ABSTRACT BOOK

British Society for Gene & Cell Therapy

BSGCT
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Saïd Business School
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Invited Speakers

KEY2

Harnessing fibroblast heterogeneity for skin regeneration

F M Watt [1]
1: EMBL Heidelberg

It has been known for many years that development, homeostasis and repair of mammalian skin depend on reciprocal signalling between the epidermis and dermis. However, the existence and functional significance of different subpopulations of dermal fibroblasts are only now being appreciated. In my talk I will describe the different types of fibroblasts that are present in fetal and adult skin and discuss ways in which they could be harnessed for tissue repair.

TL01

Lentiviral vector process development and GMP manufacturing

C Knevelman [1]
1: Oxford BioMedica

The number of exciting and high-profile products based on gene and cell therapy has increased significantly over the past few years. Consequently, advanced therapeutics now attract significant interest from the wider biotech/Pharma and investment communities. For over 25 years, Oxford Biomedica (OXB) has been a pioneer in the development of products based on lentiviral vectors, with the company being responsible for several firsts in clinical studies based on these vectors. OXB has used this broad CMC, clinical and regulatory experience and know-how to facilitate development of a scalable, serum-free suspension manufacturing process for the company's CDMO partners.

As the industry matures, technologies have struggled to keep pace with the expansion of therapies from ultra-rare to larger indications. This has driven the need for further innovation in vector production platforms the success of the industry hinges on. OXB has adopted a number of strategies to develop the next generation manufacturing processes yielding higher quantities of vector with suitable product quality attributes and acceptable cost of goods in order to maximise capacity and advance development of a diverse product portfolio in therapeutic areas which currently present significant challenges. These include process intensification through continuous processing, novel methods for vector purification, a transition towards producer cell lines. These innovative technologies provide opportunities to deliver sufficient vector quantities suitable for commercial manufacture for all indications

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TL02

Exploiting Adenovirus Biology for Advancing Adeno-Associated Viral Vector Manufacturing

W Su [1]
1: Oxford Genetics Ltd

Recombinant adeno-associated virus (AAV) vectors hold significant promise for gene therapy; however, challenges persist in achieving efficient and scalable production. To solve this AAV manufacturing challenge, we developed a novel helper adenovirus genetically engineered to deliver AAV genes, while self-inhibiting its own replication cycle to prevent the production of adenoviral structural protein upon infection in HEK293 cells. Here, we strategically inserted tetracycline repressor binding sites into the adenovirus Major Late Promoter and encoded the tetracycline repressor under its transcriptional control to allow for normal adenovirus replication in the presence of doxycycline, but only enhanced genome amplification and early gene expression (important for the 'helper' functions) in its absence.

This platform entitled 'Tetracycline-Enabled Self-Silencing Adenovirus' (TESSA®) enables robust and versatile approaches for producing AAV, offering flexibility through two distinct approaches. The process variation between the two models differs by the approach in which the gene of interest (GOI) is introduced into the HEK293 cells.

A TESSA® vector encoding Rep and Cap can be combined with either a TESSA® vector (TESSA® Duo model) or an AAV (TESSA® Pro model) to deliver the GOI. This flexibility enables the production of cost-effective AAV vectors using TESSA® in a rapid and scalable manner, yielding up to 20-fold more rAAV vectors with productivities exceeding >1E+6 vector genome copies (GC) per cell.

Importantly, AAV manufacture using the TESSA® platforms can be scaled up efficiently and economically. In 50L and 200L bioreactors, the TESSA® Duo produces yields exceeding 7E+11 and 3E+11 GC/mL of AAV6 and AAV2, respectively.

TL03

Innovation Hubs for Gene Therapies initiative in the UK

P Walser [1]
1: NHS Blood and Transplant

The Innovation Hubs for Gene Therapies (IHfGT) represent an initiative in aid of developing, innovating and advancing gene therapies in the UK through the GMP-production of viral vectors. It encompasses three centres: Kings College London, NHS Blood and Transplant's Clinical Biotechnology Centre near Bristol and the University of Sheffield's Gene Therapy Innovation and Manufacturing Centre. The Medical Research Council (MRC), the Biotechnology and Biological Sciences Research Council (BBSRC) and LifeArc created the network of hubs to innovate and, importantly, progress to clinical trial the manufacturing of viral vectors by building on existing and

new capabilities in a collaborative environment. An overview of the capabilities of the network will be presented along with highlighting of opportunities in manufacturing and funding.

INV03

Antisense approaches for the treatment of Duchenne muscular dystrophy and recent advances to address DMD brain comorbidities

A Goyenvalle [1]
1: University of Versailles saint Quentin en Yvelines

This presentation will cover the development of exon-skipping approaches for the treatment of Duchenne muscular dystrophy (DMD) and the recent possibilities to address DMD brain comorbidities. In addition to the progressive muscle weakness and degeneration which are characteristic of DMD, 50% of affected individuals have debilitating central nervous system (CNS) comorbidities, including intellectual disability, neurodevelopmental problems encompassing autism, Attention Deficit Hyperactivity Disorder and Obsessive-Compulsive Disorder. Several antisense tools including antisense oligonucleotides (ASO) and vectorized strategies (AAV-U7) have shown their therapeutic potential for DMD. Can these exon-skipping tools also address the CNS deficits associated with the lack of dystrophin in the brain?

INV05

Culture-acquired genetic changes in human pluripotent stem cells: implications for basic research and regenerative medicine

I Barbaric [1]
1: University of Sheffield

Human pluripotent stem cells are subject to mutations in vitro and in the presence of selection pressures, the variants with genetic changes that allow for improved growth outcompete their neighbours and overtake the culture. The commonly observed genetic changes in hPSCs are non-random and involve gains of (parts of) chromosomes 1, 12, 17, 20 and X, indicating that genes within these regions confer selective advantage to mutant cells. Mutations that arise in hPSCs during in vitro culture can affect their behaviour and confound experimental results. For example, variant cells often show signs of neoplastic progression, including reduced apoptosis, growth-factor independence and higher cloning efficiency. Genetic changes can also affect the propensity of hPSCs to differentiate. Altered patterns of differentiation caused by accrued genetic changes may significantly affect the use of such cell lines in applications that require the production of differentiated derivatives. With hPSC derivatives entering the clinical trials, a possibility that genetic changes may confer malignant properties to hPSCs or their differentiated progeny is a major cause of regulatory concern. In our work we are elucidating the molecular mechanisms that underlie the maintenance of the integrity of the hPSC genome, and how disruption of these mechanisms can lead to undesired genetic changes.

INV06

Gene therapy for an inherited childhood parkinsonism - dopamine transporter deficiency syndrome

S Waddington [1]
1: UCL

Dopamine transporter deficiency syndrome is caused by biallelic loss-of-function mutations in SLC6A3, encoding the dopamine transporter. Patients present with early infantile hyperkinesia, severe progressive childhood parkinsonism, and raised cerebrospinal fluid dopamine metabolites. The absence of effective treatments and relentless disease course frequently leads to death in childhood. To progress toward clinical translation, we used the knockout mouse model of this disease, that recapitulates human disease, exhibiting parkinsonism features, including tremor, bradykinesia, and premature death. Stereotactic delivery of AAV2.SLC6A3 gene therapy targeted to the midbrain of adult knockout mice rescued both motor phenotype and neurodegeneration, suggesting that targeted AAV gene therapy might be effective for patients with dopamine transporter deficiency syndrome.

INV07

Genetic therapies for retinal disease

J Kapetanovic [1]
1: University of Oxford

Inherited retinal diseases (IRDs) are the leading cause of blindness certification in the working age. Transformational advances have occurred in recent years in our ability to genetically diagnose these conditions as well as in our understanding of pathogenic mechanisms and the range of variability seen in each disorder. The vast majority are still untreatable. We currently have one licensed gene therapy treatment Luxturna for one type of RPE65-related retinal and several more are on the horizon. This talk covers the current landscape and likely future therapies including novel delivery methods using robotics.

INV08

A Self-regulating Gene Therapy for Rett Syndrome

R D Hector [1]
1: Simons Initiative for the Developing Brain, Centre for Discovery Brain Sciences, University of Edinburgh; Neurogene Inc., New York, USA

Rett syndrome (RTT) is a neurological disorder caused by loss-of-function mutations in the X-linked *MECP2* gene. Genetherapy has emerged as an attractive treatment option for RTT; however, overexpression of MeCP2 protein can lead to adverse effects. Conventional gene therapy vectors can be problematic for dosage sensitive conditions such as RTT, as uneven biodistribution leads to variable levels of transgene expression within cells. To address these challenges, we developed EXACT, a novel transgene regulation system that utilizes a self-

contained miRNA-based feed-forward loop. The EXACT system ensures more consistent transgene expression, acting as a safety measure against excessive expression levels. We demonstrated the ability of EXACT circuits to constrain expression of transgenic MeCP2 *in vitro* and *in vivo*. When compared to conventional, 'unregulated' gene therapy constructs, EXACT-regulated constructs displayed a narrower range of cellular MeCP2 expression. In addition, the degree of regulation increased with higher transgene doses. Following extensive *in vivo* testing in RTT mouse models, we identified an optimal configuration of the AAV9 construct, designated NGN-401, for self-regulated *MECP2* gene therapy. Delivery of NGN-401 to male *Mecp2*$^{-/y}$hemizygous mice via intracerebroventricular injection resulted in extended survival and efficacy, ameliorating RTT-like phenotypes. NGN-401 also demonstrated tolerability in female *Mecp2*$^{+/-}$ heterozygous mice, and in nonhuman primates, in contrast to conventional unregulated constructs, which exhibited toxic effects. These results have supported the advancement of NGN-401 into a first-in-human pediatric study in RTT (ClinicalTrials.gov, NCT05898620).

INV10

Development of genetically refined "precision virotherapies" suitable for systemic anti-cancer applications

A Parker [1]
1: Cardiff University School of Medicine

Adenoviruses represent attractive agents for translational applications from vaccines to oncolytics. As oncolytics, their ability to induce lysis and immunogenic cell death following infection of target cells is advantageous. Furthermore, they are amenable to genetic engineering, enabling therapeutic transgenes to be overexpressed from infected cells.

Most oncolytic adenoviruses that have undergone clinical translation rely on improved selectivity of replication inside tumour cells over healthy cells. This depletes the pool of therapeutic available for "on-target" activity due to virus uptake in "off-target" cells. Our lab has pioneered a different approach, studying how adenoviruses infect healthy cells naturally, and used these insights to guide the development of refined, tumour selective agents. This has been achieved by combining "detargeting" modifications the three major structural proteins to prevent native cell entry, coupled with the incorporation of effective targeting peptides into the capsid to enhance "on target" infection of cancer cells.

We developed an "Ad5$_{NULL}$" platform which can be targeted to tumour specific receptors through appropriate engineering. Our lead agent, Ad5$_{NULL}$-A20 targets carcinomas expressing αvβ6 integrin, a marker of tumour aggression, following systemic application in mouse models. Ad5$_{NULL}$-A20 (Trocept) is undergoing IND enabling studies, with clinical trials of this agent expressing a full-length immune checkpoint inhibitor expected to commence in patients with αvβ6 positive carcinomas by the end of 2024.

The tropism of the Ad5$_{NULL}$ platform can be manipulated to target alternative tumour antigens enabling targeting of αvβ6 negative tumour types such as gliomas and thus represents a versatile "plug-and-play" system for targeted therapeutic applications.

INV11

Gene therapy for rare surfactant protein deficiencies

D R Gill [1] K Miah [1] A R Ruffle [1] M A Viegas [1] E Castells [1] H Dolatshad [1]
S G Sumner-Jones [1] R Dean [1] E Thornton [2] C McDougall [2] C McLaughlin [2] Y Du [1]
A McStea [1] R J Munday [1] A M Munis [1] G McLachlan [2] S C Hyde [1]
1: University of Oxford 2: The Roslin Institute, University of Edinburgh

We have developed a lentiviral platform for gene delivery to the lung, based on recombinant Simian Immunodeficiency Virus (rSIV) pseudotyped with Sendai virus F and HN glycoproteins (rSIV.F/HN). The receptor for F/HN is present on many lung cell types and lentiviral integration is particularly beneficial for the multiple stem/progenitor cell niches in the lung, to ensure that vector expression is not lost during cell division. These features make rSIV.F/HN a promising platform for treatment of rare lung diseases.

Surfactant Protein Deficiency in term babies is ultra-rare. Pathogenic mutations in genes encoding Surfactant Protein B (SP-B), or lipid transporter ABCA3, result in severe respiratory distress and lung collapse in newborns. Mechanical ventilation is required at birth for survival and no treatment options are available to prolong life for these babies.

We are investigating the rSIV.F/HN platform to deliver SP-B and ABCA3 to progenitor alveolar type II (ATII) cells that produce lung surfactant. The target ATII cells can be transduced in surfactant cell culture models grown at the Air-Liquid Interface and *ex vivo* in human precision-cut lung slices. For *in vivo* correction, we delivered rSIV.F/HN expressing human SP-B to a conditional mouse disease model and showed extended (>11 months) survival compared with a median of 5 days in untreated control animals. We have also used ventilated neonatal piglets to model vector delivery to babies via the endo-tracheal tube. Together these data support the development of rSIV.F/HN for treatment of surfactant protein deficiency in neonates.

FB01

Rescue of lethal SP-B deficiency in a murine model using lentiviral vector-mediated gene therapy

K M Miah [1] A Ruffle [1] M A Viegas [1] E Castells [1] H Dolatshad [1] S Tariq [1]
S G Sumner-Jones [1] S C Hyde [1] D R Gill [1]
1: University of Oxford

Objectives: We assessed our SIV lentiviral vector pseudotyped with Sendai Virus F and HN glycoproteins (rSIV.F/HN) for treatment of ultra-rare Surfactant Protein-B (SP-B) deficiency. Surfactant, comprising lipids and proteins, including SP-B, promotes efficient gas exchange and prevents alveolar collapse. The lack of functional SP-B at birth leads to lethal respiratory distress in full-term infants, requiring long-term ventilation. Without lung transplantation these babies succumb to disease within the first year of life.

Results: A widely used, conditional, mouse model of SP-B deficiency was utilised. Following removal of protective Doxycycline from the diet, the disease state is induced and mice survive for a median of only 5 days (mice are humanely culled at 15% weight loss). We administered rSIV.F/HN expressing human SP-B via intranasal inhalation to the lungs of these mice and showed that the treatment prolonged survival for >11 months (compared with untreated control animals; *P*=0.0001). Our longest surviving treated animals remain alive >340 days post-disease induction. The risk of severe respiratory distress in treated animals was reduced (Hazard Ratio 9.3; 95% CI 2.9-30.2) with survival (60.6%) indistinguishable from non-disease induced littermates (74.2%) (*P*=0.5144).

Conclusions: These data demonstrate enduring survival in this mouse disease model after treatment with rSIV.F/HN expressing human SP-B to restore surfactant homeostasis in the lung. These data also support the development of this lentiviral platform as a potentially curative gene therapy for babies born with surfactant protein deficiencies. We are currently planning toxicology studies to support a clinical trial.

FB02

Generation of a *de novo* intronic junction in the *DMD* gene through CRISPR/Cas genome editing as a potential therapy for a high proportion of Duchenne Muscular Dystrophy patients

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Duchenne Muscular Dystrophy is caused by mutations across the *DMD* gene, leading to absence of dystrophin protein and giving rise to progressive muscle wasting. Different gene therapies are being investigated, such as AAV microdystrophin delivery, premature termination codon read-through and exon-skipping. Nevertheless, these therapies require repeated administration, could carry an adverse immunological risk and some are restricted by mutation specificity. Such problems may be circumvented with genome editing. We aim to express an internally truncated functional dystrophin from the endogenous *DMD* locus using a *Sa*Cas9 CRISPR system. Our gRNAs would delete exons 19 to 55 by creating a *de novo* junction between introns 18-55 and producing a ~800 kbp deletion. This strategy would eliminate ~81% of total DMD mutations. As a positive control, a cDNA construct expressing D19-55 dystrophin was generated and tested in *mdx* mice. This construct significantly increased dystrophin positive fibres by plasmid intramuscular injection, indicating that D19-55 dystrophin is expressible and has potential for beneficial effects when expressed in sufficient levels. To produce the deletion by genome editing, optimal gRNAs for each intronic site in murine *Dmd* were multiplexed into an *Sa*Cas9 construct and tested in Neuro2A and C2C12 cells. Deletion of exons 19-55 was confirmed by PCR and Sanger Sequencing. Multiplexed constructs were packaged into AAV9 and assessed *in-vivo* by local injection into 2-months old *mdx* mice. Functional efficacy was assessed by muscle electrophysiology. However, no beneficial effects were seen likely due to inability to detect a deletion in treated muscle samples with the employed techniques.

FB03

Liver-directed lentiviral gene therapy is safe and curative in Argininosuccinic aciduria

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The liver-based urea cycle enables nitrogen waste and clearance of neurotoxic ammonia. Argininosuccinic aciduria (ASA) caused by argininosuccinate lyase (ASL) deficiency is the second most common inherited urea cycle defect. Patients present either with neonatal- or late-onset

hyperammonaemia, which causes coma and death if untreated, and a high risk of severe cognitive impairment and epilepsy. Curative liver transplantation can be performed in severe cases but requires lifetime immunosuppression. We aimed to test *in vivo* lentiviral gene therapy in neonatal ASL-deficient (*Asl*$^{Neo/Neo}$) mice. *Asl*$^{Neo/Neo}$ animals received a neonatal intravenous injection of lentiviral vector at 4E10TU/kg encoding either the codon-optimised human ASL (LV.*coASL*) versus or a GFP as control. LV.*coASL*-injected animals survived for 12 weeks whilst control mice died within 4 weeks ($p<0.001$). Growth ($p<0.01$), fur coat pattern, ammonia ($p<0.001$), arginosuccinate ($p<0.001$), citrulline ($p<0.01$), and orotate ($p<0.05$) were normalised to those in wild-type mice. Significantly increased ASL expression ($p<0.01$) and activity ($p<0.05$) were observed in treated *Asl*$^{Neo/Neo}$ livers compared to controls. Lentiviral vectors present long-term transgene expression due to their ability to integrate into the host genome. We conducted safety studies to address the presence of genotoxic events driven by the lentiviral vector *in vivo*. Twenty neonatal wild-type mice received LV.*cohASL* vector intravenously versus 20 PBS-injected littermates. Pathology and integration site analysis on livers at 9 months post injection confirmed absence of tumours and clonal dominance. Overall, those preliminary studies demonstrated proof of concept of *in vivo* lentiviral gene therapy for ASA and its safety profile.

FB04

Safety and efficacy analysis of *in vivo* lentiviral gene therapy for ARC syndrome

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Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome is a severe autosomal recessive multi-system disorder resulting from mutations in *VPS33B*. The hepatocyte VPS33B deficiency results in abnormal bile secretion leading to progressive liver fibrosis. Research utilizing animal models indicates that lentiviral vectors (LV) may offer a safe option for *in vivo* gene therapy applications and can facilitate sustained transgene expression. We aimed to develop LV based gene therapy to treat ARC syndrome. To test the safety of this approach two LV vectors were tested in heterozygous *Vps33b*$^{+/-}$ knockout mice that do not show ARC phenotype:
1) LV.EFS1α.*coVPS33B* vector expressed in multiple tissues, utilising ubiquitous (EFS1α) promoter and 2) LV.LP1.*coVPS33B* liver specific, using the LP1 promoter. The neonatally treated mice were analysed at 9 months. We observed an increased incidence of hepatocellular carcinoma (3:10) in LV.EFS1α.*coVPS33B* treated mice compared to (0:10) cases observed in mice treated with the LP1 containing vectors. Integration site analysis from tumour samples revealed a decreased Shannon diversity score and a low integration site diversity. Given LV.LP1.*coVPS33B* treatments proved safe, we further investigated their efficacy in treating the ARC liver phenotype when administered to neonate *Vps33b*$^{-/-}$ knockout mice. The therapeutic effect has been assessed at 12 weeks after the mice were being fed for 8 weeks an 0.25% cholic acid diet. The results have shown normalisation to wild-type values of blood parameters of liver toxicity as well as animal survival and growth. These results bring hope to ARC syndrome patients and highlight the importance of safety screening for newly developed LVs.

OR01

Traceless delivery of prime editor ribonucleoprotein (RNP) complexes in engineered nanoparticles

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Genome editing has enormous potential to transform the future of genetic medicines, but current delivery strategies suffer from several shortcomings. By incorporating ribonucleoprotein (RNP) complexes instead of vector genomes into lentivirus-derived nanoparticles (LVNPs), we address key limitations of viral and non-viral delivery, including: (i) overcoming limited packaging capacity, (ii) preventing unwanted genomic integration, and (iii) reducing exposure to the therapeutic payload. The practical utility of this LVNP platform has been demonstrated, directing robust levels of genome editing in both primary human T cells *ex vivo* and the murine retina *in vivo* (PMID: 37678882). We also demonstrated LVNP-mediated delivery of larger base/prime editors, however, the substitution of Cas9 with larger RNP complexes caused a decline in both potency and viral yield.

With strategic emphasis on clinical applications, successful vectors should converge in terms of potency, safety, and manufacturing. To improve potency of LVNPs loaded with prime editors, we added effector domains to favour membrane anchoring during manufacturing and removed redundant viral proteins to improve LVNP packaging capacity. In addition, we incorporated protein/RNA aptamers to increase (e)pegRNA loading and recruit cognate effector proteins. Collectively, these alterations substantially improved prime editing efficacy (~8-fold to ~34% across targeted loci). We also tested prime editor configurations that lack the RNAseH domain but observed slightly reduced efficacy. Finally, we have established protocols for large-scale and cGMP-compliant manufacturing using VSV-G or Sendai F/HN pseudotypes. Collectively, these improvements in our LVNP platform will enable preclinical testing in patient-derived cells and *in vivo* models to assess potency and safety.

OR02

Developing a universal haematopoietic stem cell gene editing therapy for XIAP deficiency

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1: University College London

X-linked inhibitor of apoptosis (XIAP) deficiency is a rare, life-threatening inborn error of immunity, caused by mutations in the XIAP/BIRC4 gene. Allogeneic haematopoietic stem cell (HSC) transplantation can be curative, but graft versus host disease remains a significant problem. We have developed a therapeutic autologous HSC gene editing approach, thus avoiding the risk of alloreactivity and permitting the use of less toxic conditioning regimens.

A CRISPR/Cas9/AAV6 based gene editing strategy was used to achieve precise correction at the XIAP locus in human CD34+ cells. Edited patient cells were differentiated into monocytes and stimulated with MDP or LPS to assess XIAP dependent function. An i*n vitro* colony forming unit (CFU) assay was used to assess differentiation and engraftment potential of edited CD34+ cells.

HDR editing rates of up to 60% were achieved, without impacting cell viability. Correction of XIAP patient cells resulted in restoration of XIAP protein expression and partial restoration of TNFα secretion after stimulation with MDP in corrected patient cells. *In vitro* preservation of clonogenic and differentiation potential was shown. Analyses of off-target endonuclease activity, through sequencing of the predicted top 10 off-target sites and karyotype analysis, are underway.

This work confirms proof-of-principle for an HSC gene editing approach as a potential treatment for XIAP deficiency. High editing rates in CD34+ cells were achieved, with low cytotoxicity and preserved *in vitro* differentiation potential. High levels of targeted integration were observed in XIAP patient HSCs resulting in partial restoration of XIAP protein dependent immune function in differentiated, corrected cells.

OR03

Administration of mRNA alleviates disease biomarkers in a mouse model of pulmonary alveolar proteinosis (PAP)

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Autoimmune (a)PAP is a lung disease caused by accumulation of surfactants in the alveoli. Autoantibodies against GM-CSF prevent macrophage clearing surfactants. Lentiviral vector-mediated GM-CSF expression can correct biomarkers in GM-CSF knockout mice. However, the toxicity: efficacy window was narrow due to prolonged over-expression of GM-CSF. Transient expression of GM-CSF through mRNA delivery may improve this window. The cationic lipid GL67A has been safely used in clinical trials. We established the optimal ratio of mRNA to GL67A in *in vitro* experiments using firefly luciferase mRNA. Molar charge ratios of 1:1, 3:1 and 4:1 (mRNA:lipid) led to high-level gene expression and were subsequently assessed in mouse lung *in vivo*. Mice (n=5/group) were transfected with 10µg RNA encoding secreted Gaussian luciferase (GLux) and culled 24hr post-treatment. Control animals were treated with 80µg plasmid carrying the GLux cDNA complexed to GL67A using our established formulation of 4:1 (pDNA:lipid). A molar charge ratio of 3:1(mRNA:GL67A) was well tolerated and GLux expression following mRNA transfer was similar to pDNA in bronchoalveolar lavage fluid (BALF) (mRNA:1817±107,pDNA:2727±846 RLU/µl BALF). We next administered GM-CSF knockout mice with GM-CSF mRNA or GLux mRNA (n=8/group) complexed to GL67A(5 doses at weekly intervals, 10µg/dose). The mice recapitulate biomarkers of PAP disease including BALF turbidity. 4 days after the last dose, mice treated with GM-CSF mRNA had reduced ($p<0.05$)BALF turbidity (GM-CSF: OD_{600nm}5.74±0.33, control: OD_{600nm}2.93±0.29). In summary, mRNA/GL67A complexes transduce mouse lung efficiently and ameliorate a disease biomarker in PAP mice. Future studies will focus on analysis of additional biomarkers and assessment of toxicity.

OR04

Synthetic modified mRNA administration in a first in-human ex-situ heart failure model validates its use as a novel, effective and safe method of evaluating drug exposure and efficacy

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1: University of Cambridge 2: AstraZeneca 3: The Royal Papworth Hospital 4: The Golden Jubilee National Hospital

Modified mRNAs hold great promise for treating a wide variety of illnesses, but as of yet are not routinely used in cardiovascular medicine.

We utilized a novel modRNA in explanted recipient human hearts from transplantation to test whether it was expressed in a human model of cardiomyopathy. The modRNA was injected directly into the myocardium of explanted recipient hearts from transplantation. Two doses were injected at different sites – 1mg (high-dose) and 0.1mg (low-dose). The hearts were perfused ex-situ for 6 hours using our novel perfusion machine – the multi-organ perfuser (m0rgan). Perfusion was carried out at normothermic conditions. Expression of this mRNA was assessed after 6 hours of perfusion by mesoscale immunoassay analysis.

There were elevated levels of the protein in the subendocardium and mid-myocardium of injection sites which received our novel mRNA. Low dose mRNA resulted in an increase in protein levels in the mid-myocardium and subendocardium. High-dose mRNA resulted in significantly elevated protein levels in the subendocardium.

This study demonstrates the utility of ex-situ perfusion of explanted diseased organs from transplant recipients as a model to test novel therapeutics. It offers the ability to test therapies before clinical trials in human tissues in a safe manner. Moreover, it enables efficacy and dosing to be assessed before the clinical trial stage. We propose that this model acts as a link between preclinical and clinical studies for potential therapeutics.

OR05

Molecular characterisation of a novel knock-out mouse model in the context of MPSIVA ex-vivo gene therapy development

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Institute, Milan 6: University Vita-Salute San Raffaele, Faculty of Medicine, Milan 7: Pathology Unit, Department of Experimental Oncology, IRCCS San Raffaele Scientific Institute, Milan 8: Mouse Behaviour Facility, San Raffaele Scientific Institute, Milan 9: Osteoporosis and Bone and Mineral Metabolism Unit, San Raffaele Scientific Institute, Milan

Mucopolysaccaridosis type IVA (MPSIVA), caused by deficient N-acetylgalactosamine-6-sulphate sulphatase (GALNS), presents with skeletal, cardiac, and joint disorders, normally treated with enzyme replacement therapy. This limits GAGs accumulation without correcting the skeletal disease. Based on recent studies showing safety of the procedure and a beneficial effect on skeletal manifestations of ex-vivo haematopoietic stem progenitor cell gene therapy (HSPC-GT) in MPS type I-Hurler patients, we propose to develop a lentiviral-based HSPC-GT approach for MPSIVA. The disease, however, still lacks an animal model representative of patients' skeletal symptoms. Thus, we have generated Galns-/- mice by CRISPR-Cas9, using two gRNAs targeting Galns' intron1 and exon2, in C57BL6/J strain. After biochemical and histopathological characterisation, we selected the genotype inducing the most evident skeletal symptomatology, without off-target alterations. Our selected model appears smaller than wild-type controls, with shorter femurs and tibiae on CT-scan and decreased cortical and trabecular areas. Vacuolation in cartilaginous sections were highlighted by histomorphometry and immunohistochemistry. Molecularly, our selected mutation induces the translation of a protein from an alternative open reading frame, which appears inactive, contrarily to previously described enzyme-null mice. Furthermore, we highlighted different composition and GAGs distribution among mice, rats, and patients' cartilage, which we are evaluating by immunohistochemistry. Overall, the development of an MPSIVA murine model provides a novel means to assess the safety and efficacy of innovative therapies, which we are currently testing. Also, this model represents an alternative to the available rat model, easier to compare to previously acquired preclinical HSPC-GT data.

OR06

Towards a scalable, closed and automated platform for the production of cost-efficient allogeneic cell therapies: showcase of an exemplar iNK process

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Induced pluripotent stem cell (iPSC)-derived therapies offer promising "off-the-shelf" allogeneic treatments for numerous chronic conditions. However, their efficacy requires high doses exceeding ≥10^9 cells, which are not suited to traditional 2D processes that are typically open and poorly scalable technologies. Successful commercialization hinges on developing efficient processing platforms for large-scale manufacture. Here, we introduce a closed, scalable, semi-automated upstream process for iPSC expansion and differentiation into Natural Killer (iNK) cells using stirred tank reactors (STR). Unlike traditional methods relying on periodic settling for medium exchanges, our method employs an acoustic filtration system to continuously exchange medium and control aggregate size, crucial for expansion and differentiation while preserving pluripotency. Our results demonstrate reproducible high-density cultures (>10^6 cells/mL) with controlled aggregate diameter (100-200 μM).

In collaboration with Plasticell, we have scaled up a process for manufacturing iPSC-derived NK cells. We successfully integrated our iPSC expansion platform and enhanced Plasticell's NK differentiation process using a scalable STR system. This advancement yields a pure population of CD45+/CD56+ NK cells (>80%) with increased yield per starting iPSC compared to manual methods. These cells exhibit mature NK cell markers and demonstrate cytotoxicity against lung and breast cancer cell lines *in vitro*.

We believe our work represents the first end-to-end process for generating iNK cells in an STR system. This integrated approach accelerates patient access to transformative allogeneic therapies, marking a significant contribution to the field.

OR07

IKC159V: A next generation bicistronic gene therapy for the treatment of Geographic Atrophy

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Purpose: Geographic atrophy (GA) is an advanced form of age-related macular degeneration (AMD), that starts with atrophic lesions in the outer retina that expand to cover the macula and fovea, leading to severe vision loss over time. Despite recently approved treatments there remains a need for therapies with a greater efficacy, reduced treatment burden and without an increased risk of ocular neovascularisation. IKC159V is a bicistronic AAV vector that expresses pigment epithelium-derived factor (PEDF) to protect RPE and photoreceptors from atrophy and prevent neovascularisation, and a soluble form of complement cofactor CD46 to reduce pathological activation of the complement cascade.

Methods: Null, monocistronic (PEDF or sCD46) and bicistronic PEDF/sCD46 (IKC159V) vectors (AAV2 or AAV8) were evaluated in a co-culture angiogenesis assays, complement breakdown assays and murine models of laser-induced CNV and a sodium iodate (SI) induced oxidative stress model following intravitreal or subretinal delivery.

Results: IKC159V and the PEDF-only AAV's significantly reduced VEGF-induced angiogenesis whilst IKC159V and the sCD46-only AAV's showed C3b and C4b complement breakdown. In the laser CNV model, IKC159V superiorly reduced both permeability, lesion area and MAC (C5b9) deposition area compared to either monocistronic vectors. IKC159V was also more efficacious than other well-known complement-only gene therapies. In the SI model, PEDF was demonstrated to significantly preserve retinal functional compared to Null vector.

Conclusions: IKC159V shows greater efficacy in GA disease models than monocistronic vectors. The superior efficacy profile of IKC159V makes it an ideal candidate for clinical development to treat individuals with GA.

OR09

Development of a precision immunovirotherapy expressing a folate receptor α bispecific immune cell engager for treatment of ovarian cancer

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The biology and silent spread of ovarian cancer make systemic therapy fail rapidly while complete surgical resection is often not possible. Immunotherapies provide a much-needed alternative to standard treatment options. We developed a precision virotherapy utilising an αvβ6 integrin selective adenovirus type 5 virus, $Ad5_{NULL}$-A20 expressing a bispecific T-cell engager binding Folate receptor alpha (FLOR1), both overexpressed on ovarian cancer, to activate T-cells leading to enhanced immune mediated cancer cell regression.

The efficacy of the newly developed immunovirotherapy was tested *in vitro* in ovarian cancer lines and *ex vivo* ascites co-cultured with healthy donor peripheral blood mononuclear cells (PMBCs). T-cell activation was assessed in conjunction with IFN-gamma and TNF-alpha secretion by ELISA and cell viability assays determine cancer cell killing.

$Ad5_{NULL}$-A20.αFLOR1XCD3 was successfully developed and validated. Co-culture assays with PBMCs show an increase in T-cell activation of up to 80% following transduction with the engineered virotherapy. Increased levels of IFN-gamma and TNF-alpha was evident in cells expressing the bispecific compared to untreated cells. Cell viability assays indicate around 50% decrease in cancer cell viability following virus transduction. In addition, FOLR1 positive ascites-derived ovarian cancer models were established from patients and used for testing the efficacy of the viral vectors *ex vivo* confirming T-cell activation and cancer cell regression when treated with $Ad5_{NULL}$-A20.αFLOR1XCD3.

Results indicate $Ad5_{NULL}$-A20.αFLOR1XCD3 leads to T-cell activation and immune mediated tumour killing *in vitro* and *ex vivo*. This novel approach has the potential to become a much-needed alternative treatment option for patients with advanced ovarian cancer.

OR10

Single cell sequencing analysis of a CD28/CD40-based chimeric costimulatory antigen receptor (CoStAR™) activity reveals multiple functionally validated effector activities in CD4+ and CD8+ T cells

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Tumour infiltrating lymphocyte (TIL) therapy for treatment of metastatic melanoma, has demonstrated clinical efficacy leading to its recent FDA approval. Extending the clinical benefit to

patients with other cancers has posed a challenge. Insufficient costimulation in the tumour microenvironment can lead to T cell anergy and exhaustion, in turn leading to reduced anti-tumour activity. Our CoStAR™ platform synergises with TCR signals upon antigen engagement to enhance proliferation and effector function. To explore the underlying biology of CoStAR activity we performed a scRNA-seq analysis. T cells were engineered to express a HLA-A*02/CEA specific TCR and/or FRα-specific CoStAR. Following coculture with tumour cells bearing the cognate pMHC and CoStAR target antigens, samples were processed through a scRNA-seq workflow. Bioinformatic analysis was performed to compare TCR and/or CoStAR+ populations, with functional validation of gene signatures performed using cytokine analysis, flow cytometry and cytotoxicity assays. When compared to TCR alone stimulation, TCR+CoStAR signalling in CD4+ cells resulted in enhanced gene signatures associated with activation and cytotoxicity, and in CD8+ cells, signatures of enhanced cytotoxicity and reduced exhaustion marker expression. Several genes – predominantly costimulatory receptors – were upregulated following CoStAR signalling alone. The cytotoxicity signature in CD4+ T cells was confirmed using xCELLigence cytotoxicity assay, with TCR+CoStAR signalling associated with enhanced cytotoxicity compared to TCR signalling alone. Cytokine and chemokine production was concordant with gene expression data, with flow cytometry confirming upregulation of costimulatory receptors in CD4+ T cells upon CoStAR engagement alone. These data further support the clinical utility of CoStAR engineered TIL.

OR11

Toward combined cell and gene therapy for airway epidermolysis bullosa

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Epidermolysis bullosa (EB) is a group of rare, inherited genetic diseases characterised by fragility and blistering of epithelial tissues. Airway involvement in EB is rare but affected patients experience a high burden of morbidity and mortality. EB airway disease involves granulation tissue formation and scarring of the larynx and trachea. As the disease progresses, airway stenosis (narrowing) ensues and necessitates a tracheostomy.

At Great Ormond Street Hospital, children with diagnoses of JEB-S (n=9) or JEB-LOC (n=4) were most commonly referred to ENT services. 10/15 patients had mutations in the *LAMA3* gene, suggesting that variants in *LAMA3* confer particular susceptibility to airway disease and thus represent targets for therapy. We developed a lentiviral vector to express wildtype *LAMA3A* in cultured primary EB patient human airway basal epithelial cells. Transduction restored LAMA3 expression, enabled appropriate cellular differentiation and restored the *in vitro* cell adhesion of EB basal cells to levels comparable to non-EB cells.

To progress towards clinical application, we envisage transplantation of *LAMA3*-transduced autologous cells to the airways. We developed a surgical transplantation model through short segment tracheal resection and primary anastomosis in New Zealand white rabbits, establishing autologous epithelial cell cultures for each animal. After transduction with a lentivirus for cell tracking, ZsGreen+ cells were sorted, expanded, seeded onto fibrin sheets and wrapped around bespoke 3D-printed soft elastomer composite tubular constructs before tracheal reimplantation. Engraftment of transplanted cells was found after 4 or 10 days, suggesting the viability of stent-based airway epithelial cell transplantation.

OR12

Non-viral GM-CSF gene therapy is an effective treatment in a novel murine model of autoimmune PAP

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Autoimmune pulmonary alveolar proteinosis (aPAP) is a rare lung disease, characterised by the accumulation of surfactant in the alveoli due to anti-GM-CSF autoantibodies. We have previously shown correction of the underlying defect using non-viral gene transfer in a GM-CSF knock-out mouse model (PAP mice). This model, however, does not carry anti-GM-CSF autoantibodies present in humans. To progress clinical translation, we assessed whether efficacy may be altered in the presence of anti-GM-CSF autoantibodies. We utilised passive immunisation of PAP mice with murine anti-mGM-CSF antibodies as a novel option.

The antibody pair utilised (B2.6 and A7.39) effectively neutralises GM-CSF activity *in vitro*. Through a dose escalation in mice, we defined an antibody dose required to reach comparable levels to those seen in aPAP patients.

Here, we assessed efficacy of non-viral GM-CSF gene transfer in the presence of anti-GM-CSF antibodies. Lungs of PAP mice were transfected with phCEFI-mGM-CSF complexed to the cationic lipid GL67A (single dose or 5 weekly doses of 80 µg pDNA/mouse) (n=4-5/group). Treated mice also received 8 weekly doses of 20 µg/mice of anti mGM-CSF antibody pair at the same time and 3-weeks post therapy. PAP biomarkers (bronchoalveolar lavage fluid turbidity and surfactant deposition) were significantly ($p<0.05$) reduced 1 month after transfection in comparison to irrelevant vector treated mice. The anti-mGM-CSF antibody level in epithelial lining fluid (ELF) was in the range compared to the levels found in aPAP patients (median 100-400 µg/ml ELF).

These data suggests that non-viral based GM-CSF gene therapy may be suitable for clinical translation to treat aPAP.

Poster Presentations

P01

TROCEPT- an oncolytic virus platform engineered for αvβ6 integrin targeted tumour-localized expression of an immune checkpoint inhibitor following intravenous delivery

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TROCEPT is a novel tumour-selective replicating, oncolytic adenovirus type 5 (Ad5) rationally engineered to remove all natural tissue tropisms. This engineering addresses the main limitation of other viral therapies which infect normal tissues and are rapidly removed by the liver and are therefore limited to local delivery. TROCEPT has been further engineered to specifically bind to αvβ6 integrin which is expressed at high frequency in the majority of epithelial cancers, so that following intravenous delivery the virus targets tumour tissue where it replicates and amplifies. In addition, the TROCEPT platform encodes transgenes which enables in-tumour production of powerful therapeutic drugs. The lead programme, TROCEPT-01, encodes a fully human, full length immune checkpoint inhibitor (ICI) antibody. TROCEPT-01 is currently undergoing IND-enabling studies and is expected to enter First in Human studies in 2024 in multiple solid tumour indications. Here we demonstrate viral replication, transgene expression and oncolytic cell death following infection with TROCEPT-01 is highly selective for αvβ6 integrin positive tumour cells compared to a panel of normal human primary cells. Additionally, we show, intravenous delivery in SKOV3 ovarian human tumour xenograft mouse models results in virus delivery, replication, and transgene expression in the tumour. In conclusion, when compared to the parental Ad5 virus, the engineering of TROCEPT-01 leads to more viral delivery to the tumour, less delivery to normal tissue, reduced markers of liver toxicity and reduced inflammatory cytokine release, all of which validate the tumour selectivity and safety profile of the virus.

P02

Primary T-cell transduction performance as the guiding principle behind LV process development and platform innovation

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Lentiviral vectors (LV) are the most common vehicle for the manufacture of autologous CAR-T cells for cancer immunotherapy. The upstream component of the complex LV manufacturing process involves transient transfection of production cells by four plasmids; Rev, Gag-Pol,

Genome, and envelope (VSV-G) at an optimal ratio. Downstream processing is used to purify the produced LV through multiple steps including but not limited to anion exchange chromatography, ultra-diafiltration and filter-sterilization to produce the final drug product. Biological variability due to product differences, upstream and downstream processes play an important role in determining LV drug product potency.

Transduction titres, usually measured in an in-house cell line, is currently considered as the industry benchmark of LV batch manufacture success. Typically all early process optimisation/platform development activities are geared towards obtaining maximum LV titres on generic cell lines. However, efficient transduction of primary human T-cells by LV is critical for successful CAR-T cell generation. We will present data to show that optimising titre of vector on cell lines does not always correlate with highest vector performance on target cells such as primary T-cells in the case of CAR-T therapy. Through the development of a miniaturized LV purification and T-cell transduction workflow, we have now integrated T-cell transduction efficiency as an important Design of Experiments (DoE) parameter in our early client process development and platform innovation activities. Case studies involving finding the optimal design space for high T-cell transduction efficiency coupled with improved overall process robustness and its impact on vector functionality will be discussed.

P03

TROCEPT: a unique adenovirus for *in-tumour* expression of immunotherapies via intravenous delivery

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TROCEPT is a novel tumor-selective delivery technology based on adenovirus serotype 5 (Ad5). TROCEPT has been engineered to not enter normal human tissues by disabling (de-targeting) all three of the major capsid proteins (fiber knob, hexon and penton). The removal of normal tissue tropisms addresses the main limitations of other intravenously delivered viral therapies, which are rapidly sequestered in the liver and other healthy tissues. TROCEPT has been further engineered to specifically bind to αvβ6 integrin (re-targeting), a tumor marker expressed at high frequency on most carcinomas. The TROCEPT platform can be armed with transgenes encoding protein-based therapeutic drugs for *in-tumour* expression.

Here, we identified carcinomas with very high frequencies of αvβ6 integrin expression by performing an in-depth IHC analysis using primary tissue from a wide array of carcinomas. We further demonstrate TROCEPTs unique specificity for αvβ6 integrin positive cell entry *in vitro* and *in vivo* using mouse xenograft models. Cell entry led to the production of large quantities of functional payload, and viral replication only in tumour cells, demonstrating TROCEPTs potential therapeutic window between tumour and normal tissue. Finally, we performed a series of safety experiments in healthy mice and Syrian golden hamsters and found very little evidence of toxicity (serum cytokines, liver toxicity, weight loss and general health) compared to the parent Ad5 vector.

Taken together, these data support a safe starting dose in humans and form the basis for a preclinical package for TROCEPT-01, which encodes an immune checkpoint inhibitor.

P04

αvβ6 Integrin expression in solid tumours: a TROCEPT target for oncolytic viral therapy

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Upregulation of various integrins in tumours make them attractive targets for immunotherapeutic agents in cancer. The RGD (Arg-Gly-Asp)-binding integrin, αvβ6, is a cell-surface receptor exclusively expressed on epithelial cells. The major function of αvβ6 integrin is the activation of transforming growth factor-β, with additional roles shown to be involved in cell adhesion and migration and has also been implicated to play a pro-tumorigenic role. However, αvβ6 integrin expression has not been comprehensively studied to determine its frequency, intensity and pattern of expression in a wide range of human tumour indications and healthy tissues.

Here, the expression of αvβ6 integrin was examined across multiple solid tumours and healthy tissues at the gene and protein level. High expression intensity and a high prevalence of positively was observed in multiple tumor types of epithelial cancers including pancreatic, head and neck, bladder, lung, and oesophageal carcinomas. αvβ6 integrin was absent, or expressed weakly, in most adult healthy tissue, with the exception of strong expression in the transitional epithelium of healthy bladder and moderate expression in stomach. Furthermore, expression of αvβ6 integrin was also observed in metastatic lesions.

These data provide a first comprehensive analysis of αvβ6 integrin expression, confirming its presence in the majority of carcinomas, and absence or low expression in most healthy tissues. These data also provide key information for indication selection and a healthy tissue expression profile that should be considered for on-target toxicity for the αvβ6 integrin targeted therapy, TROCEPT, a tumour-specific oncolytic adenovirus platform, heading for clinical trials.

P05

Normothermically perfused human liver lobes containing tumours as a model to profile viral therapeutics

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Liver lobes with resident tumours are commonly resected from cancer patients. We show these lobes can now be attached to a clinically approved liver transplantation device (*metra*®, OrganOx, Oxford), creating a unique testing platform to match the scale, physiology, vasculature, and immune composition of tumours in a human patient, whilst allowing dosing, testing and sampling regimens which would not be permitted in a patient on a clinical trial. Three hemi-liver lobes containing either cholangiocarcinoma or metastatic colorectal tumours retrieved in theatre were connected to the *metra*® to mimic physiological conditions. The lobes were dosed with

$1e^{12}$ copies of an Ad11-based "traffic light" virus with UnaG green protein expression under an early viral promoter to show uptake and red fluorescent protein expression under a late viral promoter to indicate replication. Health of the lobes were maintained for up to 60 hours post-dosing, after which raised hydrodynamic vascular resistance and lactate levels signalled a loss of function. Throughout the perfusion, metrics of liver health were recorded, and blood samples were taken to profile viral clearance using QPCR and infectivity tests. Normal liver and tumour tissue samples were taken at the conclusion for QPCR, flow cytometry, ex vivo out-growth, and immunohistochemistry. Clearance of the virus showed good alignment with clinical data. Active virus was present in out-grown ex vivo sample slices with green and red fluorescence evident in tumour tissue. This model could provide useful insights into the pharmacokinetics and distribution of virus-based therapies in human liver containing tumours.

P06

Induced pluripotent stem cell derived alveolar type 2 cells as a model for testing novel gene therapies for interstitial lung diseases

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Objectives: Advances in protocols for directed differentiation of induced pluripotent stem cells (iPSC) to alveolar type 2 cells (iAT2) provide an important opportunity for researchers to access an unlimited source of cells that recapitulate the key transcripts and functions of primary AT2 cells. To facilitate development of viral vectors for AT2-related lung diseases, we examined adaptation of these 3D cultures to air-liquid interface (ALI).

Methods: A dual reporter iPSC line (BU3 NKX2-1^{GFP}/SFTPC^{tdTomato}) was differentiated towards distal lung cell fate using published protocols (Jacob et al. 2019; PMID:31732721). On day 29, iAT2 cells were dissociated from 3D spheres to single cell suspension and seeded on transwell inserts. Since modulation of CHIR99021 is known to mature the AT2 cell phenotype, the cultures were either (i) maintained in regular media (CHIR99021, KGF, dexamethasone, cAMP, IBMX) before airlifting, or (ii) CHIR99021 was removed from the media for 5 days followed by add-back for 24 hr, then airlifted. Gene expression was quantified by TaqMan assay.

Results: Day 29 differentiated iAT2 3D cultures expressed upregulated levels of AT2-associated genes *SFTPA2*, *SFTPB*, *SFTPC*, *ABCA3*, *LAMP3* and *NAPSA*, relative to undifferentiated iPSC. Significantly higher levels of AT2 genes were expressed in ALI cultures relative to 3D cultures; this was further enhanced by removal of CHIR99021 from the media for a 5 day period pre-airlift.

Conclusions: iAT2 showed increased expression of key AT2-associated genes when cultured at ALI. Studies are underway to evaluate the transduction of iAT2-ALI cultures with lentiviral vectors to advance gene therapy for genetic lung diseases.

P07

Developing targeted extracellular vesicles for therapeutic delivery in cardiovascular disease

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Background: Extracellular vesicles (EVs) are lipid-bound particles released from cells that are implicated in health and disease, including cardiovascular diseases (CVD). EVs can be exogenously loaded with therapeutic agents and modified with targeting peptides for targeted therapeutic delivery.

Methods: EVs were isolated using size exclusion chromatography from the serum of spontaneously hypertensive stroke-prone rats (SHRSP) and characterised by NanoSight. EVs were fluorescently labelled with PHK-67 or Licor NHS-800 dye and modified with one of five candidate cardiomyocyte or endothelial targeting peptides. EVs (control and targeted) were incubated with rat microvascular endothelial cells (MVECs) or rat H9c2 cardiomyoblasts to assess cell-selective uptake via confocal microscopy. For in vivo investigation, we intravenously injected modified EVs into male and female SHRSP rats and after 24 hours, animals were culled, and organs were imaged via Pearl Imaging.

Results: In H9c2 cardiomyoblasts, peptides 1 (DDTRHWG), 3 (CSTSMLKAC) and 5 (APWHLSSQYSRT) significantly increased EV uptake compared to untargeted EVs ($p=0.01$). In MVECs, peptides 2 (CSGMARTKC) and 4 (WLSEAGPVVTVRALRGTGSW) suggested a trend in increased EV uptake, compared to untargeted EVs. In vivo, although peptide 1 did not modify cardiac uptake, a significant increased uptake was seen in the kidneys while also detargeting the liver and spleen when compared to untargeted EVs ($n=6$; $p = 0.01$; 2.38-fold increase).

Summary: These initial data provide insight into the utility of targeting EVs as therapeutic delivery vectors for CVD however require further investigation and optimisation for future therapeutic studies.

P08

A bioluminescence-based lentiviral approach to identify regulators of the transcription factor *TP63* in airway basal cells

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In the human respiratory system, airway epithelial cells represent a key physical and immunological barrier. Maintenance of mucosal homeostasis is dependent on the regenerative capacity of basal stem cells. The transcription factor TP63 is a marker and known regulator of the

stem cell phenotype of basal cells in multiple epithelial tissues, including the airway. However, the mechanisms that regulate the self-renewal and differentiation of airway basal cells are not fully understood, including the role of the *TP63* gene regulatory network.

To screen for regulators of *TP63* expression, we developed an editable lentiviral reporter vector to track the activity of promoter sequences of interest. The selected promoter drives expression of firefly luciferase, while a constitutively active CMV promoter drives expression of renilla luciferase and dsRed2, to enable normalization and cell sorting, respectively.

We generated lentiviral reporter vectors driven by promoter sequences from the *TP63* (basal cells), *MUC5AC* (mucosecretory cells) and *FOXJ1* (multiciliated cells) genes, and transduced both a human bronchial epithelial cell line (HBEC3-KT) and primary human airway basal cells. In organoid assays, changes in promoter activity reflected cellular differentiation, validating our approach.

Following this, we have optimised a screening protocol to identify cytokines and growth factors that regulate *TP63* expression. We defined positive and negative control conditions for modulating *TP63* promoter activity. The results of this screen could identify approaches to promote the stem cell phenotype of airway basal cells, which may represent future therapeutic strategies to regenerate airway tissue in bioengineering or chronic lung diseases.

P09

Compound screening in primary human airway basal cells identifies Wnt pathway modulators as potential pro-regenerative therapies

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Airway basal cells are multipotent stem cells that can differentiate to replace epithelial cell types of the airway epithelium. This makes them a suitable target cell for cell and gene therapies for genetic diseases that affect the airways, such as cystic fibrosis or primary ciliary dyskinesia.

The addition of compounds that increase basal cell proliferation to a cell product could improve engraftment success by giving the modified basal cells an initial competitive advantage over host cells.

Primary human bronchial epithelial cells (HBECs) were transduced with the pHIV-Luc-ZsGreen construct to enable automated quantification of relative cell numbers. Using a 2D, 384-well cell culture assay, compounds from the ENZO chemical libraries and the Prestwick Chemical Library (FDA-approved) were screened for their ability to increase cell number.

Several hit compounds were activators of Wnt signalling. We validated the screen results in concentration-response and colony forming assays using additional HBEC donors to assess effects on cell proliferation and clonogenicity. Compounds were tested in the ‘tracheosphere’ assay, in which HBECs proliferate and differentiate to form a polarised spheroid structure containing the major cell types of the airway epithelium. Addition of hit compounds resulted in larger spheroid structures, suggesting increased proliferation, and did not inhibit differentiation to the major luminal cell types.

We additionally investigated the effects of structurally and functionally related compounds in these assays, finding that the induced Wnt activity correlated with organoid size. We envisage that Wnt activating small molecules could be developed into novel treatments to improve airway epithelial cell engraftment.

P10

Surfactant air liquid interface model of ABCA3 surfactant protein deficiency

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To aid progression of gene therapies for lung diseases to the clinic, we developed a physiologically relevant model of the lung parenchyma based on human pulmonary epithelial H441 cells. When grown at the air-liquid interface, in medium supporting polarisation, this cell culture model recapitulates the expression profile of human Alveolar Type II (ATII) pneumocytes (PMID:34977272). Our surfactant air-liquid interface (SALI) model is also sustained in long-term culture unlike primary ATII cells. We used the SALI culture system to generate a model of ABCA3 surfactant protein deficiency, a rare recessive disorder causing severe respiratory distress in babies, to progress lentiviral gene therapy for this condition. To generate homozygous ABCA3 knockout (KO) H441 cells, three separate gRNAs targeting exon 5 of *ABCA3* were designed. Submerged cells were transfected with the synthetic gRNAs and Cas9 ribonucleoprotein (RNP) complexes using lipofectamine. H441 cells were cultured for three passages and sorted for single cells using FACS. Individual clones (n=95) were genotyped using Sanger sequencing. One clone showing a frameshift mutation and premature stop codons in exon 5 also showed a complete absence of ABCA3 protein by Western blotting. The H441 ABCA3-KO cells have been grown as SALI cultures to assess the shift in expression of surfactant related genes after airlift. Functional studies include measurement of transepithelial electrical resistance (TEER), lipid dysregulation by DPPC-release assay, and the presence of surfactant organelles (lamellar bodies). In the absence of a robust murine model for ABCA3 deficiency this ABCA3-KO SALI model can be used to test genetic therapies.

P11

Commercial scale production (1000L) of AAV using the Gibco™ CTS™ AAV-MAX production system

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Until relatively recently, AAV production has taken place primarily in adherent cultures using 293T cells in the presence of fetal bovine serum (FBS). Adherent systems suffer from a number of shortcomings including difficulty in scaling up, the presence of the SV40 large T antigen in the producer 293T cell line as well as cost, consistency and regulatory considerations from the use of animal sera. To address these shortcomings, we developed the Gibco™ CTS™ AAV-MAX Helper-Free AAV Production System (AAV-MAX), an all-in-one, chemically defined, suspension-based

AAV production system that allows for scalable, high titer production of AAV viral vectors in a non-293T cell lineage. The system comprises all of the components required for scalable AAV production: a clonally-documented, 293F-derived producer cell line (VPC 2.0), a chemically-defined growth and expression medium, a production enhancer, a cationic lipid-based transfection reagent and booster, a chemically-defined complexation buffer, and a polysorbate 20-based lysis buffer.

In the present study, we demonstrate consistent AAV production and cellular kinetics of expression from ml scale in multi-well plates and shake flasks to 50-1000L scales in single-use stirred-tank bioreactors using AAV-MAX. Additionally, we provide guidance around scalable downstream purification and strategies for analytical characterization of the AAV particles for a seamless end-to-end AAV production process. These data demonstrate that AAV-MAX is a flexible platform for scalable, high-titer AAV production in a streamlined protocol that maximizes viral titers while reducing time and cost of production, thus providing a suitable platform for AAV production from basic research through clinical and commercial production.

P12

Lenticlair™ 1: A phase 1/2 trial evaluating the safety, tolerability and efficacy of an inhaled F/HN-pseudotyped lentiviral vector for CF gene therapy in people with CF ineligible for CFTR modulators

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This first-in-human Phase 1/2 trial will evaluate the safety and efficacy of BI 3720931, a third-generation lentiviral vector, in people with CF (pwCF) who are genetically ineligible for CF transmembrane conductance regulator modulator therapy (CFTRmt).

pwCF: ≥18 years; percent predicted forced expiratory volume in 1 second ($ppFEV_1$) 50–100% at screening; no recent exacerbations; ineligible for CFTRmt; naïve to prior GT, or prior exposure to non-viral/viral GT with drug-free intervals of 6/24 months. Phase 1: open-label dose-escalation trial (n=9) of single low/medium/high-dose nebulised BI 3720931 (3/group) plus standard of care (SoC) with 24 weeks' follow-up (FU). Primary endpoint: drug-related adverse events (AEs) within

24 weeks. Secondary endpoints: change from baseline $ppFEV_1$ ≥5% within 8 weeks; absolute change from baseline in $ppFEV_1$ at Week 24; dose-limiting toxicity up to Week 24. Bronchoscopy will be performed to quantify CFTR expression. Interim data from ≥8 weeks post-dose will inform Phase 2 dose selection. Phase 2 (n=27): randomised, double-blind, placebo-controlled, dose-expansion trial of single-dose nebulised BI 3720931 (2 dose levels), or placebo plus SoC (1:1:1) with 24 weeks' FU. Primary endpoint: absolute change from baseline in $ppFEV_1$ at Week 8; secondary endpoints: absolute change from baseline in $ppFEV_1$ at Week 24, and occurrence of serious and drug-related AEs up to Week 24.

The trial is estimated to begin in Q2 2024.

The trial will evaluate safety and efficacy of BI 3720931 over 24 weeks in pwCF who are ineligible for CFTRmt.

P13

Lentiviral gene therapy for fatal ABCA3 surfactant protein deficiency

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ABCA3 surfactant protein deficiency is a rare recessive disorder causing fatal respiratory distress in full-term babies, or interstitial lung disease in early childhood. Mutations in *ABCA3* interrupt lipid packaging into lamellar bodies prior to release of surfactant into the alveoli. Any surfactant produced has an altered composition making it inefficient at reducing lung surface tension. In the UK, palliative care is the only option for infants diagnosed with severe ABCA3 deficiency at birth. Here, recombinant Simian Immunodeficiency Virus (rSIV) pseudotyped with Sendai virus F and HN glycoproteins (rSIV.F/HN) was evaluated for ABCA3 expression and correction of ABCA3 deficiency. rSIV.F/HN directed dose-dependent ABCA3 expression in human H441 lung cells (MOI 1-40) and in murine lungs *in vivo* (BALB/c; n=5 per dose). Phenotypic correction was evaluated by detoxification of doxorubicin (10mM) in H441 cells engineered to overexpress ABCA3 mutations. Cell viability (MTT assay) was reduced by 5-12% in doxorubicin-treated *ABCA3*-mutant cells (compared with wild-type) but was restored by treatment with rSIV.F/HN expressing ABCA3. To mitigate potential side effects of off-target ABCA3 expression, new promoters driving alveolar-restricted gene expression were evaluated. *In vivo* imaging of luciferase expression in murine lungs (BALB/c; n=10 per group) showed expression was significantly more restricted (compared with e.g. the Ef1a-derived hCEF promoter; $p<0.0001$) with minimal loss in overall lung expression ($p>0.05$). Vector configuration will be further investigated in ABCA3-knockout murine and cell models. These studies support the potential for a lentiviral vector expressing ABCA3 in alveolar cells as a new treatment modality for ABCA3 surfactant protein deficiency.

P14

Administration of lentiviral vector doses achieving high transduction efficiency is well tolerated in mice

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We have developed rSIV.F/HN, a lentiviral vector pseudotyped with the F and HN proteins from Sendai virus, for cystic fibrosis gene therapy. We assessed whether acute toxicity occurred following administration of rSIV.F/HN into murine lungs at doses anticipated to transduce ~10% of airway epithelial cells.

C57BL/6 mice received once-daily doses of rSIV.F/HN vector expressing EGFP ($2x10^8$ transduction units (TU)/mouse) or diluent for 4 consecutive days via intranasal instillation (total $8x10^8$ TU/mouse). On days 2 or 7 after the last dose, mice were euthanised (18 of each sex/group/day). Body weight, blood (clinical pathology and cytokine analysis) and lung tissue (organ weight, histopathology, qRT-PCR and quantification of EGFP-expressing airway cells) were collected.

There were no vector-related clinical observations or mortality. Increase in body weight 7 days after the last dose was lower (0.1 g, n=8) in vector-treated female mice (n=8) than in controls (0.8 g, n=9). A number of inflammatory cytokines (RANTES, MCP-1, TNFα) were modestly (maximum threefold) elevated or decreased (VEGF, MIP1α) at one and/or both of the time points. Microscopic examination of lung tissue 2 days after the last dose, indicated minimal-to-mild perivascular-to-peribronchovascular infiltrates of primarily mononuclear cells, which returned to minimal severity 7 days after the last dose. ~35% ± SD 5.7 of airway epithelial cells expressed EGFP and levels of vector-specific mRNA were more than 3 log orders higher than endogenous CFTR mRNA.

These data demonstrate that rSIV.F/HN transduction of mouse lungs is well tolerated at doses that achieved significant transduction levels.

P15

Lenticlair™-ON: An extension trial examining long-term safety and efficacy outcomes associated with an inhaled F/HN-pseudotyped lentiviral vector for CF gene therapy in people with CF

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BI 3720931, a third-generation lentiviral vector, will be assessed in a first-in-human (FiH) Phase 1/2 trial in people with cystic fibrosis (pwCF) who are genetically ineligible for CF transmembrane conductance regulator modulator therapy. All trial participants enter an extension trial for long-term follow-up (FU; 15 years, as per regulatory guidelines) of safety and efficacy. Participants may be redosed in the extension trial to maintain efficacy (if supported by data).

Primary endpoint: occurrence of delayed adverse events (AEs) up to 15 years post-enrolment, including risks outlined by regulatory guidance documents for integrating gene therapies. Secondary endpoints: time to loss of efficacy (drop to <5% [absolute] above individual baseline in percent predicted forced expiratory volume in 1 second [ppFEV$_1$]); change in ppFEV$_1$ at 8 weeks post-redosing; time to loss of efficacy post-redosing; time to first pulmonary exacerbation from last dosing; occurrence of all AEs; evidence of plasma replication-competent lentivirus. Interim analyses: at FiH trial end, then every 2 years. Long-term FU: quarterly visits Years 1–2; annual visits Years 3–15; more frequent FU in the first 2 years after redosing. Durability of clinical effect after the FiH trial dose will inform redosing in the extension trial; efficacy and safety of redosing will be determined.

This extension trial will allow seamless rollover of the first participant from the FiH trial.

This trial will investigate long-term safety and efficacy duration in pwCF treated with at least one BI 3720931 dose, and determine efficacy and safety after redosing, as applicable.

P16

Mitigating Indel Impact: Precision Genome Editing through Homology Independent Targeted Integration (HITI)

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Objectives: The efficacy of homology-independent targeted integration (HITI) for disrupting dominant gain-of-function mutations was investigated. This was exemplified by targeting mutations in the clinically relevant *SERPINA1* gene encoding Alpha-1-antitripsin (AAT), which cause misfolded aggregates in the liver and the loss of AAT protective function in the lungs leading to Alpha-1-antitripsin deficiency (AATD).

Methods: HEK293T HITI reporter cells were transfected using *mNeonGreen* donor plasmids targeting an mCherry reporter cassette and *SERPINA1*. Editing was assessed by fluorescence imaging and droplet digital PCR. The integrated sequence was analysed using sanger sequencing of TOPO clones and next generation sequencing (NGS) of pooled genomic DNA. High resolution melting (HRM) analysis confirmed the status of indels throughout the edited sequence.

Results: HITI successfully achieved targeted gene disruption and replacement (6.3% editing) in the HITI reporter cell line at the *SERPINA1* locus. Notably, indels were restricted to donor-genome junctions, preserving donor sequence integrity and minimizing unintended mutations. Interestingly, NGS erroneously reported additional indels within the integrated donor sequence, but further analysis by HRM suggested these sequences were indistinguishable from known control sequences ($p>0.9999$).

Conclusion: This study demonstrates the use of HITI for precise gene editing of *SERPINA1*, offering the potential to address dominant gain-of-function genetic disorders such as AATD, while mitigating the risk of unintended mutations.

P17

Functional analysis of gene-edited CF variant G542X

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The Cystic Fibrosis (CF) causing variant G542X (**G**GA>**T**GA) results in premature termination of translation of the cystic fibrosis transmembrane regulator (CFTR) protein, and nonsense-mediated decay of the CFTR mRNA resulting in almost complete loss of functional CFTR protein expression. This leads to defective anion transport and the development of CF disease pathology. Currently available CF modulator therapies cannot be used to treat this variant, but an Adenine Base Editor (ABE) and guide RNA combination can convert the G542X stop codon to G542R, a variant which retains about 30% of WT activity and is amenable to modulator therapy. Plasmid DNA encoding this ABE, guide RNA, and GFP were encapsulated in lipid-based nanocomplexes, which have demonstrated reduced immunogenicity and high uptake in epithelial cells, were

delivered to human airway epithelial cells (HAEC) harboring the G542X CFTR variant. Cells with positive GFP fluorescence were then concentrated using fluorescence-activated cell sorting to achieve a cell population enriched with the G542R variant. This rendered the population amenable to treatment with modulators, and provides proof-of principle for the use of ABE to correct G542X with potential for re-engraftment. Cells were assessed for functional improvements demonstrated by improved RNA and protein abundance, increased ion transport (measured as short-circuit current), wild-type-like airway surface liquid height and pH, and decreased time required for wound repair.

P18

Cas9 nickase-mediated contraction of CAG/CTG repeats at multiple disease loci

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Expanded CAG/CTG repeats cause at least 15 different neurodegenerative and neuromuscular diseases that all remain without an effective disease-modifying treatment. Because the repeat tract size accounts for most of the variation in disease severity, contracting them presents an attractive therapeutic avenue. Here, we show that the CRISPR-Cas9 nickase targeting the CAG/CTG repeat itself leads to efficient contractions in Huntington's disease patient-derived neurons and astrocytes, as well as in myotonic dystrophy type 1 patient-derived neurons. Using single-cell DNA sequencing, PCR-free whole genome sequencing, and targeted long-read sequencing of the *HTT* locus, we found no off-target mutations above background in neurons and astrocytes. Furthermore, we delivered the Cas9 nickase and sgRNA stereotactically to a mouse model of Huntington's disease using adeno-associated viruses and found contractions accumulating in over half of the infected cells over a period of 5 months. We also found that the Cas9 nickase was prone to silencing, further improving the safety of the approach. Our results provide proof of concept for using the Cas9 nickase to contract the repeat tract safely in multiple cell types and diseases.

P19

Normothermically perfused livers as a model for profiling lentiviral pharmacokinetics and transduction

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Lentiviral-based vectors (LV) have been of special interest due to their ability to transduce non-dividing cells and to deliver large genetic payloads for long-term stable expression in target cells. For certain inherited diseases affecting the liver, LV gene therapy may offer a curative approach. However, the utility of LV for production of physiologically relevant levels of encoded protein is dependent on its stability in blood, evasion of Kupffer cell clearance, transduction of hepatocytes and integration into the host genome. At present preclinical models of appropriate scale and physiology to allow assessment of all these factors are lacking, contributing towards difficulty in translation into humans.

Normothermic machine perfusion can maintain physiological function of whole human-sized livers *ex vivo* for prolonged durations. It has already enabled prediction of human pharmacokinetics of oncology agents. Here we report the use of perfused porcine livers as a model for profiling LV pharmacokinetics and transduction. Livers were perfused for >36 hours with evidence of biochemical homeostasis. An HIV-based LV encoding GFP driven by a liver specific promoter was delivered via the portal vein and distributed into liver tissue within 4 hours. By 36 hours there was a 2-3x increase in vector copy number (VCN) which was sustained at 39 hours. Spatial distribution of vector integration was homogenous across liver tissue with mean 0.024 VCN/cell.

Furthermore, the enhancement of liver transduction offered by the application of externally focussed ultrasound was also assessed. These proof-of-concept studies are now being developed to test outputs using perfused human livers.

P20

The new chromatin opening models as a strong potential tool for recombinant production and gene therapy applications

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Our new chromatin opening models we recently developed which free from potential mutation sites that also considerably smaller (500bp) than the current UCOE models used in gene therapy and recombinant protein biotechnology studies, were tested on various cell groups, including mouse embryonic stem cells and human İPS cells.

It has been demonstrated in terms of replacing existing UCOE models that these new UCOE candidates we have developed are more efficient than the previous ones and that they are also a

safer profile model for clinical gene therapy studies since they are free from additional enhancing vector cassette areas. These new UCOE models ubiquitously shows a powerful resistance to DNA methylation- mediated silencing and also provides a higher and stable transfection profile.

To understand the potentiality of our new generation UCOE designs in gene therapy studies, it was tested whether the universal chromatin opening abilities will be retained stable of activation on human induced pluripotent stem cells by differentiating them into different tissue cell types as done before on mouse embryonic stem cells.

In the light of the obtained results, the new UCOE designs that we developed, have maintained their expression levels stably on human iPS cells before and after differentiation into three different tissue type cells. And additionally, they also showed their potential on monoclonal antibody production with CHO cells as producing mg and gr level of recombinant antibodies into two months of period in another parallel study we conducted.

Moreover, by the urgent need of vaccine development for COVID-19 during the pandemic, we aimed to produce a potential recombinant vaccine by using this new chromatin opening models of our own design. For this purpose a part of the spike and nucleocapsid gene sequences of COVID-19 were firstly cloned into our UCOE models. These UCOEs plasmids were then transferred into 293T cells along with plasmids carrying the genes that will form the lentivirus vectors (LVs). After harvesting and calculation of LV vector titers, the cloned vectors were then transfected into the CHO cells which the targeted recombinant production of the antigen proteins will be carried out. Antigenic structures were then isolated from the culture medium of CHO cells in following days for confirmation. Using HPLC and qTOF mass spectrometer methods, these structures in the medium were confirmed to be the units of spike and nucleocapsid proteins of the COVID-19 virus. In order to produce large amount of the recombinant antigens, the culture was then carried out with bioreactors in liters. At the final stage, these recombinantly produced antigen proteins were tested on rats to measure their immunogenic responses, and the study recently been completed successfully as a potential recombinant vaccine against COVID-19.

P21

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

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Achieving high upstream lentiviral vector (LV) titres is fundamental to the development and manufacture of commercially viable gene therapy products. 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 screening experiments revealed 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 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 these 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 recover log-fold-increases in titre without detriment to product quality attributes particularly for large, multiple, or complicated expression cassettes.

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 their transfer to GMP production.

P22

Targeted, non-viral delivery of gene editing technologies for the treatment of ASL deficiency

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Argininosuccinate lyase (ASL) deficiency is a rare genetic disorder caused by a deficit in the ASL enzyme. This deficiency leads to argininosuccinic aciduria, characterised by hyperammonaemia and multi-organ disorder. CRISPR technologies provide an attractive therapeutic approach for liver monogenic diseases now reaching clinical translation stage. We aim to fully restore the function of the deficient gene to improve the long-term outcomes, especially the most severe patients presenting with acute metabolic decompensation as newborn or in infancy, and reduce the overall burden on the healthcare system.

Here, 4basebio's synthetic, enzymatically produced DNA, and Hermes™ nanoparticles are combined to generate proof-of-concept data on a corrective strategy for ASL deficiency. Hermes™ nanoparticles were deployed to encapsulate Cas9 mRNA or Cas9 protein along with an sgRNA targeting the ASL gene in the human hepatocyte derived Huh7 cell line. The mRNA was produced using 4basebio's synthetic opDNA™ template. Hermes™ nanoparticles had favourable biophysical characteristics, stability, and achieved high cell viability, with knockout efficiency >95%.

To investigate a gene-integration approach using 4basebio synthetic DNA, we designed an oeDNA™ template encoding a GFP sequence with homology arms flanking the target gene. The co-delivery of template DNA along with the Cas9 mRNA/gRNA encapsulated in Hermes™ nanoparticles resulted in successful integration of the oeDNA template, and GFP expression in vitro.

The present study provides proof-of-concept for the use of Hermes™ particles to deliver RNA or Ribonucleoprotein complex (RNP) and donor DNA templates to achieve targeted integration, offering a promising approach to treat monogenic diseases such as ASL deficiency.

P23

Conquering gene editing off-targets with Gibco™ CTS™ HiFi Cas9 Protein

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Gibco™ CTS™ HiFi Cas9 Protein is the latest addition to Thermo Scientific's CRISPR/Cas9 portfolio. With this new product, we are now offering researchers working in a clinical environment a low off-target solution for their genome editing projects. It is designed and manufactured in compliance with GMP principles and to meet requirements of standards for Ancillary Materials for Cell, Gene, and Tissue-Based Products, including USP <1043>, Ph.Eur. 5.2.12, and ISO 20399.

A panel of sgRNAs targeting therapeutically relevant genes (TRAC, CD52, TRBC and B2M) was used to compare genome-wide off-target and on-target activities of CTS™ HiFi Cas9, wild-type CTS™ Cas9, and Supplier A GMP hi-fidelity Cas9. Cas9/gRNA RNP complexes were delivered to T cells or iPSC by electroporation (EP). Cells were harvested on day 3 or 11 post EP and analyzed using Attune flow cytometer for protein function or next generation sequencing (NGS) for Indel, and HDR. TEG-seq (PMID 30114933 & 37161298) was used for genome wide off-target discovery. TAV-seq was used for off-target validation, on-target editing efficiency.

At tested loci, CTS™ HiFi Cas9 achieved equivalent on target activity compared to wild-type CTS™ Cas9. TCR KO and CAR KI efficiency is as high as wild-type CTS™ Cas9. However, with CTS™ HiFi Cas9 over 90% reduction in off-target effects in primary immune cells was observed, compared to CTS TrueCut Cas9 (wild type) at tested loci. CTS™ HiFi Cas9 is supported by extensive traceability documentation—including COA, COO, Drug Master Files or Regulatory Support Files when required.

P24

Simple, rapid and robust bioluminescent cell-based assay for detecting neutralising antibodies against AAV in serum

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Adeno-associated viruses (AAV) are commonly used in gene therapy. But pre-existing immunity to AAVs can impede cellular uptake and trigger cytotoxic T-cell responses, compromising efficacy and safety. Therefore, a robust and reliable neutralising antibody (NAb) assay is necessary to determine eligibility for treatment as well as to identifying individuals for clinical trials.

Here we present a cell based NanoLuc® luciferase assay for detecting NAbs in serum. The assay improves on existing methods offering; higher sensitivity, lower MOI required (100–3000), shorter 24h assay time and detection using a standard luminometer.

We tested 60 human serum samples for NAbs against a panel of AAV serotypes 1—10 and engineered variant AAV-DJ. A large percentage of the samples had pre-existing NAbs to at least one serotype and of these, the majority displayed NAbs against multiple AAV serotypes. Fewer samples were seropositive for AAV-DJ than for any of the parental serotypes highlighting the benefit of capsid engineering. The assays broad dynamic range allows the serum samples to be categorised into 4 groups: negative, low, medium and high NAbtitres. Samples from each group were assayed for neutralising titres with ND50 values agreeing with their assigned groups.

To demonstrate robustness, we conducted a study with 40 mice divided into 4 groups of AAV9 at varying doses. This showed a strong correlation of NAblevels to the AAV viral loads.

Our NanoLuc® AAV reporter assay is fast, highly sensitive and reliable for NAb detection against AAVs in serum. Further validation is needed before clinical use.

P25

Elevate Your CAR-T Therapy with Plasmid DNA Sanger Sequencing

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Plasmid DNA sequence confirmation is a critical step in the development of CAR-T cell therapy, a revolutionary cancer treatment. This process involves genetically modifying T cells, often using lentivirus vectors, to express a chimeric antigen receptor (CAR) that targets cancer cells. The precision of this modification is vital, as any errors in the DNA sequence of the lentiviral plasmid used for genetic engineering could lead to ineffective or potentially harmful outcomes. Therefore, confirming the DNA sequence of the plasmid ensures that the correct genetic information is being introduced into the T cells. This not only maximizes the therapy's effectiveness but also ensures safety by minimizing the risk of introducing unintended genetic alterations. In the rapidly evolving field of gene therapy, such stringent quality control measures are essential for the success and reliability of treatments like CAR-T cell therapy.

P26

CRISPR/Cas9-based cell engineering approaches to boost recombinant AVV productivity

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Recombinant adeno-associated viral vectors (rAAV) have gained substantial attention following the commercialisation of new gene therapy products. However, current batch production methods utilising human embryonic kidney (HEK) 293 cells are inefficient, hindering cost-effective manufacturing. While various approaches have marginally improved rAAV yields, poor understanding of host cell biology may prevent further yield gains.

In our pursuit of increased rAAV titres, we devised a scalable, transient transfection process [VD1] using a suspension-adapted HEK cell line. Operational parameters were optimized via design of experiments (DoE) on the Sartorius Ambr@15 system, establishing an optimized design space. Scale-up to the Sartorius Ambr@250 system validated the process, yielding comparable titres to small-scale screening studies.

Additionally, we developed GMP-ready monoclonal HEK293 cell lines from the suspension-adapted HEK line. The optimised process was validated across two monoclonal lines, facilitating productivity-based ranking. Subsequent omics studies unveiled differentially enriched pathways, including antiviral defence pathways as potential cause for inefficient AAV productivity. To test this hypothesis, we initiated a proof-of-concept study targeting multiple genes in the cGAS–STINhG pathway using CRISPR/Cas9 technology to assess the impact on rAAV productivity.

Successful development of the multi-gene knockout strategy enables the generation of a CRISPR-mediated target validation platform, which can be applied to other candidate pathways identified through our omics studies. Whilst enhancing our understanding of key biological mechanisms in rAAV production, this will lead to the generation of novel, GMP-ready, engineered HEK lines with a potentially higher productivity, which can increase the industry gene therapy manufacturing capacity.

P27

High throughput pegRNA screening platform using lentiviral delivery

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Prime Editing (PE) has good flexibility in the scope for targeting pathogenic mutations, but selecting the most appropriate prime editing guide RNA (pegRNA) to correct any target remains a significant challenge. This challenge is mainly due to the large number of possible permutations in the 3' extension region of the pegRNA. To address this, a platform was developed utilising a HEK293T cell line expressing PEMax-eGFP for pooled lentiviral screening of pegRNA candidates, containing a unique barcode and synthetic DNA target sequence for high throughput interrogation of pegRNA performance. The HEK293T-PEMax-eGFP cell line was generated by lentiviral transduction and single cell clones (n=6) were expanded to ensure homogenous editing performance throughout. Plasmid transfection of enhanced pegRNA targeting the endogenous HEK3 locus identified one HEK293T-PEMax-eGFP clone that demonstrated 33% of the intended edit. The integrity of this approach was confirmed by lentiviral transduction of one library element targeting the HEK3 locus, which resulted in the desired insertion of CTT in HEK3 loci in the synthetic target (22.5%-35%) and in the endogenous loci (50%-75%). As a clinically relevant exemplar, a synthetic library was generated targeting the common F508del mutation in the CFTR locus (comprising 445 pegRNAs with 9 additional pegRNAs as positive and negative controls). Assembly of the library was confirmed with 100% incorporation and an average of 300x coverage for each library element. A series of whole library transductions has been performed and data analysis is underway to identify candidate CFTR F508del pegRNAs that could have therapeutic potential.

P29

AAV-Kir4.1 astrocyte gene therapy reduces seizure frequency in rodent models of acute and chronic epilepsy

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Kir4.1 is an ATP-sensitive inwardly-rectifying potassium ion channel encoded by the *KCNJ10* gene. It is expressed exclusively in glial cells and plays a key role in regulating spatial K^+ buffering of astrocytes. Kir4.1 loss-of-function results in increased extracellular K^+ level and impaired glutamate uptake that can cause neuronal hyperexcitability and seizures. A functional decrease in Kir4.1 expression has been reported in human acquired and genetic epilepsies, and in rodent models of epilepsy. Thus, it is hypothesized that upregulation of Kir4.1 in astrocytes will counteract pathogenic rises in extracellular K^+ and glutamate to prevent transition to seizures.

To investigate whether Kir4.1 over-expression could reduce seizure susceptibility, we injected unilaterally into the somatosensory and visual cortex with either AAV9-gfaABC1D-Kir4.1-tdTomato or AAV9-gfaABC1D-tdTomato vector. 2-3 weeks post-injection, electrographic recordings were performed in awake head-fixed mice and acute seizures induced by focal injection of chemoconvulsant (picrotoxin) to the transduced area of cortex. Mice over-expressing Kir4.1-tdTomato had fewer seizures than those expressing tdTomato alone.

Animals were made chronically epileptic by kainic acid injection into the right hippocampus. Telemetry devices were implanted to record baseline seizure frequency 2-3 weeks following acute status epilepticus. Animals were then treated with AAV-PHP.eB-gfaABC1D-EGFP-Kir4.1 or AAV-PHP.eB-gfaABC1D-EGFP vector injected into the right hippocampus. 2-4 weeks post-treatment, electrocorticography was recorded and analysed using a semi-automated program to quantify seizures. Kir4.1 over-expression significantly reduced seizure frequency normalised to baseline levels compared to GFP-treated mice. We conclude that AAV-Kir4.1 astrocyte gene therapy is effective in reducing seizure frequency in rodent models of acute and chronic epilepsy.

P30

AAV-shRNA mediated knockdown of CDKL5 to generate a novel human *in vitro* model of CDKL5 Deficiency Disorder

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CDKL5 Deficiency Disorder (CDD) is a rare developmental epileptic encephalopathy, arising due to *de novo* mutations in the gene encoding the serine-threonine kinase CDKL5. Patients with CDD generally experience early onset recurrent seizures coupled with severe developmental delay. At present there is no effective cure for CDD. Whilst recent research has highlighted key functions of CDKL5 in neuronal cells in the brain, many of these findings are based on mouse models which

do not accurately recapitulate key aspects of the human phenotype. As such, there is a need for novel human models of CDD to interrogate CDKL5 function in multiple cell types in the brain – from excitatory and inhibitory neurons to astrocytes and other glial cells. Here, we present an adeno-associated virus (AAV) -mediated short hairpin RNA (shRNA) knockdown of CDKL5 driven by the constitutive U6 promoter, in adult human cortical organotypic brain slices obtained from anonymous patients undergoing surgical removal of deep brain tumours. In human cortical organotypic brain slices transduced with the shAAV-CDKL5 vector, CDKL5 knockdown lead to functional deficits as assessed by disrupted phosphorylation of known targets of CDKL5 and network changes in extracellular field potential recordings. Thus, this novel model of CDD provides an interesting platform to interrogate both cell-type specific and network consequences of CDKL5 deficiency, and could be used in the future to screen novel pharmacological and gene therapy treatments for CDD.

P31

4basebio's proprietary enzymatic process via Trueprime™ amplification technology, manufactures high-quality, GMP-grade DNA for gene therapy and vaccine applications

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The manufacture of high-quality, GMP-grade DNA, crucial for gene therapy and vaccine applications, faces significant challenges including capacity limitations and complex sequence propagation issues. 4basebio has developed a proprietary, scalable, fully enzymatic process via our Trueprime™ amplification technology, yielding DNA at 1g/L; several orders of magnitude higher than plasmid fermentation yields. Our platform surpasses conventional plasmid fermentation, promising high-quality DNA with minimal footprint and turnaround time.

4basebio's process is size and sequence independent, ensuring high yield and purity in less than a week. 4basebio easily produces complex sequences including ITRs and homopolymeric sequences including long polyA tracts, without risk of deletion or recombination. Unlike plasmid DNA, 4basebio DNA eliminates contamination from endotoxins or host proteins, and excludes bacterial sequences such as antibiotic resistance genes.

Four types of application specific DNA are offered. opDNA™, optimized for mRNA production, enhances yields without the need for enzymatic linearization. Proinflammatory cytokine/chemokine levels in human PBMCs and in vivo protein expression are comparable to mRNA produced from linearised plasmid.

hpDNA™ can be used in the production of AAV, replacing conventional plasmid triple-transfection. Using hpDNA™, viral genome titres, Full:Empty ratios and infectivity are equivalent to plasmid controls, across a range of serotypes.

Finally, Hermes™, 4basebio's proprietary non-viral delivery system, encapsulates various payloads and can target specific cells or tissues. It offers long-term stability at 4°C, eliminating cold chain requirements. The combination of 4bb DNA or mRNA and Hermes™ nanoparticles can greatly accelerate the therapeutic development of gene therapy and vaccine programmes.

P32

Transfer of an AAV production platform from bench-scale to 50 L: experiences, challenges and learnings

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A key challenge for Adeno-Associated Virus (AAV) process development teams working at bench-scale (15 mL to 2 L) is to ensure the process is suitable for scale-up (50 L and beyond) and transfer to GMP to support clinical studies and commercial supply. Cell and Gene Therapy Catapult have recently transferred an AAV production platform from 2 L process development to 50 L MSAT and then to GMP manufacturing. An overview of the experiences and insights gained during the optimisation and scale-up work, highlighting the complexities faced during the most challenging steps of the upstream and downstream processes, will be discussed. Furthermore, the valuable lessons learned, and the solutions being implemented to address the remaining challenges effectively, will be shared.

P33

Automation and comparability of qPCR and ddPCR for AAV genome titration for in-process samples

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qPCR is one of the assays that is deployed routinely to determine genomic titre of produced AAV samples within the manufacturing process. Titre is used for estimating the effective therapeutic dose for this vector when testing it in preclinical or clinical trials. The method is complex, time consuming, and prone to human error due to the number of manual sample manipulation steps. As such, ddPCR is principally preferred as a technique for genomic titre analysis due to its high reproducibility, low sensitivity to impurities and eschewing calibration curve. However, due the availability of equipment and costs of procedures, this can deter laboratories from transferring genomic titre analysis to ddPCR. Automation has been shown to significantly reduce laboratory hands-on time, costs and errors. In this study we scripted manual steps of those two methods using an advanced liquid handler Tecan Fluent® and compared them by analyzing in process samples during AAV manufacturing. The enhanced precision resulting from reduced human error and elimination of operator-to-operator variability improved the comparability between qPCR and ddPCR methods. Our findings indicated that both techniques yielded comparable titers across sample types with the exception of TFF1. Notably, qPCR demonstrated advantages of lower consumable costs, increased throughput, and a wider dynamic range while both methods exhibited high precision and repeatability. In conclusion, the cost-effective, high-throughput nature, and widespread availability of qPCR make it a suitable choice for AAV genomic titration comparably to ddPCR when both methods are automated, achieving reliable genomic titre results for in-process AAV2 samples.

P34

Mechanistic and data driven models for optimisation and troubleshooting cell and gene therapy processes

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The process of lentiviral vector production is complex and presents unique challenges. When developing new products obtaining the optimal titre (best productivity) can be critical to ensure clinical and commercial success, therefore, optimising the plasmid ratios and culture conditions is key. Fast and efficient scale-up of manufacture and control of large-scale bioreactors presents additional challenges.

The Computer-aided Biology group at OXB has cultivated a unique modelling hub with digital and physical architecture that combines data, models and robotics. This modelling hub allows both data driven and mechanistic models to be run easily by operators.

We will present the following case studies:

Use of data driven approaches to optimise plasmid ratios based on historic data and associated metadata. Use of mechanistic models to troubleshoot large scale challenges.

In Case Study 1 – Advanced DoE Methods, we will discuss the use of data driven approaches to optimise plasmid ratios. Our platform routinely optimises 5-10 factors with the capacity for modelling 100+ variables. Here, we show the generation of a custom DoE via our unique framework, giving optimal space coverage, automatic model diagnostics, automated execution via liquid handler and in silico analysis and reporting.

In Case Study 2, we will discuss the use of a mechanistic model to troubleshoot large scale challenges. We will present an omics enforced mechanistic model of cell health, metabolism, and vector production. Our model allowed us to highlight cell growth abnormalities, recreating the phenotype at small scale and explore solutions to resolve issues.

P35

The TetraVecta™ System: A new tool kit enhancing lentiviral vector production for the next generation of gene therapies

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Lentiviral vectors (LVs) remain the most established and efficient means to deliver transgenes to non-/dividing cells. The successes of *ex vivo* gene medicines using LVs are a springboard for their wider use *ex vivo* and their development for *in vivo*. The configuration of 3rd generation LV genomic sequences has not changed for two decades. To provide an LV platform better suited to

wider use, we have generated the TetraVecta™ System with engineered LV genome sequences yielding improvements in four key areas.

Quality. The '2KO' feature eliminates aberrant RNA splicing derived from the LV packaging signal during production, common to 3rd Gen LVs. This results in simplified vector RNA profiles, ensuring only full-length vector genomic RNA is converted into cDNA in target cells. 2KO-genomes also reduce transgene expression during production, especially when paired with the TRiP System™, minimising the presence of transgene protein within LVs particles.

Safety. The polyadenylation (polyA) sequences within 3rd Gen self-inactivating (SIN)-LTRs are suboptimal; our 'sequence-upgraded polyA' (supA)-LTRs improve polyA activity by >50 fold. This imparts transcriptional insulation to integrated LVs such that cellular gene 'read-in' and transgene 'read-out' are greatly minimised. Transgene expression is also increased 2-3 fold.

Capacity. The 'MaxPax' form of the TetraVecta™ system has increased payload capacity thanks to removal of ~1kb of backbone sequence and is also rev-independent.

Production. Combining several/all of these features, alongside using novel production enhancers, simplifies the Upstream and Downstream aspects of LV production, reducing time spent on process development and often resulting in greater yields.

P36

*Rev*IT™ A Universal AAV Enhancer and *Trans*IT-VirusGEN® transfection reagent for Improved AAV Manufacturing

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With an exponential increase in the number of registered clinical trials using recombinant adeno-associated virus (AAV) for gene therapy, efficient viral vector manufacturing by transient transfection is now in high demand to ensure maximum yields and reduced costs. To support AAV production platforms, Mirus Bio developed *Rev*IT™, an innovative small molecule-based AAV enhancer, which is compatible with *Trans*IT-VirusGEN® Transfection Reagent or other transfection reagents. *Rev*IT™ increases higher genome titers 2-4X and improves percentages of full capsids in a range of suspension HEK 293 types and cell culture media when used to produce AAV2, AAV5, AAV8, and AAV9. Additionally, when combined with the *Trans*IT-VirusGEN® Transfection Reagent, this enhancer not only yields the highest titers but also allows for the use of reduced amounts of plasmid DNA which is considered a key cost-saving improvement. We also optimized *Rev*IT™ with TransIT-VirusGEN® at a 2L benchtop bioreactor which shows linear scalability. These studies also did not negatively impact cell viability. Overall, we demonstrate that the *Trans*IT-VirusGEN® Transfection Reagent in combination with *Rev*IT® AAV enhancer offers scalable transfection solutions with simplified, cost-effective workflows to produce high AAV titers without sacrificing quality. Moreover, since *Rev*IT™ can be seamlessly dropped into other transfection workflows, it can serve as a turnkey solution for established platforms requiring higher titers. Overall, Mirus offers solutions for cell and gene therapy developers by consolidating high performance and scalability to support cost-effective production to support more patient doses per manufacturing run.

P38

High-throughput purification method for adeno-associated viruses using capture plates

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High-throughput process development for AAV products is challenging; the upstream process generates an AAV containing cell lysate in which many CQAs cannot be assessed due to low titre and sample complexity. The sample volume available from small-scale USP optimisation studies, taking place in multiwell plates or bioreactor systems, prevent samples from being processed through standard DSP operation units which are low throughput and can take >10h for the purification of one sample. Capture plates implement affinity chromatographic techniques to allow for the rapid clean-up of up to 96 AAV containing lysate samples in a single process. The samples produced by capture plates have sufficient titre and purity for most analytical methods, CQA's such as percentage full capsids to be obtained from milli-scale upstream processes through characterisation techniques such as SEC-MALS, HPLC and mass photometry which have low sample volume requirements.

P39

Integrated metrology approaches for the characterization of viral vectors: From mass spectrometry to molecular assays

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Viral vectors are key components used in biotherapeutic applications including gene therapy and as vaccine delivery agents. The measurement of their critical quality attributes: identity, purity, potency, titre, safety, and stability and associated sources of analytical bias and error are essential, making the role of sound measurement science in viral vector characterization of utmost importance. This study focuses on developing and investigating the performance of methods used for the characterization of recombinant adeno-associated virus (rAAV) using a combination of mass spectrometry, PCR-based molecular assays. Mass spectrometry was employed to identify a unique peptide specific to each AAV9 viral capsid protein and a peptide present in every capsid protein, enabling absolute quantification of the viral capsid proteins using Stable Isotope Dilution (SID) and measurement of the ratio VP1:VP2:VP3 in AAV9. We developed improved digital PCR (dPCR) approaches to assess genome integrity and provide linkage information between different genome targets. Furthermore, we identified potential sources of bias and error in our developed dPCR approach, and we demonstrate the critical role of genome target selection in dPCR measurements of AAV titre, emphasizing its impact on measurement variability. Additionally, we show that multiplex dPCR can be used effectively for genome integrity assessment of AAV vectors, surpassing the limitations of current methods.

P40

A novel, perfusion-based lentiviral vector manufacturing process demonstrating increased productivity and enhanced purity

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For over 25 years Oxford Biomedica (OXB) has been a pioneer in the development of products and innovative technologies based on lentiviral vectors. To meet the forecast on vector demand for gene and cell therapies, OXB has recently introduced a next-generation transient lentiviral manufacturing process incorporating innovative process modifications which simultaneously enhance lentiviral process yields and improve vector quality attributes. This new process utilises perfusion technology to support vector production at higher cell densities than have been associated with traditional batch and fed batch production approaches for lentiviral vectors. This approach allows for significant process intensification without the need for increased bioreactor volume. Furthermore, using this technology, lentiviral particles secreted into the bioreactor, can be harvested using continuous perfusion which also allows stabilisation of generated vector at lower temperatures, leading to improved preservation of the final product.

Importantly, this process adopts a plug and play approach facilitating incorporation of small molecule enhancers, some of which have already been identified by OXB. For example, incorporation of U1 (RNA based enhancer) and I3A during vector production, using this new process, led to increased cell-specific productivity. Overall, process yield improvements from 2 to 10-fold have been demonstrated for a range of therapeutic lentiviral vectors with process scalability demonstrated in stirred tank bioreactors up to 200 L in GMP.

This new manufacturing platform offers significant benefits for clinical production of lentiviral vectors and will further enable OXB to support the continuing global demand for high quality gene and cell therapy products.

P41

Comparison of droplet digital PCR and real-time quantitative PCR for lentiviral vector titration

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We have developed recombinant simian immunodeficiency virus (rSIV) pseudotyped with Sendai virus F and HN proteins (rSIV.F/HN) for treatment of lung diseases.

To facilitate clinical advancement, we routinely produce highly purified (anion-exchange/TFF), highly concentrated (target >1e9 TU/mL) Lots of rSIV.F/HN carrying a range of transgenes. We typically use real-time quantitative PCR (qPCR) assays for titration of vector particles (VP/mL; via Reverse-Transcription (RT)-qPCR quantification of viral RNA extracted from vector Lots) as well

as transducing units (TU/mL; via quantification of integrated vector genome copies on genomic DNA extracted from cells transduced with serial dilutions of vector), essential factors for quality control and experimental dosing.

In contrast to qPCR, droplet digital PCR (ddPCR) does not require a standard curve and is reported to be more precise and reproducible. We compared qPCR and ddPCR for quantification of VP/mL and TU/mL titres (and respective P:I ratio) of n=18 crude (non-purified/non-concentrated) and n=54 highly purified/highly concentrated vector Lots. Using ddPCR we observed significantly lower VP/mL (~1.6-fold; p<0.0001), TU/mL (~2-fold; p<0.0001) and P:I ratios (~2.5-fold; p<0.0001) for purified Lots. Similarly, when determined by ddPCR, TU/mL titres of crude vector Lots (but not VP/mL titres) were also significantly lower (~2-fold; p<0.001).

Our ddPCR assays (i) showed significantly lower variability (p=0.036 & p=0.0245 for purified and crude vector Lots, respectively) compared with qPCR; (ii) avoided assay failure due to poor standard curve quality, and (iii) allowed TU/mL determination for n=6 crude Lots not quantifiable by qPCR. These data highlight the advantages of ddPCR for quantification of recombinant lentivirus.

P42

Leveraging microfluidics for reproducible high lentiviral transduction efficiency

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In cell culture bioprocesses, noise often overpowers signal. Cell culture bioprocesses suffer from batch-to-batch variability, largely due to manual multi-user interventions. This affects typical bioprocesses indispensable for cell and gene therapy applications, such as transduction. Conventional lentiviral transduction processes methods typically face challenges concerning efficiency. Often, it requires high multiplicity of infection (MOI) to reach acceptable levels of transduction efficiency, consequently resulting in high costs due the costs of viral reagents. Moreover, a high MOI can also increase risks of toxicity. A solution is needed to lower MOI while maintaining high efficiency, whilst also maintaining high precision and reproducibility.

Without precision, achieving consistently high efficiency remains a challenge. In this study, we leverage the benefits of a microfluidics-based bioreactor, designed with low fluid heights and large surface to volume ratios (S/V), where the geometric form-factor microenvironments enhance precision cell-lentivirus interactions. We demonstrate the feasibility using an exemplar CAR-T transduction on Primary T-cells with CD19 lentiviral vector. Using a design of experiment (DOE) methodology, we demonstrate that at lower MOIs, we obtain 2-3 fold transduction efficiency greater than conventional vessels, thereby contributing to lower experimental costs with a higher quality cell product.

We also demonstrate the consistency of the process in these precision bioreactors using a multi-operator experiment. We show evidence of comparability in terms of cell count, viability, expansion and transduction efficiency amongst multiple operators; therefore demonstrating the

robustness of the microfluidics-based bioreactor. Having a precision bioreactor helps de-risk issues related to interoperability, thereby ensuring reproducibility and consistency in cell data.

P43

SpyVector; a modular capsid decoration platform to boost adenovirus vaccine-induced humoral immunity

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Adenovirus (Ad) vector vaccines have been widely and successfully deployed in response to COVID-19. However, while Ad vectors are potent inducers of cellular immunity, improvement of vaccine-specific antibody responses upon homologous boosting is modest compared to other technologies. Here we describe a platform, SpyVector, enabling modular decoration of Ad capsid surfaces with vaccine antigens from a range of target pathogens, and demonstrate potent induction of humoral immunity against these displayed antigens.

Ligand attachment via a covalent bond was achieved using a protein superglue, DogTag/DogCatcher in a rapid and spontaneous reaction requiring only co-incubation of ligand and vector components. DogTag (23 amino acids) was genetically inserted into surface-exposed loops in the adenovirus hexon capsid protein to allow attachment of ligands fused to DogCatcher (a 15 kDa protein domain) on virus particles.

Capsid decoration shielded Ad particles from vector neutralizing antibodies and other undesirable capsid interactors. In both mice and NHP, Ad decorated with the receptor binding domain (RBD) of SARS-CoV-2 Spike (S) induced >10-fold higher SARS-CoV-2 neutralization titers compared to an undecorated Ad encoding S. Importantly, decorated Ad achieved equivalent or superior T cell immunogenicity against encoded antigens compared to undecorated Ad. We extend this work to develop pan-coronavirus vaccines by decorating Ad capsid surfaces with multiple RBDs from diverse coronaviruses with pandemic potential.

We propose capsid decoration as an effective strategy to improve safety, efficacy and boostability of Ad vector vaccines and therapeutics, with its customizable nature offering utility in personalized medicine, pandemic preparedness and outbreak settings.

P44

Combined biolistic and cell penetrating delivery for an effective and scalable intradermal DNA vaccine

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Physical-based gene delivery via biolistic methods involves precipitation of nucleic acids onto

microparticles and direct transfection through cell membrane by high velocity acceleration. The glycosaminoglycan (GAG)-binding enhanced transduction (GET) system exploits novel fusion peptides consisting of cell-binding, nucleic acid-condensing, and cell-penetrating domains, which enable enhanced transfection across multiple cell types. In this study, we combined chemical (GET) and physical (gene gun) DNA delivery systems and hypothesized the combination would generate enhanced distribution and effective uptake in cells.

Initial transfection studies explored formulations to determine the optimal GET-gold-DNA ratio, followed by incorporation into fireable gene gun bullets. Subsequent experiments explored physicochemical characterization, optimisation of bullets and transfection experiments in vitro (monolayers, engineered tissue) and in vivo.

Transfection experiments in cell monolayers and engineered tissue demonstrated these formulations transfected efficiently, including in DC2.4 dendritic cells. We incorporated these formulations into a biolistic format for gene gun by forming fireable dry bullets obtained via lyophilization. Extracted GET bullet contents retained their ability to mediate transfection significantly higher than standard bullet contents. Fired GET bullets in cells, collagen gels and mice showed increased reporter gene transfection compared to untreated controls, whilst maintaining cell viability. Lastly, a SARS-CoV-2 DNA vaccine with spike protein-receptor binding domain was delivered by gene gun using GET bullets. Specific T cell and antibody responses comparable to the conventional system were generated.

The non-physical and physical combination of GET-gold-DNA carriers using gene gun showed potential as an alternative DNA delivery method that is scalable for mass deployed vaccination and intradermal gene delivery.

P45

Size-exclusion chromatography with multi angle light scattering (SEC-MALS) for AAV characterisation

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A challenge for AAV manufacturing and process development is the ability to rapidly characterise quantity and quality for any serotype using at-line, in-line and off-line analytical techniques. Size-exclusion chromatography with multi angle light scattering (SEC-MALS) combines the power of light scattering technology with a size-based separation technique to provide information on molecular weight, size and quantity, with potential for use in-line or off-line. The Cell and Gene Therapy Catapult has developed and qualified an automated and high throughput SEC-MALS method with improved precision and comparable accuracy to orthogonal methods.

P46

Bad and ugly? Mechanisms and implications for payload expression in lentivirus manufacture

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When manufacturing lentivirus using HEK293 based transient transfection systems, high levels of transgene expression has long been known to occur. This high level of expression is seen even when the gene of interest is under the control of tissue specific promoters which are silent in HEK293 cells. Transgene expression during lentivirus production is understood to be problematic for production titres as the transgene may exhibit some toxicity or compete in some way with the other viral proteins. It was recently demonstrated that αCD19-CAR transgene expression during lentivirus manufacture can have an impact on potency by directing the lentivirus to off-target cells expressing CD19. This work was based on a clinical observation that CAR-T cell treatment of a B-cell leukaemia patient failed due to unintentional targeting of residual malignant B-cells. Careful *ex vivo* transduction of enriched T-cell populations mitigates this aberrant pseudotyping with the CAR-T therapy. However, aberrant pseudotyping of vector for *in vivo* CAR-T therapies will have significant impact on transduction efficiency.

LTR mediated RNA splicing of the viral genome transcript has been shown to be a principal driver of transgene breakthrough expression. We present data that supports splicing as the mechanism for transgene expression. We also present a novel approach for the modulation of vector genome splicing which can reduce transgene breakthrough expression by up to 1000-fold. This approach could not only bring benefit to reducing aberrant pseudotyping, it can also help with the production of sequences which are prone to splicing using current methods.

P47

Adeno-Associated Virus (AAV) Production: a collaborative approach to accelerate process optimisation

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Pharmaron believes that forming effective partnerships are critical to advance the chemistry, manufacturing and controls (CMC) development of viral vectors to bring these life changing medicines to patients. By combining expertise and knowledge with collaborative working with industrial and academic partners, Pharmaron has developed a scalable and robust multi-serotype AAV manufacturing platform. Effective partnering with upstream experts has enhanced their transfection system for large scale manufacture of AAV viral vectors in suspension cell culture and driven a significant increase in viral titre and infectious particles. Further, through collaborations on purification science, Pharmaron has developed a toolbox approach to purify different AAV serotypes and products. Through this development they have carefully balanced the purity of AAV vectors, the separation of genome containing (full) from genome-free (empty) AAV capsids and improved process recovery using Pharmaron's state-of-the-art AAV platform

analytics. This presentation will show how working collaboratively with strategic industrial partners is key to continuously improving Pharmaron's processes and advancing gene therapy innovation into commercial solutions.

P48

Optimisation of a high throughput residual DNA Picogreen assay for analysing lysate samples from AAV production without GFP interference

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Adeno-associated viruses (AAV) are commonly used gene therapy vectors. Production of AAV happens within a producer cell line. During the harvest stage, nuclease treatment is carried out to digest residual DNA. Regulatory guidelines recommends that residual DNA should not be above 10ng/dose within administered drugs. Assays used to measure this are PicoGreen® and residual DNA qPCR. GFP is commonly used as a reporter gene in AAV process development work. During such AAV production, the producer cell line is transfected with GFP plasmid, which leads to the expression and presence of GFP protein in lysate. This causes an interference with the measurement using the picogreen dye due to them sharing similar emission wavelengths. This results in an inaccurate reading for the residual DNA concentration.

We have developed and tested a streamline workflow to apply the WAKO purification kit for the purification of DNA in lysate samples prior to the picogreen assay. The WAKO kit purifies DNA through first solubilizing the proteins and lipids within the samples, and then precipitating the DNA with glycogen. It demonstrated good repeatability with a CV<20%, with some evidence of linearity and accuracy. The lack of signal from the purified samples in the absence of the picogreen dye suggests that GFP interference has been completely removed by the WAKO kit. Hence, the WAKO purification kit allows the measurement of residual DNA concentration from lysate samples using the picogreen assay free of GFP interference, which facilitates the high-throughput optimisation of nucleic acid removal strategies.

P49

Investigating the extracellular microenvironment as a potential target to monitor and control large-scale haematopoietic differentiation

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Allogeneic stem cell-derived therapies are being investigated for several indications. In-vitro haematopoietic differentiation of pluripotent stem cells (PSCs) holds great promise for the development of novel therapies, however in many cases requires large cell numbers per dose, necessitating scalable manufacturing approaches like automated bioreactor systems over planar

flask technologies. Despite the drive towards automation, cell therapies are inherently more prone to variability, with increased risk of batch failure and costs if processes are not sufficiently controlled. In-process analytics can help maintain, control, and reduce risks of failure; however, it is difficult to apply analytics that are used for monitoring expansion and differentiation in planar flasks to bioreactor systems.

To address these challenges, we explored the extracellular microenvironment during haematopoietic differentiation, analysing exosomal miRNAs. Several screening methods have been applied to the miRNA isolated from spent media to perform multi-parametric analysis for monitoring future large-scale differentiation processes.

Our initial proof of concept work has revealed potentially novel miRNA markers in the haematopoietic differentiation process for spent media via Illumina sequencing and NanoString Technologies. These markers have shown consistent differential expression across all the platforms assessed. In collaboration with Earlham Institute, we have successfully characterised a process for generating hematopoietic progenitor cells from iPSCs using miRNA sequencing and therefore, identified the extracellular microenvironment for monitoring large-scale haematopoietic differentiation.

P50

Non-viral delivery of CRISPR activation tools for directed differentiation of induced pluripotent stem cells

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Induced pluripotent stem cells (iPSCs) are a promising solution for allogeneic cell therapy production. To enhance their potential, cell engineering methods have been applied to modify their genome and/or promote their differentiation. This is made possible by the delivery of cell engineering tools to iPSCs, which has been demonstrated through viral and non-viral delivery methods.

To support the development of the next generation of allogeneic cell therapy, we are establishing an iPSC directed differentiation workflow based on non-viral delivery of CRISPR-mediated transcriptional activation (CRISPRa) tools, aiming at the transcriptional activation of four key factors leading to the differentiation into haematopoietic progenitors.

We initially optimised the non-viral delivery process by electroporation, achieving up to 95% transfection of a dead Cas9 (dCas9)/CRISPRa complex multiplexed with four guide RNAs for the selected transcriptional factors. Additionally, we established flow cytometry and droplet digital PCR analytics to evaluate the levels of gene activation. Next, we have implemented the workflow into a haemato-endothelium differentiation protocol to investigate the benefits of a directed differentiation strategy to improve allogeneic therapy manufacturing.

Our current work is focusing on benchmarking alternative non-viral delivery technologies including lipid nanoparticles, which we aim to implement in a full directed differentiation process in stirred tanked bioreactor. We anticipate that building the bioprocessing and analytical capabilities with innovative technologies has the potential to accelerate the development of the

next generation of allogeneic therapies as well as establish a testbed for technology developers to evaluate the performance of their solutions against industry standards.

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Developing stem cell and gene therapy for VPS33B deficiency

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Arthrogryposis Renal dysfunction and Cholestasis syndrome (ARC) is an autosomal recessive multisystem disorder caused by mutations in VPS33B or VIPAS39. Most patients die of severe infections or bleeding <18 months of age. VPS33B-VIPAR complex regulate protein trafficking and biosynthesis of specialised organelles. Bleeding defects in ARC result from abnormal platelet α-granules synthesis in megakaryocytes. Infections in ARC may be due to a defect in phagosome-lysosome and endosome-lysosome fusions in macrophages and other immune cells.

In this project we aim to develop ex vivo gene therapy to prevent fatal consequence of ARC such as bleeding and infections. Ex vivo lentiviral gene therapy of haematopoietic stem cells (HSC) has shown promise in humans and animal models of genetic disorders affecting immune and haematological systems for over two decades. We have developed a lentiviral vector LV.EF1α.VPS33B and tested it in a tamoxifen inducible ERT2-Cre-VPS33Bfl/fl mouse model. When induced post developmentally, this mouse model displays the bone marrow abnormalities mimicking those observed in ARC patients.

1 week post tamoxifen induction some of the knock-out mice were sacrificed, HSC were treated with LV.EF1α.VPS33B and infused into surviving tamoxifen induced Vps33bfl/fl-ERT2 mice after irradiation to secure engraftment. A 6-fold increase in the expression of VPS33B was detected in treated mice compared to the wild type mice with >2 vector copy number per cell. The treated mice demonstrated improvement in skin phenotype, normalisation of the spleen size and survival for the first time, providing proof of concept for this treatment approach in ARC.

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Development of a tumour selective precision immunovirotherapy expressing immune checkpoint inhibitors targeting LAG-3

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Oncolytic viruses (OV) have significant potential to induce immunogenic cell death (ICD), turning immunologically “cold” tumours “hot”. This potential can be enhanced when combined with

immune checkpoint inhibitors (ICI), such as LAG-3, which has been highlighted as a promising target for cancer therapy, as it acts as handbrake on immune cell activation following T-cell engagement and is also involved in T-cell homeostasis. However, some tumours appear refractory to this therapy, whilst systemic administration is associated with significant systemic toxicities. Combining OV and ICI into single agents capable of targeting tumour cells following intravenous delivery and mediating high level, tumour selective overexpression of ICI locally within the tumour microenvironment may enhance “on-target” and minimise “off-target” activity of immunotherapies.

Through extensive re-engineering of the viral capsid, our lab developed a tumour selective oncolytic adenovirus termed $Ad5_{NULL}$-A20 which infects cells expressing the tumour selective integrin, αvβ6. We engineered $Ad5_{NULL}$-A20 to overexpress a scFv-Fc of an anti-LAG-3 antibody. We demonstrate that in vitro, this $Ad5_{NULL}$-A20.LAG-3 readily infects αvβ6 integrin positive tumour cells, expressing and secreted the scFv-Fc. The secreted scFv-Fc is completely functional and was shown to be as effective as the LAG-3 antibody as it binds and blocks LAG-3 mediated signalling in vitro at the similar concentration.

In conclusion, our study demonstrates the potential for a precision virotherapy to selectively transduce αvβ6 integrin positive tumour cells, expression and secreting a virally encoded immune checkpoint into. This has the potential to significantly “heat up” the tumour microenvironment and therefore has significant translational potential.