CTGCT

Day 2026

ADVANCES IN CELL, TISSUE, AND GENE THERAPIES

CTGCT

CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

NATIONAL INSTITUTE OF CHEMISTRY

Ljubljana, Slovenia

2026



CTGCT Day 2026:
Advances in Cell, Tissue, and Gene Therapies
Book of Abstracts

Publisher
Centre for the Technologies of Gene and Cell Therapy
National Institute of Chemistry

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Ljubljana, Slovenia, 2026

Design and Layout
Tanja V. Pogačnik

Available free of charge

DOI 10.5281/zenodo.23667189

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID 301979907
ISBN 978-961-7238-18-1 (PDF)

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General Information

CTGCT Day 2026: Advances in Cell, Tissue, and Gene Therapies will be held on Wednesday, 11 November 2026, in the White Hall of the Grand Hotel Union Eurostars, Miklošičeva 1, Ljubljana, Slovenia.

The conference is organised by the Centre for the Technologies of Gene and Cell Therapy (CTGCT) at the National Institute of Chemistry, within the framework of the CTGCT project.

The event focuses on the latest scientific advances and translational achievements in cell, tissue, and gene therapies. It brings together researchers, clinicians, and other experts from related scientific and professional fields.

The programme comprises two morning sessions, two afternoon sessions, and a poster exhibition running throughout the day.

Further information about the conference is available on the event website: https://ctgct.si/en/ctgct-day-2026/.

Kind regards,

Organising committee
Rodrigo M. Ortiz de la Morena
Vesna Merc

Scientific committee
Mojca Benčina
Darja Marolt Presen

Conference Programme

Start	End	Speaker	Title
08:00	08:30	Registration	
08:30	08:40	Welcome and Opening Remarks	
		Session 1	**ATMP Production and Translation**
08:40	09:15	Zsolt Sebestyen, UMC Utrecht	Biomimetic strategies to guide rational immune receptor-based drug development
09:15	09:35	Luka Fajs, Bio-ReCell d.o.o.	Closed-System Non-Magnetic Cell Separation and Activation Approaches for T Cell Manufacturing
09:35	09:55	Lenka Součková, Masaryk University	From Clinical Trial to Patient Access: Practical Lessons from the First Czech Hospital Exemption for an Academic ATMP
09:55	10:15	Matija Povh, Sartorious BIA Separations d.o.o.	Improved Development of LNP Formulation for Organ Targeting using Chromatographic Tools
10:15	10:45	Coffee Break and Poster Session	**Sponsor Presentation: Omega ThermoFisher**
		Session 2	**From In Vivo Models to Treatments**
10:45	11:20	Jan Procházka, Czech Centre for Phenogenomics	Integrated Phenotyping: From Disease Mechanisms to Therapeutic Endpoints
11:20	11:40	Tomaž Martini, University of Maribor	Novel-Modality Therapeutic Opportunities in Hyperlipidemia Based on the Discovery of the Sexually Dimorphic Hepatic Cycle of Very-Low-Density Lipoproteins
11:40	12:00	Giulia Bortolussi, International Centre for Genetic Engineering and Biotechnology	HepCycle-Track Quantifies Hepatocyte Proliferation to Define Therapeutic Timing as a Precision Parameter for Gene Therapy and Genome Editing
12:00	12:20	Špela Malenšek, National Institute of Chemistry	Immunoengineering genetically encoded chimeric PCSK9 vaccine against hypercholesterolemia.
12:20	14:00	Break	**Sponsor Presentation: Sartorius**
		Session 3	**Advances in Gene, Cell and Tissue Therapies**
14:00	14:35	Massimo Dominici, University of Modena and Reggio Emilia	Engineering Cells to Defeat Refractory Solid Tumors: From MSC-Based Therapeutics to CAR-Driven Immunotherapy
14:35	14:55	Aleksandar Janev, University of Ljubljana	Human Amniotic Membrane Preparations Exert a Tumour-Selective Anti-Cancer Effect in Bladder Cancer Across 2D, 3D, and Patient-Derived Models
14:55	15:15	Petra Sušjan Leite, National Institute of Chemistry	Making One Copy Count — RNA-Based Therapeutic Strategies in CTNNB1 Haploinsufficiency
15:15	15:35	Duško Lainšček, National Institute of Chemistry	Decoupling reactogenicity from efficacy enables safer mRNA vaccines, AAV gene delivery, and in vivo base editing
15:35	16:05	Coffee Break and Poster Session	**Sponsor Presentation: Miltenyi Biotec, Labormed**
		Session 4	**Cellular Models for Mechanistic and Safety Studies**
16:05	16:40	Sebastian Diecke, Max Delbruck Center for Molecular Medicine	Pluripotent Stem Cells, Automation, Disease Modeling and beyond
16:40	17:00	Nina Krako Jakovljević, University Clinical Centre of Serbia	Altered Mitochondrial Respiratory Function and Morphology of Palmitate Induced Hepatocellular Carcinoma Cells: Model of Hepatic Insulin Resistance and Lipotoxcity
17:00	17:20	Bernarda Majc, National Institute of Biology	Overcoming glioblastoma resistance: enhancing standard and nk cell therapies through tgf-v pathway inhibition
17:20	17:40	David Morrow, EATRIS	From Innovation to Access: How Effective European Collaboration Can Transform the ATMP Landscape
17:40	17:50	**Closing Remarks**	

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Oral Presentation Abstracts

Session 1:

ATMP PRODUCTION AND TRANSLATION

BIOMIMETIC STRATEGIES TO GUIDE RATIONAL IMMUNE RECEPTOR-BASED DRUG DEVELOPMENT

Zsolt Sebestyen

University Medical Center Utrecht, Center for Translational Immunology, Utrecht, The Netherlands

Abstract

The development of novel cell and gene therapies strongly relies on the identification of new targets, understanding patient heterogeneity, defining functional signatures that predict toxicity and clinical success, and ultimately selecting the most appropriate lead design. Fit-for-purpose preclinical approaches that capture the most relevant aspects of the complexity and heterogeneity of human disease, while remaining sufficiently controlled, can play a critical role in guiding these choices. Biomimetic systems of increasing complexity provide an opportunity to bridge this gap by reconstructing key cellular, molecular, spatial and temporal features of a treatment.

Here, we explore how complementary experimental platforms, ranging from reductionist functional assays to complex multicellular and three-dimensional systems, can be used to interrogate target recognition, spatial and temporal dynamics, and efficacy–toxicity relationships. Integrating functional phenotyping with molecular and spatial profiling can reveal signatures associated with response, resistance and toxicity, while uncovering mechanisms that can guide target selection and lead optimization. Ultimately, these approaches may provide a framework to support rational design throughout cell and gene drug development, while also generating functional signatures that can inform clinical and patient selection.

CLOSED-SYSTEM NON-MAGNETIC CELL SEPARATION AND ACTIVATION APPROACHES FOR T CELL MANUFACTURING

Matic Bartol[1], Tina Fink[2], Tina Tragin[1], Ema Pleško[2], Roman Jerala[2], Hana Esih[1], Aljaž Kravanja[1], Brina Štante[1], Valentina Bianco[1], Luka Fajs[1], Mojca Jež[1]

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Abstract

Cell therapy manufacturing requires robust and reproducible methods that lower the overall production costs and help deliver faster and more accessible therapies. Sub-optimal manufacturing and labour are the key sources of increased production times and costs[1]. We describe novel approaches that decrease manufacturing time and costs by integrating two key production steps into compatible products for a closed and scalable workflow.

The Neptune™ T Cell Separation System is a pump-driven platform for enrichment of T cells from peripheral blood mononuclear cells (PBMCs). The process uses a disposable column-like bead assembly, avoiding the magnetic bead paradigm. The system enabled enrichment of CD3+ T cells with purity of over 90 % within approximately 25 minutes while maintaining high cell viability. Its closed, single-use format is designed to reduce manual handling and facilitate integration into cell-processing workflows.

The Atract™ T Cell Activation Kit uses non-magnetic particles with covalently immobilized anti-CD3 and anti-CD28 antibodies. Following activation, the particles are removed by filtration rather than magnetic separation. Studies comparing Atract™ with an established activation reagent demonstrated robust T cell activation and expansion. Atract™-activated cultures showed a higher proportion of CD8+ T cells after expansion, accompanied by increased cytotoxicity in functional assays. Higher proportion of CD8+ cells promotes higher antitumor activity, avoiding the need to artificially define the product's subtype constitution[12]. Experiments using patient samples showed comparable transduction efficiency to that achieved with the reference activation reagent.

These developments make a case for the use of workflows that do not rely on exorbitant upfront costs, high levels of operator knowledge or prolonged hands-on work. Future of cell therapy is weaved by its financial feasibility; facilities need to develop processes that pay attention to cost effectiveness and willingness-to-pay of their customers[3]. Described novel approaches could facilitate a transition to more effective processes and help accelerate the adoption of T cell therapies.

References

(1) Dias J., et. al, CAR-T cell manufacturing landscape—Lessons from the past decade and considerations for early clinical development, Molecular Therapy Methods & Clinical Development, 2024.

(2) Sommermeyer D., et. al, Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo, Leukemia, 2016.

(3) Patel N., et. al, Cost-Effectiveness of Chimeric Antigen Receptor (CAR) T-Cell Therapy for Blood Cancers: An Updated Systematic Review. PharmacoEconomics - open, 2026.

FROM CLINICAL TRIAL TO PATIENT ACCESS: PRACTICAL LESSONS FROM THE FIRST CZECH HOSPITAL EXEMPTION FOR AN ACADEMIC ATMP

Regina Demlová[1], Lenka Součková[1, 2], Zora Čechová[1], Jaroslav Štěrba[1, 3], Michal Kýr[1, 3], Peter Múdry[1, 3], Jana Kubátová[1], Lenka Zdražilová Dubská[1], Jiří Samek[1]

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[3] University Hospital Brno, Department of Peadiatric Oncology, Brno, Czech Republic

Abstract

The translation of an Advanced Therapy Medicinal Product (ATMP) from academic research and clinical development to patient access is a complex and highly regulated process. Beyond demonstrating reproducible manufacturing, characterisation, quality and safety, successful translation requires an integrated strategy connecting GMP manufacturing and quality control, clinical evidence generation, regulatory requirements, pharmacovigilance and traceability with a realistic pathway for continued patient access.

For ATMPs, centralised marketing authorisation represents the standard route to broad market access in the European Union. However, progression towards and maintenance of a marketing authorisation may be particularly challenging for academic and non-commercial developers, especially for highly individualised therapies or products addressing small and rare patient populations. Hospital Exemption (HE) provides a distinct regulatory pathway for the use of non-centrally authorised ATMPs under defined conditions. It is not a substitute for centralised marketing authorisation, but can provide an important route to patient access for eligible ATMPs manufactured and used within the conditions established by national legislation. Although HE is embedded in the European regulatory framework, its authorisation and practical implementation remain largely the responsibility of individual Member States, resulting in differences in national requirements and limited transferable practical experience.

MyDendrix™, an autologous dendritic cell-based immunotherapy for children and young adults with progressive, recurrent or primary metastatic high-risk solid tumours, provides a practical example of this transition. Its development was supported by CZECRIN and the CREATIC Centre of Excellence, integrating investigator-initiated clinical development with ATMP manufacturing, quality management and regulatory expertise. In 2026, MyDendrix™ progressed towards implementation under the Czech Hospital Exemption framework as the first product in the country. Reaching this milestone required the multidisciplinary team to address several practical questions.

The first Czech Hospital Exemption therefore provides more than a national regulatory precedent. It illustrates a broader challenge for academic ATMP development: successful clinical development and regulatory authorisation do not automatically ensure that a therapy can sustainably reach patients. For some academic ATMPs, particularly those developed for small populations and areas of high unmet medical need, sustainable translation may require pathways that connect clinical evidence, proportionate regulatory mechanisms, continued academic manufacturing, evidence generation and viable financing.

Sharing procedures, regulatory experience and lessons learned through national and European research infrastructures may help academic developers anticipate these challenges, avoid duplication of effort and develop sustainable pathways from investigator-initiated clinical trials to meaningful patient access.

Our experience identified six practical lessons for academic ATMP developers:
(1) define the post-trial regulatory and patient-access pathway early in clinical development;
(2) establish early dialogue with the national competent authority and maintain it throughout development;
(3) develop GMP manufacturing, quality control and regulatory documentation as an integrated system rather than as separate activities;
(4) clearly define responsibilities and interfaces between the manufacturer, sponsor/developer, hospital and treating physicians;
(5) establish pharmacovigilance, traceability, patient follow-up and real-world evidence generation prior to clinical administration under HE; and
(6) address the financial sustainability of patient access early, including manufacturing costs, hospital financing and potential reimbursement mechanisms.

IMPROVED DEVELOPMENT OF LNP FORMULATION FOR ORGAN TARGETING USING CHROMATOGRAPHIC TOOLS

Tristan Kovačič, Matija Povh, Jakob Murovec, Eva Slokar, Mojca Tajnik Sbaizero, Sergeja Lebar, Nika Samsa, Tjaša Leban, Ana Ferjančič Budihna, Nejc Pavlin, Mojca Bavčar, Aleš Štrancar, Jana Vidič, Andreja Gramc Livk

Sartorius BIA Separations d.o.o.

Abstract

mRNA therapeutics have significant potential for applications including cancer treatment, cell-based therapies such as CAR-T, and gene-editing approaches such as CRISPR. These applications rely on nanocarrier systems, particularly lipid nanoparticles (LNPs), for targeted and controlled delivery. However, formulation complexity and limited understanding of LNP subpopulations present major challenges during scale-up and clinical translation. These challenges are further amplified for actively targeted LNPs, where targeting ligands must be efficiently and reproducibly attached to the particle surface. In addition, protein corona formation can influence biodistribution and biological activity.

We have developed analytical and preparative chromatographic methods using convective interaction media (CIM) monolithic columns and they were applied to characterize targeted LNP heterogeneity, monitor ligand conjugation, quantify residual protein, and investigate LNP–protein corona complexes.

Chromatographic methods differentiate ligand-conjugated from unconjugated particles based on differences in surface properties. The method provided high recovery, enabled kinetic monitoring and quality control, and showed potential for polishing targeted LNP formulations. Hydrophobic interaction chromatography was used to quantify residual free protein and monitor its removal. Combining complementary chromatographic methods enabled the quantification of multiple quality attributes in complex targeted LNP formulations. An additional chromatographic method improved the extraction of LNP–protein corona complexes from plasma.

These results demonstrate that CIM chromatography can provide valuable insights into targeted LNP quality, conjugation efficiency, formulation heterogeneity, and protein corona formation, supporting process development and clinical translation.

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(1) Pavlin et al., Ion exclusion chromatography of RNA–lipid nanoparticles using polyethyleneimine-modified spongy monoliths, Journal of Chromatography B, 2025.

(2) Yoo et al., A material toolbox for engineering targeted lipid nanoparticles for RNA therapies, Materials Today, 2026.

(3) Tarab-Ravski at al., The Future of Genetic Medicines Delivered via Targeted Lipid Nanoparticles to Leukocytes, JCR, 2024.

(4) Kovačič et al., The impact of chemical reactivity on the quality and stability of RNA-LNP pharmaceuticals, Nature Reviews Chemistry, 2025.

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Že več kot 30 let ponujamo rešitve na področju kemije, molekularne biologije in mikrobiologije, farmacije, imunologije, onkologije, forenzike in diagnostike

Marcel Gätjen: Developing Streamlined, Flexible Cell Therapy Process Workflows from Upstream to Formulation and Fill-Finish Operations

Abstract
"As cell and gene therapies advance toward commercialization, flexible, scalable, and efficient manufacturing solutions are increasingly important. This presentation will explore how modular technologies can streamline cell therapy workflows, from upstream cell processing and expansion through downstream formulation and fill-finish operations. Modular systems designed for specific process steps offer greater flexibility to optimize individual operations, integrate new technologies, and increase manufacturing throughput while maintaining a compact footprint. Emphasis will be placed on how these systems enable streamlined, closed, and automated workflows by reducing manual interventions, improving process consistency, and facilitating efficient scale-up, ultimately supporting the transition from process development to clinical and commercial manufacturing."

Vabljeni, da nas obiščete na stojnici in spoznate, kako lahko naše rešitve podprejo vaše raziskave!

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Session 2:

FROM IN VIVO MODELS TO TREATMENTS

INTEGRATED PHENOTYPING: FROM DISEASE MECHANISMS TO THERAPEUTIC ENDPOINTS

Jan Procházka

Czech Center for Phenogenomics, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

Abstract

Integrated phenotyping connects clinical records with model characterisation to define interpretable therapeutic window and endpoints. Our workflow accounts for genotype–phenotype variability and species specificity by linking structural, functional and biochemical findings. In a patient-derived EHMT2 knock-in model, microCT confirmed craniofacial dysmorphology, while impaired gait and rotarod performance despite preserved grip strength supported a coordination deficit. Reduced cardiac output reflected lower heart rate with preserved ventricular function; histology revealed no developmental cardiac defects. Together, these findings identify disease-relevant phenotypes and delimit the model's translational scope. This interpretative logic extends to behavioural testing coupled with electrophysiology and metabolic profiling under defined dietary challenges, with pathology, histology and clinical biochemistry integrating functional findings to distinguish primary lesions from systemic consequences. Sustained multidisciplinary expertise converts these relationships into efficacy pipelines aligned with disease trajectory, intervention window and therapeutic mechanism. Simultaneously functional, tissue and biochemical responses strengthen evidence of therapeutic benefit, while baseline characterisation informs subsequent safety assessment. Combined with biodistribution and toxicology, this approach supports coherent regulatory packages for gene and RNA therapies, maintaining biological continuity from model characterisation to translation.

NOVEL-MODALITY THERAPEUTIC OPPORTUNITIES IN HYPERLIPIDEMIA BASED ON THE DISCOVERY OF THE SEXUALLY DIMORPHIC HEPATIC CYCLE OF VERY-LOW-DENSITY LIPOPROTEINS

Andrej Grobin, Maya Petek, Uroš Maver, Tomaž Martini

Faculty of Medicine, University of Maribor, Maribor, Slovenia

Abstract

Hyperlipidemia is a major driver of atherosclerotic cardiovascular disease, yet current therapies do not fully account for the spatial, temporal, and sex-dependent organisation of hepatic lipoprotein metabolism. Defining this organisation reveals previously unrecognised therapeutic opportunities for controlling circulating lipid levels and cardiovascular risk.

Our research identified the very-low-density lipoprotein receptor (VLDLR) as a key component of systemic lipoprotein homeostasis. We previously showed that hepatic VLDLR is enriched in the pericentral hepatic zone in mice and humans, with higher expression in female mice and young women than in males. In human liver, low VLDLR expression is associated with early coronary atherosis and an increased risk of myocardial infarction. In contrast, VLDL assembly is biased towards the periportal lobular zone, indicating that liver VLDL production and subsequent reuptake are spatiotemporally organised.

Temporal changes in VLDLR localisation, together with evidence of lipoprotein internalisation, further suggest that this spatial organisation is dynamic rather than static. We propose that the liver undergoes a rhythmic hepatic VLDL cycle, alternating between phases of VLDL production, peripheral lipid delivery, and receptor-mediated lipoprotein reuptake. The observed sexual dimorphism of hepatic VLDLR expression indicates that this cycle may differ substantially between women and men, with important implications for hyperlipidemia and cardiovascular risk.

We are now defining the regulatory mechanisms and therapeutic tractability of this cycle using mouse models, including models of different diseases, a human clinical study, while developing a perfusable liver-on-a-chip system for continuous monitoring of lipoprotein metabolism. Systems-guided loss-of-function and gain-of-function experiments will determine whether spatially or temporally controlled modulation of hepatic VLDLR and related pathways can lower circulating lipids without disrupting physiological lipid delivery to peripheral tissues.

Together, this work establishes a framework for developing novel-modality therapies that target the regulation of hepatic VLDL metabolism. Such approaches could complement conventional lipid-lowering treatments and support more precise therapeutic strategies for hyperlipidemia and atherosclerotic cardiovascular disease.

HEPCYCLE-TRACK QUANTIFIES HEPATOCYTE PROLIFERATION TO DEFINE THERAPEUTIC TIMING AS A PRECISION PARAMETER FOR GENE THERAPY AND GENOME EDITING

Gabriele Baj[1], Alessandra Iaconcig[2], Lisa Licini[3], Marco Bestagno[2], Fanny Collaud[4], Ilaria Secco[2, 5], Olayemi Joseph Olajide[2], Lorena Zentilin[2], Mauro Giacca[5], Federico Mingozzi[4], Giuseppe Ronzitti[4], Natalia Maximova[6], Danilo Licastro[7], Lorenzo D'Antiga[3], Andrés F. Muro[2], Giulia Bortolussi[2]

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Abstract

Therapeutic timing is emerging as a critical determinant of efficacy for liver-directed gene therapy, yet remains poorly defined, particularly in paediatric settings characterized by dynamic hepatocyte proliferation and ongoing liver growth. Here, we systematically investigated how hepatocyte cell cycle activity regulates the performance of AAV-mediated gene replacement therapy (AAV-GRT) and CRISPR/Cas9-mediated genome editing across defined postnatal developmental stages.

Using neonatal (P1-P5), young adult (P30), and adult (P60) mouse models representing highly proliferative, mildly proliferative, and homeostatic liver states, we performed a quantitative head-to-head comparison of episomal AAV-GRT and homology-directed repair (HDR)-based genome editing using reporter platforms as well as animal models of inborn errors of liver metabolism. We observed that AAV-GRT achieves maximal durability in post-proliferative livers, where episomal vector persistence is maintained, whereas CRISPR/Cas9 editing efficiency peaks during the neonatal stage, coinciding with active hepatocyte cycling and declines sharply before weaning.

To directly couple therapeutic efficacy with liver growth dynamics, we developed HepCycle-Track, a hepatocyte-specific proliferation-responsive reporter platform enabling real-time in vivo monitoring of cell cycle activation. Co-administration of HepCycle-Track and CRISPR/Cas9-mediated genome editing platform (GeneRide+Cas9) allowed simultaneous tracking of proliferating hepatocytes and genome editing events at different post-natal ages (P1, P3, P9, P16, P30 and P60).

To decode the molecular basis governing genome editing outcomes we are currently performing RNA-Seq analyis on sorted hepatocytes populations and spatial transcriptomics on liver slices in neonatal, juvenile and adult mice. The integration of HepCycle-Track with CRISPR-editing will directly link genome editing efficiency to hepatocyte cell cycle activity, providing mechanistic insight into the age-dependent decline in therapeutic genome targeting efficacy.

Additionaly, mouse to human cross-species transcriptomic and IHC-mediated validation identified a panel of age-

sensitive hepatic biomarkers distinguishing proliferative from homeostatic liver states, supporting translational stratification of treatment timing.

Together, our findings establish hepatocyte proliferation–guided therapeutic timing as a precision parameter for liver gene therapy and genome editing, providing a quantitative framework to optimize age-dependent therapeutic interventions for inherited liver diseases based on developmental stage, liver growth dynamics, and cellular context.

IMMUNOENGINEERING GENETICALLY ENCODED CHIMERIC PCSK9 VACCINE AGAINST HYPERCHOLESTEROLEMIA

Špela Malenšek[1, 2], Duško Lainšček[1, 4], Hana Esih[1], Sara Orehek[1], Tina Fink[1], Anja Golob Urbanc[1], Aleš Blinc[3], Roman Jerala[1, 4]

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[3] Department of Vascular Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia
[4] Centre for the Technologies of Gene and Cell Therapy, National Institute of Chemistry, Ljubljana, Slovenia

Abstract

Atherosclerosis is a leading cause of global morbidity, and current cholesterol-lowering therapies are not suitable or effective for all patients. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an attractive therapeutic target because its inhibition enhances LDL receptor (LDLR)-mediated cholesterol clearance, while PCSK9 deficiency does not cause adverse pathology. To develop a long-lasting alternative to repeated PCSK9-targeting therapies, we designed an “autologous” chimeric PCSK9 (chPCSK9) DNA vaccine. Solvent-accessible PCSK9 amino acids were transplanted onto a distant-homolog scaffold to generate conformational B-cell epitopes, while CD8+ T-cell epitopes, predicted using machine learning algorithms, were removed to reduce the risk of cytotoxic autoimmunity. Vaccination induced a humoral immune response in BALB/c and ApoE-/- mice, with antibodies effectively inhibiting the PCSK9–LDLR interaction in vitro. In ApoE-/- mice, vaccination reduced serum PCSK9 and cholesterol levels, increased hepatic LDLR abundance, and decreased atherosclerotic plaque formation. These effects persisted for at least 24 weeks, and analysis of splenocytes indicated a low propensity for PCSK9-specific cytotoxic T-cell activation. These findings provide proof of concept for chPCSK9 vaccination as a durable strategy for hypercholesterolemia and atherosclerosis and demonstrate the potential of rational vaccine design against endogenous proteins and other therapeutic targets.

References

Malenšek Š, et al, Engineering chimeric PCSK9 for a vaccine against atherosclerosis, Mol Ther Methods Clin Dev, 2025.



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Session 3:

ADVANCES IN GENE, CELL AND TISSUE THERAPIES

ENGINEERING CELLS TO DEFEAT REFRACTORY SOLID TUMORS: FROM MSC-BASED THERAPEUTICS TO CAR-DRIVEN IMMUNOTHERAPY

Ilenia Mastrolia, Giulia Grisendi, Chiara Chiavelli & Massimo Dominici

Department of Medical and Surgical Sciences (SMECHIMAI), University of Modena and Reggio Emilia, Modena, Italy

Abstract

Despite remarkable advances in cancer treatment, aggressive solid malignancies, including pancreatic and lung cancers and sarcomas, remain major therapeutic challenges. Their complex tumor microenvironments, profound cellular heterogeneity, and multiple mechanisms of treatment resistance continue to limit the efficacy of conventional and emerging therapies.

Cell and gene therapies are opening new therapeutic opportunities by transforming living cells into programmable anticancer agents capable of selectively targeting malignant tissues, delivering cytotoxic molecules, and activating antitumor immune responses. Pioneering research has established mesenchymal stromal cells (MSCs) as versatile platforms for the development of engineered cellular therapeutics. Genetically modified MSCs can serve as localized delivery systems for pro-apoptotic agents, including TRAIL-based molecules, offering the potential to enhance tumor cell killing while limiting systemic toxicity. In parallel, chimeric antigen receptor (CAR) technologies are redefining adoptive immunotherapy by enabling immune cells to recognize and eliminate tumor cells through antigen-specific mechanisms. CAR-T cells targeting solid tumor-associated antigens, including GD2, have demonstrated encouraging preclinical activity and emerging clinical potential, although tumor heterogeneity, immune suppression, and limited cellular persistence remain significant challenges. The convergence of MSC-based therapeutic delivery and CAR-engineered cellular platforms offers complementary opportunities to address tumor resistance through direct induction of cancer cell death, targeted immune-mediated cytotoxicity, and modulation of the tumor microenvironment. Together, these strategies illustrate a new generation of living therapeutics, moving beyond conventional pharmacology toward programmable, potentially more selective, and biologically integrated approaches for otherwise refractory solid malignancies.

HUMAN AMNIOTIC MEMBRANE PREPARATIONS EXERT A TUMOUR-SELECTIVE ANTI-CANCER EFFECT IN BLADDER CANCER ACROSS 2D, 3D, AND PATIENT-DERIVED MODELS

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Abstract

Bladder cancer is the ninth most commonly diagnosed cancer worldwide[1]. Based on the depth of invasion at diagnosis, it is classified as either non-muscle-invasive (NMIBC) or muscle-invasive (MIBC) bladder cancer, two categories that differ in their molecular profiles, clinical management and prognosis[2]. Human amniotic membrane (hAM) is a perinatal tissue with documented anti-tumour activity[3], but the molecular basis of this effect and its consistency across model systems remain poorly defined. Using hAM preparations, the full tissue homogenate and its derived extract, we characterised the transcriptional and functional response of bladder cancer to hAM and tested whether it is preserved as model complexity increases, from 2D monolayers and 3D spheroids to explants and organoids derived from patients with NMIBC.

In 2D models, hAM homogenate induced extensive differential gene expression in muscle-invasive (T24) and non-muscle-invasive papillary (RT4) bladder cancer cells, with only modest changes in normal porcine urothelial (NPU) cells, indicating a tumour-selective response. In both tumour cell lines, hAM downregulated immune-signalling programmes, most prominently pro-inflammatory pathways. qPCR and ELISA confirmed suppression of IL-6 and CXCL8/10/11. These effects translated to more complex models: in 3D Matrigel-embedded RT4 spheroids, hAM extract reduced spheroid formation and decreased spheroid viability, length, area, circularity and solidity. In patient-derived explants, 7-day exposure to hAM homogenate reduced tumour volume and disrupted tissue architecture. Furthermore, hAM extracts derived from independent donors reduced viability of patient-derived bladder cancer organoids. Both hAM preparations therefore retained their anti-tumour activity up to the level of patient-derived explants and organoids.

In conclusion, hAM drives a reproducible, tumour-selective anti-cancer effect that is maintained across 2D, 3D, and patient-derived models, supporting the further development of hAM-derived preparations as a tissue-based, intravesically applicable therapy for NMIBC.

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MAKING ONE COPY COUNT – RNA-BASED THERAPEUTIC STRATEGIES IN CTNNB1 HAPLOINSUFFICIENCY

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Abstract

CTNNB1 syndrome is caused by the loss of function mutations in the CTNNB1 gene that encodes β-catenin. β-catenin aids in transcription of Wnt target genes, which is critically important during early embryonic development. Among possible therapeutic approaches for supplementation of β-catenin are RNA-targeting strategies such as antisense oligonucleotides (ASOs) that can bind to mRNA and alter its processing and expression. We present various ways in which we were able to rescue CTNNB1 expression and activity including an ASO-based approach based on impairment of β-catenin degradation by modifed splicing of CTNNB1. Our results represent the basis for further development of RNA-based therapy for patients suffering from CTNNB1 deficiency.

DECOUPLING REACTOGENICITY FROM EFFICACY ENABLES SAFER MRNA VACCINES, AAV GENE DELIVERY, AND IN VIVO BASE EDITING

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Abstract

Nucleic-acid therapeutics, including mRNA vaccines, genome editors, and viral vectors, are constrained by acute innate reactogenicity that limits dosing flexibility, tissue targeting, and safety. Here we identify a conserved early innate inflammatory program, peaking approximately six hours after administration, that governs reactogenicity across mRNA formulated lipid nanoparticle (LNP), adenoviral vectors and AAV9. Using pharmacological and biochemical probes, we show that this program is driven predominantly by nucleic-acid cargo sensing, rather than lipid components, and is mechanistically separable from antigen expression, adaptive immune priming, and in vivo genome-editing efficacy. Selective modulation of this early cytokine burst achieved here using endosomal nucleic-acid sensing suppressors markedly reduces reactogenicity in multiple mouse models while preserving humoral and cellular immunity to mRNA vaccines, maintaining transgene expression, protecting blood–brain barrier integrity during stereotactic AAV9 delivery, and mitigating LNP-associated hepatotoxicity and thrombocytopenia. These findings define a unifying innate-sensing architecture for nucleic-acid therapeutics and establish that reactogenicity is not required for effective vaccination or gene delivery, enabling a broadly applicable strategy to widen the safety window of mRNA medicines, viral vectors, and in vivo genome editing.

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Session 4:

CELLULAR MODELS FOR MECHANISTIC AND SAFETY STUDIES

PLURIPOTENT STEM CELLS, AUTOMATION, DISEASE MODELING AND BEYOND

Sebastian Diecke

Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Technology Platform Pluripotent Stem Cells, Berlin, Germany

Abstract

Human induced pluripotent stem cells (iPSCs) have fundamentally transformed the study of human disease by enabling the generation of patient-specific cellular and tissue models that recapitulate pathological phenotypes in vitro. This talk presents an overview of the methodological foundations of iPSC-based disease modeling and their application to the investigation of monogenic and complex neurodegenerative disorders.

Sebastian Diecke, head of the Technology Platform Pluripotent Stem Cells at the Max-Delbrück-Center, will first introduce the biological principles distinguishing embryonic, induced, and adult stem cells, before addressing the generation of genome-edited iPSC lines and their directed differentiation into disease-relevant cell types and tissues, including two- and three-dimensional organoid and engineered-tissue systems.

The talk will present several case studies from ongoing research programs. In the context of Huntington's disease, a cohort of patient-derived and isogenic hiPSC lines with defined CAG-repeat expansions is used to generate an innervated engineered human myocardium model, combining sympathetic neural organoids with engineered cardiac tissue to investigate cardiac involvement in this neurodegenerative disorder. A second line of investigation addresses the role of glucocorticoid signaling in Parkinson's disease, demonstrating—across iPSC-derived neurons, brain organoids, and a transgenic mouse model—that moderate glucocorticoid exposure normalizes excitatory-inhibitory imbalance and reduces pathological α-synuclein accumulation.

A central methodological theme throughout is the automation of iPSC culture, differentiation, and quality control. Using a Biomek i7-based liquid handling platform, the Technology Platform has established standardized, high-throughput workflows for cell banking, confluence monitoring, and differentiation tracking, enabling the generation of large-scale, quality-controlled iPSC banks and improving the reproducibility of downstream disease models.

Finally, the talk extends beyond human disease modeling to illustrate how the same stem cell technologies are being applied to species conservation. As part of the BioRescue initiative, the generation of induced pluripotent stem cells and primordial germ cells from critically endangered species—including the northern white and Sumatran rhinoceros—is being pursued alongside classical IVF and genetic enrichment strategies, with the goal of producing viable offspring and preserving genetic diversity in populations on the brink of extinction. Comparative evolutionary analyses across primate species further contextualize how iPSC-derived models inform our understanding of lineage-specific regulatory and functional adaptations.

Collectively, these examples demonstrate the versatility of iPSC technology as a platform spanning mechanistic disease research, scalable automated workflows, and biodiversity conservation.

ALTERED MITOCHONDRIAL RESPIRATORY FUNCTION AND MORPHOLOGY OF PALMITATE INDUCED HEPATOCELLULAR CARCINOMA CELLS: MODEL OF HEPATIC INSULIN RESISTANCE AND LIPOTOXCITY

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Abstract

In the insulin resistant liver, insulin fails to suppress endogenous glucose production, and instead of storing, liver keeps releasing glucose into the circulation. Thus, leading to progression of whole body insulin resistance (IR) and consequently to type 2 diabetes (T2D). An elevation of hepatocellular lipids is the main hallmark of metabolic dysfunction–associated steatotic liver disease (MASLD) that has increased prevalence among people with T2D. Mitochondria, which have a central role in the cellular energy metabolism, have been affected in these metabolic conditions [1] alongside miscommunication between mitochondria and endoplasmic reticulum (ER) (Mitochondria Associated ER Membranes-MAM) [2]. The aim of this study is to further explore our previously characterized IR cell model [3], investigating effects of palmitate on human hepatocellular carcinoma (Huh7) cells in terms of mitochondrial function and morphology, MAM subcellular compartment and its marker mitofusin 2 (MFN2).

Huh7 cells were treated with 0.3 mM palmitate for 24 h. Protein expression was evaluated by RT-PCR, protein levels by immunoblot, mitochondrial respiration by high resolution respirometry (HRR), morphological examinations by electron and confocal microscopy. Finally, for MAM detection, nucleofection with two specific GFP split plasmids (courtesy of Prof. Tito Cali, University of Padua) was used together with MitoTracker Red staining.

Palmitate treated Huh7 cells require higher digitonin concentration for their permeabilization in order to effectively perform HRR. They show downregulation of mitochondrial function in both permeabilized and intact cells. By confocal image analysis both aspect ratio and relative fluorescence intensity of mitochondria staining were lower in palmitate treated cells. Preliminary results showed reduced level of MFN2 (one of the key MAM molecules) together with lower Akt phosphorylation and fewer mitochondria-ER contacts in palmitate treated cells.

Our cellular model replicates mitochondria and MAM alteration in liver pathophysiology, that might help determine relevant intracellular targets for T2D and MASLD translational research.

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OVERCOMING GLIOBLASTOMA RESISTANCE: ENHANCING STANDARD AND NK CELL THERAPIES THROUGH TGF-β PATHWAY INHIBITION

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Abstract

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults and is characterized by pronounced cellular heterogeneity and an immunosuppressive tumor microenvironment (TME), which limit the long-term efficacy of immunotherapy. The TGF-β signaling pathway is frequently dysregulated in GBM and contributes to tumor aggressiveness, invasion, immune evasion, and therapeutic resistance, making it an attractive therapeutic target.

We investigated the effects of the selective TGF-β type I receptor inhibitor galunisertib (Gal) on patient-derived 3D GBM spheroids, alone and in combination with standard therapy (ST). Marked interpatient and intratumoral heterogeneity in treatment response was observed. In two of six spheroid models, Gal increased sensitivity to ST. Western blot analysis did not indicate activation of the canonical SMAD-dependent TGF-β pathway, whereas increased ERK phosphorylation suggested engagement of non-canonical signaling mechanisms. Quantitative phosphoproteomics revealed extensive phosphosignaling network remodeling involving proteins associated with cell adhesion, cytoskeletal and membrane dynamics, and metabolism. Notably, NCAM1 and PCDH9 abundance increased, whereas ARHGEF26 decreased following treatment. Pathway analysis identified alterations in EGFR, MAPK/ERK, AKT, and STAT3 signaling. These findings suggest that Gal induces adaptive phosphosignaling changes that may enable alternative signaling pathways to sustain cellular plasticity and invasiveness.

Gal was further evaluated as an immunomodulatory therapy in combination with NK-92 cells and the IL-15 agonist N-803. In NIB216 spheroids, the combination of ST, NK-92 cells, Gal, and N-803 produced the greatest reduction in viability. This treatment also yielded the highest proportion of NKp30-positive NK-92 cells and the strongest NKp30 expression, indicating enhanced NK-cell activation. Collectively, these results suggest that Gal may support NK-cell-mediated antitumor activity when combined with N-803.

Overall, our findings support further investigation of TGF-β pathway inhibition in combination with compensatory pathway targeting or NK-cell-based immunotherapy to improve antitumor responses in GBM.

This research was funded by the Slovenian Research and Innovation Agency (ARIS) under project Z3-70129.

FROM INNOVATION TO ACCESS: HOW EFFECTIVE EUROPEAN COLLABORATION CAN TRANSFORM THE ATMP LANDSCAPE

David Morrow

EATRIS – European Infrastructure for Translational Medicine, Amsterdam, The Netherlands

Abstract

Advanced Therapy Medicinal Products (ATMPs) offer transformative opportunities for patients with serious and previously untreatable diseases. Yet fragmented infrastructure, manufacturing bottlenecks, divergent regulatory pathways, complex health technology assessment (HTA), and uncertain reimbursement continue to impede their development, affordability, and equitable availability across Europe. Overcoming these barriers requires more than scientific excellence: it demands a coordinated European approach spanning the entire journey from discovery to patient access.

EATRIS, the European Infrastructure for Translational Research, is uniquely positioned to help deliver this transformation. Connecting more than 175 academic and medical institutions across Europe, including 60 specialised ATMP centres across 15 countries, EATRIS acts as a strategic connector between scientific innovation, translational infrastructure, regulatory expertise, clinical development, and healthcare delivery. Through its distributed network, dedicated Coordination and Support Office, and role in EU-funded initiatives including PRECISEU and ERDERA, EATRIS identifies capability gaps, connects innovators with the expertise they need, and helps translate fragmented national resources into coordinated European capacity. Its pan-European ATMP mapping initiative provides a practical foundation for smarter investment, cross-border infrastructure access, and more efficient development pathways.

Building on this foundation, EATRIS contributes to addressing shared translational challenges through collaborative projects spanning gene therapy platforms, iPSC cell-based medicines, genome editing, CAR-T therapies, and workforce development. Its added value lies not simply in connecting institutions, but in orchestrating collaboration across disciplines, infrastructures, and development stages to turn complementary expertise into actionable solutions.

The European Biotech Act and the emergence of ATMP Centres of Excellence (CoEs) NOW provide an opportunity to embed this collaborative approach within Europe's biotechnology ecosystem. However, CoEs must not become isolated centres of excellence. A new European initiative, with EATRIS playing a connecting and critical role with ATMP leaders in the field across the EU, aims to link ATMP CoEs across the EU, break down institutional and national silos, and enable joint responses to challenges spanning manufacturing, regulatory science, HTA, reimbursement, and market access.

By connecting capabilities and aligning expertise across the full value chain, EATRIS can help Europe move from fragmented excellence to collective impact—accelerating development, reducing duplication, strengthening sustainability, and ultimately improving the affordability and equitable access of advanced therapies for patients across Europe.

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Digital transformation as a tool to accelerate translational medicine

WHAT IS EATRIS-CONNECT?

EATRIS-CONNECT isan EU-funded initiative that leverages digital transformation to accelerate translational medicine. Led by EATRIS and backed by a Horizon Europe budget of €3.9 million, this three-year project (May 2024-April 2027) brings together 21 organisations from across Europe. The project focuses on cross-border alignment and will equip the infrastructure with new digital capacities with a particular focus on AI-enabled technologies, advancing digital readiness while promoting environmentally sustainable digital transformation in EATRIS ERIC and its 15 national nodes. This project aligns with our commitment to the Digital Europe Programme's goal of making Europe both greener and more digital.

KEY OBJECTIVES

- Equip EATRIS to deliver digital services for translational medicine.
- Adapt EATRIS' digital capacities for clinical decision-making.
- Evolve the EATRIS business model in close alignment with national Research Infrastructure strategies.
- Cultivate complementarities and synergies in the digital domain across the Research Infrastructure landscape.
- Enable a more connected and efficient PerMed ecosystem.

IMPACT AND LONG-TERM VISION

EATRIS-CONNECT represents a pathway for EATRIS to evolve into a fully digital-ready infrastructure capable of addressing bottlenecks in translational medicine and expanding its role in personalised medicine. Through strengthened digital competencies, interdisciplinary collaboration, and environmentally responsible practices, the project will help secure EATRIS' long-term sustainability. By consolidating EATRIS's digital infrastructure landscape, EATRIS-CONNECT paves the way for accelerated innovation and enhanced collaboration across Europe, positioning EATRIS as a central player in the personalised medicine ecosystem.

PROJECT PARTNERS

AIBILI (Portugal), CEA-NeurATRIS (France), EATRIS-ERIC (The Netherlands), HEALTH-RI (The Netherlands), Infarmed (Portugal), Istituto Mario Negri (Italy), Italian National Institute of Health (Italy), Luxembourg Institute of Health (Luxembourg), Madrid Health Service (SERMAS) (Spain), Oslo University (Norway), Palacký University Olomouc (Czech Republic), Radboud University Medical Center (The Netherlands), Riga Stradins University (Latvia), Sofia University (Bulgaria), Trinity College Dublin (Ireland), University of Eastern Finland (Finland), University of Helsinki (Finland), University of Ljubljana (Slovenia), University of Zagreb School of Medicine (Croatia), Uppsala University (Sweden), VHIR Vall d'Hebron Instituto (Spain), Comenius University in Bratislava (Slovakia), Koç University (Türkiye).



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This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement Number 101130349.

POSTER ABSTRACTS

A NOVEL APPROACH FOR DSRNA REMOVAL ON OLIGO DT WITH ACIDIC DENATURATION

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Abstract

Double-stranded RNA (dsRNA) impurities are a major concern in the production of mRNA by in vitro transcription (IVT), as they can activate innate immune responses, induce pro-inflammatory cytokine secretion, reduce translational efficiency, and compromise therapeutic efficacy. While upstream process optimization can reduce dsRNA formation, robust downstream purification remains essential. Here, we present Acidic Denaturation coupled with Oligo dT purification (ACDT), a novel approach for dsRNA removal based on transient, controlled pH-induced disruption of hydrogen bonding within dsRNA structures.

The ACDT approach was evaluated using mRNA constructs ranging from 1,000 to 10,000 nucleotides. Following an initial Oligo dT capture step, the mRNA-containing fraction was briefly exposed to acidic conditions to denature dsRNA, followed by rapid in-line dilution and reloading onto a CIM® Oligo dT column under standard loading conditions. Denatured, truncated, and non-polyadenylated complementary RNA species were removed in the flow-through, while full-length polyadenylated mRNA was selectively captured and subsequently eluted. J2 dot blot, Lumit dsRNA detection, and Fragment Analyzer analyses demonstrated a 10–20-fold reduction in dsRNA, while maintaining or improving RNA integrity. Functional evaluation in BHK-21 and A549 cells showed enhanced transgene expression, increased cell viability, and reduced IFN-β secretion.

The ACDT approach provides a rapid, scalable, high-recovery (>90%) and solvent-free strategy for dsRNA removal, while maintaining standard Oligo dT column performance and high binding capacity (3–6 mg/mL).

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ACTIVATION OF CTNNB1 USING CRISPRA

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Abstract

CTNNB1 encodes β-catenin, an evolutionarily conserved protein with essential roles in both Wnt pathway-mediated transcription and cell adhesion. Haploinsufficiency caused by de novo variants in one allele of CTNNB1 leads to CTNNB1 syndrome, a rare neurodevelopmental disorder, while complete loss of CTNNB1 function is lethal neonatally[1]. In contrast, gain-of-function alterations of CTNNB1 can result in excessive Wnt pathway activity and contribute to cancer development.

Achieving biologically relevant levels of β-catenin is therefore important for developing potential therapeutic strategies for CTNNB1 haploinsufficiency. Since patients retain one functional copy of CTNNB1, activation of the endogenous gene using CRISPR activation (CRISPRa) represents a potential alternative to exogenous gene supplementation. Here, we designed a series of single guide RNAs (sgRNAs) targeting the promoter and enhancer regions of CTNNB1. We used the sgRNAs in combination with dCas9-VPR or dCas9-VP64 to activate endogenous CTNNB1 expression in HEK293T cells and assessed reporter activity and CTNNB1 expression using qPCR.

Preliminary screening identified 4 promising sgRNAs capable of increasing CTNNB1 expression up to 1.5-fold. These findings provide an initial proof-of-concept for CRISPRa-mediated activation of endogenous CTNNB1.

These findings provide an initial proof-of-concept for CRISPRa-mediated upregulation of endogenous CTNNB1. Ongoing work focuses on optimizing sgRNA-activator combinations and validating the most effective candidates in CTNNB1 haploinsufficient patient-derived cells. Furthermore, we will investigate the downstream effects on β-catenin protein levels and Wnt pathway target genes to ensure the rescued expression achieves therapeutic relevance without overactivation.

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ADVANCED CHARACTERIZATION OF MULTICARGO RNA-LIPID NANOPARTICLES BY 2D CHROMATOGRAPHY

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Abstract

Lipid nanoparticles (LNPs) have transformed gene therapy and vaccine development by enabling the delivery of nucleic acids and proteins. However, their complex composition and limited stability make it challenging to accurately assess encapsulation efficiency, RNA integrity, particle size, heterogeneity, and aggregation. We developed the PATfix LNP Switcher, a two-dimensional chromatography platform designed to address these analytical challenges without sample pre-treatment.

The system combines CIMac OH and CIMac SDVB columns, independent pumps, UV-Vis detection, and multi-angle light scattering (MALS). In the first dimension, LNPs bind to the CIMac OH column, while free RNA is directed to the CIMac SDVB column. MALS detects particle size and elution behaviour, whereas UV-Vis quantifies non-encapsulated RNA. In the second dimension, online particle disruption enables the detection and quantification of encapsulated mRNA and RNA-lipid adducts.

The platform successfully characterized complex LNP formulations containing dual mRNA cargos of 1 and 4 kb. It revealed distinct particle subpopulations, quantified free and fragmented RNA as well as RNA-lipid adducts, monitored temperature-dependent adduct formation, and detected aggregation.
By integrating multiple orthogonal measurements in a single injection, the PATfix LNP Switcher provides a comprehensive solution for formulation development, quality control, and stability assessment. It can improve understanding of LNP quality attributes and support the development of safer, more effective RNA-based therapeutics and vaccines.

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CD4 CYTOSOLIC DOMAIN AS AN EFFICIENT COSTIMULATORY MODULE FOR CHIMERIC ANTIGEN RECEPTORS

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Abstract

Chimeric antigen receptor (CAR) T cell efficacy depends on costimulatory signaling, typically mediated by CD28 or 4-1BB domains. However, limitations in persistence and activity remain. The natural TCR activation requires participation of the CD4 or CD8 receptors, which recruit Lck kinase to the immune synapse. Therefore, we thought that it could represent a potential alternative costimulatory module to enhance CAR signaling.

We engineered second-generation CAR constructs incorporating the cytosolic CD4 domain either as a sole costimulatory element or in combination with 4-1BB. Human primary T cells were transduced with lentiviral vectors encoding CARs targeting CD19, Her2, or BCMA. Functional evaluation included in vitro cytotoxicity assays, cytokine secretion analysis, transcriptomic and metabolic profiling, and in vivo efficacy in a xenograft lymphoma mouse model.

CD4-containing CAR T cells demonstrated enhanced cytotoxicity and overall antitumor activity compared to conventional CAR constructs across several tested antigens. Despite reduced cytokine secretion relative to controls, CD4-modified CAR T cells maintained robust anti-tumor activity. In an animal model, CD4-based CAR T cells improved tumor clearance and prolonged survival.

Incorporation of the CD4 intracellular domain enhances CAR T cell functionality, leading to improved cytotoxicity and in vivo efficacy while modulating cytokine secretion. This approach represents a potentially powerful strategy to optimize CAR design and may contribute to improved therapeutic performance across different targets.

Conflict of interest*: Several authors of the abstract are coinventors on the patent application related to the content of this abstract.

CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY (CTGCT): SOLVING THE TRANSLATIONAL GAP IN GENE AND IMMUNOTHERAPY USING IN-VIVO METHODS

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Abstract

The translation of promising biomedical discoveries into effective therapies remains constrained by a critical translation gap between the early-stage research and the actual clinical application, showing the need for dedicated pre-clinical infrastructure, including more predictive in vivo models to advance gene and cell therapy development, most importunately in an EMA and FDA compliant manner.

Founded to address this, at the Centre for the Technologies of Gene and Cell Therapy (CTGCT) we aim to serve as a research and infrastructure hub with the goal of developing innovative cell and gene therapies in a GMP & GLP compliant facility, advancing therapies on the path towards clinical trials and ultimately providing transformative treatments for patients with rare genetic diseases and cancers. This involves the production of viruses, nucleic acids, and non-viral delivery reagents for clinical therapy. A crucial step in bridging the gap between the lab and the clinic, however, is in vivo research, providing utmost important data on efficacy and safety of newly developed therapeutics.

To strengthen our preclinical pipeline, we have incorporated several assays in our animal facility to test common neurological and psychological conditions including learning ability, motor functions, anxiety/depression and sensory ability. These include state-of the art kinematic gait analysis, novel object recognition, open field, marble burying, food burying, the light-dark box test and many others. This diverse array of tests serves to produce high quality preclinical results across a diverse range of modified mouse lines, that we can support with our breeding facility.

With a combination of established biomedical assessments and behavioral studies we hope to support the continued development of new effective therapeutic solutions.

CHARACTERIZATION OF SUSPENSION PRODUCTION CELL LINES FOR LENTIVIRUS PRODUCTION

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Abstract

Lentiviral vectors (LVs) are essential tools in cell and gene therapy, and selecting a robust, scalable production cell line is a critical step in developing a manufacturing platform. Within the CTGCT project, we are evaluating several suspension cell lines for LV production. Here, we present results for Thermo Fisher's Expi, VPC and VPC2.0 cell lines. Using transient transfection, we assessed their production performance by comparing LV yield, the performance of different transfection reagents, the amount of plasmid DNA required, transfection efficiency, growth characteristics and viability, stability over successive passages, batch-to-batch reproducibility, and suitability for scale-up. These findings will inform the selection of the most suitable candidate for the development of a stable LV producer cell line.

DEVELOPMENT OF AN AAV GENE THERAPY FOR COCKAYNE SYNDROME TYPE B: A PATIENT-DRIVEN TRANSLATIONAL RESEARCH PROGRAMME

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Abstract

Cockayne syndrome type B (CSB) is an ultra-rare, severe neurodegenerative disorder caused by pathogenic variants in the ERCC6 gene, leading to progressive neurological decline, multisystem dysfunction and premature ageing. No disease-modifying treatment is currently available.

A patient-driven initiative led by the Viljem Julijan Association for Children with Rare Diseases has established an international translational research programme aimed at developing an AAV-mediated gene therapy for CSB. Through association-led fundraising, more than €2 million has been raised to support preclinical development and advance the programme towards clinical translation.

The therapeutic strategy is based on ERCC6 gene augmentation using adeno-associated viral (AAV) vectors designed to restore ERCC6 protein expression in affected tissues, with particular emphasis on the central nervous system. The large size of the ERCC6 gene presents a significant vector-packaging challenge. Multiple gene constructs with different regulatory elements were therefore engineered and evaluated to optimise ERCC6 expression and enable efficient AAV packaging.

Preclinical studies demonstrated robust neuronal expression of ERCC6 using several AAV-delivered constructs. Based on these findings, second-generation vectors with reduced cassette size and improved packaging efficiency were developed and are currently being evaluated in different CSB animal models. Parallel work includes the development of induced pluripotent stem cell-derived neuronal models, studies aimed at identifying clinically meaningful outcome measures, and preparation of toxicology studies to support regulatory translation and future first-in-human clinical trials.

This programme demonstrates how patient organisations can act as catalysts for translational research in ultra-rare diseases by mobilising research funding and enabling international scientific collaboration. The ongoing preclinical programme aims to establish the efficacy, safety and translational evidence required to advance AAV-mediated ERCC6 gene augmentation towards clinical development for CSB.

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DEVELOPMENT OF CHIMERIC ANTIGEN RECEPTOR T CELLS INCORPORATING DE NOVO DESIGNED BINDING DOMAINS FOR CANCER IMMUNOTHERAPY

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Abstract

Chimeric antigen receptor (CAR) T cell therapy harnesses autologous T lymphocytes engineered to express a synthetic receptor that redirects them to recognize and eliminate cancer cells, and has proven highly effective against hematological malignancies. Most current therapies use second-generation CARs in which antigen recognition is mediated by a single-chain variable fragment (scFv). However, the scFv domain poses significant challenges: it can be immunogenic, contribute to toxicity, and limit CAR T cell persistence. Moreover, both suboptimal and excessively high antigen-binding affinity can impair therapeutic function, and certain scFvs drive antigen-independent clustering on the T cell surface, causing tonic signaling and accelerated exhaustion. Expression instability arising from difficult-to-optimize scFv regions further constrains CAR performance. Here, we aim to replace the scFv with a de novo designed, compact antigen-binding domain generated using machine-learning–based protein design tools, and to evaluate its impact on CAR T cell therapeutic performance.

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DISULFIDE-STABILIZED CHIMERIC ANTIGEN RECEPTOR FOR ENHANCED CANCER IMMUNOTHERAPY

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Abstract

When chimeric antigen receptor (CAR) T cells engage target cells, interactions between the two cell types exert mechanical forces. These forces are mediated through the CAR antigen-binding domain, which is most often a single-chain variable fragment (scFv). scFvs typically exhibit lower stability compared to whole antibodies or T cell receptors and may be more sensitive to mechanical forces, which could affect the responsiveness of CAR T cells. We designed a new type of interaction domain stabilized by disulfide bonds and incorporated it at the termini of various scFvs to enhance their stability and resistance to mechanical forces. Primary human T cells were transduced with lentiviral vectors encoding CARs that incorporate disulfide-stabilized interaction domains. CAR T cell functionality was assessed through cytotoxicity assays against different cancer cell lines, cytokine secretion analysis, and an antitumor efficacy study in a xenograft lymphoma mouse model. Disulfide-stabilized CAR T cells showed enhanced cytotoxic activity and increased cytokine secretion compared with conventional CAR T cells in vitro, using CARs targeting three different antigens. In vivo, mice treated with disulfide-stabilized CAR T cells exhibited prolonged survival and improved tumor clearance compared with the control group. Taken together, these findings demonstrate that the disulfide-stabilized interaction domain enhances the cytotoxic activity of CAR T cells by stabilizing the antigen-binding domain. As the interaction domain is modular, this approach could be applied to diverse CARs, potentially improving their therapeutic efficacy and enhancing the efficiency of CAR T cell therapy.

ELECTROTRANSFER OF NAKED TRANS-AMPLIFYING MRNAS FOR CANCER GENE THERAPY

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Abstract

Transient gene expression and effective delivery strategies are two key challenges in mRNA therapeutics. In our study, we addressed these challenges in the field of oncology using the B16-F10 melanoma model by introducing a two-component trans-amplifying mRNA system (taRNA)[1] and electroporation as a local intratumoral delivery method. The taRNA system consisted of two mRNAs: one encoding an alphaviral replicase and the other, a transreplicon, encoding a reporter gene to enable the evaluation of transfection efficiency. The untranslated regions of the transreplicon were designed based on predicted secondary structures to ensure the specificity and versatility of the system. We demonstrated that both electric pulse parameters and the doses of the individual components influence the efficiency of mRNA delivery as well as cytotoxicity. We successfully delivered both taRNA components into tumor cells, resulting in efficient trans-amplification and elevated and prolonged transgene expression. The established optimal parameters for the naked taRNA GET system in murine B16-F10 melanoma cells provide a basis for establishing an intratumoral in vivo system using naked taRNA electrotransfer.

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ENHANCEMENT OF CHIMERIC ANTIGEN RECEPTOR ACTIVATION FOR CANCER IMMUNOTHERAPY THROUGH TRANSMEMBRANE DOMAIN OLIGOMERIZATION

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Abstract

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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EVALUATION OF UPSTREAM PROCESS PARAMETERS INFLUENCING RAAV GENOME INTEGRITY

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Abstract

Recombinant adeno-associated virus (rAAV) vectors are among the most widely used gene delivery platforms in gene therapy. While vector genome titers are commonly used to evaluate production performance, genome integrity is increasingly recognized as a critical quality attribute, since fragmented vector genomes may reduce therapeutic efficacy and compromise transgene expression. Therefore, optimizing manufacturing parameters that influence genome integrity is essential for producing high-quality rAAV vectors.

We produced rAAV vectors using triple transfection of suspension HEK293T cells. Two commercially available transfection reagents, KyFect and FectoVir, were evaluated for the production of rAAV serotype 2 and 9. Production was initially performed in 50 ml and 30 ml cultures before being miniaturized to a 3 ml scale for higher-throughput screening. Subsequently, a Design of Experiments (DoE) approach was implemented to investigate the effects of key transfection parameters on vector yield and quality. Capsid titers were determined using ELISA, while vector genome titers and genome fragmentation profiles were assessed using a multiplex digital PCR assay targeting four regions across the vector genome.

Comparative evaluation of the transfection reagents revealed serotype-dependent differences in production performance. Multiplex dPCR analysis demonstrated distinct genome fragmentation patterns in rAAV2 vectors produced with the two transfection reagents in the 30 ml production format. Vectors produced with KyFect exhibited a higher proportion of complete genomes and improved genome integrity, whereas vectors produced with FectoVir showed enrichment of specific single-target genome fragments containing only the 3' polyadenylation signal. The DoE-guided experiments identified optimal transfection parameters influencing both vector yield and the proportion of complete genomes.

This work represents the first step toward establishing a robust rAAV production platform in our laboratory. The developed workflow enables detailed characterization of vector genome fragmentation and provides a foundation for the systematic investigation of the relationships between process parameters, vector genome integrity, and vector quality.

FROM METABOLIC DYSFUNCTION TO THERAPY: A NOVEL MOUSE MODEL OF ADSL DEFICIENCY

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Abstract

Adenylosuccinate lyase (ADSL) deficiency is a rare inherited disorder of purine metabolism characterized predominantly by neurological manifestations. Impaired ADSL activity leads to the accumulation of the characteristic metabolites succinyladenosine (SAdo) and succinylaminoimidazole carboxamide riboside (SAICAr). Despite increasing knowledge of its genetic and metabolic basis, the pathophysiology of ADSL deficiency remains incompletely understood and no effective disease-modifying treatment is currently available.

Here, we established and validated a novel mouse model of ADSL deficiency that recapitulates the key biochemical hallmark of the human disease, with markedly elevated levels of SAdo and SAICAr. We are performing comprehensive phenotypic characterization of the model to define the consequences of ADSL dysfunction and determine to what extent they recapitulate the clinical manifestations observed in patients.

In parallel, we are using this model for preclinical evaluation of a therapeutic approach employing an episomal vector designed to restore functional ADSL expression. Together, our model provides a relevant in vivo platform for investigating the pathophysiology of ADSL deficiency and evaluating potential therapeutic strategies.

GENOME INTEGRITY AND EMPTY CAPSID CONTENT AS PREDICTORS OF RAAV FUNCTIONAL POTENCY: EVIDENCE FROM HEPATOCYTE TRANSDUCTION STUDIES

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Abstract

Recombinant adeno-associated virus (rAAV) gene therapy products require rigorous quality characterization, yet conventional analytical methods often fail to capture the full complexity of capsid content and genome completeness. This study systematically evaluated how viral genome fragmentation and empty capsid prevalence affect functional potency in a hepatocyte model.

Thirteen fractions of rAAV2-GFP were collected across the empty, full, and tail regions of an anion exchange chromatography (AEX) elution gradient. Genome integrity was characterized using multiplex digital PCR (3-plex and 4-plex assays), quantifying complete genomes alongside fragmented species. Capsid occupancy (full, partial, empty) was assessed by mass photometry. Six fractions with the highest titers were normalized to equivalent complete genome counts and used to transduce HepG2 hepatocytes. GFP expression was monitored kinetically at 12-hour intervals from 24 to 53 hours post-transduction via automated image analysis, and quantified at endpoint by flow cytometry.

Total viral titers ranged from ~4×10^{7} to ~2×10^{12} vg/mL; complete genome titers spanned 1×10^{7} to 1×10^{12} vg/mL, with complete genomes constituting at most 46% of all detected genome fragments. Fractions originating from the empty-capsid AEX region exhibited minimal GFP expression with negligible temporal progression. Notably, fractions from the full-capsid region produced substantially different GFP fluorescence levels despite normalization to identical complete genome counts. Flow cytometry revealed a clear inverse relationship between per-cell fluorescence intensity and the proportion of empty capsids or capsids harbouring fragmented genomes.

These results establish that both genome integrity and empty capsid content are independent determinants of rAAV functional potency in hepatocytes. Multi-parametric characterization by integrating digital PCR-based genome integrity assessment with capsid occupancy profiling is essential for accurate potency prediction and robust quality control in rAAV vector manufacturing.

LOWER COST, HIGHER PERFORMANCE: OPTIMIZED SARNA PRODUCTION AND PURIFICATION

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Abstract

Self-amplifying RNA (saRNA) is a promising vaccine platform that encodes both the target antigen and replicase, enabling intracellular RNA amplification and potentially lowering the required therapeutic dose. However, its large size presents challenges related to stability, degradation and presence of double-stranded RNA (dsRNA) impurities. Therefore, optimization of in vitro transcription (IVT) reaction step to reduce the use of expensive reagents, such as capping analog and optimization of purification step maximizing saRNA recovery and purity is essential for cost-efficient manufacturing of high quality product.

Design of experiment (DoE) approach was used to optimize the IVT reaction. Mg:NTP ratio together with co-cap concentration was identified as the main factor affecting capping efficiency. The optimized process increased saRNA yield to approximately 8 mg/mL, reduced dsRNA levels, and maintained capping efficiency above 80% while reducing co-cap consumption 6.5-fold. Subsequent Oligo(dT) chromatography followed by acidic Oligo(dT) polish step further reduced dsRNA levels. In dsRNA-sensitive A549 cells, two-step purified saRNA induced higher protein expression than saRNA purified by Oligo dT alone, supporting the importance of effective dsRNA removal. Overall, the optimized process supports cost-efficient production of high-quality, functional saRNA.

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PUS3 DEFICIENCY CAUSES A SEVERE MULTISYSTEM DEVELOPMENTAL DISORDER

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Abstract

PUS3 encodes a pseudouridine synthase involved in post-transcriptional modification of tRNAs. Biallelic pathogenic variants in human PUS3 are associated with a rare neurodevelopmental disorder characterized mainly by developmental delay, intellectual disability, microcephaly, hypotonia, and epilepsy, with additional ocular and congenital abnormalities reported in some patients. However, the broader developmental role of PUS3 remains poorly understood.

We characterized a Pus3em1 mutant mouse model to investigate the consequences of PUS3 deficiency during development. Homozygous mutants exhibited severe congenital abnormalities and died immediately after birth. Affected animals showed microphthalmia and impaired eye development, while high-resolution echocardiography revealed cardiac arrhythmias. In addition, abnormalities of hematopoiesis were observed, indicating involvement of multiple organ systems.

These findings demonstrate that PUS3 deficiency causes a severe multisystem developmental phenotype and suggest that the disease spectrum may extend beyond the primarily neurological manifestations described in patients. The Pus3em1 mouse provides a valuable model for studying disease mechanisms, identifying critical developmental windows, and evaluating future gene-replacement or other molecular therapeutic strategies.

QUANTIFICATION OF CELL ELECTRO FUSION RATE BETWEEN MESENCHYMAL STEM/STROMAL CELLS AND CHONDROCYTES USING IMAGING FLOW CYTOMETRY

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Abstract

Tissue regeneration is a rigorously regulated process which helps maintain tissue homeostasis and restores the functionality of injured tissues and organs. Mesenchymal stem/stromal cells (MSCs) play a key role in this process, with one of their mechanisms of action being cell fusion[1].

As hybrid cells created through fusion of two cells of different origin are proposed to possess key qualities of both parent cells, such as the capacity for self-renewal and terminal differentiation, they could represent an alternative for somatic cell therapies[1]. Experimentally induced cell fusion methods, such as polyethylene glycol-mediated, viral-mediated, and electrofusion, are often used to induce cell fusion in vitro. As articular cartilage possesses low intrinsic regenerative capacity, we have identified the cartilage resident cells, chondrocytes, as fusion partners for MSCs in establishing a potential hybrid cell-based advanced therapy medicinal product[2]. We hereby present a method which can be used for quantification of such cell fusion products.

We electrofused a mix of two primary human partner cells using a previously established protocol[3]. Briefly, chondrocytes were labelled with the green cytoplasmic dye CMFDA and MSCs were labelled with orange cytoplasmic dye CMRA. Cells were joined together and exposed to a train of 8 × 100 µs pulses at electric field strengths ranging from 0.8 to 1.0 kV/cm. Cells were analysed 48h post electrofusion using imaging flow cytometer Amnis ImageStream X Mk II. Analysis showed that the rate of successful cell fusion ranged from 2.9 % to 4.7 % at 0.8 kV/cm and from 3.2 % to 8.4 % at 1.0 kV/cm, indicating that the interindividual variability between cells of different donors was observable.

The presented method enables fast, effective and reproducible quantification of cell fusion products and may be adapted to other cell-fusion systems.

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VIRAL VECTOR PRODUCTION IN FIXED-BED BIOREACTORS: DEVELOPING A DOWNSCALE MODEL TO BRIDGE THE GAP BETWEEN DISCOVERY AND PROCESS SCALE-UP

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Abstract

Limited access to scalable and GMP-compliant viral-vector manufacturing constrains academic translation of gene-modified cell-based advanced therapy medicinal products (ATMPs). A survey of EU academic, nonprofit and commercial ATMP facilities identified scale-up complexity as the most frequently reported production challenge, with cost-effectiveness and regulatory compliance identified as key priorities (1).

In response, a γ-retroviral vector manufacturing platform was developed for an engineered T-cell therapy targeting haematological malignancies. The process was transferred from T175 flasks to a scale-X hydro fixed-bed bioreactor, providing a closed system with controlled culture conditions and continuous monitoring. This reduced dependence on labour-intensive flasks for viral production. The fixed-bed bioreactor supports higher production capacity and process consistency while reducing contamination risk. Following the scale-up study, a flask-based downscale model was developed and assessed for its reproducibility and comparability with the fixed-bed bioreactor. Preliminary studies with this model allowed identification of several process parameters that can affect vector functionality and yield. The settings of these parameters can be further optimized before being tested at bioreactor scale. The downscale model has shown the potential to increase the efficiency of process characterization and development by reducing the need for resource-intensive large-scale runs, while the fixed-bed bioreactor provides a platform for development of a robust, GMP-oriented production process.

References

(1) Ramesh, G., Sebestyen, Z., Deshantri, A.K. et al. Current capacity and gaps in viral vector manufacturing: a survey of centers associated with CTIWP of EBMT, NXTGEN-hightech, DARE-NL, T2EVOLVE, and GoCART coalition. Bone Marrow Transplant 61, 245–248 (2026). https://doi.org/10.1038/s41409-025-02774-0

Acknowledgements

We gratefully acknowledge the support of our funders, partners and sponsors, whose contributions helped make CTGCT Day 2026 possible.

Co-funded by the European Union, this project has received funding from Horizon Europe (grant agreement No. 101059842) and European Regional Development Fund EU under Slovenia's Cohesion Policy Programme 2021-2027 (grant agreement No. C3360-25-952005) and the UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee (grant number 10069462). Views and opinions expressed are of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Commission nor the granting authority can be held responsible for them.

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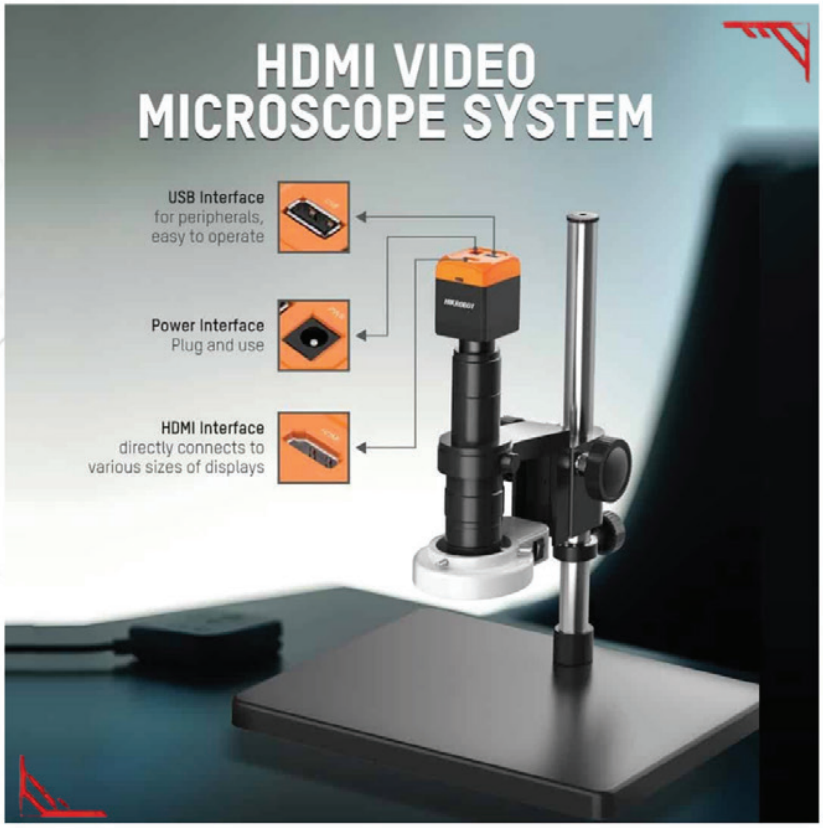
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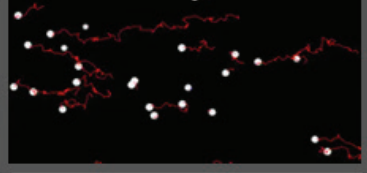


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