Presentation number: PP53 Abstract number: 73-ISABS-2026

MOLECULAR PROFILING IDENTIFIES ACTIONABLE *PTEN* MUTATION IN RECURRENT UTERINE LEIOMYOSARCOMA: A CASE REPORT SUPPORTING TARGETED THERAPY APPROACH

Žaja Sara[1], Škoprc Tonia[1], Bulić Luka[2,3,4], Brlek Petar[2,3,5], Primorac Dragan[2,3,6,7,8,1,9,10,11,12,13]

[1]School of Medicine, University of Split, Split, Croatia; [2]St. Catherine Specialty Hospital, Zagreb, Croatia; [3]School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [4]Algebra Bernays University, Zagreb, Croatia; [5]Department of Molecular Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia; [6]International Center for Applied Biological Research , Zagreb, Croatia; [7]Faculty of Dental Medicine and Health, Josip Juraj Strossmayer University of Osijek , Osijek, Croatia; [8]Eberly College of Science, The Pennsylvania State University, State College, University Park, PA, United States of America; [9]The Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, New Haven, CT, United States of America; [10]Sana Kliniken Oberfranken, Coburg, Germany; [11]School of Medicine, University of Rijeka, Rijeka, Croatia; [12]School of Medicine, University of Pittsburgh, Pittsburgh, PA, United States of America; [13]Gandhinagar Campus, National Forensic Sciences University, Gandhinagar, India

sara01zaja@gmail.com

Uterine leiomyosarcoma is a rare and highly aggressive malignancy with limited treatment options in the recurrent setting. Comprehensive genomic profiling using next-generation sequencing (NGS) is increasingly used to identify actionable mutations and guide precision oncology. We present a 47-year-old female patient diagnosed with uterine leiomyosarcoma (FIGO stage IC) in October 2022. The patient underwent multiple surgical procedures, including myomectomy followed by hysterectomy with bilateral adnexectomy and repeated cytoreductive surgeries due to recurrent disease. Systemic treatment included adjuvant chemotherapy (AI protocol), gemcitabine due to hypersensitivity to docetaxel, and subsequent targeted therapy with pazopanib. Despite treatment, the disease progressed with a pelvic recurrence measuring 29 × 29 × 37 mm. Due to further progression, molecular profiling was performed using hybrid- capture-based NGS (Illumina NovaSeq platform), enabling simultaneous DNA and RNA analysis from metastatic tissue. Molecular profiling results identified a pathogenic mutation in the *PTEN* gene, a key regulator of the PI3K/AKT signaling pathway. This finding provided a biologically rational therapeutic option using targeted therapy with the AKT inhibitor capivasertib in combination with fulvestrant. This case demonstrates the clinical value of repeat molecular profiling in advanced uterine leiomyosarcoma. Genomic analysis may significantly influence treatment decisions and represents an important step toward precision oncology in rare gynecologic malignancies.

Keywords: uterine leiomyosarcoma, next-generation sequencing (NGS), *PTEN* gene mutation, precision oncology, capivasertib therapy

AI IN DRUG DISCOVERY AND DEVELOPMENT

Presentation number: PP54 Abstract number: 63-ISABS-2026

PRESERVING HYPOXIC CONDITIONS FOR ACCURATE DRUG RESPONSE ASSESSMENT: AN OPTIMIZED FLOW CYTOMETRY APPROACH

Bernotas Paulius[1], Blanco Alfonso[2], Massa Estefania[3], Strowitzki Moritz Johannes[2,4], Piñeiro Abuín Araceli[5], Blach Justyna[6], Bernotiene Eiva[1], Cummins Eoin[7,2], Taylor Cormac[2,7,8], Uzieliene Ilona[1]

[1]State Research Institute Centre for Innovative Medicine, Vilnius, Guobų g. 4, Lithuania; [2]University College Dublin, Conway Institute of Biomedical & Biomolecular Research, Dublin, Ireland; [3]Instituto de Inmunologia Clínica y Experimental de Rosario, Rosario, Argentina; [4]Heidelberg University, Heidelberg, Germany; [5]Universidade de Vigo, Vigo, Spain; [6]University Children's Hospital of Cracow, Cracow, Poland; [7]University College Dublin, School of Medicine, Dublin, Ireland; [8]University College Dublin, Systems Biology Ireland, Dublin, Ireland

bernotaspaul@gmail.com

Hypoxia critically influences physiological and pathological processes, particularly cancer progression and drug response, yet standard in vitro analyses often fail to maintain physiological oxygen conditions, limiting translational relevance. This study aimed to develop a method for accurate cellular analysis under controlled oxygen environments using a tubing-based interface connected to a high-performance flow cytometer, which enabled more reliable investigation of hypoxia-driven cellular processes relevant to drug discovery and development. A549 lung epithelial cancer cells were cultured under hypoxic (1% O_2) and normoxic (21% O_2) conditions. A flow cytometry system with direct sampling from a hypoxia chamber was established to prevent reoxygenation. Cell viability, mitochondrial membrane potential, and intracellular hypoxia were assessed using DRAQ7, JC-1, and HypoxiTRAK probes. Results showed stable cell viability (80-95%) across conditions, while HypoxiTRAK signals confirmed that hypoxic status remained unchanged during short handling but increased significantly after 60 minutes under hypoxia and decreased upon reoxygenation. Mitochondrial membrane potential rapidly responded to oxygen shifts, with a significant increase observed within 15-60 minutes, particularly after hypoxia-to-normoxia transition, indicating fast metabolic adaptation. Even brief exposure to normoxia altered hypoxia-associated readouts. These findings demonstrate that maintaining oxygen conditions throughout analysis is essential for reliable data. The proposed approach improves physiological relevance in vitro and enhances the predictive power of drug screening models targeting hypoxia-related pathways. This research was funded by HORIZON- WIDERA-2021-ACCESS-03-01 program 382 project No. 101079489-TWINFLAG.

Keywords: hypoxia, flow cytometry, cancer, jc-1, hypoxiTRAK

Presentation number: PP55 Abstract number: 88-ISABS-2026

FIRST CRISPR/DCAS9 B-CELL BASED PLATFORM TO STUDY REGULATORY MECHANISMS OF IGG GLYCOSYLATION

Nikolić Kristina[1], Mijakovac Anika[1,2], Krajc Viktoria[1], Borošak Iwona[2], Grubeša Iva[2], Krištić Jasminka[2], Lauc Gordan[2,3], Zoldoš Vlatka[1]

[1]Faculty of Science, University of Zagreb, Zagreb, Croatia; [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia; [3]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

knikolic@biol.pmf.hr

Immunoglobulin G (IgG) is the most abundant antibody in human blood, secreted by plasma cells, and plays a central role in adaptive immunity. Its function is regulated by glycosylation, a post-translational modification in which glycans are attached to proteins. These glycans influence IgG stability, conformation, half-life, and are critically involved in inflammatory responses. Numerous studies have shown that IgG glycosylation changes across pathological conditions, but the molecular mechanisms governing this process remain poorly understood. Insights have been gained through genome-wide association studies (GWAS) of the IgG glycome, which identified genes with previously unknown roles in glycosylation pathways. To functionally validate these genes, we developed the first CRISPR/dCas9 B-cell platform with stably integrated CRISPRa and CRISPRi tools. Using PiggyBac transposition, we generated IgG-secreting stable cell lines expressing either dCas9-VPR for transcriptional activation or dCas9-KRAB for transcriptional silencing. Taking advantage of the compatibility of Sleeping Beauty transposase with this system, we established a robust protocol for gRNA addition, achieving strong activation and silencing of gene expression. As proof of concept, we targeted key glycosyltransferases, B4GALT1 and ST6GAL1, responsible for adding galactose and sialic acid, and showed that their modulation leads to significant changes in IgG galactosylation and sialylation. Using this platform, we manipulated multiple GWAS candidate genes and demonstrated their involvement in IgG glycosylation for the first time. This CRISPR/dCas9 B-cell system enables identification of novel regulatory pathways. Since glycosylation is a critical quality attribute of IgG therapeutics, these findings may support advanced glycoengineering and future drug development.

Keywords: glycosylation, IgG, CRISPR, epigenetics, glycoengineering

Presentation number: PP56 Abstract number: 57-ISABS-2026

PATIENT-SPECIFIC TRANSCRIPTOMIC RESPONSES TO CONNEXIN-43 INHIBITION IN OSTEOARTHRITIS

Pachaleva Jolita[1], Kulyar Fakhar[1], Uzieliene Ilona[1], Vaiciuleviciute Raminta[4], Kirdaite Gailute[2], Brennan Kieran[4], Blanco Alfonso[4], Mc Gee Margaret[4], Kvederas Giedrius[5], Mayan Maria[3], Bernotiene Eiva[1]

[1]Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania; [2]Department of Personalised Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania; [3]CellCOM Research Group, Center for Research in Nanomaterials and Biomedicine (CINBIO), Universidade de Vigo, Vigo, Spain; [4]Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland; [5]Faculty of Medicine, Vilnius Santaros Clinics, Vilnius university, Vilnius, Lithuania

jolita.pachaleva@imcentras.lt

Osteoarthritis (OA) is a degenerative joint disease marked by cartilage degradation, cellular senescence, and chronic inflammation, with limited treatment options. Connexin-43 (Cx43), a gap junction protein upregulated in OA, promotes harmful intercellular communication driving senescence and tissue damage, making it a promising therapeutic target. This study evaluates TUB1, a Cx43-inhibitory peptide, in human OA chondrocytes cultured under normoxia (21% O_2) or hypoxia (2% O_2) for 48 h with or without TUB1. RNA sequencing was performed; differential expression was defined as $|log2FC| \geq 1$ and adjusted $p \leq 0.05$. Oxygen alone altered 106 genes (92 upregulated, 14 downregulated), with GO enrichment indicating response to endogenous stimulus and regulation of cell proliferation, confirming oxygen shapes the transcriptome. Pooled TUB1 analysis showed limited changes; however, patient-level analysis revealed 61–1,000 differentially expressed genes per donor, indicating high variability. Under normoxia, TUB1 reduced inflammatory and chemokine signaling and increased DNA damage response and cell cycle genes. Under hypoxia, it affected collagen metabolism and cell–matrix adhesion. GO and KEGG analyses confirmed consistent effects across senescence, immune, and extracellular matrix pathways. These findings show TUB1 induces oxygen-dependent and individualized transcriptomic changes, supporting Cx43 inhibition as a candidate for personalized OA therapy.
This work was funded by project No. 101079489-HORIZON-WIDERA-2021-ACCESS-03-01 program: 382 “Twinning for Promoting Excellence, Ability and Knowledge to develop novel approaches for targeting inflammatory and degenerative age- related joint diseases” TWINFLAG and supported by the Marie Skłodowska-Curie Actions EVEREST project.

Keywords: osteoarthritis, senescence, inflammation, connexin 43, hypoxia

Presentation number: PP57 Abstract number: 79-ISABS-2026

DEVELOPMENT OF CRISPR-DCAS9 EXPRESSION SYSTEM TO INVESTIGATE ABERRANT IGA GLYCOSYLATION

Semenić Lana[1], **Lukšić Fran**[2], Josipović Goran[2], Borošak Iwona[2], Mijakovac Anika[2], Krištić Jasminka[2], Zoldoš Vlatka[3,2], Lauc Gordan[1,2]

[1]Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia; [2]Genos Glycoscience Research Laboratory, Zagreb, Croatia; [3]Faculty of Science, University of Zagreb, Zagreb, Croatia

lsemenic@pharma.hr; fluksic@genos.hr

IgA is the second most abundant immunoglobulin class in serum. In addition to providing passive immunity by neutralising pathogens at mucosal sites, it actively controls immune responses, and can drive the pathogenesis of autoimmune diseases, such as IgA nephropathy and rheumatoid arthritis. Like other antibodies, it is subject to glycosylation, a complex posttranslational modification orchestrating profound changes of protein function in health and disease. IgA1, the most abundant subclass, has two N- glycosylation and six O-glycosylation sites on each heavy chain. In cell culture, glycosylation can be modified by manipulation of gene expression using CRISPR technology. This provides a framework to test whether a gene implicated in a certain disease affects glycosylation. To study the effect of disease- associated genes on IgA1 glycosylation, we developed a system for IgA1 secretion in FreeStyle™ 293-F cells and utilised CRISPR-dCas9 to upregulate and downregulate genes of interest. We successfully designed the expression plasmid for IgA1 production, which yields enough IgA1 for LC-MS glycan analysis. Glycoprofiling of cell-secreted IgA revealed N- and O-glycosylation profiles similar to those of human derived IgA. To validate the established framework, we targeted glycosyltransferases directly involved in the process of glycosylation. We successfully upregulated and downregulated the expression of target genes which resulted in specific changes of IgA glycome composition in accordance with the function of each enzyme. Our platform represents the first cell-based CRISPR-dCas9 system to study the glycosylation of IgA. This technology will enable precise studies of aberrant IgA glycosylation, the main driver of IgA nephropathy, a debilitating condition that affects millions of people worldwide.

Keywords: IgA, glycosylation, CRISPR-dCas9, LC-MS, Golden Gate cloning

Presentation number: PP58 Abstract number: 107-ISABS-2026

EFFECT OF EMPAGLIFLOZIN ON PANNEXIN 1 AND CONNEXIN EXPRESSION IN THE KIDNEY

Svaguša Karla[1], Markotić Anita[2], Jerčić Leo[1], Vukojević Katarina[1], Filipović Natalija[1]

[1]Department of Anatomy, Histology and Embryology, School of Medicine, University of Split, Split, Croatia; [2]Department of Medical Chemistry and Biochemistry, School of Medicine, University of Split, Split, Croatia

karla.svagusa@mefst.hr

Sodium-glucose cotransporter-2 inhibitors (SGLT2i), including empagliflozin (EMPA), revolutionized diabetes treatment, yet their effects on renal intercellular communication pathways remain unexplored, potentially influencing therapeutic benefits and adverse events. This study aimed to investigate EMPA's impact on pannexin 1 (Px1) and connexin (Cx43, Cx40, Cx45) expression in key renal cell populations. Thirteen C57BL/6J mice were divided into EMPA-treated (15 mg/kg/day in 0.2% DMSO via drinking water for 4 weeks; N=7) and control (0.2% DMSO; N=6) groups. Px/Cx expression in proximal tubule epithelium (megalin+, M+), podocytes (podocin+, POD+), and endothelium (CD31+) was quantified by flow cytometry. EMPA significantly increased Cx43 in CD31+ cells ($P<0.05$) but did not affect other Px/Cx. Px1 expression was highest in PT (M+; $P<0.001$ compared to POD+ cells). Cx43 expression was significantly higher in M+ and CD31+ cells compared to POD+ ($P<0.01$ both); Cx40 expression was highest in CD31+ cells ($P<0.01$ compared to M+ and POD+), while Cx45 expression was higher in M+ ($P<0.05$) and CD31+ ($P<0.01$) compared to POD+. In M+ PT epithelium, the highest Cx43 expression was recorded ($P<0.05$ to $P<0.0001$); POD+ and CD31+ cells showed the highest expression of Cx43 and Cx40 ($P<0.01$ to $P<0.0001$ compared to Px1 and Cx45). Enhanced endothelial Cx43 may modulate vascular function/permeability, contributing to EMPA's renal benefits and risks. These findings advance understanding of SGLT2i in renal physiology/pathology for optimized therapy. This work was funded by the Croatian Science Foundation under the projects number [HRZZ-IP-2022-10-7321] and [DOK-2025-02-5516].

Keywords: empagliflozin, SGLT2 inhibitors, connexins, pannexin 1, kidney

Presentation number: PP59 Abstract number: 56-ISABS-2026

CONNEXIN 43 TARGETING FOR PRECISION BIOMARKER DISCOVERY AND REGENERATIVE MODULATION IN OSTEOARTHRITIS

Uzieliene Ilona[1,5], Lebedis Ignas[1], Sereicikaite Lina[1], Vaiciuleviciute Raminta[3], Pachaleva Jolita[1], Mayan Santos Maria[2], Fernandez Carpintero Paula[2], Lopez Inaki[2], Carneiro Alex[2], McGee Margaret[3], Brennan Kieran[3], Blanco Alfonso[3], Kirdaite Gailute[1], Kvederas Giedrius[4], Bernotiene Eiva[1]

[1]Innovative Medicine Centre, Vilnius, Lithuania; [2]University of Vigo, Biomedical Research Centre, Vigo, Spain; [3]University College Dublin, Conway Institute, Dublin, Ireland; [4]Vilnius University Santaros Hospital, Vilnius, Lithuania; [5]Nanodiagnostika ltd., Vilnius, Lithuania

ilona.uzieliene@imcentras.lt

Osteoarthritis (OA) is the most prevalent joint disease worldwide, characterized by impaired cartilage regeneration, chronic inflammation, and cellular senescence. Advancing precision medicine in OA requires identification of molecular regulators and biomarkers reflecting disease progression and treatment response. Connexin 43 (Cx43), a key mediator of intercellular communication and senescence, may influence OA pathology and extracellular vesicle (EV) signaling. This study evaluates the effects of a Cx43 inhibitory peptide (TUB1) on cartilage- and synovium-derived EVs to identify regenerative and biomarker- related signatures. Cartilage and synovium tissues obtained post-surgery were cultured as explants and treated with TUB1. Cellular proliferation, glycosaminoglycan (GAG) deposition, cytokine secretion, and gene expression were analyzed. EVs were isolated by size-exclusion chromatography and characterized for protein markers (Cx43, Pan1, CypA) using flow cytometry. TUB1 treatment reduced cellular senescence, confirmed by decreased β-galactosidase activity. Regenerative effects included reduced GAG release and improved extracellular matrix retention. Cytokine profiling indicated modulation of inflammatory signaling. Importantly, Cx43 expression decreased in EVs, suggesting that EV cargo reflects treatment-induced molecular changes and may serve as a biomarker of therapeutic response. Targeting Cx43 modulates senescence, inflammation, and EV composition, supporting its role in OA pathogenesis and regeneration. EV-based biomarker analysis combined with targeted intervention represents a promising precision medicine approach for patient stratification and monitoring treatment efficacy. The work was supported by the European Social Fund supported project according to the activity HORIZON- WIDERA-2021-ACCESS-03, title: "Twinning for Promoting Excellence, Ability and Knowledge to develop novel approaches for targeting inflammatory and degenerative age-related joint diseases".

Keywords: osteoarthritis, precision medicine, connexin43, biomarkers, extracellular vesicles