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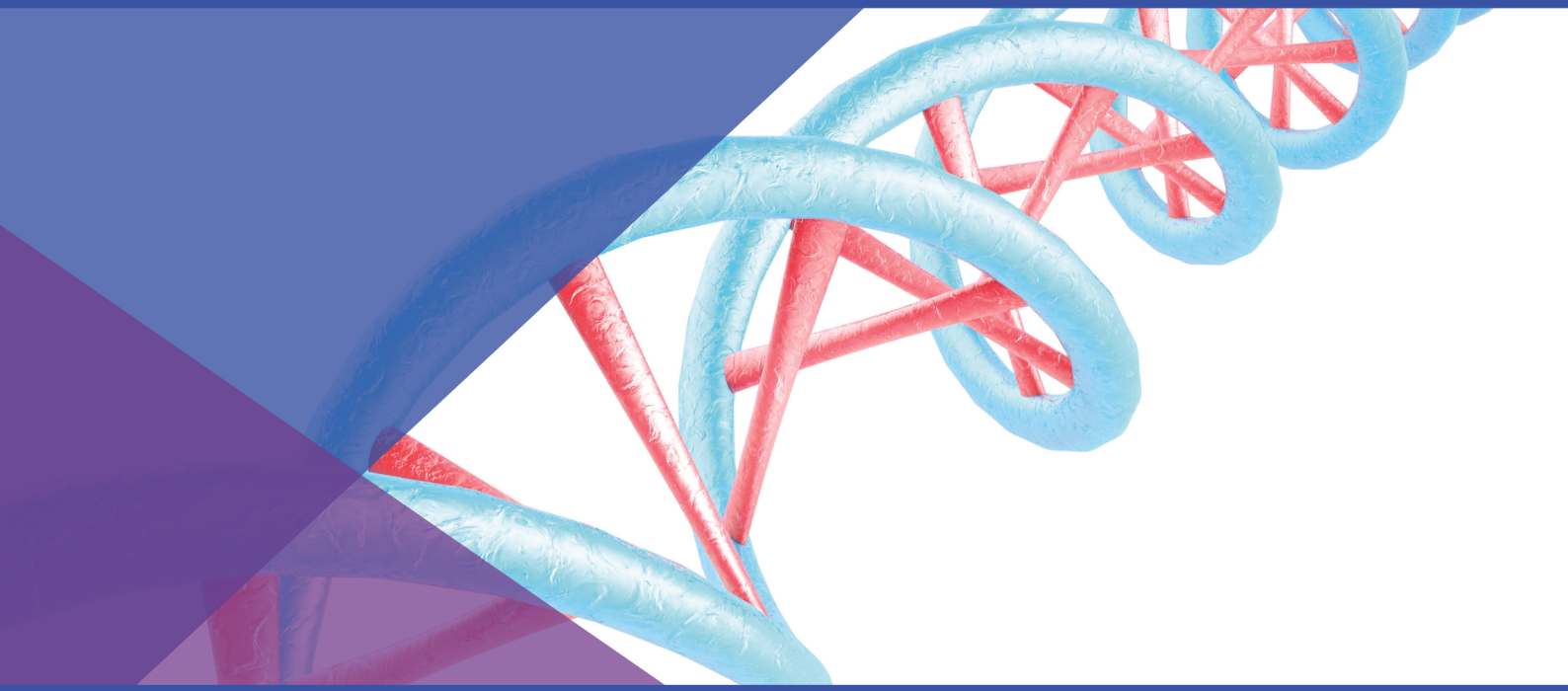
Abstracts



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Invited Speakers

INV04

Intensifying Cell Manufacture to Reduce Costs and Improve Logistics

R Thomas [1]
1: Loughborough University

Cell Therapies require sufficient manufacturing process control to guarantee clinical efficacy and sufficient scalability and economic performance to supply the target population. Despite an increasing move towards automation and engineering informed process design and control, significant improvements are still required.

Red blood cells are an example of an exceedingly high dose cell therapy, and manufacture of these cells represents a correspondingly difficult challenge. The presentation will discuss strategy and progress in establishing an ultra-high efficiency and scalable production process (in excess of 1E8 cells/mL in Litre+ stirred tank reactors) for manufactured red cells with broader applicability for development of other cell therapy manufacturing processes. Further, we will discuss the development and application of kinetic modelling tools that are required to support process development and optimisation of complex cell cultures.

INV06

Making platelets in vitro: challenges and opportunities

C Ghevaert [1]
1: University of Cambridge

The production of platelets in vitro from pluripotent stem cells (PSCs) offers the opportunity to address several issues relevant to delivery of platelets to clinical transfusion:

1. Efficiency of production and purity of the product. We show how inducing the expression of key transcription factors can lead to efficient and high purity of the platelet mother cell, the megakaryocyte (MK).
2. Ad hoc supply on demand (including in peripheral centres) using frozen MK banks as an I termediary product and bespoke engineering solution to release platelets from MKs near to the point of care.
3. Genome editing to provide universal platelets that do not express HLA Class I
4. Genome editing to produce platelets with added clinical benefit (supercharged with clotting factors and/or growth factors for tissue repair).

The challenge remain the up scaling and keeping the cost of goods with reasonable limits, the transfer to clinical production and the pre-clinical assessment in suitable animal models. To this end we are adopting novel culture approaches for the MK production informed by a process

analysis. In addition we are also promoting the release of platelets in vitro by recreating the bone marrow niche in 3D systems and promoting the quality of the end product through engineered adaptation for the downstream processing of the final product

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INV07

Developing pluripotent stem cell derived therapies for hearing loss

T Gaskell [1]
1: Rinri Therapeutics

Rinri Therapeutics was established in 2018 by Marcelo Rivolta, a Professor of Sensory Stem Cell Biology, who has dedicated his research to discovering new regenerative therapeutics for hearing loss. With a hope to tackle the unmet medical need faced by millions of people across the world and make a significant impact on both the lives of patients and healthcare systems globally. Drawing from this research, Rinri Therapeutics is now developing the world's first regenerative cell therapies for hearing loss. Rinri's therapy will have the ability to replace the dead or damaged specialised sensory cells of the inner ear that cause sensorineural hearing loss, the most common form of the condition. This presentation will outline the translational questions and steps along the way from concept towards the clinic.

INV08

Virotherapy for superficial bladder cancer: coming of age

H Pandha [1]
1: University of Surrey

Bladder cancer is the 10th most frequently diagnosed cancer worldwide with 5-year survival rate around 70%. The current first-line treatment for intermediate and high non-muscle invasive bladder cancer (NMIBC) is transurethral resection of bladder tumours followed by intravesical Mycobacterium Bovis Bacillus Calmette-Guerin (BCG) immunotherapy. This a treatment that has not changed significantly since the 1970s. Tumour recurrence rate post-BCG is still high ranging from 31% to 78% within five years. New insights into the immune microenvironment in NMIBC as well as the development of novel viral immunotherapies are set to change the standard of care, and open the door to more effective and better tolerated treatments. Oncolytic viruses have been extensively evaluated in what is a ideal clinical model of cancer gene therapy. Both non-replicating and replicating agents are beginning, finally, to make a significant impact on patient outcomes in the phase 3 clinical trial setting.

Oral Presentations

FB01

Mapping the pluripotent “breakome”: Insights into the origins of genetic variants in pluripotent stem cells.

O Laing [1] I Barbaric [1]
1: University of Sheffield

Genome Instability drives oncogenic transformation in cancer, and the same phenomenon occurs in human pluripotent stem cells.

Human Pluripotent Stem Cells (hPSC) have limitless *in-vitro* proliferative capacity and can differentiate into any somatic cell type. These features make them ideal sources of cellular material for regenerative medicine applications. However, over prolonged *in-vitro* culture, hPSC are prone to acquiring recurrent genetic changes, ranging from SNPs to gross karyotypic abnormalities. Many of the changes observed in hPSC are common to cancers, raising significant concern over the safety of hPSC-derived products in therapy.

DNA damage, followed by unfaithful repair, precludes many of the genetic changes observed in hPSC. Pluripotent cells harbour higher constitutive levels of DNA damage than their differentiated counterparts, however, the causes of this damage remain poorly characterised.

We have used INDUCE-seq to map genome-wide endogenous DNA double-strand breaks in pluripotent and differentiated cells. From these data, we have discerned differences in the distribution of DNA breaks between isogenic pluripotent and differentiated cell types and have identified pluripotent-specific DNA damage hotspots, several of which fall within regions of recurrent genetic change in hPSC. By Integrating published sequencing datasets, and contextualising damage hotspots, we identify DNA replication stress as a putative cause of DNA damage and ultimately recurrent chromosomal translocations on chromosome 1q. We hope, ultimately, this work will inform modified culture conditions to minimise the occurrence of such variants in hPSC cultures.

FB02

Purification and application of extracellular vesicles associated AAV vectors (EV-AAVs)

I Colic [1] G Massaro [1] A F Geard [1] J A Watts [2] A Benedikt [3] G R Williams [1] A A Rahim [1]
1: UCL 2: The University of Nottingham 3: Evonik Operations GmbH

The biggest hindrance to clinical application of AAVs is the host immune response that generates neutralising antibodies (NAb) following AAV administration. During vector production, a portion of AAV particles released into the cell culture media is associated with extracellular vesicles (EVs). We hypothesise that these EV-AAVs can provide complete or partial protection from NAbs, allowing for repeated administration and treatment of seropositive patients. However, due to the

similar physical properties of EVs and AAVs (i.e size and density) separating EV-AAVs from free AAV particles present in the cell culture media is quite challenging. Five different purification protocols were assessed: differential ultracentrifugation (UC), size exclusion chromatography (SEC), differential gradient centrifugation (DGC), and two combined protocols using two isolation methods, combined protocol 1 and 2 (CP1 and CP2). The presence of EVs and AAV particles was confirmed in all EV-AAV samples using different characterisation methods, however, cryo TEM imaging showed that the level of contamination of EV-AAV samples with free AAV particles was the highest in UC EV-AAV samples (most often used for isolation of EV-AAVs) and the lowest in the CP2 EV-AAV samples. Tested in vitro, CP2 EV-AAV samples showed higher transduction efficiency than AAV or UC EV-AAV when tested in the presence of NAb. Tested in vivo in naive mice (no NAb present in plasma) iv injected with 3*10^11 genome copies per animal, CP2 EV-AAV samples displayed lower transduction efficiency when compared to AAV and UC EV-AAV, but they also displayed lower immunogenicity determined by cell-based NAb assay.

FB03

Improving migration, tumour accumulation, and persistence of Tumour-Infiltrating Lymphocytes (TILs) in ovarian cancer

C Guerra [1 2] M Kalaitsidou [2] G Kueberuwa [2] R Edmondson [1] R Hawkins [2]
1: University of Manchester 2: InstilBio UK

Background: The deprived endogenous co-stimulation and hindered T-cell trafficking into the tumours constitute the principal hurdles for ovarian cancer immunotherapy. This project aims to evaluate the effect of TIL modification with the synthetic co-stimulatory antigen receptor (CoStAR) or chemokine receptors on migration towards ovarian tumours using in *vitro* and *in vivo* models.

Methods: Healthy donor (HD) T cells modified with CoStAR and CXCR2 were produced, and cytokine secretion, interleukin-2 (IL-2) and interferon-γ (IFN- γ), killing and migration capacity were measured. The expression of exhaustion and differentiation markers, and chemokine receptors in T cells, TALs (tumour-associated lymphocytes) and TILs was measured by flow cytometry.

Results: The highest chemokine receptor in HD cells was CXCR4 for both CD4 (41.6 ±2.3 %) and CD8 T-cell populations (57.3 ±16.5 %), whereas in TALs CCR5 (53.7 ±28.2 %) and CXCR4 (51.7 ±27.47 %) in the CD4 subset and CCR5 (80.5 ±36.7 %) and CXCR3 (58.6 ±28.3 %) in the CD8 T-cell population were the highest.

CoStAR demonstrated a robust functional activity after being co-cultured with OVCAR3.OKT3 cells in comparison to MOCK T cells and all cells maintain high killing capacity towards OVCAR3.OKT3 cells.

CXCR2 transduced T cells showed superior migration towards IL-8 in comparison to MOCK T cells in vitro.

Conclusion: The superior cytokine production of the CoStAR transduced T cells was demonstrated across donors. Flow panels for phenotypic characterisation of TILs were effectively developed and migration assays optimised. Data generated for CoStAR+, CXCR2+, and CoStAR+CXCR2+ modified HD cells will be compared with upcoming data from modified TILs.

FB04

Investigating the propensity of genetically variant hESCs to undergo targeted differentiation to cardiomyocytes

T J Wing [1] Y Atlasi [2] S Oh [3] I Barbaric [1]
1: University of Sheffield 2: Queen's University Belfast 3: Bioprocessing Technology Institute

Pluripotent stem cells (PSCs) are a promising tool both for regenerative medicine, as well as various research fields, e.g. in vitro disease modelling. In the field of cardiogenesis, hPSCs have been used to generate cardiomyocytes for cell replacement therapy and studying various heart-related disorders. However, a major concern in the field is the frequent and spontaneous reoccurrence of a common set of large-scale genetic aberrations, e.g. gains of chromosomes 1, 12, 17, 20 and X. Such genetic changes confer PSCs with an advantage over genetically normal PSCs. These cells are known as genetically variant (GV) PSCs. For regenerative medicine particular concerns surrounding GV PSCs arise in the potential of, implanting mutated cells which could lead to the formation of cancers in patients, impacting the ability of PSCs to efficiently differentiate, as well as altering the functionality of differentiated cell types. Here we investigated the ability of WT hPSCs and variant 1q GV counterparts to differentiate to cardiomyocytes. We showed that GV hPSCs harboring 1q gain show a block in the differentiation to cardiomyocytes under the optimized conditions of their WT counterparts. Mechanistically, we demonstrated that this apparent block in differentiation is caused by aberrant Wnt signaling in GV hPSCs. By manipulating Wnt, we were able to rescue the differentiation of GV hPSCs, but the resulting cells were phenotypically and transcriptionally distinct from their wild-type counterparts. Together, our study demonstrates that genetic changes acquired in the pluripotent state affect differentiation ability of hPSCs and the phenotype of their differentiated derivatives.

OR01

mRNA therapy restores ureagenesis and corrects glutathione metabolism dysfunction in argininosuccinic aciduria

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Argininosuccinic lyase (ASL) is a urea cycle enzyme enabling the clearance of neurotoxic ammonia. Inherited ASL deficiency results in argininosuccinic aciduria (ASA), where patients present with hyperammonaemia, chronic liver disease and neurocognitive impairment. Lack of curative therapy

and limited efficacy achieved with current standard of care asserts need for novel therapy. mRNA encapsulated in lipid nanoparticles (LNPs) has shown promise in liver inherited metabolic diseases.

We investigate the efficacy of *hASL* mRNA containing LNPs (ASL-LNP) in ASL-deficient $Asl^{Neo/Neo}$ mouse model and the involvement of glutathione dysfunction in the ASA liver pathophysiology.

Pharmacokinetics at dose of 1mg/kg following single intravenous (IV) injection of ASL-LNP in $Asl^{Neo/Neo}$ mice showed lasting efficacy for up to 7 days. Weekly IV administration from birth normalised survival, growth, fur pattern, ammonaemia, and several biomarkers including *in vivo* ureagenesis with stable isotopes. Transcriptomics analysis demonstrated the correction of liver metabolic dysfunction with a reduction from 2,600 to 7 significantly differentially expressed genes between untreated versus ASL-LNP treated mutants compared to WT littermates. Weekly IV administration of ASL-LNPs in juvenile $Asl^{Neo/Neo}$ mice significantly increased survival, growth and liver ASL activity and normalised the disease biomarkers and ureagenesis.

Reduced glutathione biosynthesis is hallmark of the chronic liver disease in ASA. *In vivo* glutathione metabolism assessed with positron emission tomography (PET) imaging using (*S*)-4-(3-^{18}F-fluoropropyl)-L-glutamate ([^{18}F]FSPG) radiotracer showed a significant improvement in ASL-LNP treated versus untreated $Asl^{Neo/Neo}$ compared to WT littermates.

This proof of concept of mRNA-LNP therapy in ASA paves the way for clinical translation.

OR02

PKC agonists as small molecule inducing agents for enhancing lentiviral vector production

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1: Oxford Biomedica (UK) Ltd.

Achieving high upstream lentivector (LV) titres is fundamental to the development and manufacture of a commercially viable gene therapy product. Product titres can vary considerably with different therapeutic transgene sequences despite being produced in the same mammalian cell system. Implementing new technologies able to recover low titres or improve high titres further is invaluable.

Induction is a key stage of the upstream LV production process that typically involves increasing the expression of LV genes in production cells with the histone deacetylase inhibitor, sodium butyrate. A series of recent screening experiments revealed to us that titres of LV products can be further increased by using an additional class of molecules known as PKC agonists alongside the induction step.

Here, we describe how, through optimisation of dosing concentration and timing, LV titres can be enhanced 2- to 9-fold with the non-tumour promoting PKC agonists prostratin and ingenol 3-angelate in a product-specific manner. Importantly, LV produced using PKC agonists have comparable or superior particle-to-infectivity ratios, and residual PKC agonists are removed from the vector product following downstream processing. Furthermore, we demonstrate that PKC

agonists act synergistically with an in-house technology based on LV RNA-targeted U1 snRNA to achieve log-fold-increases in titre without detriment to product quality attributes.

Having established the benefits of introducing PKC agonists to our platform process and in our packaging/producer cell lines, we provide insight into the mechanisms by which these agonists are acting on production cells and now intend to transfer this technology to GMP manufacturing for commercial products.

OR03

High-efficiency end-to-end bioprocessing in a microfluidic bioreactor for cell and gene therapy applications

M Kalli [1] A De Grazia [1] N Naeem [1] T Phillips-Hall [1] S Loganathan [1] D Ann Carter [1] J Kusena [1] C M Cejas [1]
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High-throughput cell culture processes destined for cell and gene therapy manufacturing require precise control of perfusion, harvesting efficiencies, growth rates, and other manipulation of biological cells. An essential key to this is streamlining *in situ* the bioprocesses involved such as seeding, transduction, expansion, perfusion, and harvest in a closed system to eliminate invasive interventions that could compromise cell viability and sterility. This can be achieved by miniaturisation of bioreactor dimensions to gain better control of the cell microenvironment in terms of media exchange and gas exchange. MicrofluidX presents a microfluidics-based bioreactor uniquely designed and focused on improving efficiency and reproducibility of cell bioprocessing, by providing a closed-system platform that can afford extreme process control, robust end-to-end process engineering and rapid process optimisation and scale-up.

In a series of exemplar processes MicrofluidX shows the end-to-end bioprocessing capabilities of its bioreactor in relatively small volumes (0.5-12mL). The unique features and functionality generates a miniaturised environment that results in improved efficiencies for seeding, expansion, transduction, and harvesting when compared to conventional cell culture devices. Moreover, MicrofluidX shows a unique scale-up strategy based on multiplexing and parallelisation of its bioreactor, which takes advantage of the miniaturised environment of the cells while increasing cell throughput. This is achieved using a unique combination of bespoke micro-engineering and understanding of small-scale cell phenomena. With these results, MicrofluidX will bring the level of control achievable at the micro-scale to large-scale GMP production.

OR04

Quantification of selective pressures experienced by human pluripotent stem cells *in vitro*

V Pisupati [1 2] I Mali [1 3] S Srinivasaraghavan [1 3] S A Mahmoud [3] M Song [1 3] D Chiarugi [3] F Merkle [1 3] R Barker [1 2]
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Maintaining the genetic stability of human pluripotent stem cells (hPSCs) is important for disease modelling and in regenerative medicine. However, hPSCs are shown to acquire non-random chromosomal abnormalities during their growth in culture, conferring the cells with a growth advantage. To identify and quantify the selective pressures experienced by hPSCs *in vitro*, we co-cultured hPSC lines in pools, in different culture conditions, with samples collected at each passage. Cell lines co-cultured in a pool grow at different rates, resulting in pool imbalance (skew). The proportion of each cell line in the pool can be identified utilising the unique genetic variation within each cell line, obtained from its whole genome sequencing (WGS), whole exome sequencing (WES), database of genotypes and phenotypes (dbGaP) information. We developed computational tools to estimate the unique variants reflecting the abundance of a cell line in the pool. By measuring the rate of skew in stem cell pools, we can screen for conditions that reduce the skew and are associated with reduced selective pressures and fewer cancer-associated mutations. A comprehensive set of culture conditions that include different hPSC culture media, substrates, dissociation methods and supplements were tested to identify the components and their impact on the genomic stability of hPSCs *in vitro*.

OR05

Investigating the causes and consequences of mitotic errors in human pluripotent stem cells – implications for cell therapy

G Gelezauskaite [1] S E McClelland [3] M Fellows [2] I Barbaric [1]
1: The University of Sheffield 2: AstraZeneca 3: Barts Cancer Institute QMUL

For human pluripotent stem cell (hPSC)-derived therapy to enter routine clinical use, it must be both efficacious and safe. However, during *in vitro* expansion, hPSCs can acquire recurrent, chromosomal abnormalities, which result in altered cell behaviour, such as increased proliferation and/or reduced propensity for differentiation, akin to cancerous cells. Chromosomally abnormal variants raise concerns surrounding tumorigenicity, and the ability to derive functional cell types for clinical use. As such, it is vital to understand the mechanisms driving variant hPSCs, to eliminate them as a therapy risk.

One likely mechanism of variant hPSC emergence is through errors in mitosis. Recent work by Barbaric and Godek labs have identified an increased mitotic error rate in hPSCs compared to somatic and differentiated cells. This suggests that the pluripotent state is associated with an impaired spindle assembly checkpoint and/or altered propensity for aneuploidy, which may be

critical for variant formation. Nevertheless, the mechanisms and consequences of mitotic errors in hPSCs have not been fully explored. In this project, we have adopted chemical and genetic (CRISPR/dCas9) manipulation to experimentally induce chromosome mis-segregation in pluripotent and isogenic differentiated cells, to study how cells respond to mitotic errors and understand how aneuploid variants arise in culture. We utilise high-content microscopy to track mis-segregating cell fate, and have successfully implemented the aforementioned techniques to derive novel aneuploid hPSC lines, to study the safety implications of chromosome-specific aneuploidies. Finally, we assess the potential bias of chromosome mis-segregation in PSCs, to further elucidate the importance of chromosome identity upon cell division.

OR06

Circumventing anti-vector immunity towards adenoviral vectored vaccines.

C M Bliss [1] J A Davies [1] M Marušková [1] L M Badder [1] M Bermingham [2] A T Baker [1] L Stack [1] M A Oliver [2] A L Parker [1]
1: Cardiff University 2: InBio

Replication deficient (RD) adenoviruses (Ad) are the most widely administered viral vectors, with licensed SARS-CoV-2 vaccines using vectors derived from human Ad type 5 (Ad5) and 26 (Ad26), and chimpanzee Ad “ChAdOx1”. Ad vectored vaccines generate robust cellular and humoral immunity, against both the transgene-encoded protein and the Ad vector itself. It’s unclear how many times a single Ad vector can be re-administered before this anti-vector immunity impairs generation of the desired transgene-specific adaptive responses. Anti-vector immunity also arises from naturally acquired Ad infections. In the absence of anti-Ad5 immunity, Ad5 is a gold-standard vector with robust vaccine immunogenicity, however widespread Ad5 seroprevalence hampers its use for the global population.

We developed novel pseudotyped Ads as RD vectored vaccines encoding SARS-CoV-2 spike protein. These vectors exhibit fiber knob swaps from low seroprevalence Ads grafted onto an Ad5 backbone. We characterised innate immune responses following intramuscular administration in mice, in addition to spike-specific adaptive responses three weeks later. We also quantified the effects of anti-vector humoral immunity against these vectors in an *in vitro* transduction assay with serum co-culture. The pseudotyped vectors exhibit many desirable vaccine characteristics as the equivalent Ad5 vector, including $CD4^+$ and $CD8^+$ T cell responses against multiple spike epitopes. Importantly, Ad fiber knob pseudotyping can substantially circumvent the direct anti-vector, humoral immunity induced through natural Ad exposure and Ad vaccination. These data indicate the adenovirus fiber knob plays a substantial role in anti-vector immunity, and can be manipulated for evasion of such responses without hampering vaccine immunogenicity.

OR07

A CD28/CD40-based chimeric costimulatory antigen receptor (CoStAR™) targeting folate receptor alpha enhances anti-tumour activity of tumour infiltrating lymphocytes.

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1: Instil Bio

Tumour infiltrating lymphocyte (TIL) therapy for treatment of refractory metastatic melanoma, has shown remarkable clinical efficacy in a number of clinical trials. However, extending the clinical benefit to patients with other cancers has posed a challenge. T cell anergy and exhaustion can be mediated by insufficient costimulation in the tumour microenvironment, in turn leading to loss of anti-tumour activity. Here, we describe the construction and functional testing of a chimeric costimulatory antigen receptor (CoStAR) which synergises with TCR signals in T cell and TILs upon antigen engagement. CoStAR consists of a tumour associated antigen specific single chain antibody fragment (scFv) fused to the signalling domains of CD28 and CD40. Transfer of a FRα specific CoStAR to T cells augments T cell activity in a manner that is strictly dependent on the provision of TCR-mediated signal 1. CoStAR also enhanced proliferation, even in the absence of exogenous IL-2. Using an *in vivo* tumour model, CoStAR improved T cell survival, enhanced control of tumour growth, and improved host survival. TIL from multiple cancer indications could be efficiently and reproducibly engineered with CoStAR, resulting in augmented activity in response to target antigen expressing cell lines and autologous tumour digest. CoStAR thus represents a novel approach to enhancing TIL activity via synthetic costimulation, and in turn increasing anti-tumour activity.

Poster Presentations

P01

Cell therapy using human pluripotent stem cells: which culture-acquired genetic changes should you monitor for?

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Genetic integrity of human pluripotent stem cells (hPSCs) is essential for their sustained use in regenerative medicine, and as a tool in modelling development and disease. Though the majority of hPSC lines derived are euploid, over the last several years it has been well established that hPSCs are subject to mutation and can acquire recurrent genetic abnormalities upon culture. The recurrent abnormalities observed commonly present as either copy number variants or gains of whole or regions of chromosomes; 1, 12, 17, 20 and X.

Despite being widely documented, changes in the frequency of recurrent chromosomal abnormalities over time and their correlation to culture conditions have not yet been investigated. Utilizing an annotated dataset of almost 20,000 karyotypes, containing cytogenetic analysis and information on the corresponding culture conditions, acquired between 2009 - 2022 we show the correlation of karyotypic changes with culture conditions used to grow hPSCs. We further identified a gain of chromosome 1q as one of the most frequent aberrations in hPSCs in feeder-free cultures and characterized the features that provide these cells with selective advantage over their wild-type counterparts. Finally, we carried out functional assays to identify a putative driver gene in the amplified region on chromosome 1q. Overall, our data reveals which recurrent genetic changes represent a current threat to applications of hPSCs in research and cell therapy and establish the molecular basis for the selective advantage of variant cells with a gain of chromosome 1q.

P02

Large-scale optimisation of human pluripotent stem cell culture conditions

V Pisupati [1] S Srinivasaraghavan [1] S A Mahmoud [1] I Mali [1] M Perez-Alcantara [3] R Barker [1] I Barbaric [2] D Chiarugi [4] F T Merkle [1]
1: University of Cambridge 2: The University of Sheffield 3: Wellcome Sanger Institute 4: Max Planck Institute for Human Cognitive and Brain Sciences

During their time in culture, human pluripotent stem cells (hPSCs) recurrently acquire mutations that compromise their utility for disease modelling and regenerative medicine, including in cancer-associated genes that are of particular concern for human cell transplantation therapies. The frequency with which these mutations recur suggest that hPSC culture conditions are sub-optimal, but the wide array of different growth conditions that are used today have complicated efforts to compare them directly and identify those that reduce selective pressures experienced by hPSCs. Here, we report a novel method for directly comparing the effects of dozens of different media, substrates, passaging methods, on the absolute and relative growth rates of hPSCs to indirectly quantify selective pressures. Having identified those conditions that minimise selective pressures, we further tested their ability to suppress the growth advantages of p53 mutant cell lines to suggest a set of more optimal growth conditions. Together, we hope these studies will promote the standardisation, reproducibility, and safety of hPSC-based cell therapies.

P03

Identification of secretome potency markers and the importance of donor selection in umbilical cord – derived mesenchymal stromal cells.

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Mesenchymal Stromal Cells (MSCs) act both in an autocrine and paracrine manner, with the consensus that several factors are required synergistically to mediate immunosuppression. Whilst their use in clinical trials has increased exponentially in recent years, successful translation has been limited due to variable results. This may be explained by donor variability and limited understanding of their mechanism of action. Here we aim to identify differences in the secretome of umbilical cord derived MSCs (UC-MSCs) between donors under *in vitro* stimulation assays and determine if this correlates with their immunosuppressive ability.

UC-MSCs from 12 different donors were stimulated with IL-1β or TNF-α and IFN-γ, culture supernatants collected, and 46 different analytes were quantified using Luminex-based assays. To assess their *in vitro* immunosuppressive potency, MSCs were co-cultured with CellTrace Violet –

stained PBMCs and suppression of CD3+ cell proliferation was measured with and without MSCs. We show that the MSC secretome changes considerably between the stimulation conditions, for example with IL-1β stimulation inducing analytes such as MMP3 ($p=0.0017$) and TIMP1 ($p=0.0452$), whilst TNF-α/IFN-γ includes IL-10 ($p=0.0003$) and MCP2 ($p<0.0001$). Analyte concentrations also varied between donor MSCs in resting, stimulated and co-culture conditions. Through multivariate analysis tools such as hierarchal clustering, principal component, and correlation analysis, we identify analytes which are significantly altered during *in vitro* immunosuppression assays, which also correlate with the variable suppression levels of MSCs. We propose several analytes including IL-6, HGF, and MCP1 to be markers of immunosuppressive potency, and be used to screen donor MSCs for clinical translation.

P04

Engineering desialylation- and CD42 cleavage-resistant platelets to optimise storage and maintain lifespan and function following transfusion.

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1: University of Cambridge 2: Emory University

Transfusion of platelets to either prevent or treat bleeding and maintain haemostasis, has been an effective therapy especially in patients undergoing chemotherapy or major surgery. Whilst an established therapy, platelet transfusion does present several difficulties, for example potential bacterial contamination due to the need to store platelet units at room temperature to prevent cold storage-induced lesions that reduce function and survival in circulation. Here, we propose to use CRISPR Cas9 editing of induced pluripotent stem cells (iPSCs), to (a) knock-out the metalloproteinase ADAM17 and two sialidases present in megakaryocytes (MKs) and (b) alter residues in CD42 where ADAM17 cleaves it. Megakaryocytes will be produced from iPSCs by a forward programming method that relies on the expression of key transcription factors under the control of an inducible cassette. The genome editing of the trigger region and mechanosensory domain of GP1BA will be performed to remove the cleavage site for ADAM17 whilst retaining the resting configuration of the mechanosensory domain and its ability to unfold when bound to vWf at high shear. We hypothesise that the platelet progeny from the engineered iPSCs would allow them to be stored at lower temperatures and for longer periods, thus reducing wastage and the risk of bacterial contamination. Additionally, by knocking out the two sialidases (*neu1* and *neu3*) we will produce platelets that maintain surface sialylation in circulation thereby prolonging their survival and haemostatic function. Finally we will perfuse labelled platelets produced in vitro in an ex vivo human spleen model to measure their survival.

P05

Development of αvβ6 integrin specific “Precision Immunovirotherapies” expressing bispecific immune cell activators

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We previously described the construction of a highly engineered, tumour-selective virotherapy, $Ad5_{NULL}$-A20, devoid of all native tropisms, but able to infect via αvβ6 integrin expressed in multiple aggressively transformed epithelial cancers. To enhance the activation of tumor infiltrating T-cells and NK cells at tumor sites we engineered the $Ad5_{NULL}$-A20 platform to express bi-specific molecules targeting either CD3 or CD16, and the tumour-antigen Epidermal growth factor receptor (EGFR) to encourage immune cell activation and redirect an effective immune response to tumour sites.

Analysis of bispecific transgene function was assessed in co-culture assays with cancer cell lines and healthy donor T-cells demonstrating significant increases in CD4+ and CD8+ T-cell activation, intracellular IFN-γ production and immune cell proliferation in response to cancer lines transduced with $Ad5_{NULL}$-A20 secreting CD3-EGFR bispecific constructs. In addition, NK cell CD107a degranulation was increased in cells secreting CD16-EGFR bi-specifics. All constructs induced immune mediated cancer killing between 1-5 days of co-culture. Furthermore, patient derived pancreatic tumour organoids transduced with oncolytic $Ad5_{NULL}$-A20 expressing CD3-EGFR or CD16-EGFR demonstrated immune mediated cell killing of cancerous cells within 48 hours of co-culture. The bispecific constructs tested demonstrated both their specificity to the target antigen and proficiency to induce activation pathways and immune mediated cell killing *in vitro*. In summary, these data demonstrate the arming of a highly tumour selective agent, $Ad5_{NULL}$-A20 to express bispecific immune cell engagers can immunologically “heat up” the tumour microenvironment. This approach has significant translational potential.

P06

Cytotoxic potential of cord blood derived NK cells correlates with phenotypic variability between donors

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Introduction: Cord blood (CB) derived natural killer (NK) cells are a promising alternative to autologous T cell immunotherapy, which provide strong anti-tumour activity with a low risk for the development of GvHD. This study aimed to characterise the phenotype, cytotoxicity, and proliferative profile of CB NK cells and detect donor characteristics that marked optimal NK cells for downstream use in immunotherapy. Pinpointing such characteristics will enable the selection of highly proliferative and cytotoxic CB NKs for use in adoptive cell therapies, reducing processing costs.

Methods: T cell-depleted CB mononuclear cells (CBMCs; n=21) were cultured in NKMACS medium with 1000 IU/ml IL-2 and 10ng/ml IL-15 for 21 days. Percentage of $CD3^{-}CD56^{+}$ cells was >95% post-expansion. Cells were phenotyped post-isolation and every 7-days subsequently. NK cell cytotoxicity against K562 leukaemia cell line was measured via bioluminescent cytolysis assay.

Results: NK cell quantification throughout expansion revealed intrinsic variability between donors that became more apparent over time (13 – 144-fold expansion at day 21). K562 cell specific lysis on days 7, 14 and 21 showed similar inter-cord variability in cytotoxicity (14 – 93%). Phenotypic profiling similarly reflected such variability in the expression of activation receptors CD16 and NKp44 throughout expansion, which were found to correlate cell specific lysis, in addition to other donor characteristics ($p<0.05$).

Conclusion: Characterisation of CB derived NK cells pre- and post- expansion has demonstrated considerable variability between donors in terms of expansion potential and cytotoxicity. Results indicate that differential marker expression correlates with the observed variation in NK cell cytotoxicity.

P07

Brain-directed AAV gene therapy corrects lethal neurodegeneration and improves locomotor behaviour in a mouse model of CLN5 Batten disease

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The neuronal ceroid lipofuscinoses (NCLs), commonly known as Batten disease, are a group of inherited lethal neurodegenerative lysosomal storage disorders. CLN5 disease is caused by mutations in the *CLN5* gene encoding a soluble lysosomal lumen protein with cysteine-based *S*-depalmitoylase activity. Children with CLN5 disease suffer progressive motor dysfunctions, vision loss, seizures and dementia, eventually leading to premature death. It is currently incurable and there is a desperate need for a novel effective therapy. Here we carried out a preclinical study of an adeno-associated virus (AAV) - mediated gene therapy in a transgenic mouse model of CLN5 disease.

A single dose of AAV9 vectors carrying the human *CLN5* gene driven by either the CAG or the synapsin promoter was administered via intracerebroventricular (ICV) injecton into neonatal Cln5 mice. Treatment efficacy was evaluated by assessment of neurodegeneration and monitoring of locomotor functions and lifespan. In a second trial, single-dose AAV9.hCLN5 was delivered ICV into juvenile early-symptomatic Cln5 mice at 4 weeks of age, to assess treatment efficacy of later stage gene therapy intervention in CLN5 disease.

Neonatal ICV administration of AAV9 expressing human *CLN5* driven by the neuronal specific synapsin promoter significantly prevented neurodegeneration, improved long-term locomotor functions and extended lifespan of the Cln5 mice. The same vector showed enhanced efficacy when delivered at a juvenile early-symptomatic stage, also resulting in long-term disease attenuation, which provides a particularly practical case for clinical translation. These results indicate that brain-directed AAV gene therapy can be a promising treatment strategy for CLN5 disease.

P08

Optimal Design of an AAV Vector is Critical to Evade Toxicity of Gene Therapy in a Mouse Model of Neuronopathic Gaucher Disease.

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Gaucher Disease is an inherited metabolic disorder caused by mutations in the *GBA1* gene. It is the most common lysosomal storage disease and can manifest with severe neurodegeneration and visceral pathology. The most acute neuronopathic form (nGD), for which there are no curative therapeutic options, is characterised by devastating neuropathology and death during infancy. In this study, we investigated the therapeutic benefit of systemically delivered AAV9 vectors expressing the human *GBA1* gene at two different doses comparing a neuronal-selective promoter with the ubiquitous chicken-β-actin promoter, on survival, neurodegeneration, motor function, biochemical markers, and visceral pathology.

High dose gene therapy resulted in extended life span of knock-out mice, normalisation of neuropathological markers and increased enzymatic activity in brain and visceral organs. While supraphysiological expression of glucosylcerebrosidase (GCase) was beneficial in ameliorating the neuropathology, signs of inflammation were present in the viscera of mice treated with the ubiquitous vectors, suggesting that elevated GCase expression may promote inflammation and have deleterious effects in the viscera. We further evaluated the effect of sustained high-levels expression of GCase driven by the CAG promoter in a 1-year long-term study. Our long-term study showed that sustained GCase expression does not fully reverse the brain pathology, as demonstrated by the signs of hyperactivity in the 1-year-old mice treated with CAG.hGBA.

Our results highlight the importance of a careful evaluation of the promoter sequence used in gene delivery vectors, suggesting a neuron-targeted therapy leading to lower GCase expression in the viscera, might be the optimal therapeutic strategy for nGD.

P09

CTx001, a gene therapy for the treatment of geographic atrophy (GA)

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GA, a leading cause of blindness, is strongly associated with genes that regulate the alternative complement pathway (AP). Complement overactivation in the choroidal compartment can be observed before the onset of GA. Poor regulation of the amplification loop results in generation of the potent opsonin C3b leading to inflammation, membrane attack complex (MAC) formation, RPE/photoreceptor cell death and vision loss. Inhibitors of the AP have shown modest efficacy to date with no vision preservation following 2 years of treatment. There is growing appreciation of the need for treatments to address complement overactivation in both retinal and choroidal compartments, separated by Bruch's membrane (BrM).

Complement Therapeutics is developing CTx001, an AAV gene therapy encoding a novel soluble protein (mini-CR1) derived from complement receptor 1 (CR1), for the treatment of GA. We show that mini-CR1 retains potent co-factor activity enabling rapid Factor I (FI) mediated cleavage of C3b with IC_{50} of 125nM. Importantly, we show mini-CR1 supports cleavage of C3b to C3dg in contrast to Factor H (FH) and FHL-1 that cleave only to iC3b at physiological conditions. Like C3b, iC3b is a potent opsonin. Mini-CR1 can traverse human BrM *ex vivo* unlike FI and FH. Mini-CR1 expression in murine eyes following subretinal CTx001 was dose proportionate, and in the laser CNV model, MAC formation was reduced by 69% relative to null vector.

CTx001 is a potent AP regulator with a highly differentiated profile and *in vivo* findings suggest it is well tolerated and pharmacologically active. Continued development is therefore warranted.

P10

A muscle-targeted AAV9 confers long-term overexpression of DOK7 in mice

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Intention becomes action where motor nerves meet muscle: the neuromuscular junction. Disjunction of these synapses underlies numerous diseases including congenital myasthenic syndromes (CMSs) and motor neuron diseases (MNDs), creating an area of therapeutic interest around the molecular pathways that govern neuromuscular junction (NMJ) stability. Several studies have shown that increasing expression of the NMJ stabilising protein Docking protein 7 (DOK7) via AAV9-based therapy can confer short-term benefits including regeneration of NMJs in models of neuromuscular disease, however this has been achieved under the control of the ubiquitous cytomegalovirus promoter.

We demonstrated that an AAV9-based therapy, targeted to skeletal muscle via the triple tandem modified muscle creatine kinase (tMCK) promoter, can instigate long-term, specific elevation of DOK7 protein in skeletal muscle. This resulted in enlargement of the pre-and post-synaptic compartments of the NMJ without affecting innervation, bodyweight, blood biochemistry, or untargeted organs, as examined by histopathology. This demonstrates a method by which gene-therapy targeted to the muscle can impact retrograde transport across the NMJ to help anchor the neuronal terminal to the muscle long-term, without the need to target motor neurons. We conclude that muscle-specific overexpression of DOK7 can be achieved safely, with the capacity to target NMJs *in vivo*.

P11

Versatile, High-Titer AAV and LV Manufacturing System using cGMP Lipid Polymer Nanocomplexes (LPNCs) for Therapeutic Applications

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Nearly 60% of cell and gene therapy programs with ongoing clinical trials seek to treat a variety of more prevalent conditions including musculoskeletal disorders, diabetes, CNS disorders, and cardiovascular diseases. These clinical trials and potential commercialization manufacturing require multiple, efficient viral vector production platforms at large-scale in a variety of cell culture platforms. Transient transfection is a flexible and easy methodology that can address this need while obtaining maximum yields and reducing costs. As a response, Mirus developed an advanced and versatile transfection formulation, *Trans*IT-VirusGEN®, consisting of a mixture of lipid and polymer enabling the formation of lipid polymer nanocomplexes (LPNCs). This novel, biomimetic technology is specifically designed for large-scale virus production to support the biopharmaceutical market's need for highly efficient recombinant AAV and LV titers in both suspension and adherent 293-derived cell types. This innovative system increases viral titers 2-10-fold over current technologies with complementary enhancers and/or complex formation solutions that provide better viral vector yields. Our data supporting the VirusGEN® platform shows i) higher titers, ii) better quality vectors (i.e., better AAV percent full capsids), iii) compatibility with various cell culture adherent and suspension systems, iv) adaptability with a turnkey easy-to-follow workflow for scalability, and v) availability in a different configuration, permitting a seamless transition from process development to GMP. These benefits make the *Trans*IT-VirusGEN® Transfection Reagent ideal for cell and gene therapy developers by consolidating versatility with performance and scalability to support cost-effective manufacturing to produce more doses per run.

P12

Rapid Optimisations for Cell & Gene Therapy Applications

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The process of lentiviral vector production is complex and presents unique challenges. Optimal production is heavily dependent on the concentration and ratios of the plasmid DNA components contributing to the transfection process. Improving titres can determine whether a new gene therapy product is commercially viable. A considerable amount of research is aimed at establishing the best titre and one of the ways to do this is by ensuring optimal transfection ratios that can improve the productivity or recovery of upstream/downstream platform processes, respectively.

This means that a multifactorial design approach is often required to address the biological complexity of the system and achieve optimised vector particle production. We have developed and optimised a cutting-edge digital and physical platform coupled with automated liquid handlers enabling:

1) Automated Design of Experiments (DoE) and multivariate systems optimisation;

2) High-throughput screening, including small molecules, siRNA, shRNA;

3) Machine Learning and Deep Learning Data Analysis;

4) Robustness and Ruggedness Testing to Support Analytical Quality by Design (AQbD) Methods.

Our platform routinely screens and models 5-10 factors. We have proven capacity for modelling 100+ factors with execution of thousands of experimental runs in a single iteration. The new platform, High-throughput Optimisation Services (HtOS), has significantly reduced manual operator time, increased throughput while meeting high demand and with improvements in precision and robustness. We will describe the capabilities and present a Case Study where the new platform was used to successfully optimise the transient transfection plasmid ratios in upstream production of lentiviral vector.

P13

Mass Photometry: A Rapid Characterisation Technique for Adeno-Associated Virus (AAV)

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Adeno-associated viruses (AAV) as viral vectors in gene therapy allow for the targeted delivery of a transgene to elicit a therapeutic effect. However, during manufacture not all AAV particles contain the intended genetic payload. Typically, some empty capsids, bearing no genetic material, are produced. The relative abundance of these empty capsids compared to those bearing the transgene (full capsids) is considered a critical quality attribute with direct impact on treatment efficacy and patient safety.

Mass Photometry is positioned to generate E:F (empty:full) capsid ratios for AAV samples whilst requiring minimal material, time and avoiding the need for expert analysts. The results obtained were comparable to results generated by, the current gold standard Analytical Ultra-Centrifugation (AUC), for E:F capsid ratio generation. Additionally, a range of commercially available purified AAV samples with known E:F capsid ratios were analysed by mass photometry with expected vs. measured E:F capsid ratios plotted. The results showed good agreement and linearity, over the range tested, with an r^2 value >0.96.

Finally, fractions were taken from various steps throughout Pharmaron's AAV downstream purification process and measured for E:F capsid ratio. The data indicated cumulative clearance of empty capsids. Using this methodology, it was possible to identify which fractions contained the lowest E:F capsid ratio with very low sample volume, concentration, and analysis time. Mass photometry can therefore help overcome the key challenge associated with material availability for AAV in-process testing especially when assessing small scale production

runs and therefore compliments Pharmaron's existing world-class AAV manufacturing and analytical capabilities.

P14

F/HN-pseudotyped lentiviral vector-mediated transduction of non-human primates

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Objectives

We have developed a lentiviral vector platform pseudotyped with the Sendai virus F and HN envelope proteins (rSIV.F/HN), including the clinical candidate BI 3720931 for cystic fibrosis (CF) gene therapy. Previously, we demonstrated efficacy in CF-patient bronchial epithelial cell air–liquid interface cultures and intestinal organoids, as well as efficient and persistent *in vivo* transduction of murine airways. Here we assess transduction efficiency and acute toxicology in non-human primates (NHPs).

Methods

Male cynomolgus monkeys received a single dose of rSIV.F/HN vector expressing green fluorescent protein (GFP) (4.2e9 transduction units) or placebo via an endotracheal tube (n=3/group), achieving lung deposition of ~25%. Toxicology was assessed by histopathology, clinical pathology, cytokine levels and changes in body and organ weight; transduction efficiency was quantified by analysing vector-specific mRNA 7 days post dosing.

Results

There were no vector-related clinical observations, mortality, or changes in body or organ weight. Clinical pathology and cytokine analyses were unremarkable. Minimal mixed-cell centriacinar inflammation was observed in 1/3 active-treated animals. Airway epithelial cell transduction efficiency was 9–12% and vector-specific mRNA levels were ~45x endogenous cystic fibrosis transmembrane conductance regulator mRNA levels.

Conclusions

This study extends our findings of rSIV.F/HN-based *in vivo* gene transfer in mice to NHPs, demonstrating transduction efficiency in the range likely to relate to clinical benefit, without toxicity. Animals treated with a higher dose are currently being analysed. These data, together with our previous murine data, support further progression of BI 3720931 towards the clinic.

P15

AAV9-Mediated Gene Therapy In A Knock-In Mouse Model Of Infantile Neuroaxonal Dystrophy

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Introduction: Infantile neuroaxonal dystrophy (INAD) is a rare and lethal paediatric neurodegenerative disease. It is caused by biallelic mutations in the *PLA2G6* gene, which codes for the enzyme calcium-independent phospholipase A2. Patients present with progressive neurological symptoms between six months and three years of age, with mortality typically occurring by 10 years old. No disease modifying treatments are available.

Methods: We conducted an in-depth characterization of the *pla2g6-inad* knock-in mouse model. Following characterization, we investigated the therapeutic potential of an AAV9.h*PLA2G6* vector administered intracerebroventricularly to neonatal and juvenile *pla2g6-inad* mice. We investigated survival, behavioural parameters, and histological analysis to assess therapeutic efficacy.

Results: The average lifespan of the model is reduced to approximately 14 weeks, with weight loss and behavioural decline from 9 weeks old. Neuropathology studies showed neuronal loss and neuroinflammation in the brain and spinal cord, along with autophagic and lysosomal accumulation. A long term-study demonstrated that neonatally administered AAV9.h*PLA2G6* gene therapy resulted in a significant improvement in all parameters measured including survival, weight, locomotor function, and neuronal counts in both the brain and spinal cord. Adult administrations to symptomatic mice have thus far shown increased survival of 21 weeks on average, improved behavioural function and ameliorated neurodegeneration.

Conclusion: This study provides novel insights into INAD disease pathology and cellular dysfunction in the CNS and suggests an AAV9-based therapy has potential to enable effective treatment of INAD. Further clinical translation studies are being undertaken with our industrial partner, Bloomsbury Genetic Therapies Ltd.

P17

Development of TetraVecta™ Lentiviral Vectors: New Technology To Improve Their Production, Quality, Safety, Capacity and Utility.

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As clinical use of Lentiviral vectors (LVVs) expands there is a need to continually improve vector production, quality, safety, capacity and potency. Here, we describe new technologies that improve these properties and underpin a new class of TetraVecta™ LVV genomes.

Our TetraVecta™ LVVs come in two core designs: [1] Rev-dependent '2KO-LVVs' and [2] Rev-independent 'MaxPax' LVVs. Both platforms harbour a modification that inactivates the major splice donor (MSD) site within the packaging sequence. This modification eliminates aberrant splicing therefore simplifies full length vRNA production.

Since MSD-inactivation alone leads to a reduction in LVV out-put titres, we have engineered different solutions to reverse this effect. 2KO-LVVs titres are maximised by the in-production co-expression of a modified U1 snRNA targeted to the LV packaging region. For MaxPax-LVVs, the attenuating effects of the MSD-inactivation are abrogated using a programmed splice event that stabilises the vRNA without contributing to overall vRNA length. Removal of RRE and most of the *gag* sequences allow MaxPax-LVVs to package ~1kb of extra transgene sequence.

Our TetraVecta™ LVVs also employ a 'sequence-upgraded polyA' or supA LTRs, providing improved transcriptional insulation for the integrated LVV in patient cells over current vectors. SupA-LTRs reduce transcriptional read-in to the cassette from adjacent chromatin by up to 50-fold whilst increasing transgene expression.

Finally, these features synergise with the 'Transgene Repression In vector Production' system (TRiP system™), which improves LV production and safety by reducing transgene expression during vector production as well as incorporation of the transgene protein into particles.

P18

Development of a rapid *in vitro* neutralising antibody assay for Adeno Associated Virus Serotypes 3, 5 and 8.

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Pre-existing neutralising antibodies (NABs) to Adeno Associated Virus (AAV) can prevent the success of gene therapy. A variety of assay have been developed to detect NAB in patients considering gene therapy including a mouse based *in vivo* transduction inhibition assay for AAV serotype 8 which requires a high multiplicity of infection (MOI) for successful *in vitro* gene transfer. The *in vivo* assay has several disadvantages, namely, the requirement for mice, cost and the long 4-6 week timeline associated with ordering and acclimatising the mice, as well as the *in vivo* phase. We have therefore developed a rapid *in vitro* assay that enables screening for NABs with a turn-around time of 2 days. Initially CMV NanoLuc and a secreted version packaged into AAV3, 5 and 8 were compared over a range of MOIs from 0.1-1000 on HEK 293T cells and incubation time from 6-24hrs. NanoLuc was selected with an MOI of 1 for AAV3 and 100 for AAV5 and 8 with a 24hr incubation time. We evaluated 38 plasma samples from healthy controls, the median 50% neutralising dilution was 837, 123 and 44 for AAV3, 5 and 8 respectively, demonstrating the high sensitivity of the AAV3 assay. A third of plasma samples were negative for neutralising antibodies, to all three serotypes, only one sample was negative for AAV5, but positive for AAV3 and AAV8. There was also a good correlation with neutralising dilution and total antibody titre measured by ELISA.

P19

CRISPR mediated upregulation of B4galnt2 as a potential treatment for Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a rare X-linked muscular disease affecting about 1:5000 new born males. It is caused by mutations on the dystrophin gene leading to a truncated protein that is degraded. DMD primarily affects skeletal and cardiac muscles. The initial signs start with difficulty in movement and inability to climb stairs which eventually lead to loss of cardiac and respiratory functions. Genetic engineering particularly the bacterial CRISPR-Cas9 system has advanced significantly in the last few years providing a platform for genome editing and regulation of gene expression. This project aims to use one of the CRISPR applications (nuclease deactivated Cas9 or dCas9) to upregulate the expression of B4galnt2. This gene encodes for an enzyme (β-1,4-N-acetyl galactosaminyl transferase-2) responsible for the glycosylation of proteins such as α-dystroglycan, laminin-α2, integrin and others which are a part of dystrophin-glycoprotein complex(DGC) present at the muscle sarcolemma. DGC is responsible for maintaining the structural integrity of muscle fibres which is crucial in DMD. Absence of B4galnt2 has shown to increase inflammation and muscle pathology in case of an injury. We hypothesise that the activation of this gene can, at least partially, compensate for the absence of dystrophin function and increase muscle functionality in vivo. To access the effect of B4galnt2 upregulation we designed RNA guides and used dCas9 in mouse myoblasts. Our data suggests that B4galnt2 can be successfully upregulated with this approach paving the way for more studies in human cells and finally in a mouse model of DMD.

P20

Development of a near-universal genome editing for Duchenne muscular dystrophy.

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Duchenne Muscular Dystrophy (DMD) is a rare recessive X-linked disease affecting 1:5000 newborn boys and is caused by mutations in the dystrophin gene. The disease is characterised by progressive muscle wasting and the substitution of muscle tissue with fat/fibrotic and connective tissue. Individuals affected by DMD generally die in their mid-thirties due to cardiac failure.

Gene editing is a powerful technology that allows scientists to make precise changes to the DNA of living organisms. The most widely used gene editing tool is CRISPR/Cas9, which uses a protein called Cas9 to cut DNA at specific locations, allowing researchers to add, remove, or modify specific genes.

In this project, I am using a gene editing application based on CRISPR/Cas9 to permanently modify the dystrophin gene. In particular, the aim is to introduce a repair template carrying a short version of dystrophin, called microdystrophin, within the 5' end of the dystrophin gene. This

study needs the careful design of the RNA guides to drive Cas (the protein cutting the DNA) in a specific region of the DNA. RNA Guides were designed and screened in cellular models. Mouse/human homology regions were targeted so that any guide found to work well in humans can be tested later in the mouse model of DMD where the functional improvement can be assessed.

P21

Novel engineered plasmids and optimized HEK293 cell line improves AAV productivity

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The need for AAV-based therapies necessitates the development of a robust GMP manufacturing platform that can be scaled and support high titres production during the manufacturing process. Traditionally AAV manufacturing for gene therapy entailed the use of labour-intensive adherent-based HEK293 processes not amenable to scale-up. Suspension adaptation of HEK293 cells has enabled more scalable processes but remains time-consuming. Low AAV productivity and lack of reproducible, commercially viable, platform processes continue to be key challenges facing AAV gene therapy developers. Lonza has responded to these challenges by establishing a robust and scalable suspension manufacturing platform for AAV production.

P22

The importance of an automated qPCR workflow for AAV genomic titre determination in a process development laboratory

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Titrating AAV by qPCR is a widely used technique that offers suitable throughput and working range to support the variety and number of samples generated in a busy process development laboratory. However, assaying hundreds of samples per month requires significant time from multiple trained operators. Despite all operators receiving formal training, variability between runs were high. Furthermore, operator errors occurred frequently which resulted in high repeat rates and delayed decision making. By automating the qPCR workflow using a Tecan Fluent®, we improved repeatability and sample turnaround times, reduced repeat rates and freed up operator time. These substantial benefits make qPCR automation indispensable for effective process development.

P23

Adeno-associated virus production platform performance for2L and 50L scales

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Adeno-Associated viruses (AAV) are targeted delivery methods for gene therapies. One of the challenges when manufacturing AAV is to optimise production for the full viral particle which contains the therapeutic gene. Catapult has developed a full upstream and downstream platform at a 2L and 50L production scale. This has been characterised by internal and external assays to assess AAV2 total particle titre, full AAV2 particle titre and impurity levels. Critical quality attribute (CQA) limits were met at both 2L and 50L scale for the TFF2 pool and external analytics show over 49% full viral particles post TFF2. Catapult's AAV downstream platform shows robustness at 2L and scalability to 50L. Future work will involve the implementation of PAT to ensure product quality, process intensification to further improve yields and assay development using novel techniques for full characterisation of AAV vectors.

P24

Developing a high order multiplex ddPCR assay for single cell vector copy number assessment of transduced T-cells

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Lentiviral vectors (LVs) are widely used for the generation of gene-modified cell therapies with their main applications in immuno-oncology and rare diseases. Yet, the variability of the transduction process may affect the consistency of the manufacturing process causing product heterogeneity. Therefore, controlling the integration of LVs into the host genome is critical to mitigate a risk for insertional mutagenesis. We have previously developed a single-cell vector copy number (VCN) assay which allowed for an in-depth measurement of the heterogeneity of vector transduction, enhancing on a population VCN analysis. However, the workflow currently used is limited by a labour-intensive droplet digital PCR (ddPCR) step, relying on several 3-plex ddPCR reactions to quantify 5 different targets in transduced single cells. In this study, we demonstrate how the ddPCR analytical burden can be streamlined with a high-order multiplexing strategy, whereby 5 targets are measured simultaneously in one single ddPCR reaction using ratio-based mixing of channel1 and channel2 fluorochrome-conjugated probes. This method improvement allows for faster and cheaper single cell VCN analysis, unlocking the potential application of this assay to a larger number of samples or higher number of single cells, enabling greater monitoring of the integration of LVs in cell therapy products. Simplification of analytical assays for the characterisation of complex cell therapy products will facilitate the adoption of novel, enhanced assays in the field, which in turn will help the design and manufacturing of safer, higher quality products.