Cell & Gene Therapy in Ireland

Maynooth University
National University of Ireland Maynooth

31 JANUARY 2025
Maynooth University
TSI Building

CONFERENCE SCHEDULE

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Welcome Message

Dear Colleagues,

We are delighted to welcome you all to the Cell & Gene Therapy in Ireland Conference 2025 here at Maynooth University. The last meeting for Cell & Gene Therapy in Ireland was held as far back as 2007/2008. Given the groundbreaking developments in this field and the fact that there are many excellent research groups working on Cell & Gene Therapy it was time to gather the Irish community to provide opportunities for networking and collaboration.

This meeting reflects the strengths across the Irish Cell & Gene Therapy community. We have invited speakers from Ireland for this inaugural meeting. Our speakers represent basic science, clinical application and access to cell therapies in Ireland with a stakeholder session focused on bringing cellular therapies to the patient.

We were delighted to receive >60 abstract submissions and >225 registrations from Institutes across the whole of Ireland. We have selected 8 short talks from many high-quality abstracts. There will be a poster session during the lunch break and there are a number of prizes for early career researchers in the oral and poster presentations.

This meeting could not have happened without the immense contribution by many people at Maynooth University including Dr. Shirley O'Dea, Prof. Aisling McMahon, Dr. Nezira Delagic, Evelina Volkova, Amanda Cardiff and many others, along with invaluable financial support from our commercial sponsors and Maynooth University.

We look forward to a great meeting, we hope that many of you can develop fruitful collaborations, grow your networks and we wish you every success in your research endeavours,

Best wishes

Karen English,
Maynooth University

(Chair of the Cell & Gene Therapy in Ireland 2025 Conference)

WE WOULD LIKE TO ENCOURAGE ALL DELEGATES TO VISIT THE TRADE EXHIBITION THROUGHOUT THE MEETING.

WE WOULD LIKE TO THANK THE FOLLOWING SPONSORS FOR THEIR GENEROUS SUPPORT IN 2025:

Conference for Cell and Gene Therapy in Ireland
Friday, January 31, 2025

Technology Science & Innovation Building, North Campus, Maynooth University, Maynooth, Co Kildare, Ireland

FRIDAY, 31ST JANUARY 2025	
08:30 – 09:25	***Conference Registration (T.S.I. Level 0, outside LT3)***
09:30 - 09:35	***Conference Open/Welcome:*** **Karen English and Shirley O'Dea** *Maynooth University*
SESSION 1:	**Cell Therapy** ***Chair: Aideen Ryan, University of Galway***
09:35 - 09:55	***"Irish Medicines: Personalized Advanced Cellular Therapeutics (IMPACT)"*** **Sakis Mantalaris** *Trinity College Dublin & NIBRT*
09:55 - 10:05	***"Targeting MAIT cells anti-tumour activity via metabolic reprogramming"*** **Ardena Berisha** *Maynooth University*
10:05 - 10:25	***"Unravelling the mechanistic underpinnings of MSC therapy"*** **Karen English** *Maynooth University*
10:25 – 10:35	***"Mechanical induction of regulatory T cells"*** **Jessica White** *Queen's University Belfast*
10:35 – 10.55	***"MSCs – Roadblocks in Manufacturing and Clinical Assessment"*** **Frank Barry** *University of Galway*

10:55 – 11:05	***"Understanding the ARDS microenvironment to enhance MSC therapy"***
	Courteney Tunstead *Maynooth University*
11:05 – 11:40	**COFFEE BREAK (ISGCT Townhall meeting, TSI128)**

SESSION 2:	**Cell & Gene Therapy** ***Chair: Shirley O'Dea, Maynooth University***

11:40 – 12:00	***"Shedding light on inherited eye disorders - elucidating causes and designing gene therapies"***
	Jane Farrar *Trinity College Dublin*
12:00 – 12:20	***"Gene Therapy in Spinal Muscular Atrophy"***
	Declan O'Rourke *Children's Health Ireland, Temple St*
12:20 – 12:30	***"Immunoengineering of UCART19 – The back story"***
	Brian Philip *NIBRT*
12:30 – 12:40	***"Examining the impact of microRNA-379 on breast cancer progression and immune cell infiltration"***
	Elan McCarthy *University of Galway*
12:40 – 13:00	***"A gene therapy based approach for glaucoma"***
	Matt Campbell *Trinity College Dublin*
13:00 – 14:00	**LUNCH AND POSTER SESSION**

SESSION 3:	**Clinical Translation & Access** ***Chair: Nicki Panoskaltsis, Trinity College Dublin***
14:00 - 14:20	***"Delivering Sustainable Pathways for the Provision of CAR-T Therapies: Legal, Ethical, and Societal Considerations"*** **Aisling McMahon** *Maynooth University*
14:20 – 14:40	***"Engineering NK cells for the treatment of cancer?"*** **Michael O'Dwyer** *University of Galway*
14:40 – 14:50	***"Safety and Preliminary Efficacy Results of ORBCEL-M in Diabetic Kidney Disease: The Phase-1b/2a NEPHSTROM Clinical Trials"*** **Steve Elliman** *Orbsen Therapeutics*
14:50 – 15:10	***"Are MSCs a REALISTic treatment for ARDS?"*** **Cecilia O'Kane** *Queen's University Belfast*
15:10 – 15:20	***"Far Behind the Curve - Addressing Ireland's Low Readiness for the Clinical Adoption of Advanced Therapy Medicinal Products"*** **Martina Hennessy** *Trinity College Dublin*
15:20 – 15:30	***"CD19 CAR T-cell Therapy and Its Impact on Antibodies Against Vaccine-Preventable Viral Infections in Lymphoma Patients"*** **Hayley Foy-Stones** *St James's Hospital*
15:30 – 15:50	***"Clinical experience of CAR-T for blood cancer in Ireland"*** **Larry Bacon** *St James's Hospital*
15:50 – 16:20	**COFFEE BREAK**

SESSION 4:	**Panel Discussion; bringing cellular therapies to the patient** ***Chair: Aisling McMahon, Maynooth University***
16:20 – 17:00	**Panelists:** **Larry Bacon** *(Consultant Haematologist, St. James's Hospital)* **Pat Byrne** *(CAR-T patient recipient)* **Nicola Gardiner** *(Chief Medical Scientist, St. James's Hospital)* **Elizabeth Higgins** *(CAR-T service co-Ordinator, St. James's Hospital)* **Owen Smith (***Professor of Child, Adolescent and Young Adult Oncology, Trinity College Dublin)*
17:00	**Close of Conference, Prize Giving and Wine Reception**

GUEST SPEAKERS

Sakis Mantalaris
Trinity College Dublin & NIBRT

Research Interests: Sakis Mantalaris is currently the Don Panoz Chair at the School of Pharmacy & Pharmaceutical Sciences at Trinity College Dublin as well as Principal Investigator at the National Institute for Bioprocessing Research & Training. Prior, he was Professor in the W.H. Coulter Department of Biomedical Engineering at the Georgia Institute of Technology (2018-2023), USA and in the Department of Chemical Engineering at Imperial College London (2000-2018). He received his PhD in Chemical Engineering in 2000 from the University of Rochester, USA. He has received several awards: the Junior Moulton Award for best paper by the IChemE (2004), Fellow of American Institute for Medical and Biological Engineering (2012), an ERC Advanced Investigator Award (2013), the Donald Medal from IChemE (2015) and the SFI Research Professor Award in 2023.

Karen English
Maynooth University

Research Interests: Karen English is a professor of Immunology at Maynooth University. Karen has been working on the fundamental understanding of the mechanisms of action of the cell based therapy mesenchymal stromal cells (MSCs) for the past 20 years. Her research is focused towards translating MSC therapy towards optimal clinical application in various inflammatory diseases. She is also collaborating with a large consortium of European researchers on gene therapy for Muscular Dystrophies through the MAGIC project. She completed her PhD at Maynooth University in 2008 and received a highly competitive Marie Curie Fellowship to carry out her postdoctoral training at the University of Oxford in the area of MSC therapy in transplant rejection. She has published >70 research articles and her work has attracted >5000 citations. Karen has obtained significant competitive research funding including an IRC Laureate Award and an SFI FFP award. In 2018, Karen was awarded the Irish Research Council Early-Career Researcher of the year award and the MU Early career research achievement award across all three faculties at Maynooth University. She is an editorial board member of Stem Cells Research & Therapy. Karen is a member of the International Society for Cell therapy (ISCT) MSC committee and gave a plenary lecture on MSCs in the Science & Innovation session at the ISCT in Vancouver in 2024.

Frank Barry
University of Galway

Research Interests: Frank Barry is Professor of Cellular Therapy and Principle Investigator at the Regenerative Medicine Institute (REMEDI), University of Galway. Here he directs a large group of researchers who focus on the development of novel therapeutic strategies in stem cell and gene therapy. He has contributed to the field of regenerative medicine by developing innovative and successful cellular therapies for the treatment of arthritic disease and chronic lower back pain. In addition, he has developed new techniques for the isolation, characterization and GMP production of stromal cells and induced pluripotent stem cells as well as advanced automated platforms for their manufacture. He sits on a number of advisory boards, including the Schroeder Arthritis Institute (Toronto), Bio-ReCell (Ljubljana), the ASCOT Trial Steering group (Oswestry) and the Center for Modular Manufacturing of Structural Tissues (Cleveland). He is a founder and director of Orbsen Therapeutics (Galway). In a career that has spanned both industry and academic research, he has been a driver in the development of cellular therapy as a biological repair strategy. His published work has received some 28,000 citations with a h-index of 71. Frank Barry was the recipient of the 2012 Marshall Urist Award for excellence in tissue regeneration research from the Orthopaedic Research Society and is a member of the Royal Irish Academy. He has coordinated several projects funded by the EU Horizon and Regional Develpopment Funds and has participated in clinical trials for the assessment of stromal cell therapy as a treatment for osteoarthritis and chronic lower back pain.

Jane Farrar
Trinity College Dublin

Research Interests: G Jane Farrar is Professor in the School of Genetics and Microbiology. Jane undertook an undergraduate degree in Genetics (1985, TCD), PhD in Human Genetics (1989, TCD) and diploma in management for scientists and engineers (1995, TCD). Jane has been actively involved for decades in education and training of TCD undergraduate and postgraduate students, was co-ordinator of the Human Genetics degree for 16 years (2005-2021) and DUTL for the School from 2013-2016. Jane has trained many PhD students and post-doctoral scientists and mentored many senior research and clinical scientists and ophthalmology fellows. Jane enjoys disseminating scientific ideas to primary and secondary school students, patient groups and the general public and regards it a privilege to disseminate truly amazing concepts in human genetics to diverse audiences.

Jane Farrar has decades of experience in the field of inherited ocular diseases focusing on retinal degenerations (IRDs) resulting in key outcomes and discoveries regarding these devastating sight loss disorders for patients, generating significant funding (approx. 20M), 160+ publications (h-index 50, google scholar), families of granted patents, two campus companies, opportunities to sit on multiple national and international boards inter alia Foundation Fighting Blindness (US), Spark Therapeutics, Royal Victoria Eye and Ear Hospital Dublin, editorial boards, organising committees for international conferences, among others. Her primary research interests are focused on elucidation of the genetic and molecular pathogenesis of ocular disorders and development of cell and gene therapies.

Declan O'Rourke
Children's Health Ireland, Temple St.

Research Interests: Declan O'Rourke was appointed Associate Director of Research for Children's Health Ireland and Deputy Director of the CHI Clinical Research Facility in 2021. His role is to coordinate and grow the advanced therapeutics medicines program at CHI. Declan is one of three Paediatric Neurologists at Ireland's largest and busiest Paediatric Neurology department at CHI Temple Street. Declan is currently Head of Department. Declan diagnoses and manages a large volume of children with acute and chronic neurological diseases including epilepsy, migraine, encephalopathies, encephalitis, neuroimmunology, critical care, stroke, acquired traumatic and infectious disorders as well as genetic and metabolic disorders. Declan runs a large General Paediatric Neurology service that has a large multidisciplinary focus. He also run an electrodiagnostic service (nerve conduction studies/ electromyography) providing diagnostic assessments for patients with neuromuscular signs and symptoms.
Declan is the lead neurologist at the largest Paediatric Neuromuscular Clinic in Ireland caring for over 400 children with Duchenne Muscular Dystrophy, Spinal Muscular Atrophy, Congenital Myopathies and Muscular Dystrophies, Myasthenia Gravis and Congenital Myasthenic Syndrome as well as hereditary motor and sensory neuropathies. Declan has been a principal investigator on several clinical trials in paediatric patients with Duchenne Muscular Dystrophy and Spinal Muscular Atrophy. Declan was co-applicant on the 2022 CHI-CRF HRB grant, an application for >€5 million designed to ensure the sustainable infrastructure for ongoing clinical trials at CHI. In 2021, Declan's team were the first group in Ireland to administer gene replacement therapy to children in Ireland.

Matthew Campbell
Trinity College Dublin

Research Interests: Matthew Campbell is Professor in Genetics and Head of Department at the Smurfit Institute of Genetics in Trinity College Dublin. He is originally from Dublin and graduated with a degree in Biochemistry from UCD in 2002 and went on to complete a PhD in 2006 at the same institution focused on understanding the role of the blood retina barrier in the degenerating retina. In the same year, he moved to Trinity College Dublin and conducted postdoctoral research with Prof Pete Humphries on the role of the blood brain/retina barriers in health and disease. In 2013, he was awarded Science Foundation Ireland's (SFI) President of Ireland Young Researcher Award (PIYRA) which allowed him to establish his own research group in TCD. Since then, he has received numerous additional awards for his research which focuses on understanding the role of the so-called blood-brain barrier (BBB) and blood retina barrier (BRB) in healthy and diseased states. In 2020 he was awarded one of Europe's most prestigious awards from the European Research Council (ERC). In the same year he was elected Science Foundation Ireland's early career researcher of the year. He also leads the SFI funded EYE-D programme, a four year €3.2M initiative which is focused on identifying novel therapeutic targets for ocular diseases. He is also a PI in the SFI funded Centre Future-Neuro. He is the founder and Director of the Neurovascular Genetics Unit at TCD and has over 20 years of research expertise in the area of blood brain and blood retina barrier biology. He sits on the scientific advisory board of the Moorfields Hospital Charity as well as the UK charity Sight Research UK.

Aisling McMahon
Maynooth University

Research Interests: Aisling McMahon is a Professor of Law at the School of Law and Criminology, Maynooth University. She previously held academic positions in Newcastle University and Durham University. Aisling's research focuses on health law, and intellectual property law, with a particular focus on the regulation of emerging health-technologies, bioethical issues related to emerging health-technologies, and the role of patents in the health context. She currently leads the European Research Council Starting Grant funded 'PatentsInHumans' project which examines the role of bioethics in the patenting/licensing of technologies related to the human body.
She previously led an Irish Research Council New Foundations funded 'Patient Access to Advanced Cancer Therapies' project which examined the landscape for provision of CAR-T therapies in Ireland. This project culminated in a policy report mapping the provision of CAR-T therapies in Ireland, highlighting key legal, ethical and societal challenges/opportunities, and made 10 policy recommendations towards developing a more sustainable system for the provision of such therapies in Ireland. She currently leads the IRC New Foundations funded 'Advanced Cancer Care: Enhancing Systems and Structures to Deliver In-House Personalised Therapies for Patients via the Hospital Exemption' project which examines the hospital exemption route for ATMPs. These projects are run in conjunction with Breakthrough Cancer Research. Aisling is regularly invited to participate in high level policy events hosted by a range of government, policy and civil society groups, and has presented her research to leading international organisations, including: the World Health Organisation, and the US National Academy of Sciences. She holds a PhD in law from the University of Edinburgh.

Michael O'Dwyer
University of Galway

Research Interests: Michael O'Dwyer MD FRCPI FRCPath is a Consultant Haematologist and Professor of Haematology at University of Galway as well as Scientific Director West North West/University of Galway Cancer Network. His current research is focused primarily on (1) how aberrant glycosylation (especially sialylation) can influence cellular adhesion, trafficking and immune evasion in multiple myeloma (MM) (2) the role of glycoimmune checkpoints in cancer and (3) use and modification of natural killer (NK) cells as cell therapy for cancer. His work on glycosylation has implicated interactions between selectins, siglecs and their ligands in disease progression, drug resistance and immune evasion in MM. These findings have provided new insights into the role of glycosylation in the progression of MM with potential for new therapeutic strategies to overcome treatment resistance. His interest in NK cells led in 2015 to the formation of a spin out company ONK Therapeutics www.onktherapeutics.com which he led as CSO until January 2023. His academic and industry work has focused on NK cell biology and function, the incorporation of novel edits to enhance cytotoxicity and persistence and the efficient, large scale manufacture of gene edited, cord-derived CAR-NK cell products. He has several granted patents covering the use of engineered NK cells for cancer.

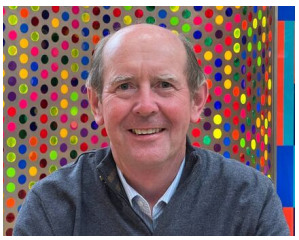
Cecilia O'Kane
Queen's University Belfast

Research Interests: Cecilia O'Kane is a Professor of Respiratory Medicine at Queen's University Belfast and Honorary Consultant Physician at Belfast Health and Social Care Trust, where she leads the pulmonary TB and non-tuberculous mycobacterial infection service. Cecilia's research interests centre around pulmonary inflammation particularly in the setting of ARDS and mycobacterial infection. She leads a translational group investigating host inflammatory response in NTM infection and testing novel therapies for ARDS in in vitro models, ex vivo human lung, healthy volunteer challenge models and clinical trials. Since August 2022 she has been co-Editor in chief of Thorax, alongside Jenni Quint and Mark Griffiths.

Larry Bacon
St. James's Hospital

Research Interests: Dr Bacon is a clinical and laboratory haematologist in St James's Hospital with specialist interest in lymphoma, acute lymphoblastic leukaemia, bone marrow transplantation (BMT) and chimeric antigen receptor T cell (CAR T) therapy.

Pat Byrne
Patient (CAR-T recipient). Pat Byrne underwent CAR-T therapy in St James hospital. Pat will share his personal experience.



Elizabeth (Liz) Higgins
St. James's Hospital

Research Interests: Elizabeth (Liz) Higgins Clinical Nurse Manager (CNM) 111, is the CAR T Service Coordinator at St James's Hospital. Liz's introduction to Haematology nursing started in Australia in 1990. When she returned to Ireland, she joined the nursing team on the Haematology in patient unit in St James's Hospital. Her roles to date have included Staff Nurse & Clinical Nurse Manger in the National Adult Bone Marrow Transplant Unit, she then moved into the role of Stem Cell Transplant Coordinator. Her current role is in CAR-T Service development and Coordination at St James's hospital.

Nicola Gardiner
St. James's Hospital

Research Interests: Nicola Gardiner is the Chief Medical Scientist in the Cryobiology Laboratory Stem Cell Facility at the National Blood Centre, St. James's Hospital. Nicola has been Scientific lead of the Cryobiology Laboratory service for the National Stem Cell Transplant Programme for 30+ years. A science graduate of Maynooth University (1990), she completed a PhD in the Haematology Department, St James Hospital as part of the transplant team. The first Irish bone marrow transplant was performed in 1984 since then the service has evolved from 6 to 200+ stem cell transplants per year. Both the number and complexity of stem cell processing for transplant has increased. Peripheral Blood Stem Cells (PBSC) have replaced bone marrow as the stem cell source of choice. Processing of products in a GMP clean room facility required a move to the National Blood Centre, obtaining a HPRA Tissue Establishment Licence (2008) and more recently international JACIE accreditation. Chimeric Antigen Receptor (CAR) T cell therapy (2020) has been an exciting new treatment option for patients with the laboratory being a critical part of the CAR T manufacturing process. Interactions with research and biopharma teams has enabled some recent research developments. The prospect of in-hospital manufacturing of cellular therapies is the next frontier to enable faster delivery of cells for the patient and increase accessibility.

Owen Smith
Childrens Health Ireland, Crumlin & St. James's Hospital

Research Interests: Professor Owen Patrick Smith, CBE, Hon FTCD, Childrens Health Ireland, Crumlin & Trinity College Dublin. Professor Owen Smith is a Professor of Child, Adolescent & Young Adult Oncology [2022] and Professor of Haematology [2002] in the School of Medicine, Trinity College Dublin. Professor Smith is Consultant Paediatric Haematologist at Children's Health Ireland at Crumlin, Chief Academic Lead to the Children Hospital Group, National Clinical Lead for Child, Adolescent & Young Adult cancers at the National Cancer Control Programme and is currently Chair of the Adolescent & Young Adult Cancer Committee of the European Society for Paediatric Oncology [2022-2025].
The co-author of more than 400 research original articles, letters, books, book chapters and papers, Professor Smith is a Fellow of the Royal College of Paediatrics and Child Health, Royal College of Pathologists, Royal College of Physicians of Dublin, Royal College of Physicians London, Royal College of Physicians Glasgow, and Royal College of Physicians Edinburgh. He is a member of numerous associations and societies, including; the Medical Research Council Childhood Leukaemia Working Party, Children's Oncology Group [USA], the International Berlin Frankfurt Munster Study Group for Childhood Leukaemia, the United Kingdom Children's Cancer Group, European Working Group on paediatric aplastic anaemia and myelodysplastic syndromes. He is an international advocate for children and adolescents with rare diseases and for expanded access to expensive drugs. In 2015, Professor Smith was conferred honorary Commander in the Most Excellent Order of the British Empire (CBE) for his life-long work on cancer in children and adolescents by Queen Elizabeth, on the advice of the Foreign and Commonwealth Office.

SELECTED ORAL PRESENTATIONS

"TARGETING MAIT CELLS ANTI-TUMOUR ACTIVITY VIA METABOLIC REPROGRAMMING."

***Ardena Berisha,** Nidhi Kedia-Mehta, Benjamin J. Jenkins, Eimear K. Ryan, Odhran Ryan, Cian Davis, Helen Heneghan, Donal O'Shea, Nicholas Jones, and Andrew E. Hogan.

Obesity Immunology Research Group, Maynooth University

***Lead Presenter: ardena.berisha.2018@mumail.ie (Postgrad Researcher (>two years))**

Mucosal Associated Invariant T (MAIT) cells are a population of unconventional T cells which play a critical role in host protection against both bacterial and viral pathogens. An emerging area of focus is their role in anti-cancer immunity, this is due to their ability to rapidly lyse target cells and produce significant amounts of effector cytokines such as IFNg. Furthermore MAIT cells are not restricted by MHC but rather recognise antigens presented the conserved MR1 molecule. These key features have highlighted their potential as an anti-cancer adoptive cellular therapy. In this study we show that MAIT cells can rapidily proflierate in vitro, and these expanded MAIT cells are armed with potent anti-cancer functions. We also show that MAIT cells express the molecular machinery to transport and metabolise lactate, a by product of glycolytic metabolism, and activating MAIT cells in the presence of exogenous sodium lactate results in remodeling of their cellular metabolism, with a significant increase in mitochondrial metabolism. Functionally this supports elevated production of effector molecules (IFNg and granzyme B). Interstingly the effect of sodium lactate on effector molecule production was greater under glucose restriction.
Building on their capacity to drive anti-cancer responses we demonstrate that exogenous sodium lactate significantly enhances MAIT cell cytotoxicity when paired with the Cibistamamb engager used to direct MAIT cell-mediated killing against colon cancer cell lines. These data collectively demonstrate that MAIT cells can utilize lactate to boost their anti-cancer effector functions via reporgrammed cellular metabolism, and this may be a novel approach for cancer immunotherapy.

"IMMUNOENGINEERING OF UCART19 – THE BACK STORY."

***Brian Philip**

NIBRT CONCEPT laboratory, Foster Avenue, Mount Merrion, Blackrock, Co. Dublin, Ireland. A94 X099

***Lead Presenter: Brian.Philip@nibrt.ie**

Adoptive cellular immunotherapies remain among the most complex drugs ever developed. Representing a combination of molecular engineering, transplant medicine and immuno-oncology, the evolving translational journey during the progression from bench to bedside highlights the challenges of developing novel immunotherapies as well as showcasing the vast therapeutic potential these 'living drugs' can achieve.

UCART19 is a combination of positive and negative immunoengineering, comprised from the genetic engineering (knock-in) of a tumour-specific anti-CD19 chimeric antigen receptor (CAR) and an associated marker-gene safety-switch, RQR8, coupled with TALEN-mediated (knock-out) tandem gene edits of the TCRα and CD52 genes to yield a universal allogeneic 'off-the-shelf' cellular immunotherapy.

Challenges arose throughout the entire process as we progressed from concept to clinic. As an entirely novel drug product, each developmental stage required the tandem design, development and optimisation of bespoke assays and models. In vitro assays evolved into increasingly complex in vivo models whilst progression toward clinical translation raised more questions than answers, reflecting known unknowns within the conversion of synthetic biology projects into drug development.

The clinical triumph of UCART19 overshadows the complexity of the drug development required to achieve clinical translation of a novel immunotherapy. Observations and lessons learned offer opportunity to avoid potential pitfalls within the development of future immunotherapies.

Successful first-in-human application of UCART19 on a compassionate use basis at Great Ormond Street Hospital paved the way for the larger phase I clinical trial: CALM - NCT02746952.

"SAFETY AND PRELIMINARY EFFICACY RESULTS OF ORBCEL-M IN DIABETIC KIDNEY DISEASE: THE PHASE-1B/2A NEPHSTROM CLINICAL TRIAL."

Elliman, Stephen J (1), Griffin, Matthew D (2), Remuzzi, Giuseppe (3), Cockwell, Paul (4), Maxwell, Alexander P (5)**,** Perico, Norberto (3), Ruggenenti, Piero Luigi (6), Introna, Martino (6), Finnerty, Andrew Anthony (7), Smythe, Jon (8)**,** Fibbe, Willem E (9), and O'Brien, Timothy (2)

1 Orbsen Therapeutics Ltd, Galway, Ireland
2 Regenerative Medicine Institute (REMEDI), School of Medicine, University of Galway.
3 Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Bergamo, Italy
4 University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom
5 Queen's University Belfast Centre for Public Health, Belfast, Belfast, United Kingdom
6 Aziende Socio Sanitarie Territoriale Papa Giovanni XXIII, Bergamo, Italy
7 National University of Ireland Galway (NUIG), Galway, Ireland
8 National Head of Cellular and Molecular Therapies, Oxford, United Kingdom
9 Leiden University Medical Center, Leiden, Netherlands

***Lead Presenter: steve.elliman@orbsentherapeutics.com (PI)**

NEPHSTROM is a randomized, placebo-controlled phase 1b/2a trial to assess safety, tolerability, and preliminary efficacy of bone marrow–derived, anti-CD362–selected, allogeneic mesenchymal stromal cells (ORBCEL-M) in adults with type 2 diabetes and progressive diabetic kidney disease.

A cohort of 16 participants at three sites was randomized (3:1) to receive intravenous infusion of ORBCEL-M (80×106 cells, n=12) or placebo (n=4) and followed for 18 months.

At baseline, clinical measurements were comparable between groups. ORBCEL-M was safe and well-tolerated. One placebo-treated participant had a quickly resolved infusion reaction (bronchospasm), with no subsequent treatment-related serious adverse events. One ORBCEL-M recipient developed low-level anti-HLA antibodies. Two ORBCEL-M recipients died 12-18 months post-infusion during follow-up of causes deemed unrelated to the trial intervention.

The median annual rate of kidney function decline after ORBCEL-M therapy compared with placebo was significantly lower by eGFR estimated by both CKD-EPI and MDRD equations. Immunologic profiling provided evidence of preservation of circulating regulatory T cells, lower natural killer T cells, and stabilization of inflammatory monocyte subsets in those receiving ORBCEL-M compared with placebo.

Findings indicate safety and tolerability of ORBCEL-M cell therapy in the trial's lowest dose cohort. The rate of decline in eGFR over 18 months was significantly lower among those receiving ORBCEL-M compared with placebo.

Mechanistic studies are ongoing and a Phase 2b/3 clinical trial is needed to determine the ORBCEL-M effect on CKD progression.

Clinical Trial registration number - NCT02585622

"FAR BEHIND THE CURVE - ADDRESSING IRELAND'S LOW READINESS FOR THE CLINICAL ADOPTION OF ADVANCED THERAPY MEDICINAL PRODUCTS."

Vicky McGrath (1), Cormac Kennedy (2), Kevin Walsh (3), **Martina Hennessy (4)**

1 Rare Diseases Ireland
2 St. James's Hospital
3 Roche Ireland
4 Wellcome HRB Clinical Research Facility

***Lead Presenter: mhenness@tcd.ie (PI)**

Presentation of the report's findings and recommendation, which includes four case studies of the deployment of four advanced therapies in Ireland. It was prepared as part of a collaboration between, TCD, Rare Diseases Ireland and Roche Ireland and highlights the need for a more comprehensive government policy approach to advanced therapies in Ireland.

"UNDERSTANDING THE ARDS MICROENVIRONMENT TO ENHANCE MSC THERAPY"

Courteney Tunstead[1,2], Evelina Volkova[1,2], Hazel Dunbar[1,2], Ian Hawthorne[1,2], Alison Bell[3,4], Bairbre McNicholas[3,4], Claudia C. Dos Santos[5], John G. Laffey[3,4] & Karen English[1,2]

[1]*Cellular Immunology Lab, Department of Biology, Maynooth University, Maynooth, Co. Kildare, Ireland.*
[2]*Kathleen Lonsdale Institute for Human Health Research, Maynooth University, Maynooth, Co. Kildare, Ireland.*
[3]*Anesthesia and Intensive Care Medicine, School of Medicine, College of Medicine Nursing and Health Sciences, University of Galway, Galway, Ireland.*
[4]*Anesthesia and Intensive Care Medicine, Galway University Hospitals, Saolta University Hospitals Groups, Galway, Ireland.*
[5] *Keenan Research Center for Biomedical Research, St. Michael's Hospital, Toronto, Canada.*

***Lead Presenter: courteney.tunstead.2018@mumail.ie (Postgrad Researcher (>two years))**

Background: Clinical trials investigating the potential of mesenchymal stromal cells (MSCs) in acute respiratory distress syndrome (ARDS) have provided disappointing results. MSCs are known to require cytokine-mediated activation signals, or licensing, in order to mediate protective effects *in vivo* and therefore MSCs may be more efficacious in the hyper-inflammatory ARDS sub-phenotype. We investigated the therapeutic efficacy of MSCs licensed with differential ARDS patient micro-environments (hyper- vs hypo-inflammatory) in a model of acute lung injury (ALI).

Methods: MSCs were exposed to 20% patient serum from COVID-19 ARDS patients for 24 hours. ARDS patient serum was segregated into hypo- or hyper-inflammatory phenotype based on IL-6 levels. The MSCs and their secretome were then screened both *in vitro* and *in vivo*.

Results: The secretome of MSCs exposed to the hyper- but not hypo-inflammatory ARDS serum reduced LPS-induced lung permeability, significantly increasing expression of tight junction genes *occludin, claudin-4* and *zo-1* in the lung epithelium in a VEGF dependent manner.

Conclusion: MSCs exposed to hyper, but not hypo, ARDS patient serum have the capacity to reduce lung permeability, due to enhanced tight junction formation, in a VEGF-dependent manner.

"EXAMINING THE IMPACT OF MICRORNA-379 ON BREAST CANCER PROGRESSION AND IMMUNE CELL INFILTRATION."

EC McCarthy (1), L Lin (1), C Pagden (1), A Jagateri1, K Wynne (2), S Hynes (3), CM Coleman (4), CP O'Neill (1), RM Dwyer (1,4)

1 Discipline of Surgery, Lambe Institute for Translational Research, University of Galway, Galway H91 YR71, Ireland
2 Systems Biology Ireland, University College Dublin, Belfield, Dublin 4, Ireland
3 Discipline of Pathology, Lambe Institute for Translational Research, University of Galway, Galway H91 YR71, Ireland
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***Lead Presenter: elan.mccarthy@universityofgalway.ie (Postdoc)**

MicroRNA-379 (miR-379) is a potent tumour suppressor, with our group and others generating compelling data supporting potential for therapeutic targeting of breast cancer progression. This study examined the impact of miR-379 on cancer hallmarks in an immune-competent breast cancer model. Mice received an orthotopic injection of either 4T1 cells, 4T1 cells transduced with miR-379 (4T1-379), or vehicle healthy controls. IVIS imaging monitored disease progression with tumours and bones harvested for proteomics and immunohistochemistry (IHC). Bone remodelling was investigated by Haematoxylin and Eosin (H+E) staining. Markers of proliferation (Ki67), angiogenesis (CD31) and immune response (CD4, CD8, F4/80) were examined by IHC. IVIS imaging revealed rapidly developing tumours with an apparent reduction in growth rate in 4T1-379 tumours, reinforced Ex Vivo with a significant reduction in tumour volume (P=0.05), Ki67 expression (P=0.01) and an apparent reduction in angiogenesis marker CD31. While there was no difference in infiltration of CD4+ (P= 0.39) and CD8+ T cells (P= 0.68), 4T1-379 tumours demonstrated a significant increase in F4/80+ macrophage infiltration (P<0.001). Further studies will include analysis of macrophage polarisation and function. H+E staining revealed significant remodelling of bone in 4T1 tumour bearing mice compared to healthy controls. Interestingly, femurs from the 4T1-379 group appeared to retain morphology more similar to healthy controls with intact growth plates and reduced loss of trabecular bone. The data demonstrate a role for miR-379 in regulation of tumour growth, macrophage infiltration and bone remodelling, all key elements of metastatic progression that represent attractive targets for therapeutic miR-379 delivery.

"CD19 CAR T-CELL THERAPY AND ITS IMPACT ON ANTIBODIES AGAINST VACCINE-PREVENTABLE VIRAL INFECTIONS IN LYMPHOMA PATIENTS."

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***Lead Presenter: foystonh@tcd.ie (Postgrad Researcher (>two years))**

Background
CD19-directed chimeric antigen receptor (CAR) T-cell therapy has proven efficacy in treating relapsed/refractory high-grade B-cell malignancies. However, these therapies induce B-cell aplasia and hypogammaglobulinemia, attributed to the on-target, off-tumour targeting of the CD19 antigen on both malignant and normal B-cells, and its impact on humoral immunity remains unclear.

Methods
We conducted a retrospective study on 12 patients who underwent fludarabine and cyclophosphamide lymphodepletion for tisagenlecleucel (n=7) or axicabtagene ciloleucel (n=5), for treating r/r DLBCL (n=11) and tFL (n=1). Serum samples were collected before and at day 100±9 post-therapy for quantitative measurement of measles, mumps, rubella (MMR) and Varicella-Zoster Virus (VZV) IgG levels.

Results
At pre- and day 100 post-CAR T-cell infusion, the seroprevalence rate for measles, rubella, and VZV IgG antibodies was 100%, and no statistical significances in titre levels were observed.

Pre-CAR T-cell therapy, 58.3% of participants (n=7) had positive anti-mumps IgG titres, 16.7% (n=2) had equivocal titres, and 25% (n=3) had negative titres. At day 100, the 9 patients who had positive anti-mumps IgG titres before therapy continued to test positive, with 2 participants shifting from equivocal to positive titres. The 3 patients who were negative pre-CAR-T remained negative. We did not observe significance between the pre- and post-titres.

Conclusion
Our preliminary study contributes evidence supporting the persistence of prior immunity against MMR and VZV following CD19 CAR T-cell therapy. Our findings highlight that our patient cohort does not require MMR or

VZV re-vaccination after CAR T-cell therapy and emphasises the importance of tailoring immunisation schedules to individuals.

"MECHANICAL INDUCTION OF REGULATORY T CELLS."

Jessica A. White (1), Muhammad Qasim (1,2), Irish Senthilkumar (2), Denise C. Fitzgerald (1) & Eoin McEvoy (2).

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Background:
Multiple sclerosis (MS) is an immune-mediated, demyelinating disease of the central nervous system (CNS). While there are no remyelinating therapies available, regulatory T cells (Treg) have been shown to promote myelin regeneration in the CNS. As such, the induction/expansion of Treg is a therapeutic goal for MS. It has recently emerged that the induction of Treg is dependent on the mechano-sensitive calcium channel, piezo1, and may be mechanically controlled. To investigate this, we employed a joint experimental-computational approach to uncover potential mechanisms for MS immunotherapy.

Methods:
Whole CD4+ T cells from C57BL/6J WT mice were cultured on polyacrylamide gels (PA) of varying stiffness in the presence of anti-CD3, anti-CD28, IL-2, TGF-β, and anti-IFN-γ. Treg activation status and FOXP3 expression were analysed by flow cytometry and applied to a computational model examining Treg induction and long-term stability.

Results:
We observed that PA gel drives the activation and differentiation of FOXP3+ Treg, increasing up to an optimal stiffness of 20 and 25 kPa. Mechanically induced Treg were also found to express IL-2 but not IL-10, and may demonstrate an increased stability of FOXP3 expression in vitro. Computationally, Treg induction was shown to biphasically respond to substrate rigidity, involving the catch slip behaviour of the anti-CD3-TCR complex, calcium influx, and nucleus shape.

Conclusion:
Together, this study uncovered a novel mechanism involved in the mechano-sensitive induction and potential stability of FOXP3+ Treg. Ongoing work is now investigating whether mechanically induced Treg can improve the efficacy of remyelination in vitro.

POSTER PRESENTATIONS

MESENCHYMAL STROMAL CELLS

01: "THE GROWTH OF HUMAN MESENCHYMAL STROMAL CELLS (HMSC) IN POROUS SILICONE MICROCARRIERS."

Christiane Schaffer (1), Ralf Portner (1), Emmet O'Reilly (2), Richard Fry (3), Richard Shor (3) and ***Denis Looby (4)**

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Porous silicone microcarriers are mechanically stable and gas permeable, therefore they can be used in high shear stirred tank and fluidised bed bioreactors, and oxygen can diffuse efficiently through the carrier matrix to the cells inside the carrier. Porous carriers are suitable for the production of secreted products, but they are not suitable for cell production, because intact cells are not fully recoverable from the carriers. In this poster, we address the growth of human hMSC cells in porous silicone microcarriers and assess their potential for process intensification and large volumetric scale-up. The hMSC cells were grown in the microcarriers in shake flask cultures, with repeated batch media feed and harvest, for 21 days. The cell density was 2 x 107 cells per ml carrier after 21 days in culture. These results are a good indicator that MSC cells can be grown to high densities in scalable fluidised or stirred tank perfusion bioreactors. Further development activities will be required, to establish and optimize hMSC cell growth and exosome production in both stirred tank and fluidized bed perfusion bioreactors. This has the potential to address some of the current manufacturing bottlenecks for stem cell therapies (low cell density and limited volumetric scale-up) and deliver commercial scale manufacturing of exosomes with reduced cost of goods.

02: "COMPARISON BETWEEN INTRALUMINAL AND LIPOPHILIC MEMBRANE DYES FOR EXTRACELLULAR VESICLES TRACKING IN CORNEAL TISSUES."

***Manon Jammes,** Monika Sypecka, Yedizza Rautavaara, Seyedmohammad Moosavizadeh, Trung Bach, Abbas Tabasi, Jiemin Wang, Neil Lagali, Thomas Ritter

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Extracellular vesicles (EVs) derived from mesenchymal stromal cells (MSCs; MSC-EVs) recently emerged as a promising approach to address corneal diseases. Exploring the uptake and biodistribution of MSC-EVs within the tissue typically involves using lipophilic membrane dyes, like PKH, inevitably increasing EV size and modifying their surface composition which potentially affect the interactions with their targets. In this context, using intraluminal dyes presents a suitable alternative to standard protocols to ensure the preservation of EV mechanism of action and the reliability of generated data.

EVs were isolated from human bone marrow MSCs using size exclusion chromatography. Fresh EVs were labelled with PKH26, CFSE or CellTrace Far Red (CTFR), and the stability of the fluorescence signal was investigated by flow cytometry after -80°C storage and/or 37°C incubation to mimic in vivo administration.

Human corneal epithelial cells (CEpiCs) and keratocytes were treated with PKH26-, CFSE- or CTFR-EVs, followed by flow cytometry and microscopy analyses.

The EV size and morphology remained unchanged after labelling with CFSE and CTFR, but was significantly increased after PKH26 labelling. CFSE-EVs exhibited signal reduction after 37°C incubation, while CTFR-EVs were impacted by -80°C storage, but both dyes were more specific than PKH26. Given the natural strong green fluorescence background in cell cultures, CFSE-EV uptake was more arduous to track in vitro than CTFR-EVs.

CTFR staining is an effective method to label EVs and track them in vitro, avoiding the structural alterations associated with PKH-like dyes. EV tracking on corneal explants after CTFR-EV topical or subconjunctival administration is currently investigated to ensure the translation of the method to in vivo studies.

03: "GENERATION OF A SAFE HARBOUR CD39 LENTIVIRAL VECTOR FOR CD362+ MSC MODIFICATION FOR THE TREATMENT OF CHRONIC LIMB THREATENING ISCHAEMIA."

***Caomhán J. Lyons (1),** Alisa Nousiainen (2), Clara Sanz-Nogués (1), Nihay Laham Karam (2), Seppo Ylä-Herttuala (2), Timothy O'Brien (1,3)

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Chronic limb threatening ischaemia (CLTI) is a chronic hyper-inflammatory condition that results from atherosclerotic blockage of vessels of the lower limb. Patients with CLTI present with pain at rest but can advance to ulceration, and amputation with a high mortality rate. Mesenchymal stromal cells (MSCs) have shown improved muscle fibre size and increased perfusion post treatment in in vivo models of CLTI. However, no therapeutic product has been developed with MSCs for CLTI to date. Our goal is to further augment the potential of MSCs by transducing them with CD39, an anti-inflammatory protein, to potentiate the anti-inflammatory effect of MSCs and accelerate limb recovery.

CD39 was PCR amplified to incorporate the restriction sites AgeI + SalI required for restriction cloning. CD39 was ligated to a novel safe integration lentiviral cloning vector or a pMini plasmid and transformed into DH5α bacteria. CD39 plasmid sequences were validated using restriction digest gel electrophoresis and Sangar sequencing.

CD39 was successfully PCR amplified using 30 cycles; however, due to cloning issues into the safe integrating vector we initially cloned the CD39 into a smaller pMini plasmid. Using a restriction digest validated CD39-pMini plasmid, we successfully cloned the CD39 fragment into the safe integrating vector. However, later Sangar sequencing revealed that the CD39-pMini plasmid used possessed a base gap and mismatch. Only 3/6 CD39-pMini plasmids generated possessed the correct CD39 sequence. Future work will clone the CD39-pMinis with the correct sequence into the safe integrating vector, and then transduce MSCs, and investigate their anti-inflammatory potential.

04: "ELUCIDATING THE IMPACT OF MACROMOLECULAR CROWDING AGENT CHEMISTRY ON MESENCHYMAL STROMAL CELL RESPONSE."

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Tissue engineered medicines require prolonged ex vivo cultures to develop implantable micro-tissues. This is due to the diluted conditions of traditional eukaryotic cell culture, where the enzymatic conversion of procollagen into collagen occurs slowly. Macromolecular crowding (MMC), a biophysical process rooted in the excluded-volume effect, reduces molecular diffusion and increases protein interaction and enzymatic processes, resulting in increased and accelerated extracellular matrix (ECM) deposition during in vitro cultures. Despite its extensive use in eukaryotic cell culture, the optimal crowder for enhanced eCM deposition in human bone marrow mesenchymal stromal cells (hBM-MSC) is still elusive. Further, it is still unclear how different crowders affect lineage commitment and fate. Herein, we evaluated the potential of three crowders (carrageenan, a sulphated polysaccharide, Ficoll™, a non-sulphated polysaccharide and polyacrylic acid, a synthetic macromolecule) in enhancing ECM deposition and their effect on hBM-MSC. Basic cellular function analyses indicated the safety of all crowders. In comparison to the control group, carrageenan significantly increased collagen types I, IV and V deposition, Ficoll™ significantly increased collagen type IV deposition and polyacrylic acid significantly increased collagen type V deposition. Mass spectrometry revealed that all crowders affected translation pathways; carrageenan influenced cell-matrix adhesion; Ficoll™ influenced ECM remodelling and immune system and polyacrylic acid influenced ECM regulation. RNA-seq analysis showed that all crowders affected, among others, ECM, organisation and remodelling pathways. Our data illustrate a crowder-dependent cell response and argues that the chemistry of the agent should be considered in the developmental cycle of therapy medicinal products that is manufactured under MMC conditions.

05: "INNOVATIVE FLUID GEL SYSTEM FOR MSC-EVS OCULAR DELIVERY."

***Seyedmohammad Moosavizadeh (1,2),** Ellen Donohoe (1,2), Jiemin Wang (1,2), Aoife Canning (1,2), Manon Jammes (1,2), Trung Bach (1,2), Abbas Tabasi (1,2), Thomas Robinson (3), Richard Moakes (3), Aideen Ryan (2,4), Liam Grover (3), Thomas Ritter (1,2)

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Corneal injuries are one of the leading causes of blindness worldwide, and effective treatment is an unmet medical need. Mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) demonstrate similar therapeutic effects as MSCs, while addressing the challenges associated to cell-therapy. Delivering MSC-EVs to the site of injury in a controlled and sustained manner may augment MSC-EVs therapeutic efficacy. In this context, gellan gum fluid gel appears as a promising delivery system for MSC-EVs which can extend the residence time of therapeutics on the cornea due to its viscoelasticity, mucoadhesive, and solid-liquid-solid phase transition features.

The MSCs were cultured and characterized. The MSC-EVs were isolated by SEC technique and characterized for size, morphology and surface biomarkers. The gellan gum fluid gel was synthesized and characterized for rheological properties. MSCs were positive for CD90, CD73, and CD44, and negative for CD45, CD11b, and HLA-DR surface biomarkers. MSC-EVs had spherical bilayer morphology with a size of ~83 nm. MSC-EVs were positive for CD9, CD63, and CD81 biomarkers. The Gellan gum fluid gels were transparent and shown to act as

solids at rest, but as fluids when a force is applied. Linear and non-linear rheology have shown their shear thinning and phase transition features.

Altogether, these results evidenced that the physical properties of gellan gum fluid gels demonstrate their potential as MSC-EV delivery systems and ocular healing materials, necessitating investigation of the therapeutic efficacy of the released-MSC-EVs in 2D and 3D in-vitro studies of corneal injury.

06: "MESENCHYMAL STEM CELL DERIVED EXTRACELLULAR VESICLES ATTENUATE B CELL ACTIVATION."

***Pamina Contreras-Kallens (1,2),** Manon Williams (3), Hannah Kimingi (1,2), Yeyu Shen (1,2), Hannah Aris (1,2), Dessi Malinova (3), Meadhbh Brennan (1,2).

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Autoimmune diseases (ADs) are characterized by B cell function dysregulation. Mesenchymal stem cells (MSCs) have been proposed as an alternative therapy for ADs, as these cells bear potent immunomodulatory capacities, particularly when exposed to inflammatory conditions known as 'licensing'.MSCs-derived extracellular vesicles (EVs) have been proposed as a safer alternative to MSCs. The aim of this study was to evaluate the effect of EVs derived from licensed and unlicensed MSCs on B cell function and activation. To do this, bone marrow murine MSCs (BM-MSCs) were cultured in EV-depleted media in either 'licensing', or in standard conditions.Their conditioned media was then collected, and the MSCs-EVs were isolated by size exclusion chromatography. EVs sampleswere characterized by NTA and BCA. For assessing the effect of EVs on B cells, primary B cells were isolated from an allogenic strain mouse and activated both in a T-cell independent and T-cell dependent way. The licensing of BM-MSCs caused the increase in their expression of both constitutive markers and inducible markers, as well as increasing their EV production, compared to standard conditions. It was found that BM-MSCs derived EVs could modulate the expression of early activation markers and the proliferation on allogeneic murine B cells. Our findings show that BM-MSC-derived EVs can modulate B cell activation and function in vitro. Additionally, MSC culture conditions influence their EV secretion profile, suggesting potential therapeutic applications in immune dysregulation diseases like ADs. Further exploration of underlying mechanisms is needed to fully harness MSC-derived EVs as immune therapies.

07: "MESENCHYMAL STROMAL CELLS ATTENUATE MIF ENHANCED TRAINED IMMUNITY."

***Molly Dunlop,** Hazel Dunbar, Karen English

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Trained immunity (TI) describes a phenomenon by which innate immune cells such as macrophages acquire an immunological memory resulting in a heightened response following exposure to a non-specific secondary pathogenic stimulus. Triggered by metabolic changes and subsequent chromatin remodeling, TI leads to elevated levels of pro-inflammatory cytokines. TI may be harmful in the context of asthma as the surge in cytokine production could further drive exacerbations in patients. Recent literature shows that increased expression of the pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) enhances this trained response in macrophages from mice containing the high expressing human MIF polymorphism, in response to the allergen

house dust mite (HDM). Recent studies have also investigated the use of mesenchymal stromal cells (MSCs) as a potential therapeutic for calming trained responses in a pre-clinical model of allergic airway inflammation. This study set out to investigate the molecular mechanisms underpinning the MSC blockade of TI in HDM-mediated enhanced trained immunity driven by high MIF expression.

Bone marrow-derived macrophages (BMDMs) from novel humanized MIF mice with either the low-expressing MIF promoter polymorphism (CATT5) or the high-expressing MIF promoter polymorphism (CATT7) or wild type (WT) litter mate controls, were trained with HDM in vitro and in vivo, MSCs were administered IV or co-cultured with BMDMs after HDM challenge prior to being exposed to a secondary heterologous stimulus on day 7. Pan methyl-transferase inhibitors show epigenetic remodeling occurs; Seahorse extracellular flux analysis shows an altered metabolic phenotype post HDM challenge.

08: "MESENCHYMAL STROMAL CELLS MODULATE IMMUNOTOLERANCE IN A MODEL OF ACUTE LUNG INJURY."

***Evelina Volkova,** Courteney Tunstead, Molly Dunlop, Claudia C. Dos Santos, John G.Laffey, Karen English

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Multiple Background: Acute respiratory distress syndrome (ARDS) can arise secondary to infection and is characterised by an acute increase in pro-inflammatory cytokines in the lung. Evidence has shown that this can lead to innate immune tolerance (IT). IT is associated with a diminished immune response to secondary infection as result of functional reprogramming of innate immune cells. This shift to a refractory innate immune state has been linked with impaired pathogen clearance and immune dysfunction. Mesenchymal stromal cells (MSCs) have been investigated as an attractive potential therapy for ARDS as they are renowned for their capacity to promote resolution of inflammation and enhance repair.

Methods: An LPS induced acute lung injury (ALI) in vivo mouse model was used to determine the role of immunotolerance in ARDS. Wild-type mice received 2mg/kg of LPS intratracheally followed by a single dose of human bone marrow-derived MSCs intravenously after 4hrs. Bone marrow-derived macrophages (BMDMs) were isolated from mice after LPS challenge and were restimulated ex vivo with various toll-like receptor agonists.

Results: We have demonstrated that ALI mice have increased pro-inflammatory cytokines TNFα and IL-6 in the bronchoalveolar lavage fluid and MSCs significantly reduced these inflammatory markers. To investigate the impact of ALI on macrophage functionality, BMDMs isolated from mice after LPS challenge demonstrated an immunotolerant phenotype in response to secondary stimulation with LPS, Pam3CSK4 and Poly(I:C) ex vivo.

Conclusions: MSCs modulate the inflammatory response associated with acute lung injury, demonstrating their potential therapeutic role to modulate immunotolerance in a model of LPS induced ALI.

09: "IDENTIFICATION AND ANALYSIS OF TRANSCRIPTOMIC CHANGES OF MSCS CELLS FROM PEOPLE WITH TYPE 2 DIABETES MELLITUS."

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Type 2 diabetes mellitus (T2DM) is a chronic disease, characterized by elevated blood sugar levels, leading to complications such as osteopathy. T2DM-induced osteopathy features reduced cortical bone mass and increased mineral density. Human bone marrow mesenchymal stromal cells (hBM-MSCs), progenitors of osteoblasts and osteocytes, play a critical role in bone homeostasis and repair. In T2DM, the number and osteogenic potential of hBM-MSCs is impaired. It was hypothesized that transcriptional activity and osteoprogenitors proportion differ in the T2DM group, with age and sex as additional contributing factors.

hBM-MSCs from people living with and without T2DM were profiled by bulk RNA-seq, supplemented with publicly deatasets (T2DM=23, Non T2DM=42). Differentially expressed genes (DEGs) were identified using DESeq2 across the T2DM, and its sex and age subgroups (18-40, 41-60 and >60 years). Deconvolution analysis, performed using BayesPrism, incorporated scRNA-seq data from Yuchen et al (2023, PMID: 37474525). Subpopulation-specific differential expression was also assessed.

In T2DM, 28 DEGs related to skeletal system development were identified on the bulk RNA data. Nine hBM-MSC subpopulations, spanning undifferentiated cells to restricted osteoprogenitors, were characterized using marker genes in the scRNA data from Yuchen et al. The osteogenesis-related gene ALPL was found to be downregulated in T2DM samples across multiple hBM-MSC subpopulations based on the deconvoluted data, although these changes were not statistically significant.

DEGs comparison within bulk RNA seq data and deconvoluted data highlighted differences between T2DM and non-diabetic groups. Subpopulation patterns aligned with previous findings, reaffirming the heterogeneity and diverse functional characteristics of hBM-MSCs.

10: "XENO-FREE MESENCHYMAL STROMAL CELLS UNDER MACROMOLECULAR CROWDING CONDITIONS AS TISSUE-ENGINEERED SKIN SUBSTITUTES."

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Chronic Skin substitutes aim to promote wound healing and closure. Currently, clinically available tissue engineered skin substitutes utilise foetal bovine serum that is associated with immune response; use skin fibroblasts that are of lower therapeutic capacity than mesenchymal stromal cells; and require prolonged in vitro cultures that are associated with high manufacturing costs. To address these limitations, herein we describe the development of a tissue engineered skin substitute using xeno-free mesenchymal stromal cells under macromolecular crowding (MMC) conditions.

Xeno-free human umbilical cord mesenchymal stromal cells (hUC-MSCs) from 3 donors were seeded at a density of 25,000 cells per cm2 on tissue culture polystyrene (TCP) and cultured without and with macromolecular

crowding (MMC) [10 μg/ml λ carrageenan (CR); 37.5 mg/ml 70 kDa + 25 mg/ml 400 kDa polysucrose cocktail (PSC1); 37.5 mg/ml 70 kDa + 25 mg/ml 400 kDa + 2.25 mg/ml 1000 kDa polysucrose cocktail (PSC2); 11.34 mg/ml 360 kDa polyvinylpyrrolidone (PVP); 50 μg/ml 75 kDa polystyrene sulfonic acid (PSSA); and 0.5 mg/ml 4000 kDa polyacrylic acid (PAA) for 4, 6 and 8 days. Cell morphology and ECM deposition was assessed via brightfield microscopy and electrophoresis and immunocytochemistry, respectively.

Among all MMC agents assessed, the PSSA induced aggregated cell morphology; CR and PAA induced the highest ECM deposition, as judged by electrophoresis and immunocytochemistry analyses. These data were confirmed among all three donors.

11: "COMPARISON OF IN-VITRO IMMUNOMODULATORY CAPACITY BETWEEN LARGE AND SMALL APOPTOTIC BODIES DERIVED FROM HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS."

***Jiemin Wang (1,2),** Seyedmohammad Moosavizadeh (1,2), Manon Jammes1, Abbas Tabasi1, Trung Bach1, Aideen E Ryan1, 2, 3*, Thomas Ritter1,2*

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Background:

Mesenchymal stromal cell (MSC) apoptosis is essential for their therapeutic effects, including immunomodulation. Previous studies have shown that MSC-derived apoptotic bodies (ApoBDs) also possess immunomodulatory properties. However, compared to small extracellular vesicles, the preparation, characterization, and biological properties of ApoBDs remain underexplored.

Results:

ApoBDs were isolated from the conditioned medium of staurosporine-induced apoptotic human MSCs and categorized into large (∼700 nm) and small (∼500 nm) groups. Both types expressed CD90, CD44, and CD73, with low levels of PD-L1, CD11b, and HLA-DR, mirroring their parental MSCs. Functional assays revealed that both ApoBDs inhibited allogeneic T-cell proliferation, with large ApoBDs demonstrating superior efficacy. In macrophage co-culture experiments, both ApoBDs polarized M1 macrophages toward an M2-like phenotype, with large ApoBDs more effectively upregulating CD163 expression. Additionally, both ApoBDs suppressed the proliferation of murine primary T cells. Furthermore, large ApoBDs exhibited enhanced macrophage uptake, as confirmed by flow cytometry and immunocytochemistry. Importantly, no cytotoxicity was observed for either ApoBD type following staurosporine treatment.

Conclusions:

Staurosporine-induced ApoBDs are non-cytotoxic and exhibit significant immunomodulatory potential in vitro. Large ApoBDs are more effective than small ApoBDs in T-cell suppression and M2 macrophage polarization, suggesting their potential as an alternative to MSC-based therapies in future studies.

12: "MICROCARRIER SCREENING AND EVALUATION OF CELL GROWTH CHARACTERISTICS FOR THE PRODUCTION OF EXTRACELLULAR VESICLES FROM AN IMMORTALIZED HUMAN MESENCHYMAL STEM CELL."

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Human mesenchymal stem cells (hMSCs) are multipotent cells with demonstrated efficacy in immunomodulation and tissue repair. However, their fragile nature poses substantial challenges to their stable production at a commercially relevant scale. Recent studies illustrated that telomerase-immortalized hMSCs (hTERT-MSCs) have high safety profiles both in vitro and in vivo and enable a long-term and stable culture of hMSCs. Microcarriers are microbeads of 90–350 μm diameters that support the growth of adherent cells in suspension mode. Culturing hMSCs on microcarriers enables a volume-dependent process scale-up.

This study evaluated the cell attachment and batch growth kinetics of an hTERT-MSC (ASC52telo) across a panel of six microcarriers. The low concentration synthemax II (SYN), enhanced attachment (EA), and collagen-coated microcarriers (CC) demonstrated acceptable hTERT-MSC attachment efficiencies of 62.5%, 85.9%, and 104.3%, respectively. Calcein-AM staining indicated that hTERT-MSCs also attached with high efficiency and spread on both the Cytodex 1 (Cyt 1) and Cytodex 3 (Cyt 3) microcarriers. Propidium iodide staining further revealed that CC, Cyt 1, and Cyt 3 may have superior hTERT-MSC recovery post-inoculation. The hTERT-MSC efficiently expanded on SYN, EA, CC, Cyt 1, and Cyt 3, albeit the cell doubling time on Cyt 1 and Cyt 3 lagged behind the other microcarriers.

These preliminary results lay the groundwork for further evaluation of hTERT-MSC expansion for scale-up and suggest the feasibility of exploring MSC-EV production on a larger scale.

13: "IMMUNOMODULATORY EFFECT OF MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES ON DENDRITIC CELLS."

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INTRODUCTION

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that link the innate and adaptive immune systems. Integrating different signals by DCs like the cytokine milieu at the inflammation site may determine the activation of DCs to either tolerance (tolerogenic DCs) or immunogenic DCs (1). Overactive DCs are implicated in inflammation-related bone loss by activating T-cells as reported in rheumatoid arthritis (RA) (2) and chronic periodontitis (3). Therefore, there is a need for therapies that can modulate DCs towards tissue-repair effectors. It has been shown that mesenchymal stromal cells (MSCs), and more recently their secreted extracellular vesicles (EVs), can influence the immune system (4). This study investigates the influence of MSC-EVs' potential to modulate dendritic cell differentiation and activation.

MATERIALS AND METHODS

Murine MSCs were cultured on tissue culture plastic in EV-depleted media for 48 hours. EVs released by the MSCs were isolated using differential centrifugation, ultrafiltration, and size exclusion chromatography (SEC). EVs were characterized by particle size and concentration using nanoparticle tracking analysis (NTA) and protein

content using bicinchoninic acid (BCA) for morphology and surface marker expression. MSC-EVs were treated on monocyte-derived dendritic cells isolated from C57BL/6 mice at increasing dosages on days 0, 3, and 6 for differentiation assay and one day after activation for activation assay.

RESULTS

The size of the isolated MSC-EVs ranged between 30 and 380 nm with the majority of EVs having the size of 130 ± 40 nm. MSC-derived EVs caused a significant dose-dependent reduction in the expression of MHC-II, CD11c, CD40, and CD86 surface markers and increased expression of CD11b surface marker and PD-L1 immune checkpoint marker.

DISCUSSION

MSC-EVs inhibited the differentiation and activation of monocyte-derived dendritic cells. To understand the underlying mechanisms behind these findings we are currently investigating the role of EV-treated dendritic cells on the activation and proliferation of T-cells. Further, we will investigate the transcriptome, miRNA, and protein cargo profiles of the murine MSC-EVs.

CAR-T CELLS AND CELL BASED IMMUNOTHERAPY

14: "*DEVELOPING IMAGE-BASED T-CELL ASSAYS TO EFFECTIVELY MONITOR APOPTOSIS AND CAR EXPRESSION"*

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Chimeric antigen receptor (CAR)-T cell therapy is a novel cellular therapeutic approach for cancer patients, including B-cell malignancies. A critical step in the CAR-T cell production process is to effectively deliver CAR genes into primary T- cells, which can be achieved through the use of viral vectors or non-viral methods. To evaluate and select effective CAR gene delivery methods and processes, it is imperative to perform analytical tests to detect and monitor cell proliferation, cell health status, and CAR gene expression. In this work, we developed an image-based method using the Cellaca® PLX Image Cytometer to quickly count T-cells, measure viability, assess apoptotic cell health, and identify CAR expression. Using this new methodology, we compared different CAR gene delivery methods, primarily focusing on non-viral methods involving electroporation. Cell viabilities were monitored daily using acridine orange / propidium iodide (AO/PI) stain and its respective dual fluorescent assay. Preliminary results showed that viabilities for all SupT1 samples decreased significantly to ~50% by day 1 following electroporation, in comparison to un-transduced SupT1 control samples, which maintained ~90%+ viabilities. These results confirmed that the introduction of plasmids, rather than the electroporation process itself, induced apoptosis and eventually cell death. Additionally, Annexin V / PI and Caspase-3 / RubyDead cell health assays were tested, and results indicated that a majority of cell death following electroporation was likely the results of apoptotic cells transitioning to the point of no return – cell death. Transduced SupT1 samples were able to fully recover, as their viabilities increased to ~90%+ by day 5 of the study. Lastly, samples were stained with APC-conjugated specific anti-CAR antibody, and SupT1 CAR expression levels were measured using the Cellaca® PLX, and results were confirmed using a flow cytometer. Utilizing this image-based method, we were able to monitor CAR expression in SupT1 cell samples on day 2, 5, 7 and compare CAR expression levels among different gene delivery methods (viral vectors or non-viral methods). With the advantages of ease of use, visual verification with captured cell images, and higher-throughput capability, the Cellaca® PLX Image Cytometer may be potentially used as a convenient benchtop system for rapid assessment of the quantity and quality of CAR-T cells, which may ultimately improve the productivity of development and manufacturability of CAR-T products.

15: "HIGH THROUGHPUT METHOD TO ANALYZE THE CYTOTOXICITY OF CAR T CELLS IN A 3D TUMOR SPHEROID MODEL USING IMAGE CYTOMETRY"

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Chimeric antigen receptor T cell therapy is an antigen dependent cellular therapy that has gained considerable traction in the field of cancer immunotherapy CAR T cell therapy involves specifically engineering T cells to attack tumor cells by binding a tumor antigen and inducing T cell activation resulting in intracellular signaling and cytokine release. Currently, there are six FDA approved CAR T cell therapies, which all target the CD19 or BCMA antigens for hematologic B cell malignancies. In the recent years, a strong focus has been placed on CAR T cell therapy discovery for solid tumors, which may better recapitulate physiological conditions, thereby potentially improving the selection of CAR construct candidates. Immune cell trafficking and immunosuppressive factors within the tumor microenvironment increase the relative difficulty in developing a robust CAR T cell therapy against solid tumors. Therefore, it is critical to develop novel methodologies for high throughput phenotypic and functional assays using 3D tumor spheroid models to better assess CAR T cell therapies against solid tumors. Recently, plate based image cytometry has emerged as a method to investigate and characterize CAR T cell functions in a high throughput manner Image cytometry has demonstrated capabilities in analyzing transduction efficiency, cell proliferation, and cytotoxicity for CAR T cell therapy. With the development of 3D spheroid models, image cytometry may provide the necessary tools and applications for CAR T cell therapy discovery geared towards solid tumors. In this work, we discuss the use of CAR T cells targeted towards PSMA, an antigen that is found on prostate cancer tumor cells, the second most common cause of cancer deaths among men worldwide. Herein, we demonstrate the use of high throughput plate based image cytometry to characterize PSMA CAR T cell mediated cytotoxic potency against 3D prostate tumor spheroids and simultaneously monitor location of the T cells in vitro. We were able to kinetically evaluate the efficacy and therapeutic value of PSMA CAR T cells by analyzing the cytotoxicity against prostate tumor spheroids. Furthermore, the T cells are fluorescently labeled with a tracer dye to visually locate the cells on the tumor spheroids. The proposed image cytometry method can overcome limitations placed on traditional methodologies to effectively assess cell mediated 3D tumor spheroid cytotoxicity and efficiently generate time and dose dependent results.

16: "COMPARISON OF CAR T CELL MEDIATED CYTOTOXICITY ASSAYS WITH SUSPENSION TUMOR CELLS USING HIGH THROUGHPUT PLATE BASED IMAGE CYTOMETERY METHOD."

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In the recent decade, chimeric antigen receptor T cell therapy harevolutionized strategies for cancer treatments due to its highly effective clinical efficacy and response for B cell malignancies. Currently, there are six CAR T cell products available in the market including Kymriah, Yescarta, Tecartus, Breyanzi, Abecma, and Carvykt. The success of CAR T cell therapy has stimulated the increase in the research and development of various CAR constructs to target different tumor types. Therefore, a robust and efficient in vitro potency assay ineeded to quickly identify potential CAR gene design from a library of construct candidates. Traditionally, in vitro CAR T cell mediated cytotoxicity is assessed using release assays such as 51 Cr (calcein (and LDH). However, release assays indirectly measure cell death via molecules released in supernatant and typically limited to only endpoint assays. In addition, handling and disposing of 51 Cr hazardous materials is less preferred Luciferase reporter assay, although highly sensitive, has similar drawbacks as the release assays. Finally, flow cytometry method can directly measure cell death and viability, but can be time consuming and requires a large number of CAR T cells when the effector to target (E T) ratio is high. Furthermore, additional steps are required for adherent cells that require trypsinization Image cytometry methodologies have been utilized for various CAR T cell mediated cytotoxicity assay using different fluorescent labeling methods, mainly due to their ease of use, ability to capture cell images for verification, and higher throughput performance. In this work, we employed the Celigo high

throughput plate based Image Cytometer to evaluate and compare two CAR T cell mediated cytotoxicity assays using GFP expressing or fluorescent dye labeled myeloma and plasmacytoma cells. Performing time and E T ratio dependent CAR T cell mediated cytotoxicity assays, the GFP based method demonstrated higher sensitivity in detecting the level of cytotoxicity when compared to the CMFDA/DAPI viability method. We have established the criteria and considerations for the selection of cytotoxicity assays that are fit for purpose to ensure the results produced are meaningful for the specific testing conditions.

17: "MODIFYING CHIMERIC ANTIGEN RECEPTOR (CAR) EXPRESSION IN T CELLS USING M6A-ASSOCIATED ELEMENTS-CONTAINING UTR SEQUENCES."

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N6–methyladenosine (m6A) is the most abundant internal modification on eukaryotic mRNA and has been implicated in a wide range of fundamental cellular processes, and several physiological processes including immunity, memory, and cancer. Most m6A modifications on eukaryote cells are located near the stop codon, in the consensus DRACH motif (D = A, G or U, R = A or G, A = m6A, and H = A, C or U). In this study we compared several combinations of this motif with well-established or newly discovered m6A-associated cis-elements in the 5' and 3' Untranslated Region (UTR) on CAR expression in T cells.

CD19-CAR mRNAs with different 5'UTR or 3'UTR sequences were in vitro-synthesized, qualified and quantified by Tape Station and qubit approach and then delivered to activated Pan-T cells by electroporation. CD19-CAR protein expression was quantified by Flow Cytometry using a CD19-CAR-specific antibody and Western blot analysis. We identified m6A-associated motifs that are positively associated with mRNA stability. In addition, several m6A-based combinations were found to increase CAR surface expression by up to 50%.

The positive impact of m6A-motifs in the UTRs on the level of CAR expression in T cells is consistent with what has been found in other mammalian cells (e.g. CHO cells). The m6A-associated motif is an exciting discovery, as it has yet to be reported. On-going work is underway to understand its cellular mechanism and gain further insight into its potential significance in RNA therapies.

18: "DYNAMIC INTERPLAY OF PROTEIN INTERACTIONS AND MODIFICATIONS – DISSECTION OF THE NLRP3 INFLAMMASOME SYSTEM."

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Introduction

The success of CAR-T therapy is influenced by multiple clinical and technical factors, including obtaining good quality starting material for commercial CAR-T manufacture. There is limited data on the immune profile of patients at the time of harvest and any impact starting cellular material may have on clinical success.

Materials & methods

MNC(A)/CAR-T starting product samples from biobanked patients (n=14) were assessed for absolute (cell/µl) and % values of T-cells (CD3/CD4/CD8), B-cells (CD19), and NK cells (CD56/CD16). The patient PET/CT scans determined whether the patient had entered complete metabolic remission (CMR) or had persistent disease (PD). We investigated the relationship with overall clinical response and the occurrence of Cytokine Release Syndrome (CRS).

Results

The 6-colour BD TBNK assay showed higher quality results across all test parameters, illustrated by the CV% obtained using control samples. Chart review demonstrated that 6 patients achieved remission (CMR) and 8 patients experienced persistent disease (PD). CRS symptoms were observed in 10 patients (n=10/14). The findings showed no statistical significance in the CD3, CD4, CD8, CD16/56, CD19 levels or CD4/CD8 ratio present in both patient cohorts ($p>0.05$).

Conclusions

The patient sample multi-parameter results showed that a higher CD4/CD8 ratio was observed in patients with an improved clinical response post-CAR-T and with the occurrence of CRS, however, these findings are not currently statistically significant. Further numbers are to determine the clinical significance of this trend. The newly validated TBNK analysis of the CAR-T starting material enables a 'snapshot' of the patient's immune status at this point of treatment.

19: "INVESTIGATING METABOLIC REPROGRAMMING OF MACROPHAGES AS A NOVEL THERAPEUTIC STRATEGY FOR ATHEROSCLEROSIS."

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Chimeric aantigen receptor (CAR) therapy has revolutionised the field of cancer immunotherapy with a range of immune effector cells including macrophages and natural killer (NK) cells now being explored to improve on CAR-T therapy1. Safety concerns and expense associated with viral vectors warrants the development of non-viral delivery systems for efficient CAR transgene delivery2. Peptide delivery systems such as RALA3 and CHAT4 are highly effective multifunctional delivery systems for delivery of nucleic acids with industry acceptable characteristics at a clinically relevant scale. In this study, RALA, CHAT and a novel CHAT derivative are investigated for delivery of nucleic acids to immune effector cells for production of cell-based therapies.

Physiochemical characterisation was performed on Peptide/mRNA nanoparticles (NPs) using a Malvern Zetasizer. Transfection efficiency of NPs delivering mRNA-GFP was assessed by FACS in a range of immune cell types and performance compared between peptides. Cell viability was investigated by PI staining and MTS assay.

Each peptide formed cationic NPs with mRNA, ideal for cellular uptake. RALA and XXX NPs transfected a range of immune effector cell lines with minimal toxicity; demonstrating the potential of these delivery peptides for non-viral production of CAR therapies.

This work provides proof of concept for the use of peptide delivery systems as transfection agents for ex vivo genetic modification of immune effector cells. Future work will evaluate transfection in NK cell lines and primary immune cells, and assessment of phenotypic functional retention following transfection with peptide delivery systems.

20: "CAR-T CLINICAL PHARMACOKINETIC/CYTOKINETIC MODELLING – TRENDS IN FDA REGULATORY APPROVAL DOCUMENTATION AND ADVANCED MODELLING APPROACHES."

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For conventional therapies, product-labelling pharmacokinetic (PK) information supports dose justification and optimisation based on dose-exposure-response (efficacy and safety) relationships in target populations. Chimeric antigen receptor (CAR)-T cell therapies frequently demonstrate exposure-response relationships. However, as CAR-T therapies have distinct PK characteristics, including an in vivo expansion phase and highly variable cell concentrations, the relationship between dose and exposure is tenuous.

The impact of potential covariates on CAR-T PK can be explored through correlating covariates with secondary PK parameters, e.g. AUC, Cmax, derived via non-compartmental (NCA) analysis. Additionally, population PK (PopPK) analysis can quantify observed PK variability in a population and identify significant covariates (partly) explaining this variability.

The goal of the current work was to assess the PK modelling and analysis approaches presented in the FDA approval documentation for six CAR-T therapies, alongside some recent suggested CAR-T PK modelling approaches presented in the literature.

NCA data was presented for all products, with a PopPK analysis clearly presented for 4/6 products. For most investigated covariates no relationship with PK parameters was found, or analyses were exploratory only. Suggested effects on AUC and Cmax from product characteristics and patient demographics/disease state were confounded by small numbers and large variability, and were insufficient to underpin dose-exposure recommendations.

Current PopPK modelling approaches have quantified, but not explained, the variability observed in CAR-T exposure. Exploration of PopPK model covariates like T-cell subpopulation phenotype and development of novel (semi-)mechanistic PK/quantitative systems pharmacology models may support more predictive model development, ultimately facilitating a personalised approach to CAR-T therapy.

21: "TNF-A PATHWAY ACTIVATION AND ALTERED MICRORNA EXPRESSION IN PERIPHERAL BLOOD-DERIVED ENDOTHELIAL COLONY-FORMING CELLS: INSIGHTS INTO DIABETES-INDUCED DYSFUNCTION."

***Yaqiong Liu,** Caomhán John Lyons, Alan Keane, Stuart McKeown, Edoardo Pedrini, Michael Creane, Nadeem Soomro, Alicja Straszewicz, Tomás Griffin, Alan Stitt, and Timothy O'Brien

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***Lead Presenter: y.liu14@universityofgalway.ie (Postgrad researcher (>2 years))**

Background: Endothelial colony-forming cells (ECFCs) are progenitors of endothelial cells and have shown angiogenic effects in pre-clinical studies in ischaemic tissues. ECFCs may represent a potential therapy for

patients who require vascular repair, including those with diabetes mellitus (DM). However, some, but not all, studies have reported a decrease in the number and impaired function of peripheral blood-derived ECFCs (PB-ECFCs) from patients with diabetes. This study aimed to compare the gene expression profiles and pathways between control and diabetic PB-ECFCs in order to understand mechanisms of diabetes-induced dysfunction.

Methods: Bulk RNA sequencing (RNA-Seq) was performed to compare the gene expression patterns of PB-ECFCs from control and diabetes groups. Subsequently, gene enrichment analysis (GSEA) was used to identify enriched pathways in PB-ECFCs from the diabetes group. To mimic the inflammatory environment of DM, PB-ECFCs were treated with TNF-α. Changes in gene and microRNA (miRNA) expression following TNF-α exposure were analysed via qRT-PCR. The proliferation, cell adhesion and tube formation ability of PB-ECFCs were assessed using Prestoblue, THP-1 cell adhesion, and in vitro Matrigel assays, respectively.

Results: We identified 66 differentially expressed genes (28 upregulated and 38 downregulated) between control and diabetic PB-ECFCs. GSEA showed that the TNF-α pathway was markedly enriched in PB-ECFCs from the diabetes group. We then exposed ECFCs to TNF-α and demonstrated activation of inflammatory pathways, which resulted in inhibition of proliferation and tube formation of control PB-ECFCs, and enhanced cell adhesion to THP-1. Additionally, we found that three miRNAs (miR-146a-5p, miR-199a-3p, and miR-199a-5p) were upregulated in control PB-ECFCs upon exposure to TNF-α.

Conclusion: In summary, this study suggested that the TNF-α pathway may play an important role in the pathophysiology of diabetic PB-ECFCs. The upregulation of miR-146a-5p and miR-199a-3p was likely a compensatory response to the inflammation induced by TNF-α. These findings may provide a rationale for the intervention of intracellular miRNAs as possible targets for improving PB-ECFC function in the inflammatory environment.

22: "UNDERSTANDING THE CLINICAL IMPORTANCE OF IMMUNE RECOVERY FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION AND CAR T-CELL THERAPY."

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***Lead Presenter: foystonh@tcd.ie (Postgrad researcher (>2 years))**

Introduction/Background:

Immune therapies such as allogeneic stem cell transplantation (allo-SCT) and chimeric antigen receptor (CAR) T-cell therapy are proven treatments for patients whose blood cancers have not responded to chemotherapy. However, up to 50% of patients relapse. The recovery of the patient's immune system, particularly T-cells, significantly influences survival rates.

Materials and Methods:

In this longitudinal study, we biobanked peripheral blood mononuclear cells from patients undergoing allo-SCT (n=20) and patients who received CAR T-cell therapy (n=19). We utilised multicolour flow cytometry to analyse CD4+ T-cells, CD8+ T-cells, mucosal-associated invariant T (MAIT) cells, invariant natural killer T (iNKT) cells, CD56+ T-cells, NK cells, monocytes, and subsets of γδ T-cells. In the CAR T-cell therapy cohort, we assessed

circulating CD19 CAR T-cells and their exhaustion marker phenotype. Statistical analysis was conducted using R Studio (V4.2.3) and GraphPad (V10.2.0).

Results:

Preliminary findings in the allo-SCT group linked slower T-cell recovery and poor outcomes, particularly CD56+ T-cells, known for their potent anti-tumour activities. In the CAR T-cell therapy group, patients achieving a complete response (CR) showed lower expression of PD-1 on the surface of CAR T-cells than those with persistent disease (PD).

Conclusion:

Our preliminary findings suggest that tracking immune profiles following allogeneic stem cell transplant could serve as valuable predictors of clinical outcomes. In the CAR T-cell therapy cohort, we observed a potential correlation between the presence of elevated PD-1 and poor clinical prognoses.

23: "γδT CELLS: POTENTIAL TARGETS FOR IMMUNOTHERAPY IN HEPATOCELLULAR CARCINOMA."

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Introduction: ɣδ T cells are innate-like immune cells enriched in the liver, representing promising immunotherapy targets due to their lack of MHC restriction. These cells display antitumour activity and hold prognostic value across most solid cancers. Hepatocellular carcinoma (HCC) accounts for 90% of liver cancer cases, with poor prognosis and high resistance to immune checkpoint inhibitors. Here, we examine the frequency and PD-1 expression of ɣδ T cells in HCC to explore their potential for enhancing existing immunotherapy.

Methods: Liver tissues from patients undergoing resection for HCC were studied. Mononuclear cells from tumour, tumour-adjacent, and distal liver samples were isolated via density gradient centrifugation and ɣδ T cell subsets were characterised using flow cytometry.

Results: Vδ2+ cells were more frequent within tumours compared to Vδ1+ and Vδ3+, appearing to infiltrate HCC tumours, while Vδ1+ cells were depleted. Intratumoral Vδ1+ and Vδ2+ subsets exhibited significant PD-1 upregulation relative to distal tissue. PD-1 expression on Vδ2+ negatively correlated with tumour size, whereas Vδ3+ cells were increased in tumours <5 cm.

Conclusions: Enhanced intratumoral frequency and PD-1 expression of Vδ2+ cells may indicate functional exhaustion, limiting tumour-killing capacity. Blocking the PD-1/PD-L1 axis could restore Vδ2+ function, enhancing proliferation, IFN-γ production, and tumour killing. Our findings support ɣδ T cell checkpoint blockade as a potential therapeutic strategy for HCC but emphasise functional differences between subpopulations. They also indicate that tumour size and ɣδ T subpopulation quantification may predict treatment response and prognosis.

GENE THERAPY

24: "CRISPR RECOMBINASES FOR TARGETED GENE THERAPY."

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The patient population for any given rare disease is quite small; however, there are over 8,000 rare diseases listed in the Orphanet database (Orphanet, 2024) representing 30 million people affected in the EU and over 300 million people worldwide. Few of these diseases are preventable or curable, most are chronic, and many result in premature death. For many of these diseases a sound genetic basis has been identified with 4,805 genes associated with 7,379 diseases (OMIM database, 2024) which has led to these diseases becoming the focus of gene-based therapies. Our research is focused on developing therapeutic gene editing using CRISPR and site-specific recombinases to enable integration of a therapeutic gene to compensate for any mutation within a target gene. Unlike homology directed repair-based systems, the use of site-specific recombinases allows for gene integration in terminally differentiated cells enabling therapeutic gene editing beyond actively dividing cells. We are currently developing a platform to integrate genes into safe harbour loci in human cells to facilitate expression of a therapeutic gene without disrupting native gene expression.

25: "DIRECTED EVOLUTION OF TRANSFER RNA THERAPEUTICS."

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Synthetic biology is a rapidly growing field with the potential to revolutionize the biotechnology industry. Synthetic biology aims to design and or evolve biomolecules with new-to-nature functions by making biology easier to engineer. Many established labs have piloted the synthetic biology design-build-test-learn paradigm to understand rules for protein expression in diverse organisms. However, an underexplored area of synthetic biology is the biogenesis and evolution of RNA. As with protein, RNA expression is prone to biases in promoter choice, and flanking genetic sequence, and many RNAs that are transcribed must be post-transcriptionally modified to promote the intended function. One RNA species with diverse functions in eukaryotes and great therapeutic potential is transfer RNA (tRNA). tRNAs are present in all cells and core to life on earth, yet we lack rules for recombinant expression of tRNA genes. tRNA-based therapeutics show promise as precision medicines to treat ~7000 known rare genetic diseases arising from premature termination codons (PTCs) within protein-coding genes. Treatment of all known PTC-derived diseases could in theory come from just 18 tRNA molecules if current limitations in delivery and PTC suppression efficiency are overcome. My lab aims to develop rules for the predictable expression and augmentation of RNA molecules with therapeutic potential in human cells.

26: "EMERGING CELLULAR AND GENE THERAPIES IN TRIGEMINAL NEURALGIA AND SALIVARY GLAND DISORDERS: A SCOPING REVIEW."

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Background: Trigeminal neuralgia (TN) and salivary gland disorders are debilitating conditions with limited therapeutic options. Emerging cellular and gene therapies offer promising avenues for regeneration and symptom management. However, the evidence base for these innovative approaches remains fragmented.

Objectives: This scoping review will aim to map existing evidence on cellular and gene therapies for TN and salivary gland disorders, identify research gaps, and inform future clinical and translational research.

Eligibility Criteria: Studies exploring cellular or gene therapies in preclinical or clinical settings for TN or salivary gland disorders will be included. Articles will be eligible if they report on therapeutic outcomes such as neuroinflammation modulation, pain relief, or glandular function restoration. Reviews, editorials, and opinion pieces will be excluded.

Sources of Evidence: An electronic search will be conducted in PubMed, Scopus, and Web of Science, supplemented by manual searches of reference lists and gray literature.

Charting Methods: The studies will be screened using software, extracting data, and chart findings using a standardized data extraction tool. Discrepancies will be resolved by consensus. Data will be synthesized narratively to provide an overview of interventions, mechanisms, and outcomes.

Results: The findings will be summarized to identify therapeutic strategies, their mechanisms of action, and their efficacy in addressing TN and salivary gland disorders.

Conclusions: This review will provide insights into the current landscape of cellular and gene therapies, highlight knowledge gaps, and propose directions for future research to advance regenerative approaches in neurotherapeutics and glandular disorders.

27: "THERAPEUTIC POTENTIAL OF MIR-379 THROUGH MODULATION OF BREAST CANCER STEM CELLS."

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Breast cancer remains a leading cause of cancer-related mortality worldwide, necessitating innovative therapeutic approaches. Our research focuses on microRNA-379 (miR-379), which is significantly downregulated in breast cancer tissues and further diminished in metastatic sites. Functional studies reveal that miR-379 suppresses tumour proliferation and growth, highlighting therapeutic potential in this aggressive disease.

This study focused on elucidating the mechanisms of action of miR-379, through proteomic analysis in a murine model of breast cancer. Differentially expressed proteins (DEPs) in miR-379-enriched tumours were identified using LC-MS/MS, revealing 600 DEPs, including 11 downregulated and 43 upregulated proteins. Functional enrichment analysis highlighted involvement in TGFβ production and TGF-β signalling pathways critical to

cancer progression. To identify the targets directly regulated by miR-379 in this proteome, target prediction analysis using bioinformatics tools such as TargetScan, miRDB, and miRTarBase was performed. Intersection analysis of predicted targets and DEPs identified the oncogene HSPA5 as a direct target of miR-379. Immunohistochemistry revealed that miR-379 enrichment reduced expression of CD44, a marker of cancer stem cells (CSCs). This suggests miR-379 modulates the HSPA5-TGF-β1-CD44 axis to inhibit stemness and tumor progression. This was borne out through an observed reduction in TGF-β1 secretion and mammosphere formation capacity of miR-379 enriched cells in vitro.

Our findings establish miR-379-5p as a potent suppressor in breast cancer through targeting the HSPA5-TGFβ1-CD44 axis, effectively disrupting CSC dynamics. This work provides mechanistic insights into miRNA-mediated tumour suppression and underscores the potential of miR-379-5p as a targeted gene therapy for breast cancer.

28: "MULTI-ATTRIBUTE ANALYSIS AND N-GLYCOMIC PROFILING OF RECOMBINANT THERAPEUTIC ADENO-ASSOCIATED VIRUSES."

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Adeno-associated virus (AAV) vector has become the leading platform for gene delivery to treat numerous genetic diseases. There are several different serotypes, each exhibiting different tissue tropism, immunogenicity, and transduction performance. Selecting the most suitable serotype for an application is critical for efficient gene delivery to target tissues. Genome divergence among different serotypes is due mainly to the hypervariable regions of the capsid proteins. Therefore, comprehensive analysis of the AAV particles is essential to ensure desired efficacy and safety. Size exclusion chromatography coupled with fluorescence and triple-wavelength ultraviolet detection, alongside incorporation of tryptophan as an internal standard offers a simple, rapid, and reliable approach for simultaneous multi-attribute analysis of AAVs. We demonstrate the capability of this approach for AAV characterization and quantification, including capsid concentration, vector genome concentration, empty to full capsid ratio, and the presence of aggregates or fragments. Additionally, the N-glycosylation profiles of capsid proteins of AAV serotypes 1 to 9 have been systemically characterized and compared using a previously developed high-throughput and high-sensitivity N-glycan profiling platform. The results show that all 9 investigated serotypes are glycosylated, with comparable profiles. The most conspicuous feature is the high abundance of mannosylated N-glycans, including FM3, M5, M6, M7, M8, and M9, that dominate the chromatograms within a range of 74 to 83%. This research lays the foundation towards gaining a better understanding of the importance of capsid proteins and their post-translational modification that may play significant roles in tissue tropism, interaction with cell surface receptors, cellular uptake, and intracellular processing.

29: "ROLE OF MICRORNA-758 IN BREAST CANCER."

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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 role of miR-758 in breast cancer progression. MDA-

MB-231 breast cancer cells were stably transduced with a lentiviral vector carrying a non-targeting control(NTC) sequence or mature miR-758 along with green fluorescent protein(GFP) to generate MDA-NTC and MDA-758 cells. Transduction success was confirmed by fluorescent imaging and RQ-PCR. Transwell membranes were employed to assess the migration capacity of MDA-NTC, MDA-758 and wild type cells in response to chemoattractants. MTS assay was performed on MDA-NTC and MDA-758 cells to investigate the impact of miR-758 on cell proliferation. Tubule formation assays were employed to investigate the impact of miR-758 on the ability of MDA-MB-231 cells to stimulate angiogenesis. Transduced cells successfully expressed GFP fluorescent signal, and qPCR analysis revealed a 180-fold increase in miR-758 expression in MDA-758 compared to MDA-NTC cells. There was no significant change in proliferation in the miR-758 enriched cells. However, MDA-758 cells demonstrated a 34% reduction in migration (P=0.04) towards chemoattractants in comparison to MDA-NTC cells. Conditioned media from MDA-758 cells also had a reduced ability to stimulate tubule formation (angiogenesis) compared to factors secreted by control cells. The data demonstrate that enrichment of aggressive breast cancer cells with miR-758 resulted in a significant decrease in migratory capacity and ability to stimulate angiogenesis, supporting a potential tumour suppressor role for miR-758.

30: "INVESTIGATING THE IMPACT OF MIR-379 ON ENDOTHELIN-1 SECRETION IN BREAST CANCER."

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Elevated circulating Endothelin-1 (EDN1) in breast cancer patients has been associated with lymph node and distant metastasis, raising interest in the potential to modulate expression via gene therapy. microRNA-379 (miR-379) has a predicted binding site in the 3' UTR of EDN1. This study aimed to determine whether miR-379 plays a role in regulation of EDN1. Breast cancer cell lines [MDA-MB-231, HCC1954; 4T1] were transduced with lentivirus to express miR-379. RNA was extracted from cells and tumour xenografts, and RQ-PCR performed targeting EDN-axis members: EDN1, endothelin converting enzyme 1 (ECE1), endothelin receptor A and B (EDNRA, EDNRB). EDN1 secretion over 72hrs was detected using ELISA. Immunohistochemistry (IHC) targeting Prepro-EDN1 was performed on tumour xenografts ex vivo. EDN1 and ECE1 were robustly expressed in all cell lines. MDA-MB-231 had robust expression of both receptors, while HCC1954 cells had negligible receptor expression. While undetectable in 4T1 cells, both receptors were strongly expressed in 4T1 tumours likely due to receptor presence in infiltrating vasculature. miR-379 enrichment had no impact on EDN1 secretion in vitro. The amount of EDN1 secreted after 72 hours varied significantly between cell lines and did not correlate with mRNA levels. Both 4T1-379 and HCC1954-379 ex-vivo tumours showed a significant reduction of Prepro-EDN1 compared to respective control tissues. This supports a role for miR-379 in regulation of translation of the precursor protein, for which it has a predicted binding site. Understanding the interplay between genes involved in EDN1 processing and uptake will further elucidate the functional relevance in disease progression.

31: "DESIGN OF EXPERIMENT (DOE) ENABLED SCALE-UP OF ADENO-ASSOCIATED VIRUS (AAV) PRODUCTION."

***Bogdanovic, A (1,2),** Whelan, J (2,3), McDonnell, S (2), Donohue, N (1), Glennon, B (1,2)

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Adeno-associated virus (AAV) therapies are produced in a process called triple transient transfection into a single cell line where three plasmids are combined with a transfection reagent that undergoes interactions with DNA. This process brings challenges associated with process performance in terms of titre and product quality along with challenges during scale-up.

A 2-stage DoE was used to select optimum conditions for triple transfection using FectoVir-AAV and a HEK293F cell line. The three plasmids allowed for production of an AAV5 capsid containing the transgene encoding green fluorescent protein (GFP). A screening design was executed to identify factors, including amount of DNA, plasmid ratios, volume of FectoVir-AAV, complexation volume, complexation time and cell culture density, with the potential to have significant effects on transfection efficiency, genome titre, capsid titre, and ratio of full to empty capsids. Analysis indicated that DNA amount, complexation time, volume of FectoVir-AAV and the ratio of pTransgene demonstrated the strongest effect and were taken forward to the second stage, which implemented a central composite design to identify optimum conditions for triple transfection.

The optimum conditions resulted in ~76% transfection efficiency, genome titre of ~1.3 x 10^11 vg/mL, capsid titre of ~2.2 x 10^11 capsids/mL and a percentage of full capsids of ~60%. The conditions were further verified in the laboratory at both 125 mL shake flask, 3L shake flask and 10 L Wave reactor-scales.

32: "The immunoregulatory capacity of SHP1 in human health and disease; and its implications in systemic lupus erythematosus."

***Sarah Harkin**, Dr Marion Butler

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The protein tyrosine phosphatase SHP1 has been widely recognised for its importance in immunoregulation. SHP1 activation is required to limit immune exacerbations and does this by reducing pro-inflammatory cytokines. SHP1(-/-) mice develop severe autoimmunity, paired with uncontrolled levels of inflammation. Similarly, reduced phosphorylation/activation of SHP1 has been associated with increased T-cell proliferation in autoimmune diseases such as systemic lupus erythematosus (SLE). The role of SHP1 downstream of various toll-like receptors (TLRs) has been investigated, however, its role downstream of TLR7 has been largely neglected. Due to the major implications of SHP1 in TLR7-driven diseases such as SLE, we sought to investigate the impact of SHP1 in TLR7 signalling.

Stable SHP1-knock-down (KD) CAL-1 plasmacytoid dendritic cells, that constitutively express TLR7, but not TLR8, were generated using an shRNA lentiviral KD approach to investigate the impact of SHP1 deficiency on the TLR7 pathway. Naïve or SHP1-KD CAL-1 cells were then stimulated with the TLR7/8 agonist, R848, and supernatants were harvested to assess cytokine levels by-way-of ELISA. In parallel, cell lysates were profiled by Western blot analysis to examine how SHP1 affects transcription factor activation downstream of TLR7.

SHP1-KD in CAL-1 cells enhanced the production of both pro-inflammatory cytokines and IFNβ downstream of TLR7. In addition to this, we also saw increased activation of the major transcription factors involved in this signalling pathway; specifically in the NF-κB pathway. These results provide preliminary evidence that SHP1-KD has the potential to exacerbate disease and could be a potent inducer of autoinflammation downstream of TLR7 signalling.

CLINICAL APPLICATIONS AND ACCESS

33: "THE EU PHARMACEUTICAL PROPOSALS AND THEIR IMPLICATIONS FOR THE HOSPITAL EXEMPTION ROUTE FOR THE PROVISION OF ATMPS IN EUROPE."

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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 where these are tailored (custom-made) for individual patients (this is the so-called hospital exemption route). Although exempt from the requirements of a marketing authorisation, the manufacturing of these products is authorised by the relevant competent authority in each EU Member State. 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. The proposed EU pharmaceutical package, if adopted in its current form, sets out a series of proposals which would impact the current operation of the hospital exemption route in Europe.

This paper considers the current operation of the hospital exemption pathway and how it may be impacted by the proposed EU Pharmaceutical legal reforms, focusing in particular on: 1) proposals relating to the collection of data and reporting requirements for hospital exempt ATMPs 2) proposals relating to the quality, safety and efficacy data required for hospital exempt ATMPs; and 3) proposals relating to cross-border exchange of hospital exempt ATMPs. The paper will reflect on the responses of various industry, medical and patient groups to these legal proposals, and contestations within these. It will also assess more broadly, the potential implications for access to ATMPs for patients under the hospital exemption route in Europe.

34: "PREPARING FOR THE FUTURE OF ADVANCED THERAPIES: A QUALITY IMPROVEMENT APPROACH."

***Gerry Hughes,** Cormac Kennedy, David Kevans, Derval Reidy, Jeremy Towns, Edel O Dea, Kishor Santhosh, Caitriona Ryan

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Background

Advanced therapies offer transformative potential for rare diseases, targeting conditions at the genetic level to halt or mitigate progression. The Wellcome-HRB Clinical Research Facility (CRF) at St James's Hospital, Dublin, has been at the forefront of delivering advanced therapies since 2018, hosting clinical trials and facilitating treatments for patients requiring these innovative interventions.

Aim

To evaluate the advanced therapies trials and treatments hosted by the CRF and develop a quality improvement action plan for enhancing service delivery.

Methods

A mixed-methods quality improvement approach was employed, incorporating:

- Evidence synthesis on the readiness of the Irish healthcare system to adopt advanced therapies.

- A needs assessment with CRF staff to identify service improvement opportunities.

- Internal and external stakeholder engagement.

- Implementation of the "Plan-Do-Study-Act" (PDSA) cycle for iterative quality improvement.

Results

A scoping review protocol was published (review itself submitted as separate abstract). The needs assessment identified a critical requirement for the CRF's aseptic compounding unit to manage diverse advanced therapy modalities. Stakeholder consultations and a literature review informed the establishment of a dedicated oversight committee to conduct risk assessments and develop handling protocols for new advanced therapy modalities.

Conclusion

This initiative highlights the critical role of quality improvement in enhancing the capacity of clinical research facilities to safely and effectively deliver advanced therapies. Continued investment and collaboration are essential to address evolving challenges and meet the needs of patients and clinicians.

Funding: Supported by Pfizer, Grant #76768559.

35: "CLINICAL ADOPTION OF ADVANCED THERAPIES IN IRELAND: A SCOPING REVIEW.

***Gerry Hughes, Isabella Devine, Clarice O Brien, David Mockler, Cormac Kennedy, David Kevans, Derval Reidy, Jeremy Towns, Edel O Dea, Kishor Santhosh, Caitriona Ryan**

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Background

Advanced therapy medicinal products (ATMPs) represent a paradigm shift in treating rare and refractory diseases. However, their adoption by healthcare systems remains challenging due to infrastructural, regulatory, and operational gaps.

Aim

This scoping review aimed to evaluate the readiness of the Irish healthcare system to adopt ATMPs and identify barriers and facilitators influencing their integration.

Methods

Joanna Briggs Institute Scoping Review methodology. A comprehensive search across peer-reviewed journals, government reports, industry publications, and patient organization outputs was conducted. Inclusion criteria focused on literature examining ATMP adoption specific to Ireland. Data were synthesized narratively and presented in tabular formats to map barriers, facilitators, and strategic opportunities.

Results

Ten articles were identified. Facilitators include Ireland's robust biopharmaceutical manufacturing capabilities and collaborative networks. Barriers encompass limited clinical trial infrastructure, misaligned reimbursement

models, insufficient healthcare workforce training, and underdeveloped digital health systems. Regulatory delays and high upfront costs further impede ATMP accessibility. Strategic recommendations highlight the need for dedicated advanced therapy centres, public-private partnerships, and innovative reimbursement frameworks to foster ATMP adoption.

Conclusion

While Ireland demonstrates potential for ATMP adoption due to its biopharmaceutical strengths, significant gaps in clinical infrastructure, workforce readiness, and regulatory frameworks hinder progress. Addressing these gaps through strategic investment and systemic reforms will be pivotal in ensuring equitable access to these transformative therapies. Future studies should explore operational readiness within patient-facing institutions to optimize ATMP delivery.

Funding: Supported by Pfizer, Grant #76768559.

36: "KNOWLEDGE TRANSLATION STRATEGY FOR ADVANCING ADVANCED THERAPIES AT THE WELLCOME-HRB CLINICAL RESEARCH FACILITY."

Gerry Hughes, Cormac Kennedy, David Kevans, Derval Reidy, Jeremy Towns, Edel O Dea, Kishor Santhosh, Caitriona Ryan

Wellcome - HRB Clinical Research Facility at St James's Hospital, Trinity College Dublin School of Medicine

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Pre- Background

Knowledge translation (KT) bridges the gap between research and clinical practice, ensuring that innovations, such as advanced therapies, reach patients effectively. The Wellcome-HRB Clinical Research Facility (CRF) at St James's Hospital plays a pivotal role in supporting advanced therapy clinical trials. However, a structured KT strategy is essential to maximize the CRF's impact on patients, clinicians, and the wider community.

Aim

To design and implement a specified-purpose knowledge translation strategy for the CRF.

Methods

A mixed-methods approach was employed, including a literature review and qualitative interviews with patients and clinicians.

Results

Key outputs included:

- Identification of a suitable KT framework from the literature.
- Clinician and public engagement events:

o A Clinical Research Summer School introducing and reinforcing the CRF role, including in supporting advanced therapies

o A CRF 10-year anniversary event featuring a patient panel discussion

o Display of a commissioned Lego sculpture at the Trinity College Dublin Child Health Research Festival

- o Development of a patient guidance document and video to assist patients navigating the CRF
- o Patient-facing podcast episode highlighting the CRF's role and its focus on advanced therapies
- o Establishment of a grants oversight committee with patient representation as a strategic advisory group

Conclusion

This initiative demonstrates the importance of a targeted KT strategy in enhancing the CRF's visibility, fostering stakeholder engagement, and advancing the adoption of advanced therapies. These efforts have created a scalable model for integrating research into practice while ensuring patient involvement.

Funding: Supported by HRB, Grant #KTA-2022-011

37: "THE NEW EU SUBSTANCES OF HUMAN ORIGIN (SOHO) REGULATION (REGULATION 2024/1938) – IMPLICATIONS FOR ATMPS?"

***Sinéad Masterson**

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Regulation (EU) 2024/1938 on the standards of quality and safety for substances of human origin (SoHO) intended for human application was adopted in June 2024. SoHO include any substance collected from the human body, including substances which may be transfused (e.g. blood) or transplanted (e.g. heart valves/haemopoietic stem cells). SoHO are integral to the manufacture of advanced therapy medicinal products (ATMPs). Thus, the new SoHO regulation has significant relevance for ATMP developers and this is the core focus of this paper.

Three key aspects of the new regulation are considered: 1) it has an extended scope of application, e.g., only the donation, collection and testing of SoHO when intended for manufacture as an ATMP was covered by the previous framework. Under the new regulation, donor registration, donor history review and medical examination, testing of donors, collection, as well as storage, release, distribution to the manufacturer and import and export will be covered. 2) It provides a mechanism for harmonizing the classification of borderline products i.e. products that may fall between different regulatory frameworks. This will involve seeking an opinion on the regulatory status of a SoHO product from the newly established SoHO Co-Ordination board (SCB). 3) It will provide for more regulatory oversight in terms of registration and reporting requirements to the national SoHO competent authority and newly established EU SoHO Digital Platform.

There is a transitional period for the new regulation until 2027, however, understanding its implications for the use of SoHO should be a priority for ATMP developers.

38: "REGULATORY READINESS LEVEL: A TOOL TO ENHANCE EARLY REGULATORY ADOPTION IN ACADEMIC ATMP DEVELOPMENT."

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The number, diversity and complexity of cell and gene therapies (Advanced Therapy Medicinal Products [ATMPs]) currently in development across a wide range of therapeutic areas is giving increasing hope to patients(1). Whilst academia is recognised as a source of these therapies(2,3), difficulties arise due to a lack of understanding and knowledge of rigorous regulatory requirements needed to gain clearance to market innovative products(4, 5,6). There is a need for tools to aid academic developers to understand the complex ATMP regulatory space. Whilst Technology Readiness Level (TRL) tools exist, they are simplistic in nature and do not provide the necessary detail to facilitate this process. An expansion of the TRL in the form of a Regulatory Readiness Level (RRL) tool, has been developed by researchers in TU Dublin, as a method for guiding researchers through the appropriate regulatory framework. Initial validation work was applied to a platform in development (Advanced Tubular Prototypes for Cell Regeneration) to assess its compliance to the required standards and regulations. A review of the platform using the RRL tool was carried and indicated necessary tasks required in regulatory compliance to be followed and to maintain a clear, transparent record of supporting documentation for future regulatory assessments.

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39: "COMPARATIVE STUDY OF GOOD MANUFACTURING PRACTICE REQUIREMENTS FOR DECENTRALISED MANUFACTURING OF ADVANCED THERAPY MEDICINAL PRODUCTS (ATMP): A STUDY OF EUROPEAN MEDICINES AGENCY (EMA) AND MEDICINES AND HEALTHCARE PRODUCTS REGULATORY AGENCY (MHRA) REGULATIONS AND GUIDANCES."

Vinicius Beal Boufleur, **Elaine Harris**

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Advanced Therapy Medicinal Products (ATMPs) stand at the forefront of healthcare innovation, offering potential solutions for individuals with unmet medical needs. Decentralised manufacturing of ATMPs emerges as a strategy to streamline production lead times and integrate healthcare systems by localising manufacturing activities at point-of-care facilities. This research delves into the Good Manufacturing Practice (GMP) regulations governing decentralized ATMP manufacturing in the European Union and the United Kingdom.

A comprehensive literature review and interviews with Subject Matter Experts revealed that both regions adhere to the directives outlined in EudraLex Volume 4, Part IV, for ATMP manufacturing; therefore, a direct comparative analysis was not performed. However, it was observed that discrepancies exist in the interpretation of these regulations among EU Member States.

Further examination identified some key differences in the regulatory frameworks for ATMPs between the EU and the UK, particularly in the Exception Pathways offered to developers. While the UK offers fewer pathways, it maintains comparable product and trial availability to the EU, suggesting potential efficiencies.

This work identified challenges associated with decentralised manufacturing, including GMP compliance, personnel management, communication, equipment and facility requirements and ATMPs' unique nature. Overcoming these challenges necessitates enhanced stakeholder awareness, developing effective communication channels, investment in closed and automated systems, and a nuanced understanding of ATMP intricacies.

Despite hurdles, decentralised manufacturing presents a promising avenue for widening ATMP accessibility to patients. Bridging regulatory and logistical gaps will be imperative to fully harness ATMPs' potential in healthcare systems, emphasizing the importance of collaborative efforts and continued regulatory support.

40: "IMPACT OF THE NEW SUBSTANCE OF HUMAN ORIGIN REGULATIONS ON CELL THERAPIES."

***Ewa Pociask,** Elaine Harris

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***Lead Presenter: Postgrad researcher (first 2 years), elaine.harris@tudublin.ie**

Advanced Therapy Medicinal Products (ATMPs) are a novel medical treatment derived from substances of human origin (SoHOs), including cells, genes and tissue of human origin. Cell therapies, a subset of ATMPs, work by repairing, replacing and restoring damaged cells within the body, potentially offering long-term healthcare solutions.

In the European Union, SoHOs are currently regulated under the Blood Directive (Directive 2002/98/EC) and the Tissue and Cells Directive (Directive 2004/23/EC), together known as the BTC Directives or BTC legislation to establish quality and safety standards. Due to medical and technological advancements, an evaluation of the current legislation done in 2019 revealed various gaps that have emerged in the existing legislation.

To address these gaps, the European Commission proposed a new regulatory framework called the Substance of Human Origin regulations, which have been adopted and are expected to be in full effect by 2027.

This study examines the impact of the new Substance of Human Origin regulations on the development and manufacture of cell therapies, analysing the changes to the current legislation and discussing their potential to reshape the ATMP sector.

41: "NIBRT CONCEPT FACILITY - A CORE FACILITY FOR BIOTHERAPY DEVELOPMENT."

***Brian Philip,** Clair Gallagher, Julia Serna

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The rapid evolution of technology arises concurrent with recent advances in immunotherapies. Funded with Science Foundation Ireland (SFI) and IDA Ireland support, the recently opened CONCEPT facility offers access to advanced technologies and expertise which can extend beyond the means of academics and small biotech companies. Exploiting the capacity and potential of human immunity, immunotherapies remain among the most complex drugs ever developed. Often simply described as CAR T cells, chimeric antigen receptor T cells represent a convenient oversimplification for the complexity of a drug product comprised from predominantly genetically engineered T lymphocytes but likely includes a wide range of haematological effector lineage residuals, each possessing a phenotypic plasticity capable of independent responsiveness within the physiological environment. Further biological variation arises based on the donor source of immune effectors. Autologous therapies remain

constrained by donor (patient) specific limitations and variations. Although development of allogeneic immunotherapies aims to resolve such limitations, batch-to-batch intra- and inter-donor variations remain an intrinsic element of these 'living drugs'. Advanced platforms such as the Bruker Lightning and IsoSpark systems offers opportunity for single cell investigations supporting immunotherapy drug design and development through to retrospective interrogation following clinical application whilst access to manufacturing platforms such as the Lonza Cocoon and Cytiva Xuri bioreactors offers opportunity for scale-up production and engineering runs. Potential applications afforded by further platforms will also be presented.

42: "DISINFECTION WITHOUT HARSH CHEMICALS FOR A SAFER CGMP CELL AND TISSUE PRODUCTION ENVIRONMENT."

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Chemical disinfectants/sterilants are a massive risk to cells in culture. Antibiotics damage mitochondria, altering cell metabolism, gene regulation, and impairing T cell function. Control of the physical attributes of the cell handling atmosphere offers the promise of microbial control without the use of antibiotics or chemical biocides. The Xvivo System is a closed cell incubation and handling environment charged with sterile-filtered dry gases (O2, CO2, N2). Particles, temperature, and relative humidity (RH) are under continuous monitoring and control. Previous data showed a microbe-dependent reduction in microbial viability under desiccating conditions in cell handling areas. It also showed that microbes were immobilized where they dried. Here we test the ability of disinfectants that are less risky to cells to remove dried-on microbes from the cell handling chamber. These studies showed that compared with SporKlenz, a harsh sporicidal disinfectant, that plain sterile water was equally effective at desiccated microbial removal. In media runs, no mock cell cultures were contaminated and no CFU were found on chamber surfaces even though inoculated coupons, harvested at intervals, still showed viable B. subtilis spores present (n=4 independent runs). Controlling the physical attributes, particularly humidity, of the closed cell handling environment allows for reduction or even elimination of use of harsh chemical disinfectants which, in turn, reduces risks to cultured Immunotherapeutics.

43: "MONITORING L-TYROSINE DISSOLUTION USING THE CANTY PHARMAFLOW PARTICLE SIZING SYSTEM."

***Claire Boles,** Jessica Whelan, Roderick Jones

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L-Tyrosine, a critical amino acid in cell culture media, supports protein synthesis and cellular metabolism. However, its slow dissolution kinetics and low solubility at neutral pH pose challenges for preparing concentrated media essential for bioprocesses. Increasing demands for higher cell densities and product titres in intensified processes have exacerbated the need for effective L-Tyrosine dissolution strategies. These issues highlight the need for robust process analytical technology (PAT) to monitor and determine dissolution endpoints.

The feasibility of developing the Canty PHARMAFLOW system as a PAT method for tracking the dissolution of L-Tyrosine in an aqueous solution was investigated. The Canty system uses high-resolution imaging coupled with image analysis software to analyse particle size and shape distributions on the basis of multiple metrics. L-

Tyrosine was dissolved at multiple pHs in the range of 2 to 10. The rate of dissolution varied with pH due to the changes in the saturation concentration. The dissolution process was monitored with the Canty PHARMAFLOW system.

Multiple metrics from the Canty such as particle minor diameter and bounding rectangular diagonal indicated the progression and endpoint of dissolution as it varied with pH. These metrics showed the decrease in particle size as dissolution progressed until reaching steady state when dissolution endpoint was reached. This was confirmed by both offline HPLC-determined liquid phase concentration and visual inspection results.

Future studies will provide additional insights into the influence of environmental variables on dissolution behaviour and endpoint determination. Offering a significant step forward in optimizing dissolution processes for bioprocess media preparation..

CELL ENGINEERING AND REGENERATIVE MEDICINE

44: "OPTIMIZING ENDOTHELIAL COLONY-FORMING CELL CULTURE USING HUMAN PLATELET LYSATE: A XENOGENEIC APPROACH."

Christine Ayu Lagonda (1), Caomhán John Lyons (1), Michael Creane (2), Timothy O'Brien (1,3)

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Endothelial colony-forming cells (ECFCs) are essential for blood vessel formation and hold great potential for vascular regenerative therapies. However, despite significant research progress, transitioning ECFC-based therapies to clinical trials remains challenging. A key limitation lies in the lack of a fully xenogeneic manufacturing approach.

This study investigates the potential of human platelet lysate (HPL), produced in-house at the Centre for Cell Manufacturing Ireland (CCMI), as a xenogeneic culture medium replacement of foetal bovine serum (FBS) to facilitate the use of ECFC for clinical applications. To optimize ECFC growth and performance, we evaluated heparin sodium salt, a vital additive that prevents fibrin gel formation and enhances cellular activity in HPL-based systems, at various concentrations. An optimal heparin concentration was defined as a concentration which inhibits gel formation while minimising impact on ECFC cell growth. ECFCs proliferation in 10% HPL with heparin were compared with those grown in 10% and 2% FBS in EGM-2 media.

Our preliminary results (N=1) showed that heparin concentrations between 0.5 IU/ml to 1.5 IU/ml are effectively prevent any gel formation due to coagulation without compromising the ECFC growth. Moreover, ECFCs cultured with 10% HPL indicated the superior growth compared to those cultured in either 2% or 10% FBS in EGM-2 media. These results suggest the feasibility of a xenogeneic approach using HPL, highlighting its impact on ECFC proliferation.

Future research will focus on doing subsequent experiments by expanding the sample size (N=3) to validate and build on the preliminary findings.

45: "DEVELOPMENT OF HUMANIZED, FUNCTIONAL, TISSUE ENGINEERED IN VITRO MODEL OF HEALTHY MYOCARDIUM."

***Meenakshi Suku (1,2),** Jack F. Murphy (3), Sara Corbezzolo (1,2) , Manus Biggs (2), Giancarlo Forte (4), Irene Turnbull (5), Kevin D. Costa (5), Lesley M. Forrester (6), Michael G. Monaghan (1,2)

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The myocardium is a contractile tissue that hosts multiple cell types that work in unison to maintain a steady supply of oxygen and nutrients throughout the body. Furthermore, research over the past decade has significantly improved our understanding of the interaction between cardiomyocytes and immune cells, illuminating the importance of cardiac resident macrophages in maintaining homeostasis within the myocardium. In particular, macrophages in the heart perform a number of critical functions such as facilitating electrical conduction, capture and elimination of cardiac exophers and routine immunosurveillance. Here, we generate a tissue engineered model of healthy myocardium, with an innate immune response capability for patient-specific drug screening, regenerative medicine and drug discovery applications.

Using induced pluripotent stem cell (iPSC) derived cardiomyocyte (iCM) and macrophage (iMac) sources, we demonstrate excellent integration of the two cell types in co-culture. We show that iMacs resemble a cardiac resident macrophage phenotype in the presence of iCMs, while iCMs exhibit improved maturation in the presence of iMacs. Using a 3D engineered heart tissue (EHT) model, we verify improved tissue maturation and function with iMac integration.

46: "COMPARING HEART-TISSUE DERIVED EXTRACELLULAR VESICLES OF ZEBRAFISH AND RAT."

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***Lead Presenter: kommerir@tcd.ie (Postdoc)**

Heart attacks or myocardial infarction (MI) can lead to immediate death or partial damage of the heart tissue, even if appropriate treatment is administered. In survivors, currently available medications provide symptomatic relief with life-long intervention to control blood pressure and cholesterol levels. Several years of research have reported various therapeutic interventions that deliver cells, genes, small biomolecules, and acellular biomaterials to achieve cardiac regeneration in mammals. Rather than improving cardiac function, the existing approaches fail to replace the fibrous scar with a functional myocardium. Extracellular vesicles are nanocarriers of biomolecules that exchange valuable information between cells in tissue microenvironments. In addition to circulating EVs, vesicles bound to the extracellular matrix can be considered better representatives of the local tissue microenvironment under pathological conditions. In this study, we isolated and characterized matrix-bound nanovesicles (MBV) from super-regenerative Zebrafish hearts and non-regenerative rodent (rat) hearts. Zebrafish possess an extraordinary ability to regenerate functional heart tissue after losing 20-25% of their heart ventricle. MBVs are isolated by digesting the heart tissue in collagenase and are differentially centrifuged followed by ultracentrifugation. Vesicles were characterized for size distribution and morphology using a nanoparticle analyser (NTA) and transmission electron microscopy. The uptake and metabolic activity of MBV-treated HeLa cells were evaluated using imaging and MTT assays. The heart MBVs of both zebrafish and rats are comparable in size and shape. Both MBVs were cytocompatible and taken up by the HeLa cells. Future studies will characterize the cargo molecules using proteomics and RNA sequencing experiments.

47: "MACROMOLECULAR CROWDING INCREASES EXTRACELLULAR MATRIX DEPOSITION BY MODULATING IN VITRO COLLAGEN BIOPROCESSING."

***Laura Trujillo Cubillo, Dimitrios Zeugolis**

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***Lead Presenter: laura.trujillocubillo@ucdconnect.ie (Postgrad Researcher (>two years))**

Macromolecular crowding (MMC) is a biophysical phenomenon that significantly influences molecular diffusion and enhances protein-protein binding and enzymatic activities. The use of MMC agents in vitro has been shown to increase extracellular matrix (ECM) deposition, with carrageenan (CR) proving to be the most effective. However, the different CR subtypes and their impact on collagen bioprocessing and turnover mechanisms remain unexplored.

We assessed different CR molecules at varying concentrations on human dermal fibroblasts (25000 cells/cm²). The effect of the optimal MMC agent on collagen bioprocessing was evaluated by quantifying procollagen C-proteinase (PCP) and procollagen-I C-propeptide (PICP) in cell sheets and culture media. The degree of collagen crosslinking was evaluated via lysyl oxidase (LOX) activity and ECM turnover was analyzed through matrix metalloproteinases (MMPs) expression. Cell-cell and cell-ECM interactions were observed though integrin-beta and connexin 43.

Supplementation with λ-CR (50 µg/ml) resulted in a 4.8-fold increase in collagen I deposition by day 8. MMC significantly increased PICP levels in cell sheets and culture media, while PCP levels on the cell culture media were significantly higher for -MMC, indicating that MMC increases ECM deposition by promoting collagen processing in vitro. LOX levels were significantly higher in +MMC cell sheets, accounting for collagen crosslinking and maturation. MMPs quantification showed increased ECM remodelling under MMC. Finally, MMC led to increased expression of integrin beta responsible for increased cell-ECM interaction. This further demonstrates the mechanisms behind MMC and its potential to efficiently recreate biological processes in vitro, resulting in more biologically relevant environments.

48: "REFINING THE METHODOLOGY FOR CHARACTERIZING AND ISOLATING CYTOKINE-SECRETING HUMAN REGULATORY T CELLS (TREG) DURING EX VIVO EXPANSION."

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***Lead Presenter: q.ou1@universityofgalway.ie (Postgrad Researcher (>two years))**

Aim: Current clinical trials use polyclonally expanded regulatory T cells (Tregs) with potential for diverse cytokine profiles, either anti- or pro-inflammatory. This study investigated three key cytokines (IL-10, IL-17, and IFN-γ) in Tregs during ex vivo expansion and explored the potential for depleting IFN-γ-secreting Tregs.

Methods: Peripheral blood-derived mononuclear cells from healthy volunteers were isolated, and FACS-purified Tregs (CD4+CD25+CD127-) or their subpopulations (CD45RA+, CD45RA-CD39-, CD45RA-CD39+) were obtained and expanded with anti-CD3/CD28 Dynabeads, IL-2, and rapamycin. Expanded Tregs were then stimulated with PMA/ionomycin, analyzed by 12-color flow cytometry panel, or enriched by IFN-γ Secretion Assay and magnetic columns.

Results: Cytokine-secreting cells (IFN-gamma, IL-10, and IL-17) compromised distinct populations within expanded Treg. They were predominantly Helios- cells within CD45RA-CD39- and CD45RA-CD39+ cells, rather than within CD45RA+ cells, either before or after ex vivo expansion. PMA/ionomycin stimulation and further magnetic enrichment allowed good purity of IFN-y-depleted Treg by using LD column (less than 1% IFN-y+ cells) and good purity of IFN-y-secreting by LS column (>70%). However, exposure to PMA/ionomycin induced instability of Foxp3 expression. In expanded CD45RA-CD39- Tregs, five-day Dynabead stimulation resulted in low but still detectable IFN-gamma-secreting cells (1%) which could be magnetically depleted. IFN-γ-secreting characteristics persisted during further expansion after enrichment. Suppression potency to CD4+ and CD8+ T cells was similar between IFN-y-enriched and IFN-y-depleted Treg.

Conclusion: Polyclonally-expanded human Treg subpopulations have exhibit heterogenous cytokine secretion patterns. Enriching or depleting IFN-γ-secreting Tregs through magnetic separation is technically feasible but its benefits for potency of expanded Treg require further study.

49: "A NOVEL MODEL FOR VASCULAR DISEASES: IPSC-DERIVED DIFFERENTIATION INTO VASCULAR ENDOTHELIAL CELLS."

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Endothelial cells form a monolayer lining the inner surface of blood vessels, where they play pivotal roles in regulating vascular permeability, inflammatory responses, and angiogenesis. Dysfunction of endothelial cells is a central pathological mechanism underlying various vascular diseases, including diabetic vascular complications. Induced pluripotent stem cells (iPSCs), which are pluripotent and self-renewing cells derived from reprogrammed adult somatic cells, offer significant potential for differentiation into multiple cell types, including endothelial cells. In this study, we developed an efficient protocol for differentiating iPSCs into endothelial cells using small molecule compounds. Initially, iPSCs were directed to differentiate into mesodermal cells using CHIR-99021. Subsequently, a combination of small molecule compounds, including BMP4, VEGF and bFGF, was applied to promote further differentiation into endothelial cells. The differentiated cells were confirmed to express key endothelial markers, such as CD31, VE-cadherin, vWF, and eNOS, and demonstrated mature endothelial cell functions in vitro, including tubule formation and cell adhesion. This optimized protocol establishes a robust and scalable platform for applications in drug screening, toxicity assessment, cell transplantation, and the investigation of vascular disease pathogenesis.

50: "PRODUCTION OF ADENO-ASSOCIATED VIRUS CONTAINING PRO-APOPTOTIC TRAIL IS ENHANCED BY SHRNA INTERFERENCE."

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Adeno-associated virus (AAV) is a widely used therapeutic gene vector for treating genetic diseases and delivering cytotoxic genes in cancer therapy. For instance, TNF-related apoptosis-inducing ligand (TRAIL) binds to DR4 and DR5, inducing tumour cell death. However, producing AAV with cytotoxic genes poses challenges, as these genes are toxic to producer cells, reducing cell viability and production yield.

This study presents a novel AAV production system addressing this challenge. A viral genome containing a transcriptional fusion of gfp and TRAIL was cloned into the pAAV plasmid. An anti-gfp shRNA cassette was then inserted into the backbone of pAAV-gfp-TRAIL. It was hypothesized that anti-gfp shRNA would suppress gfp-TRAIL mRNA expression, preventing TRAIL production in HEK293 producer cells.

Compared to the control with scrambled shRNA, anti-gfp shRNA significantly improved producer cell viability and virus yield. HEK293 cells transfected with anti-gfp shRNA exhibited markedly reduced GFP expression compared to those transfected with scrambled shRNA. Furthermore, DR5 expression was significantly lower in anti-gfp shRNA-transfected cells, correlating with decreased TRAIL expression.

These results demonstrate that TRAIL expression impairs producer cell viability and AAV yield, while anti-gfp shRNA effectively restores viability and production, albeit not to the wildtype pAAV-gfp level (no TRAIL present). Targeting gfp instead of TRAIL simplifies readout and enables the plasmid's use for generating AAV containing other cytotoxic genes.

51: "SIMULATION OF THE CRYOPRESERVATION PROCESS AND ITS EFFECTS ON CHO-K1 CELLS BY A HYBRID MODELLING APPROACH."

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Cryopreservation is the fundamental method for banking and long-term storage of cell lines in research and industry. Despite its broad application, limited fundamental process understanding has resulted in the development of cryopreservation protocols relying primarily on empirical studies.

This study focuses on the development of a serial hybrid model in MatlabR2022a® for predicting quality attributes (i.e., cell viability measurements by trypan blue and flow cytometry; as well as mitochondrial potential changes) as a function of a number of process parameters, namely cooling rate, freezing volume, DMSO concentration, viable cell density and initial cell viability.

The validation of the hybrid model was performed in two stages upon 1 mL control volume (cryovial's scale). Firstly, the validation of the 1st principle model with sample of CHO-K1 cells at 1×10^7 cells/ml (cooling rate of 1 °C/min) indicated relative prediction residuals below 1.8 %. Subsequently, the partial validation of the hybrid model on DMSO concentration and viable cell density resulted in root mean squared error of approximately 11 %.

The next step of this work includes the data generation for mapping the remaining process variables (i.e., cooling rate and freezing volume) across the design space of interest and their effects on the system's performance, with subsequent training and validation of the hybrid model. The hybrid model will then be applied to optimize the cryopreservation process in order to maximize the viability and mitochondrial potential of the cells post-thaw.

52: "'IMPROVING SUP-TRNA THERAPEUTICS WITH DIRECTED EVOLUTION."

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Nonsense mutations, also known as premature termination codons (PTCs), are responsible for approximately 10-15% of known human genetic disorders. PTCs result in early termination of protein translation and loss of protein function. Currently, there is no approved treatment to help patients suffering from PTC-derived genetic disorders such as forms of Cystic Fibrosis. One strategy is to provide diseased cells with a mechanism to decode PTCs and install the correct amino acid, thereby restoring protein expression. Transfer RNAs (tRNAs) can be engineered to suppress PTCs through rational design of the anticodon thus creating "suppressor" or "sup-tRNAs". Delivery of naive sup-tRNAs produced by in vitro translation can restore some protein expression in cell lines and mouse models, however, the response is not robust or long-lasting. Directed evolution is a laboratory method that mimics biomolecular evolution by natural selection. Here we are applying directed evolution methods to engineered sup-tRNAs. Our immediate goal is to create a high-throughput quantitative assessment of sup-tRNA PTC suppression efficiency. Through robust control of sup-tRNA expression and activity, we hope to evolve sup-tRNAs for enhanced PTC decoding, using PTC mutations in the Cystic Fibrosis transmembrane conductance regulator (CFTR): Y122X, G542X, R112X, and W1282X as a test case. We hope by overcoming some of the unique challenges sup-tRNA therapeutics pose due to their complex structure and diverse modifications, we can bring this class of therapy a step closer to the clinic.

53: "CHAMBER-SPECIFIC PHOTONIC PROFILING OF HUMAN IPSCS-DERIVED CARDIOMYOCYTES FOR UNDERSTANDING PHENOTYPIC DEMANDS."

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Background: Human induced pluripotent stem cells (hiPSCs) can be exploited to develop patient-specific cell lines and isogenic disease models, but the differentiation process requires a careful analysis of the quality of the obtained cells and an evaluation of their metabolic profile. Two-photon fluorescence lifetime imaging microscopy (2P-FLIM) is an emerging alternative to more commonly used invasive techniques, as it allows profiling the metabolic fingerprints of cells through the autofluorescence lifetime of NAD(P)H and FAD coenzymes.

This work is focussed on the metabolic evaluation of chamber-specific hiPSC-derived cardiomyocytes (iCMs) with 2P-FLIM, highlighting metabolic patterns during and after iCMs differentiation.

A modified differentiation protocol allowed to obtain chamber-specific cardiomyocytes from hiPSCs that were further purified with glucose-deprived media supplemented with lactate: faster spontaneous beating resulted in atrial iCMs and after lactate selection. To confirm that atrial and ventricular iCMs were obtained, the presence of specific genes and markers were investigated wit RT-PCR and Flow Cytometry analysis.

Cardiomyocytes contractility frequency was evaluated performing calcium-transients imaging, and imaging live cells in a heated chamber. Calcium transients were analyzed with an in-house built MATLAB program, allowing to further investigate chamber-specific characteristics of the obtained cardiomyocytes. 2P-FLIM was performed at each step of the 8-days differentiation and during the purification with lactate-supplemented media and it showed a significant increase in NADH lifetime at the beginning of the differentiation (from 1 ns up tp 2.5 ns) and after lactate selection treatment.


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