CTGCT DAYS 2025

Abstract Book

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Publisher:

Centre for the Technologies of Gene and Cell Therapy

National Institute of Chemistry

Editors:

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Issued by:

Centre for the Technologies of Gene and Cell Therapy

National Institute of Chemistry

Ljubljana, 2025

Price: Free

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID 248743427
ISBN 978-961-7238-13-6 (PDF)

CTGCT CONFERENCE – CELL THERAPY

DAY 1 – 15 September

13:00	**Registration**	
13:45	Gregor Anderluh Roman Jerala Mojca Benčina	Introduction
14:00	Felix Wensveen	Why we get sick and how to get better; opportunities for targeting sickness metabolism in non-communicable disease.
14:30	Jürgen Kuball	Uncovering the Spatial Regulation of γδ T Cells: Toward Receptor-Guided Immunotherapy
15:00	Nicola Maciocia	Development of CAR-T cells for T cell cancers
15:20	Robert Zorec	From understanding astroglial lysosomal fusion to pleiotropic targeting of cancer antigens with immunohybridomas: The first innovative biological medicine for advanced cancer therapy in Slovenia
15:40	Tiago Santos	Sponsored Talk: Functional Single-Cell Analysis to Advance CAR-T and TCR-T Therapies through Beacon Discovery
16:00	**Coffee Break**	
16:30	General Assembly for CTGCT Partners (Closed Session)	
18:00	**End of Day 1**	

DAY 2 – 16 September

08:00	**Registration**	
08:30	Natalia Marek-Trzonkowska	Optimal preclinical models and cancer heterogeneity-challenges for cancer immunotherapy
09:00	Tina Fink	Optimizing CAR architecture for improved T cell performance
09:20	Ines Papak	From One Cell to a Tumor: The Story of $CD45^{+}$ Cells in NSCLC
09:40	Jiri Eitler	Dual-CAR NK cells targeting PD-L1 and ErbB2/HER2 eliminate solid tumor cells and prevent immune escape due to antigen loss
10:10	**Poster Session /Coffee Break**	
10:45	Kavitha Lakshmi Anke Fuchs	Automated GMP-Compatible Production of Universal CAR Tregs for Precision Immunomodulation
11:05	Ramesh Ganesh	THE FUTURE OF VIRAL VECTORS: A survey among Centers Associated with CTIWP of EBMT,

		NXTGEN Hightech, DARE-NL, T2EVOLVE, and GoCART Coalition
11:25	Vesna Spasovski	Stem Cell-Based Therapy and Bioactive Hybrid Nanocarriers in Osteoarthritis Treatment: Distinct Pathways toward Tissue Repair
11:55	Regina Demlová	Bridging Research and Care: CREATIC CoE's Academic Development of Cell-Based ATMPs for Paediatric and Rare Conditions
12:25	**Break**	
14:00	Tanja Jesenko	From bench to bedside in Slovenia: Phase I trial of phIL12 intratumoral gene electrotransfer in basal cell carcinoma
14:25	Damjan Osredkar	Shaping the Future of Rare Disease Care: Connecting Patients and Innovation
14:50	Matjaž Sever	Academic CAR T-Cell Manufacturing: Implications for Clinical Implementation and Access
15:15	Viktor Glaser	Repurposing base editors for targeted knock-in and simultaneous knockouts to generate multiplex-edited allogeneic CAR T cells with minimal translocations
15:35	Polona Šafarič Tepeš Seth Plancer	Hospital-embedded innovation: U.S. experience in advancing translational medicine across system
16:00	**Poster Session / Coffee Break**	
16:30	Moderator: Gregor Cuzak Participants: Leila Amini, Tanja Jesenko, Jürgen Kuball, Lenka Součková, Suzana Vidic	ROUND TABLE I: Regulatory and Clinical Pathways for Gene and Cell Therapy
18:00	**End of Day 2**	
19:00	Dinner for speakers	

GENE H CONFERENCE – GENE THERAPY

DAY 1 – 17 September

8:00	**Registration**	
08:30	Ajda Lenardič	Interspecies Generation of iPSC-derived Functional Muscle Stem Cells as a potential Duchenne Muscular Dystrophy Treatment
08:50	Robert Torrance	Functional restoration of immune defects in STAT1 gain-of-function immunodeficiency following gene editing
09:10	Tina Lebar	Homology-guided engineering of tyrosine recombinase nucleotide specificities
09:30	Frank Buchholz	Engineering Designer-Recombinases for therapeutic genome editing
10:00	Emma Morris	Gene Editing for Inborn Errors of Immunity
10:30	**Sponsored talk: Thermo Fisher Scientific / Coffee Break**	
11:00	Ana Dolinar Češarek	Co-Delivery of Non-Encapsidated DNA by rAAV: Is This a Potential Biosafety Concern?
11:20	Oscar Wilkins	Precision therapeutics for ALS and FTD
11:40	James Sleigh	Targeting receptor tyrosine kinases to restore axonal transport in genetic peripheral neuropathy.
12:00	Luis Pereira de Almeida	Gene Therapy Strategies for Spinocerebellar Ataxia Type 3: Preclinical Advances in Allele-Specific Silencing and Gene Inactivation
12:30	**Coffee Break**	
13:00	Moderator: Nik Prebil Participants: Zora Čechová, Boštjan Čeh, Andrej Klemenc, Mojca Pečar, Luis Pereira de Almeida	ROUND TABLE II: Bridging Innovation and Commercialization in Gene and Cell Therapy
14:30	**End of Day**	

We would like to thank everyone involved in organising and supporting the events held during the CTGCT Days 2025. In no particular order here are listed the institutions:

The CTGCT Project and its partner institutions:

The Gene H Project and its partner institutions:

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2nd CTGCT Conference

CTGCT Conference – Cell Therapy

15-16 September 2025

Book of Abstracts

Organised by:

Centre for the Technologies of Gene and Cell Therapy

National Institute of Chemistry, Slovenia

Why We Get Sick: How the Immune System Modulates Our Systemic Metabolism in Context of Disease

Speaker: Felix Wensveen[1]

1. Department of Histology & Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Being sick makes you feel miserable. Whereas we experience this as a pathology, after all, we're feeling bad, in fact it is not. It is a carefully orchestrated and highly conserved physiological response that is mediated by the immune system. In response to an infection, or another serious threat to the body, the immune system alters systemic metabolism, which is what we experience as being sick. Apparently, this misery somehow helps your body better deal with the underlying cause of the immune response. Surprisingly, very little is known about how or why this happens, but recent discoveries have shed more light on its cause. In this presentation, I will highlight some key mechanisms of how the immune alters systemic metabolism and how it helps the anti-pathogenic immune response. Importantly, I will elucidate how targeting of some of these systems may help alleviate key negative aspects of non-communicable diseases such as cancer, thus contributing to the efficacy of therapy and quality of life of patients.

Uncovering the Spatial Regulation of γδ T Cells: Toward Receptor-Guided Immunotherapy

Speaker: Jürgen Kuball[1]

1. University Medical Centre Utrecht, Utrecht, The Netherlands

Gamma delta (γδ) T cells are a unique type of immune cell that can help the body detect and destroy cancer. However, their exact role in solid tumors has remained unclear, partly because traditional laboratory models do not fully reflect how these cells behave in the human body (Beringer et al., Nature Reviews Cancer, 2025). In this talk, I will present a comprehensive strategy to study γδ T cells directly in patients with colorectal cancer, using matched samples from blood, healthy colon tissue, primary tumors, and liver metastases. We identified distinct receptors that guide γδ T cells either into tumors or toward effective cancer cell killing—making these defined receptors promising tools for novel immunotherapies. Overall, this research provides important new insights into how γδ T cells function across different tissue environments and opens new avenues for improving cancer treatment by leveraging the power of these specialized immune cells and their receptors.

Development of CAR-T Cells for T Cell Cancers

Speaker: Nicola Maciocia[1,2]

Authors: Paul Maciocia[1,2], Nicola Maciocia[1,2]

1. Department of Haematology, University College London Hospitals, London, UK
2. UCL Cancer Institute, University College London, London, UK

Chimeric antigen receptor (CAR) T-cell therapy has transformed the treatment landscape for B cell cancers, but translating this success to T cell malignancies poses distinct challenges. The paucity of truly tumour-restricted antigens leads to a risk of fratricide during manufacture, and of prolonged T cell aplasia. However, early phase studies hint at feasibility. TRBC1-targeted CARs have achieved metabolic complete responses in peripheral T cell lymphoma; while anti-CD7 and anti-CD5 CAR-T have led to >90% complete responses in T cell leukaemia. However, important challenges remain including infectious toxicity and high relapse rates. In this talk we will explore the present and future options for CAR-T therapy in this highly challenging group of diseases.

From Understanding Astroglial Lysosomal Fusion to Pleiotropic Targeting of Cancer Antigens with Immunohybridomas: The First Innovative Biological Medicine for Advanced Cancer Therapy in Slovenia

Speaker: Robert Zorec[1]

Authors: Helena H.Chowdhury, Danaja Kuhanec, Ena Sanjković, Rebeka Dajčman, Kaja Belko Parkel, Erika Rus, Samo Pirnat, Urška Černe, Carla Marion, Urša Adamič, Primož Runovc, Miha Pate, Zala Smole, Julijan Vršnik, Anemari Horvat, Tomaž Mark Zorec, Matjaž Stenovec, Jernej Jorgačevski, Maja Potokar, Nina Vardjan, Mateja Gabrijel, Matjaž Jeras, Marko Kreft & Robert Zorec

1. University of Ljubljana, Faculty of Medicine, Institute of Pathophysiology, Laboratory of Neuroendocrinology – Molecular Cellular Physiology; CELICA, Biomedical Centre, Lab Cell Engineering, Ljubljana, Slovenia

We aim to understand how subcellular vesicle traffic, membrane fusion, second messenger signalling, cytoskeletal dynamics and single cell metabolism contribute to ageing and diseases to translate the findings. In the field of NEUROLOGICAL INDICATIONS, we have developed novel animal models (mice, Drosophila) and small molecules targeting neuroglial aerobic glycolysis, antigen presentation in neurodegeneration by engaging novel orphan G-protein coupled receptors. In the domain of CANCER, which shares aerobic glycolysis with neuroglia, and based on electrophysiological studies of lysosomal fusion, we have developed autologous cell-based immunotherapy (an ATMP), based on immunohybridomas (i.e. hybrids of immune and cancer cells) to treat prostate cancer, available for patients in Slovenia, with technology applicable to treating solid tumours, including triple-negative breast cancer (ongoing preparations for the clinical trial), and rare diseases. In the lecture, we will emphasise the need to address the biology and pathophysiology of multiple targets in cancer, a pleiotropic approach, by using heterologous lysosomal fusion through electrofusion of cells and their organelles.

Sponsored Talk: Functional Single-Cell Analysis to Advance CAR-T and TCR-T Therapies through Beacon Discovery

Speaker: Tiago Santos[1]

1. Brucker Cellular Analysis

The development of effective CAR-T and TCR-T therapies depends on the ability to rapidly identify and select immune cells with potent, specific, and durable anti-tumor activity. Beacon Discovery is a next-generation platform enabling high-throughput, multiparametric analysis of live single cells—capturing dynamic cytotoxicity, cytokine secretion, and surface phenotype in real time. By providing rapid, functional insights into engineered cell products, Beacon streamlines candidate selection, validation, and optimization with unmatched resolution. This presentation will showcase real-world applications, including Prof. Eichmüller's work on patient-specific TCR discovery and Prof. Zamora's studies on CAR-T functionality, illustrating how Beacon accelerates the path from concept to clinic in advanced cell therapy development.

Technologies for cell and gene therapies Workshops & Demonstrations

Workshop

CTGCT DAYS 2025

Date:
September 15-17, 2025

Place:
CTGCT Days 2025 - Best Western Premier Hotel Slon, Slovenska cesta 34, 1000 Ljubljana, Slovenia

Topic:
Novel Technologies for CGT research

Contact:
Lara Mlakar, +386 40 544 547, mlakar@accela.eu

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Program 15.09.2025

Forenoon

1. Technology presentation: Introducing Cytek Aurora Evo, the New Standard of Full Spectrum Cytometry (9:00-9:30)
2. Hands-on cell sorting session: Easily sort large and fragile cells with WOLF Gentle Cell Sorter (9:30-10:15)
3. Technology presentation: Precise and efficient Extracellular Vesicles purity detection with ExoPlorer Nano Flow Cytometer (10:15-10:45)
4. Mantis – technology presentation and instrument setup (10:45-11:30)
5. Technology presentation: Introducing VERLO: Image-Guided & Gentle Cell Sorter (11:30-12:00)

Afternoon

6. IsoSpark – technology presentation (13:00-13:30)
7. Hands-on Spectral Flow Cytometry session: Cytek Aurora- managing highly autofluorescent cells and overlapping dyes (14:15-15:15)
8. Orion – technology presentation (15:15-15:40)
9. Tempest – technology presentation and instrument setup (16:00-17:00)

Presentation included in CTGCT scientific program:

Functional Single-Cell Analysis to Advance CAR-T and TCR-T Therapies through Beacon Discovery (15:40-16:00). Access is based on registration at CTGCT Conference.

Abstract: The development of effective CAR-T and TCR-T therapies depends on the ability to rapidly identify and select immune cells with potent, specific, and durable anti-tumor activity. Beacon Discovery is a next-generation platform enabling high-throughput, multiparametric analysis of live single cells—capturing dynamic cytotoxicity, cytokine secretion, and surface phenotype in real time. By providing rapid, functional insights into engineered cell products, Beacon streamlines candidate selection, validation, and optimization with unmatched resolution. This presentation will showcase real-world applications, including Prof. Eichmüller's work on patient-specific TCR discovery and Prof. Zamora's studies on CAR-T functionality, illustrating how Beacon accelerates the path from concept to clinic in advanced cell therapy development.

Tiago Santos, PhD, MBA - Market Development Executive**, Bruker Cellular Analysis**

Program 16.09.2025

Forenoon

1. Hands-on Nano Flow Cytometry session: Precise and efficient Extracellular Vesicles purity detection with ExoPlorer Nano Flow Cytometer (9:00-10:00)
2. Technology presentation: Elevate you Spectral sorting experience for small and large particles with Cytek Aurora CS. (10:10-10:45)
3. Mantis – technology presentation and instrument setup (10:45-11:30)
4. Orion & Cyto Finder – technology presentation (11:30-12:30)

Afternoon – Wine meets Technology (French and Italian selection)

5. Beacon Discovery – technology presentation (12:30-13:30)
6. Hands-on Spectral Flow Cytometry sessions: Cytek Aurora- managing highly autofluorescent cells and overlapping dyes (13:00-14:00)
7. F.A.S.T. – technology presentation and instrument setup (14:30-16:15)
8. Hands-on cell sorting session: Easily sort large and fragile cells with WOLF Gentle Cell Sorter (16:15-17:15)

Optimal Preclinical Models and Cancer Heterogeneity - Challenges for Cancer Immunotherapy

Speaker: Natalia Marek-Trzonkowska[1,3]

Authors: Ines Papak[1], Martyna Muszczek[1], Artur Piróg[1], Jakub Faktor[1], Anna Biernacka[1], Zuzanna Urban- Wójciuk[1], Magdalena Szczepanowska [2], Magdalena Sitkiewicz [2], Witold Rzyman[2], Sachin Kote[1], Natalia Marek- Trzonkowska[1,3]

1. International Centre for Cancer Vaccine Science, University of Gdansk, Gdańsk, Poland
2. Department and Clinic of Thoracic Surgery, Medical University of Gdańsk, Gdańsk, Poland
3. Laboratory of Immunoregulation and Cellular Therapies, Department of Family Medicine, Medical University of Gdańsk, Gdańsk, Poland

Cancer is characterized by genome instability and high diversity even in patients with the particular type of cancer. Therefore, there is need for novel and individualized anti- cancer strategies. Our team have been working on immune therapy of non-small cell lung cancer (NSCLC). NSCLC accounts for 85% of all lung cancer cases and is the leading cause of death among both men and women with malignancies. NSCLC is also characterized by high tumour mutational burden, being a perfect target for immunotherapy.

Our anti-cancer strategy takes under consideration diversity of cancer cells within the tumour and their interactions with immune cells. We also verify the impact of in vitro and in vivo models on repertoire of peptides presented by MHC class I molecules of cancer (immunopeptidome). Finally, we identify cancer neoantigens in the context of NSCLC therapy and diagnostic.

In the current study freshly retrieved cancer and healthy lung samples have been processed and analysed with mass spectrometry for identification and quantification of peptides presented by MHC class I molecules. Then, 2D and 3D cell cultures have been established. Simultaneously, the same cancer samples have been implanted into NSG mice to establish patient derived xenograft models (PDX). After tumour growth the immunopeptidome of NSCLC from in vivo and in vitro models has been analysed. In parallel peripheral blood mononuclear cells (PBMC) and tumour infiltrating lymphocytes (TILs) have been phenotyped, expanded and tested in functional assays against autologous cancer cells.

We have observed that cancer cells derived from the same patient but expanded in different model differed significantly in terms of repertoire of MHC class I peptides. Nevertheless, we have identified several cancer unique peptides which were shared by the primary tumours and the models. These peptides seem to be promising candidates for development of antigen specific T cells which will serve as a tool against lung cancer. We have also identified 3 major types of cancer cells present within NSCLC tumours, including population with stem cell like features. The latest was not recognized by T cells due to lack of expression of HLA-A and -B molecules.

Our observations clearly demonstrate that cancer model chosen for preclinical studies affects repertoire of peptides presented by MHC class I molecules of the tumour. In vitro and in vivo testing of anti-cancer therapies is a standard pathway towards the clinical trial. Nevertheless, without understanding how particular model affects the cancer biology and immunogenicity, we have low chances to defeat this disease. Our observations also suggest that one type of immune

cells cannot be an efficient weapon against all subsets of cancer cells. However, purposeful selection of target neoantigens is probably the future of immunotherapy.

Acknowledgements. This work was supported by the following projects:

1. “International Centre for Cancer Vaccine Science” carried out within the International Research Agendas Program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund
2. “Science for Welfare, Innovations and Forceful Therapies (SWIFT)”, project no. FENG.02.01-IP.05-0031/23; funded by European Funds for Smart Economy 2021-2027 (FENG) Priority FENG.02 Innovation-friendly environment, Measure FENG.02.01 International Research Agendas
3. “Enhancing Cancer Vaccine Science for New Therapy Pathways (CANVAS)” funded by the European Commission, within Horizon Europe Twinning program; HORIZON-WIDERA-2021-ACCESS-03, grant agreement no. 101079510
4. “Diagnostics of NSCLC- algorithm (DiaNA), project no. 2024/ABM/03/KPO; funded funded Medical Research Agency, National Recovery and Resilience Plan, Component D Efficiency, Accessibility and Quality of the Health System, Investment D3.1.1

Optimizing CAR Architecture for Improved T Cell Performance

Speaker: Tina Fink[1]

Authors: Tina Fink[1], Ema Pleško[1], Duško Lainšček[1, 2], Roman Jerala[1, 2]

1. Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia
2. Centre for the Technologies of Gene and Cell Therapy, Ljubljana, Slovenia

Chimeric antigen receptor (CAR) T cell therapy has changed the treatment landscape for haematological malignancies. Nevertheless, further improvements in CAR design are required to achieve greater efficacy, persistence and applicability in solid tumours. Here we describe a novel CAR configuration that incorporates a signaling domain derived from a native component of the T-cell receptor (TCR) complex. This domain functions as an additional or alternative costimulatory module, introduced into the cytoplasmic region of CAR constructs targeting CD19, BCMA or HER2.

Positioned proximal to the transmembrane domain, the novel signaling element was evaluated in combination with or as a replacement for canonical costimulatory domains such as 4-1BB or CD28 alongside the CD3ζ activation domain. CAR T cells engineered with this modification showed significantly improved activation, proliferation, cytokine secretion and cytotoxic function in vitro. In vivo, these modified CAR T cells demonstrated superior tumor control in xenograft models compared to clinically validated CARs containing 4-1BB.

Strikingly, the functional improvement conferred by the TCR-derived domain was consistent across multiple antigen targets and donor backgrounds, indicating the versatility of the platform. This study highlights a previously untapped signaling element as a promising strategy to enhance the efficacy of CAR-T cells and expand the therapeutic potential in various cancer indications.

From One Cell to a Tumor: The Story of CD45, Cells in NSCLCS

Speaker: Ines Papak[1]

Authors: Ines Papak[1], Natalia-Marek Trzonkowska[1,2], Anna Biernacka[1], Artur Pirog[1], Jakub Faktor[1], Martyna Siewiera[1], Alicja Snarkowska[1], Katarzyna Dziubek[1], Monikaben Padariya[1]

1. International Centre for Cancer Vaccine Science, University of Gdansk, Gdansk, Poland
2. Laboratory of Immunoregulation and Cellular Therapies, Department of Family Medicine, Medical University of Gdańsk, Gdansk, Poland

Lung cancer remains the leading cause of cancer-related death worldwide. In non-small cell lung cancer (NSCLC), early tumor spread contributes to a five-year survival rate of less than 15%. The tumor microenvironment (TME) in NSCLC exhibits a high degree of cellular heterogeneity, which continues to hide the molecular mechanisms underlying tumorigenesis and metastatic progression.

In this study, we isolated and characterized a population of undifferentiated, stem-like NSCLC cells marked by the expression of CD45, a protein typically associated with immune lineages. In vivo xenograft experiments demonstrated that only these undifferentiated cells were capable of forming tumors. Interestingly, tumor formation was accompanied by the presence of epithelial and mesenchymal markers while CD45 expression was lost, indicating a high degree of phenotypic plasticity. Mass spectrometry and flow cytometry further revealed altered expression of HLA class I molecules in this cell population.

Understanding the unique characteristics of these cells and their role in tumor initiation, metastasis, and immune evasion may offer new therapeutic opportunities in NSCLC. Additionally, these findings raise important questions about early detection, use of CD45 as exclusion marker and mechanisms of disease spread in lung cancer.

References:

(1) Ishizawa K. et al., CD45,CD326, Cells are Predictive of Poor Prognosis in Non–Small Cell Lung Cancer Patients, Translational Cancer Mechanisms and Therapy, November 15, 2019.
(2) Sun Z. et al., Circulating CD45,EpCAM, Cells as a Diagnostic Marker for Early-Stage Primary Lung Cancer, Frontiers in Medical Technology, Volume 4, 2022.

Dual-CAR NK Cells Targeting PD-L1 and Erbb2/HER2 Eliminate Solid Tumor Cells and Prevent Immune Escape Due to Antigen Loss

Speaker: Jiri Eitler[1,2,3]

Authors: Jiri Eitler[1,2,3], Idan Ben-Horin[4], Kristin Freudenberg-Jahn[1,2], Paola Ortiz-Montero[1,2], Wiebke Rackwitz[1,2], Liliana R. Loureiro[5], Nivedha Murali Shankar[1,2], Corinna Opitz[1], Lydia Krutz[1,2], Thomas Gutbrod[1,2], Pranav Oberoi[4], Aline Häcker[4], Dirk Jäger[10], Stephan R. Künzel[1,2], Kristina Hölig[1,2], Anja Feldmann[5], Michael Bachmann[5,6,7], Winfried S. Wels[4,8,9], Torsten Tonn[1,2,3]

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3. German Cancer Consortium (DKTK), Partner Site Dresden, Dresden, Germany
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6. National Centre for Tumor Diseases (NCT), University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany
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9. German Cancer Consortium (DKTK), partner site Frankfurt/Mainz, Frankfurt am Main, Germany
10. Department of Medical Oncology, National Centre for Tumor Diseases Heidelberg, University Hospital Heidelberg, Im Neuenheimer Feld 460, 69120 Heidelberg, Germany

Natural killer (NK) cells engineered with chimeric antigen receptors (CARs) have shown promising efficacy against B-cell malignancies. In solid tumors, target antigen expression is more heterogeneous, which can lead to treatment resistance and immune escape (1, 2). Such cancers are often also characterized by an immunosuppressive tumor microenvironment and expression of the immune checkpoint molecule PD-L1.

To overcome these hurdles, we generated CAR-NK cells carrying dual CARs which simultaneously target PD-L1 and the tumor-associated antigen ErbB2 (HER2). NK-92 cells were transduced with lentiviral PD-L1.CAR and ErbB2.CAR vectors, and antitumor activity of the resulting dual CAR-NK cells against cancer cells of various solid tumor origins was evaluated in vitro and in vivo.PD-L1/ErbB2.CAR NK-92 cells exhibited high and specific cytotoxicity against breast, ovarian, pancreatic, gastric and lung cancer cell lines expressing PD-L1, ErbB2, or both targets. Similar results were obtained in 3D spheroid tumor models and with primary cancer cells. At the molecular level, double-positive targets stimulated downstream CAR signalling pathways in an additive manner. When PD-L1 or ErbB2 were blocked with antibodies mimicking antigen loss, PD-L1/ErbB2.CAR NK-92 cells, but not NK cells carrying a single CAR, retained high antitumor activity. Likewise, PD-L1/ErbB2.CAR-NK cells outperformed single-target CAR variants in a breast cancer xenograft model.

Our data demonstrate that dual targeting of PD-L1 and ErbB2 enhances the efficacy of CAR-NK cells against otherwise difficult-to-treat tumors, and counteracts immune escape due to antigen

loss. These results may aid clinical translation of this strategy as an effective treatment approach for solid tumors.

References:

(1) Eitler J, et. al, CAR-mediated targeting of NK cells overcomes tumor immune escape caused by ICAM-1 downregulation. Journal for ImmunoTherapy of Cancer, 2024.

(2) Eitler J, et. al, Inability of granule polarization by NK cells defines tumor resistance and can be overcome by CAR or ADCC mediated targeting. J Immunotherapy Cancer, 2021.

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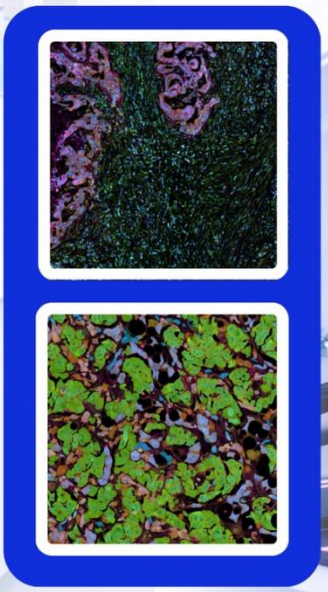

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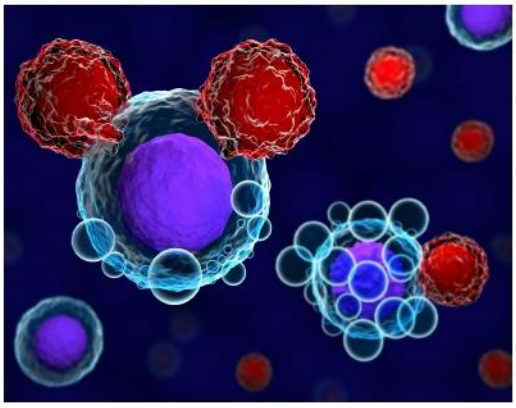

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Automated GMP-Compatible Production of Universal CAR Tregs for Precision Immunomodulation

Speakers: Kavitha Lakshmi[1,2], Anke Fuchs[1,2,11]

Authors: Kavitha Lakshmi[1,2], Alexandra von Jutrzenka-Trzebiatowski[3], Liliana Loureiro[3], Karla Elizabeth González Soto[3], Katja Peter[3], José Manuel Marín Morales[1,2], Samikshya Santosh Nirmala[1], Nicole Berndt[3], Claudia Arndt[2,3], Yueyuan Hu[1,4,] Jing-Wun Li[1], Claudia Peitzsch[1], Anna Taubenberger[2,5,] Rebekka Wehner[6], Hinrich Abken[7], Kristina Hölig[8,9], Ezio Bonifacio[1], Martin Bornhäuser[10], Michael Bachmann[3,6,11], Anja Feldmann[3,6,11], Anke Fuchs[1,2,11]

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3. Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Dresden, Germany
4. DKMS Stem Cell Bank, Deutsche Knochenmarkspenderdatei (DKMS), Dresden, Germany
5. Biotechnology Center, Center for Molecular and Cellular Bioengineering (CMCB), TUD Dresden University of Technology, Dresden, Germany
6. National Center for Tumor Diseases (NCT), NCT/UCC Dresden, a Partnership Between DKFZ, Faculty of Medicine and University Hospital Carl Gustav Carus, TUD Dresden University of Technology and Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Germany
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Background: Regulatory T cell (Treg) therapy has shown safety and early signs of efficacy in promoting immune tolerance in diseases like graft-versus-host disease (GvHD). CAR-engineered Tregs improve targeted activation and suppression compared to standard polyclonal Tregs, but their use is limited by high production costs and single antigen specificity. To overcome this, we used the RevCAR system—a universal CAR platform that relies on an inert peptide and separate adapter molecules (RevTM) for flexible targeting. As a proof of concept, we focused on carcinoembryonic antigen (CEA), found in the gastrointestinal (GI) tract, to treat GI acute GvHD. Importantly, the universal nature of this system makes it suitable for many other immune-mediated diseases.

Methods: To support future clinical use, we developed a GMP-compliant process using automated systems. Tregs were enriched from leukapheresis using CliniMACS Plus and sorted for purity with MACSQuant Tyto. Cells were then modified and expanded using the CliniMACS Prodigy to produce clinically relevant Treg numbers. Final products were tested for purity, stability, target specificity and ability to suppress immune responses.

Results: In five manufacturing runs, Tregs with an average starting purity of 94% (range: 90.5%–97.6%) expanded to an average of 602 million cells (range: 52.6–1,522.5 million), enough for a dose of 8.6 million cells/kg for a 70 kg patient. Final products stayed highly pure (average 91.9%)

and showed efficient gene transfer. Expanded Tregs mostly had a central memory phenotype with expression of key functional markers. Even under inflammatory conditions, they maintained FOXP3 and Helios, produced few inflammatory cytokines. Importantly, they were specifically activated by the target antigen and demonstrated dose-dependent suppression.

Conclusion: We developed a scalable, GMP-ready process to produce stable, functional RevCAR Tregs—supporting future off-the-shelf therapies for a range of immune-mediated diseases.

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The Future of Viral Vectors: A Survey among Centers Associated with CTIWP of EBMT, NXTGEN Hightech, DARE-NL, T2EVOLVE, and GoCART Coalition

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Advanced therapy medicinal products (ATMPs) hold great potential for treating complex diseases, and viral vectors are mainly used for producing them [1]. Several viral vector–based ATMPs have been approved globally, particularly for cancer [2, 3]. Among these, chimeric antigen receptor (CAR) T-cell therapies represent the most established application, with all approved products

engineered using lentiviral or γ-retroviral vectors [4, 5]. However, large-scale production of GMP-grade viral vectors remains a significant bottleneck. Most academic institutions and hospitals rely on contract development and manufacturing organizations for vector supply, which delays research and clinical translation.

To assess current practices and challenges in viral vector production for ATMPs, we conducted a survey among facilities affiliated with the EBMT, NXTGEN Hightech, DARE-NL, T2Evolve, GoCART Coalition, and commercial ATMP developers. The survey was distributed to academic, non-profit, and commercial ATMP facilities. It collected data on viral vector usage, production technologies, and manufacturing practices. Respondents were also asked to identify technological needs to guide future innovation.

A total of 92 valid responses were received, representing centers across 33 countries. Development stages of viral vector–based gene therapies ranged from laboratory research to pre-GMP, clinical trials, and commercial production. Thirteen facilities reported in-house viral vector manufacturing, mainly for ex vivo cell modification (n=10), with lentiviral vectors (n=9) and HEK293 derivatives (n=11) being most commonly used.

We will report during the meeting on reported key advantages and limitations of upstream production technologies, along with areas requiring improvement. We also report on mapping results of downstream processing methods and current or desired online/offline process parameters.

The survey highlights the growing role of academic and hospital-based facilities in viral vector production, particularly for autologous therapies. It demonstrates broad geographic engagement in cell and gene therapy development and reinforces the central role of viral vectors in advancing ATMPs across both academic and clinical settings.

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Stem Cell-Based Therapy and Bioactive Hybrid Nanocarriers in Osteoarthritis Treatment: Distinct Pathways toward Tissue Repair

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During the last two decades, regenerative medicine has witnessed the high potential of stem cells in the treatment of various diseases. Extensive research has delineated some remarkable features of stem cells derived from specific organs and niches, leading to conclusions about the mechanisms through which they perform their functions. The discovery of specific molecules through which stem cells exert their effects has enabled the use not of stem cells per se, but of their products, to reduce inflammation and pain, activate the body's intrinsic mechanisms for healing, enabling the reduction of tissue damage and leading to recovery.

To entrap stem cell-derived products and make them more accessible and usable by target tissues, both natural and artificial nanocarriers have been considered. Small extracellular vesicles are natural membranous vesicles released by cells that play a crucial role in cell-to-cell communication. Among artificial nanocarriers, liposomes are the most widely explored for targeted drug delivery systems. Their composition and size can be tailored to specific needs and cell types.

Here, the results of a seven-year longitudinal study of knee osteoarthritis patients treated with mesenchymal stem cells will be presented. Furthermore, the establishment of an in vitro model of osteoarthritis and its potential use in exploring the effects of molecules captured in natural or artificial nanocarriers will be discussed. Finally, the formation of hybridosomes - nano-sized lipid vesicles associated with bioactive phytochemicals from spruce needle homogenate, and their potential in delivering these anti-inflammatory molecules to target cells will be discussed.

Bridging Research and Care: CREATIC CoE's Academic Development of Cell-Based ATMPs for Paediatric and Rare Conditions

Speaker: Regina Demlová[1,2]

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CREATIC CoE (Central European Advanced Therapy and Immunotherapy Centre) is a Horizon Europe Teaming for Excellence project, granted in December 2022. Masaryk University has established a strategic partnership with the Fraunhofer Institute for Cell Therapy and Immunology IZI, Leipzig University, and Copenhagen University, and in January 2024 launched the R&D CREATIC Centre of Excellence focused on Advanced Therapy Medicinal Products (ATMPs), with a particular emphasis on paediatric and rare diseases. In collaboration with the European Clinical Research Infrastructure Network (ECRIN-ERIC), CREATIC creates a unique and structured environment that supports the full continuum of translational research, from early development to clinical implementation. This integrative model strengthens CREATIC's position on the European scientific landscape as a hub for innovation, regulatory expertise, and patient-centred clinical research.

CREATIC's academic pipeline currently includes two proprietary cell-based products under clinical phase of development: FlyCellix, Mesenchymal Stem Cell medicinal product targeting Epidermolysis Bullosa patients, and MyDendritte, designed to harness the immunomodulatory potential of dendritic cells in refractory paediatric cancers. Both products exemplify CREATIC's mission to address unmet medical needs through non-commercial, academically driven research, while ensuring rigorous scientific and ethical standards. The Centre leverages its close ties with academic hospitals, regulatory authorities, and patient communities to foster agile, yet compliant pathways for early-phase clinical trials.

By actively engaging in European partnerships and contributing to cross-border infrastructures, CREATIC bridges the gap between basic research and clinical care. Its work not only accelerates the availability of innovative therapies for vulnerable patient populations but also contributes to the development of a sustainable model for academic ATMP development. This model aims to empower national health systems and academic institutions to play a more central role in the future landscape of advanced therapies across Europe.

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From Bench to Bedside in Slovenia: Phase I Trial of phIL12 Intratumoral Gene Electrotransfer in Basal Cell Carcinoma

Speaker: Tanja Jesenko[1]

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The phase I clinical trial investigated intratumoral gene electrotransfer (GET) of the phIL12 plasmid, an antibiotic resistance-free gene therapy designed to stimulate local IL-12 expression and avoid systemic toxicity. Conducted in patients with basal cell carcinoma, the trial demonstrated excellent safety, with no serious adverse events and only mild, transient effects such as edema and pain. Quality of life remained stable throughout treatment. The therapy successfully induced local IL-12 and IFN-γ production and triggered strong immune activation, including increased infiltration of immune cells. By activating the immune system directly in the tumor microenvironment, phIL12 GET can overcome key limitations of systemic cytokine therapies. Overall, the trial lays the foundation for future phase II studies, anticipated as combination of phIL12 GET with local ablative approaches such as radiotherapy or electrochemotherapy.

This clinical trial represents a key contingency of the SmartGene.si project, highlighting the culmination of efforts by a multidisciplinary consortium dedicated to advancing gene therapy for cancer treatment in Slovenia. The SmartGene.si consortium brought together leading institutions and organizations, including the Institute of Oncology Ljubljana, COBIK, Iskra PIO, Jafral, University of Ljubljana and University Medical Centre Ljubljana. Through this collaboration, the project focused on developing, producing and evaluating phIL12 plasmid. By combining preclinical research, biotechnological process development, clinical expertise, and industrial production capabilities, the consortium not only initiated Slovenia's first gene therapy clinical trial but also laid the groundwork for a sustainable platform to develop and transition innovative therapies into clinical practice.

Shaping the Future of Rare Disease Care: Connecting Patients and Innovation

Speaker: Damjan Osredkar[1,2]

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Gene replacement therapies have successfully advanced into clinical practice and have demonstrated considerable promise as safe and effective disease-modifying treatment strategies for selected patient populations. While the number of currently approved gene therapies remains limited, ongoing advancements in molecular biology and clinical research strongly indicate that this field is poised for substantial growth in the near future. Despite these advancements, several critical challenges remain, including concerns about long-term safety, variable therapeutic efficacy, durability of treatment effects, high financial costs, and complex ethical considerations. Furthermore, the treatment of rare genetic disorders—often with limited preclinical data—poses additional hurdles for clinical translation and regulatory approval. A clear and open conversation between patients, doctors, researchers, and the public is important to help ensure these new treatments are accepted, accessible, and used ethically.

Academic CAR T-Cell Manufacturing: Implications for Clinical Implementation and Access

Speaker: Matjaž Sever[1,2]

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Chimeric antigen receptor (CAR) T-cell therapy revolutionized treatment of hematologic malignancies. Its widespread adoption is constrained by centralized manufacturing models that are costly, can be slow, and inaccessible to many patients. Academic, point-of-care (POC), and distributed CAR T-cell production approaches offer promising alternatives to address these limitations while maintaining safety and efficacy.

Academic institutions had a critical role in early CAR T-cell innovation, especially for rare and pediatric cancers. However, broader clinical translation is challenged by limited commercial interest, regulatory difficulties, and fragmented infrastructure. POC manufacturing—meaning that CAR T cells are produced within or near the treating hospital—can reduce vein-to-vein time, improve cell viability and efficacy, and lower production costs. Automated, closed-system platforms such as CliniMACS Prodigy and Lonza Cocoon demonstrated feasibility, achieving turnaround times of 7–14 days and manufacturing success rates above 85%. Despite these advantages, POC implementation requires significant investment in facilities, trained personnel, and quality control systems. Regulatory frameworks can be an obstacle, as current licensing models are not optimized for decentralized production.

Distributed manufacturing extends POC principles across networks of regional or academic centers, enabling scalable production and preserving local access. Success depends on harmonized protocols, centralized vector sourcing, and good comparability data. Regulatory agencies are interested in pathways to support decentralized models, but concerns about oversight, liability, and product consistency remain.

Cost is a major barrier to CAR T-cell therapy. Commercial products can cost $400,000 per treatment, excluding hospitalization. In contrast, academic and POC models demonstrated production costs as low as $35,000–$50,000, depending on vector availability and infrastructure used. Other funding strategies, including mixed charity and commercial investment, may support development for low-return indications.

Safety and efficacy of academic and POC CAR T-cell therapies are comparable to commercial products in early-phase trials. Toxicity profiles and response rates are similar. However, broad validation and long-term data are needed. Accessibility remains important concern, with geographic and socioeconomic differences leading to different patient access. Distributed models can help overcome these obstacles but require coordinated support systems and adequate policy.

Academic, point-of-care, and distributed CAR T-cell manufacturing models offer comparable and cost-effective alternatives to centralized production. These approaches have the potential to expand access, accelerate innovation, and improve equal access — especially for underserved populations — if supported by regulators, proper investment, and collaboration in clinical research.

Repurposing Base Editors for Targeted Knock-in and Simultaneous Knockouts to Generate Multiplex-edited Allogeneic CAR T Cells with Minimal Translocations

Speaker: Viktor Glaser [1,2,3]

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Novel genetic engineering technologies can enhance the efficacy and accessibility of cellular therapies for cancer and autoimmune disorders. Unlike semi-randomly integrating vectors such as lentiviruses or transposon systems, CRISPR-Cas enables precise genome editing and eliminates the need for viral vector production. CRISPR-Cas has been used to insert chimeric antigen receptors (CARs) into genes of the TCR/CD3 complex, enhancing T cell functionality through physiological CAR expression1–3. Additional gene knockouts can improve persistence, potency, and support allogeneic applications. However, conventional multiplex editing induces DNA double-strand breaks (DSBs), which raises safety concerns including off-target effects and chromosomal rearrangements4.

To address these limitations, we developed a base editor-mediated knock-in (BEKI) platform that repurposes the Cas9 nickase domain of adenine or cytosine base editors to mediate homology-directed repair (HDR) via two spaced nicks on opposed DNA strands. By optimizing guide RNA design - including PAM orientation, nick distance, and base editing targets - we achieved efficient CAR integration at the TRAC, CD3ζ, and CD3ε loci. Insertion efficiency was further enhanced using HDR-promoting small molecules and Cas-target sequences (CTS) on donor templates5. The BEKI system facilitates simultaneous base editing, enabling multiplex gene disruption alongside CAR knock-in. Triple-edited CAR T cells (CAR-knock-in with RASA2 and FKBP12 knockouts) showed >20% CAR+ cells and >90% base editing efficiency. Genotoxicity profiling by CAST-seq and digital droplet PCR revealed markedly reduced chromosomal translocations compared to conventional Cas9-based editing. To enable allogeneic use, we further silenced the B2M and CIITA genes to abrogate HLA class I and II expression, efficiently generating quintuple-edited CAR T cells. Preliminary results using a humanized mouse model indicate that these HLA-edited cells exhibited improved engraftment.

In summary, BEKI enhances the safety of multiplexed engineered cell therapies by minimizing DSB-associated genotoxicity. The streamlined workflow presents a major advantage for scalable, clinically compliant manufacturing of allogeneic CAR T cells.

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Hospital-Embedded Innovation: U.S. Experience in Advancing Translational Medicine Across System

Speakers: Polona Šafarič Tepeš[1], Seth Plancer[2,3]

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Cell and gene therapies are reshaping modern medicine, yet their translation from discovery to patient care remains one of the most formidable challenges. In the United States, Northwell Health has developed a hospital-embedded model through the Feinstein Institutes for Medical Research, where laboratory innovation, clinical demand, and commercialization are tightly integrated. Supported by institutional and extramural funding, and powered by MD–PhD partnerships, this approach aligns research with patient priorities while navigating IRB oversight, HIPAA, and FDA compliance.

Northwell's ROSE Program for endometriosis exemplifies this pathway. Over more than a decade, with sustained support from Northwell Venture Partners, studies of menstrual effluent–derived stromal cells evolved into a validated, non-invasive diagnostic. This long arc, iterative validation, venture investment, and hospital infrastructure, illustrates how the U.S. has advanced high-risk, high-reward projects with sustained institutional commitment. The same blueprint is applied to cell and gene therapies, where regulatory rigor and clinical proximity are essential.

The U.S. ecosystem is further strengthened by accelerators such as NIH RadX, ARPA-H, NSF I-Corps, and VentureWell, which provide regulatory coaching, commercialization scaffolds, and weekly PI strategy sessions to help researchers bridge discovery and translation. Another distinguishing feature is the role of private practices. Fertility, oncology, and pain clinics accelerate translation by enrolling patients rapidly, initiating feasibility trials with fewer barriers, and generating real-world evidence. Their agility complements the regulatory strength of large hospitals, creating a hybrid model that blends speed with compliance.

Slovenia's healthcare environment is structured differently, with fewer private practices and more centralized coordination. Yet this offers distinct advantages, particularly as CTGCT builds GMP-grade infrastructure and national networks. Adapting analogs of U.S. accelerators while leveraging CTGCT's centralized strengths could create a balanced model where hospitals provide regulatory rigor and private partners supply agility. Having worked within a hospital-embedded system at Northwell and private clinics, I see opportunities to adapt strategies that unite hospital governance with private sector agility, respecting local structures, while accelerating the safe delivery of advanced therapies.

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Abstracts of Posters

CTGCT Conference – Cell Therapy

Method Matters: Evaluating Isolation Techniques for Accurate Characterization of Tissue-Derived EVs in NSCLC

Presenter: Anna Biernacka[1]

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Recent breakthroughs in oncology have come with the development of cell therapies and immunotherapies, which utilize patients' autologous immune cells to fight cancer. However, tumour cells can evade immune surveillance by creating an immunosuppressive tumour microenvironment. A key mechanism used by tumour to establish its tolerance and favourable microenvironment is the secretion of small extracellular vesicles (sEVs), including exosomes. Tumour-derived EVs (TEVs) have been shown to profoundly affect immune cell function. Therefore, in-depth characterization of TEVs is critical not only for understanding tumour progression, but also for identifying strategies to counteract cancer-induced immunosuppression.

Most studies on TEVs have focused on sEVs isolated from conditioned media of cancer cell lines. However, these may significantly differ from sEVs present in the native tumour microenvironment. While direct isolation of sEVs from tumour tissue offers a more biologically relevant approach, such material is often heavily contaminated with serum components, apoptotic bodies, and intracellular vesicles. Thus, selecting an appropriate method that ensures high purity is essential for reliable downstream analyses.

In this study, we compared six EV isolation methods from non-small cell lung cancer (NSCLC) tissue: ultracentrifugation, iodixanol density cushion, and four commercial kits based on vesicle precipitation, membrane affinity, immunoaffinity, and phosphatidylserine enrichment. Using fluorescence nanoparticle tracking analysis and proteomic profiling, we assessed the yield and purity of isolated EVs. While the iodixanol density cushion method resulted in the highest purity, ultracentrifugation provided the second-best purity with significantly better yield. Overall, ultracentrifugation offers the most effective balance between sample purity and isolation efficiency for tissue-derived EVs, making it a practical choice for TEV studies in solid tumours such as NSCLC.

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IL-10: A Key Regulator of Endolysosomal Biogenesis and Proteolytic Activity in Myeloid Cells

Presenter: Tomislav Kostevc[1,2]

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IL-10 is a pleiotropic cytokine with highly context-dependent effects – suppressing hyperinflammation while potentially weakening protective immunity. Despite intensive development of IL-10-based cell therapies recently, its mode of action remains poorly understood. To this end, we set out to investigate how IL-10 shapes intracellular processes in myeloid cells, a key target population of this cytokine.

Human monocytes cultured with GM-CSF and IL-4 in the presence of IL-10 differentiated into $CD1c^{-}CD14^{+}CD141^{+}CD163^{+}$ cells with remarkably high HLA-DR expression, reaching levels typically seen in control cells only following TLR stimulation. To identify the processes underlying this phenotype, we performed exploratory RNA-seq and whole-cell proteomics. Both approaches revealed IL-10-driven upregulation of endolysosomal machinery, including LAMP1 and proteolytic enzymes such as cathepsins and legumain. Consistent with these findings, transmission electron microscopy showed a striking, time-dependent accumulation of endosome-like vesicles in IL-10-treated cells, which was 20-fold higher than in control cells and correlated with increased LAMP1 expression. Western blot analysis confirmed that IL-10 boosted both cathepsin expression and maturation. In line with this, using a dye-quenched BSA assay, we observed enhanced global proteolytic activity, which was further validated by the activity-based probe BMV109, specifically demonstrating elevated activity of cysteine cathepsins. Finally, antigen-presentation assays showed that IL-10-conditioned cells provoked markedly weaker recall responses from autologous memory T cells; immunopeptidomics corroborated this by revealing a broader, less focused peptide repertoire that may dilute immunodominant epitopes.

Our findings identify a novel role for IL-10 in driving endolysosomal reprogramming of myeloid cells. By enhancing vesicle accumulation, boosting protease activity, and inducing high HLA-DR expression, IL-10 modulates antigen presentation and T cell priming. These mechanistic insights inform both the therapeutic promise and the safety considerations of IL-10-based cellular interventions.

Intravenous Lipid Emulsions Affect Jurkat Cells: A Deformability Cytometry Study

Presenter: Lija Fajdiga[1]

Authors: Lija Fajdiga[1], Špela Zemljič[1], Jure Derganc[1]

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Intravenous lipid emulsions (ILEs) are an essential component of parenteral nutrition and are widely administered to critically ill patients. In addition to their nutritional function, the fatty acids in ILEs have immunomodulatory properties that can influence the activity and viability of lymphocytes and other blood cells [1,2]. This is particularly significant in the context of cell-based immunotherapies, such as CAR-T cell therapy, where interactions between immune cells and circulating lipids may influence treatment efficacy. Despite their routine clinical use, the effects of ILEs on immune cells at a cellular level are not fully understood.

In this study, we investigated how two commonly used ILEs, SMOFlipid (a mixed oil emulsion) and Omegaven (a fish oil emulsion), affect Jurkat cells, a widely used model of human T lymphocytes. We used deformability cytometry (DC) as the primary analytical tool. While DC is traditionally used to assess cell mechanical properties [3], we extended its application by introducing two novel, label-free analytical approaches: deep neural network-based image classification to evaluate cell viability and stress responses; and morphological analysis to quantify intracellular lipid droplet (LD) accumulation.

Our results show that Omegaven significantly reduces cell viability compared to SMOFlipid, while only minimally affecting cell stiffness. This was confirmed by MTS assays and machine learning–based image analysis. DC imaging revealed prominent LD accumulation in treated cells, while Seahorse metabolic profiling showed no significant changes in energy metabolism. Taken together, these results suggest that excess fatty acids were stored rather than utilized.

These findings demonstrate the usefulness of DC for the label-free, multiparametric analysis of immune cell state and morphology. They also highlight the importance of considering ILE-induced alterations when developing and administering T cell-based immunotherapies.

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Proteomic Interrogation of the T Cell Subset Activation Dynamics Reveals Subset Specific Membrane Signatures and Pathway Landscapes

Presenter: Jakub Faktor[1]

Authors: Jakub Faktor[1], Ines Papak[1], Anna Biernacka[1], Sachin Kote[1], Natalia Marek-Trzonkowska[1]

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T cell activation involves tightly coordinated events, frequently initiated at cell membrane propagated through downstream signaling (1). Here, we apply fractionated SWATH-MS proteomics to elucidate temporal remodeling of membrane-associated biomarkers during T cell activation, complemented by pathway analysis of cytosolic and membrane fractions, revealing divergent dynamics across CD4+ and CD8+ naïve and memory subsets. This strategy offers mechanistic insight potentially supporting future efforts to tune T cell function. Additionally, tracking alterations in T cell activation pattern represents diagnostic and prognostic potential in oncology.

Comprehensive, time resolved proteomic profiling of membrane and cytosolic fractions reveals distinct activation dynamics between T cell subsets. We quantified 2782 proteins in cytosolic fraction and 3109 proteins in membrane fraction (qvalue < 0.01) with each fraction showing an expected compartment-specific protein enrichment. Statistical analysis identified known and novel membrane T cell activation markers such as e.g. VASP (Vasodilator-stimulated phosphoprotein), SGMR1 (Sigma non-opioid intracellular receptor 1), ITA6 (Integrin alpha-6) distinguishing activated T cell subsets at different stages based on MS intensities. Pathway-level insights were obtained through temporal, cell type specific Gene Ontology (GO) enrichment analysis via Gene Set Enrichment Analysis (GSEA) revealing 6 functional clusters: immune modulation, cell cycle dynamics, cellular communication, migration, adhesion, metabolism and intracellular structural organization. Notably, activated CD8+ memory T cells exhibited strong activation of glucocorticoid response and steroid hormone pathways which have not been previously reported. We also revealed a temporal, antagonistic interplay between "cortical cytoskeleton organization" and "intermediate filament organization" in CD8+ memory cells, potentially representing a dynamic immune synapse modulation.

By integrating activated T cell membrane marker with pathway signaling analysis our approach may contribute to the rational design of subset and stage specific T cell manipulations. Moreover, monitoring of fine alterations in T cell activation pattern has robust diagnostic potential and can be used in screening diagnostics in oncology.

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Development of a Platform for Studying NSCLC Immunopeptidome in Tumors and Patient-Derived Models – Optimization for CANVAS Project

Presenter: Martyna Siewiera[1]

Authors: Martyna Siewiera[1], Elżbieta Chruściel[1], Zuzanna Urban-Wójciuk[1], Anna Biernacka[1], Ines Papak[1], Katarzyna Dziubek[1], Łukasz Arcimowicz[1,2], Artur Piróg[1], Jakub Faktor[1], Sachin Kote[1], Natalia Marek-Trzonkowska[1,3]

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The term 'immunopeptidome' refers to a set of peptides that are presented on a surface of major histocompatibility complex (MHC) proteins, which in humans are called human-leukocyte antigens (HLA). These peptides are recognized as 'self' or 'non-self' by T-cells, in which case an immune reaction is triggered. This mechanism could be exploited to target cancer cells which present aberrant peptides, different from body's immunopeptidome (1). So far no effort has been made to study the antigens presented via MHC complexes (here termed pHLA-I) in different cell models and their parental tumor, although it seems very important for testing immunotherapies and precision medicine.

The aim of the CANVAS project is to select a non-small cell lung cancer (NSCLC) model that best mimics the parental tumor in the context of immunopeptidome repertoire with a special focus on neoantigens – aberrant peptides absent on healthy cells.

The tested models are: traditional 2D culture (monolayer), scaffold-free 3D culture and patient-derived xenografts of NSG mice (PDX). Before the main experiment it was important to take into account the limitations of immunopeptidomic studies: it requires a lot of material to identify potential neoantigens through tandem mass spectrometry.

The poster presents optimization process concerning an optimal protocol development for establishment of patient-derived primary cell cultures, medium composition and a proof of concept experiment results, suggesting PDX models are seemingly different from their parental tumors, especially in the context of pHLA-I originating from genes like ACTG1, GAPDH, HBB, OGN, SERPINH1 and others.

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Fresh View on Cancer Biomarker Discovery: Plasma/Tissue Peptidomes and NK cell Populations – Project DIaNA

Presenter: Artur Pirog[1]

Authors: Artur Pirog[1], Ines Papak[1], Jakub Faktor[1], Anna Biernacka[1], Zuzanna Urban-Wójciuk[1], Monikaben Padariya[1], Martyna Siewiera[1], Sachin Kote[1], Natalia Marek-Trzonkowska[1,2]

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Using large scale -omic techniques for biomarker discovery was proven to be much harder than anticipated. Extreme dynamic range of protein concentrations in plasma proteome and high variability of plasma peptide content and metabolome makes identification of genuine biomarkers challenging. Here we propose two novel approaches that may enable to identify otherwise missed markers in lung cancer.

Project DIaNA aims on using two orthogonal approaches for identification of biomarkers for lung cancer risk assessment and diagnostics. First approach relies on plasma/serum peptidome quantification combined with identification of potential marker peptides directly from healthy and tumor tissue samples. Here, a combined plasma and tissue peptide sequence library is used to perform an enhanced quantification of plasma peptidome, augmenting it for biomarker discovery. Preliminary results identified 4 peptides that are simultaneously specific for tumor tissue and cancer patient plasma.

Second part of the project aims at analysis of the distribution of NK and T-cells subpopulations in cancer patients and healthy people using multichannel flow cytometry. Current results, obtained after unsupervised machine learning analysis of raw results obtained from NK cells, strongly suggest existence of cancer-specific differences of diagnostic potential.

Our current task is to evaluate already detected peptide markers, identify novel candidates, and confirm differences in cell populations on large cohort (>300 healthy and cancer patient samples). Additionally, selected peptides will be quantified in ~2000 plasma samples using targeted LC/MS approach. Promising markers will be further analyzed in terms of their applicability in real-world diagnostic procedures.

Inspecting the Natural Killer Cell - Glioblastoma Crosstalk in Advanced in Vitro Models

Presenter: Anamarija Habič[1,2]

Authors: Anamarija Habič[1,2], Tina Kolenc Milavec[1,2], Bernarda Majc[1], Pia Žižek[1], Špela Kladnik[1], Metka Novak[1], Barbara Breznik[1]

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Glioblastoma (GB) is the most common and aggressive type of brain cancer in adults with poor patient prognosis. Despite standard treatment (surgical resection, radiotherapy and chemotherapy with temozolomide), the disease almost ultimately recurs. Natural killer (NK) cell-based immunotherapy may fulfil the urgent need for more efficient therapeutic options. NK cells are innate lymphoid cells that can eliminate a spectrum of target cells without prior sensitization. Importantly, it has been demonstrated that glioblastoma stem cells (GSCs) – the drivers of GB recurrence – are susceptible to NK cell-mediated cytotoxicity (1). Nevertheless, the highly immunosuppressive GB tumor microenvironment may hamper the anti-cancer activity of NK cells. A deeper understanding of the GB-NK cell crosstalk is needed to develop effective NK cell-based therapeutic approaches and prevent or revert potential resistance mechanisms. We explored NK cell responses against GB in three distinct in vitro model systems. Firstly, spheroids were prepared from either GSCs or differentiated GB cells and co-cultured with NK-92 cells. Although NK-92 cells more efficiently infiltrated spheroids of differentiated GB cells, their cytotoxicity was higher against GSC spheroids. A number of immunomodulating cytokines have been detected in spheroid and co-culture supernatants and their profiles differed significantly between the two types of spheroids. Secondly, higher NK-92 cell attraction of differentiated GB spheroids (compared to GSC spheroids) was confirmed in a dynamic microfluidic platform mimicking the blood flow and influx of immune cells into the tumors. Thirdly, the ability of NK-92 cells to detect and eliminate GB cells was also investigated in advanced co-cultures of GB spheroids infiltrated into cerebral organoids. Altogether, our research highlights important factors in the GB-NK cell communication and contributes to a clearer picture of the complex crosstalk between GB and NK cells.

Acknowledgements: Funding: ARIS program P1-0245, ARIS projects N3-0394, NC-25002, J3-4504 and EU Horizon project CutCancer (101079113).

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Advancements in Lentiviral Purification: From High Throughput Screening to Scalable Solutions

Presenter: Luka Bevc[1]

Authors: Luka Bevc[1], Ana Pavšič Rijavec[1], Valentina Novak[1], Sandra Potušek[1], Ivana Petrović Koshmak[1], Maja Leskovec[1], Hana Jug[1], Aleš Štrancar[1]

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Lentiviruses are commonly used in cell and gene therapy due to large insert capacity and capability to integrate in both dividing and non-dividing cells. The use of LVs, and number of gene therapies involving them, have steadily increased over the years. As such, the need for better downstream processes, with increased impurity clearance and infective LV recovery, has become evident. With chromatographic step being essential for scalable and cost-effective downstream process, we tested monolithic columns for purification of lentivirus.

Good initial results have been achieved on CIMmultus QA. To further improve the recovery of the downstream process, we determined the factors and conditions, which contribute the most to downstream recovery, by using Design of Experiments (DoE) approach. To increase throughput of the DoE studies, we used CIM QA 0.05 mL Monolithic 96-well Plates (6 μm), which significantly speed up the testing process, as well as decreased variables which occur from day-to-day work. Results indicated starting NaCl concentration in lentiviral load has the most profound effect on lentiviral recovery, with increased NaCl concentration leading to better downstream recovery. Using DoE and high throughput testing with plates, we could improve the infective lentiviral recovery from 20% up to 90%. The process was then successfully transferred to 1mL QA (6 μm) column, using conditions, which can be scaled up to manufacturing columns. On 1mL column, 70% average infectious recovery was achieved, with over 2000-fold decrease in host-cell protein (HCP) content. Final average HCP and hcDNA contents were calculated to be 490 ng of HCP and 230ng of hcDNA per dose of 10^{9}TU. Additionally, particle composition analysis demonstrated removal of incomplete particles during chromatography and enrichment of complete lentiviral particles, from 34% in clarified harvest, to 51%. When tested for capacity it was determined to be 3E+12 vp/mL of QA monolith (6 μm).

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Mass Spectrometry-Based Mapping of the PD-1 Interactome Reveals Cross-Species Therapeutic Targets

Presenter: Katarzyna Dziubek[1]

Authors: Katarzyna Dziubek[1], Jakub Faktor[1], Kiran Bharat Lokhande[2,3], Ashish Shrivastava[2], Ines Papak[1], Elzbieta Chrusciel[1], Magdalena Pilch[1], Theodore Hupp[1,4], Natalia Marek-Trzonkowska[1,5], Ashutosh Singh[2], Maciej Parys[6], Sachin Kote[1]

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The role of PD-1 immune checkpoint receptor in T cell exhaustion is well established, but little is known about non-canonical PD-1 signalling in immune cells. Contradictory reports also exist regarding PD-1 function in cancer cells, where it may either suppress or promote tumour growth depending on the cancer type. Studying the PD-1 interaction network is therefore critical for understanding its role in cancer. It may uncover new therapeutic targets to enhance the efficacy of PD-1 immunotherapy or mitigate PD-1–mediated CAR-T cell exhaustion.

To map the PD-1 interactome, we isolated PD-1–interacting proteins in U2OS human osteosarcoma cells using PD-1 pull-down followed by quantitative LC-MS/MS analysis. The most promising candidate targets were selected for validation of the interaction with PD-1 by western blotting and proximity ligation assay. We confirmed the tyrosine kinase receptor AXL as a novel PD-1–interacting partner, with additional candidates under evaluation. Notably, AXL inhibition has previously shown benefit in NSCLC patients receiving immune checkpoint blockade, yet no mechanistic link between PD-1 and AXL has been reported. Sequence alignment analysis revealed marked homology between PD-1 and AXL across species. We experimentally confirmed the PD-1/AXL interaction in canine osteosarcoma, a spontaneously occurring cancer in dogs. For prospective therapeutic targeting, we characterized the interacting residues of PD-1 and AXL by molecular modelling and molecular dynamics. We observed significant conservation of these residues across species, supporting the biological relevance of the PD-1/AXL interaction.

This work establishes a platform for mapping PD-1 interactions across cell types and species to guide therapeutic target discovery. We shed light on the unexplored PD-1 interactome in cancer cells, with implications for non-canonical PD-1 signalling in immune cells. Ultimately, our findings hold promise for improving the efficacy of immunotherapy and mitigating PD-1–mediated CAR-T cell exhaustion in both humans and animals with naturally occurring cancers.

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Development of Chimeric Antigen Receptor T Cells Incorporating De Novo Designed Binding Domains for Cancer Immunotherapy

Speaker: Tjaša Mlakar[1,2]

Authors: Tjaša Mlakar[1,2], Tadej Satler[1], Arvind Kumar Gupta[1], Helena Gradišar[1], Duško Lainšček[1,3], Roman Jerala[1,3]

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Chimeric antigen receptor (CAR) T cell therapy, which employs autologous T lymphocytes engineered to express a synthetic receptor, has proven highly effective in treating various hematological cancers. These engineered CAR T cells are able to recognize and eliminate target cells via binding on the target antigen on cancer cells and consequently triggering an immune response. Most current therapies include second-generation CARs where the antigen binding occurs via single-chain variable fragment or scFv. However, the scFv domain presents significant challenges, including immunogenicity, toxicity and thus limited persistence of CAR T cells. Furthermore, both low and excessively high antigen-biding affinity can impair therapeutic function and some scFv domains can also cause antigen-independent clustering on T cells, resulting in tonic signaling and faster exhaustion. Expression issues due to poor modification of scFv regions further limit CAR functionality. Our study aims to replace the scFv domain with a de novo designed, small antigen-binding domain created using machine-learning based protein design tools and assess its impact on CAR T cell therapy performance.

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Enhancement of Chimeric Antigen Receptor Activation for Cancer Immunotherapy Through Transmembrane Domain Oligomerization

Presenter: Hana Vokač Križaj[1]

Authors: Hana Vokač Križaj[1], Tina Fink[1], Duško Lainšček[1], Roman Jerala[1]

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Chimeric antigen receptor (CAR-T) cell therapy offers a potent approach for treating malignancies and other disorders that necessitate targeted cell elimination. While this platform has demonstrated significant success in treating B-cell leukemias and lymphomas and is being explored for autoimmune diseases and solid tumors, its efficacy is constrained by factors such as adverse effects, limited tumor penetration, and antigen escape but also lower efficacy in comparison to the natural TCR/CD3 complex.

Our investigation focused on augmenting the performance of second-generation CARs by modifying their transmembrane domains (TMDs) and hinge regions. We specifically examined how the oligomerization state of CARs influences their activation. Our results demonstrate that a higher-order oligomerization of the TMD significantly enhances the function of second-generation CARs, as validated by both in vitro and in vivo studies. We have established that the strategic insertion of a GXXXG motif within specific regions of the TMD can bolster CAR activation. Furthermore, we determined that the interaction between the hinge and the TMD is a critical parameter to consider during the rational design of next-generation CARs.

Our high-throughput screening identified a TMD that forms a trimer-dimer complex as the most effective candidate. This novel construct demonstrated superior performance compared to the standard second-generation CAR in a preclinical animal cancer model, resulting in enhanced tumor-killing activity and improved overall in vivo tumor clearance. This optimization strategy provides a compelling strategy for enhancing CAR-T cell therapeutic efficacy.

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ROUND TABLE I:
Regulatory and Clinical Pathways for Gene and Cell Therapy

16 September 2025, 16:30-17:30

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Moderator: Gregor Cuzak, General Secretary, ZOPS

Zveza organizacij pacientov Slovenije

Participants:

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2. Lenka Součková – ECRIN European Correspondent for Czechia, Masaryk University
3. Jürgen Kuball – Chair of the Department of Haematology, Cancer Centre, UMC Utrecht
4. Tanja Jesenko – Senior Research Fellow, Institute of Oncology Ljubljana
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Interspecies Generation of iPSC-derived Functional Muscle Stem Cells as a potential Duchenne Muscular Dystrophy Treatment

Speaker: Ajda Lenardič[1]

Authors: Ajda Lenardič[1], Seraina A. Domenig[1], Joel Zvick[1], Monika Tarnowska-Sengül[1], Nicola Bundschuh[1], Regula Furrer[2], Falko Noé[1,3], Christine Ling Li Trautmann[1], Adhideb Ghosh[1,3], Giada Bacchin[1], Pjeter Gjonlleshaj[1], Xhem Qabrati[1], Evi Masschelein[4], Katrien De Bock[4], Christoph Handschin[2], Ori Bar-Nur[1]

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Satellite cells, the stem cells of skeletal muscle, possess remarkable regenerative potential and are considered promising candidates for cell-based therapies in muscle diseases such as Duchenne muscular dystrophy (DMD). However, their clinical use is limited by low yields from donor or autologous tissue and rapid loss of stemness during in vitro expansion. To address these limitations, our laboratory explores strategies for production of more engraftable, accessible and/or expandable muscle cells for therapeutic purposes (1–4). In a recent study, we investigated whether satellite cells could be produced in sufficient numbers for direct transplantation within allogeneic or xenogeneic animal hosts (1). To this end, CRISPR/Cas9-corrected induced pluripotent stem cells (iPSCs) derived from DMD mice were injected into mouse blastocysts engineered to ablate their endogenous satellite cells or into rat morulae, and transferred into foster mothers. After development and birth, the resulting mouse–mouse chimeras exclusively harbored iPSC-derived satellite cells. Similarly, rat–mouse chimeras generated from the xenogeneic injections harbored mouse satellite cells. iPSC-derived satellite cells were collected from both types of generated chimeras and transplanted into the muscles of DMD mice, either directly or after in vitro expansion. Remarkably, these iPSC-derived satellite cells restored dystrophin expression, contributed to the recipient's satellite cell pool, and ameliorated functional muscle force decline after repeated tetanic contractions. Collectively, our study demonstrates the feasibility of generating therapeutically competent satellite cells across divergent species, opening the possibility of producing human muscle stem cells in large animal hosts for regenerative medicine applications.

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Functional Restoration of Immune Defects in STAT1 Gain-Of-Function Immunodeficiency Following Gene Editing

Speaker: Rob Torrance[1]

Authors: Rob Torrance[1], Kate Orf[1,2], Nathan White[3], Alexander McKenna[1], Claire Booth[3,4], Adrian Thrasher[3], Thomas Fox[1,2,5], Pietro Genovese[5], Siobhan O. Burns[1,6], and Emma C. Morris[1,2,6,7]

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Hypothesis: Gain-of-function (GOF) mutations in the STAT1 gene result in a rare, combined immunodeficiency with a severe clinical phenotype. The only curative treatment option is allogeneic haematopoietic stem cell (HSC) transplantation (allo-HSCT) which carries high risks of morbidity and mortality for STAT1 GOF patients. Autologous gene therapy may be curative without the immunological complications associated with allo-HSCT. We hypothesise that gene editing of STAT1 GOF mutations in patient T cells and HSCs will restore normal STAT1 function.

Methods: Guide RNAs specifically targeting the p.T385M mutation were electroporated into T cells and HSCs in conjunction with mRNA encoding an adenine base editor. The degree of editing was determined by Sanger sequencing of target site amplicons. Functional analyses were performed in edited cells using flow cytometry to assess total STAT1, phosphorylated STAT1 (pSTAT1) and IL-17 production before and after stimulation. Real time quantitative PCR (RT-PCR) was used to measure transcript levels of key interferon stimulated genes (ISGs). Next-generation sequencing of on- and off-target loci was performed using an Illumina MiSeq instrument.

Results: Base editing of p.T385M STAT1 GOF patient T cells resulted in 99.8% + 0.4% (n=4) reversion of the patient mutation to the wild-type sequence, with an associated statistically significant ($p<0.005$) restoration of total STAT1 and pSTAT1 to healthy control levels, and a concurrent rescue in ISG expression. Up to 100% allelic correction was achieved following base editing of HSCs from p.T385M STAT1 GOF patients. Levels of editing were maintained in HSC-derived monocytic progeny and corresponded to functional correction of total STAT1 and pSTAT1 in these cells. Importantly, initial experiments indicate that base editing does not induce any unintended on-target or off-target DNA aberrations, and that base edited HSCs retain engraftment potential.

Conclusions & Future Directions: Here, we show functional restoration of STAT1 gain-of-function following gene editing in patient T cells and HSCs. To our knowledge, this is the first case of base editing being used to successfully correct gain-of-function mutations causing inherited immunodeficiency. Future work will include further on-target genotoxicity studies, investigations of engraftment potential in humanised mouse models, as well as experiments utilising artificial

thymic organoids to assess functional rescue in edited HSC-derived T lymphocytes. In-vivo chimerism experiments in a validated STAT1 GOF murine model are also underway.

Acknowledgements: With thanks to the MRC for funding this project (MR/N013867/1).

Homology-Guided Engineering of Tyrosine Recombinase Nucleotide Specificities

Speaker: Tina Lebar[1]

1. Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia

The ability to manipulate large DNA fragments is a defining feature of next-generation genome editors. Tyrosine recombinases represent attractive tools due to their capacity to mediate precise rearrangements of large DNA segments without inducing double-stranded breaks. However, their broader application is limited by their complex DNA recognition and poor programmability.

We investigated the DNA-binding specificity of a diverse family of tyrosine recombinases with previously undocumented activity in human cells. Comparative sequence analysis and structural homology revealed a small DNA-binding motif within the compact enzyme structure. Its transplantation between orthologous enzymes yielded hybrid enzymes with predictable shifts in partial target preference, and high-throughput functional characterization guided computational prediction of pseudo-targets in the human genome. Finally, we demonstrate precise, scarless insertion of 5-kilobase DNA cargo into multiple native human loci without the need for pre-installed recognition sites. These findings pave the way toward rational design of tyrosine recombinases with diverse specificities and support their utility as modular, programmable DNA editing tools.

Engineering Designer-Recombinases for Therapeutic Genome Editing

Speaker: Frank Buchholz[1]

1. Medical Systems Biology, UCC, Medical Faculty and University Hospital Carl Gustav Carus, TU Dresden, Germany

Many genetic mutations that cause human diseases have been identified over the last decades. Recent breakthroughs in the field of genome editing now provide a genuine opportunity to establish innovative gene and cell therapy approaches to repair DNA lesions to replace, engineer or regenerate malfunctioning cells in vitro, or directly in the human body. However, most of the recently developed genome editing technologies introduce double stranded DNA breaks at a target locus as the first step to gene correction. These breaks are subsequently repaired by one of the cell intrinsic DNA repair mechanisms, typically inducing an abundance of random insertions and deletions (indels) at the target locus. Ideally, therapeutic genome editing should, however, be efficient and specific, without the introduction of indels.

Site-specific recombinases (SSRs) allow genome editing without triggering cell intrinsic DNA repair pathways as these enzymes fulfill both cleavage and immediate resealing of the processed DNA, allowing precise, predictable and efficient genome editing in vivo. We use substrate-linked directed evolution coupled with rational design and AI to program SSRs to target therapeutically relevant human genomic sites. Examples of engineered recombinases with therapeutic potential will be presented.

Gene Editing for Inborn Errors of Immunity

Speaker: Emma C Morris[1]

1. UCL Institute of Immunity & Transplantation, London, UK

I will discuss latest advances in gene editing and gene therapy for inborn errors of immunity. I will present pre-clinical data from my research group describing novel gene editing approaches for the treatment of rare monogenic diseases including CTLA4 Insufficiency, STAT1 GOF Immunodeficiency, APDS1 and APDS2. I will also discuss results from recent phase I clinical gene therapy trials in the setting of inborn errors of immunity.

Co-Delivery of Non-Encapsidated DNA by rAAV: Is This a Potential Biosafety Concern?

Speaker: Ana Dolinar Češarek[1]

Authors: Ana Dolinar Češarek[1], Mojca Janc[1], Maja Štokelj[2], Alja Štern[3], Katja Kološa[3], David Dobnik[1,4]

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4. Niba Labs d.o.o., Ljubljana, Slovenia

In recent years, treatment of several genetic diseases has become possible due to the ongoing development of new gene therapies. Recombinant adeno-associated viruses (rAAVs) are widely used in gene therapy field due to their advantages over other viral vectors, namely safety, broad tissue tropism, and ability to support long-term expression.

During rAAV manufacturing and purification, multiple critical attributes are routinely monitored to ensure product quality, including potency, identity, purity, and safety. One significant safety concern is the presence of residual host cell DNA (hcDNA) in the product. Previous work from our group demonstrated that hcDNA can be detected across multiple fractions of rAAV preparations (1). Following that, a preliminary study revealed that non-encapsidated DNA remains associated with the rAAV capsid surface even after DNase I treatment. Similarly, recent findings by researchers from France showed that non-encapsidated miRNA contaminants can be found in purified rAAV products (2).

However, it remains unclear whether such capsid-associated contaminants are delivered into host cells during rAAV transduction and whether they are transcriptionally or translationally active. To address this, we investigated whether capsid-associated DNA fragments can be co-delivered into host cells and expressed into functional protein.

We produced rAAV2 viral particles encoding the green fluorescent protein (GFP) using triple transfection method and purified them on CIMmultus® monolith columns. Afterwards, we incubated rAAV particles with DNA fragments encoding the red fluorescent protein (RFP) and transduced HepG2 cells with this mixture. We assessed the presence of DNA fragments in cells and evaluated the potential expression of encoded fluorescent proteins GFP and RFP using microscopy, fluorometry and flow cytometry.

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Precision Therapeutics for ALS and FTD

Speaker: Oscar Wilkins[1,2]

1. UCL Queen Square Motor Neuron Disease Centre, Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, UCL, London, UK
2. The Francis Crick Institute, London, UK

Gene therapies have the potential to revolutionise the treatment of neurological disorders such as ALS and FTD. However, developing payloads that effectively rescue pathology without significant toxicity is a major challenge. In our group, we are working to address this by developing both candidate gene therapies and safety mechanisms to limit off-target expression. I will present our "TDP-REG" technology, which embeds the coding sequences of gene therapies into novel cryptic exons, thus ensuring that expression is limited to cells with TDP-43 pathology. This approach can be applied to any gene therapy coding sequence. Furthermore, I will provide an update on our progress applying TDP-REG technology to a promising candidate gene therapy, towards our ultimate goal of designing a safe and efficacious therapeutic for people suffering from ALS and FTD.

Targeting Receptor Tyrosine Kinases to Restore Axonal Transport in Genetic Peripheral Neuropathy

Speaker: James N. Sleigh[1,2]

1. Department of Neuromuscular Diseases and UCL Queen Square Motor Neuron Disease Centre, Queen Square Institute of Neurology, University College London, London, UK
2. UK Dementia Research Institute, University College London, London, UK

Axonal transport is the process whereby motor proteins actively traverse the microtubule network to bi-directionally deliver diverse cargoes along axons. Anterograde transport connects the cell body to axon terminals and is essential for transporting organelles and key mRNA transcripts for local protein synthesis; retrograde transport enables transfer of crucial survival molecules secreted by distal target cells to the cell body. Indeed, produced by terminal Schwann cells and skeletal muscles, the neurotrophins are a family of neurotrophic factors that signal through Trk receptors found on axon terminals to maintain neuronal homeostasis and function. Upon internalisation, Trk-neurotrophin complexes are sorted into signalling endosomes for long-range retrograde transport to the cell body, where they elicit pro-survival gene transcription. We have developed a technique that uses an atoxic binding fragment of tetanus neurotoxin (HCT) to visualise the in vivo axonal transport of signalling endosomes within the intact sciatic nerve of anaesthetised mice. After injection into muscle, fluorescently-labelled HCT is internalised at the neuromuscular junction prior to being loaded into signalling endosomes. Using this approach, we have identified perturbances in the in vivo axonal transport of signalling endosomes at early stages in several mouse models of peripheral nerve diseases, including amyotrophic lateral sclerosis (ALS) and Charcot-Marie-Tooth disease (CMT). Through further intravital imaging and neuropathological assessments, we have discovered mechanisms underlying these trafficking perturbances and created alleviating therapeutic approaches. Recently, we generated gene therapies that selectively boost the availability of the neurotrophin BDNF and the neurotrophic factor GDNF within muscles; these approaches ameliorate pathology in two different forms of neuropathy caused by mutations in the aminoacyl-tRNA synthetase genes, GARS1 (CMT2D) and YARS1 (DI-CMTC). In my talk, I will present some of our latest data showing that targeting receptor tyrosine kinases in skeletal muscle can restore axonal transport and treat peripheral neurodegeneration.

Gene Therapy Strategies for Spinocerebellar Ataxia Type 3: Preclinical Advances in Allele-Specific Silencing and Gene Inactivation

Speaker: Luis Pereira de Almeida[1,2,3,4,5]

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2. Centre for Innovative Biomedicine and Biotechnology (CIBB), Vectors, Gene and Cell Therapy Group, University of Coimbra, Coimbra, Portugal
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5. GeneT, Centre for Excellence in Gene Therapy in Portugal, University of Coimbra, Coimbra, Portugal

Spinocerebellar ataxia type 3 (SCA3), or Machado-Joseph disease, is an autosomal dominant neurodegenerative disorder caused by CAG repeat expansion in the ATXN3 gene, resulting in toxic gain-of-function of mutant ataxin-3 and progressive neuronal dysfunction. Despite intensive research, no disease-modifying treatments are currently available. Recent preclinical advances have focused on gene therapy strategies that directly target the underlying genetic cause of SCA3, including allele-specific silencing and permanent gene inactivation.

In one promising approach we developed recombinant adeno-associated viral (rAAV) vectors to deliver artificial microRNAs (miRNAs) designed for selective silencing of the mutant ATXN3 allele. In mouse models, intra-cerebellar and intracisterna magna administration of rAAV-miATXN3 constructs achieved robust and sustained suppression of mutant ATXN3, reduced protein aggregation, decreased neuroinflammation, and significant improvements in motor function. Transcriptomic analyses further revealed a shift toward a healthier, wild-type molecular profile in treated animals. Extending these findings to a non-human primate (NHP) model, intracisterna magna delivery of AAV9-miR-ATXN3 enabled targeted transgene expression in affected brain regions, dose-dependent silencing of mutant ataxin-3, and prevention of SCA3-associated neuropathology, demonstrating translational potential.

A complementary strategy leveraged on CRISPR-Cas9 genome editing to permanently inactivate the ATXN3 gene. Delivery of rAAV-CRISPR-Cas9 constructs in mouse models led to a marked reduction in mutant protein aggregates and preservation of neuronal function. Early, widespread brain transduction via cerebrospinal fluid administration in neonatal mice successfully preserved locomotor function, highlighting the therapeutic promise of permanent gene inactivation.

Collectively, these studies provide compelling preclinical evidence that both allele-specific silencing using rAAV-delivered artificial miRNAs and CRISPR-Cas9-mediated gene inactivation are viable gene therapy strategies for SCA3. The successful translation of these approaches in relevant animal models paves the way for future clinical development targeting the root cause of this currently untreatable disorder.

Abstracts of Posters

GENE H Conference – Gene Therapy

Advance Quantification of Nucleic Acid Impurities in Recombinant Adeno-Associated Viral Vectors Using Nanopore Sequencing and Multiplex Digital PCR

Presenter: Mojca Janc[1,2]

Authors: Mojca Janc[1,2], Ana Dolinar Češarek[1], Tjaša Jakomin[1], David Dobnik[1,3]

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Recombinant adeno-associated viruses (rAAVs) have undoubtedly revolutionized the gene therapy field and drastically changed the lives of many families. However, the optimal outcomes of AAV treatment can still be challenged by various factors. One such factor originates from production, as the final products can contain heterogeneous fragments of encapsidated nucleic acid impurities that originate either from plasmids or production cells. Comprehensive characterization is essential to predict the potential side effects of these impurities.

In our study, we combined advances from two analytical approaches, namely next-generation sequencing and multiplex digital PCR, for a comprehensive assessment of plasmid- or host cell-derived full-length genes in AAV samples. The Oxford Nanopore Technologies platform was first used to identify potential problematic DNA impurities. Their length was assessed but could be underestimated due to potential fragmentation during library preparation and short-read bias. To precisely determine the length of these impurities and thereby more accurately predict their side effect potential, several dPCR targets were tested simultaneously in a newly developed multiplex dPCR. This approach enables the detection of three distinct fragments (complete gene, left half of the gene, and right half of the specific gene) to determine the completeness of the sequence. Our study focused on a set of rAAV samples produced via triple transfection in HEK293T cells and purified in two consecutive ultracentrifugation runs in a CsCl gradient. We tested the presence of complete antibiotic resistance gene (ampR), host cell E1A gene, and SV40 large T antigen. Results revealed the presence of complete genes and their fragments in the tested samples.

The combination of these two methods significantly enhanced current impurity profiling approaches. Insights gained from the study could lead to optimization of production and ensure higher purity and safety of rAAVs products.

Erythrocyte Membrane-Derived Vesicles for Safe and Efficient siRNA Delivery in Gene Silencing Therapy

Presenter: Nina Kostevšek[1,2]

Authors: Nina Kostevšek[1,2], Giulia Della Pelle[1,2], Tim Božič[3], Jernej Šribar[1], Boštjan Markelc[3]

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2. Jožef Stefan International Postgraduate School, Ljubljana, Slovenia
3. Department of Experimental Oncology, Oncology Institute, Ljubljana, Slovenia

Erythrocytes are the most abundant circulatory cells and can be isolated in large quantities, making them an attractive, low-cost source for therapeutic delivery systems. In this study, we developed a neutral siRNA delivery system using red blood cell-derived membrane vesicles (EMVs). We selected siRNA for its small size and established clinical relevance. As a proof of concept, we loaded anti-TdTomato siRNA into EMVs and demonstrated their safety, efficiency, and affordability in vitro and in vivo using a melanoma model (1).

We optimized EMV purification and storage protocols. Vesicle morphology was characterized by TEM and cryo-TEM. siRNA-loaded EMVs were assessed for nuclease resistance, release kinetics, and RNA interference activity in vitro. For in vivo evaluation, Cy5-labeled siRNA-EMVs were further stained with Vybrant-DiO and injected into tumor-bearing mice (1.5 mg/kg; n=19). Mice were sacrificed at 2, 24, and 48 hours post-injection. Blood and major organs were collected for confocal imaging and RNA quantification (RT-PCR).

siRNA was successfully encapsulated within EMVs, as confirmed by freeze-fracture TEM and STED confocal microscopy. The vesicles protected siRNA from RNAse A degradation and achieved ~80% gene silencing at just 0.3 nM in vitro—outperforming HEK293 and Neuro2a-derived EVs. Efficacy was consistent across various cell lines (CT26, B16F10, NHLF), suggesting a copy number–dependent mechanism. In vivo, siRNA-EMVs achieved ~60% silencing of TdTomato expression in melanoma-bearing mice and remained detectable in circulation 48 hours post-injection.

We developed a scalable, low-cost, and effective siRNA delivery platform based on erythrocyte membranes. Future directions include tumor-targeted delivery and the co-loading of imaging agents for theranostic applications.

Acknowledgements: Funding information: The study was supported by the Slovenian Research Agency ARIS (program numbers P2-0084, P3-0003, project numbers PR-10482, J3-14531 and J4-50150).

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Accelerating AAV Processing: Scalable, Serotype-Agnostic Process Eliminating 2-steps (DNase and TFF) and Optimized for Physiological-Like Conditions

Presenter: Rok Žigon

Authors: Rok Žigon[1], Sara Drmota Prebil[1], Mirjam Krašna[1], Janja Merkelj Koren[1], Tomaž Švigelj[1], Maja Štokelj[1], Andrej Mihevc[1], Ivana Petrovič Koshmak[1], Petra Dekleva[1], Sandra Potušek[1], Melita Semič[1], Maja Leskovec[1], Ažbe Žnidaršič[1], Teja Nusdorfer[1], Aleš Štrancar[1]

1. Sartorius BIA Separations, Ajdovščina, Slovenia

Adeno-associated virus (AAV) production for gene therapy applications has become increasingly important, yet the downstream processing (DSP) of AAV remains a complex and cumbersome series of steps, each contributing to reduced overall yield and extended processing time. Current methodologies generally consist of seven or more individual steps: DNase treatment > Clarification > TFF1 > Capture step > TFF2 > Polishing step > Formulation and filtration. The resulting low yields and prolonged timelines hinder the scalability and efficiency of AAV production, posing significant challenges to manufacturing. This study proposes a streamlined alternative to traditional AAV downstream processing, focusing on reducing the number of steps while simultaneously improving both recovery and process efficiency. We demonstrate a novel approach comprising just five integrated steps shown to work on various serotypes (AAV2, AAV8, AAV9).

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ASO-Mediated Inhibition of MyD88L265P-dependent signaling as a Therapeutic Approach for Waldenström's Macroglobulinemia

Presenter: Peter Pečan[1,2]

Authors: Peter Pečan[1,2], Duško Lainšček[1,3], Roman Jerala[1,3], Mateja Manček Keber[1,3]

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MyD88 is an adaptor protein involved in the signaling pathways of Toll-like receptors (TLRs) and the Interleukin-1 receptor family, which are crucial for innate immune responses. Its somatic gain-of-function mutations, in particular MyD88 Leu265Pro (MyD88L265P), are implicated in lymphoid malignancies such as Waldenstrom's macroglobulinemia (WM), where more than 90% of patients have been shown to carry the mutation. MyD88L265P promotes lymphoma cell survival by upregulating the expression of Bcl-xL and activating Burton tyrosine kinase (BTK), while also having an effect on the bone marrow microenvironment by transmission of MyD88L265P via extracellular vesicles (EVs) to propagate inflammation. Our research explores a gene therapy strategy using antisense oligonucleotides (ASOs) to induce exon skipping of MyD88, resulting in a dominant negative isoform (MyD88 short, MyD88S) that inhibits MyD88L265P signaling. In vitro, our ASOs successfully altered exon splicing to produce the short isoform and nearly completely inhibited IL-1β signaling. Experiments on the malignant B-cell line MWCL-1, which harbors the L265P mutation, demonstrated significant cytokine secretion inhibition and apoptosis induction. Notably, ASOs outperformed current small molecule therapies, including ibrutinib and venetoclax, in these aspects. We also tested efficient in vivo delivery using lipid nanoparticles (LNPs) conjugated to anti-CD38 antibodies, achieving targeted localization in the spleen and lymph nodes. Our findings highlight the therapeutic potential of ASOs for WM treatment.

Crispr-Based Prime Edit Strategy for Correction of CTNNB1 Mutations

Presenter: Vida Forstnerič

Authors: Vida Forstnerič[1,2], Jure Bohinc[1], Duško Lainšček[(1,2)], Greta Junger[1], Špela Miroševič[3,4], Damjan Osredkar[5,6], Roman Jerala[1,2]

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CTNNB1 syndrome is a rare monogenic neurodevelopmental disorder caused by heterozygous loss-of-function mutations in the CTNNB1 gene, which encodes β-catenin, a key effector of the Wnt signaling pathway. There is currently no targeted therapy for this condition. Here, we explore the application of CRISPR Prime Editing as a precision genome-editing strategy to correct pathogenic CTNNB1 mutations without inducing double-stranded DNA breaks. We designed and screened multiple prime editing (PE) guide RNAs (pegRNAs) targeting two clinically relevant CTNNB1 point mutations. Editing efficiency and fidelity were evaluated in vitro using a reporter system. We further improved the system via coiled-coil based tethering of the nickase and reverse transcriptase subunits of the PE system and showed improved function with several tested pegRNAs. To facilitate screening on a relevant cell model, we additionally developed and characterized lipid nanoparticle (LNP) formulations functionalized for efficient uptake by human induced pluripotent stem cells (iPSCs). Delivery of mRNA via functionalized LNPs resulted in successful cellular uptake and nuclear localization, supporting their potential use in stem cell-based disease modeling and correction.

Our findings highlight the feasibility of Prime Editing for allele-specific correction of CTNNB1 mutations and establish a foundation for the development of a non-viral, clinically scalable delivery platform for therapeutic genome editing in neurodevelopmental disorders.

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Design of a Modular miRNA-Sensing Based Genetic Circuit

Presenter: Archismita Kundu[1,2]

Authors: Archismita Kundu (1,2), Tina Fink (1,3), Roman Jerala (1,3)

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MicroRNAs (miRs) are small endogenous non-coding RNAs that post-transcriptionally regulate gene expression by binding to target mRNAs. Their expression profile changes dynamically in response to cellular conditions, making them powerful real-time biomarkers that reflect the physiological state of a cell (1,2). This responsiveness positions miRNAs as promising diagnostic tools, where specific miRNAs can be detected as indicators of disease or cellular activity (3). Current miRNA sensing strategies often depend on loss-of-signal outputs, which can limit their applicability in dynamic or live-cell contexts (4,5). To overcome this, we present a synthetic miR sensor platform that produces a quantifiable positive signal, overcoming key limitations of conventional systems. Our design employs a bidirectional promoter to drive simultaneous expression of a reporter protein containing a protease cleavage site and a mammalian-compatible protease regulated by the target miRNA, enabling miRNA-dependent cleavage. In the absence of the target miR, protease expression remains high, leading to reporter cleavage and low signal. However, when the miR is present, it suppresses protease production, allowing accumulation of the intact reporter and generating a positive signal. We generated the sensor constructs using molecular cloning and validated them in HEK cells, confirming miRNA overexpression via qPCR and quantifying reporter activity using dual luciferase assays. This novel platform offers enhanced specificity, tunability, and translational potential for real-time live-cell imaging and therapeutic monitoring.

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Splitting the Adenine Base Editor using Coiled-Coils Increases Editing Efficiency via mRNA Delivery

Presenter: Jure Bohinc[1,2]

Autors: Jure Bohinc (1,2), Duško Lainšček (1,3,4), Roman Jerala (1,3,4)

1. Department of Synthetic Biology and Immunology, National Institute of Chemistry, Ljubljana, Slovenia
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Recent advancements in CRISPR-based gene editing technologies predominantly involve RNA-guided nucleases coupled with DNA modifying enzymes. Notable among these are Base Editors, comprising either a deactivated Cas9 or a Cas9 nickase linked to a deaminase enzyme (1,2) and Prime Editors, integrating a Cas9 nickase with reverse transcriptase, among others (3). However, the substantial size of these fusion proteins presents challenges for delivery via vectors such as AAVs, necessitating the development of novel delivery approaches. In recent years, following the COVID pandemic lipid nanopartice (LNP) delivery of mRNA saw a massive increase in research and development. LNPs are well-defined in their composition, straightforward to characterize and assess their quality. They do not have packaging size limits and are less immunogenic than AAVs or other viral delivery systems. In clinics they have been used to deliver siRNA and mRNA vaccines. Our research introduces a method for constructing split editor proteins that can increase DNA editing capabilities. This is achieved through the use of synthetic coiled-coil motifs that reassemble the split proteins non-covalently within the cell (4,5). Using this technique we developed an adenine base editing system, where the nickase and deaminase components are separated and linked to dimerizing coiled-coil motifs. We package the editor mRNA into LNPs and deliver it to mammalian cells. Tests on various reporters and genomic sites show that our dimerizing split adenine base editor not only preserves but, in some cases, enhances editing efficiency. These findings suggest that our novel split adenine base editor is a promising addition to the existing arsenal of base editing tools, with potential applications in correcting clinically relevant mutations. Further, we are extending this innovative approach to include other advanced genome editors.

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ROUND TABLE II:
Bridging Innovation and Commercialization in Gene and Cell Therapy

17 September, 13:00–14:00

Organised in collaboration with Slovenia Biotech Hills.

Moderator: Nik Prebil, Director of Slovenia Biotech Hills

Participants:

1. Zora Čechová – Regulatory Researcher in Cell & Gene Therapies for Rare Diseases, Centre of Excellence CREATIC, Masaryk University
2. Luis Pereira de Almeida – Principal Investigator, Centre of Excellence GeneT, University of Coimbra
3. Boštjan Čeh, Labena's Head of Business Development, President of Biotech Hills
4. Mojca Pečar – Member of the Slovenian Intellectual Property Office, Ministry of the Economy, Tourism and Sport
5. Andrej Klemenc – Member of the Minister's Office, Ministry of Finance