xenografts in NSG mice using luciferase-expressing T98G cells. Ex vivo 400µm agarose-embedded tumor slices were cocultured with CAR-T cells for 5 days. Both CAR-T demonstrated bioluminescence reduction and pro-inflammatory cytokine secretion. In order to reproduce the tumor microenvironment and predict the immunosuppressed effect, we generated iTregs by inducing the transcription factor Foxp3 superexpression in CD4+ T lymphocytes. These cells were evaluated in a multicellular system comprising tumor spheroids and CAR-T cells. Importantly, we confirmed that iTregs did not demonstrated cytotoxic effect against the spheroids at the same time that suppressed the CAR-T proliferation. Indeed, CAR.GD2/GITRL cells demonstrated increased capacity to diminished the spheroids compared to CAR.GD2, confirming the less susceptibility of the immunosuppression driven by iTregs. These findings indicate that GITRL coexpression increases CAR-T cell resistance to exhaustion and immunosuppressive signals. Furthermore, we validate tumor slice and spheroid coculture models with iTregs as highly predictive platforms to assess CAR-T efficacy in solid tumor microenvironments.

**Keywords:** CAR-T cells; glioblastoma; immunosuppression

## **Abstract 156 | Poster | Poster Session Day 2**

### **Lipid Nanoparticle-Based mRNA Delivery for Adenine Base Editing Therapy in Recessive Dystrophic Epidermolysis Bullosa.**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** ROBERTHA MARIANA RODRIGUES LEMES; Ricardo Weinlich

**Institution:** Hospital Israelita Albert Einstein — São Paulo-SP

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare and severe monogenic skin disorder caused by loss-of-function mutations in the COL7A1 gene, resulting in deficient type VII collagen (C7) expression. The lack of functional C7 compromises dermal-epidermal integrity, resulting in chronic wounds, recurrent blistering, severe pain, progressive fibrosis, and life-threatening complications. Although recently approved gene therapies have represented a major advance for RDEB treatment, their widespread clinical application remains limited by the need for repeated administration, dependence on viral vectors, and invasive delivery procedures. Therefore, safer, minimally invasive, and non-viral gene delivery strategies capable of achieving efficient and durable therapeutic outcomes are urgently needed. This study proposes a topical gene-editing platform based on lipid nanoparticles (LNPs) for the delivery of adenine base editor (ABE) mRNA targeting the COL7A1 c.5047C>T mutation, the predominant pathogenic variant in the Brazilian RDEB cohort. To establish and optimize the experimental workflow, enhanced green fluorescent protein (eGFP) mRNA was initially used as a reporter to optimize LNP composition and transfection efficiency in cell models. A library of 40 LNP formulations was generated by systematically varying ionizable lipids, sterols (cholesterol or  $\beta$ -sitosterol), helper lipids (DSPC or DOPE), and PEG-lipids (DMG-PEG or DSG-PEG), while maintaining a fixed molar ratio of 50:38.5:10:1.5. Each formulation, containing 200 ng of eGFP mRNA/well, was evaluated in HEK-293T and BJ-hTERT cells to identify candidates with high mRNA delivery and low cytotoxicity. Transfection efficiency, eGFP expression, and cell viability were quantified by flow cytometry. Formulations were characterized for particle size, polydispersity index, zeta potential, and mRNA encapsulation efficiency by DLS and electrophoresis. The screening identified three leading LNP formulations enriched in  $\beta$ -sitosterol, DOPE, and DMG-PEG. Of these, formulation '35' showed the best overall performance, exhibiting suitable physicochemical characteristics, high transfection efficiency, robust eGFP expression, and low cytotoxicity. Dose-response studies (12.5–200 ng mRNA/well) identified 50 ng/well as the optimal dose, while optimization of the nitrogen-to-phosphate (N/P) ratio demonstrated that an N/P ratio of 6 maximized transfection efficiency and protein expression without compromising cell viability. Collectively, these findings establish an optimized LNP formulation for efficient and safe mRNA delivery, providing a robust foundation for the encapsulation and topical administration of ABE mRNA targeting pathogenic COL7A1 mutations. This non-viral platform represents a promising strategy for developing next-generation gene-editing therapies for RDEB and may overcome key limitations of current viral-based approaches, improving the long-term treatment of this debilitating genetic disorder

**Keywords:** LNP, mRNA delivery, ABE e RDEB

## Abstract 157 | Poster | Poster Session Day 2

### Generation and expansion of CAR-NK cells using different transposon systems

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Paula Daniela Souza Alves; EMMANUEL ARTHUR ALBUQUERQUE ARAGAO; LUIZA DE MACEDO ABDO; MARTÍN BONAMINO

**Institution:** Instituto Nacional de Câncer — Rio de Janeiro-RJ

Autologous CAR-T cell therapy is highly effective against certain hematologic tumors but raises concerns regarding safety and cost. CAR-NK92 cells, which have been tested in clinical trials for CAR-based therapies, offer the advantage of allogeneic use and a lower risk of adverse effects. The NK-92mi clone is an IL-2- independent NK cell line that reduces costs associated with expansion and genetic modification, making it a practical off-the-shelf CAR carrier. In line with efforts to reduce costs related to viral vector scalability, this study tested two transposon/transposase-based systems — Sleeping Beauty (SB) and piggyBac (PB) — to generate anti-CD19 CAR-NK92mi cells. In the SB system, we used PT3, PT4, and an innovative minimized version of the PT4 backbone vector called a minicircle (PT4-MC). These transposons were combined with the SB100x transposase, while the PB system was used with the PBase transposase. To generate anti-CD19 CAR<sup>+</sup> cells,  $1 \times 10^6$  NK92mi cells were allocated to each condition (mock, PT3, PT4, PT4- MC, and PB) and electroporated using the 2b Nucleofector (Lonza) with the NK92 program. Subsequently, cell viability and anti-CD19 CAR expression were assessed by flow cytometry. As expected, NK92mi cells were highly sensitive to electroporation, and viability across all conditions was reduced to approximately 20% of the initial count. Moreover, while the other plasmids showed ineffective transfection, the PT4-MC condition achieved a stable 5% CAR<sup>+</sup> NK92mi cell frequency. Thus, the use of PT4-MC for transfection shows improved efficiency and transgene stability. NK cells possess innate immune mechanisms that hinder the transfection process; therefore, our results suggest that the reduced size of minicircle may offer an advantage in the genetic modification of NK cells. Enrichment assays for CAR-NK92mi cells are currently underway, along with in vitro and in vivo cytotoxicity tests against B-cell cancer lines and a xenograft mouse model, respectively.

**Keywords:** Natural Killer cells; non-viral gene delivery; chimeric antigen receptor; immunotherapy

## **Abstract 158 | Poster | Poster Session Day 2**

### **MMP-9 Gene Silencing Mediated by Ionizable Lipid-Polymeric Nanoparticles: A Strategy to Attenuate Tumor Burden and Metastasis in Triple-Negative Breast Cancer Models**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Pedro Henrique Rodrigues; Andrea Teixeira-Carvalho; Breno Francisco Figueiredo Maia; Carolina Fontanella-Araújo; Eloy Ramos Seixas; Kelly Alves Bicalho; Marco Túllio Rodrigues Alves; Pedro Luís Araújo Antônio; Pedro Pires Goulart Guimarães

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Breast cancer is the second most prevalent neoplasm and the fourth leading cause of cancer-related mortality worldwide. Among its subtypes, triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor, progesterone receptor, and HER2 expression, is distinguished by its aggressive behavior, high metastatic potential, limited therapeutic options, and poor prognosis. In this context, matrix metalloproteinase-9 (MMP-9) represents an important molecular target, as its overexpression within the tumor microenvironment is associated with extracellular matrix degradation, tissue remodeling, tumor invasion, and metastatic dissemination. Although small interfering RNA (siRNA) enables the specific silencing of genes involved in tumor progression, such as MMP-9, its therapeutic application is limited by low stability, bioavailability, and cellular uptake. Therefore, ionizable lipid-polymeric nanoparticles (LNPs) represent promising platforms for the intracellular delivery of siRNA, providing improved biocompatibility, protection of genetic material, and enhanced delivery efficiency of these nucleic acids. This study aims to evaluate the therapeutic effects of LNPs carrying MMP-9-targeting siRNA (siRNA-MMP-9@LNPs) in in vitro and in vivo models of TNBC. The LNPs will be formulated using microfluidic technology and characterized according to their physicochemical properties and encapsulation efficiency. In murine 4T1-Luc2 cells, LNP uptake, MMP-9 silencing, and the effects on cell viability, migration, and invasion will be evaluated. Metastatic TNBC models will be established in female BALB/c mice and monitored by bioluminescence imaging following treatment. Molecular, flow cytometric, and histopathological analyses will be performed to assess MMP-9 expression and the therapeutic impact on tumor progression and metastasis in vivo. The LNPs exhibited an approximate hydrodynamic diameter (HD) of 115 nm, a polydispersity index (PdI) ranging from 0.08 to 0.12, and a zeta potential ( $\zeta$ ) ranging from  $-13.0$  to  $-15.0$  mV, indicating favorable characteristics for this intracellular siRNA delivery. Following treatment of 4T1-Luc2 cells with siRNA-MMP-9@LNPs at concentrations of 10, 50, and 100 nM for 12 or 24 hours, greater gene silencing was observed at 50 and 100 nM, with an approximate reduction of 70% after 12 hours and 80% after 24 hours. None of the concentrations or exposure times evaluated resulted in cytotoxicity. The evaluation of additional LNP concentrations and the standardization of the in vivo models are currently underway. The developed platform is expected to promote MMP-9 silencing and reduce tumor progression and metastasis in TNBC, highlighting its potential as an RNA interference-based therapeutic strategy.

**Keywords:** Triple-negative breast cancer; Ionizable lipid-polymeric nanoparticles; siRNA; MMP-9;

## **Abstract 159 | Poster | Poster Session Day 2**

### **LIMB-GIRDLE MUSCULAR DYSTROPHY SUBTYPE 2G/R7 MODELS EXHIBIT INCREASED CELL DEATH, IMPAIRED MUSCLE MATURATION, AND OXIDATIVE STRESS**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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**Background:** Limb-girdle muscular dystrophy R7 (LGMD-R7, formerly LGMD2G) is a rare autosomal recessive neuromuscular disorder caused by biallelic pathogenic variants in the TCAP gene, which encodes telethonin. Telethonin is a structural sarcomeric protein that maintains Z-disc integrity by anchoring titin to the contractile apparatus. In addition to its structural role, accumulating evidence indicates that telethonin regulates intracellular pathways involved in myogenic differentiation, mechanotransduction, apoptosis, and cell survival. However, the molecular mechanisms driving muscle degeneration in LGMD-R7 remain poorly understood due to the absence of representative disease models. **Objective:** To generate TCAP knockout cellular models using CRISPR-Cas9 and investigate the effects of telethonin deficiency on skeletal muscle differentiation, cell survival, and redox homeostasis. **Methods:** Pathogenic variants were introduced into exon 2 of the TCAP gene in C2C12 myoblasts using CRISPR-Cas9. Myogenic differentiation was monitored by light microscopy and Western blot analysis of fast and slow myosin heavy chain (MHC) isoforms. Apoptosis, necrosis, and viability were quantified by flow cytometry, while oxidative stress was evaluated by antioxidant enzyme activity. **Results:** CRISPR-Cas9 generated two TCAP-deficient cell lines (T4 and T11). Both clones exhibited impaired myotube formation and reduced fast and slow MHC expression. Flow cytometry demonstrated increased apoptosis and necrosis in both clones, whereas reduced viability was observed only in T4. Both models also displayed altered antioxidant enzyme activity, indicating disrupted redox homeostasis. **Conclusions:** We established two TCAP knockout cellular models that reproduce key pathological features of LGMD-R7. Telethonin deficiency impaired myogenic differentiation, increased cell death, and disrupted redox balance, providing valuable platforms for mechanistic studies and preclinical therapeutic development.

**Keywords:** Limb-girdle muscular dystrophy; LGMD2G/R7; CRISPR; Cell differentiation; Cell death.

## **Abstract 160 | Poster | Poster Session Day 2**

### **Functional Validation of a Chicken-Derived Anti-CLDN18.2 IgY Antibody as a Translational Tool for Gastric Cancer**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Thaíssa Vitória Portal Rodrigues; Amanda Caroline Linhares Rodrigues; André Salim Khayat; Fernando Barbosa Tavares; Jaqueline Diniz Pinho; Livia Erika Carlos Marques; Paulo Pimentel de Assumpção; Rommel Rodríguez Burbano; Samir Mansour Moraes Casseb

**Institution:** Oncology Research Center, Hospital University João of Barros Barreto, Belém, Pará-Brazil; Instituto de Saúde e Produção Animal, Universidade Federal Rural da Amazônia, Belém, Pará, Brazil; Molecular Biology Laboratory, Ophir Loyola Hospital, Belém, Pará, — Belém-PA

**Introduction:** Gastric cancer is among the malignancies with the highest mortality rates, especially in advanced stages. Loss of epithelial polarity, associated with altered tight junction proteins such as claudins, plays a role in tumor progression. Claudin 18.2 (CLDN18.2) has attracted interest because of its expression in gastric mucosa and presence in tumors, with low expression in most normal tissues, making it a promising target. **Objective:** To obtain, characterize, and validate a CLDN18.2-specific IgY antibody produced by immunization with the PCNI peptide, evaluating its affinity and expression in gastric adenocarcinoma cell lines for translational applications. **Materials and Methods:** Adult White Leghorn hens were immunized with the PCNI peptide, corresponding to the extracellular loop of CLDN18.2, combined with Montanide adjuvant, in three doses on days 0, 14, and 21 (CEUA Protocol No. 1326191222). Eggs were collected before and after immunization. IgY antibodies were extracted from yolks, purified using Amicon® filters, and quantified. Protein integrity was assessed by SDS-PAGE, and immune response by Dot Blot, Western Blot, and ELISA. In ELISA, anti-CLDN18.2 IgY affinity was compared with commercial polyclonal anti-CLDN18 as positive control. Specificity was assessed by immunofluorescence in MKN45, ACP03, and AGP01 cell lines. Statistical analyses used ANOVA followed by Tukey's post hoc test, considering  $p \leq 0.05$ . **Results:** Purification with Amicon® filters resulted in recovery greater than 60%, preserving antibody integrity, as confirmed by SDS-PAGE, with bands corresponding to light (~27 kDa) and heavy (~67 kDa) chains. Dot Blot demonstrated an increase in immune response, whereas Western Blot confirmed IgY specificity and integrity. ELISA indicated strong affinity of anti-CLDN18.2 IgY for the PCNI peptide, with statistically significant differences at 1395 µg/mL and 2739 µg/mL compared with the positive control ( $R = 0.98$ ;  $p < 0.0001$ ). Immunofluorescence confirmed antibody expression and specificity in the three cell lines, with selective staining in MKN45, ACP03, and AGP01 and no signal in negative controls. Compared with commercial anti-CLDN18 antibody, the produced IgY showed equivalent reactivity, validating functionality in metastatic and primary gastric cancer cells. **Conclusion:** To the best of our knowledge, this may be the first chicken-derived IgY antibody directed against CLDN18.2. The antibody showed high specificity and reactivity equivalent to commercial anti-CLDN18, highlighting its potential as an immunological tool for diagnostic, experimental, and translational applications in gastric cancer. These findings may also support the detection and validation of CLDN18.2 expression in studies involving target-directed immunotherapies and engineered cellular therapies.

**Keywords:** Gastric Cancer, Claudin 18.2, IgY Antibody

## **Abstract 161 | Poster | Poster Session Day 2**

### **Multicompartment Immune Architecture Distinguishes Responders from Non-Responders Following Anti-CD19 CAR-T Cell Therapy**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Ana Luiza Sionti Pacheco

**Institution:** A. C. Camargo — São Paulo - SP

**Introduction:** Anti-CD19 CAR-T cell therapy has transformed the treatment of relapsed and refractory B-cell malignancies, yet clinical responses remain heterogeneous. Increasing evidence suggests that therapeutic efficacy depends on coordinated immune remodeling rather than CAR-T persistence alone. Interactions among CAR-T cells, endogenous T cells, NK cells, B cells, and myeloid populations may generate immune signatures associated with treatment outcome. **Objective:** To identify multicompartment immune signatures associated with response after anti-CD19 CAR-T cell therapy. **Methods:** Peripheral blood samples (CAAE: 65750722.3.0000.5432) were prospectively collected before and after CAR-T infusion. Patients were stratified according to clinical response. Multiparametric flow cytometry included CD45, CD3, CD4, CD8, anti-CD19 CAR, CD56, CD16, CD69, CD62L, CD45RO, TIGIT, CD19, CD14, CD25, CD127, and HLA-DR. Immune profiling integrated CAR-positive and endogenous T cells, NK cells, B cells, and monocytes to evaluate differentiation, activation, inhibitory receptor expression, antigen presentation, and humoral compartments. **Results:** Responders and non-responders displayed distinct baseline immune profiles characterized by differences in T-cell differentiation, activation/exhaustion, NK phenotypes, monocyte HLA-DR expression, and lymphocyte composition. After infusion, immune remodeling remained heterogeneous. Responders preserved balanced CD4/CD8 homeostasis with coordinated remodeling of CAR-positive and endogenous T-cell compartments, accompanied by dynamic changes in memory differentiation and inhibitory receptor expression. In contrast, non-responders developed multicompartment immune dysregulation involving adaptive, innate, and myeloid compartments. No single immune subset distinguished clinical outcome; instead, integrated multicompartment immune signatures differentiated responders from non-responders. **Discussion:** Rather than isolated immune populations, our findings support a systems immunology model in which clinical response is associated with coordinated immune remodeling. Integrated changes involving CAR-positive T cells, endogenous T cells, NK cells, B-cell aplasia, and monocyte activation indicate global immune network reorganization. Preserved immune architecture despite variable CAR-T expansion suggests that treatment efficacy depends on coordinated adaptive, innate, humoral, and myeloid responses. Although exploratory because of the limited cohort size, these findings support further investigation of multicompartment immune signatures as biomarkers of CAR-T response. **Conclusion:** Clinical response after anti-CD19 CAR-T therapy appears to depend on coordinated multicompartment immune remodeling rather than isolated immune alterations. Systems-level immune signatures may provide a framework for biomarker development and response monitoring following CAR-T therapy.

**Keywords:** CAR-T cells; therapeutic response; flow cytometry; immune biomarkers.

## **Abstract 162 | Poster | Poster Session Day 2**

### **CAR T-Cell Therapy for Triple-Negative Breast Cancer: Targeting carbonic anhydrase IX**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** RENATA SCHMIEDER PIVETTA; ELOAH RABELLO SUAREZ

**Institution:** UNIFESP — SÃO PAULO- SP

**Introduction.** Triple-negative breast cancer (TNBC) is a highly aggressive malignancy that remains resistant to conventional therapies. Although chimeric antigen receptor (CAR) T-cell therapy has transformed the treatment landscape for hematologic malignancies, its clinical efficacy in solid tumors, such as TNBC, is currently limited. TNBC frequently expresses both carbonic anhydrase IX (CAIX) and programmed death-ligand 1 (PD-L1), presenting themselves as two promising targets for CAR-based therapeutic strategies, but are still many barriers to overcome. Challenges restricting the efficacy of CAR T cells against solid tumors includes the antigenic heterogeneity of the tumor cells and the frequent exhaustion of T cells driven by the immunosuppressive tumor microenvironment. Furthermore, the intrinsic migratory profile of T cells severely limits their ability to effectively infiltrate and circulate within the highly dense tumor stroma. **Objectives.** Evaluate in vitro the antitumor effects of different CAR T cells targeting CAIX capable of releasing anti-PD-L1 antibodies, or capable of secretion of cell migration modulators (CMM) in the tumor microenvironment. These CAR cells were constructed with either CD28 or 4-1BB as costimulatory domains. **Methods.** The lentiviruses were generated via transient transfection of GAG-Pol, VSVG, Rev, and CAR-coding plasmids. Viruses were concentrated, titrated, and transduced into T cells purified from healthy donor blood mononuclear fractions. The resulting CAR cells were assessed for transduction and viability levels. These CAR cells were co-cultured with Hs578T cells to evaluate their cytotoxicity potential. **Results.** We observed persistent CAR transduction in T cells and cytotoxicity of 80% against TNBC cells in vitro with CAIX-targeted CAR-T cells capable of releasing anti-PD-L1 antibodies, compared to the control group. Also, previous results showed Anti-CAIX CAR T cells secreting anti-PD-L1 IgG improved the overall survival of NSG mice in about 20 days compared to the control group in a TNBC orthotopic model. Anti-CAIX/MMC CAR-T cells cytotoxicity against Hs578T in vitro are still in evaluation. **Conclusions.** The anti-CAIX CAR T cell therapy inducing immune checkpoint blockade by anti-PD-L1 secretion is an effective approach for Hs578T TNBC cells and should be tested associated to the Anti-CAIX/MMC CAR-T cells and also in other TNBC models. CAAE; 71055617.3.0000.5594/UFABC; CTNBio/UFABC 9560/2025; CEUA CIPE A.C. Camargo Cancer Center 088/21; FAPESP n. 2023/03631-3, n.2025/19984-8; CAPES 001.

**Keywords:** CAIX, PD-L1, TNBC.

## **Abstract 163 | Poster | Poster Session Day 2**

### **Ex vivo CAR-T cell expansion with microbial metabolites to generate a metabolically fit cellular product with enhanced cytotoxic potential for treating GD2 tumors**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Karolina Santos Esteves

**Institution:** Ribeirão Preto Blood Center, Ribeirão Preto Medical School, University of São Paulo — Ribeirão Preto-SP

The therapy with T lymphocytes modified to express a chimeric antigen receptor (CAR-T) for tumor treatment, despite great success against hematological neoplasms, still faces some challenges that limit its efficacy, especially in solid tumors. In this context, the search for generating a cellular product enriched in memory cells, a phenotype widely correlated with better clinical outcomes, emerges as an attractive strategy. Within the exploited alternatives, there is CAR-T cells' expansion media modification, aiming to reduce terminal differentiation and enhance T cell metabolic fitness, such as using microbiota metabolites associated with better antitumoral responses in the culture medium. Inosine is a nucleoside that can replace glucose as a carbon source for T lymphocytes. This molecule promotes a wide epigenetic and metabolic reprogramming in CAR-T cells, favoring stemness retention and improving their antitumoral activity in pre-clinical models. On the other hand, butyrate is a short-chain fatty acid produced by commensal gut microbiota, and its circulating levels are positively correlated with better CAR-T cell therapy clinical outcomes. This molecule promotes a metabolic rewiring in T cells, favoring oxidative phosphorylation, as well as inducing stem-like phenotypes and reducing exhaustion. In recent clinical data, higher levels of circulating butyrate were associated with better response to CAR-T treatment. Thus, this project aims to evaluate the combined effect of inosine and butyrate in generating a CAR-T cell product enriched in memory cells with enhanced metabolic fitness and cytotoxic potential towards GD2+ tumors. To this end, T lymphocytes from healthy donors were transduced with a lentiviral vector for CAR expression, and expanded for a total of 10 days, under interleukins 7 and 15 stimuli, in inosine or glucose containing-media, with or without butyrate supplementation. To determine optimal butyrate supplementation, 3 concentrations (0.5, 1, and 1.5 mM) and 3 time periods (24 h, 48 h, and 72 h) in T cells during the last expansion days were evaluated. Those cells were analyzed for viability via annexin-V and 7-AAD staining, showing that higher concentrations of butyrate were associated with reduced T lymphocyte viability. Furthermore, when CAR-T cells were treated with butyrate at 0.5 mM, a tendency towards an increase in CD62L+ cell frequency was observed, with a higher frequency in the central memory (CD45RA-CD62L+) subpopulation, and more pronounced effects noted in the combined inosine and butyrate treatment. Future steps will focus on determining the optimal time period for butyrate conditioning, based on memory T-cell phenotypes, cytotoxic activity in coculture assays, and the final product metabolic phenotype. Next, the in vivo antitumoral action of the combined inosine and butyrate pre-treatment of CAR-T cells will also be evaluated.

**Keywords:** CAR-T cells; inosine; butyrate; metabolic fitness

## **Abstract 164 | Poster | Poster Session Day 2**

### **Heterogeneous Myeloid Remodeling and Cytokine Profiles During Cytokine Release Syndrome Following Anti-CD19 CAR-T Cell Therapy**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Mariana Soares Gomes

**Institution:** A.C.CAMARGO CANCER CENTER — São Paulo-SP

**Background:** Cytokine release syndrome (CRS) is the most common immune-mediated toxicity associated with anti-CD19 chimeric antigen receptor (CAR) T-cell therapy and a major cause of early morbidity. Although cytokine overproduction is its hallmark, monocytes increasingly recognized as central mediators of systemic inflammation. Whether patients with CRS reflects a shared inflammatory program or comprises distinct myeloid and cytokine endotypes remains unclear. **Objective:** To characterize circulating myeloid remodeling and systemic cytokine signatures associated with CRS following anti-CD19 CAR-T cell therapy and to identify distinct inflammatory endotypes through integrated immunophenotypic and soluble biomarker profiling. **Methods:** Patients receiving anti-CD19 CAR-T cells were prospectively enrolled in an ethics committee-approved protocol (CAAE: 65750722.3.0000.5432). Peripheral blood mononuclear cells (PBMCs) were analyzed by multiparameter flow cytometry. Monocyte subpopulations were defined based on CD14 and HLA-DR expression, alongside additional lineage markers. Serum concentrations of IL-6, IL-10, IL-8, TNF, IL-1 $\beta$ , and IL-12p70 were quantified using cytometric bead array (CBA). Longitudinal analyses integrated myeloid immunophenotypes with circulating cytokine profiles. **Results:** Patients with CRS and clinical response showed expansion of circulating CD14<sup>+</sup> monocytes following infusion (16.50% to 21.72%), whereas patients without CRS showed a reduction in the monocytic compartment (43.00% to 22.95%). HLA-DR expression declined in both groups, but remained relatively preserved in CRS patients (83.55% to 70.25%) compared with non-CRS patients (92.95% to 61.30%). Cytokine responses were highly heterogeneous, with IL-6 ranging from 9.21 to 2,861.49 pg/mL and IL-10 from 1.98 to 4,257.46 pg/mL. Distinct cytokine signatures emerged, including concurrent IL-6/IL-10 elevation and IL-8/TNF-predominant profiles, whereas IL-1 $\beta$ /IL-12p70 remained low in most samples. **Discussion:** Expansion of CD14<sup>+</sup> monocytes and the relative preservation of HLA-DR supports coordinated myeloid remodeling rather than generalized monocyte deactivation following CAR-T infusion. The heterogeneous cytokine patterns further indicate that CRS comprises biologically distinct inflammatory endotypes rather than a uniform cytokine response. Concurrent IL-6 and IL-10 elevation may reflect simultaneous inflammatory and regulatory immune activation, whereas IL-8/TNF-predominant profiles may indicate enhanced myeloid recruitment and activation. These findings support an integrated systems immunology framework for understanding CRS heterogeneity. **Conclusion:** CRS following anti-CD19 CAR-T therapy is associated with coordinated myeloid remodeling and heterogeneous cytokine signatures rather than a single inflammatory profile. Integrated immunophenotypic and cytokine profiling may improve biological stratification and inform future precision immunomodulatory strategies.

**Keywords:** CAR-T cell therapy; Cytokine release syndrome; Monocytes; Cytokines e Inflammatory endotypes.

## Abstract 165 | Poster | Poster Session Day 2

### Effect of in vivo gene editing on bone alterations in MPS I mice

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Cáren Nunes de Oliveira

**Institution:** 1. Laboratório Células, Tecidos e Genes do Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil. 2. Programa de Pós-Graduação em Genética e Biologia Molecular da Universidade Federal do Rio Grande do Sul (UFRGS), Departamento de Genética, Porto — Porto Alegre - RS

Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused by alpha-L-iduronidase (IDUA) deficiency, causing glycosaminoglycan (GAG) storage within cells. Patients with MPS I present skeletal abnormalities, including short stature and dysostosis multiplex. Bone alterations remain largely unresponsive to current therapies. Previously, we have treated MPS I mice with a lipid nanoparticle (LNP) carrying the components of the CRISPR-Cas9 system in plasmids, and showed long term benefits on tissue enzyme activity and GAG levels in multiple organs. However, we have not examined the effect of the treatment on bone alterations. Therefore, the aim of this study was to characterize morphological, compositional, and biomechanical bone alterations in a murine MPS I model and evaluate the efficacy of CRISPR/Cas9-mediated gene editing. All analysis were carried in six-month-old wild type mice, untreated MPS I (Idua -/-) mice and neonatal CRISPR/Cas9-mediated gene editing treated mice. Study was approved by ethics committee of HCPA. MPS I mice exhibited significantly higher load-at-failure values but reduced flexural strength compared with WT controls in the 3-point bending test ( $p < 0.05$ ), suggesting biomechanical alteration in the bones of MPS I mice. X-ray analysis showed increase femur width in the MPS mouse model. Quantitative densitometry confirmed significantly reduced cortical and trabecular bone mineral density, while Raman spectroscopy revealed no significant alterations in mineral/matrix composition. CRISPR/Cas9 gene editing significantly reduced bone width. However, it failed to reduce skeletal storage burden or restore normal mechanical properties. MPS I mice exhibited marked morphological, compositional, and biomechanical skeletal abnormalities. Although gene editing partially improved specific morphological features, restoration of normal bone mechanical properties and correction of skeletal glycosaminoglycan storage remain major therapeutic challenges.

**Keywords:** Mucopolysaccharidosis type I; bone biomechanics; skeletal pathology; UA- HNAc(1S) biomarker; CRISPR/Cas9.

## Abstract 166 | Poster | Poster Session Day 2

### Diagnostic accuracy in the assessment of measurable residual disease in B-cell acute lymphoblastic leukemia using flow cytometry panels: A systematic review

**Category:** Manufacturing, quality control, clinical studies and science communication

**Authors:** KATHERINE JAZMIN HUAMAN PRADA; Julia Luisa Moya Naranjo; Luisa Ariana Challco Macedo; Ricardo Mafalky Rodríguez Torres

**Institution:** School of Medical Technology - Universidad Nacional Mayor de San Marcos — LIMA

**Background:** B-cell acute lymphoblastic leukemia (B-ALL) is a fast-progressing hematological malignancy, making rapid monitoring vital for survival (1). Measurable residual disease (MRD) assessment is crucial to evaluate therapeutic response, predict relapse, and guide treatment after chemotherapy or stem cell transplantation (2). Multi-parametric flow cytometry (MFC) is highly favored for its rapidity and accessibility over molecular assays. However, targeted immunotherapies like anti-CD19 (blinatumomab, CAR-T cells), anti-CD22 (inotuzumab ozogamicin), and anti-CD20 (rituximab) heavily alter or cause the loss of targeted lineage antigens on leukemic blasts (3,4). These antigen modifications complicate conventional MFC gating strategies, increasing the risk of false-negative MRD results. Therefore, synthesizing the diagnostic accuracy of various MFC antibody panels under modern therapeutic shifts is essential. **Objective:** To systematically synthesize scientific evidence regarding the diagnostic accuracy of flow cytometry panels for measurable residual disease (MRD) detection in patients with B-cell acute lymphoblastic leukemia (B-ALL). **Methods:** This systematic review and meta-analysis followed the PRISMA guidelines and was registered in PROSPERO (CRD420251241173). Electronic searches were conducted in PubMed, Scopus, ScienceDirect, and Wiley Online Library for diagnostic accuracy studies published between 2010 and 2025. Search strategies combined MeSH terms and text words for B-ALL, flow cytometry, and residual neoplasm. Original studies evaluating MRD in bone marrow or peripheral blood samples that reported sensitivity and specificity data were included. Studies acquiring fewer than 1,000,000 events by MFC were excluded to prevent low-sensitivity bias. Document screening and data extraction were performed independently by two reviewers using the Rayyan platform, achieving a 99% initial consensus. Risk of bias was evaluated using QUADAS-2, and certainty of evidence was assessed via GRADE. **Results:** From 4,277 records, 10 primary studies were selected. Meta-analysis showed a pooled specificity of 0.953 (95% CI: 0.904-0.978), confirming high accuracy to identify patients without MRD. Pooled sensitivity was 0.867 (95% CI: 0.733-0.939); its moderate heterogeneity was tied to protocol variations and event counts. While increasing antibodies/fluorochromes positively influenced specificity, sensitivity depended strictly on LOD and processing methods, notably bulk lyse. **Conclusion:** MFC provides solid performance for B-ALL clinical stratification. Via GRADE, evidence certainty was MODERATE due to methodological variability. While its 0.953 specificity safely prevents over-treatment, its 0.867 sensitivity requires strict protocol standardization and bulk lyse optimization to reduce false negatives under immunotherapy antigen shifts. Homogeneous future study designs are necessary to strengthen precision estimators.

**Keywords:** Minimal Residual Disease; Flow Cytometry; Precursor B-Cell Lymphoblastic Leukemia-Lymphoma; Diagnostic Accuracy; Meta-Analysis.

## **Abstract 167 | Poster | Poster Session Day 2**

### **Comparative Evaluation of CAR-T Cell Delivery Routes for Enhanced Expansion, Persistence, and Antitumor Efficacy in Solid Tumors**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** IGOR DE OLIVEIRA SANTOS; EMMANUEL ARTHUR ALBUQUERQUE ARAGAO; LUIZA DE MACEDO ABDO; MARTÍN BONAMINO

**Institution:** INSTITUTO NACIONAL DE CANCER - INCA — Rio de Janeiro - RJ

**Background:** CAR-T cell therapy has demonstrated high efficacy in hematological malignancies. However, its application to solid tumors remains limited by challenges in T cell trafficking, localized infiltration, and persistence within the immunosuppressive microenvironment. Evaluating alternative administration routes, such as intraperitoneal (I.P.) delivery, presents a strategic approach to bypass systemic limitations and maximize effector cell concentration at the disease site. **Objectives:** To evaluate and compare the impact of different delivery routes, intraperitoneal (I.P.), intravenous (I.V.), and subcutaneous (S.C.) on CAR-T cell expansion, systemic vs. regional persistence, trafficking, and overall antitumor efficacy in preclinical models. **Methods:** Immunodeficient mouse models were used to evaluate and compare administration routes (I.P., I.V., S.C., and peritumoral). First, baseline CAR-T expansion and trafficking kinetics were quantified in non-tumor-bearing mice using longitudinal bioluminescence imaging (BLI). Second, in solid tumor-bearing models established with the human ovarian adenocarcinoma cell line (SKOV3) (subcutaneous xenografts, mean initial volume ~75 mm<sup>3</sup>), tumor growth kinetics (total flux) and localized anti-HER2 CAR-T effector cell accumulation were monitored following I.P., I.V., or peritumoral treatment. **Results:** In non-tumor-bearing models, longitudinal BLI demonstrated that I.P. administration induces higher CAR-T cell expansion and prolonged local accumulation compared to systemic I.V. or S.C. routes. In subcutaneous solid tumor-bearing models, I.P. delivery achieved higher local effector cell infiltration and sustained tumor growth suppression, resulting in significantly lower total tumor flux compared to intravenous infusion. Ongoing functional assays from peritoneal lavage, spleen, and bone marrow flush are currently being conducted to confirm the sustained activation profile of I.P. delivered cells. **Conclusion:** Intraperitoneal delivery significantly enhances CAR-T cell expansion, local infiltration, and tumor control compared to systemic intravenous administration in solid tumor models. These findings highlight local delivery as a highly effective translational strategy to overcome delivery barriers in solid malignancies.

**Keywords:** CAR-T cell therapy; HER2; SKOV3 ovarian cancer; Intraperitoneal delivery; Cellular expansion and persistence; Preclinical models.

## **Abstract 168 | Poster | Poster Session Day 2**

### **Development of an in vitro immunosuppression assay using natural and induced regulatory T cells for functional evaluation of CAR-T cells**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Raissa Raminelli Rezende

**Institution:** Fundação Hemocentro de Ribeirão Preto — Ribeirão Preto - SP

T cell therapy using lymphocytes engineered to express chimeric antigen receptors (CAR) has shown high remission rates in patients with leukemias and lymphomas (1, 2). However, its efficacy against solid tumors, which account for the majority of cancer-related deaths, remains limited. This is mainly due to the highly immunosuppressive tumor microenvironment (TME), which is enriched with inhibitory molecules and regulatory immune cells, particularly regulatory T cells (Tregs) (3). Although Tregs play a critical role in immune homeostasis, their accumulation in the TME impairs antitumor responses and is associated with poor prognosis in cancers such as glioblastoma (4). Given the limited availability of in vitro models that accurately recapitulate this immunosuppressive niche, we developed of a co-culture assay incorporating natural Tregs (nTregs) or induced Tregs generated through ectopic Foxp3 expression (iTregs) (5, 6), to assess their ability to suppress anti-GD2 CAR-T cell activity against glioblastoma cells, which high levels of the GD2 antigen. To generate iTregs, CD4+ T cells were isolated from peripheral blood mononuclear cells (PBMCs) by immunomagnetic selection. These cells were subsequently transduced with a lentiviral vector encoding Foxp3 and a truncated CD271 (tCD271) selection marker, followed by positive selection of tCD271-expressing cells. The phenotype of both iTregs and nTregs was characterized by flow cytometry evaluating the expression of CD4, CD25, CTLA-4, GITR and FOXP3. The suppressive activity of iTregs on effector T cells and CAR-T cells was evaluated using a CFSE-based proliferation assay. Co-cultures were established at different Teff:Treg ratios (1:2, 1:1, 2:1, 4:1 and 8:1). T cell proliferation was quantified by flow cytometry after 72 hours. A significant reduction in T cell proliferation was observed as the proportion of Tregs increased, confirming their suppressive activity. Furthermore, in vitro coculture assays were performed using anti-GD2 CAR-T cells, T98G glioblastoma cells expressing luciferase and nTregs isolated from PBMCs by immunomagnetic selection (anti-CD4/CD25). Cocultures were set in duplicates at a 1:1 CAR-T:T98G ratio, and Tregs were added at 0.25 and 0.50 ratios relative to CAR-T cells. Tumor cell death was assessed by quantifying bioluminescence emitted by tumor cells using the IVIS Lumina system at 0h and 72h. After 72 hours, wells containing Tregs showed significantly less tumor cell death, demonstrating a suppressive effect on CAR-T antitumor activity. These results confirm the immunosuppressive effect of nTregs on anti-GD2 CAR-T cell activity against glioblastoma in vitro. The suppressive activity was also observed in coculture assays using iTregs at a CAR-T ratio of 1:2, employing the same iTreg ratios used in the proliferation assay. Thse systems offers a relevant model to study Treg-mediated inhibition and may support the development of strategies to enhance CAR-T efficacy in solid tumors.

**Keywords:** Immunosuppression; Regulatory T cells (Tregs); CAR-T cells

## **Abstract 169 | Poster | Poster Session Day 2**

### **Short-chain fatty acids mediated metabolic and epigenetic reprogramming enhances CAR-T cell antitumor activity**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Bianca Tiemi Toma

**Institution:** Hemocentro de Ribeirão Preto - Faculdade de Medicina de Ribeirão Preto (FMRP - USP)  
— Ribeirão Preto

Chimeric antigen receptor (CAR)-T cell therapy has been successful against certain hematologic malignancies. However, comparable clinical outcomes in solid tumors remain limited by the immunosuppressive and hostile tumor microenvironment (TME). Improved CAR-T cell persistence and cytotoxicity have been associated with enhanced metabolic fitness, epigenetic remodeling, as well as distinct T cell differentiation states. In this context, this study aimed to enhance the antitumor activity of anti-GD2 and anti-CD19 CAR-T cells through metabolic and epigenetic modulation mediated by the short-chain fatty acids (SCFAs) acetate and butyrate. To address this, CAR-T cells targeting GD2 or CD19 obtained from peripheral blood mononuclear cells were activated with anti-CD3/CD28 magnetic beads and expanded for 8 days in RPMI with IL-2. In the last 48 hours, cells were treated with acetate (Ace: 15 mM), butyrate (But: 0.5 mM) or both (Ace + But). Memory phenotype, mitochondrial mass and membrane potential, as well as acetylated H3K27 were assessed by flow cytometry. Metabolic profiling was performed using seahorse assay. CAR-T cells were cocultured with T98G or Raji cells expressing luciferase over 24-hour rounds at a 1:2 effector-to-target ratio. Tumor lysis was determined by bioluminescence imaging using IVIS and IFN- $\gamma$  secretion was assessed by ELISA assay. The results showed that SCFA-treated GD2 CAR-T cells exhibited higher cytotoxicity against T98G cells (Ace: 64.3%; But: 68.8%; Ace + But: 75%) than those non-treated (52.1%), along with enhanced levels of IFN- $\gamma$  secretion. Similarly, SCFA-treated CAR-T CD19 cells displayed greater cytotoxicity against Raji cells (non-treated: 11.9%; Ace: 32.4%; But: 25.5%; Ace + But: 29.1%), and this effect was maintained after a second antigen exposure (non-treated: 29.5%; Ace: 46.4%; But: 39.4%; Ace + But: 47.7%). Notably, enhanced cytotoxicity was preserved even under low-glucose and chemically induced hypoxic conditions (non-treated: 49.8%; But: 64.9%; Ace + But: 58%). Functionally, the increased antitumor activity was associated with elevated H3K27 acetylation levels (MFI Control: 18,592; Ace: 22,686.; But: 22,004; Ace + But: 24,511). Moreover, butyrate treatment increased mitochondrial membrane potential, maximal oxygen consumption and spare respiratory capacity, as well as glycolytic capacity, indicating an enhanced bioenergetic capacity and metabolic fitness. These results suggest that acetate and butyrate enhance the therapeutic potential of different CAR-T cell constructs in both solid and hematologic malignancies, through epigenetic and metabolic reprogramming. Thus, this study highlights the immunomodulatory role of gut microbiota-derived metabolites in T cells, supporting their potential use in CAR-T cell therapy and other adoptive immunotherapies.

**Keywords:** CAR-T; Immunometabolism; Short-chain fatty acids

## **Abstract 171 | Poster | Poster Session Day 2**

### **CRISPR/Cas9-Mediated TGFBR2 Editing in NK-92 Cells Establishes a Modular Platform for TGF- $\beta$ -Resistant CAR NK-Cell Engineering**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Leticia Maria Ribeiro Bassi

**Institution:** Hemocentro de Ribeirão Preto — Ribeirão Preto-SP

Transforming growth factor-beta (TGF- $\beta$ ) is a major immunosuppressive mediator of the tumor microenvironment and can impair natural killer (NK)-cell cytotoxicity, degranulation, cytokine production, and metabolic fitness. Therefore, engineering NK cells to resist TGF- $\beta$ -mediated inhibition may improve the activity of adoptive NK-cell therapies. Here, we are developing a modular NK-cell engineering platform based on disruption of the transforming growth factor-beta receptor II gene (TGFBR2), designed to subsequently incorporate of antigen-specific CAR constructs. As an initial application, this strategy will be evaluated using a CD19-directed CAR for the targeting of CD19-positive B-cell malignancies. NK-92 cells were selected as an initial engineering platform because their homogeneous and continuously expandable nature enables reproducible gene editing, clone selection, and standardized functional characterization before translation to primary NK-cell sources. NK-92 cells were edited using CRISPR/Cas9 targeting TGFBR2. Editing efficiency was assessed by flow cytometry. Wild-type and TGFBR2-edited NK-92 cells were exposed to recombinant TGF- $\beta$ 1 at 10 ng/mL for 48 hours. Functional assay was evaluated by co-culture with K562 cell, including cytotoxicity and CD107a degranulation assays. Metabolic activity was assessed using Seahorse Real-Time Cell Metabolic Analysis. CRISPR/Cas9 editing generated a TGFBR2-edited NK-92 population with 70–80% editing efficiency. TGFBR2 knockout NK-92 cells maintained their cytotoxic activity after gene editing and no significant difference was observed between wild-type and TGFBR2-edited NK-92 cells. Following 48 hours of TGF- $\beta$ 1 exposure, cytotoxicity against K562 cells was reduced in both wild-type and TGFBR2 knockout NK-92 cells. This finding may be explained by the heterogeneous nature of the edited cell population, in which 20–30% of cells retained TGFBR2 expression. In contrast, degranulation analysis exhibited a reduction in wild-type following TGF- $\beta$ 1 treatment, whereas TGFBR2-edited NK-92 cells maintained their degranulation capacity under the same conditions. Seahorse analysis showed distinct bioenergetic responses. Metabolic profiling shows that the TGFBR2-edited NK-92 cell pool exposed to TGF- $\beta$ 1 exhibited lower oxygen consumption (OCR) and extracellular acidification (ECAR) than the other experimental groups. Additional analyses are required to determine whether these differences reflect altered mitochondrial function, glycolytic activity, or metabolic adaptation. Collectively, these findings indicate that TGFBR2 disruption does not impair NK-cell cytotoxicity, otherwise the heterogeneous nature of the edited cell population may have influence in results. This study establishes a genetically edited NK-92 platform to screen strategies against TGF- $\beta$  immunosuppression before validation in primary sources. Future steps will evaluate sorting enrichment cells against and CD19-positive malignancies and solid tumors.

**Keywords:** Natural Killer cells, gene knockout, TGFBR2, CRISPR/Cas9, CAR-NK immunotherapy.

## **Abstract 172 | Poster | Poster Session Day 2**

### **Characterizing the Cellular Specificity and Expression Profile of Recombinant Adeno-Associated Virus (rAAV) of Mixed Serotype 1/2 in the Pigmented Rat Retina**

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Cosme Filipe Costa Figueredo; Gabriel Pinheiro Alves da Silva; HILDA PETRS SILVA; THAÍS COSTA PORTO MARINHO; Victor Guedes de Araujo; VINICIUS DE TOLEDO RIBAS

**Institution:** 1. Gene Therapy and Viral Vectors Laboratory, Carlos Chagas Filho Institute of Biophysics, UFRJ, Brazil. 2. Laboratory of Neurobiology, Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte. — RJ MG

**Introduction:** Optic neuropathies, such as glaucoma, are characterized by the progressive loss of retinal ganglion cells (RGCs) and their axons, making it the leading cause of irreversible blindness worldwide. Currently, there is no cure, only some treatments that are minimally effective and do not reverse neuronal loss. In this context, recombinant adeno-associated virus (rAAV) vectors have been used as a tool for various experimental approaches, and are already used in gene therapy drugs for the retina. There are different rAAV vector serotypes, both wild-type and novel capsids generated in the laboratory. Each serotype presents a distinct cellular tropism, enabling a wide range of biotechnological strategies aimed at different tissues and systems. Thus, understanding the transduction profile of a vector serotype in the study model is the first step toward developing more efficient therapeutic strategies. In addition to vector tropism, transduction efficiency also depends on the promoter present in the construct. **Objective:** The aim of this study is to evaluate the transduction profile of the mixed serotype 1 and 2 rAAV vector, with the human synapsin (hSyn) neuronal promoter, which drives the expression of the red fluorescent protein (mCherry), in the retina of pigmented rats. **Methods:** To test this, one-month-old Lister Hooded rats of both sexes were used. Intravitreal injections of 5 µL of the rAAV1/2-hSyn-mCherry vector are performed (CEUA/UFRJ No. 115/21). After four weeks, the eyes are collected, fixed overnight in 4% paraformaldehyde, and dissected. The retinas are then processed and analyzed by immunofluorescence using markers for the different retinal cell types and mCherry, to assess the vector's transduction profile. **Results:** The project is ongoing, and it is expected that mCherry expression will be observed both in retinal flat-mounts, to assess the transduced area of the tissue, and in cross-sections, to analyze the different cell subtypes reached. Furthermore, the intravitreal route of administration targets the vectors to RGCs, cells affected in glaucoma and of interest to the laboratory. Characterizing the transduction profile of this vector will directly contribute to the implementation of studies focused on RGCs.

**Keywords:** Viral Vectors, Transduction Profile, Retina, Intravitreal Injection

## Abstract 173 | Poster | Poster Session Day 2

### Gene therapy in gastric cancer cell lines from the State of Pará, Brazil.

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Samara Silveira da Cruz; Amanda Correa Soares Pimentel; Amanda de Nazaré Cohen Paes; Débora Monteiro Carneiro do Vale; Márcia Mariana Figueiredo Barbosa; Rommel Mario Rodriguez Burbano

**Institution:** Universidade Federal do Pará — BELÉM-PA

Gastric cancer is one of the most aggressive neoplasms worldwide and is the second most frequent among men and the fifth among women in the North region of Brazil. Its histological classification divides it into two subtypes, diffuse and intestinal, with distinct morphology, epidemiology, pathogenesis and clinical behaviour. Overexpression of the MYC gene is recurrent in gastric carcinogenesis, and as tumour staging advances the number of MYC alleles increases. In Belém, Pará, our research group established and immortalized four gastric cell lines from patients from the State of Pará: ACP02 (diffuse-type tumour), ACP03 (intestinal type), AGP01 (Paraense Gastric Ascites 1) and MNP01 (Paraense Normal Mucosa 1). We silenced the MYC protein in these cell lines, analysed and identified overexpressed genes capable of regulating MYC and, among the genes analysed, we selected the CDC25B gene for gene silencing by CRISPR-Cas9, with subsequent analysis of proliferation, migration and cell invasion, and sequencing of the target region to assess the efficacy of the technique. The statistical methods used were ANOVA and Tukey's test. As a result of the experiments, blocking CDC25B caused mitotic catastrophe, resulting in complete loss of viability of the cell lines within the first 24 hours after inactivation by CRISPR/Cas9. Wild-type cells showed a strong positive correlation with time in all cell lines, indicating progressive cell growth over time, as these are gastric cancer cells (AGP01:  $r = 0.927$ ,  $p = 0.0077$ ; ACP02:  $r = 0.973$ ,  $p = 0.0011$ ; ACP03:  $r = 0.948$ ,  $p = 0.0040$ ). For CDC25B silencing, the correlation was strongly negative in the ACP01 ( $r = -0.993$ ,  $p = 0.073$ ) and ACP03 ( $r = -0.997$ ,  $p = 0.052$ ) cell lines, indicating a consistent reduction in cell number over time, although the p value is slightly above the conventional threshold of 0.05. As a consequence, the subsequent proliferation, migration and cell invasion assays could not be performed, demonstrating the critical role of this gene in maintaining tumour viability and implying its value as a potential therapeutic target. This new evidence on the involvement of the CDC25B gene in gastric carcinogenesis provides important advances for understanding the aetiology and pathophysiology of these tumours and opens new perspectives for the development of more effective gene and pharmacological therapies.

**Keywords:** CRISPR Cas9; Gene silencing, Gene therapy

## **Abstract 174 | Poster | Poster Session Day 2**

### **Differential Functional Remodeling of CD4<sup>+</sup> and CD8<sup>+</sup> T Cells Following Anti-CD19 CAR-T Cell Therapy**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Larissa Campos de Souza

**Institution:** A.C. Camargo Cancer Center — São Paulo SP

**Introduction:** Anti-CD19 chimeric antigen receptor T-cell (CAR-T) therapy drives immune remodeling beyond the infused product, affecting endogenous T cells. An integrated functional profile of cytokines, cytotoxicity, activation, and immune checkpoints may broaden assessment of post-CAR-T immune changes. Yet, coordinated functional remodeling of circulating CD4<sup>+</sup> and CD8<sup>+</sup> T-cell compartments after anti-CD19 CAR-T remains poorly defined. **Objective:** To define longitudinal remodeling of circulating CD4<sup>+</sup> and CD8<sup>+</sup> T cells before and after anti-CD19 CAR-T by integrating activation, cytotoxicity, cytokines, and immune checkpoints. **Methods:** Peripheral blood was collected from patients with relapsed/refractory diffuse large B-cell lymphoma before anti-CD19 CAR-T infusion and 30 days after therapy, under a Research Ethics Board-approved protocol (CAAE: 65750722.3.0000.5432). Peripheral blood mononuclear cells (PBMCs) were density-gradient isolated and stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin to assess function. Stimulated cells were stained and assessed by high-dimensional multiparameter flow cytometry (FACSymphony A5). Analyses of circulating CD4<sup>+</sup> and CD8<sup>+</sup> T cells included activation, immune checkpoints, cytotoxic mediators, and cytokine production. This approach captured complementary effector and regulatory features in both T-cell compartments. Paired analyses compared baseline and day 30 after CAR-T infusion. **Results:** Functional profiling showed substantial remodeling of the circulating T-cell pool 30 days after anti-CD19 CAR-T. After polyclonal stimulation, CD4<sup>+</sup> and CD8<sup>+</sup> T cells showed enhanced function, with increased IFN- $\gamma$  and TNF production and dynamic changes in granzyme B expression. CD8<sup>+</sup> cells showed greater cytotoxic potential, whereas CD4<sup>+</sup> cells displayed a TNF-dominant profile. Activation marker expression increased after therapy, with differential remodeling of PD-1, TIGIT, and TIM-3 expression. **Discussion:** Early immune recovery involved coordinated functional remodeling of the circulating T-cell pool in the first month after CAR-T therapy. Enhanced cytokine production and cytotoxic potential after polyclonal stimulation indicate preserved functional capacity despite heterogeneous immune checkpoint remodeling. CD4<sup>+</sup> and CD8<sup>+</sup> T cells followed distinct functional trajectories, suggesting differential remodeling of helper and cytotoxic programs during early recovery. These findings suggest immune checkpoint expression alone may not fully reflect functional competence after CAR-T therapy. **Conclusion:** Anti-CD19 CAR-T drives coordinated functional remodeling of circulating CD4<sup>+</sup> and CD8<sup>+</sup> T-cell compartments during early immune recovery. Distinct cytokine production and cytotoxic profiles, despite heterogeneous immune checkpoint remodeling, support preserved functional competence rather than global dysfunction. These findings support integrated functional immune profiling to monitor immune reconstitution after CAR-T therapy.

**Keywords:** CAR-T cell therapy; Diffuse large B-cell lymphoma; T-cell immune reconstitution; Immune checkpoints; T-cell function

## **Abstract 175 | Poster | Poster Session Day 2**

### **Mapping CAF Spatial Heterogeneity In PDAC Through SpheroMap Cytometry: Insights Into Hypoxia And Tumor Cell Context**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Sara Evelyn de Castro Silva

**Institution:** Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo. — Ribeirão Preto, SP.

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense desmoplastic stroma largely composed of cancer-associated fibroblasts (CAFs), which play central roles in tumor progression, immune exclusion, and therapy resistance. CAFs comprise functionally distinct and reversible phenotypes, including myfibroblastic CAFs (myCAFs) and inflammatory CAFs (iCAFs); however, their spatial distribution across hypoxic and normoxic tumor regions remains poorly understood. Here, we established heterotypic PDAC spheroid models to identify and quantify the distribution of myCAF-like cells across hypoxic and normoxic compartments using SpheroMap Cytometry, a spatial flow cytometry approach that enables the quantitative assessment of cell localization within tumor-stroma spheroids. CAPAN-1 cells, derived from a PDAC metastasis, or MIA PACA-2 cells, derived from a primary pancreatic tumor, were cocultured with CellTrace-labeled HS-5 stromal cells at a 20:80 tumor-to-stroma ratio, totaling 20,000 cells per well. Spheroids were generated in ultra-low-attachment 96-well plates in the presence of methylcellulose. After 48 h of aggregation, spheroids were incubated with Image-iT Green Hypoxia, a fluorescent probe that accumulates in hypoxic cells, enabling spatial mapping of cells dissociated from spheroids. At 72 h, they were enzymatically dissociated and stained for CD73 and CD140b to identify myCAF-like cells. Our results showed a robust enrichment of CD73<sup>+</sup>/CD140b<sup>+</sup> myCAF-like cells within the hypoxic core. In CAPAN-1/HS-5 spheroids, CD73 and CD140b co-expression reached 68.27% in the hypoxic compartment, compared with 40.70% in the normoxic compartment ( $p < 0.0001$ ). Similarly, in MIA PACA-2/HS-5 spheroids, co-expression reached 54.77% in the hypoxic compartment, compared with 9.12% in the normoxic compartment ( $p < 0.0001$ ). When the two tumor cell line models were compared, CAPAN-1 spheroids showed a greater enrichment of CD73<sup>+</sup>/CD140b<sup>+</sup> myCAF-like cells within the hypoxic compartment than MIA PACA-2 spheroids (68.27% vs. 54.77%,  $p = 0.0018$ ). The most pronounced difference was observed in the normoxic compartment, where CAPAN-1 spheroids contained 40.70% CD73<sup>+</sup>/CD140b<sup>+</sup> myCAF-like cells, compared with 9.12% in MIA PACA-2 spheroids ( $p < 0.0001$ ). Our results suggest that hypoxia strongly enriches CD73<sup>+</sup>/CD140b<sup>+</sup> myCAF-like cells within PDAC spheroids, while revealing that the spatial distribution of this phenotype is markedly influenced by the tumor cell context. By enabling the quantitative mapping of stromal phenotypes across hypoxic and normoxic compartments, SpheroMap Cytometry opens new avenues for the preclinical evaluation of CAF-targeting therapies and supports the development and selection of more effective, context-specific treatment strategies for PDAC. This study was funded by the São Paulo Research Foundation (FAPESP), Brazil, grant number 2025/16434-7, and the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES).

**Keywords:** PDAC; Stroma; CAF; Spheroid; SpheroMap Cytometry

## Abstract 176 | Poster | Poster Session Day 2

### **hMAX gene therapy reshapes early mitochondrial, epigenetic, and glial stress responses in a rat model of ocular hypertension.**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Victor Guedes de Araujo; HILDA PETRS SILVA; Rodrigo Martins Barros

**Institution:** Gene Therapy and Viral Vectors Laboratory, Carlos Chagas Filho Institute of Biophysics, UFRJ, Brazil. — Rio de Janeiro-RJ

Glaucoma is a progressive optic neuropathy marked by irreversible retinal ganglion cell (RGC) loss. Ocular hypertension (OHT), its main risk factor, engages stress pathways, including mitochondrial dysfunction, epigenetic dysregulation, and neuroinflammation. rAAV2-mediated overexpression of hMAX was shown to protect RGC against glaucomatous degeneration (1), yet the early responses underlying this neuroprotection remain uncharacterized. As the central hub of the MYC/MAX/MXD network, elevated MAX could shift the competitive balance toward activating MYC-MAX dimers. This might displace repressive MAX-MXD complexes, which otherwise dock the SIN3A scaffold to deliver catalytic HDAC2 for gene silencing. In turn, MYC-MAX activity may drive TFAM- and NRF1-dependent mitochondrial biogenesis, potentially easing cellular stress and glial reactivity. To evaluate whether these mechanisms underlie hMAX neuroprotection, we investigated how OHT and hMAX gene therapy modulate markers of mitochondrial integrity (VDAC, ND4), epigenetic regulation (HDAC2), and glial reactivity (GFAP, Iba1). To this end, one-month-old, Lister Hooded rats received intravitreal injection (5  $\mu$ L, 10E13 vg/mL) of rAAV2-smCBA-hMAX vectors. Four weeks later, OHT was induced by full-circle limbal plexus cauterization (2). Uninjected OHT animals served as control. At 1, 3, and 7 days post-injury, animals were euthanized, retinas were dissected, and immunofluorescence was performed for VDAC, ND4, HDAC2, GFAP, and Iba1. Images were acquired by confocal microscopy and mean fluorescence intensity was quantified in ImageJ. Statistics were performed in GraphPad Prism. In untreated OHT retinas, VDAC was persistently elevated (68% at D1, 104% at D3, 59% at D7; relative to naive retinas, n=4), whereas ND4 remained stable initially but fell by 27% at D7. HDAC2 increased only late (55% at D7), while GFAP+ projections and Iba1+ occupied area revealed glial and microglial activation from D3 (76- and 6-fold, respectively) and maintained at D7 (93- and 3.5-fold). In contrast, hMAX-treated OHT retinas showed an attenuated, transient profile: VDAC was unchanged at day 1, rose only transiently (49% at D3), and normalized by day 7. ND4 was preserved early and increased at D3 (68%) and D7 (94%). HDAC2 was reduced early (47% at D1) and normalized thereafter. Glial responses were delayed and dampened, with GFAP rising only late (79-fold at D7) and Iba1 showing a transient increase (5-fold at D3) before returning to baseline. Thus, hMAX gene therapy converts a sustained OHT stress signature into a transient one. Our findings are consistent with the proposed MYC/MAX/MXD mechanism: early HDAC2 suppression and rising ND4 suggest relieved epigenetic repression and enhanced mitochondrial biogenesis, easing cellular stress (normalizing VDAC) and dampening secondary glial reactivity. These results are a first step, identifying early mechanisms of hMAX neuroprotection that remain correlative and need further validation.

**Keywords:** rAAV; neuroprotection; glaucoma

## Abstract 177 | Poster | Poster Session Day 2

### Ionizable Lipid-polymeric Nanoparticles for the Treatment of Osteoporosis: RANKL Gene Silencing via siRNA in Osteoblasts

**Category:** Vectors and gene delivery systems (viral and non-viral)

**Authors:** Carolina Fontanella de Araújo; Andrea Teixeira-Carvalho; Breno Francisco Figueiredo Maia; Eloy Ramos Seixas; Kelly Alves Bicalho; Maiara Fonseca C. de Oliveira; Marco Túlio Rodrigues Alves; Pedro Henrique Rodrigues; Pedro Luís Araújo; Pedro Pires Goulart Guimarães

**Institution:** Fiocruz Minas - Instituto René Rachou — Belo Horizonte - MG

Osteoporosis is a major public health concern, with projections estimating 2.6 million hip fractures worldwide in 2025 and 6.25 million by 2050. The disease mainly affects older adults, particularly postmenopausal women, due to estrogen deficiency, which increases osteoclast activity and bone resorption. Receptor Activator of Nuclear Factor kappa-B Ligand (RANKL) plays a central role in osteoclastogenesis by binding to RANK receptors on osteoclast precursors. After menopause, estrogen deficiency leads to RANKL overexpression, promoting osteoclast recruitment and accelerating bone loss. Current treatments, including hormone replacement therapy, calcium and vitamin D supplementation, bisphosphonates, and denosumab, are effective but may cause significant adverse effects. Therefore, new therapeutic strategies are needed. Ionizable lipid nanoparticles (LNPs) have emerged as promising platforms for nucleic acid delivery and gene therapy. This project aims to develop ionizable lipid-polymeric nanoparticles capable of delivering small interfering RNA (siRNA) targeting RANKL into murine osteoblasts to reduce RANKL expression and inhibit osteoclastogenesis. Murine MC3T3-E1 pre-osteoblasts obtained from the Rio de Janeiro Cell Bank (BCRJ) were used. Two LNP formulations containing different siRNA sequences were produced at the Laboratory of Biophysics of Nanostructured Systems (UFMG) using microfluidic technology and designated siRNA-RANKL@LNP1 and siRNA-RANKL@LNP2. Cells will be incubated with different nanoparticle concentrations to identify the most effective dose for gene silencing. After treatment, RNA extraction, cDNA synthesis, and RT-qPCR analysis will be performed to evaluate RANKL gene expression. Cell viability was assessed using the alamarBlue assay to investigate potential cytotoxicity. Both LNP formulations were successfully prepared and characterized, presenting physicochemical properties suitable for intracellular siRNA delivery. The average hydrodynamic size was 71.25 nm for siRNA-RANKL@LNP1 and 71.62 nm for siRNA-RANKL@LNP2, with polydispersity indices of 0.2838 and 0.3073, respectively. Zeta potential values were 1.7861 mV and 2.7437 mV, while the apparent pKa values were 5.26 and 5.01 for LNP1 and LNP2, respectively. Encapsulated siRNA concentrations reached 165.3 ng/μL for LNP1 and 160.6 ng/μL for LNP2. Cell viability assays demonstrated no cytotoxicity, even at higher nanoparticle concentrations. These preliminary findings indicate that the developed LNPs possess appropriate physicochemical characteristics and biocompatibility for intracellular siRNA delivery. Ongoing experiments will determine the most efficient nanoformulation for RANKL silencing and evaluate nanoparticle internalization. Overall, this platform shows promising potential as an RNA interference-based therapeutic strategy for osteoporosis by targeting osteoclastogenesis.

**Keywords:** Osteoporosis, Bone resorption, RANKL, siRNA, Ionizable lipid-polymeric nanoparticles

## Abstract 178 | Poster | Poster Session Day 2

### rAAV2-Mediated FEZF2 Overexpression as a Neuroprotective Strategy in an Experimental Rat Model of Glaucoma

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Gabriela Silva Almeida; HILDA PETRS SILVA; MARIANA SANTANA DIAS; THAÍS COSTA PORTO MARINHO; Victor Guedes de Araújo; VINICIUS DE TOLEDO RIBAS

**Institution:** Federal University of Rio de Janeiro — Rio de Janeiro

**Introduction:** Glaucoma comprises a heterogeneous group of optic neuropathies and is the leading cause of irreversible blindness worldwide. It is characterized by the progressive degeneration of retinal ganglion cells (RGCs) and their axons, with no cure available to date. Current therapies are limited to pharmacological and surgical interventions that control the main risk factor, elevated intraocular pressure (IOP). However, these strategies do not act on the underlying neurodegeneration and fail to prevent progressive RGC loss. This has driven growing interest in approaches promoting RGC neuroprotection and regeneration. The transcription factor FEZF2 plays a key role during development, particularly in axonogenesis, acting as a major molecular regulator of neuronal circuit organization, axon guidance, and neuronal differentiation. We therefore hypothesized that overexpressing a pro-neuronal, axon-growth-associated developmental factor such as FEZF2 could reactivate embryonic molecular programs, fostering an environment favorable to axon growth and RGC neuroprotection under injury. **Methods:** To test this, we investigated the therapeutic potential of rAAV2-mediated FEZF2 overexpression in an ocular hypertension (OHT) glaucoma model induced by full-circle cauterization of the limbal plexus. One-month-old Lister Hooded rats of both sexes were randomized into three groups and received a single intravitreal injection (5  $\mu$ L) of saline or rAAV2-hSyn-FEZF2 ( $1.0 \times 10^{13}$  vg/mL). Four weeks later, OHT was induced. Thirty days post-injury, retinal structure was assessed in vivo by optical coherence tomography (OCT), and visual function by optomotor response (OMR) and photopic negative response (PhNR) from full-field flash electroretinogram. Retinas were then collected for anti-RBPMS immunofluorescence to quantify RGC density. Images were acquired by confocal microscopy; data are shown as mean  $\pm$  SEM and analyzed in GraphPad Prism 8.0. **Results:** After OHT induction, rAAV-FEZF2 preserved higher RGC densities relative to saline ( $1749 \pm 162$  vs  $1012 \pm 100$ , respectively;  $n=4$ ;  $p<0.05$ ). This structural benefit, however, did not translate into a comparable functional gain. The OMR threshold under FEZF2 was only slightly higher than in saline-treated eyes ( $0.52 \pm 0.01$  vs  $0.45 \pm 0.03$  cpd;  $n=3-4$ ;  $p=0.06$ ), and electrical function was not preserved, as PhNR amplitude did not differ significantly from saline ( $44.1 \pm 4.8$  vs  $39.0 \pm 5.6$   $\mu$ V;  $n=3-4$ ;  $p=0.11$ ). **Conclusion:** Our results indicate that rAAV2-mediated FEZF2 overexpression promotes morphological preservation of RGCs in an IOP-driven glaucoma model. This work indicates that overexpressing a pro-neuronal transcription factor is a promising strategy for developing gene therapy approaches aimed at RGC neuroprotection in experimental glaucoma.

**Keywords:** Glaucoma, rAAV, RGCs, Gene Therapy and FEZF2

## **Abstract 179 | Poster | Poster Session Day 2**

### **Heterotypic Spheroid, 3D Model for Immunotherapy Studies for Triple Negative Breast Cancer**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Samuel Iwao Maia Horita

**Institution:** Fiocruz — Rio de Janeiro - RJ

**Introduction:** Triple-negative breast cancer (TNBC) is an aggressive subtype lacking estrogen, progesterone, and HER2 receptors, which limits treatment options and contributes to poor prognosis and high mortality. Bispecific antibodies (BsAbs) emerge as promising immunotherapeutic agents for TNBC, as they can simultaneously target tumor antigens and activate immune cells. However, in vitro validation of BsAbs remains challenging due to the need for complex models that mimic tumor-immune interactions. **Objective:** This project aims to develop a complex heterotypic spheroid model with tumor and immune components to validate the therapeutic effects of BsAbs. **Results and Methodology:** For the initial characterization of the spheroid model, fibroblasts, endothelial cells, breast epithelial cells (non-tumor), and GFP-tagged TNBC cells were used. The model was maintained on the magnetic apparatus in a mixed medium (1:1:1 ratio) for 3 or 5 days. H&E staining demonstrated good cohesion and viability. Immunofluorescence confirmed cytokeratin-expressing epithelial cells, CD31-positive endothelial cells, and acellular regions. To incorporate immune components, Jurkat T lymphocytes were introduced to 3-day-old spheroids at three concentrations ( $1.5 \times 10^5$ ,  $3 \times 10^5$  and  $1.5 \times 10^6$ ) and co-cultured for an additional 2 days. Anti-CD4 labeling showed that  $1.5 \times 10^6$  cells yielded the highest lymphocyte infiltration. Immunofluorescence of sectioned spheroids following Jurkat T cell treatment revealed a reduction in GFP-positive tumor cells, alongside increased Ki-67 and decreased cleaved caspase-3 in the remaining tumor cell population. Finally, in a separate functional assay using luciferase-expressing MDA-MB-231 cells within the spheroids, luciferin bioluminescence enabled rapid quantification of tumor cell viability, demonstrating a significant reduction in tumor viability upon Jurkat T cell treatment in the heterotypic model. **Conclusion:** Heterotypic spheroids demonstrate promising characteristics for evaluating BsAbs: structural cohesion, preserved viability, successful infiltration of functional T lymphocytes, and the capacity for rapid quantitative readout of tumor cell killing via bioluminescence following immune engagement.

**Keywords:** TBNT, CAR-T, immunotherapies

## **Abstract 180 | Poster | Poster Session Day 2**

### **IL-7 and IL-15 culture drives T cells out of quiescence without TCR activation while preserving a less differentiated phenotype and metabolic fitness for CAR-T manufacturing.**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Millena Brandão

**Institution:** Hemocentro de Ribeirão Preto, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP) — Ribeirão Preto - SP

CAR-T cell therapy is a major advance in immunotherapy, achieving high response rates in hematologic malignancies. However, many patients relapse or become refractory, and efficacy against solid tumors remains limited. One contributing factor may be the conventional manufacturing process, which relies on strong T-cell activation via anti-CD3/anti-CD28 before gene transfer. Although this enhances proliferation and lentiviral transduction, it also promotes differentiation, metabolic remodeling, and may impair long-term persistence. We hypothesized that culturing T cells with IL-7 and IL-15, without TCR-mediated activation, would preserve a less differentiated phenotype and metabolic fitness. Preliminary results identified 5 days of IL-7/IL-15 culture as optimal for transduction, leading to cell-cycle, phenotypic, and metabolic characterization. PBMCs were obtained from four healthy platelet donors (D0). Non-activated T cells were purified by negative selection and cultured with IL-7/IL-15 without TCR stimulation. Activated PBMCs underwent overnight monocyte depletion, then stimulation with anti-CD3/anti-CD28 beads on D1. Both groups were cultured until D6, with sampling on D1, D4, and D6 for flow cytometry (cell cycle: Hoechst/Pyronin Y; proliferation: CFSE; differentiation: CD45RA/CD62L/CD95; activation: CD69/CD25; mitochondrial potential: MitoTracker) and metabolic profiling (Seahorse XF T Cell Metabolic Profiling Kit) on D6. Both groups showed cell-cycle progression from D1 to D6, but activated cells exited quiescence (G0) more markedly and proliferated more (lower CFSE retention). TCR activation increased effector memory T cells (TEM) among CD3+/CD8+ populations and drove progressive CD69/CD25 upregulation, while non-activated cells stayed phenotypically stable and predominantly CD69-CD25- throughout. Non-activated cells retained a higher proportion of functionally preserved mitochondria across culture, whereas activated cells showed a transient drop at D4. Seahorse analysis revealed a shift toward a more energetic metabolic profile in activated cells, characterized by increased glycolytic activity (ECAR). However, non-activated T cells maintained comparable mitochondrial respiratory capacity, as evidenced by similar spare respiratory capacity between groups. These results demonstrate that culture with IL-7/IL-15 is sufficient to drive T cells out of the quiescent state (G0), required for lentiviral transduction. Furthermore, this protocol preserves a less differentiated phenotype and mitochondrial respiratory capacity comparable to activated cells. Although TCR-mediated activation promoted greater proliferation, it was also associated with transient mitochondrial alterations. Thus, the IL-7/IL-15-based strategy represents a promising approach for CAR-T cell production, enabling gene transfer while preserving functional fitness, persistence potential, and therapeutic efficacy.

**Keywords:** CAR-T cells, CAR-T cell manufacturing, IL-7/IL-15, T cell characterization.

## **Abstract 181 | Poster | Poster Session Day 2**

### **Development and application of biofunctional surfaces for the study of axonal regeneration in the motor circuitry of rodents in vitro.**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Samuel Iwao Maia Horita

**Institution:** UFRJ — Rio de Janeiro

The motor circuit involves communication between two populations of motor neurons. Primary motor neurons have somas in layer V of the motor cortex and project axons to the spinal cord. Secondary motor neurons, also known as motoneurons, have somas in the ventral horn of the spinal cord and innervate effector muscles via their axons. Multiple axonal injuries can affect both populations of neurons, resulting in debilitating consequences for affected patients, especially in cases involving primary motor neuron damage. Neuronal degeneration and regeneration differ between the central and peripheral nervous systems due to cellular and molecular differences in the microenvironments surrounding these neurons. Various strategies have been employed to promote regeneration, but individual therapies do not provide long-lasting results. One alternative strategy is to use surfaces coated with extracellular matrix elements, such as laminin or a modified version of this protein called poly(laminin) (laminin at pH 4). In this study, in vitro cultures of cortical and spinal neurons were examined under the following conditions: laminin at pH 7, laminin at pH 4 (poly(laminin)), and a control condition using Poly-D-Lysine. We developed a new culture system based on a rectangular silicone chamber filled with PCL filaments functionalized with laminin or poly(laminin). Cortical or spinal tissue fragments were cultured in this system. The tissue fragments adhered well to the filaments and exhibited extensive cell migration and neuriteogenesis. This process was enhanced by the combination of PCL and laminin in both tissue types. Additionally, both monolayer cells and tissue fragments were characterized for the presence of the Lin28a protein, which was found to be expressed in neurons under all conditions and was also observed in spinal neurons from adult mice. Additionally, the presence of the Lin28a protein was characterized in both monolayer cells and tissue fragments. This protein was found to be expressed in neurons under all conditions and was also observed in spinal neurons from adult mice. Thus, the developed model indicated that PCL filaments biofunctionalized with laminin-modified surfaces promote adhesion, migration, and neuriteogenesis. This represents a promising strategy for in vitro models studying neuronal regeneration.

**Keywords:** Regeneration 2. Biomaterials 3. Motor neurons 4. Spinal cord

## Abstract 182 | Poster | Poster Session Day 2

### Neuroprotective Gene Therapy with AAV2-NGN2 in a Rat Model of Glaucoma

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Thaís Costa Porto Marinho; COSME FILIPE COSTA FIGUEREDO; GABRIELA SILVA ALMEIDA; HILDA PETRS SILVA; MARIANA SANTANA DIAS; RAFAEL LINDEN; Victor Guedes de Araujo; VINICIUS DE TOLEDO RIBAS

**Institution:** Gene Therapy and Viral Vectors Laboratory, Carlos Chagas Filho Institute of Biophysics, UFRJ, Brazil; Laboratory of Neurogenesis, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro; Laboratory of Neurobiology, Department of — Rio de Janeiro-RJ

**Introduction:** Glaucoma is the leading cause of irreversible blindness worldwide. It encompasses a heterogeneous group of optic neuropathies defined by the progressive degeneration of retinal ganglion cells (RGCs) and their axons. Currently, no cure exists; available treatments are restricted to pharmacological interventions and surgical procedures designed to manage intraocular pressure (IOP), the elevation of which remains the primary modifiable risk factor for disease progression. Consequently, there is a profound interest in developing novel therapeutic approaches focused on neuroprotection and neuronal regeneration. Neurogenin-2 (NGN2) is an essential, early-stage transcription factor in neurodevelopment, playing a critical role in the differentiation of retinal progenitor cells into neurons, including RGCs. Furthermore, NGN2 is involved in axonal guidance and neuronal circuit formation during development. In this context, we hypothesize that the overexpression of pro-neuronal factors essential for developmental axonal growth, such as NGN2, can recapitulate the embryonic environment to promote axonal extension and RGC neuroprotection. **Methods:** To test this, the present study evaluated the therapeutic potential of rAAV2-mediated NGN2 expression in driving cellular neuroprotection using a rat model of ocular hypertension (OHT)-induced glaucoma. One-month-old Lister Hooded rats of both sexes were randomly assigned to three groups and received a 5  $\mu$ L intravitreal injection of saline, rAAV2-hSyn-mCherry ( $1.0 \times 10^{10}$  vg/ $\mu$ L), or rAAV2-hSyn-NGN2-hSyn-mCherry ( $1.0 \times 10^{10}$  vg/ $\mu$ L). Four weeks post-injection, ocular hypertension (OHT) was induced via full-circle limbal plexus cauterization. After 30 days of injury, we analyzed in vivo retinal structure by optical coherence tomography (OCT) and function by electroretinogram, followed by retinal harvest and preparation for immunohistochemistry with anti-RBPMS antibody for quantification of retinal ganglion cell (RGC) density. Images were acquired by confocal microscopy, and statistics were performed in GraphPad Prism 8.0. **Results:** We showed that NGN2 overexpression attenuated optic nerve head cupping compared to the glaucoma group. Preliminary photopic electroretinography data indicated a trend toward an increase in Photopic Negative Response (PhNR) amplitude in the OHT+NGN2 group, suggesting a potential mitigation of RGC functional decline. Additionally, we observed a significant increase in RGC density within the GCL of the OHT+NGN2 group relative to the OHT group (OHT  $1012 \pm 100.7$ , N=6; OHT+NGN2  $1561 \pm 62.17$ , N=5). **Conclusion:** Taken together, our findings suggest that NGN2 promotes RGC survival in an experimental model of glaucoma. Ultimately, this work provides a more comprehensive characterization of NGN2-mediated neuroprotection, offering a foundation for future gene therapy strategies targeting RGC loss in glaucoma.

**Keywords:** Glaucoma, rAAV, RGCs, Gene Therapy and NGN2

## **Abstract 183 | Poster | Poster Session Day 2**

### **Establishment of a biotechnological process for the production and characterization of spheroids derived from human umbilical cord mesenchymal stromal cells as a new platform for studying the pathophysiology and treatment of COPD/emphysema**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Arthur Carmona Vilar; Eder Zucconi; Isabella da Silva Carvalho; João Tadeu Ribeiro-Paes; Lincoln Gozzi Moro; Lucas Pires Guarnier; Mariane Secco; Vinícius Augusto Simão

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Chronic Obstructive Pulmonary Disease (COPD) is a multifactorial and heterogeneous lung condition characterized by chronic respiratory symptoms due to abnormalities in the airways and alveoli that cause persistent and progressive airflow obstruction. COPD ranks as the third leading cause of death worldwide, representing a major public health problem. Despite significant advances in clinical management, an effective therapy—other than palliative care—has not yet been achieved. In this context, cell therapy using monolayer (2D) cells emerges as a promising therapeutic strategy. 2D cell cultures represent a classic experimental model widely used in toxicology and genotoxicity studies; however, they are limited in their ability to mimic paracrine and extracellular matrix (ECM) interactions and, therefore, do not adequately reproduce in vivo tissues in vitro, compromising the translation of results. In light of this, three-dimensional (3D) cultures, such as spheroids, provide a microenvironment more closely resembling in vivo tissue, promoting paracrine communication and recapitulating cellular functions in a more physiological manner. Among the various cellular sources, human umbilical cord stromal cells (UC-MSCs) stand out for their high proliferative capacity and low immunogenicity. Thus, this study aims to establish and characterize a reproducible methodology for obtaining UC-MSC spheroids, with the goal of developing a platform for studies on the pathophysiology and cell therapy of COPD. To this end, the UC-MSCs were phenotypically characterized by flow cytometry, and the spheroids were formed in 96-well U-bottom plates coated with 2% agarose, with 10,000 cells per well. Morphometric, histological, immunofluorescence, cell viability, and differentiation analyses were performed to characterize the culture. The spheroids ( $n = 8$ ) exhibited volumetric compaction by the third day of culture, remaining stable with a sphericity index close to 1 through the 14th day analyzed. Histologically, the spheroids exhibited organization and internal integrity, as confirmed by H&E staining. The immunofluorescence assay demonstrated the maintenance of the stromal progenitor phenotype through the expression of nestin. The adipogenic differentiation assay was validated by positive staining of lipid vesicles (Oil Red O), and chondrogenic differentiation was validated by the accumulation of extracellular matrix proteoglycans (Toluidine Blue). Cell viability assessed by the Live/Dead assay ( $n=3$ ) showed a decline by the third day (63%) and recovery by the 21st day analyzed (85%). Cell viability assessed by MTT ( $n=4$ ) demonstrated statistically significant metabolic activity ( $p=0.0028$ ). The results obtained allow us to conclude that the spheroids formed from UC-MSCs exhibited histological and phenotypic characteristics consistent with three-dimensional MSC culture, providing the basis for new research approaches in the pathophysiology and treatment of COPD.

**Keywords:** COPD; tridimensional cell culture; UC-MSC; characterization; cell therapy.

## **Abstract 184 | Poster | Poster Session Day 2**

### **Use of umbilical cord mesenchymal stromal cells as a new therapeutic approach in a preclinical model of pulmonary emphysema induced by exposure to cigarette smoke**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Isabella da Silva Carvalho; Arthur Carmona Vilar; Eder Zucconi; João Tadeu Ribeiro-Paes; Lincoln Gozzi Moro; Mariane Secco; Rafael Guilen de Oliveira; Vinícius Augusto Simão

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Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous lung condition characterized by chronic respiratory symptoms (dyspnea, cough, and exacerbations) resulting from abnormalities in the airways (bronchitis) and/or alveoli (emphysema). The main environmental factors include tobacco smoke, pollution, and occupational exposure to toxic gases and particles. From an epidemiological perspective, COPD represents a serious public health problem, being the third leading cause of death worldwide. Despite significant advances in the treatment of COPD, an effective clinical cure has not been achieved, and the available approaches are palliative. In this context, stem cell therapy represents a potentially promising therapeutic approach for COPD. Umbilical cord-derived mesenchymal stromal cells (UC-MSCs), in particular, have attracted considerable interest in the treatment of COPD, as they exhibit greater proliferative and differentiation capacity and lower immunogenicity, favoring the use of UC-MSCs in allogeneic transplants. In this context, the present study aimed to evaluate the therapeutic efficacy of UC-MSCs in a murine model of pulmonary emphysema induced by exposure to cigarette smoke. To this end, male Swiss strain mice were exposed to cigarette smoke three times a day for 60 consecutive days. Seven days after the end of exposure, animals in the treatment group ( $n = 9$ ) received an intravenous infusion of  $5 \times 10^5$  cells/kg, while the control group ( $n = 10$ ) received no therapeutic intervention. After euthanasia, the lungs were collected and processed for histological analysis. Hematoxylin and eosin staining was used to determine the mean linear intercept (Lm), a morphometric marker of alveolar destruction, while Orcein staining allowed for the quantification of elastic fibers (EF) in the tissue, indicating the integrity of the elastic matrix. The UC-MSCs were previously characterized by flow cytometry and in vitro cell differentiation assays. Based on the results obtained, a statistically significant reduction in Lm was observed in the treated group compared to the control group, both in the right lung (Mann-Whitney,  $p=0.0008$ ) and in the left lung (Student's t-test,  $p<0.0001$ ). A greater number of elastic fibers was also observed in animals treated with UC-MSCs compared to the control group (Student's t-test,  $p=0.0433$ ). The isolated cells exhibited morphological characteristics typical of UC-MSCs, the ability to differentiate into chondrogenic and adipogenic lineages, and an immunophenotypic profile consistent with mesenchymal cells. Given these findings, the results corroborate the efficacy, in a murine model, of UC-MSCs in regenerating histopathological changes associated with emphysema, as evidenced by reduced alveolar destruction and greater preservation of the pulmonary elastic matrix. In summary, the results obtained support the proposal that the use of UC-MSCs represents a potential therapeutic approach in an experimental model of COPD.

**Keywords:** COPD, Cell therapy, UC-MSC, Murine model, Heterologous transplantation

## Abstract 185 | Poster | Poster Session Day 2

### A CRISPR/Cas9-Generated HEK293 Model of Mucopolysaccharidosis Type II

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** André Hennigen

**Institution:** Universidade Federal do Rio Grande do Sul — Porto Alegre - Rio Grande do Sul

Mucopolysaccharidosis type II (MPS II, Hunter syndrome) is a rare X-linked lysosomal storage disease caused by iduronate-2-sulfatase (IDS) deficiency. Impaired degradation of heparan and dermatan sulfate leads to progressive glycosaminoglycan (GAG) accumulation within lysosomes, disrupting cellular homeostasis. MPS II affects multiple organs, often with neurological involvement and shortened survival. Enzyme replacement therapy (ERT) improves some manifestations but does not reach the central nervous system or reverse established sequelae. Cellular models help investigate disease mechanisms and screen potential therapies. We generated and characterized an IDS-knockout HEK293 model. Cells were transfected with an all-in-one CRISPR/Cas9 plasmid encoding GFP and an sgRNA targeting IDS exon 3. After 48 h, single GFP-positive cells were sorted into 96-well plates, clonally expanded, and screened by Sanger sequencing. The selected HP18 clone carried c.261\_266del6/c.259\_261del3 alterations. IDS activity was measured by fluorimetry, while specific GAG-derived oligosaccharides were quantified by tandem mass spectrometry (UHPLC-MS/MS). LysoTracker Red-99, MitoTracker Green, H2DCFDA, and acridine orange were analyzed by flow cytometry to assess lysosomal enlargement, mitochondrial mass, oxidative stress, and autophagy, respectively. Data are mean  $\pm$  SD. IDS activity was higher in wild-type (WT) cells ( $116.702 \pm 87.503$  nmol/h/mg protein) than in HP18 ( $2.936 \pm 1.556$ ;  $n = 8$ /group;  $p = 0.0079$ , Welch's  $t$  test). Conversely, GAG levels were higher in HP18 ( $10.000 \pm 7.071$  fmol/mg protein) than in WT ( $0.625 \pm 1.768$ ;  $n = 8$ /group;  $p = 0.0010$ , Mann-Whitney U test). DCF fluorescence was also higher in HP18 ( $1.089 \pm 0.010$ ) than in WT ( $1.029 \pm 0.024$ ;  $n = 3$ /group;  $p = 0.0165$ , Student's  $t$  test), indicating increased intracellular oxidation. MitoTracker Green showed a trend toward higher fluorescence in HP18 ( $3.054 \pm 0.224$ ) than in WT ( $2.576 \pm 0.228$ ;  $n = 3$ /group;  $p = 0.0606$ ). LysoTracker Red fluorescence was almost twice as high in HP18 cells ( $2586.5 \pm 342.9$ ) as in WT cells ( $1370.0 \pm 162.6$ ), but statistical significance could not be established with two replicates per group ( $p = 0.3333$ , Mann-Whitney U test). Acridine orange did not differ between groups ( $p = 0.3921$ ). The higher MitoTracker signal may reflect compensatory mitochondrial biogenesis or accumulation of dysfunctional mitochondria due to impaired turnover; either could contribute to the higher DCF signal. The LysoTracker result is compatible with increased lysosomal content, but requires confirmation in new experiments with more replicates and confocal analysis of lysosomal number and morphology. The IDS knockout clone reproduces the primary biochemical features of MPS II and may help investigate secondary cellular mechanisms and screen candidate treatments, including novel ERT and gene-based approaches. Financial Support: CNPq, CAPES, FAPERGS and FIPE-HCPA.

**Keywords:** Hunter syndrome; mucopolysaccharidosis; lysosomal storage disease; CRISPR/Cas9; knockout cell model

## **Abstract 186 | Poster | Poster Session Day 2**

### **Transient Small-Molecule Conditioning Enhances Both Fresh Cytotoxic Potency and Post-Thaw Recovery and Effector Fitness in Human NK Cells**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Matheus Henrique dos Santos

**Institution:** Fundação Hemocentro de Ribeirão Preto, Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo — Ribeirão Preto

The research on clinical translation of adoptively transferred NK cell products relies mainly on modifying NK cells to enhance its effector functions, however a major barrier is the functional loss following cryopreservation, a manufacturing step required for off-the-shelf products<sup>1</sup>. We investigated whether transient exposure to a small-molecule pharmacological agent during ex vivo expansion enhances NK cell cytotoxic function and, separately, whether the same brief exposure applied immediately before cryopreservation improves post-thaw cell fitness. NK cells were isolated from healthy donor PBMC and expanded using irradiated K562-mbIL15-mbIL21 feeder cells for 7 or 14 days. In one arm, cells at day 6 or day 13 at culture were treated for 24 hours with the agent, in a specific range of doses. After drug wash out, the cells were assessed for cytotoxicity against tumor cell line K562, degranulation (CD107a), Granzyme B/Perforin, surface receptors, mitochondrial potential/mass, supernatant IFN-gamma/TNF and lysosome staining. In a second arm, the cells received the same treatment immediately before cryopreservation; post-thaw viability, cytotoxicity, mitochondrial profile, and proliferative recovery (live-cell imaging) were compared to untreated-frozen controls. Extracellular flux analysis (Seahorse XF24) was performed on separate day 14 cultures. Both day 7 and day 14 treated cells showed increased killing of K562 cells, however without a rise in CD107a, Granzyme B, or Perforin. The cells also showed elevated levels of TNF-alpha in co-culture supernatant, besides no difference of IFN-gamma. They showed an activating-skewed receptor shift (CD11a, CD62L, NKp44, NKp46, DNAM-1 up; NKG2A, PD-1 down), and higher mitochondrial membrane potential and mass. No difference were observed in lysosomal staining. These results showed similarly across doses rather than scaling with dose. Day 14 Seahorse data showed a parallel plateau: basal extracellular acidification rate and glycolytic ATP rate elevated at all doses, with modestly reduced oxidative ATP fraction. Pre-freeze treatment of cultures at day 7 and 14 improved post-thaw outcomes: higher viability, higher cytotoxic capacity, healthier mitochondrial profiles, and greater proliferative recovery than untreated-frozen controls, with the cells frozen at day 7 showing higher overall recovery. Transient exposure to this small-molecule agent enhances NK cell cytotoxic potency independent of increased granule content, and the same exposure applied before cryopreservation improves post-thaw viability, function, and proliferative recovery. This dual effect together with the overall mitochondrial mass and membrane potency suggests a shared mitochondrial/bioenergetic and receptor-remodeling program may underlie both enhanced potency and cellular resilience. These findings support this conditioning approach as a manufacturing strategy to improve potency and cryopreservation robustness of adoptive NK cell products.

**Keywords:** NK cell; cryopreservation; pharmacological modulation

## Abstract 188 | Poster | Poster Session Day 2

### Suppressing Inflammation to Enable Repair: Gene-Engineered Chondrocytes Expressing IL-4 and TGF- $\beta$ 1 for Osteoarthritis

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Maria Mascarenhas; Camila Cohen Kaleka; Cynthia Alves Bartolomeo; Luana D'Mambro; Moisés Cohen; Roberta Sessa Stilhano

**Institution:** Faculdade de Ciências Médicas da Santa Casa de São Paulo — São Paulo-SP

**Introduction:** Osteoarthritis (OA) is the most prevalent joint disease and a leading cause of chronic pain and disability, yet no therapy durably regenerates articular cartilage or halts progression. Chronic low-grade synovial inflammation—driven by IL-1 $\beta$ , IL-6 and TNF- $\alpha$ —perpetuates cartilage degradation, whereas IL-4 (anti-inflammatory) and TGF- $\beta$ 1 (pro-chondrogenic) are chondroprotective. We propose an ex-vivo gene therapy in which human chondrocytes are engineered to express IL-4 or TGF- $\beta$ 1, aiming to dampen synovial inflammation while stimulating chondrocyte proliferation and matrix synthesis. **Objective:** To establish and validate a lentiviral platform for IL-4 and TGF- $\beta$ 1 overexpression in human chondrocytes (C-20/A4), and to validate an in-vitro inflammatory model, as a proof of concept for OA. **Methods:** Transfer plasmids pAIP-hIL4-co and pHAGE-TGFB1 were amplified and confirmed by restriction digestion (ApaI; XhoI) and gel electrophoresis. Lentiviruses were produced in HEK-293T by calcium-phosphate transfection (Stilhano et al., 2017) under BSL-2. Working puromycin (selection) and LPS (inflammatory stimulus) concentrations were defined by MTT (24–72 h). LV-TGF- $\beta$ 1 was titrated by GFP-positive area (ImageJ). Chondrocytes were transduced and selected by puromycin resistance (IL-4) or GFP sorting (TGF- $\beta$ 1). LPS-induced TNF- $\alpha$  and IL-6 mRNA was quantified by RT-qPCR (RPL35 reference). **Results:** Both plasmids showed the expected restriction patterns. Lentivirus production was successful (GFP+ HEK-293T). Puromycin cytotoxicity was dose- and time-dependent (48-h IC<sub>50</sub>  $\approx$  1.6  $\mu$ g/mL); 4  $\mu$ g/mL was chosen for selection. GFP titration of LV-TGF- $\beta$ 1 was viral-load-dependent (up to  $\sim$ 7.25% GFP+ area). Transduction with non-concentrated supernatant gave low efficiency ( $\sim$ 6.5% by flow cytometry), as expected; puromycin eliminated non-transduced controls while sparing IL-4-transduced cells, and sorting enriched GFP-high/low TGF- $\beta$ 1 populations. By RT-qPCR, LPS up-regulated TNF- $\alpha$  ( $\sim$ 11–17-fold) and IL-6 ( $\sim$ 2–8-fold) mRNA at 24 h versus unstimulated controls, broadly dose-related, confirming a pro-inflammatory transcriptional response and validating the inflammatory model. **Conclusion:** We established a reproducible ex-vivo lentiviral platform to engineer human chondrocytes expressing IL-4 or TGF- $\beta$ 1, obtained selected/enriched modified populations, and validated an LPS-driven inflammatory model in chondrocytes. This proof of concept supports ongoing functional assays (ELISA of IL-4/TGF- $\beta$ 1 and cytokines; transwell inflammation-modulation) and replication in primary human nasoseptal chondroprogenitors, advancing a combined cell-and-gene strategy for OA.

**Keywords:** gene therapy; osteoarthritis; basic research; cell therapy.

## **Abstract 189 | Poster | Poster Session Day 2**

### **Gene Therapy for Osteoarthritis and Focal Chondral Lesions in Large Animal Models: A Systematic Review and Meta-Analysis**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** MARIA MASCARENHAS; Luana D'Mambro; Maria Eduarda Amaral; Roberta Sessa Stilhano; Vinicius Monnerat

**Institution:** Faculdade de Ciências Médicas da Santa Casa de São Paulo — São Paulo-SP

**Purpose:** Osteoarthritis (OA) and focal chondral lesions are debilitating joint disorders characterized by progressive cartilage degradation and chronic pain. Current treatments offer limited symptomatic relief without reversing joint damage. Gene therapy has emerged as a promising disease-modifying strategy, providing sustained, targeted intra-articular delivery of therapeutic molecules. This systematic review and meta-analysis evaluate the efficacy and safety of gene therapy for OA and focal chondral lesions specifically in translational large animal models. **Materials & Methods:** We systematically reviewed the literature for in vivo and ex vivo gene therapy interventions evaluated exclusively in medium and large animal models (e.g., rabbits, dogs, sheep, minipigs, and horses). Applying strict inclusion and exclusion criteria, 23 studies were included in the final analysis (12 focusing on OA models and 11 investigating focal chondral lesions). Data regarding vector characteristics, molecular targets, and clinicopathological outcomes were extracted and analyzed. **Results:** The analyzed studies utilized viral (e.g., AAV, adenovirus, lentivirus) and non-viral vectors targeting anti-inflammatory (e.g., IL-1Ra, IL-10) and pro-chondrogenic (e.g., FGF-2, TGF- $\beta$ , SOX9, IGF-1) pathways. Gene therapy interventions significantly improved macroscopic, biochemical, and histological scores across both disease models compared to controls. Target gene overexpression consistently reduced cartilage breakdown, enhanced type II collagen synthesis, and decreased pro-inflammatory cytokines in synovial fluid. In osteochondral defect models, therapeutic gene delivery significantly improved subchondral bone reconstruction and mitigated perifocal OA development. The therapies were well-tolerated, with only mild, transient local immune responses reported for specific vectors. **Conclusion:** Gene therapy demonstrates significant efficacy as a disease-modifying intervention for OA and focal chondral lesions in translational large animal models. These findings strongly support the continued clinical development of targeted intra-articular gene delivery for joint preservation.

**Keywords:** gene therapy; osteoarthritis; basic research; cell therapy.

## **Abstract 190 | Poster | Poster Session Day 2**

### **Genetically engineered conditional HoxB8 progenitors as a versatile platform for studying hematological malignancies**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** Vitor Leonardo Bassan; Fabíola Attié de Castro

**Institution:** School of Pharmaceutical Sciences of Ribeirão Preto - University of São Paulo (FCFRP-USP) - Brazil — Ribeirão Preto - SP

**Introduction and aims:** HoxB8 cells are conditionally immortalized murine hematopoietic progenitors. They are maintained in a perpetually proliferative, undifferentiated state through the activity of an estrogen-dependent HoxB8 fusion protein. When beta-estradiol is withdrawn, the HoxB8 fusion protein is inactivated, prompting the cells to differentiate into distinct myeloid populations upon exposure to specific hematopoietic growth factors. Consequently, these cells provide a versatile experimental platform for investigating the mechanisms regulating hematopoietic differentiation and their disruption in diverse models, including hematological malignancies. Here, we investigated genetically engineered HoxB8 progenitors as an in vitro model to characterize the effects of BCR::ABL1, the oncogenic fusion that drives chronic myeloid leukemia (CML), on myeloid differentiation and cellular phenotype. **Methods:** Murine bone marrow-derived hematopoietic progenitors were transduced with MSCV-ERHBD-HoxB8 and maintained in the presence of beta-estradiol, stem cell factor (SCF), and Fms-like tyrosine kinase 3 ligand (Flt3L). HoxB8 progenitors were subsequently transduced with pMIG-BCR::ABL1-GFP or the corresponding empty vector (EV). Myeloid differentiation was induced by  $\beta$ -estradiol withdrawal and exposure to lineage-supporting cytokines, including GM-CSF and SCF/Flt3L. Cell proliferation and viability, morphological changes, immunophenotypic differentiation, and expression of PDE4D, a phosphodiesterase enzyme implicated in oncogenic cell survival, were evaluated throughout the differentiation process. **Results:** BCR::ABL1-expressing HoxB8 progenitors exhibited increased proliferative capacity and viability compared with EV controls. BCR::ABL1 expression was also associated with accelerated myeloid differentiation and altered distribution of differentiated cell populations, including a reduced frequency of dendritic cells. Moreover, BCR::ABL1+ cells exhibited increased PDE4D expression, indicating that altered cyclic nucleotide signaling accompanies the BCR::ABL1-driven phenotype and may contribute to the enhanced survival and differentiation changes observed in this model. **Conclusions:** Collectively, these findings demonstrate that genetically engineered HoxB8 progenitors provide a flexible and experimentally tractable platform for modeling oncogene-driven perturbations of hematopoiesis. By combining conditional control of myeloid differentiation with genetic manipulation, this system enables mechanistic investigation of leukemia-associated alterations in cell survival and lineage differentiation and may provide a valuable platform for identifying and evaluating potential therapeutic targets.

**Keywords:** hematology; immunology; genetic engineering; chronic myeloid leukemia; cell culture; transfection; leukemia

## **Abstract 191 | Poster | Poster Session Day 2**

### **Spatial Mapping of CAR-T Cell Infiltration and Activation in Hypoxic Lymphoma Spheroids by SpheroMap Cytometry**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** GIOVANA BEATRIZ CAPUZ MORETO

**Institution:** Regional Blood Center of Ribeirão Preto, Ribeirão Preto Medical School, University of São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil — Ribeirão Preto - SP

The efficacy of CAR-T cell therapy depends not only on tumor recognition and cytotoxicity but also on the ability of engineered T cells to infiltrate and remain functional within the tumor microenvironment (TME). Hypoxia, stromal interactions, and spatial constraints may influence these processes, yet conventional in vitro assays provide limited information on regional CAR-T cell behavior. To address this limitation, we applied SpheroMap Cytometry, a 3D spatial flow cytometry approach that combines tumor spheroids with hypoxia-based compartmentalization to resolve immune-cell phenotypes in the hypoxic core and normoxic periphery. Heterotypic spheroids were established with CD19<sup>+</sup> Raji lymphoma cells and HS-5 mesenchymal stromal cells at a 1:4 ratio ( $4 \times 10^4$  cells/spheroid) in ultra-low attachment U-bottom 96-well plates. At 24 h, anti-CD19 CAR-T cells (~48% transduction) or untransduced T cells (UTD) were added at  $4 \times 10^4$  cells/spheroid. Cultures were untreated or exposed to Dasatinib, a Src/Lck inhibitor, or vehicle control. Hypoxic regions were identified using Image-iT Green Hypoxia, followed by spheroid dissociation at 48 h and flow cytometric assessment of viability, CD3, CAR, CD25, and CD19. Microscopy demonstrated compact spheroid formation and development of a defined hypoxic core. CAR-T cells showed greater infiltration than UTD cells in untreated spheroids (40% vs 23%) and substantially reduced the residual CD19<sup>+</sup> tumor population (1% vs 11%), demonstrating CAR-dependent antitumor activity within the 3D model. Compartment-resolved analysis revealed that 76% of infiltrating T cells localized to the hypoxic core. Within this region, 48.9% of CD3<sup>+</sup> cells were CAR<sup>+</sup>, compared with 33.4% in the normoxic periphery, showing a higher frequency of CAR-expressing cells in the hypoxic compartment. Pharmacological interruption of CAR signaling with Dasatinib reduced CD3<sup>+</sup> infiltration from 40% to 20% and increased residual CD19<sup>+</sup> tumor cells from 1.16% to 18%. Dasatinib also decreased the proportion of CAR<sup>+</sup> cells in the hypoxic core from 48.9% to 30.9%. This inhibition was accompanied by a marked reduction in CD25 expression among CAR<sup>+</sup> cells, from 68.9% to 26.5% in hypoxia and from 44% to 14% in normoxia. In untreated cultures, higher CD25 expression was observed in hypoxic than normoxic CAR-T cells, suggesting enhanced activation within the tumor-enriched core, potentially reflecting the combined influence of antigen engagement and local hypoxic stress. Collectively, these results demonstrate that SpheroMap Cytometry enables spatially resolved assessment of CAR-T cell infiltration and activation across distinct regions of the TME. This platform provides a scalable strategy to investigate CAR-T function and pharmacological modulation within spatially organized, hypoxic 3D tumor models, supporting its application in the preclinical evaluation of next-generation cell therapies. This study was financed by FAPESP, Brazil (Process #2022/12856-6), and CAPES.

**Keywords:** CAR-T cells; Hypoxia; 3D tumor spheroids

## **Abstract 192 | Poster | Poster Session Day 2**

### **Genotype-phenotype correlation and modifying factors in recessive dystrophic epidermolysis bullosa - a cross-sectional observational study in a Brazilian cohort.**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** MEL DOMINICI SANFINS; Jeanine Magno; Laurent Ketlen Leão Viana; PRISCILA KEIKO MATSUMOTO MARTIN; Raymundo Machado de Azevedo Neto

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Epidermolysis bullosa (EB) comprises a heterogeneous group of inherited disorders characterized by skin and mucosal fragility, resulting in blistering and erosions following minor mechanical trauma. Recessive dystrophic epidermolysis bullosa (RDEB), one of the most prevalent EB subtypes in Brazil, is caused by biallelic pathogenic variants in the COL7A1 gene. These variants lead to reduced or absent type VII collagen expression and result in a broad spectrum of clinical manifestations and disease severity. This study aims to investigate correlations between COL7A1 variants and the clinical manifestations of individuals with RDEB, as well as to explore potential genetic, demographic, and clinical factors that may contribute to phenotypic variability. This is an observational study combining retrospective data from the DEBRA Brasil registry with information obtained through a standardized cross-sectional clinical questionnaire. Demographic characteristics, clinical manifestations, disease complications, and treatment-related variables are being assessed. Clinical data have already been collected from 90 individuals with RDEB and are currently being integrated and compared with their molecular genetic findings. Descriptive and inferential statistical analyses will be performed to investigate associations between genotypes and clinical outcomes. Regression models will also be applied to identify potential factors associated with disease severity, with statistical significance defined as  $p < 0.05$ . The study involves minimal risk and is being conducted in accordance with applicable ethical principles. All participants, or their legal guardians, provided informed consent, and all data are stored and analyzed in anonymized form. This study is expected to improve the understanding of genotype-phenotype relationships in a genetically diverse Brazilian RDEB cohort. The findings may support more accurate prognostic assessment, individualized clinical follow-up, genetic counseling, patient stratification, and the development of targeted therapeutic strategies.

**Keywords:** Colágeno Tipo VII; Correlação Genótipo-fenótipo; Doenças Genéticas Raras; Epidermólise Bolhosa Distrófica Recessiva; Heterogeneidade Fenotípica

## **Abstract 193 | Poster | Poster Session Day 2**

### **A High-Content Screening Coculture Platform to Evaluate MicroRNA-Mediated Stromal-Immune Modulation**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** Larissa Ignácio da Silva

**Institution:** Fundação Hemocentro de Ribeirão Preto — Ribeirão Preto- SP

The tumor microenvironment (TME) is a dynamic ecosystem in which stromal and immune cells interact to regulate cellular behavior and immune responses. Stromal cells can actively modulate immune cells through soluble factors, extracellular matrix components and other intercellular signals. MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression capable of altering cellular phenotypes and intercellular communication. However, the functional consequences of miRNA modulation in stromal cells and their impact on immune cell responses remain incompletely understood. Here, we established a coculture-based functional screening model to evaluate whether candidate miRNAs introduced into HS-5 stromal cells could modulate NF- $\kappa$ B reporter activity in cocultured Jurkat T cells. HS-5 stromal cells were reverse-transfected with 16 individual pre-miRNAs. After 48 h, Jurkat NF- $\kappa$ B-eGFP reporter cells were preactivated with PMA/ionomycin for 2h and added to the HS-5 cultures. Controls conditions were included. High-content screening (HCS) was performed at 0, 6, 12 and 24 h after coculture establishment. Automated image analysis was used to identify Jurkat cells and quantify NF- $\kappa$ B reporter activation based on the percentage of eGFP-positive cells and eGFP mean fluorescence intensity (MFI). Cells were classified as eGFP-positive using a fluorescence cutoff of 0.048, established from the 95th percentile of the non-activated control distribution at 0 h. Data were analyzed by two-way ANOVA followed by multiple comparisons against the negative control. The coculture system showed a clear temporal response, with low reporter activity at 0 h (13–20% eGFP-positive cells), a marked increase at 6 h (approximately 60–75%), sustained activation at 12 h, and a decline at 24 h. Activated and non-activated controls showed clearly distinguishable responses. Two-way ANOVA revealed a highly significant effect of time ( $F = 10237.24$ ,  $p < 0.0001$ ), accounting for 90.28% of the variance, as well as a significant treatment-by-time interaction ( $F = 21.59$ ,  $p < 0.0001$ ), accounting for 3.62% of the variance and indicating that treatment effects varied across the activation kinetics. A significant overall treatment effect was also observed ( $F = 5.65$ ,  $p < 0.0001$ ). eGFP positivity and MFI provided complementary measures of reporter activity, suggesting that treatment-associated differences may involve changes in both the proportion of responding cells and reporter signal magnitude. This study establishes an image-based HS-5/Jurkat NF- $\kappa$ B-eGFP coculture platform for functional evaluation of candidate miRNAs. The robust temporal response and significant treatment-by-time interaction demonstrate its capacity to detect differences in the dynamics of immune-cell NF- $\kappa$ B activation following stromal miRNA modulation. This model provides a quantitative proof of concept for candidate prioritization and subsequent expansion to larger miRNA libraries and mechanistic validation.

**Keywords:** MicroRNAs, Tumor Microenvironment, High-Content Screening, Post-Transcriptional Regulators

## Abstract 194 | Poster | Poster Session Day 2

### Transient CD62L<sup>+</sup>CD38<sup>+</sup> NK-Cell States Precede Adaptive Immune Reconstitution After Allogeneic Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia.

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Gabriel Andrade de Paula

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**Introduction:** Allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative treatment for acute myeloid leukemia (AML). Natural killer (NK) cells are among the earliest lymphocytes to recover after transplantation and contribute to antiviral immunity and graft-versus-leukemia (GVL) activity. However, numerical recovery does not necessarily reflect phenotypic maturation or functional reconstitution. CD62L and CD38 may identify transitional NK-cell phenotypes during immune reconstitution, but their dynamics after allo-HCT remain poorly understood. **Objective:** To characterize CD62L/CD38-defined NK-cell phenotypes during immune reconstitution following allo-HCT for AML and evaluate their relationship with T-cell recovery. **Methodology:** Peripheral blood samples from four AML patients undergoing allo-HCT were collected before transplantation and on days +30, +90, +180, and +360; one patient had no D+30 sample. Peripheral blood mononuclear cells were analyzed by multiparameter flow cytometry. NK cells were identified as viable CD45<sup>+</sup>CD3<sup>-</sup>CD56<sup>+</sup> lymphocytes and classified into four phenotypic states according to CD62L/CD38 co-expression: CD62L<sup>+</sup>CD38<sup>+</sup>, CD62L<sup>+</sup>CD38<sup>-</sup>, CD62L<sup>-</sup>CD38<sup>+</sup>, and CD62L<sup>-</sup>CD38<sup>-</sup>. T-cell recovery was assessed by CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> frequencies, and NK/T-cell ratios were calculated. Analyses were descriptive and exploratory. **Results:** Early immune reconstitution was characterized by transient expansion of the CD62L<sup>+</sup>CD38<sup>+</sup> NK-cell phenotype between D+30 and D+90. This population increased from 1.35% to 52.0%, from 13.9% to 48.4% at D+30, and from 5.96% before transplantation to 79.5% at D+90 in representative patients. Subsequently, this phenotype declined at D+180 and D+360, with progressive redistribution toward CD62L<sup>-</sup>CD38<sup>+</sup> and CD62L<sup>-</sup>CD38<sup>-</sup> phenotypes. NK/T-cell ratios demonstrated early NK predominance, with median NK/CD3, NK/CD4, and NK/CD8 ratios increasing from 0.28, 0.63, and 1.11 before transplantation to 3.11, 7.18, and 17.65 at D+30, followed by decline to 0.05, 0.12, and 0.18 at D+360. The magnitude of early NK predominance varied across patients. **Discussion:** NK-cell reconstitution following allo-HCT involves a transient CD62L<sup>+</sup>CD38<sup>+</sup> phenotype preceding broader T-cell recovery. NK/T-cell ratios reflect early NK predominance but are influenced by T-cell depletion and therefore should not be interpreted as surrogates for absolute immune reconstitution. The subsequent decline of CD62L<sup>+</sup>CD38<sup>+</sup> cells together with the emergence of CD62L<sup>-</sup>CD38<sup>+</sup> and CD62L<sup>-</sup>CD38<sup>-</sup> phenotypes suggests progressive phenotypic maturation and inter-patient heterogeneity during immune reconstitution. **Conclusion:** Early immune reconstitution after allo-HCT is characterized by transient expansion of CD62L<sup>+</sup>CD38<sup>+</sup> NK-cell phenotypes, followed by progressive phenotypic diversification. Combined assessment of NK-cell phenotypes and NK/T-cell ratios provides a broader framework for evaluating immune reconstitution and warrants validation in larger cohorts.

**Keywords:** Natural killer cells; allogeneic hematopoietic stem cell transplantation; immune reconstitution; CD62L; CD38.

## Abstract 195 | Poster | Poster Session Day 2

### DEVELOPMENT OF AN IMMUNE VHH LIBRARY FROM LLAMA IMMUNIZED WITH RAJI CELLS FOR TARGETING B-CELL MALIGNANCY MEMBRANE PROTEINS VIA BRASIL CELL-PANNING

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Joao Duarte de Paula Coutinho Marques; Andrea queiroz maranhao; Carla Celedonio; MARTÍN BONAMINO; Maurício Tilburg

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Monoclonal antibodies and derived single-domain antibodies (VHH/nanobodies) are powerful immunological tools to be applied in different areas of research and immunotherapies, including B-cell malignancies. Camelid nanobodies combine high stability, small molecular weight, and high tissue penetration. In silico sequence alignment revealed about 65% identity between the extracellular domains of human CD19, CD20, and CD22 compared to their alpaca/lama orthologs, supporting the justification for provoking a robust cross-species humoral immune response. This study aimed to generate and validate an immune VHH library derived from a llama immunized with human B-cell lymphoma cells (Raji line), for selection of nanobodies on whole intact cells using the BRASIL (Biopanning and Rapid Analysis of Selective Interactive Ligands) method. A llama glama was immunized with whole Raji cells ( $2 \times 10^7$  cells/immunization) emulsified in Montanide adjuvant. Sera titers were evaluated by indirect ELISA against cytosolic and membrane protein fractions. Due to an initial predominant response against cytosolic proteins, subsequent booster injections were optimized using purified Raji membrane protein extracts (150 ug ). Target antigen presence (CD19, CD20, and CD22) in the membrane fraction was confirmed by dot blot. Humoral response maturation was monitored via cell-based ELISA against intact cells. Peripheral blood mononuclear cells were collected for RNA extraction, cDNA synthesis, and VHH gene amplification to construct the immune VHH library. Combining intact cells and purified membrane protein boosters successfully redirected the immune response toward surface antigens, achieving a serum titer of 1:10,000 against membrane extracts and 1:32,000 in cell-based ELISA against intact Raji cells. To select surface-specific nanobodies, cell lines with differential target expression, including wild-type and engineered K562, NALM-6, and Raji cells, were established. VHH fragments will be used to build the phage library and the BRASIL strategy will be used to select specific nanobodies, relying on organic phase differential centrifugation to isolate cell-bound phage from unbound particles in a single step, minimizing background noise and cell loss during subtractive and positive selection rounds. The immunization strategy combining whole Raji cells and membrane extracts generated a sufficient titer immune response against Raji cells. The VHH library to be built, coupled with established differential cell lines (K562, NALM-6, and Raji) and the BRASIL cell-panning method, provides a high-efficiency platform to select specific nanobodies against immunogenic B-cell surface proteins, such as CD19, CD20, and CD22.

**Keywords:** Nanobodies, B-cell, Cancer, VHH Library

## Abstract 196 | Poster | Poster Session Day 2

### A High-Content Screening Approach to Assess the Impact of Hypoxia on CAR-T Cell Function Using a 2D Tumor-Stroma Model

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** João Pedro Zanon Muraroli

**Institution:** Regional Blood Center of Ribeirão Preto, Ribeirão Preto Medical School, University of São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil — Ribeirão Preto-SP

The tumor microenvironment (TME) imposes structural and metabolic barriers that compromise CAR-T cell efficacy in solid tumors and in hypoxic niches of hematological malignancies, with the hypoxia-stroma axis being a central driver of this resistance. However, conventional 2D cytotoxicity assays lack the ability to quantify CAR-T performance across oxygen gradients in this context. We used Coverslip Hypoxia High-Content Screening (CH-HCS) to spatially quantify the impact of hypoxia on CAR-T cell function across distinct B-cell lymphoma/leukemia lineages. A total of  $10^4$  cells/well umbilical cord mesenchymal stromal cells (UC-MSCs) were co-cultured with CD19+ Raji, Jeko-1 or Nalm6 cells ( $10^4$  cells/well, eFluor 670-labeled), together with anti-CD19 CAR-T cells or untransduced T cells ( $2 \times 10^4$  cells/well, CellTrace Violet-labeled) in 96-well plates, in quintuplicate. Radial hypoxia gradients were generated using a 5-mm glass coverslip (CS); in parallel, Raji/CAR-T co-cultures were performed without UC-MSCs to assess the stromal contribution to cytotoxicity. Whole-well images (25-field montages) were acquired at 0, 12, 24 and 48h on an ImageXpress Micro XLS HCS system and analyzed using CellProfiler and KNIME. Four concentric regions of interest (ROIs) relative to the CS — InnerCore (severe hypoxia), OuterCore (moderate hypoxia), Periphery (transition zone) and Outside (normoxia) — enabled spatially resolved quantification of tumor and effector cell death (SYTOX Green). Data were analyzed by two-way ANOVA with Sidak's post-test. In Raji co-cultures, tumor death in normoxia increased over time, from ~30% at 12h to >60% at 48h, while hypoxic regions (Inner/OuterCore) remained below 10% throughout ( $p < 0.0001$ ); the Periphery zone showed an intermediate increase (~10 to ~30%). Jeko-1 showed a similar pattern, with ~70% death in normoxia against ~20% in hypoxia at 48h ( $p < 0.01$ ); in Nalm6, CAR-T activity was nearly abolished under hypoxia despite ~65% tumor death in normoxia ( $p < 0.0001$ ). The Raji/CAR-T co-culture without UC-MSCs showed no significant ROI-dependent differences in tumor death, reaching 50–60% at 48h even in hypoxic ROIs, suggesting that the mesenchymal stromal component contributes to establishing hypoxia-driven immunosuppression of CAR-T cytotoxicity. Raji co-cultured with untransduced T cells likewise showed no ROI-dependent differences, with negligible cytotoxicity across all regions, confirming antigen-driven CAR-T specificity. These findings show that hypoxic gradients selectively impair CAR-T cytotoxicity while preserving specificity, and that the mesenchymal stromal component contributes to hypoxia-driven immunosuppression, reinforcing its relevance in this model. The CH-HCS platform offers a robust preclinical tool to screen CAR-T constructs for cytotoxicity and persistence under hypoxia-driven immunosuppression. This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil. Process Number #2022/12856-6; and CAPES.

**Keywords:** CH-HCS, Hypoxia, CAR-T, 2D model, TME

## Abstract 197 | Poster | Poster Session Day 2

### Optimization of Primary CAR-NK Manufacturing Identifies Pseudotype-Specific Responses to Antiviral Pathway Modulation

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Renata Nacasaki Silvestre; Maria Vitória Oliveira de Souza; Mário Soares de Abreu Neto; Péricles Natan Mendes da Costa; Rafaela Rossetti; Rodrigo do Tocantins Calado de Saloma Rodrigues; Tárík Reis Heluy; VIRGINIA PICANCO E CASTRO

**Institution:** Fundação Hemocentro de Ribeirão Preto — Ribeirão Preto-SP

**Introduction:** Efficient expansion and genetic modification of primary natural killer (NK) cells remain major challenges for scalable CAR-NK manufacturing. Here, we systematically evaluated GMP-compatible culture conditions and lentiviral transduction strategies to establish a translational workflow for primary CAR-NK cell production. **Methods:** Peripheral blood mononuclear cells were recovered from leukoreduction filters from two donors, and NK cells were isolated by magnetic negative selection. Cells were expanded in AIM V, NK MACS, or CTS NK-Xpander medium supplemented with IL-2 and 5% CTS Immune Cell Serum Replacement, with irradiated feeder cells added every 7 days. Expansion, immunophenotype, glucose consumption, and lactate production were monitored. Based on expansion performance, CTS NK-Xpander was selected for CAR engineering. NK cells were activated with IL-1 $\beta$  and IL-15 and transduced on day 2 with anti-CD19 lentiviral vectors pseudotyped with BaEV (MOI 0.5) or VSV-G (MOI 10). To investigate whether transient modulation of antiviral restriction could enhance transduction, cyclosporin H (8  $\mu$ M) and 5Z-7-oxozeaenol (1.2  $\mu$ M) were evaluated alongside untreated controls. CAR expression was assessed by flow cytometry 5 and 8 days after transduction. **Results:** CTS NK-Xpander markedly outperformed AIM V and NK MACS, supporting 4.4- to 17.4-fold NK-cell expansion by day 17, whereas expansion in the other media remained  $\leq$ 1.6-fold. Expanded cultures retained high NK-cell purity, with CD3-CD56+ cells representing 94.6-96.2% of endpoint cultures. Glucose consumption and lactate production were comparable among culture conditions. Lentiviral transduction was strongly dependent on vector pseudotype and construct. For VSV-G-pseudotyped vectors, baseline CAR expression was low in both donors (0.1-2.9%) but consistently increased following pharmacological modulation, reaching 5.0-11.7%. In contrast, BaEV-EF1 vectors achieved substantially higher baseline CAR expression (17.8-20.9%), with no improvement following pharmacological treatment (12.2-19.1%). A BaEV-SFFV construct evaluated in one donor showed a modest increase from 10.0% to 12.9% following treatment. **Conclusion:** CTS NK-Xpander provided the most effective culture platform for primary NK-cell expansion while maintaining high NK-cell purity. Lentiviral engineering showed a pronounced pseudotype-dependent pattern: transient antiviral pathway modulation consistently enhanced VSV-G-mediated CAR expression but provided no consistent benefit to BaEV-pseudotyped vectors. These findings indicate that strategies used to enhance primary NK-cell transduction should be tailored to the vector pseudotype and construct rather than applied universally, and provide a framework for further optimization of scalable CAR-NK manufacturing.

**Keywords:** CAR-NK cell, lentiviral vector, cell culture, CAR-NK manufacturing

## **Abstract 199 | Poster | Poster Session Day 2**

### **Development of a 3D Bioprinted Cardiomyocyte Culture with CRISPR-Cas13-Mediated Modulation of EGLN1 as a Strategy to Induce Angiogenesis**

**Category:** Tissue engineering, biomaterials and advanced in vitro models

**Authors:** JULIANA DIAMANTINO; CYNTHIA SILVA BARTOLOMEIO; ERONILDO ALVES PINTO JÚNIOR; ROBERTA SESSA STILHANO YAMAGUCHI

**Institution:** Departament of Physiological Sciences, Faculdade de Ciências Médicas da Santa Casa de São Paulo (FCMSCSP) — São Paulo-SP

**Introduction:** Ischemic heart disease is the leading cause of death worldwide. After infarction, the peri-infarct myocardium remains hypoperfused, and revascularizing this border zone is critical to limit adverse remodeling and preserve contractile function. Under normoxia, PHD2 (encoded by EGLN1) hydroxylates HIF-1 $\alpha$  and targets it for degradation, restraining angiogenesis. CRISPR-Cas13 cleaves EGLN1 mRNA and stabilizes HIF-1 $\alpha$  without permanent genome editing. **Objectives:** To develop an alginate-gelatin bioink for cardiac bioprinting and to assess whether EGLN1 targeting by CRISPR-Cas13 in bioprinted hiPSC-derived cardiomyocytes (hiPSC-CMs) induces angiogenic behavior in surrounding endothelial cells. **Methods:** Four bioinks combining two polymer concentrations (Alg 3.6%/Gel 5.45%; Alg 4%/Gel 6%) with two solvents (supplemented DMEM or 0.9% saline) were characterized by rheology (sol-gel transition, amplitude and frequency sweeps, flow curves). Cytocompatibility was assessed in H9C2 cardiomyoblasts by MTT (24 and 48 h extracts) and Live/Dead (24 h and 7 days), quantified in Fiji/ImageJ. hiPSC-CMs transduced with a lentiviral vector carrying RfxCas13d and an EGLN1-targeting gRNA (L1C3) were bioprinted in the selected bioink. Pro-angiogenic potential was assessed by bead sprouting: HUVEC-coated Cytodex 3 microcarriers in fibrin gels were co-cultured with control or L1C3 constructs for 96 h under normoxia, without exogenous VEGF. **Statistics:** one-way ANOVA with Šídák, Mann-Whitney, and Fisher's exact test. **Results:** The solvent was the dominant determinant of gelation temperature (24.7-25.3 °C in DMEM vs 23.3-23.5 °C in saline), with an optimal printing window of 20-22 °C. C1 (Alg 4%/Gel 6%, DMEM) outperformed its saline counterpart in both assays (MTT at 48 h,  $p=0.0004$ ; Live/Dead at 7 days, median viability 87.85% vs 72.99%,  $p=0.0004$ ) and was selected for hiPSC-CM bioprinting. In the bead sprouting assay, 12.0% of beads co-cultured with L1C3 constructs displayed vascular sprouts (9/75 beads, 16 fields), whereas none were observed in controls (0/53 beads, 8 fields; Fisher's exact test,  $p=0.0103$ ), with sprouts oriented toward the construct. **Conclusions:** C1 proved suitable as a cell carrier, and bioprinted constructs containing hiPSC-CMs targeting EGLN1 via CRISPR-Cas13 recruited endothelial sprouting under normoxia, without exogenous VEGF, indicating that encapsulated cells remain secretory and act on surrounding tissue through a paracrine route. Molecular confirmation of knockdown with biological replicates, validated reference genes, and thermally controlled bioprinting remain the next steps.

**Keywords:** bioprinting, hiPSC-derived cardiomyocytes, CRISPR-Cas13, angiogenesis, alginate-gelatin

## **Abstract 200 | Poster | Poster Session Day 2**

### **Age- and sex-associated depletion of the human skeletal stem cell pool and loss of subset-defining markers during ex vivo expansion: implications for potency-defined SSPC-based bone therapies**

**Category:** Mesenchymal stromal cells, extracellular vesicles and cell-derived products

**Authors:** Rhayra Braga Dias; Danielle Cabral Bonfim

**Institution:** UNIVERSIDADE FEDERAL DO RIO DE JANEIRO — RIO DE JANEIRO

The success of regenerative bone therapies depends on identifying cell populations with high therapeutic potential, as the clinical outcome is dictated by the intrinsic biological properties of the starting material. While scaffold technologies have advanced, the cellular component remains a primary bottleneck for clinical translation. Although human skeletal stem and progenitor cells (hSSPCs) are leading candidates for bone repair, their use is hindered by marked heterogeneity arising from intrinsic (age, sex) and extrinsic (in vitro expansion) factors, which precludes the a priori prediction of the in vivo potency of a given culture and limits clinical scalability. To generate potency-defined hSSPC products, we quantified these subsets in clinical bone sources and evaluated their molecular stability during expansion using PDPN, CD146, CD164, and CD73, markers that discriminate multipotent skeletal stem cells (SSCs) from committed osteo- (OP) and chondroprogenitors (CPs). Lineage-depleted marrow from acetabular reamings (n=19) were analyzed by FACS. Sorted PDPN<sup>-</sup> CD146<sup>+</sup> (OP-rich) and PDPN<sup>+</sup> CD146<sup>-</sup> (SSC/CP-rich) fractions were expanded, and their immunophenotypic and transcriptomic profiles were compared to those of standard BMSCs using FACS and Clariom D microarrays. We observed a significant age-associated depletion of the SSC pool in donors >60 years-old compared to the 40-60 group (0.03% vs 0.16% of the non-hematopoietic compartment of the bone marrow,  $p = 0.0177$ ). Females showed more severe niche exhaustion, with significantly fewer SSCs ( $p = 0.0391$ ) and OPs ( $p = 0.0437$ ) than age-matched males. Expansion caused rapid convergence of PDPN and CD146 expression in both OP and SSC/CP cellular fractions, resulting in a common PDPN<sup>+</sup> CD146<sup>+</sup> phenotype, followed by a progressive decline in PDPN expression ( $p < 0.001$ ). This rendered the sorted fractions immunophenotypically identical to BMSCs by passage 3. Transcriptomic profiling, however, revealed persistent divergence in gene expression profiles: 3D PCA fully separated SSC/CP, OP and BMSC samples at passage 3, and SSC/CPs retained higher GREM1 expression along with enrichment for bone mineralization, ossification and extracellular matrix organization. Together, these findings show that the hSSC pool declines with age and is smaller in females, and that sorted hSSPC fractions lose their surface identity in culture while retaining their molecular identity. For hSSPC-based advanced therapy products (ATPs), this has direct regulatory implications: the markers used to select the starting material cannot confirm product identity after expansion, and donor age and sex influence the composition of the starting material. Defining culture-stable identity markers and donor eligibility criteria is therefore essential to establish the identity, purity and potency specifications required for batch release and clinical translation of potency-defined hSSPC products.

**Keywords:** skeletal stem cells; aging; sex differences; ex vivo expansion; advanced therapy products.

## Abstract 201 | Poster | Poster Session Day 2

### A quantitative model-based approach to predict fetal hemoglobin levels in erythroid cells derived from base-edited CD34+ cells targeting HBG-175

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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Reactivation of fetal hemoglobin (HbF) is a promising therapeutic strategy for sickle cell disease (SCD), as increased HbF can reduce sickle hemoglobin polymerization and ameliorate disease manifestations. Adenine base editors (ABEs) targeting the -175 promoter region of the gamma-globin genes HBG1/2 can induce HbF, but editing at the intended A5 is often accompanied by bystander editing at nearby adenines, generating heterogeneous profiles. We asked whether ABE8e-NG editing frequencies at this region quantitatively predict HbF, and whether this generalizes across ABEs and experimental settings. Colony-forming assays (CFAs) were established from single CD34+ cells from healthy donors edited using the ABE8e-NG editor and cultured for 14 days in 96-well plates coated with a layer of mesenchymal cells. On day 14, individual erythroid colonies were collected and analyzed for HbF levels and editing. Cell lysates were analyzed by high-performance liquid chromatography (HPLC) to quantify hemoglobin fractions, while editing at the target (A5) and bystander (A3 and A11) sites was quantified by amplicon-based next-generation sequencing (NGS). HbF and editing profiles were characterized across 46 colonies from three independent experiments. HbF levels ranged from 0 to 90.2%, with substantial heterogeneity in A3, A5, and A11 editing. Mixed-effects modeling identified A5 editing as positively associated with HbF ( $\beta = 0.0389$ , 95% CI 0.0333-0.0444,  $p < 0.001$ ) and A3 editing as negatively associated with HbF ( $\beta = -0.0157$ , 95% CI -0.0241 to -0.0074,  $p < 0.001$ ), while A11 did not provide additional predictive information. These results were used to derive an equation for estimating HbF from A3 and A5 editing frequencies. Model coefficients remained stable across bootstrap iterations, supporting the robustness of the model, and repeated five-fold cross-validation demonstrated good predictive performance. The model was subsequently evaluated using datasets generated from different experimental systems after editing CD34+ cells using ABE8e-NG or ABEmax-NG, an alternative tool that does not generate the A3 and A11 bystander edits. These datasets, from both healthy and SCD donors, included CFAs, in which editing and HbF were measured in day-14 erythroid colonies; erythroid differentiation assays, with editing measured at day 5 and HbF at day 18; and xenotransplantation, with editing and HbF measured in human CD235a+ cells from NBSGW bone marrow 16 weeks after transplantation of edited CD34+ cells. The model remained applicable for predicting HbF from HBG-175 editing profiles across different time points, experimental systems, donor backgrounds, and editing contexts. Together, these findings demonstrate that editing-based prediction can be applied to diverse experimental settings and may provide a practical means of estimating HbF induction from editing outcomes when direct HbF measurement is not readily available.

**Keywords:** sickle cell disease, gene therapy, base editing, fetal hemoglobin, gamma-globin

**Abstract 202 | Poster | Poster Session Day 2**  
**An innovative platform for 4th-generation off-the-shelf**  
**CAR-macrophage production for cancer treatment**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

**Authors:** Luana Correia Croda Catharino; Elisa Lie Martines Matsumura; Livia Furquim de Castro; Rafael Ribeiro Almeida; Rodrigo Nalio Ramos; Vanderson Rocha

**Institution:** Instituto de Ciências Biomédicas, Universidade de São Paulo — São Paulo-SP

Solid tumors remain largely unresponsive to chimeric antigen receptor (CAR)-T cell therapy due to poor tumor trafficking/infiltration and the immunosuppressive tumor microenvironment (TME). CAR-macrophages (CAR-M) have emerged as an innovative alternative, given the macrophages' enhanced tumor infiltration capacity and ability to acquire a pro-inflammatory phenotype, enabling tumor cell phagocytosis, antigen presentation to T lymphocytes, among other effector functions. In this context, our group has established a platform to generate second-generation (2nd-gen) CAR-M from umbilical cord blood hematopoietic progenitor cells. These engineered macrophages demonstrated potent antigen-specific phagocytosis coupled with NF- $\kappa$ B nuclear translocation, infiltration into 3D tumor spheroids, and tumor control in zebrafish larvae models (1). However, emerging clinical data for 2nd-gen CAR-M indicate only modest therapeutic efficacy against solid tumors (2). Therefore, to improve therapeutic outcomes and promote durable antitumor immunity, this project explores CAR-M as modulators of the TME, enhancing their capacity to recruit and activate adaptive immune cells, thereby bridging innate and adaptive immune responses. We developed a fourth-generation (4th-gen) CAR-M engineered to secrete a cytokine "X", capable of improving immune cell recruitment, and to overexpress an innate immune receptor "Y", whose activation leads to the TME remodeling through the combination of multiple immunomodulatory effects. Human and murine cell lines were transduced to validate transgene expression and function. A luciferase reporter assay showed increased "Y" receptor pathway activation in 4th-gen vector-transduced cells following "Y" receptor agonist stimulation, although this effect was not restored in murine "Y" receptor KO cells. Preliminary generation of allogeneic CAR-M from UCB hematopoietic progenitor cells showed lower transduction efficiency with the larger 4th-gen vector, but no phenotypic differences during differentiation in comparison to 2nd-gen CAR-M. Functionally, 4th-gen CAR-M displayed a dose-dependent, approximately two-fold increase in "Y" receptor agonist-induced expression of its main immunomodulatory cytokine compared with 2nd-gen CAR-M. Ongoing studies are evaluating tumor cell killing, cytokine "X" secretion and further functional validation of the engineered CAR-M.

**Keywords:** CAR-Macrophages, solid tumors, immunotherapy.

## Abstract 203 | Poster | Poster Session Day 2

### Fast-Analyzr-BE: an automated workflow for base editing quantification and haplotype profiling in gene therapy research

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** PAULO ALFONSO SCHUOFF

**Institution:** Hospital Israelita Albert Einstein, São Paulo/SP, Brazil; Instituto Nacional de Terapia Gênica - INTERGEN/INCT — São Paulo, SP

Base editors (BEs) have emerged as versatile genome editing technologies that enable precise single-nucleotide conversions without inducing DNA double-strand breaks. Cytosine base editors (CBEs) mediate C•G-to-T•A conversions, whereas adenine base editors (ABEs) enable A•T-to-G•C transitions. Amplicon-based next-generation sequencing (NGS) is a standard approach for assessment of base-editing outcomes with high sensitivity and resolution. CRISPResso2 is the most common tool to quantify editing outcomes from amplicon sequencing data, but preparing batch files and extracting target-base conversions, indel, and haplotype frequencies from its outputs can still require custom scripting or substantial manual processing. Here, we present Fast-Analyzr-BE, an automated and reproducible computational workflow that complements CRISPResso2 for ABE and CBE base-editing experiments. Implemented in Bash and R and distributed as an executable shell script, Fast-Analyzr-BE reduces the number of manual steps required to configure, execute, and summarize base-editing experiments involving multiple samples and/or targets. The workflow comprises three main stages: (i) user-friendly batch file generation, (ii) execution of CRISPResso2 analyses, and (iii) automated post-processing and consolidation of results into standardized outputs. The pipeline automates batch input generation and validation, runs CRISPResso2 across multiple samples, performs strand-aware extraction of target-base conversion frequencies, and consolidates read counts, editing, indel and haplotype frequencies into standardized, analysis-ready tables and visualizations. Its haplotype module transforms CRISPResso2 allele-frequency data into haplotypes defined by user-selected editing positions, enabling quantitative analysis of the co-occurrence of intended and bystander edits within the same reads. We validated Fast-Analyzr-BE using synthetic paired-end amplicon datasets simulating non-edited samples, single and multiple edited haplotypes, background substitutions, indels, and low-quality reads, in both ABE and CBE contexts and for forward and reverse guide orientations. Observed editing, indel, and haplotype frequencies showed complete concordance with the expected values ( $R^2 = 1$ ), which was also maintained across spacer lengths from 16 to 24 nucleotides, for which Fast-Analyzr-BE automatically sets the CRISPResso2 quantification window. Benchmarking with varying sequencing depth, number of guides, and number of samples showed predictable scaling of runtime, with haplotype-level analysis adding minimal computational overhead relative to position-wise analysis. Thus, Fast-Analyzr-BE provides a reproducible workflow for quantitative and haplotype-resolved analysis of base editing data, facilitating analysis across experimental scales without requiring extensive experience with CRISPResso2 outputs or custom coding.

**Keywords:** Sequence analysis, Alignment, Software, Data analysis, Next-generation sequencing

## Abstract 205 | Poster | Poster Session Day 2

### Metformin induces autophagy level changes in heterozygous and CRISPR-edited TSC2 primary fibroblasts

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

**Authors:** GABRIEL MORALES CHIAPIN; Arthur Bandeira de Mello Garcia; Clevia Rosset; GUILHERME BALDO; Guilherme Danielski Viola; Ivan da Silva; Patricia Ashton Prolla

**Institution:** Hospital de Clínicas de Porto Alegre — Porto Alegre-RS

**Introduction:** Tuberous Sclerosis Complex (TSC) is a genetic disorder caused by pathogenic variants in TSC1 or TSC2 genes, leading to mTORC1 hyperactivation and autophagy inhibition. Loss-of-heterozygosity (LOH) may worsen the dysregulation of these pathways and may trigger tumorigenesis. The standard treatment with mTORC1 inhibitors, such as rapamycin, presents limitations, suggesting that alternative therapies must be investigated. In this study we aimed to evaluate the autophagy pathway status in haploinsufficient and knockout TSC2 cells, and explore the role of metformin in regulating autophagy levels. **Methods:** Primary fibroblast cultures were established from skin biopsies of a patient with the TSC2 germline pathogenic variant c.1008T>G (NM\_000548) and from an unaffected family member. CRISPR gene editing was used to induce LOH in a pool of the heterozygous cells. Heterozygous, gene-edited and wild-type cells were treated for 12 hours with rapamycin and metformin as well as vehicle control (DMSO) and Bafilomycin A1 for autophagy flux evaluation. Autophagy levels were assessed by flow cytometry using acridine orange staining and MAP1LC3 (LC3) immunofluorescence. mTORC1 activation was evaluated via immunofluorescence detection of phosphorylated S6K. **Results:** Flow cytometry analysis indicated that autophagic cell proportion did not vary between treatments and DMSO in wild-type cells. Conversely, the treatments showed an increased number of autophagic cells compared to DMSO in heterozygous cells: rapamycin (16.2% vs 6.5%,  $p=0.006$ ) and metformin (17.6% vs 6.5%,  $p=0.002$ ). In CRISPR-edited cells, both treatments increased the proportion of autophagic cells compared to heterozygous cells: rapamycin (27.1% vs 16.2%,  $p<0.001$ ) and metformin (27.2% vs 17.6%,  $p<0.001$ ). LC3 immunofluorescence analysis revealed that heterozygous and gene-edited metformin-treated cells had a higher number and smaller autophagosomes in comparison to DMSO and rapamycin treatment. In addition, in gene-edited cells, metformin treatment induced lower activity of mTORC1, as indicated by reduced p-S6K immunofluorescence, showing similar results to rapamycin treatment. For heterozygous and CRISPR-edited cells, bafilomycin treatment significantly decreased LC3-II levels compared to rapamycin treatment while treatment with rapamycin plus bafilomycin increased total LC3-II levels compared to bafilomycin alone, suggesting that the treatment may increase the synthesis of autophagy-related membranes instead of provoking alterations in autophagy flux. **Conclusions:** Our study supports the role of TSC2 haploinsufficiency in autophagy dysregulation. This dysregulation is amplified by LOH. Furthermore, we demonstrate that metformin induces autophagy in TSC2-deficient and haploinsufficient cells, which is phenotypically distinct from that observed in direct mTORC1 inhibition by rapamycin.

**Keywords:** CRISPR/Cas9, Metformin, mTORC1, Rapamycin, Tuberous Sclerosis Complex.

## **Abstract 206 | Poster | Poster Session Day 2**

### **Optimization of the Anti-BCMA CAR Antigen-Recognition Domain: Comparative Evaluation of Murine, Humanized, and Harmonized scFv in CAR-T Cells for Multiple Myeloma**

**Category:** Adoptive cell immunotherapy (CAR-T, CAR-NK, TIL and antigen-specific T cells)

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**INTRODUCTION:** Multiple myeloma (MM) is a hematological malignancy characterized by clonal plasma cell expansion in the bone marrow. CAR-T cell immunotherapy targeting B-cell maturation antigen (BCMA) has shown high clinical efficacy, but limitations remain, including those related to the CAR single-chain variable fragment (scFv) immunogenicity, self-aggregation, and tonic signaling. This project proposes the development and comparison of anti-BCMA CAR-T cells containing murine (MUR), humanized (HU), and less aggregative harmonized (HA) scFv to evaluate their effects on function, persistence, exhaustion, and antitumor activity. **METHODOLOGY AND RESULTS:** The MM cell lines U266 and RPMI 8226 were phenotypically characterized by flow cytometry. U266 showed high BCMA expression and was positive for both CD138 and CD38, whereas RPMI 8226 also expressed CD38. CD19 was negative in both cell lines. Based on its high BCMA expression, U266 was selected for further experiments, and a GFP+ U266 cell line was generated for in vitro functional evaluation. Peripheral blood mononuclear cells (PBMCs) from healthy donors, obtained from INCA Blood Bank leukoreduction filters, were used to generate CAR-T cells by electroporation with the MUR, HU, and HA sleeping beauty transposon system, in addition to the MOCK negative control (cells just submitted to electroporation without any vector). Initially, PBMCs were activated and electroporated on the same day. On eight day, CAR expression was 20.8% (HU) and 7.86% (HA) in the first donor; in the second donor, 5.04% (MU), 13.4% (HU), and 4.78% (HA). In a second experiment, PBMCs were activated and electroporated two days later. On eight day, 2.41% (MU), 21.3% (HU), and 27.0% (HA) were observed in the first donor, and 6.06% (MU), 12.7% (HU), and 16.0% (HA) in the second donor. CAR-T cells from this experiment were evaluated for cytotoxicity against GFP+ U266 cells up to five days of co-cultivation using Incucyte® approach to determine residual GFP+ target cells. A reduction in GFP + cells was observed for all three constructs, corroborating the cytotoxic activity for all constructs CAR-T cells. At the last time point evaluated (day five), CAR-T cells promoted approximately 64–70% inhibition of tumor growth compared with the MOCK control. **CONCLUSION:** Preliminary results demonstrate the feasibility of generating anti-BCMA CAR-T cells with different scFv constructs and established the experimental model. All three constructs showed CAR expression and cytotoxic activity against U266 cells, allowing further comparative investigation of their functional characteristics. **PERSPECTIVES:** CAR-T cells will be evaluated for memory and exhaustion phenotypes, proliferation, cytokine production and cytotoxic activity. Subsequently, a murine MM model will be established to evaluate in vivo safety, therapeutic efficacy, tumor burden, and survival.

**Keywords:** Cancer, multiple myeloma, CAR-T cell

## Abstract 207 | Poster | Poster Session Day 2

### CRISPR/Cas9 Targeting of MGMT Enhances Temozolomide Sensitivity in T98G Glioblastoma Models

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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Glioblastoma is a highly aggressive neoplasm of the central nervous system, distinguished by its rapid growth potential, molecular diversity, invasive nature, tendency to recur, and resistance to treatment. Temozolomide (TMZ) serves as the primary chemotherapeutic drug for its therapy, functioning via DNA methylation, which includes the formation of O<sup>6</sup>-methylguanine (O<sup>6</sup>-MeG), a modification that can initiate DNA damage responses and apoptosis. The O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) protein directly facilitates the repair of this damage and is a primary mechanism linked to TMZ resistance. This study assessed TMZ response through modification of the MGMT gene using the CRISPR/Cas9 mechanism in knock-out/wild type T98G glioblastoma cells. Initially, capillary sequencing detected two variants in the MGMT gene, including a synonymous and a missense, with no documented correlation to protein function loss. The assessment of cellular viability by the MTT test revealed significant resistance of the wild-type T98G cell line to TMZ, with an IC<sub>50</sub> of 1507 µM following 72 hours of exposure. At sub-IC<sub>50</sub> doses of 139.5, 279, and 558 µM, flow cytometry demonstrated a gradual rise in beginning apoptosis, culminating at 78.03 ± 2.98% at the 558 µM concentration. In the three-dimensional spheroid model, this same concentration facilitated a more significant reduction in compaction and disaggregation of the structure, whereas the lower concentrations exhibited less marked alterations. For the modification of MGMT, sgRNAs directed at exons 2 and 3 were used. The T7 Endonuclease I experiment revealed indels for the sgRNA targeting exon 3, but the sgRNA for exon 2 exhibited no detectable editing under the assessed circumstances. The concurrent application of both sgRNAs did not enhance editing. The cell population transfected with the sgRNA targeting exon 3 exhibited a decrease in the TMZ IC<sub>50</sub> from 1507 to 855.5 µM, indicating an estimated 43% reduction, which implies increased sensitivity to the chemotherapeutic following MGMT editing. Nevertheless, due to the heterogeneous nature of the cell population, it remains essential to isolate and characterize clones originating from individual cells, ensuring confirmation of biallelic editing and the absence of expression or function of the MGMT protein, in order to definitively establish the correlation between gene knockout and increased sensitivity to temozolomide.

**Keywords:** Glioblastoma, Temozolomide, Chemoresistance, MGMT, CRISPR/Cas9.

## **Abstract 208 | Poster | Poster Session Day 2**

### **Tumor Suppressor Potential of miR-10a and miR-10b in Localized and Metastatic ccRCC**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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**INTRODUCTION:** Renal cell carcinoma (RCC) accounts for approximately 85–90% of all kidney cancers. Its most common subtype, clear cell renal cell carcinoma (ccRCC), represents nearly 75% of cases and is characterized by high mortality and recurrence rates due to resistance to conventional therapies. MicroRNAs such as miR-10a and miR-10b are downregulated in this tumor type, suggesting a tumor suppressor role in ccRCC. MicroRNA mimic-based restoration has demonstrated antitumor effects in several malignancies. **OBJECTIVE:** This study aimed to investigate the tumor suppressor effects of miR-10a and miR-10b through their induction, both individually and in combination, in the metastatic clear cell renal cell carcinoma cell line CAKI-1 and the localized clear cell renal cell carcinoma cell line CAKI-2, focusing on migration, invasion, proliferation, apoptosis, and the expression of target genes associated with tumor progression. **METHODOLOGY:** CAKI-1 and CAKI-2 cell lines were transfected with miR-10a and miR-10b mimics, separately and simultaneously, using Lipofectamine™ RNAiMAX. Transfection efficiency was confirmed by qPCR. Cell migration was assessed using the wound healing assay, whereas invasion was evaluated using Transwell chambers coated with Matrigel. Cell proliferation was analyzed by flow cytometry. In addition, the expression of VEGFA, HAS3, and CD44 was evaluated by qPCR. Statistical analyses were performed using GraphPad Prism software. **RESULTS:** qPCR analysis confirmed a significant increase in miR-10a and miR-10b expression following transfection. In the metastatic CAKI-1 cell line, co-transfection with miR-10a/b significantly reduced cell invasion and promoted a trend toward reduced migration within the first 24 hours. Furthermore, a decrease in the proliferative cell population (Ki67<sup>+</sup>) was observed, while apoptosis levels were also modulated following treatment. In the localized CAKI-2 cell line, no significant changes were observed in cell invasion or migration. However, increased cell proliferation was detected after individual treatments, while co-transfection increased apoptosis. Gene expression analysis revealed reduced HAS3 expression following treatment with miR-10a and miR-10a/b, as well as increased CD44 expression after miR-10a/b co-transfection. **CONCLUSION:** Restoration of miR-10a and miR-10b expression was able to modulate processes associated with tumor progression in clear cell renal cell carcinoma, with distinct effects between metastatic and localized cell lines. These findings suggest that the activity of these microRNAs is dependent on the biological context and molecular characteristics of each cellular model, highlighting the role of these microRNAs in the regulation of tumor progression-related processes in ccRCC.

**Keywords:** microRNA-10a, microRNA-10b, clear cell renal carcinoma, migration, invasion, proliferation

## **Abstract 209 | Poster | Poster Session Day 2**

### **The role of Neurogenin 2 in the neuroprotection and regeneration of human neurons derived from induced pluripotent stem cells**

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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Neurodegenerative disorders comprise chronic neurodegenerative diseases and traumatic injuries of the central nervous system, resulting in progressive damage to neurons and permanent neurological deficits, since axons are affected early and do not regenerate. Neuroprotective strategies aimed at reducing neurodegeneration and promoting axonal regeneration and synaptogenesis are therefore needed. Considering the pro-neuronal role of Neurogenin-2 (NGN2) — a transcription factor involved in cortical neuron development and in neuronal differentiation — this project aims to evaluate the neuroprotective and regenerative potential of NGN2 overexpression by means of rAAV vectors in human neurons derived from induced pluripotent stem cells (iPSCs). The culture of human iPSC-derived neurons is a new project in the laboratory, and its implementation required a collaboration with Prof. Fabíola Ribeiro, from the Department of Biochemistry, for training in this culture technique. Three different cell lines were provided for this purpose: 7889SA, NSB690 and NSB553. During cell expansion, the NSB690 line showed early differentiation and the NSB553 line showed gliogenic behaviour, and both were discarded. The 7889SA line showed no differentiation problems and was selected for future experiments. To ensure the best cell adhesion and cell survival until the end of the culture period, different types of coating were tested. The combinations of poly-L-ornithine + laminin and polyethyleneimine + laminin were tested, and the best substrate association was the poly-L-ornithine + laminin coating, on which the cells differentiated without any loss of cell viability. To test the plating cell density, baseline concentrations for differentiation were established. NSCs were plated at densities of 50, 100 and 200 thousand cells per well for 8 weeks. It was observed that 50 thousand cells showed a low rate of cell death and that processes were easily visible, suggesting an optimal density. Finally, to characterize the differentiated cells in culture, immunocytochemistry was performed at different time points to characterize the progression of neural stem cell differentiation into neurons and astrocytes on a weekly basis, using the following antibodies: PAX6 for NSCs, GFAP for astrocytes and MAP2 for mature neurons. To ensure transduction efficiency in human neurons, different viral serotypes were tested, namely AAV1/2.NGN2, AAV2.EGFP and AAV6.mCherry. The vectors were added to cultures already at 2 weeks of differentiation and kept until 8 weeks of differentiation. The serotype that best transduced human neurons was AAV6. For NGN2 overexpression, the AAV6.NGN2 vector was produced in collaboration with the Viral Vector Production Centre of UFMG for future analyses.

**Keywords:** Regeneration, iPSCs, Neurogenin-2

## **Abstract 210 | Poster | Poster Session Day 2**

### **RANKL EXPRESSION KINETICS IN OSTEOBLASTS AND PROSTATE ADENOCARCINOMA CELLS FOR EVALUATING GENE SILENCING VIA siRNA ENCAPSULATED IN IONIZABLE LIPID-POLYMERIC NANOPARTICLES**

**Category:** Vectors and gene delivery systems (viral and non-viral)

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Prostate adenocarcinoma (CaP), the leading cancer in men in Brazil in 2024 according to World Health Organization data, progresses to bone metastases (BM) in approximately 80% of advanced cases. The morbimortality associated with this complication stems from RANKL overexpression by osteoblasts stimulated by tumor cells affecting RANK/RANKL signaling. Current treatments, based on bisphosphonates and immunobiologicals, present high costs, frequent adverse effects, and limited efficacy in overall survival. **OBJECTIVES:** Therefore, the objectives were: (1) to determine the expression kinetics of RANKL in osteoblast cultures (SaOs-2), prostate adenocarcinoma cells (PC3-Luc2), and their coculture for subsequent gene silencing via lipid-polymeric nanoparticles (LNPs) encapsulating siRNA-RANKL; and (2) to formulate and characterize LNPs containing siRNA-RANKL. **METHODOLOGY:** RNA from SaOs-2, PC3-Luc-2, and their coculture at 10%, 20%, and 30% PC3-Luc-2 proportions was extracted after 24h, 48h, and 72h to quantify RANKL expression via  $\Delta\Delta C_t$  in RT-qPCR. For LNP formulation, two distinct siRNA-RANKL sequences were used: RANKL1 and RANKL2. LNPs were synthesized via microfluidics (3:1 ratio) by mixing an ethanolic phase (7C1/C14PEG2K, 10:1) and an aqueous phase (10 mM citrate buffer, pH 3.0). Encapsulation efficiency, hydrodynamic diameter (HD), polydispersity index (PDI), and zeta potential (ZP) were evaluated for both LNP populations. **RESULTS:** After culture at the determined time points, RT-qPCR revealed the highest increase of RANKL in the PC3-Luc-2/SaOS-2 coculture (30% PC3-Luc-2) after 72 hours compared to the control group at the same time point, highlighting the potential of tumor cells to positively modulate pro-osteolytic pathways in the bone microenvironment. Regarding the formulations, LNPs 1 and 2 presented, respectively, an HD of 67.68 nm and 80.27 nm, a PDI of 0.189 and 0.214, a ZP of 6.11 mV and 3.79 mV, and siRNA concentrations of 107 ng/ $\mu$ L and 195 ng/ $\mu$ L. The LNPs will be used to treat cell lines at the optimal RANKL mRNA expression time point. **CONCLUSION:** In addition to representing a paradigm shift compared to conventional therapies, the platform offers the possibility of functionalization, suggesting higher efficacy and lower systemic toxicity.

## Abstract 211 | Poster | Poster Session Day 2

### IDENTIFICATION OF TRANSCRIPTOMIC TARGETS TO PREVENT AND TREAT METASTATIC TRIPLE NEGATIVE BREAST CANCER

**Category:** Genome editing and gene therapy for genetic, rare and oncological diseases

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Based on World Health Organization data, breast cancer (BC) was the second most incident malignancy and fourth in mortality worldwide in 2022. Triple-Negative Breast Cancer (TNBC) is characterized by hormone receptor and HER2 loss, limiting standard chemotherapeutic options. TNBC exhibits an aggressive profile with rapid visceral, neural, and bone metastasis, severely worsening survival and prognosis. **OBJECTIVE:** This study aims to identify key transcriptomic targets differentially expressed in TNBC metastasis compared to primary tumors. **METHODOLOGY:** A bioinformatic review analysis was conducted through a public international data consortium and an interactive analytic tool. On September 18, 2026, a systematic search was performed in NCBI GEO DataSets using the query: ("triple negative breast cancer" OR TNBC) AND (primary AND metastasis) AND ("Expression profiling by high throughput sequencing" OR "microarray"). Inclusion criteria required human samples, compatibility with GEO2R, and a minimum of 10 samples per dataset. Cell line models, PDX studies, and non-TNBC cohorts were excluded. The datasets were individually analyzed via GEO2R (adjusted p-value < 0.05) and findings were compiled in a broad table listing prominent molecular upregulated or downregulated genetic targets. **RESULTS:** Three datasets met the criteria, focusing on lymph node metastasis (LNM) — the primary route of breast cancer cell dissemination — and lung metastasis (LM), the predominant metastatic site for TNBC. The analysis encompassed 72 primary, 28 LNM, and 4 LM samples. A distinct microRNA expression signature emerged: miR-139-3p, miR-601, miR-136 and miR-498 were downregulated more than two times in LNM (adj.p.value = 0.458), whereas miR-451a was discovered to be upregulated in the pulmonary site compared to primary tumor (p-value ~ 0.001). **DISCUSSION:** Lymph node downregulated miRNAs are involved in tumoral suppression, modulating DNA damaging, cell proliferation pathways inhibiting metastatic potential and repairing oncogenes as BRCA1 and PTEN associated molecules. In opposition, miR-451a is usually downregulated in primary TNBC - therefore, the upregulation may suggest a progression and invasive phenotype for secondary site survival. **CONCLUSION:** Replacing downregulated miRNA or silencing upregulated ones are trending strategies in oncologic therapeutics. The findings must be deeper explored and validating those data experimentally is necessary in order to prime an applicable metastasis prevention and treatment strategy.