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Book of Abstracts

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Oral Presentations

O.01

Metabolic reprogramming enhances stem-like and functional properties of lung cancer-derived TILs

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Abstract

Introduction

Lung cancer remains the leading cause of cancer-related death, driven by late-stage diagnosis and therapy resistance. Adoptive cell therapy (ACT) using tumour-infiltrating lymphocytes (TILs) offers durable responses in some cancers, but its success in lung cancer is limited by T-cell exhaustion and metabolic dysfunction. Nicotinamide riboside (NR), a precursor of NAD^+, supports mitochondrial activity and may restore T-cell energy balance. This study aimed to optimise expansion of lung cancer-derived TILs with improved metabolic resilience, stem-like properties, and anti-tumour function.

Materials and Methods

Tumour samples from lung cancer patients were cultured with IL-2 for 15 days, with or without NR supplementation. Expanded TILs were analysed by flow cytometry for CD3, CD4, CD8, CD39, CD69, PD-1, LAG-3, and TIM-3. Cytokine secretion (IFN-γ, TNF-α, IL-2, granzyme B) was measured following CD3/CD28/CD137 stimulation or autologous tumour co-culture. SIRT1 and C1QBP expression were quantified by qPCR as markers of NR-induced metabolic enhancement, while TCF-7 and IL-7R were assessed as indicators of a stem-like, memory-progenitor TIL phenotype.

Results

TILs were successfully expanded from 53 lung cancer patients. Strong proliferation correlated with higher $CD4^+$ support and enrichment of stem-like $CD39^-CD69^-CD8^+$ subsets. Elevated LAG-3 expression associated with reduced proliferation. NR supplementation enhanced TIL quality by expanding stem-like $CD8^+$ subsets, reducing PD-1, upregulating SIRT1, C1QBP, TCF-7, and IL-7R, and increasing TNF-α, IL-2, IFN-γ secretion, indicating improved metabolic, stem-like, and effector function.

Conclusion

Metabolic reprogramming with NR promotes a less differentiated, metabolically resilient, and tumour-reactive TIL phenotype, supporting its potential to enhance TIL-based immunotherapy for lung cancer.

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O.02

Mesenchymal stromal cells influence the epigenetic landscape of trained macrophages in a clinically relevant model of allergic asthma in vivo

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Abstract

Few therapeutic strategies have been developed to manage asthma, a chronic disease affecting over 300 million people globally where standard treatments are ineffective for severe asthmatics. The innate immune system plays a significant yet complex role in asthma, influencing both development and progression of disease. Recent work from our lab suggests the bone marrow (BM) serves as an untapped reservoir shaping innate responses in allergic asthma through epigenetic imprinting of haematopoietic stem and progenitor cells (HSPCs). This process is known as trained immunity, a phenomenon that involves the metabolic and epigenetic rewiring of innate immune cells leading to enhanced innate responses upon exposure to secondary stimuli.

Using a murine model of allergic airway inflammation, BM macrophages were assessed following intravenous administration of MSCs. Integrated epigenetic and transcriptomic profiling revealed that in response to house dust mite alone, macrophages acquire pro-inflammatory profiles influenced by deposition of H3K4me3 at gene promoters of inflammatory cytokines, a process abrogated by MSC administration. The gain/loss of H3K4me3 is mirrored in macrophages restimulated with LPS ex vivo, where cells isolated from the MSC cohort fail to induce pro-inflammatory phenotypes. Functionally, MSC administration significantly attenuates development of severe disease in the lung, shown through the reduction of Th2 cytokines in bronchoalveolar lavage fluid.

Our findings highlight that HSPCs in the BM play a central role in driving immune dysregulation in asthma and demonstrate that MSC therapy not only modulates disease of the airway but also through the epigenetic reprogramming of innate immune progenitors in the BM.

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O.03

Enhanced retinal pigment epithelial cells as a delivery vehicle for retinal disease

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Abstract

Age-related macular degeneration (AMD) is a progressive retinal disease and a leading cause of irreversible vision loss, affecting more than 200 million people worldwide. The condition arises from dysfunction and degeneration of the retinal pigment epithelium (RPE), leading to photoreceptor loss and central vision impairment. As AMD advances, two clinical forms emerge. The exudative or wet form accounts for about 15% of cases and is driven by abnormal vascular endothelial growth factor (VEGF) signalling, while the non-exudative or dry form represents most cases and is associated with chronic inflammation and complement activation. Although anti-VEGF therapies such as aflibercept have transformed the management of wet AMD, they require frequent intravitreal injections and do not prevent disease progression. Furthermore, no approved treatments currently exist for atrophic AMD in Europe.

To address these challenges, we generated iPSC-derived RPE cells transduced with adeno-associated viral (AAV) vectors to express and secrete aflibercept and soluble CD59 (sCD59), a complement inhibitor that blocks membrane attack complex (MAC) formation. These modified RPE cells produced stable and sustained levels of both therapeutic proteins *in vitro*. The secreted proteins reduced MAC formation and prevented pathological endothelial permeability *in vitro*. Subretinal transplantation of the enhanced RPE cells into the JR5558 mouse model of spontaneous choroidal neovascularization significantly reduced vascular leakage and lesion area.

These findings demonstrate a gene- and cell-based approach for durable, localised delivery of therapeutic proteins that target both angiogenic and complement-mediated pathways. This strategy offers a promising route for long-term treatment of both wet and dry AMD.

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O.04

Engineering T cells with synthetic promoter sensors that respond to exhaustion and improve cellular immunotherapy

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Abstract

Cellular Immunotherapy using CAR T cells is a successful example of a synthetic biology approach to improve the outcome of blood malignancies. Unfortunately, it remains challenging to target solid tumors, and one of the reasons is that T cells develop exhaustion (Tex). These Tex are characterized by the upregulation of specific transcription factors (TFs). Strategies to prevent or reverse Tex are extremely needed. We generated and screened a library of 80+ newly engineered synthetic promoters (SPs) sensing six TFs upregulated in Tex. We validated our best SP targeting NR4A2 TF in an *ex vivo*Tex model following T CD8 cell engineering, and it showed outstanding progressive and differential kinetic induction. By coupling the sensors to actuators - T cell fitness and migration molecules (IL-12p70 and CCL21) - we demonstrated the controlled and reversible release of therapeutics when T cells progress towards the exhaustion state. Once co-engineered with NR4A2 sensors and anti-HER2 CAR and co-cultured with HER2+SKOV3 ovarian cancer cells, CD8+ T cells showed specific and reversible activation of the SPs and release of IL-12p70. In an *in vivo* melanoma xenograft tumor model, the sensors for T exhaustion showed induced and specific activity locally in the tumor. Collectively, we developed a tunable and versatile sensing platform to engineer T cells that operate locally within the TME and, once coupled to the release of therapeutic molecules, have the potential to overcome T cell exhaustion and improve cellular immunotherapy.

O.05

SPARCL1 overexpression modulates the repair and protective properties of the MSCs' secretome

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Abstract

Mesenchymal stem/stromal cells (MSCs) have attracted strong interest as potential therapeutcis for multiple disease or injury situations, in great part due to their relative ease of isolation and their ability to act as immunomodulators. Despite the abundance of promising pre-clinical evidence suggesting that MSCs may be effective in cardiac therapies, the translation to successful clinical trials has been minimal. We previously identified the matricellular protein Sparcl1 (also known as hevin) as being one of the major secreted factors playing a role in MSCs-mediated cardiac repair. Here, we describe the generation and characterisation of immortalised MSC lines overexpressing Sparcl1. The accumulation of Sparcl1 protein in the medium was quantified over time to determine the optimum conditions for conditioned medium (CM) preparation. The Sparcl1_MSC secretome was compared to the parental MSC secretome in its ability to modulate T-cell response and to protect cardiomyocytes and MSCs from hypoxic/ischemic induced death. We found that Sparcl1 overexpression significantly enhanced the capacity of the secretome to protect against oxidative stress, without altering immunosuppressive properties on activated T cells and pro-inflammatory macrophages. This work confirms the critical role of Sparcl-1 as a mediator of cardioprotection.

O.06

Identification of Therapeutic Responses of Mesenchymal Stromal Cells Retrieved from Murine Osteoarthritic Joints

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Abstract

Osteoarthritis (OA) is a widespread and debilitating joint disease characterized by synovial inflammation, cartilage degeneration and chronic joint pain. Mesenchymal stromal cells (MSCs) have shown therapeutic efficacy for diseases with a strong inflammatory profile, including OA. However, the disease-specific mechanisms of action remain unaddressed. In this study, we aimed to characterize the disease-induced profile of MSCs following exposure to the *in vivo* OA environment.

Murine syngeneic GFP + bone marrow-derived MSCs (BM-MSCs) were delivered via intra-articular injection in a mouse collagenase-induced osteoarthritis (CIOA) model (n = 8). BM-MSCs were retrieved by cell sorting on days 14 and 56, following whole mouse knee digestions. The retrieved cells were expanded in culture and characterized based on their phenotype, immunomodulatory effects on lymphocytes and macrophages, and transcriptomic profile.

Retrieved BM-MSCs (1.33%) had minimal effects on lymphocyte proliferation but induced macrophage anti-inflammatory activity. Surviving retrieved BM-MSCs activated several pathways, with their secretome impacting immune system regulation and extracellular matrix organization, correlating with the disease stage. Data comparing the transcriptomic profiles of retrieved and *in vitro*-licensed BM-MSCs suggested a chondroprogenitor profile and identified BRINP3 as a novel factor in MSC function for potential OA modulation.

The beneficial effects of BM-MSCs in OA post-local delivery could be attributed to a specific subset of cells able to resist the micro-inflammatory milieu and contribute to cartilage healing and suppression of associated synovial inflammation. Furthermore, data suggest a paradigm of environmentally guided plasticity associated with MSCs upon local delivery in both early and late OA.

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O.07

Assessing the impact of parent cell genetic modifications to generate visually traceable derivative EVs on EV characteristics and function

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Abstract

Extracellular vesicles (EVs) are nanoparticles emitted as a means of intercellular communication. In particular, mesenchymal stromal cell (MSC)-derived EVs exhibit traits (e.g., pro-angiogenic, immunomodulatory) ideal for tissue regeneration applications. Due to their small size, EV uptake by cells and biodistribution are difficult to assess, and many options for generating traceable EVs experience complications (e.g., non-specific tagging, radioactive elements and special facilities required, short half-life of fluorescent (FL) probes). Genetic modification (GM) of parent cells with PalmtdTomato (FL probe) or PalmGRET (FL and bioluminescent (BL) probe) generates EVs with strongly bright FL and/or BL membranes able to be quantified and visualized over long periods of time. Here, we assessed any other impacts that GMs to parent cells may have had on derivative EV properties. EVs were obtained from a murine bone marrow-derived MSC cell line (D1 ORL UVA cells; ATCC, Germany). Cells were naïve, FL (PalmtdTomato), or FL/BL (PalmGRET). EVs were harvested from each cell type by culturing cells in EV-depleted media for 48 h, and isolating EVs from conditioned media via serial centrifugation then size exclusion chromatography. All EV types were characterized according to the 'Minimal information for studies of extracellular vesicles (MISEV) 2023,' with traits of the different EV types compared. PalmtdTomato and PalmGRET EVs were also analyzed via super resolution microscopy and microplate reader. Additionally, the impact of EVs on D1 cell proliferation was evaluated and compared between EV types. Thus far, no differences have been observed between EV characteristics or functionality across groups.

O.08

Upstream process development for the biomanufacture of therapeutic MSC-derived extracellular vesicles.

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Abstract

MSC-derived Extracellular Vesicles (MSC-EVs) are emerging as a promising cell-free alternative to traditional Mesenchymal Stem Cell (MSC) therapy in regenerative medicine, offering enhanced safety profiles, ability to cross physiological barriers more readily and increased stability. Despite the advantages, there are many challenges remaining to be overcome including a process platform for scalable cost-effective production.

The focus of this work is the development of an upstream process platform for the production of therapeutic MSC-EVs. The ASC52telo hTERT immortalized adipose derived MSC cell line has been used. In order to move towards a scalable, GMP-ready process, the EV production process was transitioned from 2D T-flasks to 3D microcarrier-based cultures and scaled to 1 L. A single media formulation, suitable for both growth and collection phases of the process which is xeno-free has been developed to simplify operations, improve volumetric productivity and act as an enabler for continuous or semi-continuous operation. In order to characterize the impact on product quality, a novel flow cytometry based method on the Beckman Coulter Cytoflex-Nano was developed and used in conjunction with a panel of assays for analysis of the MSC cell phenotype, culture process parameters and EV characteristics.

On the basis of growth area, to date a greater than 5 fold improvement in EV productivity has been achieved.

O.09

Cell-selective delivery of nucleic acids for the treatment of glioblastoma

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Abstract

Glioblastoma (GBM) is an aggressive malignant tumour of the central nervous system. Despite current standard of care comprising surgical resection with radio- and chemotherapy, prognosis remains poor, with median survival around 15 months[1]. Gene therapy offers promise for GBM treatment, but delivery is hindered by the blood–brain barrier (BBB) and the diffuse, heterogeneous nature of GBM. CHAT is a multifunctional peptide for non-viral delivery of nucleic acids[2]. Here we have modified CHAT with a targeting sequence to produce NeuroCHAT, designed to specifically interact with receptors over-expressed on GBM cells and facilitate cell-selective delivery of novel therapeutics for GBM.

NeuroCHAT nanoparticles (NPs) were formulated with mRNA-GFP and physicochemical properties assessed using DLS, TEM and encapsulation efficiency. Transfection efficiency in U87-MG GBM cells and NCTC-929 fibroblast cells was analysed using FACS analysis and confocal microscopy. Cell viability was measured using alamarBlue assay.

NeuroCHAT produced cationic, spherical NPs (~61.27 d.nm) with >80% encapsulation efficiency. NeuroCHAT resulted in a 5-fold increase in transfection efficiency in U87-MG cells after transfection, compared with unmodified CHAT NPs. Cell viability was over 80%, indicating minimal toxicity.

NeuroCHAT enhanced gene delivery to U87-MG cells when compared to unmodified CHAT, with no toxicity in vitro. Further work will investigate NP penetration in a BBB transwell, before moving to in vivo studies investigating biodistribution, safety and efficacy. These initial results indicate the potential of NeuroCHAT for cell-selective delivery of nucleic acids specifically to GBM cells, with the potential to enhance treatment outcomes through efficient delivery of novel therapeutics.

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O.10

Revolutionizing viral vector production: Biodegradable hyperbranched poly (β-amino ester)-based transfection technology boosts yields and efficiency

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Abstract

Transient transfection using polyethyleneimine (PEI) is widely used to generate lentiviral (LV) vectors for CAR-T manufacturing but is limited by functional titres, process inflexibility and safety concerns. We developed BrPERfect®, a biodegradable hyperbranched poly(β-amino ester) (HPAE) transfection platform optimised for LV production and evaluated its impact on downstream CAR-T functionality.

Across four LV products produced in 125 mL shake flasks, BrPERfect increased infectious LV titres to 210–254% of PEI while maintaining comparable host-cell DNA (HCD) per transducing unit. In 3 L bioreactors, BrPERfect doubled LV harvest titres and reduced HCD per 10^7 TU by ~50%, supporting higher upstream productivity without increasing purification burden. LV drug substance manufactured with BrPERfect showed a 26% higher functional titre than PEI controls with similar residual HCD.

Using three donors and a four-point MOI matrix (0.5–4), we compared CAR-T cells generated with LVs produced by BrPERfect or PEI. Cell viability, proliferation and CAR expression kinetics were comparable between groups. However, at MOI 2, real-time killing assays demonstrated enhanced cytotoxicity for CAR-T cells transduced with BrPERfect-produced LVs (mean 76.9% vs 41.3% target-cell killing; ~1.9-fold increase), indicating improved functional potency at equivalent dose.

These data establish BrPERfect as a scalable, GMP-compatible alternative to PEI for LV manufacturing, enabling higher functional yields and more potent CAR-T products. The same HPAE platform, validated for targeted transfection of hard-to-transfect cells, provides a foundation for future delivery strategies.

O.11

Modifying Plasmids and Inverted Terminal Repeats improves Adeno-associated Virus Production and Performance

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Abstract

Introduction

Recombinant Adeno-associated Virus (rAAV) is a preferred vector in gene therapy due to its broad tissue tropism and good safety profile. However, manufacturing challenges, namely high production costs, is an inhibitory factor in widespread adoption. The most common approach for rAAV production involves triple transfection of HEK293 cells with 3 plasmids: pTransgene, pRep/Cap and pHelper. Sufficient plasmid production is challenging and can account for up to 40% of total batch costs.

This work aimed to increase plasmid production yields by replacing the origins of replication and antibiotic resistance markers. Furthermore, Inverted Terminal Repeat (ITR) sequences in pTransgene were modified, as it is thought that the strong secondary structures may interfere with plasmid production.

Methods

rAAV plasmids were modified by replacing backbone elements and targeted deletions within the ITRs. These plasmids were transfected into HEK293 cells before collecting and quantifying rAAVs via qPCR and ELISA. CHO cells were transduced with rAAVs to measure transgene expression by flow cytometry.

Results

A 140% increase in pHelper production was achieved, while the most successful ITR variant increased pTransgene plasmid yields by 57%. Transgene expression also increased by 28%, without reducing rAAV yields or transduction efficiency.

Discussion

Improvements in plasmid production will reduce rAAV production costs, whereas the observed increase in transgene expression could drive efficiency in gene therapy manufacturing as fewer rAAVs can achieve the same effect. Overall, these results offer a promising avenue to reduce production overheads to make gene therapy more accessible.

O.12

Can AAV vectors train the innate immune response?

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Abstract

Myopathies arise from monogenic genetic mutations, such as in Duchenne Muscular Dystrophy (DMD) where mutations in the *DMD* gene affect muscle architecture and integrity. While these conditions are rare, they affect patient quality of life and result in significant morbidity. Ongoing gene therapy research aims to resolve genetic mutations using adeno-associated viral (AAV) vectors which offers targeted delivery of genetic material and long-term gene expression. However, clinical trials have reported severe inflammatory responses following systemic administration of these therapies which highlight scope for further research in this area.

Innate immune cells are subject to reprogramming that can amplify early host defences to subsequent immunological challenges. This has been described for live-attenuated vaccines but has yet to be investigated for gene therapies.

Our studies aim to characterise whether AAV vectors can modulate innate immune responses using donor peripheral blood mononuclear cells. Monocytes were exposed to capsid particles from AAV vector serotypes 2, 5, 8, and 9 before differentiation into macrophages. Following a rest period, cells were re-stimulated with heterologous stimuli. Inflammatory responses following secondary stimulus were assessed by changes in activation markers and cytokine analysis for IFNα_2, TNF-α, IL-6 and IL-10. Our findings suggest that AAV vector training modifies macrophage inflammatory responses to heterologous stimuli.

These studies reveal under-appreciated interactions between gene therapy vectors and early responder inflammatory responses. Furthermore, understanding how AAV vectors elicit lasting effects on innate immunity will inform therapeutic strategies to increase safety profiles for future applications.

O.13

Enhancing AAV Production Yield for Pro-Apoptotic Gene Therapy Using an shRNA Strategy

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Abstract

Adeno-associated virus (AAV) is an important gene delivery vector with growing applications in genetic diseases and has great potential to deliver pro-apoptotic or cytotoxic genes as cancer therapy. However, producing AAV with these genes poses a major challenge: the encoded proteins can damage or kill producer cells during vector production, reducing cell viability and significantly lowering viral yield.

In this study, we developed a post-transcriptional knockdown strategy using short hairpin RNA (shRNA) to suppress expression of a pro-apoptotic gene during AAV production. TNF-related apoptosis-inducing ligand (TRAIL) was selected as the gene of interest (GOI). A *gfp-TRAIL* fusion construct was cloned between ITRs in a pAAV plasmid, and anti-*gfp* shRNA cassettes were inserted in the plasmid backbone. We hypothesized that shRNA-mediated silencing of *gfp-TRAIL* mRNA would prevent TRAIL expression in producer HEK293 cells, preserving cell viability while enabling efficient AAV packaging.

HEK293 cells transfected with the anti-*gfp* shRNA construct showed markedly reduced GFP expression, improved viability, and increased AAV yield compared with cells transfected with a scrambled shRNA control. Additionally, surface expression of death receptor 5 (DR5), which decreases when TRAIL is overexpressed, was significantly higher in anti-gfp shRNA-transfected cells compared to scrambled shRNA condition, confirming effective suppression of TRAIL during production.

These results demonstrate that TRAIL expression negatively impacts producer viability and AAV production, and that shRNA interference can restore both viability and yield, although not to wild-type levels. Targeting *gfp* rather than the GOI streamlines validation and supports broader application of this strategy for other cytotoxic transgenes.

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O.14

New gene(-editing) therapies and Justice – the challenges of access, equity and sustainability

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Abstract

Contemporary genomic technology is making significant advances in the development of human therapeutics. These achievements create new challenges concerning access, equity, and justice. In response, there are prominant calls for greater access and equity to such interventions. This presentation critically examines these calls, and argues for a broader 'justice in healthcare and innovation' approach—one that situates demands for equity in access to gene and gene-editing therapies within a wider non-exceptionalist framework of justice in healthcare.

The presentation draws on interdisciplinary insights from distributive justice in bioethics and political theory, with insights from the current technical and regulatory developments. I will consider Health Technology Assessments and explore the evolving landscape of payment and reimbursement models. Technical variables, such as ex vivo versus in vivo approaches, are examined, as are the high cost of robust clinical trials. The assessment also considers the idea versus reality of intellectual property and incentivising innovation, and the role of public funding in basic research.

Some discussions in 'justice in access' seem well placed to productively combine new empirical considerations with previous health and distributive justice debates. Conversly, some prominent calls for greater equity risk relying on overly idealised and exceptionalist assumptions regarding justice in exceptionally high-cost scientific-medical innovation.

I conclude that an expanded framework of ‘justice in healthcare and innovation’ provides a more realistic and sustainable basis for demands of equity and access to new genomic therapies. I reflect on new challenges emerging from the U.S., particularly the long-term sustainability of any call for justice.

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O.15

Immune Exhaustion Signatures In CD19 CAR T-Cell Recipients

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Abstract

Introduction:

Chimeric antigen receptor T-cell therapy is effective for relapsed/refractory high-grade B-cell malignancies. Despite response rates reaching up to 50%, it remains unclear what factors differentiate responders from non-responders. T-cell exhaustion, driven by prolonged antigenic stimulation, may influence treatment outcomes. This study presents preliminary findings on exhaustion marker dynamics following CD19 CAR T-cell therapy.

Materials and Methods:

Nineteen patients treated with CD19 CAR T-cell therapy were studied. Peripheral blood mononuclear cells were collected at 1, 3, and 6 months following infusion. Flow cytometry quantified circulating CD19 CAR T-cells and the expression of TIM-3, PD-1, BTLA, CTLA-4 and LAG-3. Data analysis was performed using FlowJo (V10.8.1) and GraphPad (V10.2.0).

Results:

Patients received Kymriah® (n=14) and Yescarta® (n=5). At 6 months, 12 (63%) achieved complete response (CR), while 7 (37%) had persistent disease (PD). Mean CD19 CAR T-cell percentages declined from 2.92% to 1.82% between months 1 and 6, with absolute counts similarly decreasing. No significant differences in CD19 CAR T-cell numbers or percentages were observed between CR and PD ($p>0.05$). TIM-3 exhibited the highest levels of expression on CD19 CAR T-cells, followed by PD-1, BTLA, CTLA-4 and LAG-3. PD-1 expression on CD19 CAR T-cells was notably higher in the PD group, which was statistically significant at 3 months post-CD19 CAR-T cell therapy ($p=0.02$).

Conclusions:

CD19 CAR T-cells declined over time, and exhaustion markers showed dynamic expression patterns.

Elevated PD-1 expression at 3 months in the PD groups suggests a potential prognostic biomarker.

O.16

Opportunities and challenges of the use of the hospital exemption in the early-stage development of novel cellular therapies

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Abstract

Introduction: Advanced Therapy Medicinal Products (ATMPs), particularly novel cellular therapies, represent a significant technological advancement in medicine. These transformative therapies offer personalised and potentially curative treatments for rare, ultra rare and life-threatening diseases. However, their development and clinical use are often limited by regulatory inconsistencies and manufacturing, logistics, and access challenges. Aim: This research explores the role of the Hospital Exemption (HE) pathway within the European Union (EU) in facilitating early access to personalised novel cellular therapies for patients with unmet medical needs where, there are no alternative treatments available. Methodology: A mixed-methods approach was employed to investigate the regulatory variations across the EU, manufacturing and logistical challenges, and ethical concerns associated with the use of the HE pathway. The research also examined unique opportunities that the HE pathway could provide to drive innovation and improve patient access, particularly for therapies developed in academic settings. Results: The main findings of this research highlight the need for harmonised regulations, improved transparency, and standardised data collection across Member States for the HE pathway. Conclusion: Based on these findings, the research provides recommendations to optimise the HE pathway's potential in supporting early-stage development of novel cellular therapies while maintaining patient safety and public trust.

Poster Presentations

P.01

AI-Driven Morphology Profiling to Monitor MSC Functional States

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Abstract

Mesenchymal stromal cells (MSCs) therapeutic efficacy largely stems from immunomodulatory and anti-inflammatory properties. Cellular morphology profiles dynamically reflect MSC functional states, suggesting that image-based approaches can serve as non-invasive tools to predict cell potency. This study explores an image-based artificial intelligence (AI) analysis using brightfield microscopy images to predict MSC functional abilities, aiming toward future applications in MSC manufacturing and quality control for standardized, rapid, and cost-effective potency assessment.

Human MSCs were licensed with inflammatory cytokines for 24-72 hours to enhance their immunosuppressive ability. At each point, MSCs were imaged using brightfield and F-actin fluorescence microscopy, alongside immunofluorescent staining for Intercellular Adhesion Molecule 1 (ICAM-1) and Indoleamine 2,3-dioxygenase (IDO). Functional assay quantified L-Kynurenine concentration in the supernatant as a surrogate marker of IDO enzymatic activity. Morphological feature profiles were extracted from F-actin-stained images using CellProfiler. Dimensionality reduction was applied, and top informative morphological features were selected for predicting MSC functional abilities and compared with those derived from brightfield images segmented through an in-house built AI model.

Licensing induced a time-dependent increase in ICAM-1 and IDO expression, as well as L-Kynurenine production, which was associated with distinct morphological changes in MSCs. These changes were accurately predicted by both CellProfiler-assisted fluorescence image analysis and AI-assisted label-free image analysis. These findings support the feasibility of AI-driven morphology profiling as a real-time approach for monitoring MSC function. Future work will extend this framework to improve MSC potency prediction directly from label-free images, providing a foundation for morphology-based quality control in cellular medicine manufacturing.

P.02

Use of DESI-MSI to Visualize and Analyse Spatial Lipidomics of Human Bone Marrow Mesenchymal Stromal Cells in Culture

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Abstract

Desorption electrospray ionization mass spectrometric imaging (DESI–MSI) is a label-free, non-destructive imaging technique that enables in-situ evaluation of metabolites and lipids in cells and tissue sections. It provides spatial information and quantification of metabolites on the cell surface and therefore has the capacity to provide important information regarding metabolic markers and lipid signals. We have applied DESI-MSI as a novel analytical tool for characterisation of cultured mesenchymal stromal cells (MSCs) with applications in process monitoring, potency assays and quality release tests. In one particular application, we have employed this approach to study the effects of media composition on cells, thereby providing a rapid and information-rich strategy for media development.

Bone marrow-derived MSCs were expanded in serum-containing and xeno-free media and analysed on a DESI-MSI/Xevo-QTOF platform (Waters) to obtain lipidomic data in the 50-1200mz range. Mass spectral data were analysed using the web-based open-source MetaboAnalyst platform and showed clear differences in the lipidomic signatures between cells expanded under the 2 conditions. Further work is underway to apply this strategy in MSC-T cell and MSC-macrophage interactions.

This work provides confirmation of the utility of this approach in precision metabolomics applications in cell processing and cell-cell interactions.

P.03

High-Throughput and AI driven formulation using Microfluidic Platform for Accelerating Nanomedicine Development

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Abstract

Lipid nanoparticles (LNPs) have emerged as a critical drug delivery system particularly in RNA therapeutics. However, conventional microfluidic systems for LNP synthesis and screening suffer from low throughput, large sample waste of expensive materials and/or cost-contamination. Here, we present a high-throughput microfluidic platform designed to overcome these limitations, enabling rapid and efficient LNP preparation. The platform uses 8 parallel flow focusing channels for fast and efficient formulation screening which is compatible with 96-well plate. Benchmarking studies of one of the machines, MiNano-Form at a trial partner site validate its ability to synthesize high-quality LNPs (<100 nm size, PDI < 0.2 and >85% encapsulation efficiency) which is comparable with market leaders. The patented mixing design allows for precise nanoparticle synthesis with minimal reagent consumption (<400 µL) and lower flow rates, significantly reducing costs while improving efficiency. The system has been developed for 4 years and tested in CDMO, trial labs and gene therapy companies, and will be launched in 2026. Along with artificial intelligence, the formulation optimization will be demonstrated for the treatment of cancer and genetic dieasese. The benifits of AI-driven formualtion solutions will be shared with our Nanomind AI software and high throughput microfluidic formulation platform.

P.04

Bridging research to GMP manufacturing: scalable cell therapy automation through the Solupore® Integrated platform

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Abstract

Autologous cell therapy access remains limited by high costs, manual operations, and complex workflows. GMP-compliant production requires integrated, automated, and scalable technologies that enhance reproducibility and reduce cost per dose. The Solupore® Integrated Cell Therapy Manufacturing platform represents a next-generation solution that supports manual research scale development through to process automation and commercial scale-out.

The Solupore® Integrated platform is a consumable-based and digitally automated system that unifies activation, transfection, transduction, and expansion within a closed process architecture. A GMP-aligned core controller manages process automation, data integrity, and traceability under 21 CFR Part 11 compliance, supporting a seamless transition from research use to GMP manufacturing. The platform enables parallel batch processing for efficient and scalable production. Platform performance was assessed using a model 7-day CAR-T process.

The system consistently produced >2.5×10^9 viable cells and >1×10^9 CD19-CAR$^+$ cells from 1.5×10^8 CD3$^+$cell input, achieving >40% CAR expression and approximately 17-fold expansion. The resulting cells exhibited a stem-like phenotype with low exhaustion and potent cytotoxicity at a multiplicity of infection (MOI) of 1. Integrated transfection capability within the same closed workflow uniquely enables gene editing and other cell engineering workflows. The architecture supports up to 350 batches per integrated system annually, incorporating automated sensing, closed-loop control, and electronic batch records to ensure process robustness and GMP alignment.

These results demonstrate that the Solupore® Integrated platform delivers a scalable, automated, and regulatory-compliant manufacturing approach that can accelerate clinical translation, enhance product consistency, and improve the economic feasibility of advanced cell therapies.

P.05

Expansion of human T cells using TheraPEAK® AmpliCell® Cytokines

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Abstract

The cell and gene therapy landscape has seen significant advancements, as evidenced by recent approvals of novel therapeutics and a robust pipeline of developmental programs targeting various indications. The development of clinical-grade products for cell and gene therapy heavily relies on the availability of cGMP-compliant high-quality raw materials used during the manufacturing process, to allow consistent manufacturing of robust drug products that are safe for patient use.

To address this need, we are introducing Lonza's TheraPEAK AmpliCell Cytokines, which are GMP-grade, animal-component free, xeno-free products that are produced in human cell lines thus enabling these proteins to express the appropriate post-translational modifications characteristic of human cytokines. To demonstrate the expansion and downstream application of TheraPEAK AmpliCell Cytokines, we cultured healthy human peripheral blood mononuclear cells (PBMC) or enriched T cells in either serum-containing conditions or in serum-free culture, supplemented with either IL-2 alone or IL-7 and IL-15 cytokines.

We demonstrate that TheraPEAK AmpliCell Cytokines support the expansion of highly viable T cells, exhibiting preferred stem-like T_{SCM} or central T_{CM} characteristics. Transduction efficiency was similar for the different cytokine conditions, and CAR-T cells demonstrated robust cytotoxicity upon co-culture with tumor cells. Notably, during the co-culture with NALM6 cells, we observed the enrichment of CD19-CAR T cells demonstrating preferential expansion of antigen-specific tumor-killing T cells. Altogether, our data demonstrate that TheraPEAK AmpliCell Cytokines are able to support robust expansion of genetically modified T-cell products that exhibit strong phenotype and function.

P.06

Guide to GMP manufacturing of pDNA for viral vector manufacturing

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Abstract

Plasmid DNA is a critical starting material in the production of viral vectors and nucleic acid based vaccines. Although platform processes for large-scale pDNA manufacturing have been established since the 1990s, ambiguity remains regarding specific quality and documentation requirements despite existing regulatory guidance.

This presentation provides practical guidance for sponsors planning to engage CDMOs for GMP pDNA production. The discussion will focus on the planning, execution, and support of multi-plasmid manufacturing campaigns—such as those required for viral vector systems that use several helper plasmids and a transgene plasmid. For this session, a scenario involving up to four plasmids will be considered.

Key topics include the establishment and testing of master cell banks, understanding CDMO platform pDNA process flows, timelines, and assessing how CDMO process options can affect yield and purity. The presentation will also outline essential documentation requirements before and after manufacturing, regulatory expectations, and strategies to ensure compliance and readiness for release.

Finally, preparatory activities that can streamline CDMO engagement—such as understanding analytical package selections, small-scale process evaluation, and document readiness—will be highlighted. Attendees will gain an understanding of critical planning elements and decision points that influence successful GMP pDNA manufacturing campaigns and collaboration with CDMOs.

This session will be valuable for organizations seeking to establish or outsource pDNA manufacturing, offering a framework to align technical, regulatory, and operational expectations with CDMO partners.

P.07

How to Design an Autologous CAR-T Manufacturing Facility

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Abstract

CAR-T cell therapy disrupted the cancer treatment landscape with the first commercial approval in 2017 for Novartis's Kymriah. Several CAR-T products have been approved since then with the focus of the early commercial approvals being speed to market. All commercially approved CAR-T therapies to date have been autologous products, meaning that the starting material is drawn from the patient (apheresis). This brings significant challenges with respect to the cell viability and batch to batch variability.

This field is rapidly evolving with respect to the available process technology, allowing the initially very manual processes to now be produced on integrated and fully automated manufacturing platforms. These advances in process technology have produced a shift in focus from speed to market to process optimisation, with the decision on "when to close" and "when to automate" being key factors in facility design requirements. This presentation will walk through the key decisions and operational philosophies associated with designing a flexible and agile facility, to suit production launch requirements as well as potentially unknown future needs. Case studies will be presented to highlight optimised facility design examples that also consider sustainable design requirements.

Meeting high production demands requires a scale-out approach as the batch size is fixed at one patient. This approach combined with the variability in starting material brings challenges in determining the required equipment quantities to meet the throughput requirements whilst also "right sizing" your facility. This presentation will demonstrate how these factors can be addressed through process simulation for optimised facility designs.

References

This abstract is based on Arcadis's project experience designing cell therapy manufacturing facilities for our C&GT clients. The case studies presented in this presentation will be based on real examples of facility designs.

P.08

Successful collection & processing of patient cells for Chimeric Antigen Receptor T (Car-T) Cell Therapy: The St James Hospital Experience.

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Abstract

Introduction/Background:

Chimeric Antigen Receptor (CAR) T cell therapy has proven to be very effective in treatment of relapsed /refractory CD19 positive disease. The modification of the autologous T cells requires effective collection, processing and shipment of starting material. We reviewed the first 155 patient collections for CAR T therapy.

Materials and Methods: Mononuclear cells were collected by apheresis MNC(A) under mandatory regulations of the Tissue Establishment HPRA Authorisation. 155 patients underwent MNC (A) product collection (November 2021-August 2025). Patient indications for CAR T were DLBCL (n=109), NHL (n=4), Richter's CLL (n=2), PMBL (n=6), FL (n=26) and ALL (n=8). Apheresis was performed on Spectra Optia Apheresis system, using MNC programme. No adverse patient reactions occurred during apheresis.

Results: 142/155 (91%) patients had single day collection and 13 patients required a second day collection. Patient PB FBC and CD3 counts were performed on the day. All MNC (A) products were assessed for sterility, TNC and CD3 content prior to shipment. 148 Final CAR-T products were received post manufacture with Median TAT of 28 days (19-35). 4 manufacturing failures and 3 cancellations during manufacturing occurred. CAR T Products are verified and stored in vapour phase LN2 while patients receive lymphodepletion. 127 Final CAR T products were thawed at the bedside and infused in this patient cohort to date.

Conclusions: We have successfully collected, processed and shipped MNC (A) products for over 155 patients. The criteria for commercial CAR T Manufacture was met and only 4 manufacturing failures have occurred.

P.09

Redirecting MAIT Cells against Melanoma Through PD-1 Blockade and Bispecific T Cell Engagers

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Abstract

Mucosal-associated invariant T (MAIT) cells are unconventional T cells that play essential roles in host defence against bacterial and viral pathogens. Recently, studies have demonstrated their capacity for rapid target cell lysis and robust production of effector cytokines, including Granzyme B and IFN-γ, highlighting their anti-cancer capacity. In addition, MAIT cells are not restricted by classic MHC molecules and do not induce graft-versus-host disease. These features position MAIT cells as promising candidates for adoptive cellular immunotherapy.

In this study, using multi-parameter flow cytometry, we report a significant reduction in circulating MAIT cells in a cohort of patients with metastatic melanoma, with reduced cytotoxic function indicated by decreased degranulation, Granzyme B, and IFN-γ. PD-1 signalling drives these defects. However, after six months of anti-PD-1 therapy, MAIT cell numbers and functions were restored, highlighting their responsiveness to immunotherapy.

The loss of MAIT cells is associated with poor outcomes in metastatic melanoma, so we next explore their potential as a cellular therapy. We demonstrate that MAIT cells can be redirected via clinically relevant bispecific T cell engagers to kill metastatic melanoma cells *in vitro*. In addition, the MAIT cell secretome directly enhanced melanoma cell line death. Finally, we demonstrate that IFN-γ within the MAIT cell secretome induced tumour sensitisation to cell death pathways and robustly activated NK cells, amplifying downstream anti-tumour responses.

Collectively, these findings highlight MAIT cells as a potential novel approach for cancer immunotherapy and provide a rationale for further investigation of MAIT cells in cancer treatment.

References

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P.10

Cytokine-Induced Memory-Like NK Cells as a Novel Immunotherapeutic Strategy for Oesophageal Adenocarcinoma

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Abstract

Oesophageal adenocarcinoma (OAC) is a poor-prognosis cancer with a 5-year survival rate of less than 25%. Response rates to chemo-radiotherapy are approximately 30% and new therapeutics are urgently needed. The current response rate to available immunotherapies stands at less than 25%. The use of Natural Killer (NK) cells as adoptive cellular therapy faces a number of challenges, including persistence in vivo, and effective tumour homing and infiltration. The emergent NK cell subset, cytokine-induced memory-like (CIML) NK cells, may be capable of greater efficacy and persistence in the solid tumour microenvironment. Here, we propose to investigate the therapeutic potential of CIML NK cells in OAC.

We have developed a robust and reproducible protocol for the development of CIML NK cells from healthy donor blood. Furthermore, we have shown that this population contains high frequencies of the reprogrammed memory-like cells, eML NK cells. Phenotyping data show that CIML NK cells, and eML NK cells in particular, have a highly activated phenotype. Upon restimulation, CIML NK cells produced higher levels of cytotoxicity marker Granzyme B (GzmB) and cytokines TNF-α and IFN-γ. Importantly, NK cell killing activity against K562 cells was conserved between control NK and CIML NK cell populations, suggesting CIML NK cells do not become functionally exhausted following culture and re-stimulation.

Future work will examine CIML NK cell migration towards OAC tumour. We will interrogate their potency within the OAC tumour microenvironment and identify whether specific pathways can be targeted to maximise CIML NK cell infiltration and killing of OAC tumours.

P.11

Testing EV-Based Therapeutic Strategies in Physiologically Relevant In Vitro Lung Adenocarcinoma Models

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Abstract

Introduction

Lung adenocarcinoma (LUAD), the most prevalent subtype of non-small cell lung cancer, remains a major contributor to cancer-related mortality [1]. Systemic therapies often fail due to poor tumour-targeting, resistance, and toxicity [2-4]. Our project investigates a novel inhalable gene therapy approach using mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) loaded with p53-targeting microRNAs and aimed at local administration, using physiologically-relevant LUAD models.

Methods

We employed three-dimensional (3D) air–liquid interface (ALI) mono- and co-cultures of LUAD cells, with or without lung fibroblasts, previously established in our lab [5-6]. Fluorescently labelled MSC-EVs (containing no therapeutic cargo) were applied apically or basolaterally, and uptake was assessed via confocal microscopy. Ten bioengineered MSC-EV formulations targeting the p53 pathway, identified through conventional 2D *in-vitro* testing, were then tested for their cytotoxic potency. Combination treatments with cisplatin or doxorubicin were also evaluated.

Results

Apical exposure resulted in clear MSC-EV penetration into the 3D cultures, whereas basolateral exposure produced minimal uptake. No significant changes in cell viability were observed, confirming MSC-EVs' biocompatibility. However, no significant cytotoxic/adjuvant effect was observed for the ten therapeutic MSC-EV candidates tested.

Discussion/Conclusion

Our findings on the MSC-EVs uptake support the feasibility of localised administration via inhalation. Our ongoing systematic review of lung cancer breathing parameters will inform future nebulizer design for inhaled EV administration. Furthermore, our negative results for the ten therapeutic MSC-EV candidates highlight the importance of using physiologically-relevant 3D cultures as screening platforms for gene therapy in LUAD, and underscores the need to optimise EV cargo loading.

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P.12

MSC-EVs as immunomodulators of B cell activation

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Abstract

Introduction: Mesenchymal stromal cells (MSCs) exert potent immunomodulatory effects largely mediated by their secretome, which includes extracellular vesicles (EVs). EVs released from MSCs stimulated with pro-inflammatory cytokines such as IFN-γ and TNF-α ("licensed" MSCs) display distinct molecular cargos and enhanced immunoregulatory properties compared with those from unlicensed MSCs. While most studies have focused on phagocytic cells, the effects of MSC-EVs on B cells remain poorly understood. This study investigated how licensing and EV dosage influence MSC-EV modulation of B cell activation.

Methods: The murine bone marrow–derived MSC line (D1 MSCs) was cultured under standard or licensing conditions. EVs were isolated by tangential flow filtration followed by size-exclusion chromatography and characterized by nanoparticle tracking analysis, BCA protein quantification, and spectral flow cytometry. Primary murine B cells were activated in vitro using T cell–dependent (αIgM+CD40L) or CD40L alone, and T cell–independent (CpG) stimuli, and treated with different doses of licensed or unlicensed MSC-EVs.

Results: Licensing altered D1 MSC surface marker expression and increased EV yield without affecting size or protein content. EV uptake by activated B cells, confirmed using PalmtdTomato reporter EVs, was dependent on the activation state of B cells. Dose-dependent effects were observed: low EV doses reduced, whereas high doses enhanced activation marker expression. Licensed MSC-EVs more effectively modulated CpG-driven activation and antigen presentation, while MSC-EVs did not affect B cell proliferation.

Conclusion: MSC licensing, EV dosage, and B cell activation context collectively shape immunomodulatory outcomes, underscoring the need for standardized and optimized MSC-EV–based therapeutic strategies.

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P.13

The Role of Exosomes in Managing Dry Mouth: A Scoping Review

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Abstract

Background

Dry mouth (xerostomia) severely affects oral physiology, taste, and quality of life, with current therapies providing only temporary relief for example artificial saliva. Exosomes, cell-derived extracellular vesicles are emerging as biologically active carriers capable of promoting salivary gland regeneration and immune modulation. Although exosome-based research has expanded rapidly, there is currently no evidence synthesis of how exosomes have been explored to restore salivary gland function or manage dry mouth. There are different types of studies that vary in methodology, and lack standardized evaluation. This scoping review will bridge this evidence gap by systematically mapping available clinical research on exosome applications in xerostomia. Understanding exosomes therapeutic potential will help shape next-generation regenerative approaches for dry mouth management.

Methods

This scoping review will be conducted following the Joanna Briggs Institute (JBI) methodology. Searches will be performed across PubMed, Scopus, Web of Science, and Embase to identify clinical studies investigating the use of exosomes in salivary gland regeneration or xerostomia management. Data will be charted to extract exosome sources, isolation methods, delivery mechanisms, and reported biological effects.

Results

It is expected that the review will identify studies demonstrating the ability of exosomes derived from mesenchymal stem cells and salivary gland epithelial cells to enhance acinar regeneration, promote angiogenesis, and modulate inflammation.It is expected that considerable heterogeneity will be found in exosome isolation and delivery protocols.

Conclusion

This review will provide an evidence-based overview of exosome applications in managing dry mouth. The findings are expected to highlight the promise of exosome-based therapeutics

P.14

Enhancing sup-tRNA readthrough of nonsense mutations with directed evolution

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Abstract

Nonsense mutations, also known as premature termination codons (PTCs), are responsible for approximately 10-15% of known human genetic disorders, such as Cystic Fibrosis. PTCs result in early termination of protein synthesis and loss of protein function. Currently there are no approved therapies for patients suffering from PTC-derived genetic disorders. Transfer RNAs (tRNAs) can be engineered to recognize PTCs and promote the incorporation of specific amino acids to restore protein expression and function. These engineered tRNAs are referred to as suppressor tRNAs (sup-tRNAs) and show promise as an RNA or gene therapy for many rare genetic disorders. While delivery of naive sup-tRNAs produced by *in vitro* transcription can restore partial protein expression in cell line and mouse models, the response tends to be modest and transient. We aim to improve protein recovery through directed evolution of sup-tRNAs for enhanced PTC readthrough. To date, we have developed a high-throughput sensor for quantifying PTC suppression efficiency in mammalian cells. With this sensor, we are now developing directed evolution selection conditions to evolve sup-tRNA genes. Specifically, we are investigating delivery of sup-tRNA gene variant libraries as an RNA or a DNA encoded gene therapy. Improved sup-tRNA variants will progress to a cell line model of Cystic Fibrosis containing homoallelic knock-in of known PTCs in CF patients.

P.15

Targetting EHD4 stabilizes the endothelial barrier in permeability-driven retinal disease

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Abstract

Purpose:
Dysregulated endothelial barrier function contributes to the progression of age-related macular degeneration (AMD) and diabetic retinopathy (DR). Although neutralizing vascular endothelial growth factor (VEGF) has established its role as a key driver of edema, alternative strategies are needed to stabilize the retinal barrier. In this project, we propose targeting the membrane remodeling gene EH Domain Containing 4 (EHD4) to enhance endothelial barrier integrity and thereby control vascular homeostasis.

Methods:
EHD4 role was examined using siRNA-mediated knockdown in primary human retinal endothelial cells (HRECs). RNA-Seq analysis was performed in bEnd.3 cells following Ehd4 silencing to assess endothelial identity markers and trafficking regulators. For in vivo validation, C57BL/6 mice were anesthetized, and the left eye was injected with 100 ng VEGF + 0.5 nmol non-target siRNA, while the right eye received 100 ng VEGF + 0.5 nmol si-Ehd4. Retinal thickness was measured by OCT at baseline and 48 h post-injection.

Results:
EHD4 knockdown preserved endothelial cell identity in both HRECs and bEnd.3 cells, with mRNA and RNA-Seq data showing maintained expression of CD34, TIE2, and VEGFR2, associated with enhanced endothelial-stabilizing signaling. RNA-Seq analysis revealed that Ehd4 suppression altered expression of multiple regulators involved in receptor and junction trafficking, supporting a mechanism in which si-Ehd4 prevents barrier dysfunction by modulating VEGFR2 and junctional trafficking dynamics. In vivo, co-injection of VEGF/si-Ehd4 reduced the retinal structural changes and suppressed vitreous hyperreflective foci.

Conclusion:
Targeting EHD4 preserves endothelial markers and modulates receptor and junctional turnover, representing a potential strategy to preserve retinal vascular integrity across degenerative conditions.

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P.16

An in vivo pipeline for screening gene therapies in neovascular retinal diseases

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Abstract

Introduction

Neovascular retinal diseases, such as Diabetic Retinopathy and Age-related macular degeneration, are a huge burden on healthcare, due to an ever ageing population, leading to severe vision loss. Using retinal neovascularisation murine models, we wish to establish a pipeline process for screening and assessing the stabilisation of retinal vascular permeability and prevention of new blood vessel growth using gene and cell therapy-based approaches.

Methods

JR5558 mice, known for spontaneous intraretinal lesions, were treated with therapeutic targets via oral, systemic, or intravitreal routes at varying doses and time points. Fundus fluorescein angiography (FFA) was used to assess lesion characteristics before and after treatment. In addition, we injected C57BL/6J intravitreally with VEGF in the presence or absence of therapeutic targets to determine changes in retinal thickness by optical coherence tomography (OCT).

Results

Dosing JR5558 mice systemically and locally resulted in significant decreases in total lesioned area when compared to vehicle-treated controls. The effect on total lesioned area was observed when testing various therapeutic approaches that target different neovascular disease mechanisms. In our VEGF-challenge model, efficacy was also observed with the therapeutic targets in reducing retinal thickness and permeability.

Conclusion

Our findings suggest that the JR5558 mouse and VEGF-challenge model complement each other as good models of neovascular retinal disease pathology. Each model showed efficacy with various therapeutic targets that act on the different pathways linked to vision loss. Therapeutic strategies acting to reduce retinal vascular permeability and stabilise intraretinal lesions are beneficial in conditions such as Diabetic Retinopathy or Macular Edema.

P.17

Elucidating Pathological CSF1R Signalling to Inform Therapeutic Development in ALSP

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Abstract

Introduction / Purpose

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare neurodegenerative disorder caused by heterozygous pathogenic variants in CSF1R, a receptor tyrosine kinase essential for myeloid function and neurovascular integrity. There is a significant unmet therapeutic need in ALSP, and the disease represents a compelling candidate for gene-targeted therapeutic strategies, as its monogenic nature suggests that correcting CSF1R dysfunction may be achievable. However, developing such therapies requires a clear understanding of variant-specific signalling defects and their downstream consequences.

Methods

We investigated the functional consequences of disease-associated CSF1R variants using a combination of in vitro signalling assays and transcriptomic profiling of patient-derived immune cells.

Results

Our findings reveal variant-specific effects on receptor signalling and identify transcriptional changes in pathways relevant to immune regulation and vascular stability. These results reinforce the role of aberrant CSF1R activity in blood–brain barrier dysfunction and highlight signalling pathways that could inform the design of future gene-based interventions.

Discussion / Conclusion

By clarifying how CSF1R dysfunction shapes peripheral and central immune changes, this work provides a foundation for future cell and gene therapy strategies for ALSP, illustrating the importance of mechanistic insights in guiding therapeutic development for monogenic neurodegenerative disorders.

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P.18

Investigation of microRNA-758 Targets in Breast Cancer

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Abstract

Although advances in medicine have improved breast cancer outcomes, advanced disease remains incurable. MicroRNA(miRs) are post-transcriptional regulators of gene expression that play an important role in normal physiology and cancer. This study aimed to investigate the targets of miR-758 in breast cancer progression. MDA-MB-231 and HCC-1954 breast cancer cells were stably transduced with lentiviral vectors carrying either a non-targeting control (NTC) sequence or mature miR-758. Both vectors also contained a green fluorescent protein (GFP) marker, which, along with RQ-PCR, was used to confirm transduction success. The resulting cell lines (MDA-NTC, MDA-758, HCC-NTC, and HCC-758) were then subjected to proteomic profiling to explore mechanistic changes following miR-758 overexpression. Additionally, cell migration was evaluated with Transwell assays, comparing the responses of MDA-NTC and MDA-758 cells to chemoattractants. The transduced cells exhibited successful GFP expression. Meanwhile, qPCR analysis demonstrated a dramatic upregulation of miR-758, with approximately 6,000-fold and 3,000-fold increases in MDA-758 and HCC-758 cells, respectively, compared to the NTC controls. Proteomic profiling further identified over 169 (2.59%) and 41 (0.63%) differentially expressed proteins in the two miR-758 transduced cell lines ($Log2FC > 1$, Adjusted $p\text{-value} < 0.05$). Enrichment analysis indicated that these proteins were associated with mesenchymal migration. Consistently, MDA-758 cells showed a significant 34% reduction in migratory capacity toward chemoattractants relative to MDA-NTC cells ($P = 0.04$). Collectively, this study establishes that miR-758 overexpression suppresses migration in aggressive breast cancer cells. This anti-migratory phenotype, coupled with corresponding alterations in the proteome, supports miR-758 as a tumour suppressor in this context.

P.19

Impact of microRNA-379 on the Endothelin-axis in Breast Cancer

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Abstract

Introduction:

Elevated circulating Endothelin-1 (EDN1) in breast cancer patients has been associated with distant metastasis, raising interest in gene therapy to modulate expression. EDN-1 production, secretion, and uptake requires sequential steps involving proteins of the EDN-axis. microRNA-379 (miR-379) has a predicted binding site in the 3' UTR of EDN1. This study aimed to explore the impact of miR-379 on proteins responsible for EDN1 processing in breast cancer.

Methods:

Breast cancer cell lines [MDA-MB-231, HCC1954; 4T1] were transduced with lentivirus to express miR-379 or a Non-Targeting Control (NTC). Immunohistochemistry targeting Prepro-EDN1 was performed on tumour xenografts and quantified via QuPath. EDN1, Endothelin Converting Enzyme-1 (ECE1), Endothelin Receptor A and B (EDNRA/B) were analysed by western blot and RQ-PCR respectively. EDN1 and Big-EDN1 secretion were detected using ELISA.

Results:

4T1-379 and HCC-379 tumours showed a significant reduction of Prepro-EDN1 staining compared to respective control tissues. PCR analysis showed robust EDN1 and ECE1 expression in all cell lines. Receptor A and B mRNA expression was relatively high in MDA-MB-231, while not detected in HCC1954 and 4T1 cells. Western blot confirmed full EDN-axis protein expression in MDA-MB-231 and HCC1954 lysates with a reduction of Prepro-EDN1 and EDNRB in HCC-379 lysates. miR-379 had no impact on EDN1 secretion in vitro. Big-EDN1 secretion was significantly higher from MDA-379 than MDA-NTC (P=0.004).

Conclusion:

These results demonstrate a role for miR-379 in repressing PreproEDN1 and EDNRB. Understanding regulation of EDN-1 axis proteins and the compensatory interplay may reveal important therapeutic targets in the breast cancer-bone metastatic cascade.

P.20

Development of a Framework for Recombinant Adeno-Associated Virus (rAAV) Production and Purification

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Abstract

Recombinant adeno-associated viruses (rAAV) are a promising class of therapeutics for gene therapy treatment. The current manufacturing processes for rAAV therapies are expensive, time-consuming and their manufacture has not been standardized across the industry. Many different production systems and purification methods are used resulting in high production costs and processes that lack flexibility, scalability and quality, and is limiting patient access to rAAV-mediated gene therapy. This work describes the development of a framework for rAAV process development, with an emphasis on scalable, GMP-ready technologies.

A 2-stage design of experiment (DoE) approach was used to optimize triple transfection to produce AAV5 using FectoVir-AAV and a HEK293F cell line. This consisted of an initial screening design to identify significant factors followed by a central composite design to optimize these factors. Once optimum conditions were identified, conditions were verified at 125 mL shake flask, 3 L shake flask and 10 L Wave reactor scales demonstrating reproducible performance across each scale achieving genome titres of ~1.3 x 10^{11} vg/mL and 60+ % full capsids.

For chromatography-focused rAAV purification, a process development workflow, moving from 96-well plates to TECAN and AKTA platforms, has been proposed to streamline development of a 2-step serotype agnostic downstream platform. POROS™CaptureSelect™AAVX affinity resin (ThermoFisher) has been selected as the initial model resin to develop and demonstrate the proposed approach.

This framework provides a structured, stepwise approach to the development of a scalable rAAV purification platform ensuring that an efficient, high yield, cost-effective and serotype agnostic process is developed.

P.21

Improving Recovery After Spinal Cord Injury: A Molecular Approach

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Abstract

Spinal cord injury (SCI) affects 250,000–500,000 people worldwide each year representing a significant medical and economic burden (1). SCI results in full or partial loss of sensation and function below the level of injury. SCI usually arises from traumatic events that cause neuronal death and inflammation. These lead to the formation of a glial scar, which blocks axonal regeneration (2). The glial scar is produced by reactive astrocytes. Chondroitin sulfate proteoglycans (CSPGs) are major inhibitors of axonal growth within the glial scar, these consist of core proteins with attached glycosaminoglycan chains. Their synthesis is carried out by enzymes such as xylosyltransferases (XT1 and XT2) and chondroitin sulfate glycosyltransferases (CSGAL1 and CSGAL2), which sequentially initiate and elongate the chains (3)(4)(5).

This study explores whether silencing four key enzymes in CSPG biosynthesis (XT1, XT2, CSGAL1, CSGAL2) using lentiviral vectors to deliver short hairpin RNA (LV-shRNA) can reduce CSPG production and promote axonal growth.

Using Neu7 cells, a rat astrocyte model, XT2 and CSGAL2 mRNAs were reduced approximately 70% and 90% respectively by their targeting LV-shRNAs. Protein analysis by Western blot and immunocytochemistry are ongoing. CSPG levels in the knockdown cells will be measured using lectin flow cytometry and CSPG-specific antibodies. The functional consequences of a reduction in CSPG levels will be assessed using dorsal root ganglion cells. Future work will involve organotypic slice lesion models and in vivo validation of the lentiviral knockdown.

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P.22

Establishment of a Physiologically Relevant In Vitro Model of Type 2 Diabetes-Induced Osteopathy

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Abstract

Type 2 diabetes mellitus (T2DM) is associated with increased fracture risk despite elevated bone mineral density, suggesting impaired osteoblast function. Osteoprogenitors are precursors of osteoblasts and osteocytes, responsible for bone homeostasis. Their behaviour under diabetic-like metabolic conditions is compromised. Many in-vitro models of diabetes employ glucose concentrations ≥25 mM, far exceeding levels observed even in severe diabetes, obscuring disease-relevant responses and limiting clinical relevance.

Here we aim to establish and validate an in-vitro model of T2DM-induced osteopathy using human osteoblasts (hFOBs). Although traditionally expanded at 18mM glucose, here they were cultured under a clinically relevant glucose concentration (8 mM) that more accurately reflects the human hyperglycaemia. FOBs were maintained under proliferative conditions (34°C) or induced to differentiate at 39°C, with or without osteogenic supplements (dexamethasone, ascorbic acid, β-glycerophosphate), over 35 days with weekly sampling. Alkaline phosphatase activity was assessed via BCIP/NBT staining and pNPP assay. Matrix mineralisation was monitored via Alizarin Red S staining and calcium quantification. Morphological changes were observed, reminiscent of primary cell osteogenesis. Expression of osteogenic markers RUNX2, ALP, COL1A1, OCN, and BSP were assessed with qRT-PCR.

Time-dependent upregulation of osteogenic marker transcription was consistent with published primary osteoblast patterns, supporting the suitability of hFOBs for modelling osteogenesis under physiological glucose levels. Culturing hFOBs under clinically relevant hyperglycaemia (8 mM) rather than their standard 18mM provides a platform for investigating diabetes-related osteogenic impairment. Future work will incorporate inflammatory cytokines to model T2DM-associated osteogenic dysfunction and support development of targeted gene-therapy strategies for T2DM-induced osteopathy.

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P.23

Stable correction of the R553X CFTR mutation through adenine base editor delivery by virus-like particles

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Abstract

Cystic Fibrosis (CF) is a life-limiting autosomal recessive disorder caused by loss-of-function mutations in the CFTR gene, which encodes a chloride channel critical for epithelial ion and fluid transport. The CF-causing R553X mutation introduces a premature termination codon (PTC) in CFTR, that results in complete loss of CFTR protein expression in affected individuals. This study investigates a CRISPR-based adenine base editing approach to correct the R553X mutation and restore functional CFTR expression.

We delivered the editing machinery in R553X-16HBEge human bronchial epithelial cells using engineered base editor virus-like particles (BE-eVLPs) - self-assembling, non-replicating vectors. Editing efficiency and stability were quantified through next-generation sequencing, and CFTR protein restoration evaluated by Western blotting.

BE-eVLP transduction achieved ~93% correction of R553X alleles, with corresponding recovery of CFTR protein to ~90% of wild-type expression levels. Editing efficiency was sustained for over two months post-transduction, with the proportion of corrected alleles increasing to ~99% over time. This increase suggests a proliferative advantage for corrected cells, consistent with restoration of functional CFTR.

Our results demonstrate efficient repair and recovery of CFTR in a human CF cell line. Future studies include in vivo functional validation in primary airway epithelial cells. As R553X and other PTC CF-causing mutations do not respond to existing CFTR modulator therapies, base editing via BE-eVLPs represents a potential therapeutic approach, addressing an unmet clinical need in CF treatment.

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P.24

Engineering circular RNAs for selective protein expression in dysfunctional endothelial cells.

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Abstract

The success of the messenger RNA (mRNA)-based vaccines against COVID-19 proved the clinical potential of RNA technology for protein production in vivo. Circular RNA (circRNA) is a new class of single-stranded RNA with a closed-loop structure that improves its stability[1]. In contrast to mRNA, circRNA is translated via Internal Ribosome Entry Site (IRES)-mediated translation that often relies on the assistance of factors known as Internal Ribosome Entry Site Trans-Acting Factors (ITAFs) which may vary in their expression depending on the cell type and cell state[2,3]. IRES engineering could provide an avenue for tissue-specific expression by making circRNA the preferred RNA for the ribosome in a cell under stress conditions such as hypoxia and inflammation, relevant in high-medical-need diseases such as sepsis or cancer[4].

Here, we investigated a library of 40 viral and cellular IRES sequences for their ability to drive robust and specific circRNA translation.

We found that Group IV viral IRES displays robust secreted luciferase activity in primary human endothelial cells (ECs) at 24 h after transfection with comparable protein expression levels to m1ψ-modified mRNA. This data was correlated with an in-silico analysis of the IRES candidates to predict potential ITAFs binding the circRNA, with Group IV viral IRES showing the highest number of interactions with RNA-Binding Proteins (RBPs). Furthermore, we observed significant differences in translation between circRNA and mRNA in ECs under hypoxia and other stress conditions. These results demonstrate the great potential for circRNA in therapeutic applications to overcome the challenges that mRNA technology poses.

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P.25

Preclinical evaluation of EXH-001: safety, expression, and efficacy of AAV-based MMP3 gene therapy for glaucoma

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Abstract

Glaucoma is primarily driven by ocular hypertension resulting from increased resistance to aqueous humour (AH) outflow. Gene therapies targeting the anterior chamber offer a promising strategy for reducing intraocular pressure (IOP) while avoiding the adherence challenges associated with topical medications. We previously demonstrated that intracameral delivery of AAV9 expressing matrix metalloproteinase-3 (MMP3) enhances outflow facility, lowers IOP, and reduces extracellular matrix (ECM) density in murine models, and is well tolerated in non-human primates (NHP).

Here we extend this work by evaluating ocular safety, MMP3 expression dynamics and efficacy following intracameral administration of an AAV-based MMP3 gene therapy, in African Green monkeys. Adult NHPs were randomized to receive contralateral injections of vehicle or one of three AAV doses. Across all groups, AAV was well tolerated through 14 weeks. No systemic or ocular adverse findings were observed. Longitudinal ophthalmic examinations showed no structural abnormalities and revealed no retinal functional deficits.

Aqueous humour analysis demonstrated dose-dependent, transient increases in MMP3 levels following administration, with peak expression at early time points and subsequent decline, consistent with expected AAV expression kinetics and potential adaptive tissue responses. Histopathology identified no AAV–related findings. Sustained MMP3 expression for over 1 year correlated with over 50% increased aqueous outflow 7-10 months post injection of AAV.

Together, these preclinical data demonstrate that intracameral AAV-mediated MMP3 delivery is safe, produces measurable MMP3 secretion, and results in a clinically relevant increase in outflow through the anterior chamber. This supports further development as a therapeutic candidate for lowering outflow resistance in glaucoma.

P.26

Optimizing NMNAT Genes for Disease-Independent NAD^+ Augmentation Therapy in Dry AMD

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Abstract

Age related macular degeneration (AMD), a common multifactorial disorder, is a leading cause of blindness in people over 65-years. Dry AMD, representing ~85% of AMD cases, has few therapeutic options and those available have limited efficacy. Mitochondrial dysfunction, impaired metabolism and chronic inflammation are important early drivers of disease. We have investigated novel overarching therapeutic approaches modulating dysregulated cellular pathways in dry AMD that are independent of specific targets. In principle these candidates represent therapies that may be broadly applicable to dry AMD. Nicotinamide mononucleotide adenylyltransferase (NMNAT) enzymes catalyze the final step of NAD^+ biosynthesis, a critical cofactor for metabolism, DNA repair, and mitochondrial homeostasis. Reduced NAD^+ levels are linked to neurodegeneration and aging, making NMNAT restoration a compelling therapeutic strategy. For example, NMNAT1 and NMNAT2 have established neuroprotective and anti-inflammatory roles, acting through pathways including SIRT1 activation, PARP modulation, and axonal protection.

Codon-optimized NMNAT genes were designed and engineered into adeno associated virus (AAV) for gene delivery. NMNAT coding sequences were optimized to augment transgene expression, among other features. Expression of optimized transgenes was confirmed by NAD^+ fluorometric quantification and western blot analyses. Furthermore, optimised NMNAT constructs are being evaluated in multiple AMD cell models, including primary retinal pigment epithelial cells, simulating aspects of the human disorder. Findings thus far suggest significant beneficial effects of these therapeutic strategies and support further development of optimized NMNAT genes as promising disease-independent gene therapy candidates for dry AMD, offering a metabolic rescue strategy that may overcome some limitations of current, target-specific approaches.

P.27

Genetically modified human pluripotent stem cell-derived beta cell differentiation and drug screening for the treatment of Type 1 diabetes mellitus

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Abstract

Type 1 diabetes mellitus (T1DM) is characterized by autoimmune destruction of pancreatic β cells, leading to insulin deficiency and hyperglycemia. Transplantation of functional insulin-producing cells derived from human induced pluripotent stem cells (hiPSCs) represents a promising regenerative approach; however, inflammation-mediated β cell loss remains a critical barrier to long-term success. This study aims to identify small molecules from traditional Chinese medicine (TCM) that can protect hPSC-derived β cells from interleukin-1β (IL-1β)-induced inflammatory injury. Candidate compounds were selected through literature mining in PubMed, focusing on molecules traditionally used for diabetes treatment and previously reported to protect other cell types, such as chondrocytes, from IL-1β or related cytokine stress. In parallel, an established β cell differentiation protocol was reproduced in our laboratory to generate insulin-producing cells from hiPSCs, with ongoing efforts to validate their identity and functionality. To facilitate preliminary screening, rat insulinoma INS-1 cells were employed as an alternative β cell model to evaluate cytoprotective effects of selected TCM compounds under inflammatory conditions. The expected outcomes include identification of one or more TCM-derived molecules capable of mitigating cytokine-induced β cell damage, thereby providing new candidates for future cell therapy enhancement or combination drug development. This work lays the groundwork for integrating traditional medicine with stem cell-based regenerative strategies in T1DM treatment.

P.28

Generation of equine induced pluripotent stem cells from cells of embryonic, perinatal and adult tissues

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Abstract

Regenerative therapies are quickly expanding to equine patients because of their importance as sporting and companion animals. Furthermore, aligning with a One Health concept, veterinary medicine offers a unique platform for preclinical studies. While mesenchymal stem/stromal cells (MSCs) therapies are already used in treating horses, strategies involving induced pluripotent stem cells (iPSCs) are underdeveloped. iPSCs present great potential for therapy and disease modelling, but their consistent generation in horses requires is inconsistent. We compare the reprogramming potential of equine cells from tissues of three developmental origins: prenatal (embryo-derived MSCs, eMSCs), perinatal (cord blood-derived MSCs, CB-MSCs) and adult (articular chondrocytes, ACs). Two reprogramming methods and different culture conditions were tested. Pluripotent gene expression was analyzed at different time-points to reveal transcriptomic changes associated with reprogramming. The generated equine iPSCs (eqiPSCs) were characterized by alkaline phosphatase (AP) staining, expression of pluripotent genes and proteins, three-germ layer differentiation and karyotype. Equine CB-MSCs and ACs were only partially reprogrammed and could not be efficiently expanded in culture. Only eMSCs generated putative eqiPSCs that met the cellular, molecular and functional criteria of pluripotent cells. Equine eMSCs showed higher proliferation and basal expression of pluripotent genes compared to CB-MSCs and ACs, and showed the highest upregulation of pluripotent genes while reprogramming. The developmental stage of the starting cell, as well as the culture conditions used, strongly influences their reprogramming potential in equine species.

P.30

Transcriptomic Dysregulation of Bone Marrow Mesenchymal Stromal Cells in Type 2 Diabetes Mellitus

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic disease, characterized by elevated blood sugar levels, leading to osteopathy. Notably, the risk of osteopathy in individuals with T2DM is influenced by sex2. Bone marrow mesenchymal stromal cells (BM-MSCs) play a crucial role in bone regeneration and were widely studied for their therapeutic potential in regenerative medicine [3,4]. T2DM-induced osteopathy is associated with impaired bone quality, with the reduced number and diminished differentiation capacity[5]. However, the transcriptomic mechanisms driving these impairments remain unclear.

BM-MSCs from 27 donors (13 individuals with T2DM; 14 without T2DM), were expanded for 1 passage to bulk RNA sequencing. Gene expression profiles were analyzed all donors, and within sex-specific subgroups. Differentially expressed genes (DEGs) were identified using DESeq2, common DEGs were identified by UpsetR. Gene set enrichment analysis (GSEA) was conducted on the ranked log2 fold-change values to identify differentially regulated pathways.

People with T2DM and non-T2DM were highly similar on a global transcriptomic level, however, 82 DEGs were identified in the overall donors with T2DM, 195 DEGs in female donors with T2DM and 115 DEGs in male donors with T2DM during sex-specific comparisons ($|\log_2 FC| \geq 2$, $p < 0.05$), with 3 genes common to all groups. Interestingly, from male and female T2DM donors exhibited distinct gene expression patterns within the osteoclast differentiation pathway.

In conclusion, this study provides insights into the transcriptomic dysregulation of BM-MSCs in T2DM, and potential mechanisms. This finding underscored the impact of sex-specific differences on BM-MSC transcriptome regulation, inspiring the targeted therapeutic strategies development.

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P.31

Investigating the Effects of Cold Atmospheric Plasma on Mesenchymal Stem Cells

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Abstract

Mesenchymal Stem Cells (MSCs) are distinguished by their ability to self-renew, differentiate into specialised cells, modulate immune response and migrate to injury sites [1]. Cold atmospheric plasma (CAP) is ionized gas composed of a unique cocktail of excited atoms, electromagnetic fields, UV radiation and reactive oxygen and nitrogen species (RONS). CAP's application extends to several fields, particularly in medicine where it is used for wound healing, disinfection and modulating cellular responses leading to changes in proliferation and differentiation [2]. This study aims to investigate the effect of CAP on MSC function, stemness and cell-cell communication.

ASC52telo-hTERT MSCs (ATCC) were treated using a helium-based Atmospheric Pressure Plasma Jet device (J-Plasma, Apyx Medical). Reactive species were characterised by measuring total oxidative species, H_2O_2, nitrites and nitrates generated in dH_2O at different exposure times and distances. Cell responses were quantified as metabolic activity and cell mass using resazurin and crystal violet, respectively. Further analyses included quantification of intracellular ROS, expression of stemness markers and analysis of the cellular secretome.

MSCs demonstrated a treatment-time-dependent response to direct cold plasma. At shorter exposures (<30sec), cells retained their metabolic activity, while longer treatments progressively reduced viability. Cold plasma did not alter the cells' differentiation but initial results from conditioned media treatments suggest changes to the secretion of soluble factors and/or extracellular vesicles which impact recipient cells. These findings may provide insights into the role of MSCs in cold plasma-assisted wound healing while also advancing our general understanding of the effects of RONS on MSCs.

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P.32

Combinatorial Cell Therapy Using CD362+Mesenchymal Stromal Cells (MSCs) and Endothelial Colony-Forming Cells (ECFCs) for the Treatment of Critical Limb-Threatening Ischaemia (CLTI)

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Abstract

Critical limb-threatening ischaemia (CLTI) represents the most severe form of peripheral arterial disease and is associated with high rates of amputation and mortality. Current treatments focus on revascularisation procedures to bypass obstructive atherosclerotic lesions. However, a substantial portion of patients are unsuitable for these interventions due to extensive vascular damage or comorbidities, and amputation often remains the only treatment option. Cell-based therapies offer a promising alternative by promoting neovascularisation and tissue repair. While evidence exists that single-cell therapies may be beneficial in preclinical models, clinical outcomes have been modest. This study investigates a combinatorial approach exploiting the synergistic potential of endothelial colony-forming cells (ECFCs) and mesenchymal stromal cells (MSCs) to enhance vascular and muscle regeneration in CLTI.

Umbilical cord CD362+MSCs (Orbcel-C®) and ECFCs were transplanted into mice subjected to hindlimb ischaemia as single or combined modality. Blood flow recovery was assessed longitudinally using Laser Doppler Imaging. Muscle samples were collected 28 days post-surgery for histological and muscle weight analysis. The combinatorial cell therapy significantly enhanced blood flow recovery compared to single-cell approach. Furthermore, the combinatorial cell therapy significantly reduced ischaemia-induced muscle mass loss. The muscle mass ratio was significantly correlated with the degree of ischaemia-induced muscle damage assessed histologically.

These findings highlight the therapeutic potential of combined MSC and ECFC therapy to address the multifactorial pathology of CLTI. By harnessing the complementary paracrine and vascular structural contributions of these progenitor populations, this dual-cell therapy offers a relevant approach to restore perfusion and promote tissue repair in patients with CLTI.

P.33

A novel marker, REM-1, isolates mesenchymal stem/stromal cells with tri-lineage potential from the bone marrow of multiple species

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Abstract

There continues to be strong interest in the therapeutic utility of mesenchymal stem/stromal cells (MSC), with many active clinical trials underway, despite the fact that they are an ill-defined heterogeneous population of cells characterised mainly by their cultured phenotype and differentiation potential. To date, there is still a lack of useful selection markers to purify naïve MSCs directly from bone marrow, as known markers tend to isolate all cells capable of forming fibroblastic colonies (CFU-F), without distinguishing between cell populations which demonstrate full trilineage potential and those which demonstrate only osteogenic and/or adipogenic potential. The few existing markers are mostly either human-specific (STRO-1, W8B2, CD271, STRO-3, CD200 etc) or murine specific (Sca-1, nestin, PDGFRa etc) with little overlap between the species.

Here we show that REM-1, a novel marker associated with the primary cilium, is a good candidate for isolating naïve MSCs from different species. Moreover, this marker separates MSCs capable of tri-lineage differentiation from the cell population with only osteo-adipogenic potential. The REM-1 isolated MSC population also expresses more stemness genes than either the REM-1 negative fraction or unsorted cells. In addition, based on the extremely high species conservation of this marker, we speculate that it will isolate MSCs from bone marrow of all species, resulting in a uniquely defined multispecies MSC isolation tool. In the context of selecting MSCs with specified chondrogenic potential, this marker has value in isolating cells for cartilage repair and for the treatment of arthritic disease.

P.34

Validating a methodology to isolate ALP+ osteoprogenitors using flow cytometry

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Abstract

Within bone, mesenchymal stromal cells (BM-MSCs) maintain bone homeostasis and stimulate repair following injury. Tissue non-specific alkaline phosphatase (ALP) expression in vitro and in vivo is correlated to osteogenic potential and serves as a validated marker of osteogenic differentiation. This project is interested in ALP+ osteoprogenitor quantity and transcriptional activity in the context of Type 2 Diabetes Mellitus (T2DM). T2DM is a chronic, metabolic disease characterized by insulin resistance and hyperglycaemia resulting in a myriad of vascular complications. Our team was the first to establish reduced number of ALP+ MSCs isolated from the bone marrow of humans living with T2DM compared to age matched controls (AMC) determined by ALP+ stained colony-forming osteoblast and colony-forming fibroblast assays. This project aims to create and validate a methodology enabling the sensitive, quantitative assessment of osteoprogenitor number and support downstream transcriptional interrogation of ALP+ and ALP- MSC subsets using fluorescence activated cell sorting (FACS) coupled with an optimized TRIzol RNA isolation protocol. A positive control cell line (LentiX 293T) was created using lentiviral overexpression of TNAP. Antibodies targeting classical MSC phenotypic markers were titrated, then utilized to identify and sort CD90+, CD73+, CD105+, CD45- and TNAP+ MSCs. Validation of this approach in human primary BM MSCs captures variability in ALP expression between 1-40%. Four RNA isolation methodologies were compared by Agilent TapeStation, identifying an optimal protocol resulting in RIN^e values above 8 indicating its integrity. Together, this workflow enables the identification, quantification and isolation of ALP+ MSCs, supporting subsequent molecular analysis.

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P.35

Cryopreservation agent comparison study using HomeBrew and Stem-CellBanker® for cryopreservation of a human Adipose-Derived Mesenchymal Stromal Cell bank

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Abstract

Cryopreservation of cellular products enables long-term storage allowing for flexible manufacturing and delivery. A robust cryopreservation protocol is essential to preserve the viability and functionality of cells. Therefore, choosing a suitable cryopreservation agent is crucial for cell bank generation.

The aim of this study is to compare the effect of HomeBrew (10% Dimethyl Sulfoxide, 85% α-Minimum Essentials Medium, and 5% Human Platelet Lysate) and Stem-CellBanker® on the cryopreservation of a human adipose-derived stromal cell (hADSC) bank.

Viability, recovery, growth, phenotype and metabolic health of the cells were examined. hADSCs were cryopreserved in HomeBrew or Stem-Cell Banker®. Cells were cryopreserved using a liquid nitrogen-free controlled-rate freezer at -2°C/minute and transferred to a -150°C freezer. Recovery and viability were calculated post-thaw and growth, morphology, phenotype and metabolic activity were assessed.

Cell recovery and viability were significantly higher in the Stem-CellBanker® group when compared to the HomeBrew group. The Stem-CellBanker® group also demonstrated superior growth and a lower population doubling time. Cells cryopreserved in Stem-CellBanker® showed higher metabolic activity, albeit not statistically significant. Both groups met the recommended phenotypic criteria as described by the International Society for Cellular Therapy.

This proof-of-concept study demonstrates that Stem-CellBanker® provides optimal cryopreservation conditions for the generation of a hADSC bank. As an off-the-shelf product, Stem-CellBanker® also provides the advantage of consistency and reproducibility. In future studies, additional samples will be tested using a broader range of cryopreservation agents to further evaluate the effect of cryopreservation conditions when generating cell banks.

P.36

Oxygen-Dependent Immunomodulatory and Regenerative Properties of Canine Mesenchymal Stromal Cells: Implications for Pulmonary Vascular Repair

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Abstract

Pulmonary arterial hypertension (PAH) is a progressive vascular disease characterized by endothelial dysfunction and aberrant vascular remodeling, leading to increased right-ventricular afterload and life-threatening outcomes.

Regenerative medicine, particularly mesenchymal stromal/stem cell (MSC) therapy, offers a promising approach to restore vascular homeostasis through paracrine, immunomodulatory and angiogenic mechanisms. In veterinary medicine, canine MSCs have been isolated, expanded, cryopreserved and applied in early clinical settings, highlighting their translational relevance both for companion-animal care and as large-animal models.

Hypoxia is a key driver of pulmonary vascular injury, endothelial dysfunction and endothelial to mesenchymal transition (EndMT). Understanding how oxygen tension modulates canine MSC behavior and secretory activity is therefore crucial to optimize their therapeutic potential.

This study investigates the regenerative and immunomodulatory properties of canine umbilical cord- (UC) and adipose tissue-derived (AT) MSCs in the context of pulmonary vascular repair. Three independent UC- and AT-MSC isolates were characterized. UC-MSCs demonstrated clonogenicity, strong cell-cell adhesion, rapid migration and trilineage differentiation. AT-MSCs displayed comparable profiles, though with variable proliferation rates (PDT <1–2 days). Under 5% O_2 hypoxia, UC-MSCs displayed slightly increased viability (MTT assay), reduced ROS levels (H_2DCFDA staining) and transient mitochondrial depolarization (JC-1 assay). The immunomodulatory effects of MSC-conditioned medium (normoxia vs. 2% O_2 hypoxia) were evaluated on canine M1-polarised macrophages via CD206/MHC-II expression (flow cytometry, RT-PCR) and indoleamine 2,3-dioxygenase (IDO) activity. These findings provide preliminary insights into oxygen-dependent immunomodulatory responses of canine MSCs, supporting further studies on their role in pulmonary vascular regeneration.

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P.37

Mesenchymal stromal cell therapy attenuates acute lung inflammation

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Abstract

Mesenchymal stromal cells (MSC) have been widely investigated as cell-based therapies for immune-mediated disorders. Their capacity to resolve inflammation and promote repair make MSCs a promising therapeutic candidate for acute respiratory distress syndrome (ARDS). ARDS is characterised by diffuse pulmonary damage and cellular infiltrate. High mortality rates are a consequence of limited therapeutic strategies. MSC therapies are well-tolerated although therapeutic efficacy is observed in only 40% of ARDS patients thus, highlighting the need for further mechanistic understanding.

To investigate MSC efficacy, a lipopolysaccharide induced acute lung inflammation (ALI) *in vivo* mouse model was used. LPS was administered intranasally followed by a single intravenous dose of human bone marrow-derived MSCs in wild-type mice. After 48 hours bronchoalveolar lavage fluid (BALF) and lung tissue were harvested for cytokine quantification and flow cytometric analysis. Bone marrow cells were isolated, differentiated to bone marrow-derived macrophages (BMDMs) and were subjected to secondary immunological challenge *in vitro*.

LPS challenge significantly elevated TNFα, IL-6, IL-1β, IFNγ and neutrophil elastase levels in BALF. Robust cellular infiltration predominantly mediated by neutrophils characteristic of ALI was determined in both BALF and lung tissue. BMDMs from LPS-challenged mice produced significantly enhanced levels of pro-inflammatory cytokines following heterologous immunological challenge. MSC administration significantly reduced levels of pro-inflammatory cytokines, cellular infiltration and neutrophil activity in ALI. We show that MSCs alleviate acute lung inflammation and these studies will provide mechanistic insight critical for understanding MSC-based therapy in ARDS.

P.38

Brinp3 function test in Osteoarthritis

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Abstract

Background: Osteoarthritis is a widespread disease which costs millions of expense and heavy life burden to patients. Although there are many methods to relieve the painfulness, there is no remedy to cure this disease, while MSCs become a promising method. Our previous work identified Brinp3 as a new cytokine factor by MSCs and showed Brinp3 may be helpful in osteoarthritis. This study aims to test the function of Brinp3 in osteoarthritis.

Method: Choose randomly 3 MSC or iMSC donors and differentiate these MSC/iMSC donors to chondrocytes, add Brinp3 in experimental group, after 28days differentiation, test the GAG synthesis. Stimulate monocytes and T cells in fresh blood, check TNF-a secretion of monocytes and proliferation rates of T cells by flow cytometer.

Results: In chondrogenesis assay, it seems that Brinp3 did not accelerate the synthesis of GAG during differentiation. In monocyte assay, there seems to have a trend that Brinp3 could slightly decrease the secretion of TNF-a by monocytes but there is no significant difference. Also, in T cell assay, we could not see the difference.

Conclusion: Brinp3 was identified by our previous work by local injection of MSCs into mouse knee joints, but through chondrogenesis assay, monocyte assay and T cell assay, data did not support the differentiation function or immunomodulatory function of Brinp3 in osteoarthritis.

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P.39

Immunomodulatory role of mesenchymal stem cell-derived extracellular vesicles on dendritic cells

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Abstract

Dendritic cells (DCs) are professional antigen-presenting cells that regulate immunity and tolerance, but their overactivation has been linked to inflammatory diseases, including inflammation-related bone loss and autoimmune diseases. Mesenchymal stem cells (MSCs) possess immunomodulatory potential mediated through their paracrine factors, including secreted extracellular vesicles (EVs). This study explored how MSC-EVs influence DC differentiation, activation and function.

Murine MSC-EVs were isolated using differential centrifugation, ultrafiltration, and size exclusion chromatography. EVs were characterized by nanoparticle tracking analysis, bicinchoninic acid, transmission electron microscopy and flow cytometry for particle concentration, protein levels, morphology and surface marker expression. Monocyte-derived DCs from C57BL/6 mice were cultured in the presence of MSC-EVs at increasing dosages (0, 4 x $10^{9,}$ 8 x 10^{9} and 2.25 x 10^{10} particles per mL) with or without stimulation with LPS and CpG. DC phenotype and cytokine secretion were analyzed using flow cytometry. To further understand the mechanism of action of the MSC-EVs, DCs were transfected with abundant EV cargo miRNA, and their activation phenotype was analysed.

MSC-derived EVs significantly reduced the expression of MHC-II, CD11c, CD40, and CD86 surface markers and increased the expression of CD11b surface marker and PD-L1 immune checkpoint marker. Transfecting monocyte-derived dendritic cells with murine miRNA miR-21a-5p, miR-125b-5p and miR-let-f7-5p significantly reduced their expression of MHC-II, CD80, CD86 and CCR7. This indicates that the mechanism of action of MSC-EVs may, in part, be through miRNA-mediated regulation of immune signalling. Further profiling of their transcriptome and protein cargo will give insights into the molecular mechanisms underlying DC regulation.

P.40

The Septic Microenvironment Attenuates MSCs' Enhancement of Pulmonary Epithelial Wound Repair

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Abstract

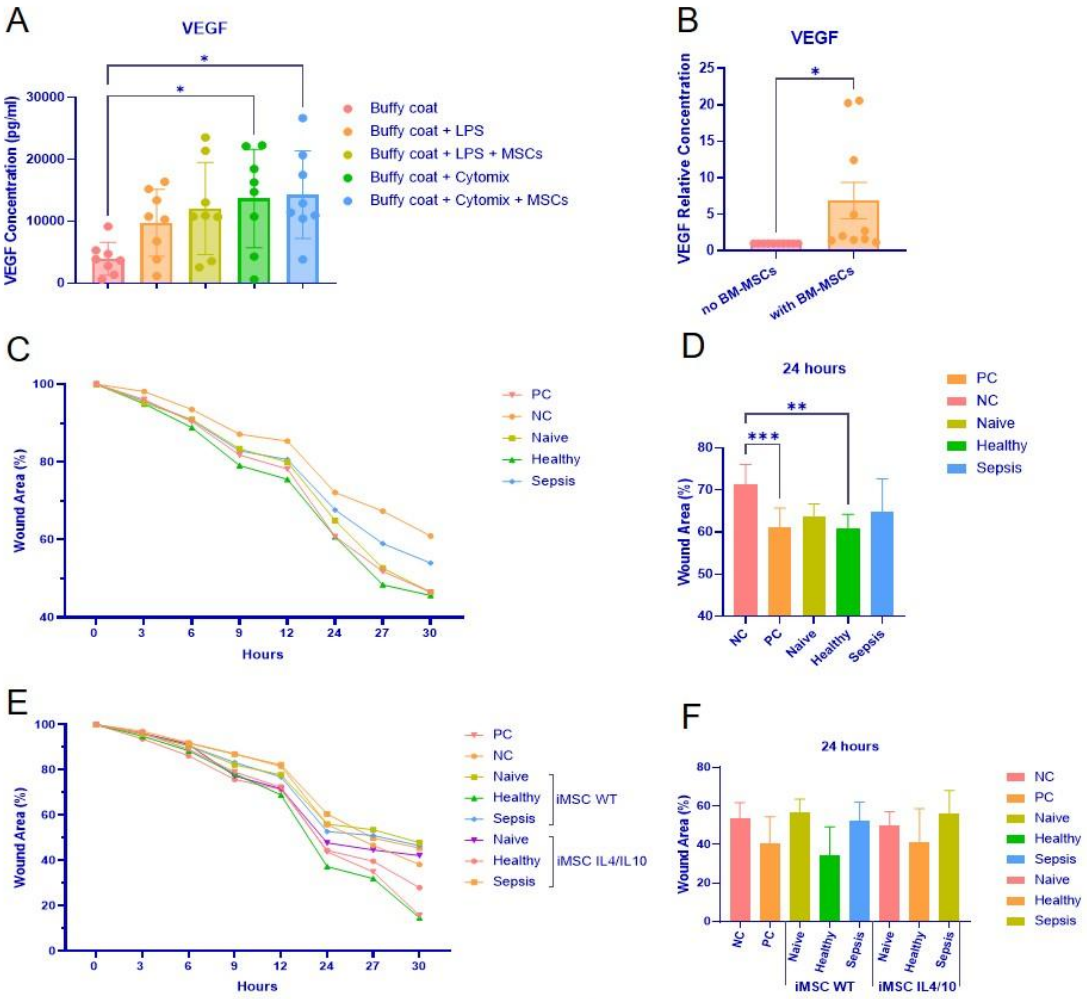

Fig 1: VEGF concentration in plasma increased after adding BM-MSCs in buffy coat stimulated with/out LPS/ cytomix(A). VEGF concentration in plasma increased after adding BM-MSCs in sepsis blood(B). CM from healthy-serum-stimulated BM-MSCs enhanced wound closure, while CM from sepsis-serum-stimulated BM-MSCs showed less beneficial effect (C, D). CM from healthy-serum-stimulated iMSCs WT and iMSCs IL-4/IL-10 enhanced wound closure (E, F). Cytomix: 50ng/ml TNF-α, IL-1β, IFN-γ. PC: RPMI Complete Media. NC: Serum free MEM + complete RPMI (3:1).

INTRODUCTION: Sepsis, a life-threatening organ dysfunction often stemming from lung injury, affects nearly 50 million people globally each year. While mesenchymal stem cell (MSC) therapies show therapeutic potential, their efficacy is limited by complex pathophysiology of the septic microenvironment. This study investigated how preconditioning MSCs with healthy or septic serum influences their wound-healing potential.

METHODS: Bone marrow-derived MSCs (BM-MSCs) were co-cultured with LPS/cytomix-stimulated buffy coats (n=8) and sepsis blood (n=10) for 24hours to generate septic plasma. BM-MSCs, induced pluripotent stem cell-derived MSCs (iMSCs-WT), and iMSCs overexpressing IL-4/IL-10 were cultured with serum from healthy donors or sepsis patients. Conditioned media (CM) was collected. The functional effect of CM on alveolar repair was assessed using an A549 epithelial cell wound healing (scratch) assay, wound closure was quantified by measuring relative wound width at 3-hour intervals.

RESULTS: VEGF concentration significantly increased in plasma from buffy coats regardless of LPS/cytomix stimulation and sepsis blood co-cultured with BM-MSCs. Functionally, CM from healthy-serum-stimulated BM-MSCs enhanced wound closure, while CM from sepsis-serum-stimulated BM-MSCs showed less beneficial effect. CM from healthy-serum-stimulated iMSCs-WT and iMSCs IL-4/IL-10 enhanced wound closure, but there was no difference between iMSCs-WT and iMSCs IL-4/IL-10; CM from sepsis-serum-stimulated iMSCs-WT and iMSCs IL-4/IL-10 showed no beneficial effect and no significant difference.

CONCLUSION: These findings indicate pro-healing capacity of MSCs is more effective in a healthy serum microenvironment and is diminished by the septic environment. This alveolar repair function appears independent of an anti-inflammatory cytokine milieu.

P.41

PPARδ antagonism improves the wound healing and anti-inflammatory properties of MSCs in an in vitro model

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Abstract

Background/Purpose: The use of MSCs as a therapeutic approach is a major area of interest in ARDS research. In contrast to the significant results found in preclinical research, clinical trials have yet to show similar efficacy of MSCs in ARDS. Previous studies have demonstrated that antagonising peroxisome proliferator-activated receptor delta (PPARδ), a nuclear receptor protein in charge of the metabolism of the cell, improved the efficacy of MSCs in an inflammatory environment. Thus, this study aims to investigate PPARδ's effect on MSC functionality in an in vitro model.

Methods: Bone marrow-derived MSCs were cultured for 24 hours with commercially synthetised PPARδ agonist and antagonist, as well as cytomix (IL-1β, TNF-α, IFN-γ (10ng/ml)) to mimic the inflammatory microenvironment, for 6 hours. The cells were then washed and incubated with fresh serum-free media for 24 hours before collecting the conditioned media (CM). The CM was analysed using ELISA and various functional assays such as scratch wound assay, anti-inflammatory assay, and phagocytosis assay.

Results: After stimulation with the PPARδ agonist, there was an increase in ANGPTL-4, a protein linked to PPARδ activation, as well as a decrease after stimulation with the antagonist. Antagonising PPARδ was shown to enhance wound healing, anti-inflammatory, and phagocytic properties of the MSCs, as confirmed by the functional assays conducted.

Conclusion: Overall, these results suggest that blocking PPARδ can improve MSC function in an inflammatory environment. This research highlights the potential of targeting PPARδ to modify MSCs and enhance their therapeutic effects.

P.42

MUSE Cells Provide a Cell-Therapy–Based Approach to combat AMR Clinical Pathogens *In Vitro*

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Abstract

Pneumonia remains a major global health concern, with high morbidity and mortality and treatment largely limited to antibiotics. Multilineage-differentiating Stress-Enduring (MUSE) cells are a distinct subpopulation of MSCs with immunomodulatory and regenerative properties that may enhance host defense, reduce inflammation, and promote lung repair. Beyond the known antimicrobial activity of MSCs, their ability to modulate immune responses and support tissue regeneration positions MUSE cells as a promising strategy for preventing pneumonia-related complications such as persistent inflammation. This study investigates the therapeutic and immunomodulatory effects of MUSE cells as a novel approach to pulmonary infection.

MUSE cells were isolated from human lipoaspirate–derived MSCs using MACS, and marker expression was confirmed by flow cytometry. Cells were cultured in serum-free media for 24 hours to obtain conditioned media (CM), which was tested in an A549 pulmonary epithelial injury model through cell viability, wound-healing, inflammatory response assays, and cytokine quantification by ELISA. To evaluate antimicrobial activity, CM was added to defined concentrations of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae*, and bacterial proliferation was measured by spectrophotometry.

Unstimulated MUSE cells displayed enhanced properties compared with conventional MSCs. Their effects resembled those of cytokine-activated MSCs, inhibiting bacterial growth and improving outcomes in epithelial cell assays. CM improved inflammatory markers, reduced bacterial growth, and supported cell metabolism compared with injury controls.

These findings show that naïve MUSE cells can mitigate key indicators of injury and inflammation in pulmonary infection and represent promising candidates for further investigation, particularly in pneumonia involving antimicrobial-resistant bacteria.

P.43

Development of a robust process to isolate and manufacture human Adipose-Derived Mesenchymal Stromal Cells suitable for technology transfer to a good manufacturing practice environment

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Abstract

Mesenchymal stromal cells (MSCs) are spindle-shaped immunomodulatory cells that have multipotent differentiation capacity. They are characterised by their ability to adhere to plastic, differentiate and express typical cell surface markers. MSCs can be easily isolated from lipoaspirate for various therapeutic applications.

The aim of this study is to isolate and manufacture human adipose-derived stromal cells (hADSCs) from lipoaspirates shipped at ambient temperature for 72 hours and to determine its suitability for technology transfer into a Good Manufacturing Practice (GMP) environment.

Lipoaspirates from two donors were shipped at ambient temperature and processed after 72 hours. Sterility testing was performed prior to processing. Lipoaspirates underwent centrifugation and wash steps to eliminate oil and blood layers. Collagenase was used to digest the tissue and isolate the stromal vascular fraction (SVF), which was concentrated downstream by red blood cell lysis and filtration. Cell viability, morphology and phenotype were examined. Mycoplasma, sterility and endotoxin testing were performed to ensure safety of the isolated cells.

SVF demonstrated high viability post-isolation, and the cells were negative for mycoplasma, bacterial growth and endotoxin following expansion for one passage. Morphology was spindle-like and consistent with hADSCs. Phenotypic assessment confirmed hADSCs as described by the International Society for Cellular Therapy acceptance criteria for cell surface marker expression.

This study has demonstrated a robust aseptic process for the isolation of hADSCs from lipoaspirate tissue in a good laboratory practice environment. This process is now ready for technology transfer to a GMP facility which will enable manufacturing of hADSCs for clinical application.

P.44

Media matters: culture medium influences the biological potential of mesenchymal stromal cells (MSCs)

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Abstract

Despite sustained efforts in clinical testing, the number of market authorisations for mesenchymal stromal cell (MSC) therapies remains limited. A look at clinical trial registries demonstrates the diversity of MSC expansion protocols with variabilities in cell source, isolation method and expansion medium, among other culture variables, making it impossible to compare study outcomes and highlighting a lack of standardised manufacturing protocols and an absence of biologically meaningful release assays.

We analysed the influence of five widely used culture media formulations on the colony-forming potential, proliferation kinetics, trilineage differentiation potential, extensive cell surface marker expression and immunomodulatory potential of human bone marrow-derived MSCs (BM-MSCs). Although all cell preparations expressed the standard MSC surface markers (CD73, CD90 and CD105), one-third of the 243 markers tested exhibiting variable expression profiles. In addition significant differences in the biological attributes of BM-MSCs such as clonogenicity, proliferation, differentiation propensity and immunomodulatory capacity were revealed in response to the composition of the culture medium. Principal component analysis demonstrated that BM-MSCs isolated and expanded in a proprietary xeno- and serum-free medium displayed the most consistent cell phenotypes with little variability between donors compared to platelet lysate and foetal bovine serum-containing media. These data highlight the importance of manufacturing details and implies that the standard surface markers conceal the biological differences between preparations.

P.45

ATMP manufacturing barriers, the skills needed to manufacture them and the knowledge needed to support ATMP facility Inspections

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Abstract

Introduction:

Advanced Therapy Medicinal Products (ATMPs) are an emerging class of treatments with transformative potential across a wide range of diseases. Despite their promise, manufacturing barriers continue to restrict scalability and delay patient access. Addressing these challenges requires both specialised skills among manufacturing personnel and enhanced knowledge among regulatory staff to ensure effective oversight.

Purpose:

This study investigates:

- The key obstacles hindering ATMP manufacturing.
- The essential skills required for manufacturing personnel.
- Whether existing biopharmaceutical expertise can be adapted to meet ATMP-specific requirements.
- The knowledge needed to support regulatory inspections of ATMP facilities.

Methods:

- A survey to capture industry perspectives on manufacturing barriers and skills needs.
- Development of a Manufacturing Skills Heatmap.
- A survey to determine the interest in customed training courses for manufacturing personnel and regulators.

Results:

The study revealed:

- Barriers: Regulatory complexity and technical scalability issues among other barriers.
- Skills gaps: Specialised competencies such as in aseptic techniques and digital skills beyond traditional biopharmaceutical expertise as well as soft skills.

- Knowledge needs: Regulatory staff require an understanding of manufacturing processes, while manufacturers benefit from reciprocal regulatory awareness.
- Educational outcomes: Development of two accredited Level microcredentials with Atlantic Technological University (ATU), one tailored for manufacturing personnel and the other designed for regulatory personnel.

Conclusion:

This research highlights the critical barriers to ATMP/CGT manufacturing and underscores the necessity of tailored skills and knowledge for both production and regulatory inspection. The creation of ATU-accredited training programs demonstrates a tangible step toward strengthening workforce capability and regulatory preparedness in the ATMP sector.

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UC-ECFCs Exhibit Tolerogenic Effects by Reducing T Cell Activation and Enhancing T reg Proliferation under IL2 Condition

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Abstract

Umbilical cord blood-derived endothelial colony-forming cells (UC-ECFCs) are endothelial progenitor cells capable of generating new blood vessels and supporting vascular regeneration. Their vasculogenic and reparative potential makes them promising candidates for therapeutic angiogenesis, particularly in graft revascularization and ischaemic conditions such as chronic limb-threatening ischaemia (CLTI). However, the clinical use of allogeneic UC-ECFCs requires a better understanding of their immunogenicity and immunomodulatory properties.

In this study, we investigated the immunological interactions between UC-ECFCs and T cells. UC-ECFCs, either unstimulated or pre-treated with interferon-gamma (IFN-γ, 100 µg/mL) for 24 hours, were co-cultured with peripheral blood mononuclear cells (PBMCs) at a 1:1 ratio for 2 or 4 days, in the presence or absence of interleukin-2 (IL-2). T cell activation was assessed by CD25 and CD69 expression on Day 2, and proliferation by Day 4. Additionally, IFN-γ-stimulated UC-ECFCs were analysed for CD80 and CD86 expression to determine co-stimulatory potential.

T cell activation occurred only with IL-2 stimulation, and co-culture with UC-ECFCs reduced the frequency of activated T cells compared to IL-2 alone at day 2, indicating early suppression of activation. By Day 4, activation markers normalized, and proliferation was observed only under IL-2-supplemented conditions. Proliferated CD4+ T cells displayed an increased FoxP3+ fraction, suggesting a regulatory phenotype.

Overall, UC-ECFCs did not promote any T cell activation or proliferation but instead exhibited immunosuppressive properties, supporting their potential as safe allogeneic cell therapy candidates for ischaemic and chronic inflammatory diseases.

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P.47

Developing an MRI protocol to analyse limb recovery after MSC and ECFC therapy in a hindlimb-ischaemia model

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Abstract

Magnetic resonance imaging (MRI) is a powerful in vivo imaging technology with extensive use in neuroimaging. To date there is little research on MRI's potential for imaging the lower limb. MRI has potential to monitor recovery in vivo in response to ischaemia which results in inflammation, dysregulated muscle fibre organisation, and reduced blood flow. We aimed to develop a protocol for using MRI technology for a hind-limb ischaemia (HLI) mouse model which will facilitate monitoring recovery after treatment with our future mesenchymal stromal cell and endothelial colony forming cell based therapeutics, without the need for animal sacrifice at each timepoint.

Six male Balb/c nude mice were subjected to MRI on days -3,+3, and +7 with single ligation of the femoral artery on day 0. Scans were performed for muscle fibre orientation (DW-EPI), inflammation (FLAIR), muscle anatomy (3D-GRE), and vessel anatomy (3D-Multi-TOF).

MRI distinguished a significantly increased inflammation in the ischaemic limb at day 3 and 7 ($P<0.0001$, 0.0001 respectively) with a corresponding increase in muscle volume ($P<0.0001$, 0.0001 respectively). However, the muscle volume change was not indicative of muscle weights at day 7, which instead displayed a trend decrease in weight with ischaemia. This difference was likely due to the replacement of lost muscle mass with less dense water.

MRI shows to be sufficiently sensitive to monitor inflammation and muscle volume across time, suggesting that MRI is a suitable method to assess therapeutic recovery after HLI. Future analysis will determine whether MRI can monitor angiogenic recovery post therapeutic intervention.

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Dual-chamber device enables ambient temperature transport and real-time monitoring of therapeutic cells

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Abstract

Cell therapies offer the ability to target malignant cells, regenerate damaged tissue and restore physiological function. While cryopreservation remains the gold standard for cell shipment, the freeze-thaw process can compromise viability, functionality and overall therapeutic potency. Transporting fresh cells at ambient temperatures (15-25°C) avoids cold-chain logistics and eliminates cryodamage; however, current approaches are limited by shorter shipment windows and vulnerability to hypoxic stress. To overcome these constraints, we developed an oxygen-supplying ambient transport device with integrated sensors to support cell health and enable real-time monitoring during shipment. In 48-hour pilot studies, surrogate models for diabetes and CAR-T products maintained viability and functionality, outperforming out-of-thaw controls. Here, we expand this work by numerically evaluating oxygen transfer and oxygen durability, or how long cells can be sustained in the device. A finite-element computational framework, validated against experimental sensor data, predicts optimal cell-loading densities based on their oxygen consumption rates. Using this model, we further optimised the device to extend transport timeframes to maintain cells for 120 hours, a realistic target for global distribution. This workflow highlights the capabilities of this ambient transport system for long-range shipments. Predictive cell-loading densities and shipment durations ensure the cells are sustained throughout transit, while integrated sensors monitor in-transit behaviour to indicate cell health and readiness for use upon arrival.

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Study of iPS-derived MSCs in ARDS

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Abstract

Mesenchymal stromal cells (MSCs) have demonstrated benefit in pathogenic acute respiratory distress syndrome (ARDS) models and are in clinical trials, but allogeneic MSCs have several manufacturing and consistency disadvantages. Recently, an iPS-derived MSCs (iMSCs) has been developed by a novel mRNA method generating mutation-free, clinically viable cells. The main aim of this study is to characterise the iMSCs in *in vitro* potency assays and test them in an animal model of bacterial ARDS.

Cells were cocultured with activated PBMCs at different iMSC:PBMC ratios, and PBMC proliferation and cytokine secretion was detected by flow cytometry and ELISA, respectively. Conditioned media was collected and it was incubated with 3 different bacterial clinical isolates for 24 hours. 10^9 CFUs/animal of *Escherichia coli* was administered to Sprague-Dawley rats intratracheally. 10^7 cells/kg were administered 30 min after the bacterial instillation, and full animal assesment was done after 48 hours of the bacterial instillation.

iMSCs can reduce T-cell proliferation and the secretion of proinflammatory cytokines and increase the secretion of anti-inflammatory cytokines. Furthermore, their derived conditioned media can inhibit the growth of 3 different bacterial clinical isolates. In our *in vivo* preclinical model, induced by intratracheal *Escherichia coli* instillation, iMSCs have been administered shortly after the bacterial instillation. The iMSC administration improves several outcomes, including lung static compliance and bronchoalveolar lavage white blood cell counts.

In conclusion, iMSCs are good candidates for further preclinical studies in bacterial ARDS, and cell pre-activation strategies should be studied for further enhance their therapeutical properties.

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P.50

The application of quality by design (QbD) for cell therapies: opportunities and barriers.

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Abstract

Introduction: CAR-T cell therapies have achieved unprecedented success in treating late-stage cancers like leukaemia. Despite their clinical potential, CAR-T cell therapies face significant challenges during their development and manufacturing phases. The biological complexity of living cells and the incomplete understanding of their mechanisms of action present significant manufacturing, regulatory and scaling challenges. Aim: To address these barriers, this research explores the application of quality by design (QbD) principles in enhancing process understanding, control, and efficiency in autologous CAR-T cell therapy manufacturing. . Methodology: This study involves a comprehensive literature review, semi-structured interviews with subject matter experts (SMEs), and a survey of industry professionals. Results: A review of current best practice outlines the potential of QbD and digital tools, such as process analytical technology (PAT), automation, digital twins, and artificial intelligence (AI) to enhance manufacturing robustness and product quality. The SME interviews identified common barriers such as variability of patient-specific starting materials, lack of standardisation, inconsistency and unsuitability of existing regulatory frameworks. Survey results further showed cost concerns and availability of real-time monitoring tools limit the effective implementation of QbD. Discussion: Appropriate application of QbD can provide enhanced knowledge of quality target product profiles (QTTPs), critical quality attributes (CQAs), critical process parameters (CPPs), design space, and control strategies and this provides a sound science- and risk-based framework for enhancing consistency, efficacy, and scalability in cell therapy manufacturing. Conclusion: This research provides insights into how integrating QbD and digitalisation can help to overcome manufacturing and regulatory barriers in autologous CAR-T cell therapies.

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Advancing cell and gene therapies through a decentralised manufacturing approach. Evaluating regulatory approaches in the UK, EU, and US

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Abstract

Introduction: Advanced Therapy Medicinal Products (ATMPs) are transforming the healthcare landscape, by shifting the focus from managing symptoms to potentially curing diseases and genetic disorders. However, the unique nature of their starting materials presents several challenges including intricate manufacturing processes, high development and production costs, and complex logistical and regulatory hurdles. A decentralised manufacturing strategy may offer way to both reduce production costs and increase availability of these therapeutics. Recognising the potential of this approach, the UK's Medicines and Healthcare product Regulatory Agency (MHRA) has announced introduced a regulatory framework dedicated to point-of-care manufacturing for ATMPs. Aim: This study explores these guidelines, comparing them to existing approaches in the EU and US, and evaluates whether this innovative approach could overcome the barriers limiting access to these therapies. Methodology: Data for this study was gathered through a literature review, a case study and conduction of interviews with subject matter experts (SMEs). Results: The findings show regulators are keen to understand this approach, but greater regulatory alignment is still required. While decentralised manufacturing offers clear benefits such as reduced vein-to-vein timelines and lower contamination risks through the use of automated closed systems, significant hurdles still remain for the implementation of this approach. These include inconsistent regulations across regions and the potential for inequality of access. Conclusion: Despite these challenges, decentralised manufacturing represents a significant step forward for ATMP manufacture options. Adoption of this approach should be encouraged to accelerate more research and development and to facilitate increased patients access to ATMPs.

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Trends in reporting uncertainty quantification in recent CAR-T mechanistic modelling research in the context of EMA and ICH reporting guidance for model-informed drug development.

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Abstract

Introduction/Purpose: Regulatory acceptance of mechanistic models in model-informed drug development (MIDD) depends on the potential model risk. Recent ICH guidance and EMA concept papers include a focus on uncertainty quantification (UQ), to quantify model predictive performance risk. As living drugs, chimeric antigen receptor (CAR)-T cell pharmacokinetics, or cytokinetics (CK), differ from conventional drugs with in vivo expansion and high inter-individual variability in CAR-T CK. Recently there have been notable advances in developing complex (semi-) mechanistic models to predict CAR-T products' clinical CK.

The aim of this pilot project is to investigate the reporting of UQ in recent publications on advanced CAR-T CK mechanistic models.

Methods: This study is broadly based on methods presented in EUPAS 1000000717 (https://catalogues.ema.europa.eu/node/4620/administrative-details) but focused on research publications of CAR-T CK mechanistic models. Recent review citations were consulted to identify a convenience sample of contemporary (since 2023) relevant papers. Aspects considered were: 1) predictive performance measures presented; 2) whether UQ results were explicitly discussed.

Results: Six publications were identified, none of which included specific results/discussion sections relating to UQ, or quantifying model risk/impact. All studies included some predictive performance data (e.g. goodness of fit, simulated and observed plots, %MSE/RSE, sensitivity analysis).

Discussion/conclusion: Publications generally focused on presenting advanced modeling approaches with varying levels of predictive performance information. However, these preliminary results suggest that, given increasing regulatory UQ emphasis and the role of research publications in informing MIDD, explicit reference to UQ should become standard in research outputs predicting CAR-T CK.

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The Hospital Exemption Route in Europe: A Critical Analysis of the Current Regulatory Landscape and Impact of Proposed EU Pharmaceutical Package Reforms on Patient Access?

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Abstract

Article 28(2) of Regulation 1394/2007/EC provides a European regulatory pathway for the provision of advanced therapy medicinal products (ATMPs) prepared on a non-routine basis under the exclusive responsibility of a medical practitioner for individual patients in cases of unmet need (the so-called *hospital exemption route*). Products made under the hospital exemption route are exempt from the requirements of a marketing authorisation in Europe. The manufacturing of products under the hospital exemption route must be authorised by the relevant national competent authority in each EU Member State, and differences in the implementation of Article 28(2) across EU Member States has led to a divergence of approaches on the hospital exemption route in practice. Accordingly, the proposed EU pharmaceutical package, if adopted in its current form, sets out a series of proposals related to the operation of the hospital exemption route in Europe.

This paper examines the operation of the hospital exemption pathway under Regulation 1394/2007EC, and specifically, the key changes around this route proposed by the EU Pharmaceutical Package reforms. In doing so, it critically analyses the potential implications of these reforms for the development and access to ATMPs for patients under the hospital exemption route in Europe, including considering stakeholder responses to these. The paper makes the case for a nuanced approach to the hospital exemption route, arguing, that EU reforms should have as their core objective the promotion of ethically robust patient centered pathways for the delivery of ATMPs in Europe.

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CONCEPT: A core facility for early-stage biotherapy development.

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Abstract

CONCEPT is a core facility dedicated to advancing and accelerating early-stage biotherapy research and development. By providing access to state-of-the-art instrumentation at CONCEPT, researchers can interrogate new disease biomarkers, investigate mechanisms of action, explore novel therapeutic targets, and generate research-grade Biologics, Cell, Gene, and RNA therapies for in vitro and pre-clinical studies. Our approach is modular, facilitating access to individual instruments or complete end-to-end workflows tailored to your needs, with pricing options designed to enable academic research as well as industry resourcing.

Our dedicated scientific team offers comprehensive support throughout the process from streamlined workflow design, protocol development, and process optimisation. Together, we can drive innovation forward.

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