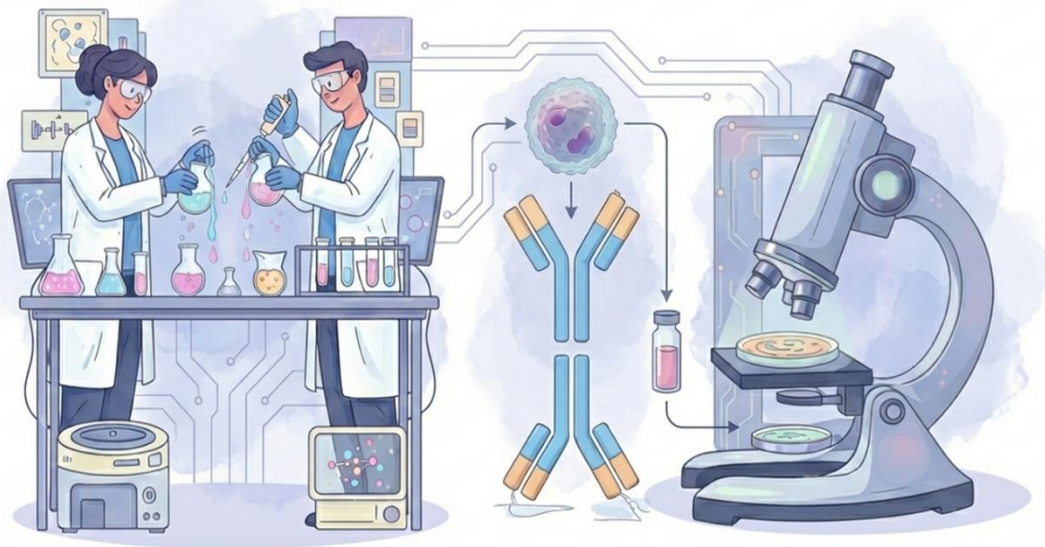




## THE ANNUAL MEETING OF THE CROATIAN IMMUNOLOGICAL SOCIETY 2026

---



1<sup>ST</sup> TO 3<sup>RD</sup> OCTOBER, 2026  
HOTEL TOMISLAVOV DOM,  
MEDVEDNICA NATURE PARK,  
ZAGREB

## Welcome letter

Dear colleagues, dear friends,

It is my great pleasure and privilege to welcome you to the 30th Annual Meeting of the Croatian Immunological Society with international participation (HID).

This year's meeting is particularly meaningful to me, as it marks the end of my term as President of HID. Over the past years, I have had the honor of working with you and contributing to the development of our Society and the advancement of immunology in Croatia. The HID Annual Meeting has always been much more than a scientific gathering. It is a place where collaborations are initiated, young scientists are encouraged, and friendships are built. I am especially pleased to see the continued engagement of young researchers and students, whose enthusiasm and creativity are essential for the future of our field. The 30th anniversary is an opportunity to look back with gratitude at all those who have contributed to our immunological community, while also looking forward to new challenges and opportunities. Our Society has an important role in promoting collaboration between basic, translational and clinical immunology and in supporting the next generation of immunologists.

I sincerely thank all members of HID, our invited speakers, participants, organizers and sponsors for their contribution to this meeting. I am especially grateful to all my colleagues for their support throughout my term, and to Dr. Lidija Milković, Secretary of HID, for her invaluable help and dedication. As I complete my term, I look forward to welcoming the new President of our society. I am confident that HID will continue to grow as a strong professional community and a platform for excellent science and collaboration.

It has been a great honor to serve as President of the Croatian Immunological Society. I am grateful for your trust, support and friendship, and for all that we have achieved together.

I wish you all a successful meeting, creative discussions, new collaborations, and an enjoyable time together.

With best regards

Mariastefania Antica



**THE ANNUAL MEETING OF THE CROATIAN IMMUNOLOGICAL SOCIETY 2026**

**Hotel Tomislavov Dom, Medvednica Nature Park, Zagreb, 01. - 03.10.2026.**

---

**ORGANIZED BY**

**CROATIAN IMMUNOLOGICAL SOCIETY**

Bijenička cesta 54, Ruđer Bošković Institute, Zagreb

President: Mariastefania Antica, Zagreb

Vice-President: Vanda Juranić Lisnić, Rijeka

Secretary: Lidija Milković, Zagreb

**ORGANIZING COMMITTEE**

Felix M. Wensveen, Rijeka

Bojan Polić, Rijeka

Danka Grčević, Zagreb

Stipan Jonjić, Rijeka

Alan Šućur, Zagreb

Asja Stipić Marković, Zagreb

Tomislav Kelava, Zagreb

Alenka Gagro, Zagreb

Ines Mrakovčić-Šutić, Rijeka

Astrid Krmpotić, Rijeka

## PROGRAM

Thursday, October 1<sup>st</sup>, 2026.

**14:00-24:00** HOTEL CHECK-IN  
**14:30-15:15** REGISTRATION  
**15:15-15:30** OPENING CEREMONY

*Chairs: Vanda Juranić Lisnić & Bojan Polić*

**15:30-16:15** ***Invited Lecture - MARCO DE ANDREA***, Department of Public Health and Pediatric Sciences, University of Turin Medical School, Turin, Italy; CAAD Center for Translational Research on Autoimmune and Allergic Disease, University of Piemonte Orientale, Novara Medical School, Novara, Italy  
***Cytomegalovirus infection rewires cellular senescence to promote inflammation***

---

**16:15-17:15** *Selected Oral Presentations - SESSION 1*

**16:15 Marina Babić Čač**, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia - ***Regulation of Lymphocyte Responses During Neuroinflammation***

**16:30 Lucija Mušak**, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia - ***Environmental modulation of CD4<sup>+</sup> T-cell responses and disease outcome in CNS autoimmunity***

**16:45 Iva Vladić**, Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia - ***IFN-γ Drives Cerebellar Glutamatergic Synapse Loss and Motor Dysfunction Following Congenital Cytomegalovirus Infection***

**17:00 Pavao Planinić**, Department of Physiology, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina - ***Mechanisms of Bone Loss in Murine Models of Cholestatic Liver Disease***

---

**17:15 - 17:45** COFFEE BREAK

*Chairs: Felix Wensveen*

**17:45-18:30** ***Invited Lecture - HRVOJE LALIĆ***, Department of Physiology and Immunology, University of Zagreb School of Medicine; Croatian Institute for Brain Research, University of Zagreb School of Medicine; Department of Laboratory Immunology, Clinical Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Croatia  
***Monocytes in chronic graft-versus-host disease***

**18:30-19:15**      *Invited Lecture - IVA HAFNER BRATKOVIČ*, Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia; EN-FIST Centre of Excellence Ljubljana, Slovenia; Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia  
*Inflammasome-centered synthetic immunology*

---

**Friday, October 2<sup>nd</sup>, 2026**

*Chairs: Ines Mrakovčić-Šutić & Alan Šučur*

**9:00-9:45**      *Invited Lecture - MAJA COKARIĆ BRDOVČAK*, Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia  
*Cytomegalovirus-based vaccine vectors as a platform for durable systemic and mucosal immunity*

---

**9:45-10:45**      *Selected Oral Presentations – SESSION 2*

**9:45 Hana Musil**, School of Medicine, University of Zagreb, Zagreb, Croatia  
*- Endothelial Glycocalyx Injury in Pediatric IgA Vasculitis: Potential Biomarkers of Gastrointestinal and Renal Involvement*

**10:00 Sara Aničić**, Dept. of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia - *Notch signaling negatively regulates human osteoclastogenesis in vitro and is downregulated in osteoclast progenitors from patients with rheumatoid arthritis*

**10:15 Ozana Jakšić**, Dept. of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia - *Migration properties and osteoclastogenic potential of circulating osteoclast progenitors in patients with type 1 diabetes mellitus*

**10:30 Marta Radošević**, Dept. of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia - *Increased inflammatory cytokines and metabolic changes are associated with increased bone-resorption markers in STZ-induced type 1 diabetes mouse model*

---

**10:45 – 11:15**      **COFFEE BREAK SPONSORED BY GOREA PLUS**

---

|                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                    | <i>Chairs: Mariastefania Antica &amp; Tomislav Kelava</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>11:15-12:00</b> | <b><i>Invited Lecture - LUDOVICA CACOPARDO</i></b> , Department of Information Engineering and Research Center 'E. Piaggio' - University of Pisa, Pisa, Italy<br><b><i>Engineering the thymic tissue</i></b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>12:00-12:45</b> | <b><i>Selected Oral Presentations - SESSION 3 - Bright Spark</i></b><br><br><b>12:00 Lucija Šakota Planinić</b> , Faculty of Pharmacy, University of Mostar, 88000 Mostar, Bosnia and Herzegovina - <b><i>STING Shapes Neuroinflammation and Neurodevelopmental Gene Expression During Congenital CMV Infection</i></b><br><b>12:15 Sara Priselac</b> , Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia - <b><i>Modeling osteoclast progenitor transendothelial migration and differentiation in a 3D microenvironment</i></b><br><b>12:30 Ozren Majstorović</b> , Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia - <b><i>Adiponectin Receptor Signaling Regulates CD8<sup>+</sup> T Cell Function</i></b> |
| <b>12:45-14:45</b> | <b>BREAK for networking, sponsor exhibition, poster viewing, etc.</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                    | <i>Chair: Danka Grčević</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>14:45-15:15</b> | <b><i>Invited Lecture - JOŠKO MITROVIĆ</i></b> , Division of Clinical Immunology, Allergology and Rheumatology, Department of Internal Medicine, Dubrava University Hospital, Zagreb, Croatia; University of Zagreb School of Medicine, Zagreb, Croatia; University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia<br><b><i>Extra-Articular Manifestations of Rheumatoid Arthritis</i></b>                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>15:15-16:00</b> | <b><i>Invited Lecture - EFIS - IL Lecture Award - VIVIANNE MALMSTRÖM</i></b> , Department of Medicine, Division of Rheumatology, Karolinska Institutet, Stockholm, Sweden; Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden<br><b><i>Understanding and interfering with autoimmunity - antigen specificities, lymphocyte repertoires and effector functions in rheumatoid arthritis</i></b>                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>16:00-18:00</b> | <i>Chairs: Alan Šučur &amp; Ilija Brizić</i><br><b>POSTER SESSION</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>19:00</b>       | <b>GALA DINNER</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

Saturday, October 3<sup>rd</sup>, 2026

9:00-10:00 GENERAL ASSEMBLY OF THE CROATIAN IMMUNOLOGICAL SOCIETY

*Chairs: Tomislav Kelava & Felix Wensveen*

10:00-10:45 **Invited Lecture - VEDRANA JELENČIĆ**, Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia  
***Immune sensing of stress: from stress ligands to tissue dysfunction***

10:45-11:45 *Selected Oral Presentations – SESSION 4*

10:45 **Emma Bellulovich**, Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia - ***High PVR protein expression marks clear cell renal cell carcinoma with metastatic spread***

11:00 **David Čičić**, Division of Hematology, Department of Internal Medicine, University Hospital Dubrava, Zagreb, Croatia - ***Disentangling Lipid Metabolism and Monocytic Differentiation in Venetoclax Resistance in Acute Myeloid Leukemia***

11:15 **Alojzija Brčić**, Department of Physiology and Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia - ***Metabolic control of myeloid differentiation: distinct roles of RRM2 and RRM2B in acute myeloid leukemia***

11:30 **Klara Bardač**, Laboratory for Cell Biology, Department of Physiology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia - ***Hydroxyurea-Induced Monocytic Differentiation in Acute Myeloid Leukemia: Evidence for Mechanisms Beyond RRM2 Suppression and Cell-Cycle Arrest***

11:45-12:15 COFFEE BREAK

12:15-13:00 AWARD CEREMONY AND CLOSING REMARKS

## Invited Lecture

***Cytomegalovirus infection rewires cellular senescence to promote inflammation*****Marco De Andrea<sup>1,2</sup>**

<sup>1</sup>Department of Public Health and Pediatric Sciences, University of Turin Medical School, Turin, Italy;

<sup>2</sup>CAAD Center for Translational Research on Autoimmune and Allergic Disease, University of Piemonte Orientale, Novara Medical School, Novara, Italy

Cellular senescence is increasingly recognized as an important component of virus–host interactions, yet its contribution to inflammation and disease remains poorly defined. In this lecture, I will discuss virus-induced senescence (VIS) as a conceptual framework to understand how viruses reshape host-cell stress responses, cell-cycle control, and secretory programs, focusing on human cytomegalovirus (CMV) as a model DNA virus.

Using primary endothelial cells and murine infection systems, we investigate how CMV alters the fate of infected cells and how this may contribute to inflammatory pathology in vivo. CMV infection triggers a DNA damage-associated, p16INK4a-linked senescence program together with strong activation of inflammatory and innate immune pathways. While senescence-associated growth arrest is largely restricted to infected cells, inflammatory signaling extends to surrounding bystander cells. CMV-induced endothelial inflammation is also associated with cytoplasmic chromatin accumulation and includes a STING-sensitive cytokine component.

These findings support a model in which CMV-induced senescence is not simply a by-product of infection, but part of a broader host–pathogen interaction that promotes local inflammatory circuits and may contribute to systemic disease associations. More broadly, VIS may help explain how DNA viruses drive chronic inflammatory outcomes through common host pathways.

## Invited Lecture

### *Monocytes in chronic graft-versus-host disease*

**Hrvoje Lalić<sup>1,2</sup>**

<sup>1</sup>*Department of Physiology and Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia.*

<sup>2</sup>*Department of Laboratory Immunology, Clinical Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia.*

Chronic graft-versus-host disease (cGvHD) is a major long-term complication of allogeneic hematopoietic stem cell transplantation, and its heterogeneous clinical presentation and variable organ involvement make biological characterization difficult. Beyond adaptive immunity, monocytes and macrophages are increasingly recognized as contributors to persistent inflammation, immune dysregulation and tissue remodeling.

We analyzed peripheral blood monocyte subsets by flow cytometry and monocyte-related cytokines and chemokines by a multiplex bead-based immunoassay in 101 allogeneic transplant recipients: 31 with newly diagnosed cGvHD, 38 with long-standing cGvHD and 32 without cGvHD. Associations with disease activity, severity and organ involvement were assessed.

Classical monocyte counts were increased in long-standing cGvHD and correlated with disease severity. Intermediate monocytes were associated with pulmonary involvement in newly diagnosed disease and with liver involvement in the overall cohort. CD14++HLA-DR- monocytes were also increased in cGvHD. CXCL10 was markedly elevated in newly diagnosed disease and declined with disease duration, whereas IL-6 and IL-8 correlated with severity in long-standing disease; MCP-1 was increased in the overall cGvHD cohort. IL-10 and IL-17A were associated with pulmonary and oral involvement, respectively, in newly diagnosed cGvHD. These associations were largely independent of major transplantation-related and demographic factors.

Monocyte-associated immune responses appear to differ across the clinical course of cGvHD and patterns of organ involvement. Integrating cellular and soluble markers of the monocyte response may improve biological characterization of cGvHD and support the development of clinically informative biomarker combinations.

## Invited Lecture

*Inflammasome-centered synthetic immunology*

Elvira Boršić Mlinarič<sup>1</sup>, Taja Železnik Ramuta<sup>1</sup>, Sara Orehek<sup>1</sup>, Roman Jerala<sup>1</sup> and **Iva Hafner Bratkovič**<sup>1-3</sup>

<sup>1</sup>Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia;

<sup>2</sup>EN-FIST Centre of Excellence Ljubljana, Slovenia

<sup>3</sup>Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Inflammasomes are central components of early immune responses. Upon sensing infection or disrupted tissue homeostasis, inflammasome sensors lead to the assembly of inflammasomes and activation of inflammatory caspases. Caspase-1 subsequently proteolytically activates proinflammatory cytokines IL-1 $\beta$  and IL-18 and gasdermin D into its N-terminal pore-forming domain that causes pyroptosis. Proinflammatory cytokine release combined with immunogenic cell death (ICD) induces potent inflammatory responses. Cancer is an example of a disease where the immune system fails and several types of tumors downregulate different cell death pathways. Using synthetic biology combined with mechanistic insight into inflammasome assembly, we are currently developing inflammasome-inspired approaches to provide efficient priming of immune responses in cancer immunotherapy.

One of the approaches we developed is cytokine-armed pyroptosis. To induce pyroptosis, we utilized designed, tightly regulated gasdermin D variants with different pore-forming capabilities and diverse modes of activation, representing a toolbox of immunogenic cell death (ICD) inducers. We demonstrated that the electrogenic transfer of ICD effector-encoding plasmids into mouse melanoma tumors, when combined with intratumoral expression of cytokines IL-1 $\beta$ , IL-12, or IL-18, enhanced anti-tumor immune responses. Careful selection of immunostimulatory molecules is, however, imperative as a combination of IL-1 $\beta$  and IL-18 antagonized the protective effect of pyroptosis by IFN $\gamma$ -mediated upregulation of several immunosuppressive pathways. We show that the intratumoral introduction of armed pyroptosis provides protection against distant tumors and proves effective across various tumor types without inducing systemic inflammation in preclinical mouse models.

Inflammasome-mimicking approaches are tunable and tumor-agnostic strategies to enhance antitumor responses.

# Invited Lecture

## *Cytomegalovirus-based vaccine vectors as a platform for durable systemic and mucosal immunity*

**Maja Cokarić Brdovčak<sup>1</sup>**, Jelena Materljan Franki<sup>1</sup>, Marko Šustić<sup>1,2,3</sup>, Karmela Miklič<sup>1</sup>, Magdalena Medved<sup>1</sup>, Stipan Jonjić<sup>1</sup>, Ilija Brizić<sup>1</sup>, Astrid Krmpotić<sup>4</sup>

<sup>1</sup>Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

<sup>2</sup>Department of Cardiology, Clinical Hospital Center Rijeka, Rijeka, Croatia

<sup>3</sup>Department of Internal Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

<sup>4</sup>Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Current COVID-19 vaccines face challenges such as waning immunity and limited mucosal protection, which contribute to reinfections and continued viral transmission. These limitations highlight the importance of developing next-generation vaccines that can induce strong tissue-specific and systemic immune responses. Cytomegalovirus (CMV) represents a promising vaccine vector due to its ability to elicit robust and durable immunity.

In this study, we generated murine CMV (MCMV)-based vaccine vectors expressing the SARS-CoV-2 Spike (S) protein using either a wild-type or immunologically attenuated viral strain. By comparing intraperitoneal (IP) and intranasal (IN) immunization with both MCMV vectors, we show that IP administration induced higher, long-lasting S-specific antibody levels in serum. Both routes elicited strong systemic S-specific CD8<sup>+</sup> T cell responses; however, CD8<sup>+</sup> T cell responses induced by different immunization routes differed notably in their kinetics and phenotype. IP immunization with both vectors generated higher frequencies of S-specific CD8<sup>+</sup> T cells in the blood, spleen and lungs, and the majority of cells were KLRG1<sup>+</sup> and CD127<sup>-</sup>, characteristic of effector-like T cells. Importantly, IN immunization with a recombinant MCMV vector expressing NKG2D ligand RAE-1 $\gamma$  resulted in robust accumulation of lung-resident S-specific CD8<sup>+</sup> T cells, exceeding the levels induced by licensed COVID-19 vaccines.

Overall, our findings demonstrate the potential of CMV-based vaccine vectors to fine-tune immune responses through the combined optimization of vector design and the delivery route. By leveraging these variables, it is possible to influence both the magnitude and the quality of the immunity induced.

## Invited Lecture

*Engineering the thymic tissue*Francesco Fontana<sup>1</sup>, Arti Ahluwalia<sup>1</sup>, **Ludovica Cacopardo<sup>1</sup>**<sup>1</sup>*Department of Information Engineering and Research Center 'E. Piaggio' - University of Pisa, Pisa, Italy*

The thymus has a pivotal function in the generation of immunocompetent T cells. Despite this function strictly relies on its complex extracellular matrix, to date, the organization of its 3D mesh structure, as well as its mechanical properties have been poorly investigated.

In this context, the multimodal and multiscale characterization of the thymus performed by our group provides researchers with a portfolio of mechanical and structural data for engineering this organ.

According to the measured specification, gelatin gels were engineered using chaotropic salts, obtaining 3D scaffolds able to mimic thymic fibrous network as well to match its viscoelastic behavior.

Both the mechanical and structural portfolio and the thymus-mimetic gels pave the way for future human-relevant in vitro studies or clinical applications.

## Invited Lecture

### *Extra-Articular Manifestations of Rheumatoid Arthritis*

**Joško Mitrović**

<sup>1</sup>*Division of Clinical Immunology, Allergology and Rheumatology, Department of Internal Medicine, Dubrava University Hospital, Zagreb, Croatia*

<sup>2</sup>*University of Zagreb School of Medicine, Zagreb, Croatia*

<sup>3</sup>*University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia*

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized primarily by chronic inflammation and progressive joint damage. However, persistent immune activation may affect multiple organs and substantially contribute to morbidity and mortality, primarily through cardiovascular and pulmonary involvement, as well as anaemia, infections, osteoporosis, amyloidosis, and depression. Cardiovascular and pulmonary involvement are among the most clinically relevant extra-articular manifestations of RA and are major contributors to the increased mortality observed in these patients. Chronic systemic inflammation, autoimmunity, endothelial dysfunction, altered lipoprotein function, and both traditional and non-traditional cardiovascular risk factors interact to promote accelerated atherosclerosis, microvascular dysfunction, and myocardial injury. In addition, RA is associated with an increased risk of heart failure, in which myocardial inflammation, fibrosis, and microvascular dysfunction may play important roles.

Interstitial lung disease (RA-ILD) represents another major and potentially life-threatening complication of RA. Genetic susceptibility and environmental exposures, particularly smoking, may promote respiratory epithelial injury, protein citrullination, and local autoimmunity. Subsequent activation of inflammatory and profibrotic pathways leads to interstitial remodeling and progressive pulmonary fibrosis. Importantly, RA-associated ILD may remain clinically silent, highlighting the importance of identifying patients at increased risk and considering appropriate screening and early diagnostic evaluation. Together, cardiovascular disease and RA-ILD illustrate how persistent systemic inflammation and autoimmunity extend the burden of RA well beyond the joints. Early recognition of these and other extra-articular manifestations is essential for improving prognosis and reducing mortality in patients with RA.

Invited Lecture - EFIS - IL Lecture Award

*Understanding and interfering with autoimmunity  
– antigen specificities, lymphocyte repertoires and effector functions in rheumatoid  
arthritis*

**Vivianne Malmström**

*Department of Medicine, Division of Rheumatology, Karolinska Institutet, Stockholm, Sweden  
Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden*

Autoimmunity to modified self, i.e. citrullinated proteins, are a hallmark of rheumatoid arthritis (RA) and are influenced by both genetic and environmental risk factors. This autoimmunity predates the inflammatory joint disease and hence allows the identification of individuals at risk of a future RA diagnosis. RA is hereby a disease which can be studied in various stages, including before diagnosis.

We have studied citrulline-specific B and T cells at different stages of disease and in various tissues. Based on epitope mapping, lymphocyte repertoires and effector functions, our data implicate profound T cell – B cell cross-talk driving chronic immune responses. This suggests that disrupting such a forward loop should be beneficial. Indeed, severe cases of autoimmune diseases have been successfully treated with immune-targeting therapy leading to so-called immune reset (by CAR-T cell therapy or bi-specific T cell engagers). I will discuss a different approach focusing on targeting the precise autoimmunity, which would have the benefit of maintaining protective immunity.

## Invited Lecture

### *Immune sensing of stress: from stress ligands to tissue dysfunction*

**Vedrana Jelenčić**

*Department of histology and embryology, Faculty of Medicine, University of Rijeka*

Cells undergoing transformation, infection, metabolic challenge or other forms of stress can alter their expression of stress-associated ligands, thereby becoming visible to the immune system. NK cells and other innate lymphocytes are particularly equipped to detect such changes through an array of activating receptors, including NKG2D and NCR1. Yet, stress recognition is not a simple receptor–ligand interaction. Rather, immune responsiveness emerges from the integration of multiple signals and is shaped by receptor tuning, cellular plasticity and the surrounding tissue environment.

Our work has explored how stress recognition is regulated at the level of NK-cell activating receptors and how the remarkable plasticity of NK cells enables them to respond to altered cells despite major changes in their receptor repertoire. These studies have expanded from classical tumour and infection surveillance towards understanding how immune cells recognize stress within tissues and how stress–ligand-mediated immune sensing can influence tissue responses. Together, these findings support a broader view of immune surveillance in which stress-associated signals connect changes in cellular state with immune recognition. Understanding how these signals are integrated and interpreted across different biological contexts may provide new insights into the complex relationship between cellular stress, immune responses and tissue function.

*Selected oral presentations – SESSION 1**S1-01 Regulation of Lymphocyte Responses During Neuroinflammation*

Christoforos Dimitropoulos<sup>1,2</sup>, Lucija Mušak<sup>3</sup>, Klara Ivančić<sup>3</sup>, Alba del Rio Serrato<sup>4,5,6</sup>, Oliver C. Knight<sup>1,2</sup>, Christina Stehle<sup>1,2</sup>, Timo Ruckert<sup>1,2</sup>, Bojan Polić<sup>3</sup>, Carmen Infante-Duarte<sup>4,5,6</sup>, **Marina Babić<sup>3</sup>\***, Chiara Romagnani<sup>1,2\*</sup>

\* These authors contributed equally.

<sup>1</sup>Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin and Cluster of Excellence ImmunoPreCept, Institute of Medical Immunology, Augustenburger Platz 1, 13353 Berlin, Germany; <sup>2</sup>Innate Immunity, German Rheumatism Research Centre – a Leibniz Institute, Berlin, Germany; <sup>3</sup>Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia; <sup>4</sup>Experimental and Clinical Research Center, a cooperation between the Max Delbrück Center for Molecular Medicine in the Helmholtz Association and Charité – Universitätsmedizin Berlin, Berlin, Germany; <sup>5</sup>Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, ECRC – Experimental and Clinical Research Center, Berlin, Germany; <sup>6</sup>Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany.

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS) characterized by inflammation, demyelination, oligodendrocyte loss, and axonal injury. While autoreactive CD4<sup>+</sup> T cells are considered key drivers of pathology, the contribution of antigen-independent bystander T cells to neuroinflammation remains largely unexplored.

We identified a conserved population of circulating NKG2D<sup>+</sup>ID2hi Th1 cells present in both mice and humans and enriched in patients with autoimmune diseases, particularly MS. Using experimental autoimmune encephalomyelitis (EAE) combined with single-cell RNA sequencing, we characterized NKG2D<sup>+</sup>CD4<sup>+</sup> T cells accumulating in the inflamed CNS and uncovered two distinct functional states within this population. Alongside antigen-specific effector cells, we detected a population of bystander-like ID2hi CD4<sup>+</sup> T cells that lack signatures of recent TCR engagement and transcriptionally resemble circulating counterparts present at steady state. Strikingly, these antigen-independent bystander cells retain strong inflammatory potential where NKG2D signaling amplifies proinflammatory programs in both antigen-specific and bystander CD4<sup>+</sup> T cells, promoting myeloid cell recruitment and exacerbating demyelination during EAE. Together, our findings challenge the view that CNS inflammation is driven solely by autoreactive lymphocytes and identify NKG2D as a key checkpoint enabling bystander CD4<sup>+</sup> T cells to amplify neuroinflammatory responses.

*Selected oral presentations – SESSION 1*

***S1-O2 Environmental modulation of CD4<sup>+</sup> T-cell responses and disease outcome in CNS autoimmunity***

**Lucija Mušak<sup>1</sup>**, Christoforos Dimitropoulos<sup>2</sup>, Chiara Romagnani<sup>2</sup>, Marina Babić<sup>1</sup>

<sup>1</sup>*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*

<sup>2</sup>*Institute of Medical Immunology, Charité Universitätsmedizin Berlin, Berlin, Germany*

Autoimmune diseases such as multiple sclerosis are driven by dysregulated activation of autoreactive T cells. However, antigen-specific responses occur within a broader inflammatory environment that can also activate non-cognate “bystander” T cells. Increasing evidence suggests that bystander CD4<sup>+</sup> T-cell activation can shape immune responses during infection and inflammation through cytokine-mediated, TCR-independent mechanisms. Although this phenomenon has been primarily studied in infectious settings, its contribution to autoimmune responses remains poorly understood. Peripheral factors, including microbial exposure and diet, can further shape immune cell function and influence susceptibility to disease.

Using the experimental autoimmune encephalomyelitis (EAE) model, combined with transcriptomic approaches and extensive flow cytometry, we now focus on understanding how dietary cues modulate the early CD4<sup>+</sup> T cell dynamics during the initiation of autoimmune responses and influence subsequent disease development.

We hypothesize that environmental signals alter the inflammatory context in which T-cell priming occurs, thereby affecting both antigen-specific and bystander CD4<sup>+</sup> T-cell responses.

Preliminary dietary intervention studies indicate that diet composition can modulate disease susceptibility and the magnitude of antigen-specific CD4<sup>+</sup> T-cell responses. Together, these findings support a role for peripheral environmental cues as important, yet incompletely understood, regulators of early immune activation and autoimmune disease initiation.

*Selected oral presentations – SESSION 1****SI-O3 IFN- $\gamma$  Drives Cerebellar Glutamatergic Synapse Loss and Motor Dysfunction Following Congenital Cytomegalovirus Infection***

Daria Kveštak<sup>1</sup>, **Iva Vladić<sup>1</sup>**, Elisabetta Menna<sup>2</sup>, Andrea Mihalić<sup>1</sup>, Dubravka Karner<sup>1</sup>, Tihana Lenac Roviš<sup>1</sup>, Michela Matteoli<sup>2</sup>, Stipan Jonjić<sup>1</sup> and Ilija Brizić<sup>1</sup>

<sup>1</sup>Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

<sup>2</sup>Laboratory of Pharmacology and Brain Pathology, Humanitas Clinical and Research Center, Milano, Italy

Congenital human cytomegalovirus (cHCMV) infection of the brain is associated with a wide range of neurodevelopmental sequelae. Using neonatal infection of mice with mouse cytomegalovirus (MCMV) as a model that recapitulates many aspects of cHCMV infection, we have previously shown that activated natural killer (NK) and type 1 innate lymphoid cells (ILC1) in the neonatal brain drive immunopathology rather than controlling infection, with interferon- $\gamma$  (IFN- $\gamma$ ) released by these brain-infiltrating cells driving altered cerebellar development. Here we show that this developmental impairment is accompanied by a marked reduction in glutamatergic synapse density in the cerebellum, which in turn underlies deficits in motor coordination in infected mice. Blockade of IFN- $\gamma$  preserved glutamatergic synapse density and prevented the development of motor coordination deficits, demonstrating that the host immune response, and IFN- $\gamma$  specifically, is a major driver of infection-induced synaptic pathology and motor dysfunction. These findings highlight the potential for therapeutically modulating the antiviral immune response to limit immune-mediated brain injury while preserving its protective, antiviral functions.

## Selected oral presentations – SESSION 1

### SI-O4 Mechanisms of Bone Loss in Murine Models of Cholestatic Liver Disease

**Pavao Planinić<sup>1</sup>**, Marta Radošević<sup>2,3</sup>, Ivo Krešić<sup>1</sup>, Sara Aničić<sup>2,3</sup>, Darja Flegar<sup>2,3</sup>, Alan Šučur<sup>2,3</sup>, Petra Turčić<sup>5</sup>, Vedran Katavić<sup>2,4</sup>, Nataša Kovačić<sup>2,4</sup>, Danka Grčević<sup>2,3</sup>, Antonio Markotić<sup>1</sup>, Ivan Čavar<sup>1</sup>, Tomislav Kelava<sup>2,3</sup>

<sup>1</sup>Department of Physiology, School of Medicine, University of Mostar, 88000 Mostar, Bosnia and Herzegovina; <sup>2</sup>Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia; <sup>3</sup>Department of Physiology, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia; <sup>4</sup>Department of Anatomy, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia; <sup>5</sup>Department of Pharmacology, Faculty of Pharmacy and Biochemistry, University of Zagreb, 10000 Zagreb, Croatia.

**Objectives:** Cholestatic liver diseases are frequently associated with bone degeneration, but the underlying pathophysiological mechanisms, as well as adequate animal models to study this complication are not defined. In the present study we have investigated bone loss in two common dietary murine cholestatic models: a diet supplemented with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) or with 1-naphthylisothiocyanate (ANIT).

**Methods:** Female C57BL/6 mice were fed a standard diet, a DDC-supplemented diet or ANIT-supplemented diet for 4–8 weeks. Bone changes were assessed using micro-computed tomography (μCT). Bone marrow osteoclast progenitors (OCPs) were characterized by flow cytometry (CD45+Ly6G-CD3-B220-NK1.1-CD11b<sup>lo</sup>CD115<sup>+</sup>). Sorted OCPs were cultured in osteoclastogenic medium (RANKL/M-CSF) with varying DDC or ANIT concentrations, and osteoclast differentiation was assessed by TRAP staining.

**Results:** Development of liver fibrosis in both models was confirmed by Sirius red staining and increased expression of Col1a1, Acta2 and Krt19 genes. Furthermore, μCT analysis revealed reduced cortical thickness in both models compared to control group. However, analysis of trabecular bone showed a bone loss only in ANIT model, whereas DDC-model was surprisingly associated with an increase in BV/TV and Tb.N. parameters. Analysis of peripheral OCPs revealed a consistent increase in frequency of CD45+Ly6G-CD3-B220-NK1.1-CD11b<sup>lo</sup>CD115<sup>+</sup> population in ANIT model, whereas the results in DDC model were variable. In vitro experiments showed strong inhibitory effect of DDC on osteoclast differentiation.

**Conclusion:** Our findings highlight model-specific mechanisms linking liver disease to changes in bone morphology, with ANIT model better mimicking clinical findings.

*Selected oral presentations – SESSION 2*

***S2-O1 Endothelial Glycocalyx Injury in Pediatric IgA Vasculitis: Potential Biomarkers of Gastrointestinal and Renal Involvement***

**H. Musil<sup>1</sup>, A. Kozmar<sup>2</sup>, N. Kifer<sup>1,3</sup>, D. Grguric<sup>1,3</sup>, M. Frkovic<sup>1,3</sup>, M. Sestan<sup>1,3</sup>, M. Jelusic<sup>1,3</sup>**

<sup>1</sup>*School of Medicine, University of Zagreb, Zagreb, Croatia*

<sup>2</sup>*Department of Laboratory Diagnostics, University of Zagreb School of Medicine, University Hospital Centre Zagreb, Zagreb, Croatia*

<sup>3</sup>*Department of Pediatric Immunology, Rheumatology and Allergology, University Hospital Centre Zagreb, Zagreb, Croatia*

*Introduction:* IgA vasculitis (IgAV) is the most common childhood vasculitis and may involve the gastrointestinal (GI) tract and kidneys. Endothelial glycocalyx injury contributes to vascular permeability and endothelial dysfunction and may reflect disease severity.

*Objectives:* To evaluate serum and urine glycocalyx-related biomarkers in children with IgAV and their associations with disease manifestations and activity.

*Methods:* Serum and urine Syndecan, Glypican and Spectrin concentrations were measured at disease onset and after 6 months in children with IgAV and healthy controls. Associations with GI and renal involvement, relapses, and laboratory parameters were analyzed using nonparametric and correlation analyses.

*Results:* Forty-nine children with IgAV and 17 healthy controls were included. Nephritis occurred in 20 (40.8%), severe GI manifestations in 16 (32.7%), and severe skin involvement in 7 (14.3%). Serum Spectrin was significantly higher in IgAV than controls ( $p=0.0001$ ), while serum Glypican also differed between groups ( $p=0.035$ ). Lower baseline serum and urine Syndecan levels were associated with severe GI manifestations (2.77 vs 4.28 ng/mL,  $p=0.007$ ; 0.71 vs 1.61 ng/mL,  $p=0.010$ ), as was 6-month urine Syndecan (0.48 vs 1.56 ng/mL,  $p=0.012$ ). Lower baseline urinary Syndecan was associated with combined proteinuria and hematuria ( $p=0.042$ ). Higher Spectrin was associated with hematuria, relapse number, and creatinine.

*Conclusion:* Glycocalyx-related biomarkers are altered in pediatric IgAV and show distinct associations with GI and renal involvement. Syndecan may reflect glycocalyx depletion, while Spectrin may indicate persistent endothelial and cellular injury. This work was supported by the Croatian Science Foundation under project number HRZZ-IP-2024-05-6848 and NPOO 10106-25-2897 (GUT-VAS), funded by EU-NextGenerationEU.

## Selected oral presentations – SESSION 2

### ***S2-O2 Notch signaling negatively regulates human osteoclastogenesis in vitro and is downregulated in osteoclast progenitors from patients with rheumatoid arthritis***

**Sara Aničić<sup>1,2</sup>**, Mihovil Plečko<sup>3</sup>, Marta Radošević<sup>2</sup>, Ozana Jakšić<sup>1,2</sup>, Korina Pervan<sup>2</sup>, Ivo Krešić<sup>4</sup>, Sara Priselac<sup>2,5</sup>, Tomislav Balen<sup>2,5</sup>, Marina Ikić Matijašević<sup>6</sup>, Darja Flegar<sup>1,2</sup>, Tomislav Kelava<sup>1,2</sup>, Nataša Kovačić<sup>2,5</sup>, Alan Šućur<sup>1,2</sup>, Maša Filipović<sup>1,2</sup>, Danka Grčević<sup>1,2</sup>

<sup>1</sup>Dept. of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>2</sup>Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Croatia; <sup>3</sup>Dept. of Orthopedic Surgery, University Hospital Centre Zagreb, Croatia; <sup>4</sup>Dept. of Physiology, University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina; <sup>5</sup>Dept. of Anatomy, University of Zagreb School of Medicine, Croatia; <sup>6</sup>Dept. of Clinical Immunology, Rheumatology, and Pulmonology, University Hospital "Sveti Duh", Zagreb, Croatia

**Background and Aim:** Rheumatoid arthritis (RA) is a joint-affecting autoimmune disease, characterized by chronic inflammation, as well as local and systemic bone loss mediated by increased osteoclast activity. Our aim was to investigate whether the Notch-pathway, an established regulator of hematopoietic cell fate decisions, may act as a negative modulator of peripheral blood and bone-marrow monocyte-derived osteoclast progenitor (OCP) differentiation into multinucleated bone-resorbing osteoclasts.

**Methods and Results:** Peripheral OCPs (CD45+CD15–CD3–CD19–CD56– CD11b+CD14+) were identified in RA patients (n=39) and control subjects (n=38) within classical/intermediate monocyte subsets, and analyzed for their expression of Notch-pathway components using flow-cytometry and qPCR. OCPs were expanded in RA (p<0.001), in parallel with elevated serum level of resorption marker CTX-I (p<0.05). RA OCPs in peripheral blood and bone-marrow exhibited reduced surface and gene expression of Notch receptors (NOTCH1 and NOTCH2; p<0.05), showing inverse correlation with the disease activity score DAS28(ESR) (p=–0.491, p<0.05). Expression of Notch-pathway components has also been confirmed in periarticular bone-marrow (n=11) of RA patients by immunohistochemistry. FACS-sorted OCPs differentiated into multinuclear TRAP+ osteoclasts and formed resorption pits on calcium phosphate-coated plates, in parallel with the upregulation of osteoclast-differentiation genes. Immobilized Notch-ligands, especially DLL1, suppressed osteoclast formation in vitro and neutralizing anti-Notch 1 antibodies partially reversed this effect, indicating that Notch 1 mediates ligand-induced suppression of osteoclast differentiation.

**Conclusions:** These findings identify Notch signaling as a negative regulator of human osteoclastogenesis and suggest that reduced Notch receptor expression in RA OCPs may contribute to enhanced osteoclast formation and pathological bone loss.

**Funding:** HRZZ-IP-2022-10-2285, NPOO-10106-25-3013

*Selected oral presentations – SESSION 2****S2-O3 Migration properties and osteoclastogenic potential of circulating osteoclast progenitors in patients with type 1 diabetes mellitus***

**Ozana Jakšić<sup>1,2</sup>, Maša Filipović<sup>1,2</sup>, Marta Radošević<sup>1,2</sup>, Sara Aničić<sup>1,2</sup>, Korina Pervan<sup>1</sup>, Ivo Krešić<sup>3</sup>, Tomislav Balen<sup>1,4</sup>, Sara Priselac<sup>1,4</sup>, Darja Flegar<sup>1,2</sup>, Alan Šučur<sup>1,2</sup>, Tomislav Kelava<sup>1,2</sup>, Nataša Kovačić<sup>1,4</sup>, Ivana Kraljević<sup>5,6</sup>, Valerija Bralić Lang<sup>7,8</sup>, Danka Grčević<sup>1,2</sup>**

<sup>1</sup>Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>2</sup>Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>3</sup>Department of Physiology, University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina; <sup>4</sup>Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>5</sup>Department of Endocrinology, University Hospital Centre Zagreb, Zagreb, Croatia; <sup>6</sup>Department of Internal Medicine, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>7</sup>Specialist Family Medicine Practice, Zagreb, Croatia; <sup>8</sup>Department of Family Medicine, University of Zagreb School of Medicine, Zagreb, Croatia

**Background and Aim:** Type 1 diabetes (T1D) is associated with increased fracture risk reflecting impaired bone quality, but the mechanism is not fully revealed. Chronic inflammation in T1D may enhance osteoclast activity by priming monocyte-derived osteoclast progenitors (OCPs), potentially contributing to skeletal fragility. We aimed to characterize phenotypic, migratory, and osteoclastogenic properties of circulating OCPs in T1D patients.

**Methods:** Peripheral blood from healthy controls (n=39) and patients with T1D (n=32) was analyzed by flow-cytometry and LEGENDPlex. OCPs (CD45+CD15–CD3–CD19–CD56–CD11b+CD16–CD14+) were evaluated for their expression of CCR2, TLR4, and CD115, receptors implicated in monocyte migration, inflammatory response, and osteoclastogenesis. FACS-sorted OCPs underwent Transwell-migration toward CCL2 or LPS gradient, with CCR2 or TLR4 blockade. Osteoclastogenic potential was tested under standard (RANKL/M-CSF) and inflammatory (TGF- $\beta$ /TNF- $\alpha$ ) conditions. Osteoclast maturation was confirmed by morphology and TRAP-expression, while their resorptive capacity was evaluated on calcium phosphate-coated plates.

**Results:** Patients with T1D had a higher proportion of classical monocytes (comprising OCPs) than controls (27.7% [IQR 22.6–35.8] vs 18.4% [12.3–26.2]; p<0.05), with higher serum CCL2. More than 80% of OCPs expressed CCR2 and TLR4, with increased CCR2, TLR4, and CD115 in T1D. CCL2 induced robust OCP migration, markedly reduced by CCR2 blockade, whereas LPS-mediated migration was subtle. Both standard and inflammatory osteoclastogenic conditions efficiently generated mature multinucleated TRAP+ osteoclasts, capable of resorbing mineralized matrix.

**Conclusions:** Our findings indicate expansion and functional priming of peripheral OCPs in T1D and support the CCR2–CCL2 axis as a potential mechanistic link between systemic inflammation and skeletal fragility.

**Funding:** HRZZ-IP-2022-10-2285, HRZZ-DOK-2025-02-2318

## Selected oral presentations – SESSION 2

### ***S2-O4 Increased inflammatory cytokines and metabolic changes are associated with increased bone-resorption markers in STZ-induced type 1 diabetes mouse model***

**Marta Radošević<sup>1,2</sup>**, Maša Filipović<sup>1,2</sup>, Darja Flegar<sup>1,2</sup>, Ozana Jakšić<sup>1,2</sup>, Sara Aničić<sup>1,2</sup>, Tomislav Balen<sup>1,3</sup>, Pavao Planinić<sup>4</sup>, Tomislav Kelava<sup>1,2</sup>, Sanja Novak<sup>5</sup>, Nataša Kovačić<sup>1,3</sup>, Danka Grčević<sup>1,2</sup>

<sup>1</sup>Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>2</sup>Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>3</sup>Department of Anatomy, University of Zagreb School of Medicine, Croatia; <sup>4</sup>University of Mostar School of Medicine, Mostar, Bosnia and Herzegovina; <sup>5</sup>Department of Physiology and Cell Biology, University of Arkansas for Medical Sciences, Little Rock, AR

**Background:** Type 1 diabetes mellitus (T1D) has been associated with bone loss and increased risk of pathological fractures, although the mechanism is not fully understood. We propose that metabolic changes and inflammatory environment may contribute to altered kinetics of bone-marrow and peripheral myeloid populations, which include osteoclast progenitors (OCPs).

**Aim:** We investigated changes in bone metabolism in parallel with serum cytokines and OCP subsets in a streptozotocin (STZ)-induced T1D mouse model.

**Methods and Results:** T1D was induced in male mice by STZ administration (50 mg/kg i.p., 5 days) and confirmed by fasting hyperglycemia, impaired glucose tolerance, and pancreatic histological changes. Flow-cytometric analysis of bone-marrow- and spleen-derived CX3CR1+ OCPs (CD45+CD3-B220-NK1.1-CD11b/+CD115+) showed higher migratory properties and induction of markers associated with osteoclast activity (RANK, CCR2, CD64, TLR4, CD27) in T1D compared to controls. Differentiation of FACS-sorted T1D CX3CR1+ OCPs under osteoclastogenic conditions (M-CSF/RANKL) demonstrated increased osteoclast formation. ELISA/EIA revealed elevated systemic bone resorption markers (TRAcP5b, CTX-I) for up to three months following STZ-treatment, which positively correlated with glycemic levels. LEGENDPlex assay indicated increased serum concentrations of inflammatory cytokines TNF- $\alpha$ , CCL2, IL-1 $\beta$ , and decreased anti-inflammatory IL-10, which negatively correlated with hyperglycemia. SCENITH assay, used to measure major metabolic pathways, indicated altered energy dependence across different cell populations, including monocytes, T and B lymphocytes, presumably as a result of inflammation.

**Conclusions:** We concluded that in the STZ-induced T1D mouse model metabolic changes and destruction of pancreatic  $\beta$ -cells contribute to local and systemic inflammation that increase OCP activity and enhance bone resorption.

**Funding:** HRZZ-IP-2022-10-2285, NPOO-10106-25-3013

*Selected oral presentations – SESSION 3 - BRIGHT SPARK**S3-SBI STING Shapes Neuroinflammation and Neurodevelopmental Gene Expression During Congenital CMV Infection*

**Lucija Šakota Planinić<sup>1,2</sup>**, Iva Vladić<sup>2</sup>, Ivana Bertović<sup>2</sup>, Fran Krstanović<sup>2</sup>, Andrea Mihalić<sup>2</sup>, Stipan Jonjić<sup>2</sup>, Berislav Lisnić<sup>2</sup>, Ilija Brizić<sup>2</sup>

*<sup>1</sup>Faculty of Pharmacy, University of Mostar, 88000 Mostar, Bosnia and Herzegovina*

*<sup>2</sup>Center for proteomics, Faculty of Medicine, University of Rijeka, 51000 Rijeka, Croatia*

Congenital cytomegalovirus (CMV) infection is the most common congenital infection and a leading infectious cause of long-term neurological disability, causing structural and functional abnormalities in the developing central nervous system (CNS) that manifest as hearing loss, intellectual disability, and cerebral palsy. Although inflammation contributes to CMV-associated neuropathology, the underlying mechanisms remain poorly understood. The stimulator of interferon genes (STING) pathway is a key regulator of innate antiviral immunity and type I interferon (IFN-I) responses. Here, we investigated the role of STING in the pathogenesis of congenital CMV infection using a mouse model and mouse cytomegalovirus (MCMV) infection.

We found that IFN-I signaling was essential for controlling MCMV infection in newborn mice, as IFN-I receptor-deficient mice succumbed to infection and showed increased viral levels in the brain and other organs. While STING was dispensable for MCMV control in adult mice, it contributed modestly to viral control in neonates. Despite this modest antiviral role, STING deficiency substantially altered the neonatal brain immune landscape, with enhanced microglial activation, increased NK and CD4<sup>+</sup> T-cell responses, and altered cytokine profiles, consistent with a transcriptomic signature of heightened inflammation. Notably, multiple genes associated with synaptic assembly were dysregulated in STING-deficient brains, linking STING-dependent immune regulation to pathways relevant to neurodevelopment. Pharmacological STING activation with the agonist DMXAA reduced MCMV replication both in vitro and in vivo, suggesting a therapeutic potential for boosting this pathway. Together, these findings identify STING as a regulator that limits neuroinflammation and preserves neurodevelopmental gene programs during congenital CMV infection, offering a potential mechanistic link between antiviral immunity and the neurological sequelae of congenital CMV disease.

## Selected oral presentations – SESSION 3 - BRIGHT SPARK

### *S3-SB2 Modeling osteoclast progenitor transendothelial migration and differentiation in a 3D microenvironment*

**Sara Priselac**<sup>1,2</sup>, Darja Flegar<sup>1,3</sup>, Maša Filipović<sup>1,3</sup>, Ozana Jakšić<sup>1,3</sup>, Sara Aničić<sup>1,3</sup>, Marta Radošević<sup>1,3</sup>, Ivo Krešić<sup>1,4</sup>, Tomislav Balen<sup>1,2</sup>, Tomislav Kelava<sup>1,3</sup>, Danka Grčević<sup>1,3</sup>, Alan Šušćur<sup>1,3</sup>, Nataša Kovačić<sup>1,2</sup>

<sup>1</sup>Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>2</sup>Department of Anatomy, University of Zagreb School of Medicine, Croatia; <sup>3</sup>Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>4</sup>Department of Physiology, School of Medicine, University of Mostar, 88000 Mostar, Bosnia and Herzegovina.

**Introduction:** Systemic and local bone resorption in rheumatoid arthritis is mediated by increased recruitment, migration and homing of a circulating monocytic subset comprised of osteoclast progenitors (OCPs) to the affected joints, leading to increased osteoclast differentiation and activation. Modelling this process in a standard cell culture does not reflect the complexity of joint tissues, particularly the osteochondral compartment. We have previously established a hydrogel-based 3D model of the osteochondral compartment, using 2% decellularized adipose tissue-derived extracellular matrix (adECM), mesenchymal cells and bone fragments, that may be used to model OCP trafficking and osteoclast activity.

**Results and methods:** To model transendothelial and tissue migration of OCPs, adECM was placed on a transwell membrane, and lined with human umbilical vein endothelial cells. PBMCs, a population containing OCPs, were placed in the top compartment, while the bottom compartment contained 10-100ng/mL CCL2, which dose-dependently induced transendothelial migration of PBMCs, and their migration through the hydrogel towards the bottom compartment.

To create a 3D model of osteoclast differentiation and activity, 4x10<sup>4</sup> FACS-sorted OCPs [CD45posCD3negCD19negCD56negCD15negCD14posCD11bpos] were mixed with 50µl adECM or adECM containing decellularized bone fragments, and osteoclastogenesis was induced by 100ng/mL RANKL. TRAP staining of fixed constructs on culture day 19 was used to visualize multinuclear mature osteoclasts, found adjacent to bone fragments. Culture supernatant was collected at each medium exchange. Calcium concentration remained stable over the culture period, consistent with low-level resorption activity.

**Conclusion:** adECM allows the creation of endothelial barriers, supports OCP migration and differentiation, and is suitable for 3D in vitro modelling of osteoclast development and function.

**Funding:** This work was supported by the European Union through National Recovery and Resilience Plan under grant agreements NPOO.C3.2.R3-I1.04.0093. and NPOO.C3.2.R2-I1.06.

*Selected oral presentations - SESSION 3 - BRIGHT SPARK****S3-SB3 Adiponectin Receptor Signaling Regulates CD8<sup>+</sup> T Cell Function***

**Ozren Majstorović<sup>1</sup>**, Sanja Mikašinović<sup>1</sup>, Inga Kavazović<sup>1</sup>, Karlo Mladenić<sup>1</sup>, Tamara Turk Wensveen<sup>2,3</sup>, Felix M. Wensveen<sup>1</sup>

<sup>1</sup>*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

<sup>2</sup>*Center for Diabetes, Endocrinology and Cardiometabolism, Thalassotherapia Opatija, Opatija, Croatia*

<sup>3</sup>*Department of Internal Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

Adiponectin is a highly abundant adipose tissue-derived hormone whose circulating levels inversely correlate with adiposity. Although adiponectin receptors (AdipoRs) are widely expressed across immune cell populations, their contribution to CD8<sup>+</sup> T-cell biology remains incompletely defined. Here, we investigated the role of AdipoR signaling in regulating CD8<sup>+</sup> T-cell responses.

Following in vitro activation, OT-1 TCR-transgenic CD8<sup>+</sup> T cells exhibited the highest AdipoR expression during the effector phase at both the transcript and protein levels. Genetic ablation of AdipoR signaling in OT-1 T cells (adiponectin-receptor deficient cells; ARcKO) resulted in impaired cellular accumulation during the early stages of activation compared with wild-type (WT) controls, an effect that was largely independent of glucose or glutamine availability.

This competitive disadvantage persisted during the effector and memory phases in in vitro co-culture assays and was associated with impaired survival. Consistently, ARcKO OT-1 T cells exhibited reduced persistence following co-transfer with WT cells into congenic recipient mice infected with recombinant murine cytomegalovirus (mCMV-OVA), while loss of AdipoR signaling also altered cytokine responses. Collectively, these findings establish adiponectin receptor signaling as an important regulator of CD8<sup>+</sup> T-cell function by promoting cellular fitness and supporting effector responses.

Given that circulating adiponectin concentrations are reduced in obesity, impaired adiponectin signaling may contribute to the diminished antiviral CD8<sup>+</sup> T-cell responses and reduced antiviral capacity observed in this population.

## Selected oral presentations – SESSION 4

### *S4-O1 High PVR protein expression marks clear cell renal cell carcinoma with metastatic spread*

Paola Kučan Brlić<sup>\*1</sup>, **Emma Bellulovich<sup>\*1</sup>**, Mijo Golemac<sup>1</sup>, Ante Jakšić<sup>2</sup>, Dean Markić<sup>2</sup>, Karmela Miklić<sup>1</sup>, Suzana Malić<sup>1</sup>, Emina Babarović<sup>3</sup>, Gordana Đorđević<sup>3</sup>, Vesna Šupak Smolčić<sup>4</sup>, Berislav Lisnić<sup>1</sup>, Pini Tsukerman<sup>5</sup>, Guy Cinamon<sup>5</sup>, Stipan Jonjić<sup>1</sup>, Tihana Lenac Roviš<sup>1</sup>

<sup>1</sup>Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; <sup>2</sup>Department of Urology, Clinical Hospital Center Rijeka, Rijeka, Croatia; <sup>3</sup>Clinical Department of Pathology and Cytology, Clinical Hospital Center Rijeka, Rijeka, Croatia; <sup>4</sup>Clinical Department of Laboratory Diagnostics, Clinical Hospital Center Rijeka, Rijeka, Croatia <sup>5</sup>Nectin Therapeutics LTD, Jerusalem, Israel

**Background:** The poliovirus receptor (PVR/CD155) is a checkpoint molecule and a promising therapeutic target currently being investigated in combination with other immune checkpoint inhibitors. PVR exists as membrane-bound PVR and the secreted PVR. Although PVR is highly expressed across multiple malignancies, its prognostic and predictive value in renal and bladder cancers remains underexplored.

**Methods:** PVR expression was quantified by immunohistochemistry in bladder and renal cancer tissues. Matched serum samples were analyzed using a custom enzyme-linked immunosorbent assay to determine total circulating PVR levels. To distinguish isoforms, we generated an antibody specific to the secreted isoform and developed an isoform-selective assay.

**Results:** Circulating PVR levels were elevated in patients with both bladder and renal cancer; however, serum levels did not correlate with tumor tissue PVR expression, either at the total or isoform-specific level. In bladder cancer, tissue PVR expression was associated with higher tumor grade and lymphovascular invasion. In renal cancer, expression was subtype-dependent, with only a subset of clear cell carcinoma samples showing tissue positivity. Importantly, reduced overall survival in clear cell carcinoma was linked to PVR expression, and rare cases with high levels consistently progressed to metastatic disease.

**Conclusion:** In renal tumors, a subtype-specific pattern emerged: PVR expression was generally low in clear cell carcinoma but enriched in papillary carcinoma. Notably, in the clear cell cohort all cases with high expression were associated with metastatic progression. These findings highlight PVR as a potential prognostic marker in clear cell carcinoma tissue and support its further investigation as a biomarker and therapeutic target in renal cancers.

*Selected oral presentations – SESSION 4****S4-02 Disentangling Lipid Metabolism and Monocytic Differentiation in Venetoclax Resistance in Acute Myeloid Leukemia***

**David Cicic<sup>1</sup>**, Lara Divjak<sup>2</sup>, Klara Bardac<sup>3</sup>, Alojzija Brcic<sup>3</sup>, Hrvoje Lalic<sup>3,4</sup>, Coralie Alcazar<sup>5</sup>, Sirisha Natani<sup>6</sup>, Nadira Durakovic<sup>2,7</sup>, Radovan Vrhovac<sup>2,7</sup>, Fanny Granat<sup>5</sup>, Dora Visnjic<sup>3</sup>, Jean-Emmanuel Sarry<sup>5</sup>, Paolo Gallipoli<sup>6</sup>, Ozren Jaksic<sup>1,7</sup>, Vilma Dembitz<sup>3</sup>

<sup>1</sup>Division of Hematology, Department of Internal Medicine, University Hospital Dubrava, Zagreb, Croatia;

<sup>2</sup>Division of Hematology, Department of Internal Medicine, University Hospital Centre Zagreb, Croatia;

<sup>3</sup>Department of Physiology and Croatian Institute for Brain Research, University of Zagreb School of Medicine, Croatia; <sup>4</sup>Division of Laboratory Immunology, Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia; <sup>5</sup>Université de Toulouse, Centre de Recherches en Cancérologie de Toulouse, Centre Hospitalier Universitaire de Toulouse; <sup>6</sup>Barts Cancer Institute, Queen Mary University of London, London, UK; <sup>7</sup>Chair for Internal Medicine, University of Zagreb School of Medicine, Zagreb, Croatia

Venetoclax resistance remains a major challenge in acute myeloid leukemia (AML), and its metabolic determinants are incompletely understood. We investigated whether lipid metabolism is associated with ex vivo venetoclax response in primary AML samples.

In a pilot study, 22 primary AML samples were tested for venetoclax sensitivity, 14 of which were profiled by RNA sequencing. Resistant samples showed enrichment of cholesterol metabolism, adipogenesis and fatty acid metabolism pathways. To assess reproducibility, we analysed six independent public datasets (three proteomic and three transcriptomic; n=42–115), correlating venetoclax response with gene or protein expression and performing pre-ranked gene set enrichment analysis. Cholesterol-related pathways, including Cholesterol Homeostasis, Cholesterol Metabolism and LDL Clearance, were enriched in resistant samples across all six cohorts. Triglyceride, glycerolipid and adipogenesis signatures showed a similar but less consistent association, whereas fatty acid oxidation showed the opposite pattern. However, monocytic differentiation, an established determinant of venetoclax resistance in AML, was strongly associated with resistance (Spearman  $\rho=0.41-0.73$ ), and several leading-edge lipid-associated genes, including CD36, LDLR and LRP1, are established markers of monocytic differentiation. To elucidate these associations, we established a prospective cohort integrating immunophenotyping, whole-exome sequencing, proteomics, metabolomics, lipidomics and ex vivo venetoclax testing. Preliminary data from the first 33 patients show no association between venetoclax sensitivity and FAB subtype, FLT3 or NPM1 status, or blast maturation. Plasma total cholesterol, LDL and HDL were comparable, whereas resistant patients had significantly lower plasma triglycerides and a trend towards higher CD36 expression on CD45-low blasts.

Further functional and multi-omic studies will determine whether lipid metabolism independently contributes to venetoclax resistance or primarily reflects monocytic differentiation.

## Selected oral presentations – SESSION 4

### *S4-O3 Metabolic control of myeloid differentiation: distinct roles of RRM2 and RRM2B in acute myeloid leukemia*

**Alojzija Brčić<sup>1</sup>**, Klara Bardač<sup>1</sup>, Hrvoje Lalić<sup>1</sup>, Vilma Dembitz<sup>1</sup>, Tomislav Smoljo<sup>1</sup>, Romana Penker<sup>2</sup>, Giovanni Rodriguez Blanco<sup>2</sup>, Dora Visnjic<sup>1</sup>

<sup>1</sup>Department of Physiology and Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

<sup>2</sup>Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

Perturbation of nucleotide metabolism and the resulting replication stress can promote myeloid differentiation, but the mechanisms determining cellular responses to nucleotide stress remain unclear. We investigated the role of ribonucleotide reductase (RNR), a key regulator of deoxyribonucleotide homeostasis, in differentiation induced by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr), the dihydroorotate dehydrogenase inhibitor brequinar, and low-dose cytarabine. Differentiation, cell viability, cell-cycle responses, and expression of the RNR subunits RRM2 and RRM2B were assessed in AML cell lines, and their functional roles were examined by siRNA-mediated depletion. Associations with replication-stress signaling were examined using a published proteomic dataset of primary AML samples.

All three agents induced myeloid differentiation, while RNR adaptation differed between cellular backgrounds. In p53-mutant U937 cells, differentiation-inducing treatments increased RRM2 expression, whereas p53-wild-type MOLM-13 cells preferentially induced the p53-responsive RRM2B isoform. RRM2 depletion enhanced differentiation in U937 cells without substantially affecting viability, while in MOLM-13 cells it reduced survival and impaired differentiation. In contrast, RRM2B depletion had little effect on MOLM-13 differentiation or viability. In published primary AML proteomic data, CHEK1 Thr279 phosphorylation correlated with RRM2 abundance ( $p = 0.49$ ,  $p = 1.4 \times 10^{-4}$ ) and the RRM2/RRM2B ratio ( $p = 0.424$ ,  $p = 0.00127$ ), but not with RRM2B.

These findings reveal context-dependent roles of RRM2 and RRM2B in responses to nucleotide stress and associate RRM2-dominant RNR composition with checkpoint signaling in primary AML. Differential RNR adaptation may influence whether nucleotide stress promotes myeloid differentiation or compromises cell survival.

*Selected oral presentations – SESSION 4****S4-O4 Hydroxyurea-Induced Monocytic Differentiation in Acute Myeloid Leukemia: Evidence for Mechanisms Beyond RRM2 Suppression and Cell-Cycle Arrest***

**Klara Bardač<sup>1</sup>, Alojzija Brčić<sup>1</sup>, Hrvoje Lalić<sup>1,2</sup>, Radovan Vrhovac<sup>3,4</sup>, Vilma Dembitz<sup>1</sup>, Dora Višnjić<sup>1</sup>**

*<sup>1</sup>Laboratory for Cell Biology, Department of Physiology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>2</sup>Department of Laboratory Immunology, Clinical Department of Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia; <sup>3</sup>Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb, Zagreb, Croatia; <sup>4</sup>Department of Internal Medicine, University of Zagreb School of Medicine, Zagreb, Croatia*

In hematological malignancies, hydroxyurea (HU) is used for cytoreduction and is best known for inhibiting ribonucleotide reductase (RNR), thereby impairing DNA synthesis and causing S-phase arrest. Although HU has been reported to induce differentiation in AML cell lines, the mechanisms remain incompletely understood. To elucidate the molecular and cellular mechanisms underlying myeloid differentiation, three AML cell lines (U937, MOLM-13, and THP-1) and primary samples from seven patients with AML were investigated.

HU-treated U937 cells were characterized by multiparametric flow cytometry, May-Grünwald-Giemsa and  $\alpha$ -naphthyl acetate esterase (NSE) staining, with functional assessment of phagocytosis and dihydrorhodamine-123 (DHR123) fluorescence. After 72 hours, HU induced monocytic differentiation characterized by increased CD11b, CD14, and CD64 expression, morphological maturation, NSE positivity, increased cellular complexity and lysosomal content, enhanced phagocytic activity, and increased DHR123 fluorescence consistent with higher intracellular reactive oxygen species (ROS) levels. HU also induced CD11b and CD64 in MOLM-13 and THP-1 cells, with cell-line-specific cell-cycle arrest. MOLM-13 cells were more sensitive to HU-induced cytoreduction than U937 cells, and the lines have distinct baseline profiles of RNR subunits RRM2 and RRM2B. However, baseline RRM1, RRM2, and RRM2B abundance did not correlate with HU sensitivity in publicly available proteomic data from primary AML samples. RRM2 silencing neither mimicked nor modified HU-induced differentiation. Nucleoside supplementation and N-acetylcysteine attenuated differentiation in U937 without reversing HU-induced cell-cycle arrest, while effects were weaker in MOLM-13. Primary AML samples cultured ex vivo on HS-5 stromal cells showed HU-induced increases in CD11b and CD64 in a subset of cases.

HU therefore promotes monocytic differentiation across AML models through mechanisms extending beyond RRM2 suppression and replication-associated cell-cycle arrest. The response is also detectable in primary AML samples, supporting relevance beyond established cell lines.

## POSTER SESSION

| No. | Presenter                       | Title                                                                                                                                                |
|-----|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| P1  | <b>Hana Musil</b>               | <i>Distinct Clinical Phenotypes of IgA Vasculitis Identified by Cluster Analysis</i>                                                                 |
| P2  | <b>Hana Musil</b>               | <i>Clinical Relevance of Serum Calprotectin in Non-Systemic Juvenile Idiopathic Arthritis – an International Multicenter Prospective Pilot Study</i> |
| P3  | <b>Hana Musil</b>               | <i>Serum Calprotectin Across Pediatric Inflammatory Diseases: Diagnostic and Monitoring Potential</i>                                                |
| P4  | <b>Ana-Zorica Pavičić</b>       | <i>Direct effects of incretin receptor agonists on metabolic stress and NKG2D ligand expression in 3T3-L1 adipocytes</i>                             |
| P5  | <b>Inga Kavazović</b>           | <i>CD8 T cells as a prognostic marker for Head and Neck Squamous Cell Carcinoma progression</i>                                                      |
| P6  | <b>Rajna Koren</b>              | <i>Immune system-mediated changes in amino acid metabolism enhance the immune response during viral infections</i>                                   |
| P7  | <b>Sanja Skok</b>               | <i>The role of epinephrine on CD8 T cell biology</i>                                                                                                 |
| P8  | <b>Lara Melvan</b>              | <i>Increased susceptibility to viral infection in MASLD is associated with dyslipidemia</i>                                                          |
| P9  | <b>Tomislav Glavan</b>          | <i>Interferon-gamma and interleukin-1 trigger viral infection-induced hypothermic response</i>                                                       |
| P10 | <b>Ivo Krešić</b>               | <i>Monocyte-to-moDC differentiation does not discern ankylosing spondylitis and psoriatic arthritis</i>                                              |
| P11 | <b>Sara Šrepfler</b>            | <i>Cell-Type-Specific Type I Interferon Signaling Shapes Cytomegalovirus Control in the Brain</i>                                                    |
| P12 | <b>Petra Gulan</b>              | <i>Antibody Properties Shape the Performance of Immunoaffinity Chromatography for Vesicular Stomatitis Virus Purification</i>                        |
| P13 | <b>Karlo Mladenčić</b>          | <i>MASLD compromises antiviral immunity of NK cells in the context of mCMV infection</i>                                                             |
| P14 | <b>Sanja Mateljak Lukačević</b> | <i>Towards an ex-vivo model that discriminates virus replication in tumor and healthy tissue</i>                                                     |
| P15 | <b>Ivana Bertović</b>           | <i>Pathogenesis of MCMV infection in the adrenal gland</i>                                                                                           |
| P16 | <b>Jelena Železnjak</b>         | <i>MCMV altered-self MHC I molecules and their impact on adaptive immune responses</i>                                                               |

|     |                             |                                                                                                                                                                 |
|-----|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| P17 | <b>Vilma Dembitz</b>        | <i>Unfolded protein response signalling in SCD inhibitor-induced lipotoxicity in acute myeloid leukemia</i>                                                     |
| P18 | <b>Darja Flegar</b>         | <i>Microbial drivers of bile acid circadian rhythmicity in mice: correlating diurnal shifts in bile acids with microbial enzymes shaping the bile acid pool</i> |
| P19 | <b>Marina Čunko</b>         | <i>Targeting PVR/CD155 as an Alternative and Complementary Immune Checkpoint Strategy in Triple-Negative Breast Cancer</i>                                      |
| P20 | <b>Ruža Frkanec</b>         | <i>Peptidoglycan-Based Biomimetic Nanomaterials Reveal Lectin Recognition in DAP-Type Bacteria: Toward Rational Design of Antimicrobial Therapeutics</i>        |
| P21 | <b>Lucija Ivandić</b>       | <i>Assessment of an In Vitro Model for the Evaluation of VSV Oncolytic Activity</i>                                                                             |
| P22 | <b>Mariastefania Antica</b> | <i>From Stem Cells to Thymic Organoids - The Potential of <i>Artocarpus tonkinensis</i></i>                                                                     |
| P23 | <b>Sanja Mikašinović</b>    | <i>IGF-1 receptor signalling promotes CD8 memory T cell formation and enhances tumour control in vivo</i>                                                       |

### *P1 Distinct Clinical Phenotypes of IgA Vasculitis Identified by Cluster Analysis*

N. Kifer<sup>1</sup>, D. Kifer<sup>2</sup>, H.Musil<sup>1</sup>, M. Sestan<sup>1</sup>, S. Srsen<sup>3</sup>, M. Frkovic<sup>1</sup>, A. Gagro<sup>4,5</sup>, M. Jelusic<sup>1</sup>

<sup>1</sup>Department of Pediatric Immunology, Rheumatology and Allergology, School of Medicine, University of Zagreb, University Hospital Center Zagreb, Zagreb, Croatia

<sup>2</sup>Department of Biophysics, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

<sup>3</sup>Department of Pediatrics, University of Split School of Medicine, UHC Split, Split, Croatia

<sup>4</sup>Department of Pediatric Pulmonology, Allergology, Immunology and Rheumatology, Children's Hospital Zagreb, Zagreb, Croatia

<sup>5</sup>Medical Faculty Osijek, University of Osijek, Osijek, Croatia

**Introduction:** IgA vasculitis (IgAV) is generally self-limiting, but gastrointestinal (GI) and renal involvement may lead to acute and chronic complications.

**Objectives:** To identify distinct IgAV subgroups based on clinical and laboratory characteristics using cluster analysis.

**Methods:** We included 798 patients diagnosed with IgAV/IgAVN according to EULAR/PRINTO/PRES criteria between 2009 and 2024 at three Croatian pediatric clinics. Variables included joint, GI and renal involvement, rash, CRP, ESR, C3, C4, stool blood, and hematuria. Cluster analysis was performed using Ward's D linkage. Relapse rates and age at diagnosis were compared between clusters.

**Results:** Among 798 patients, 50% were male and median age at diagnosis was 6.4 years (IQR 4.5–8.6). Five distinct subgroups were identified. The first cluster showed the highest burden of multi-organ involvement, with universal renal disease and frequent arthritis and GI manifestations. The second primarily showed elevated CRP with occasional GI involvement, without arthritis or renal disease. The third, largest cluster was characterized by GI involvement and arthritis. The fourth included arthritis with elevated ESR and CRP, while the fifth predominantly showed isolated arthritis. The renal involvement cluster had significantly more relapses (32% vs. 14%,  $p < 0.001$ ) and a higher proportion of patients aged  $\geq 7$  years (59% vs. 36%,  $p < 0.001$ ). The fourth cluster had the fewest relapses and youngest patients.

**Conclusion:** Cluster analysis revealed five clinically distinct IgAV subgroups. Renal involvement was associated with older age and higher relapse rates, supporting tailored monitoring based on early clinical presentation.

This work was supported by the Croatian Science Foundation under project number HRZZ-IP-2024-05-6848.

***P2 Clinical Relevance of Serum Calprotectin in Non-Systemic Juvenile Idiopathic Arthritis – an International Multicenter Prospective Pilot Study***

**H. Musil<sup>1</sup>, M. Šestan<sup>1,2</sup>, A. Lončar Vrančić<sup>3</sup>, M. Sparchez<sup>4</sup>, D. Turudić<sup>1,2</sup>, M. O. Erkan<sup>5</sup>, O. Basaran<sup>5</sup>, B. Sener<sup>6</sup>, A. Cochino<sup>7</sup>, V. Bittermann<sup>8</sup>, D. Misikova<sup>9</sup>, T. Dallos<sup>9</sup>, J. Anton<sup>10</sup>, S. Ozen<sup>5</sup>, D. Rogić<sup>3</sup>, M. Jelušić<sup>1,2</sup>**

<sup>1</sup>School of Medicine, University of Zagreb, Zagreb, Croatia; <sup>2</sup>Department of Paediatrics, University Hospital Centre Zagreb, Zagreb, Croatia; <sup>3</sup>Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia; <sup>4</sup>Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania; <sup>5</sup>Department of Paediatrics, Division of Rheumatology, Hacettepe University Faculty of Medicine, Ankara, Türkiye; <sup>6</sup>Department of Infectious Diseases and Clinical Microbiology, Hacettepe University Faculty of Medicine, Ankara, Türkiye; <sup>7</sup>Department of Clinical Immunology, National Institute for Mother and Child Health Alessandrescu-Rusescu, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania; <sup>8</sup>Department of Pediatric Rheumatology, SJD Barcelona Children's Hospital Passeig Sant Joan de Déu, Barcelona, Spain; <sup>9</sup>2nd Department of Paediatrics, National Institute of Children's Diseases, Comenius University Medical School in Bratislava, Bratislava, Slovakia; <sup>10</sup>Department of Paediatrics, Division of Rheumatology, SJD Barcelona Children's Hospital Passeig Sant Joan de Déu, Barcelona, Spain

**Objectives:** To evaluate the association of serum calprotectin (sCal) with disease activity in non-systemic JIA (nsJIA) and explore its utility in differentiating treatment and remission cohorts. **Methods:** This international multicenter prospective pilot study included patients with nsJIA divided into Treatment (TC) and Remission cohorts (RC), followed every 3 months at tertiary pediatric rheumatology centers. Disease activity was evaluated using JADAS-27 ESR and CRP. SCal was measured using the automated Gentian Calprotectin Immunoassay (GCAL®). Associations were analyzed using Spearman's correlation, and ROC analysis assessed discrimination between cohorts.

**Results:** A total of 323 visits from 128 patients were analyzed (mean age at diagnosis  $7.5 \pm 5.37$  years). SCal significantly correlated with JADAS-27 ESR ( $p=0.357$ ), JADAS-27 CRP ( $p=0.36$ ), active joint count ( $p=0.321$ ), physician global assessment ( $p=0.313$ ), parent/patient global assessment ( $p=0.285$ ), and ESR ( $p=0.212$ ; all  $p<0.001$ ). Correlations were stronger in the TC than RC. ROC analysis showed moderate discrimination between cohorts (AUC=0.624); a cut-off of 1.89  $\mu\text{g/mL}$  achieved 91.9% specificity

**Conclusion:** SCal showed stronger associations with clinical and laboratory measures of inflammatory activity, particularly in the TC, supporting its role as a biomarker of active inflammation in non-systemic JIA. Exploratory ROC analyses suggest potential utility in differentiating active disease from remission. Extended prospective follow-up of this pilot cohort is currently in progress.

Supported by the Croatian Science Foundation under project number HRZZ-IP-2024-05-6848 and NPOO 10106-25-2896 (JIA-BARRIER-MARKERS), funded by EU-NextGenerationEU

### *P3 Serum Calprotectin Across Pediatric Inflammatory Diseases: Diagnostic and Monitoring Potential*

M. Šestan<sup>1</sup>, H. Musil<sup>1</sup>, D. Grgurić<sup>1</sup>, A. Lončar Vrančić<sup>2</sup>, D. Rogić<sup>2</sup>, M. Frković<sup>1</sup>, M. Jelušić<sup>1</sup>

<sup>1</sup>*School of Medicine, University of Zagreb, Department of Pediatric Immunology, Rheumatology and Allergology, University Hospital Centre Zagreb, Zagreb, Croatia*

<sup>2</sup>*Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia*

**Background and Aims:** To compare serum calprotectin levels in children with sJIA, pJIA, cSLE, IgAV, IBD, and IL-1-mediated AIDs, and evaluate its role as a diagnostic and discriminative marker.

**Methods:** This prospective study included 208 children: 31 sJIA, 62 pJIA, 18 cSLE, 76 IgAV, 15 IBD, 6 AID, 20 healthy controls. Serum calprotectin was measured using a turbidimetric assay (GCAL®, Gentian AS). Differences between groups were analyzed with the Kruskal-Wallis test ( $p < 0.05$ ).

**Results:** Median calprotectin levels (yg/ml) were: sJIA: 2.30 (0.50–110.61), pJIA: 1.19 (0.30–19.27), cSLE: 1.39 (0.31–6.29), IgAV: 1.99 (0.46–21.76), IBD: 1.62 (0.40–5.80), AID: 1.26 (0.56–232.5), and Controls: 1.00 (0.57–1.43). Children with sJIA and IgAV had significantly higher levels than controls ( $p < 0.01$ ). Significant differences were also found between sJIA and pJIA ( $p = 0.004$ ). By disease activity, median levels were: sJIA (active): 9.47 (2.22–110.61) yg/ml vs. remission: 1.08 (0.50–2.06) yg/ml, pJIA (active): 2.5 (0.30–19.27) yg/ml vs. remission: 1.03 (0.40–4.71) yg/ml, cSLE (active): 3.77 (1.00–6.29) yg/ml vs. remission: 1.21 (0.31–3.54) yg/ml, IgAV (active): 3.53 (0.46–21.76) yg/ml vs. remission: 1.25 (0.46–5.34) yg/ml. Active phases of sJIA, pJIA, cSLE and IgAV had significantly higher calprotectin levels than their respective remission phases ( $p < 0.05$ ). Active disease patients had higher calprotectin than controls, while remission patients had levels similar to controls.

**Conclusions:** Serum calprotectin reflects systemic inflammation and may help distinguish active disease from remission across several pediatric inflammatory diseases. Further studies are needed to validate its clinical utility.

This work was supported by NPOO 10106-25-2897 (GUT-VAS) and NPOO 10106-25-2896 (JIA-BARRIER- MARKERS) funded by EU-NextGenerationEU.

***P4 Direct effects of incretin receptor agonists on metabolic stress and NKG2D ligand expression in 3T3-L1 adipocytes***

**Ana-Zorica Pavičić<sup>1</sup>, Tamara Turk Wensveen<sup>2,3,4</sup>, Felix M. Wensveen<sup>1</sup>**

<sup>1</sup>Dept. of Histology & Embryology, University of Rijeka faculty of medicine, Rijeka, Croatia

<sup>2</sup>Dept. of internal medicine, University of Rijeka faculty of medicine, Rijeka, Croatia

<sup>3</sup>Center for Diabetes, Endocrinology and Cardiometabolism, Special Hospital for Medical Rehabilitation of the Heart and Lung Diseases and Rheumatism Thalassotherapy Opatija, Ul. maršala Tita 188/1, 51410 Opatija, Croatia

<sup>4</sup>Department of Clinical Medicine, Faculty of dental medicine and health, Josip Juraj Strossmayer University of Osijek, Crkvena 21, 31000 Osijek, Croatia

**Background:** Metabolic stress in parenchymal cells is an important driver of chronic inflammation associated with metabolic disease. Previous work in our lab demonstrated that lipid accumulation, induces expression of stress ligands in adipose tissue, which initiates inflammation and contributes to insulin resistance. However, it remains unknown whether incretin-based therapies act directly on metabolically stressed adipocytes and ameliorate stress ligand expression.

**Aim:** To investigate the effects of semaglutide, tirzepatide, and retatrutide on 3T3-L1 adipogenesis and determine whether these agents alter expression of the NKG2D ligands MULT-1, RAE-1, and H60 in mature adipocytes.

**Methods:** 3T3-L1 cells were differentiated into adipocytes in the presence of semaglutide, tirzepatide, or retatrutide either throughout adipogenesis, or after this process was completed. Intracellular lipid accumulation was shown using BODIPY 493/503 dye and stress ligands were labeled with antibodies and analyzed by flow cytometry.

**Results:** Preliminary data demonstrate that all incretin mimetics reduce lipid content of adipocytes in a concentration-dependant way, with a most potent effect for retatrutide, both when they were applied during and after adipogenesis. Surface H60, but not Rae1 or MULT1 expression was reduced in the presence of incretin mimetics, though we did not observe a consistent linear dose-response pattern.

**Conclusions:** Incretin receptor agonists may directly influence adipocyte lipid content and H60 expression in a compound- and concentration-dependent manner. These findings extend previous observations linking metabolic stress to NKG2D-mediated immune activation. Additional experiments are required to confirm these effects and determine their relationship to lipid accumulation and immune cell activation.

***P5 CD8 T cells as a prognostic marker for Head and Neck Squamous Cell Carcinoma progression***

Mia Klapan<sup>1,2</sup>, Ita Hadžisejdić<sup>2,3</sup>, Bojan Polić<sup>4</sup>, Felix M Wensveen<sup>4</sup>, Blažen Marijić<sup>1,2</sup>,  
**Inga Kavazović<sup>4</sup>**

<sup>1</sup>*Department of Otorhinolaryngology, Head and Neck Surgery, Clinical Hospital Center Rijeka, Rijeka, Croatia.*

<sup>2</sup>*Faculty of Medicine, University of Rijeka, Rijeka, Croatia.*

<sup>3</sup>*Clinical Department of Pathology and Cytology, Clinical Hospital Center Rijeka, Rijeka, Croatia.*

<sup>4</sup>*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.*

Head and neck squamous cell carcinoma (HNSCC) is a highly heterogeneous malignancy associated with poor clinical outcomes and limited response to current immunotherapeutic approaches. Although immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have improved outcomes in a subset of patients, response rates remain limited, highlighting the need for additional biomarkers that better reflect the functional state of anti-tumour T cells. This ongoing study aims to characterise the phenotypic and functional properties of CD8 T cells in HNSCC and identify immune features associated with disease progression and clinical outcome. Treatment-naïve HNSCC patients undergoing surgery at KBC Rijeka are being prospectively recruited, with paired tumour tissue and peripheral blood collected for analysis. Tumour-infiltrating lymphocytes and peripheral blood mononuclear cells are being characterised by multiparameter flow cytometry, focusing on CD8 T-cell differentiation, activation and exhaustion-associated markers, including PD-1, LAG-3, TIM-3 and TIGIT. Functional capacity is assessed following ex vivo stimulation by measuring IFN $\gamma$ , TNF and IL-2 production together with granzyme B expression, while healthy donor PBMCs serve as reference controls. Initial study activities have focused on establishing and optimising standardised workflows for tumour tissue processing, TIL isolation and flow-cytometry analysis, alongside prospective patient recruitment and sample collection. This approach will enable paired comparison of tumour-infiltrating and circulating CD8 T-cell populations and their integration with clinical data and patient follow-up, with the ultimate goal of identifying candidate immune biomarkers of HNSCC progression and potential therapy response.

This research is supported by a donation from the Adris Foundation.

***P6 Immune system-mediated changes in amino acid metabolism enhance the immune response during viral infections***

Sanja Mikašinović<sup>1</sup>, **Rajna Koren<sup>1</sup>**, Martina Brnjić<sup>2</sup>, Felix M Wensveen<sup>1</sup>, Bojan Polić<sup>1</sup>, Marko Šestan<sup>1</sup>

<sup>1</sup>*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*

<sup>2</sup>*Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia*

Everyone knows infections make us feel awful — tired, achy, dramatic, questioning all life choices. But behind the scenes, your body is actually running a highly coordinated metabolic “battle mode.” The immune system starts bossing around organs, telling them how much sugar, fat, and fuel to release, all to starve the pathogen and keep you alive. Feeling sick is basically your metabolism saying: “Hold on, I’m busy fighting.” We understand this pretty well for glucose and lipids. But amino acids? Those tiny building blocks of life? Viral infections mess with them too — we just barely know how or why. It’s like discovering your immune system has been rearranging the furniture without telling you. So, we decided to investigate. Using fancy immunology tools and metabolic wizardry, we’re mapping out the weird, multi-organ circuits that control amino acids during viral infection. And because effective antivirals are still... let’s say “aspirational,” we also tested whether flipping these circuits on purpose could stop viruses from replicating. In short: we’re figuring out how your body secretly rewires amino acids during infection — and whether we can use that trick to outsmart viruses.

*P7 The role of epinephrine on CD8 T cell biology*

**Sanja Skok<sup>1</sup>**, Rajna Koren<sup>1</sup>, Bojan Polić<sup>1</sup>, Felix M. Wensveen<sup>1</sup>, Sanja Mikašinović<sup>1</sup>, Marko Šestan<sup>1</sup>

<sup>1</sup>*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*

CD8 T cells are cytotoxic T cells which play a central role in antiviral and antitumor responses. Once the effector CD8 T cells clear an infection, a small pool of these cells is able to survive as memory cells. Immunological memory is a key feature of the adaptive immune system, giving us long-term protection against infectious agents. Understanding how the body regulates the generation, survival, and function of effector and memory CD8 T cells can help us develop more efficient vaccines and immunotherapies. One way of regulating the immune response is through the sympathetic nervous system (SNS). We want to uncover how adrenergic receptors shape the birth and behavior of memory CD8<sup>+</sup> T cells — basically, how stress-linked signals whisper instructions that influence which T cells become long-lasting defenders and how well they perform when danger returns. By probing this neuroimmune crosstalk, we aim to reveal whether epinephrine-driven cues fine-tune the quality, durability, and recall power of memory CD8<sup>+</sup> T-cell responses.

***P8 Increased susceptibility to viral infection in MASLD is associated with dyslipidemia***

**Lara Melvan<sup>1</sup>, Karlo Mladenčić<sup>1</sup>, Felix M. Wensveen<sup>1</sup>, Bojan Polić<sup>1</sup>, Maja Lenartić<sup>1</sup>**

*<sup>1</sup>Dept. of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a highly prevalent comorbidity of metabolic syndrome, associated with obesity, insulin resistance, type 2 diabetes, and dyslipidemia. Emerging clinical evidence suggests that individuals with MASLD are more prone to severe viral infections, although the underlying mechanisms remain poorly understood. MASLD is characterized by profound lipid remodeling. Beyond their metabolic roles, these lipids can modulate immune responses and support different stages of the viral life cycle, suggesting that dysregulated lipid metabolism may compromise antiviral defense through both immune-mediated and direct virus-host interactions.

Our preliminary data from a steatosis-steatohepatitis diet (SSD) mouse model show that these mice have a higher viral burden in spleen, liver, and visceral adipose tissue upon murine cytomegalovirus infection. Analysis of the lipid profile of these animals revealed that, similar to human MASLD, various lipid species were affected, with ceramides among the most significantly enriched lipid species. In vitro infection of mouse embryonic fibroblasts with mCMV showed that ceramides were potent enhancers of viral replication. Conversely, pharmacological inhibition of de novo ceramide synthesis significantly reduced viral burden in both cell lysates and supernatants, indicating impaired viral replication and overall virus production. Importantly, inhibition of de novo ceramide synthesis also restores antiviral control in visceral adipose tissue in vivo.

Together, these findings identify dysregulated ceramide metabolism as a potential mechanistic link between MASLD and impaired antiviral defense. Defining this relationship may reveal novel lipid-targeted strategies to improve antiviral immunity in metabolically vulnerable populations.

***P9 Interferon-gamma and interleukin-1 trigger viral infection-induced hypothermic response***

**Glavan T<sup>1</sup>, Lenartić M<sup>1</sup>, Etemaj E<sup>1</sup>, Ljesar M<sup>1</sup>, Wensveen FM<sup>1</sup>, Polić B<sup>1</sup>**

*<sup>1</sup>Dept. of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*

Core body temperature is prone to changes during infection. While a fever response is typically seen, sometimes an opposite, hypothermic response is triggered. The mechanism of fever has been well documented, but the mechanism of infection-induced hypothermia still remains unclear. Using acute MCMV and Flu infection models, we observe a hypothermic response in later stages of viral infection. Furthermore, we show that mice with total body deficiency of IFN- $\gamma$  or IL-1 receptor had a marked reduction of such response. In knockout mice lacking T cells, and wild-type mice depleted of macrophages or TRPV1+ sensory neurons we observed a similar finding, pointing to these cells as potential drivers. We found that in vitro differentiation of CD8 T cells at hypothermic temperatures suppresses cell proliferation, IFN- $\gamma$  and TNF- $\alpha$  production. These findings were confirmed in vivo by using heated animal cages to prevent hypothermic response and measuring cytokine production of isolated lymphocytes. We propose that infection-induced hypothermic response is a strategy of disease tolerance and a part of feedback loop which suppresses immune cell function in order to limit immune-mediated tissue damage during an overwhelming immune response to viral infection.

***P10 Monocyte-to-moDC differentiation does not discern ankylosing spondylitis and psoriatic arthritis***

**Ivo Krešić<sup>1</sup>**, Pavao Planinić<sup>1</sup>, Sara Aničić<sup>3,4</sup>, Marta Radošević<sup>3,4</sup>, Sara Priselac<sup>4,5</sup>, Tomislav Balen<sup>4,5</sup>, Katerina Z. Petrović<sup>4</sup>, Danka Grčević<sup>3,4</sup>, Tomislav Kelava<sup>3,4</sup>, Nataša Kovačić<sup>4,5</sup>, Marina I. Matijašević<sup>2</sup>, Ivan Ćavar<sup>1</sup>, Alan Šućur<sup>3,4</sup>

<sup>1</sup>Department of Physiology, School of Medicine, University of Mostar, 88000 Mostar, Bosnia and Herzegovina; <sup>2</sup>"Sveti Duh" University Hospital, Zagreb, Croatia; <sup>3</sup>Department of Physiology, School of Medicine University of Zagreb, 10000 Zagreb, Croatia; <sup>4</sup>Laboratory for Molecular Immunology, Croatian Institute for Brain Research, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia; <sup>5</sup>Department of Anatomy, School of Medicine University of Zagreb, 10000 Zagreb, Croatia

**Background:** Spondyloarthropathies, such as ankylosing spondylitis (AS) or psoriatic arthritis (PsA), are known for involvement of monocyte-derived dendritic cells (moDCs), yet their functional and phenotypical properties in these diseases aren't fully explored.

**Methods:** CD3<sup>-</sup>CD14<sup>+</sup> monocytes and autologous CD3<sup>+</sup> lymphocytes from healthy donors and patients with active AS or PsA were FACS-sorted, then cultured for five days spontaneously or with cocktail containing M-CSF, IL-4 and TNF- $\alpha$ . Subsequently, these cells were phenotyped by flow cytometry (markers CD14, CD16, CD1a, CD1c, CD141, CD163, CD206, CD209, CD40, CD80, CD86, HLA-DR). pHrodo uptake was measured as percentage-positive cells and median fluorescence intensity (MFI). Antigen presentation was evaluated by 8-day co-culture with CD3<sup>+</sup> lymphocytes using CEFT pool, MOG pool, or PMA/ionomycin, with intracellular cytokines in CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Analysis used paired Wilcoxon tests with FDR correction and PERMANOVA. **Results:** Compared to spontaneous cell immunophenotype, the cocktail upshifted CD209 39 to 69% and CD1c 6 to 15%, downshifting CD14 90 to 75%, CD206 73 to 52% and CD16 33 to 14%, (all corrected  $p < 0.001$ ). Particle uptake fell to 0.37 of spontaneous by MFI (95% CI 0.29–0.44;  $p < 0.001$ ), while percentage-positive uptake dropped from 96 to 83%, indicating fewer particles per cell, not fewer cells. Co-culture was antigen-specific, as most cytokine measurements differed among CEFT and MOG (corrected  $p = 0.013$ ), with CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup> rising 0.05 to 0.69%. PERMANOVA showed no difference between controls, AS and PsA at baseline or in differentiation response.

**Conclusion:** The monocyte-to-moDC transition remains unaltered in AS or PsA, with a fall in particle uptake per cell. T-cell response to these cells is antigen-specific with the disease impact likely reliant on function, rather than differentiation.

**P11 Cell-Type-Specific Type I Interferon Signaling Shapes Cytomegalovirus Control in the Brain**

Andrea Mihalić<sup>1#</sup>, Lucija Šakota<sup>1,2#</sup>, Fran Krstanović<sup>1#</sup>, **Sara Šrepfler<sup>1</sup>**, Ulrich Kalinke<sup>3</sup>, Stipan Jonjić<sup>1,4</sup>, and Ilija Brizić<sup>1\*</sup>

<sup>1</sup>Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

<sup>2</sup>Faculty of Pharmacy, University of Mostar, Mostar, Bosnia and Herzegovina

<sup>3</sup>Institute for Experimental Infection Research, TWINCORE, Centre for Experimental and Clinical Infection Research, a joint venture between the Helmholtz Centre for Infection Research and the Hannover Medical School, Hannover, Germany

<sup>4</sup>Department of Biomedical Sciences, Croatian Academy of Sciences and Arts, Rijeka, Croatia

#Authors contributed equally to this work

Type I interferons (IFN-I) are central regulators of antiviral immunity, directly suppressing viral replication and orchestrating innate and adaptive immune responses. Human cytomegalovirus, a  $\beta$ -herpesvirus, is a leading cause of congenital infections and is associated with severe, often irreversible neurological sequelae. Using mouse cytomegalovirus (MCMV) infection of newborn mice as a model of congenital infection, we show that IFN-I signaling is required for viral control and host survival. To determine its cell-type-specific role in the infected central nervous system (CNS), we generated mice lacking IFN-I signaling selectively in neurons, astrocytes, or microglia. Early IFN-I signaling in microglia was essential for limiting MCMV replication in the CNS and for upregulating microglial MHC class I expression, consistent with a role in shaping local CD8<sup>+</sup> T-cell responses. In contrast, absence of IFN-I signaling in neurons or astrocytes did not alter the immune response or impair viral control. Collectively, our findings identify early microglial IFN-I signaling as a critical, cell-type-specific mechanism controlling cytomegalovirus infection and antiviral T-cell immunity in the brain.

***P12 Antibody Properties Shape the Performance of Immunoaffinity Chromatography for Vesicular Stomatitis Virus Purification***

**Petra Gulan<sup>1</sup>**, Dubravko Forčić<sup>1</sup>, Beata Halassy<sup>1</sup>, Adela Štimac<sup>1</sup>

<sup>1</sup>Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia

Vesicular stomatitis virus (VSV) is an enveloped RNA virus with promising applications in oncolytic virotherapy and vaccine development, creating a need for efficient and selective downstream purification strategies. Immunoaffinity chromatography (IAC) offers highly selective virus capture; however, efficient purification requires a balance between strong virus binding and subsequent recovery under conditions that preserve viral integrity and infectivity.

Polyclonal anti-VSV IgG antibodies of guinea pig and human origin were immobilized on hydrazide-activated monolithic columns and evaluated for virus capture and recovery. Although both columns exhibited high binding capacity, successful native elution was achieved only with human anti-VSV antibodies, which, in contrast to the guinea pig anti-VSV antibodies, exhibited substantially lower specific neutralization potency.

Elution was performed under physiological conditions using high-molarity amino acid solutions. Among the tested eluents, arginine was the most efficient, enabling high recovery of total and infectious virus particles. Process analysis revealed near-complete removal of host cell DNA following benzonase treatment, while residual host cell proteins co-eluted with virus particles, likely due to their association with virus particles and/or extracellular vesicles.

This study demonstrates that our innovative and patent-protected “native elution” immunoaffinity chromatography, originally developed for mumps virus, can be adapted for VSV purification. These findings suggest that the performance of VSV immunoaffinity chromatography depends not only on efficient virus capture but also on the characteristics of the immobilized antibodies. Specific neutralization potency may contribute to the balance between VSV capture and recovery, highlighting antibody selection as a critical parameter in the development of efficient IAC purification processes.

***P13 MASLD compromises antiviral immunity of NK cells in the context of mCMV infection***

Karlo Mladenčić<sup>1</sup>, **Lara Melvan**<sup>1</sup>, Felix M. Wensveen<sup>1</sup>, Bojan Polić<sup>1</sup>

<sup>1</sup>*Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a hepatic manifestation of metabolic syndrome encompassing a spectrum of liver conditions ranging from steatosis to steatohepatitis, fibrosis, and cirrhosis. Emerging evidence suggests that MASLD patients have increases susceptibility to severe viral infections, but the underlying mechanisms remain poorly understood.

To investigate whether MASLD impairs antiviral immunity, we induced MASLD in mice using a steatosis and steatohepatitis diet (SSD). Following murine cytomegalovirus (mCMV) infection, SSD-fed mice exhibited higher viral loads in tissues compared with normal chow diet (NCD)-fed controls. In parallel, hepatic natural killer (NK) cells, key mediators of early antiviral defense, showed reduced production of IFN- $\gamma$  and Granzyme B in SSD-fed mice upon infection. Notably, NK cell depletion abolished the difference in hepatic viral load between SSD- and NCD-fed mice, indicating that NK cells dysfunction contribute to impaired viral control in the liver of SSD-fed animals.

Our previous work linked metabolic stress in the liver to immune dysfunction through chronic engagement of the NKG2D activating receptor by stress-induced NKG2D ligands on hepatocytes. To determine whether this pathway contributes to impaired antiviral defense in MASLD, we used mice lacking NKG2D receptor on immune cells. NKG2D deficiency abolished the difference in viral load between SSD- and NCD-fed mice, implicating that MASLD-impairs NKG2D signalling in mCMV infection. Consistent with these findings, hepatic NK cells isolated from mCMV infected SSD-fed mice displayed reduced cytotoxicity against NKG2D ligand-expressing YAC-1 cells compared to control animals.

Together, these findings identify impaired NKG2D-dependent NK cell cytotoxicity as a potential mechanism linking MASLD to defective antiviral immunity and impaired tissue-level control of viral replication.

*P14 Towards an ex-vivo model that discriminates virus replication in tumor and healthy tissue*

**Sanja Mateljak Lukačević<sup>1,2</sup>**, Tihana Kurtović<sup>1,2</sup>, Melita Perić-Balja<sup>3</sup>, Snježana Ramić<sup>3</sup>, Beata Halassy<sup>1,2</sup>

<sup>1</sup>Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia

<sup>2</sup>Center of Excellence for Virus Immunology and Vaccines, CERVirVac, Zagreb, Croatia

<sup>3</sup>University Hospital Centre Sestre Milosrdnice, University Hospital for Tumours, Zagreb, Croatia

Oncolytic viruses owe their selectivity toward tumor cells to enhanced replication compared to healthy cells, which results from differences in the functioning of various intracellular signaling pathways involved in antiviral defense. However, a major challenge is measuring this difference in the laboratory, that is, selecting an appropriate “healthy control” for a given type of tumor cell or tissue type.

Here, we present preliminary results from the development of an assay designed to demonstrate stronger viral efficacy in tumor tissue than in healthy tissue. We use an ex vivo/in vitro model in which fragments of breast cancer tissue and fragments of healthy breast tissue from the same patient are incubated in the presence of the virus. Viral replication is then assessed by determining the amount of virus produced after several days of incubation. Since tissue fragments originating from the same source are not identical in composition or size, they are expected to introduce variability into the method.

Following incubation, the amount of virus present in both the surrounding medium and the homogenized tissue is measured. Results are expressed as the ratio of the viral titre in the homogenate to that in the supernatant, which minimizes inherent differences among individual tissue fragments. We have observed that this parameter yields lower values in healthy tissue than in tumor tissue. The observed difference likely reflects slower and more restricted viral replication in healthy tissue, as opposed to relatively unhindered replication in tumor tissue.

*P15 Pathogenesis of MCMV infection in the adrenal gland*

**Ivana Bertović<sup>1</sup>**, Marija Mazor<sup>1</sup>, Jelena Železnjak<sup>1</sup>, Tina Ružić<sup>1</sup>, Magdalena Medved<sup>1</sup>, Maja Cokarić Brdovčak<sup>1</sup>, Martina Brnjić<sup>1</sup>, Jelena Tomac<sup>2</sup>, Stipan Jonjić<sup>1</sup>, Vanda Juranić Lisnić<sup>1</sup>, Berislav Lisnić<sup>1</sup>

<sup>1</sup>Center for Proteomics, Faculty of Medicine, University of Rijeka

<sup>2</sup>Department of Histology and Embryology, Faculty of Medicine, University of Rijeka

Murine cytomegalovirus (MCMV) is an established model for studying beta-herpesvirus infections and host-pathogen interactions. The adrenal gland, a central organ in stress adaptation and hormone synthesis, is a target of MCMV infection, yet the mechanisms underlying viral pathogenesis and immune-endocrine interactions in this tissue remain poorly defined. To investigate these mechanisms, mice were intravenously infected with MCMV, and adrenal viral replication was assessed by viral titration and immunohistological detection of IE1-positive cells. Local immune responses were characterized by cytokine profiling and immune cell depletion, while endocrine function was evaluated by measuring adrenal hormone production. The role of type I interferon signaling was examined using mice deficient in the type I interferon receptor. Viral titers in the adrenal glands peaked at day 5 post-infection, with IE1-positive cells predominantly localized to the adrenal cortex. Despite robust viral replication, adrenal hormone production remained preserved. Adrenal cytokine levels generally reflected viral loads in infected organs; notably, IL-6 was consistently absent, correlating with the lack of corticosterone induction during infection. In the absence of type I interferon signaling, viral replication was markedly enhanced and accompanied by increased adrenal hormone secretion and induction of proinflammatory cytokines, including IFN- $\gamma$ , TNF, CCL2, and IL-6. Depletion studies identified NK cells and CD8<sup>+</sup> T cells as key mediators of viral control, whereas CD4<sup>+</sup> T cells were required for establishment of viral latency. Together, these findings demonstrate that type I interferon signaling is critical for limiting MCMV replication and maintaining adrenal hormonal homeostasis, while distinct immune cell populations exert tissue-specific roles in viral control and latency.

*P16 MCMV altered-self MHC I molecules and their impact on adaptive immune responses*

**Jelena Železnjak<sup>1</sup>**, Magdalena Medved<sup>1</sup>, Ivana Bertović<sup>1</sup>, Maja Cokarić Brdovčak<sup>1</sup>, Marija Mazor<sup>1</sup>, Ilija Brizić<sup>1</sup>, Berislav Lisnić<sup>1</sup>, Stipan Jonjić<sup>1</sup>, Lars Dölken<sup>2,3</sup>, Vanda Juranić Lisnić<sup>1</sup>

<sup>1</sup>Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

<sup>2</sup>Institute for Virology and Immunobiology, Julius-Maximilians-Universität-Würzburg, Würzburg, Germany

<sup>3</sup>Institute for Virology, Hannover Medical School (MHH), Hannover, Germany

Cytomegalovirus (CMV) is a widespread  $\beta$ -herpesvirus that can persist in its host for a lifetime. One of its main strategies to evade the immune response involves modulation of major histocompatibility complex class I (MHC I) molecules, thereby affecting the responses of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. Among the viral proteins involved in this process, MATp1, encoded by the most highly expressed transcript of murine CMV (MCMV), partners with m04 protein, to modulate MHC I expression, ensuring effective inhibition of NK cells via inhibitory Ly49 receptors. However, our preliminary data indicate that CMV utilizes MATp1 not only to regulate NK cell responses, but also to modulate antigen presentation, potentially reducing the quality of peptides presented by MHC I molecules. Consistent with this, in vivo infection model with the wild-type MCMV exhibits a weaker CD8 T-cell response compared to a MCMV lacking MATp1, suggesting a role for MATp1 in CTL-mediated immune evasion.

The aim of this study is to elucidate the mechanisms by which MATp1 modulates MHC I molecules and to investigate how peptide quality affects the adaptive immune response. Since CMV is being explored as a platform for vaccine development, unraveling its immune evasion strategies could advance antiviral approaches. This is particularly important for HCMV-specific adoptive T-cell therapy (ACT), which utilizes virus-specific CTLs to control infection in immunocompromised patients. Therefore, an improved understanding of how CMV manipulates MHC I and antigen presentation may lead to more precise therapeutic protocols and optimized CD8 T-cell responses.

***P17 Unfolded protein response signalling in SCD inhibitor-induced lipotoxicity in acute myeloid leukemia***

Damir Lisjak<sup>1</sup>, Klara Bardac<sup>1</sup>, Alojzija Breic<sup>1</sup>, Hrvoje Lalic<sup>1,2</sup>, Dora Visnjic<sup>1</sup>, Celine Philippe<sup>3</sup>, Paolo Gallipoli<sup>4</sup>, **Vilma Dembitz<sup>1</sup>**

<sup>1</sup>Department of Physiology and Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia; <sup>2</sup>Division of Laboratory Immunology, Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia; <sup>3</sup>University of Rennes, INSERM U1242, Rennes, France; <sup>4</sup>Centre for Haemato-Oncology, Barts Cancer Institute, London, UK

**Background:** Stearoyl-CoA desaturase (SCD) catalyses monounsaturated fatty acid synthesis and is a promising therapeutic target in acute myeloid leukemia (AML). SCD inhibition induces lipotoxicity and antileukemic effects, but how fatty acid imbalance kills cells remains unclear. In solid tumors, saturated fatty acids can induce endoplasmic reticulum (ER) stress and unfolded protein response (UPR)-driven apoptosis.

**Methods:** We studied UPR involvement in SCD inhibitor (SCDi) toxicity in SCDi-sensitive MOLM-13 and MV-4-11 and resistant OCI-AML3 cells. PERK and IRE1 activation was assessed from downstream targets (DDIT3/CHOP, XBP1). Functional contribution was tested by pharmacological inhibition of PERK and IRE1 and siRNA knockdown of PERK and CHOP in MV-4-11 cells; PERK was overexpressed in OCI-AML3 cells to test whether increased PERK activity confers SCDi sensitivity.

**Results:** SCD inhibition markedly increased DDIT3/CHOP and XBP1 expression in MOLM-13, with more modest induction in MV-4-11 cells. PERK or IRE1 inhibition partially reduced SCDi toxicity in MOLM-13, but PERK inhibitor toxicity in MV-4-11 cells and non-specific suppression of IRE1-associated targets complicated interpretation. Consistently, PERK or CHOP knockdown did not affect SCDi toxicity in MV-4-11 cells, and PERK overexpression in OCI-AML3, confirmed to activate the PERK arm without inducing IRE1 targets, failed to increase SCDi sensitivity. Interestingly, PERK overexpression increased sensitivity to exogenous oleate, which strongly induced PERK expression in MOLM-13 and, less so, MV-4-11 cells.

**Conclusions:** Although SCD inhibition activates UPR components, PERK/CHOP activation alone is insufficient to determine SCDi sensitivity. Instead, PERK may support adaptation to monounsaturated fatty acid overload and lipid homeostasis. Further work should separate PERK and IRE1 contributions to SCDi-induced lipotoxicity.

***P18 Microbial drivers of bile acid circadian rhythmicity in mice: correlating diurnal shifts in bile acids with microbial enzymes shaping the bile acid pool***

**Darja Flegar<sup>1,2</sup>**, Gabriel S.S. Tofani <sup>1,3\*</sup>, Kardokh Kaka Bra<sup>1,2\*</sup>, Monica Cazzaniga<sup>1,2</sup>, Madhuriben H. Panchal<sup>4</sup>, Cara M. Hueston<sup>1,2</sup>, Marcus J. Claesson<sup>1,2</sup>, Jonathan R. Swann<sup>4</sup>, John F. Cryan<sup>1,3</sup>, Cormac G.M. Gahan<sup>1,2,5</sup>

<sup>1</sup>Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland; <sup>2</sup>Department of Microbiology, University College Cork, Cork, Ireland; <sup>3</sup>Department of Anatomy & Neuroscience, University College Cork, Cork, Ireland; <sup>4</sup>School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK; <sup>5</sup>School of Pharmacy, University College Cork, Cork, Ireland

\*Contributed equally

**Introduction:** The roles of bile acids (BA) have expanded from digestion of lipids to signaling molecules that connect metabolic and immune functions of the host with gut microbiota, specifically influencing immune homeostasis of the intestines, liver and the central nervous system. Disruption of circadian rhythms by modern lifestyle, unhealthy dietary habits and stress has also been linked to impaired metabolic health. BA synthesis is subject to strict diurnal regulation. In addition, circadian oscillations are present in gut microbiota that enzymatically transform bile acid species. Therefore, in this study we focused upon the potential for microbial alterations of bile acid signatures over the 24-hour cycle in normal SPF mice under strict light/dark cycles.

**Methods:** Mouse groups were sampled at Zeitgeber Times (ZT) ZT0, ZT6, ZT12 and ZT18. Bile acid signatures were established using UPLC-mass spectrometry. Gut microbiota was analyzed by 16s rDNA and shotgun sequencing. Host gene expression from ileum, cecum, colon and liver was measured using qRT-PCR. Additionally, we optimised an in vitro assay to evaluate BSH activity in the same caecal microbiota samples.

**Results:** We verified diurnal oscillations of BA species levels in the contents of the large intestine and blood of mice measured at 6-hour intervals. Analyses revealed an elevation of unconjugated bile acids at ZT6 concomitant with increased abundance of taxa with potent bile salt hydrolase (BSH) deconjugating activity (including *Lactobacillus* species). Follow-up with shotgun metagenomic analysis of the caecal microbiota revealed nuances in the abundance of particular Bsh and BAI orthologues which may provide insights into taxa/genes of importance for regulating microbial diurnal alterations in BA signals. In vitro BA digestion assay demonstrated how BSH activity can be modulated by riboflavin, and that this effect might be more pronounced at a specific time of the day.

**Conclusion:** Our study describes the complex time-dependent environment in which BA species are produced in mice and can be used to guide further investigations in this area. Diet can affect BSH activity directly or indirectly (through changes in microbiome), which could also be involved in disease pathophysiology.

***P19 Targeting PVR/CD155 as an Alternative and Complementary Immune Checkpoint Strategy in Triple-Negative Breast Cancer***

**Marina Čunko<sup>1</sup>**, Paola Kučan Brlić<sup>1</sup>, Marija Mazor<sup>1</sup>, Ema Bellulovich<sup>1</sup>, Karmela Miklič<sup>1</sup>, Suzana Malić<sup>1</sup>, Mijo Golemac<sup>1</sup>, Pini Tsukerman<sup>2</sup>, Guy Cinamon<sup>2</sup>, Stipan Jonjić<sup>1</sup>, Tihana Lenac Roviš<sup>1</sup>

<sup>1</sup>*Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia*

<sup>2</sup>*Nectin Therapeutics LTD, Jerusalem, Israel*

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype with limited targeted treatment options. Although PD-1/PD-L1 immune checkpoint blockade benefits selected patients, heterogeneous responses and disease progression highlight the need for additional therapeutic strategies.

Poliovirus receptor (PVR/CD155) is an immunoregulatory molecule frequently overexpressed in solid tumors, with high expression in TNBC associated with poor survival. NTX1088, our first-in-class anti-PVR monoclonal antibody designed to enhance antitumor immunity, has demonstrated preclinical activity and is currently being clinically investigated as monotherapy and in combination with the PD-1 inhibitor pembrolizumab in patients with advanced solid tumors. Previous studies demonstrated anti-PVR activity across human tumor cell models in vitro. In addition, PVR targeting inhibited tumor growth in a humanized A549 xenograft model, a setting in which PD-1 blockade showed limited efficacy due to the PD-L1-negative tumor phenotype.

Here, additional anti-PVR monoclonal antibodies were characterized, including blocking antibodies and antibodies recognizing PVR epitopes accessible within pre-existing PVR-antibody complexes. In parallel, growth kinetics of the MDA-MB-231 TNBC xenograft model were established in vivo. We next investigated PVR targeting in this PD-L1-positive model, where PVR blockade could complement or provide an alternative to PD-1-directed therapy. Established tumors retained substantial PVR expression in vivo. Importantly, a blocking anti-PVR monoclonal antibody effectively controlled MDA-MB-231 tumor growth, with greater activity than PD-1 blockade in this experimental setting.

These findings support PVR as an independent and complementary immune checkpoint target in TNBC and provide a basis for further development of PVR-directed therapeutic strategies.

***P20 Peptidoglycan-Based Biomimetic Nanomaterials Reveal Lectin Recognition in DAP-Type Bacteria: Toward Rational Design of Antimicrobial Therapeutics***

**Ruža Frkanec<sup>1</sup>**, Marcela Šišić<sup>1</sup>, Anne Imberty<sup>2</sup>, Antonija Tomić<sup>3</sup>, Andrea Usenik<sup>4</sup>, Vladislav Tomišić<sup>4</sup>, Leo Frkanec<sup>3</sup>

<sup>1</sup>Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia

<sup>2</sup>CERMAV-CNRS F38041 Grenoble Cedex 9, France

<sup>3</sup>Department of Organic Chemistry and Biochemistry, Institute Ruđer Bošković, Zagreb, Croatia

<sup>4</sup>Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia

Biomimetic nanomaterials that replicate natural biological systems provide versatile platforms for biomedical applications, including targeted drug delivery and innovative approaches to personalized medicine. The peptidoglycan monomer (PGM; GlcNAc–MurNAc–L-Ala–D-isoGln–mesoDAP( $\epsilon$ -NH<sub>2</sub>)–D-Ala–D-Ala) represents a structurally defined, minimal fragment of DAP-type bacterial cell walls, characteristic of Gram-negative pathogens such as *Escherichia coli*. Here, we present a comprehensive study of design and synthesis of biomimetic nanomaterials functionalized with PGM and the investigation of their interactions with selected lectins to gain mechanistic insights into glycan recognition processes relevant to host-pathogen interactions. Using a combination of molecular modelling, isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), and agglutination assays, we demonstrate that strong binding to wheat germ agglutinin (WGA) arises from cooperative interactions between the GlcNAc–MurNAc glycan and the adjacent peptide stem. Immobilization of WGA on a biochip enabled real-time SPR analysis and determination of the dissociation constant ( $K_d = 9.3 \times 10^{-4}$  M), which is in excellent agreement with the value obtained by ITC ( $1.2 \times 10^{-3}$  M). The PGM–lectin binding landscape, validated by both computational and experimental approaches, highlights the potential of the peptidoglycan monomer as a precise glycomimetic model. Our findings establish a robust platform for exploring selective lectin-carbohydrate interactions in bacterial recognition, with possible applications in the development of artificial lectins for diagnostic and therapeutic use. This model further enables the rational design of peptidoglycan-targeting therapeutics, particularly next generation antimicrobials with high specificity. Understanding how host-derived or synthetic lectins interact with this structure offers a promising route toward novel antimicrobial strategies.

*P21 Assessment of an In Vitro Model for the Evaluation of VSV Oncolytic Activity*

**Lucija Ivandić**, Erika Gamulin, Maja Lang Balija, Dubravko Forčić, Maja Jagušić, Beata Halassy

*Centre for Research and Knowledge Transfer in Biotechnology, University of Zagreb, Zagreb, Croatia*

Oncolytic virotherapy is a promising therapeutic approach in cancer treatment, with several viruses from different families demonstrating potential for selective tumor cell destruction. The aim of this study was to establish and optimize an experimental in vitro system for the evaluation of viral oncolytic activity, using vesicular stomatitis virus (VSV) as a model virus.

Preliminary experiments were performed using continuous cell lines derived from human breast carcinoma, as well as feline and canine mammary carcinoma and spheroids prepared from them. Following VSV infection, three complementary parameters of oncolytic activity were evaluated: viral replication in 2D cell cultures, and cell viability and induction of apoptosis in 3D cell cultures. Viral replication was assessed by determining infectious virus titers in culture supernatants, while cell viability was evaluated using PrestoBlue™ in 3D tumor spheroids. VSV-induced apoptosis was assessed using a Caspase-Glo® 3/7 assay.

VSV replicated in all tested mammary tumor cell lines and induced strong reduction in cell viability and measurable activation of apoptosis in 3D cell cultures. Among the tested human mammary tumor cell lines, differences in susceptibility to VSV were observed, with some cell lines showing greater sensitivity to infection.

These results demonstrate the feasibility of the established experimental setup and provide a basis for further optimization of infection conditions, including multiplicity of infection and sampling time points, for comparative evaluation of different oncolytic viruses.

**P22 *From Stem Cells to Thymic Organoids - The Potential of Artocarpus tonkinensis***

D. V. Delfino<sup>1</sup>, Ildikó Bódi<sup>2</sup>, M. Matulic<sup>3</sup>, L. Heckel<sup>4</sup>, Trinh Thi Thuy<sup>5</sup>, **Mariastefania Antica<sup>1,3,4</sup>**

<sup>1</sup>Foligno Nursing School and Section of Pharmacology, Department of Medicine and Surgery, University of Perugia, Perugia, Italy; <sup>2</sup>Department of Anatomy, Histology and Embryology, Semmelweis University, 1085 Budapest, Hungary; <sup>3</sup>Department of Biology, Faculty of Science, University of Zagreb, Horvatovac 102, 10 000 Zagreb, Croatia; <sup>4</sup>School of Dental Medicine, University of Zagreb, Zagreb, Croatia; <sup>5</sup>Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam

Thymic epithelial cells (TECs) are key components of the thymic microenvironment, providing essential signals for thymus organization, regeneration, and T-cell development. The generation of functional thymic tissue *in vitro* represents an important goal for understanding human thymus biology and for developing novel therapeutic strategies for patients with impaired thymic function. Three-dimensional (3D) culture systems offer a promising approach to recapitulating key features of the human thymic microenvironment and studying the cellular interactions required for T-cell development. We have previously identified a novel population of epithelial stem cells in the human thymus and established robust methods for their isolation. These cells retain epithelial characteristics, providing a promising cellular source for human thymic tissue generation. Treatment with *Artocarpus tonkinensis* extract markedly enhanced their proliferation and expansion and promoted the formation of large numbers of epithelial spheroids. Importantly, co-culture of these spheroids with hematopoietic stem cells (HSCs) resulted in the development of complex thymic organoids containing both epithelial and hematopoietic components. These structures provide a physiologically relevant 3D model of the human thymic microenvironment and represent, to our knowledge, the first human thymic organoid system generated from this newly identified population of thymic epithelial stem cells. Our stem cell-based system provides a novel platform for studying human thymic development, epithelial-hematopoietic interactions, and T-cell differentiation, as well as for evaluating potential thymic regenerative therapies. Ultimately, this approach may contribute to the development of functional or “off-the-shelf” thymic tissue for patients with congenital or acquired thymic dysfunction.

Funding: EU support NextGenerationEU, through the National Recovery and Resilience Plan (NPOO) (SFZG-07-2025), Faculty of Science, University of Zagreb, NextGenerationEU through the Faculty of Science, University of Zagreb, CERRM EU Grant Agreement KK01.1.1.01.0008.

P23 IGF-1 receptor signalling promotes CD8 memory T cell formation and enhances tumour control *in vivo*

**Sanja Mikašinović<sup>1</sup>**, Ozren Majstorović<sup>1</sup>, Marko Šestan<sup>1</sup>, Bojan Polić<sup>1</sup>, Felix M. Wensveen<sup>1</sup>

<sup>1</sup>*Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Croatia*

The interplay between the immune and endocrine systems is essential for maintaining metabolic homeostasis and protecting tissues during infection. When a viral infection occurs, the immune system induces metabolic changes that contribute to “sickness behavior”, enhancing the body’s ability to combat the pathogen. Moreover, endocrine hormones are significant players in shaping immune cell activity, ensuring their energy consumption aligns with systemic availability. Despite their importance, many immunomodulatory effects of hormones remain poorly understood.

Insulin and insulin-like growth factor 1 (IGF-1) are structurally similar hormones that can cross-react with each other’s receptors. We previously demonstrated that insulin stimulation enhances cytokine production by CD8 T cells. However, this effect was also observed in CD8 T cells deficient in the insulin receptor (IR), indicating that insulin does not regulate the antiviral CD8 T cell response through IR. Instead, our data revealed that CD8 T cells in the memory phase express high levels of the IGF1R, which binds insulin with low affinity. IGF-1 stimulation enhanced IFN $\gamma$  production and glycolytic activity in memory CD8 T cells. Furthermore, IGF-1 signaling was required for the generation of functional memory CD8 T cells *in vivo*. Mice lacking both IR and IGF1R in CD8 T cells exhibited impaired memory responses and failed to control B16 melanoma growth.

Together, these findings identify IGF-1R signaling as an important regulator of CD8 memory T cell formation and tumor control *in vivo*. Disruption of hormonal control of the antiviral response in the context of metabolic disease might explain increased susceptibility to pathogens in individuals with metabolic disorders, such as diabetes.



---

*NOTES:*



*NOTES:*

Gold Sponsors:



REPUBLIKA HRVATSKA  
Ministarstvo znanosti,  
obrazovanja i mladih



European Federation of  
Immunological Societies

Silver Sponsors:



**GOREA**  
PLUS



**Jasika®**

PUBLISHER: Croatian Immunological Society  
EDITING & DESIGN: Lidija Milković



[www.hid.hr](http://www.hid.hr)